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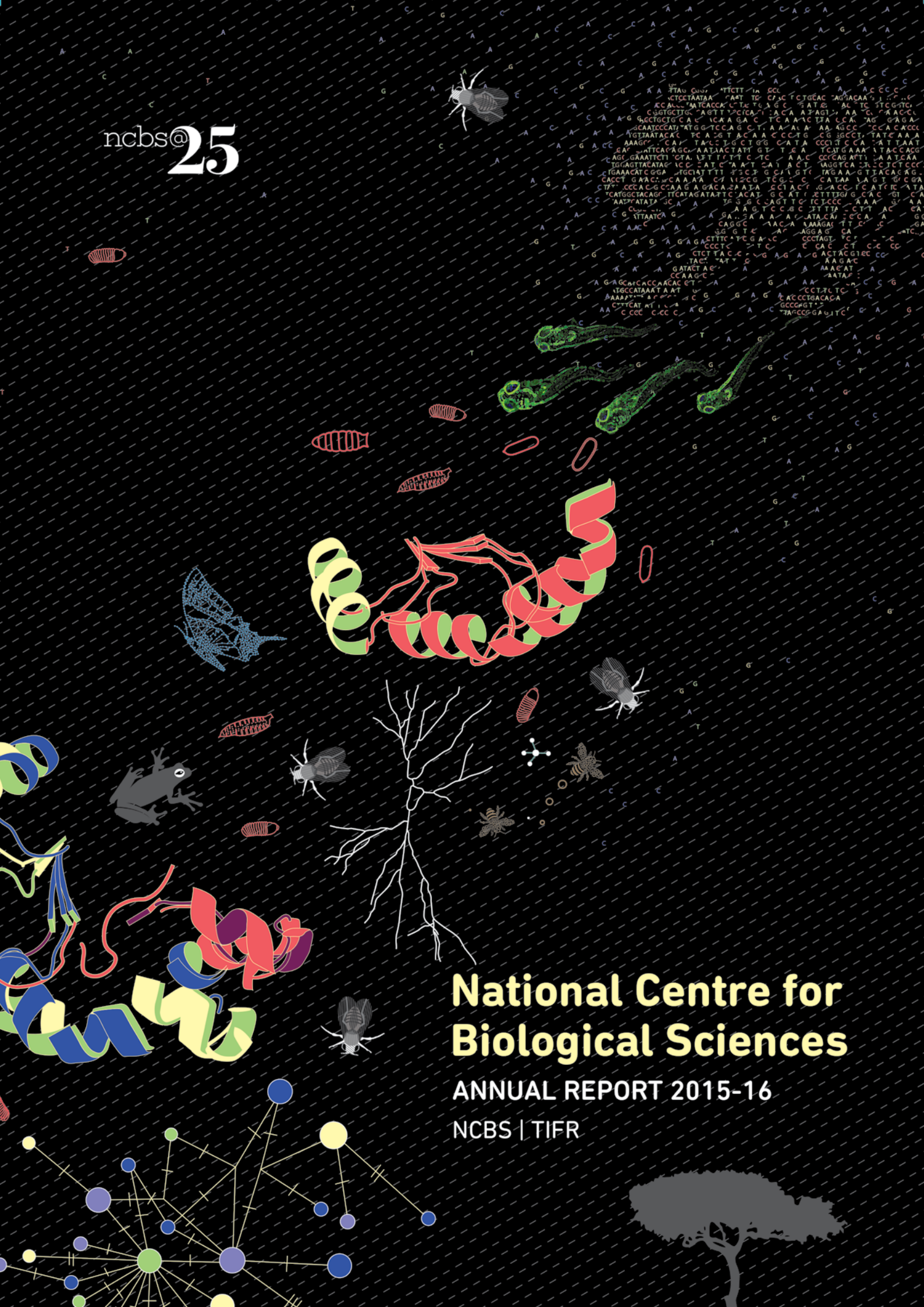
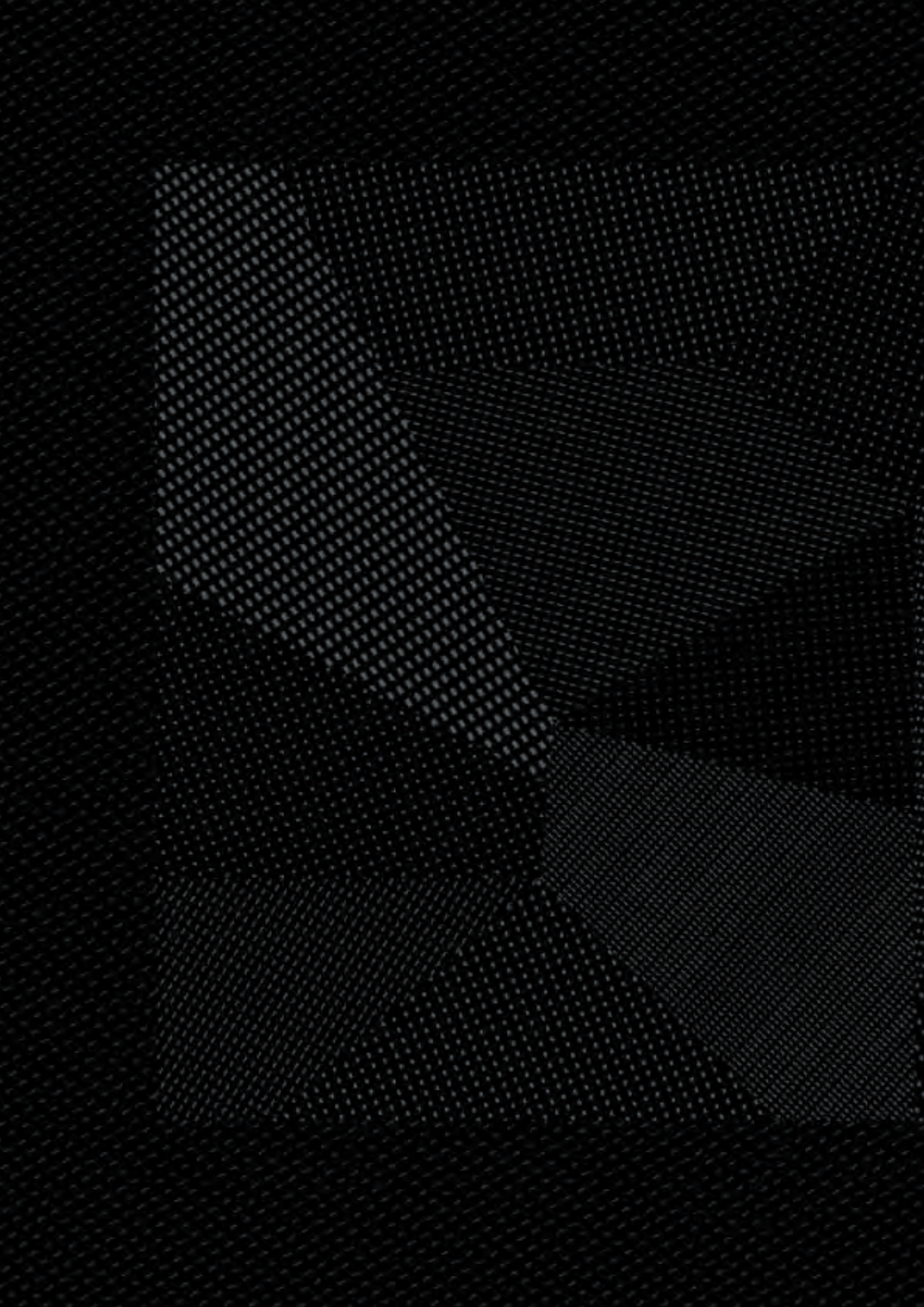


National Centre for Biological Sciences

ANNUAL REPORT 2015-16

NCBS | TIFR



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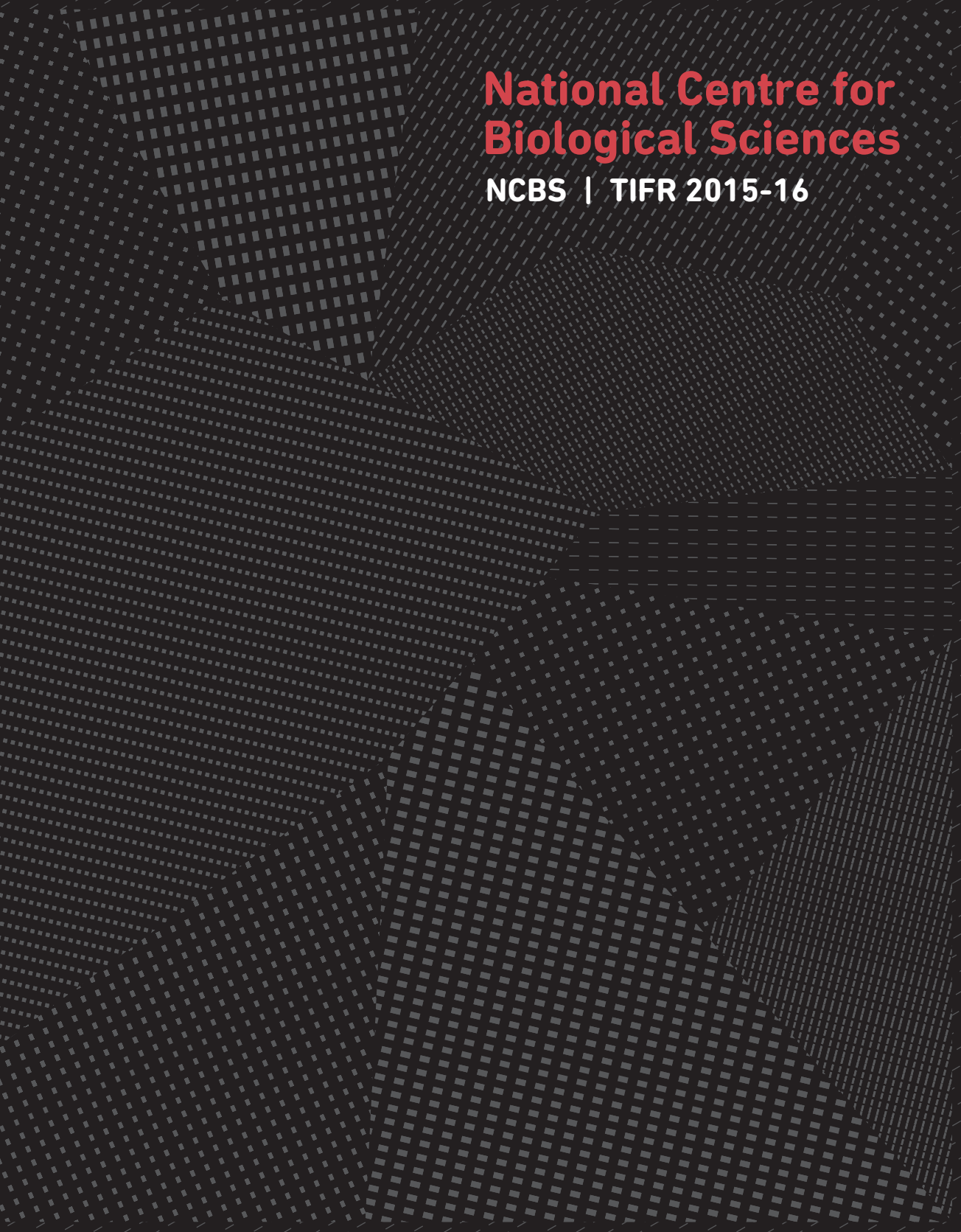

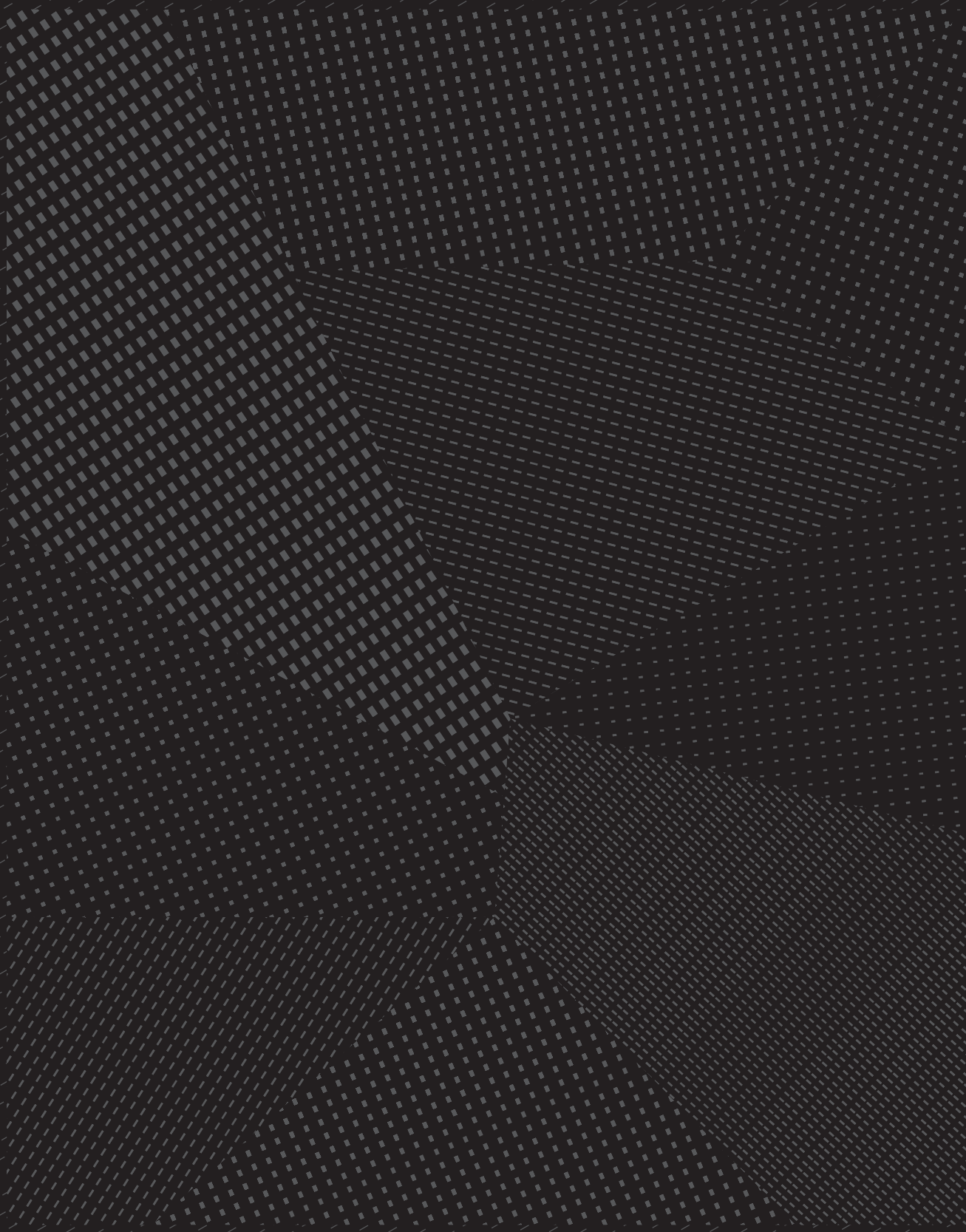

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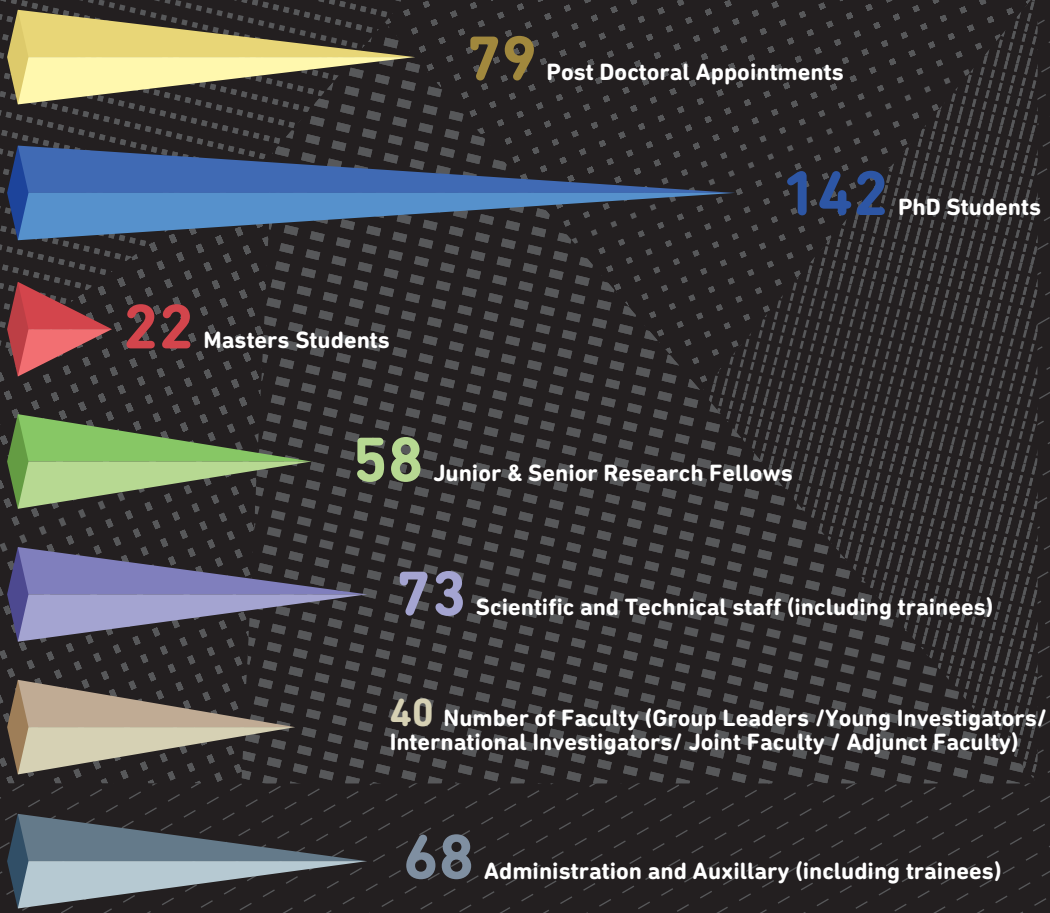


Director's Note

This year NCBS will commemorate its Silver Jubilee. There is a huge buzz on the campus with a number of Conferences, and Workshops spanning the range of biology, occurring throughout the year on the campus. This will culminate in our customary Annual Talks, 11-14th Jan 2017, when we plan to have a celebration for our Silver Jubilee.

As we reach this milestone, we are better placed to acknowledge and appreciate our past. This means documenting and archiving our inception and history, from the distant rumblings of creating a Molecular Biology Unit at TIFR in Bombay to Obaid's (Obaid Siddiqi, the founder of NCBS) decision to expand Biology outside Colaba, to the approval of the NCBS 'project' by the Union cabinet on 22nd October 1991. Archives@NCBS has now begun in earnest, and is also fueling a sizable exhibition on the History of NCBS put together by the Science and Society program. We look forward to opening this exhibition during our Annual talks.

Our past is also encoded in our Alumni. We now have a sizable number of individuals who have been through our portals; students, post-doctoral fellows and also some faculty spread all over the world. An Alumni association was formed earlier this year, and we will have our second Alumni meeting in December, where we hope many of our far flung NCBS'ites will descend on us for old times' sake, and to help record our history.

To commemorate our Silver Jubilee, we are also expanding many aspects of our Public Engagement and Outreach. With the success of Moth Day where Sanjay (Sane) and his colleagues put together a wonderful day showcasing some exciting experiments to our wider community, we are expanding this to build up Open days for high school students. We are creating a Museum space for more programmatic engagement with our community. We strongly feel that the study of any biological system demands an understanding of its

contingent history, and to communicate the excitement of biology we need to tell a story about our past. A Science Museum will serve to tell stories about our biological past, with the passage of time etched in physical rocks and metaphorical fossils. We are also putting in place a Collections facility, to document our unique biological diversity, and create a type repository, so needed in this country for the scientific study of our biogeography.

This year hosts a feast of science that spans our full range in the campus (https://www.ncbs.res.in/events). In addition, a special seminar series on Ecology and Evolution organized by Deepa Agashe is in full swing with a number of exciting talks that address this growing area at NCBS. This is accompanied by a view from a historical, social and ecological perspective of what we have done to our immediate neighbourhood, and by extension our planet. Mahesh Rangarajan with Jayshree Ratnam and Ajith Kumar from our Wild Life and Conservation Science program, in collaboration with Aparna Banerjee at Science and Society have curated a series of discussions on the Future of Nature, to culminate in a book next year.

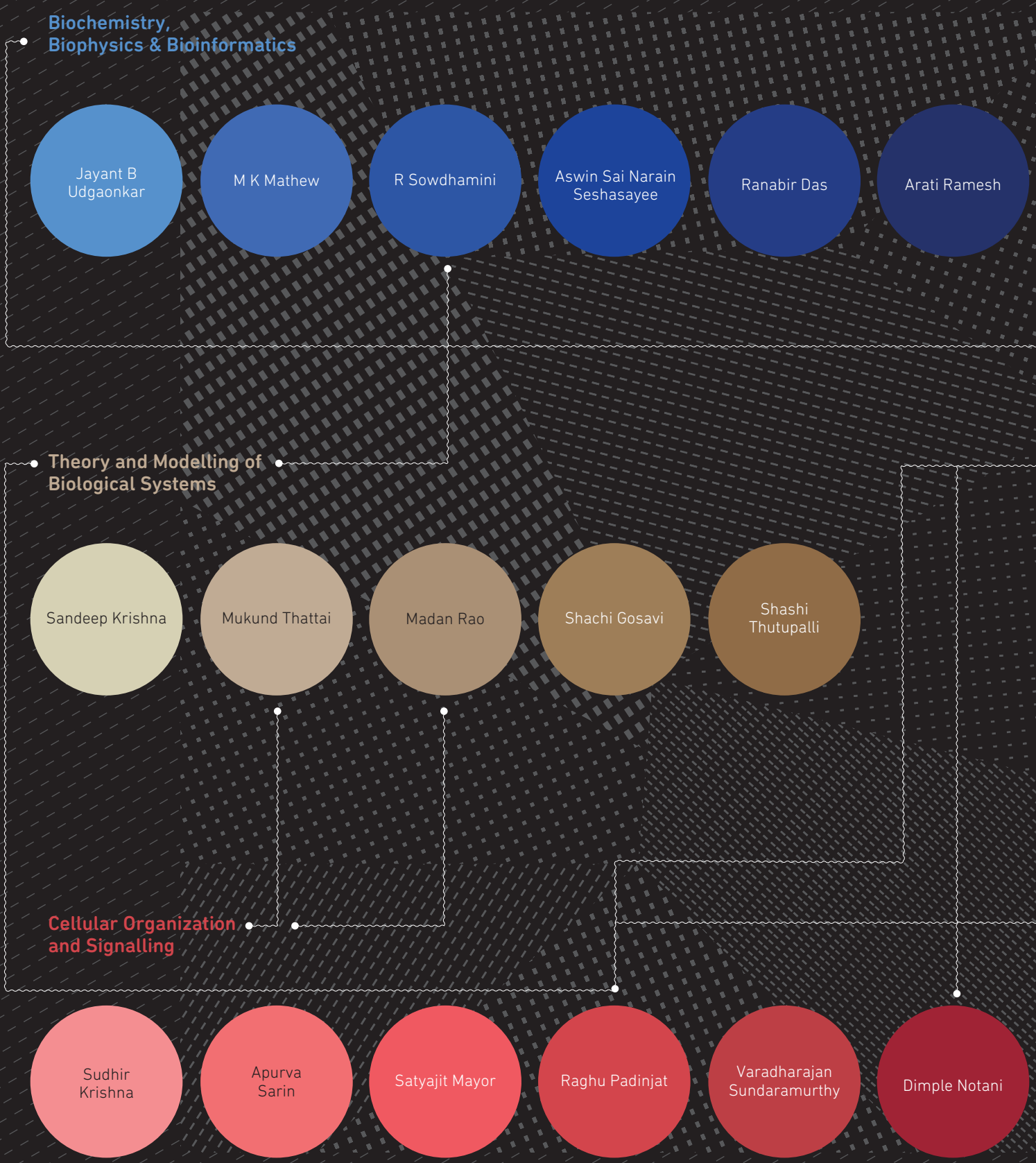
With the establishment of a credible scientific culture, we have also been able to embark on a new and ambitious Program on Chemical Ecology. Inspired by K. S. Krishnan along with Mani Ramaswami and P. Balaram and their explorations of native cone snail species and toxins, Chemical Ecology has become a major magnet for collaborations, especially for our colleagues just North of us in the University of Agricultural Sciences, and a little further North, in the North Eastern limit of India. Given the interest already generated, I am confident in the coming years this program will drive research at our array of field stations, now spread all across the diverse ecosystems of our country.

Closer to home, we are beginning to form a new ecosystem for biological research on the Campus, from fundamental research in individual laboratories, to thematic research with a more translational focus and encompassing a potential for technology development and entrepreneurship in the Life Sciences, 'The Bengaluru Life Sciences Cluster'. This is now under the strategic administrative stewardship of Mr. Knight Paul Pandian, former Financial Advisor to the DBT and Ministry of Earth Sciences. Many challenges, not the least being able to steer a steady ship in seas of inter-departmental angst, loom large but if we can ensure that the three entities NCBS, inStem and CCAMP are in this for the long and productive haul, there is a lot going for this concentration of science and scientists in the North of Bengaluru. Under this cluster initiative, a Big Data and Cryo EM facility for structural biology is underway, which will put our campus on the map with a major Structural Biology Initiative.

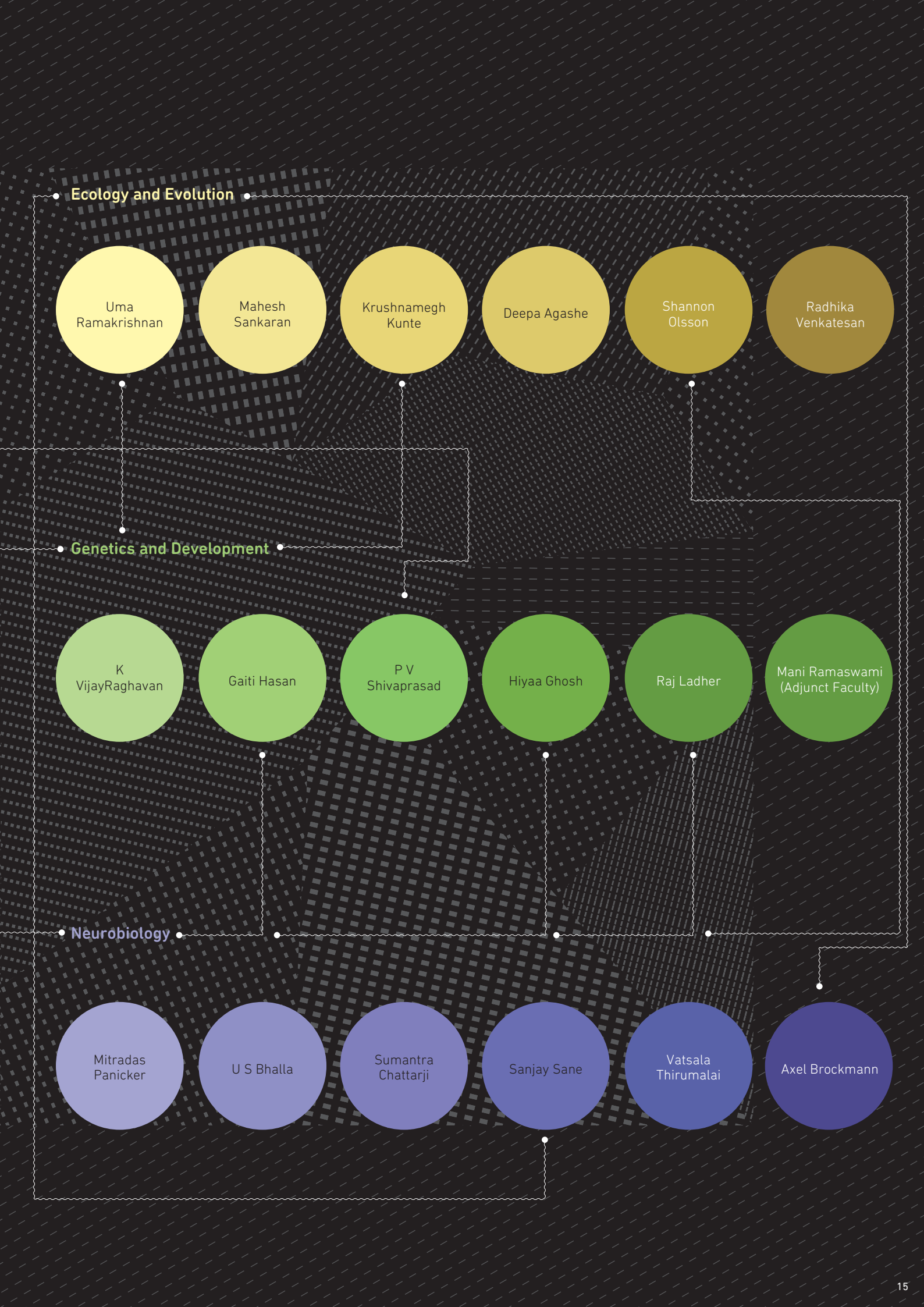
This cluster has also facilitated a major multi-institutional programme linking NCBS, inStem, NIMHANS, and CMC Vellore, garnering support from the DBT, and based on accelerating the application of stem cell technology in human disease to engage with Blood and Brain disorders (ASHD) [https://www.ncbs.res.in/adbs/]. The Brain Disorders programme, anchored at NCBS by Raghu Padinjat and in collaborations with inStem, clinicians at NIMHANS have come together to take on a programme of national dimensions on accelerating discovery in brain disorders using stem cells (ADBS). Over the past years, five clinics at NIMHANS have engaged with patients with familial incidence of clinically diagnosed mental illnesses, and have recruited these families to a major longitudinal study. Deep clinical phenotyping, blood samples, stem cells and genomic information at different levels are being collected from these individuals, and unaffected family members, to develop a long term study of the causes and consequences of these disorders. Support from Kris Gopalakrishnan (Infosys co-founder) and the The Pratiksha Trust set up by Kris and Sudha, have made it possible for us to take on these challenging initiatives.

Our Silver Jubilee year marks a new effort on our side to develop a major Endowment fund for our campus. We are seeking Endowment funds for institution building as well as providing flexible support for a number of initiatives to commemorate this landmark year. With 25 (crores) for 25 (years) as our slogan I am sure in Bengaluru alone we will be able to raise this modest goal from the Friends of Science in this Science City. This is just a beginning and we hope to capitalize on our success this year to create a long-term sustainable fund for the campus.

SATYAJIT MAYOR
Director, NCBS



Several of our investigators have research interests spanning multiple areas of biology and only a few of their affiliations is given here.


Ecology and Evolution
Uma Ramakrishnan
Mahesh Sankaran
Krushnamegh Kunte
Deepa Agashe
Shannon Olsson
Radhika Venkatesan
Genetics and Development
K VijayRaghavan
Gaiti Hasan
P V Shivaprasad
Hiyaa Ghosh
Raj Ladher
Mani Ramaswami (Adjunct Faculty)
Neurobiology
Mitradas Panicker
U S Bhalla
Sumantra Chattarji
Sanjay Sane
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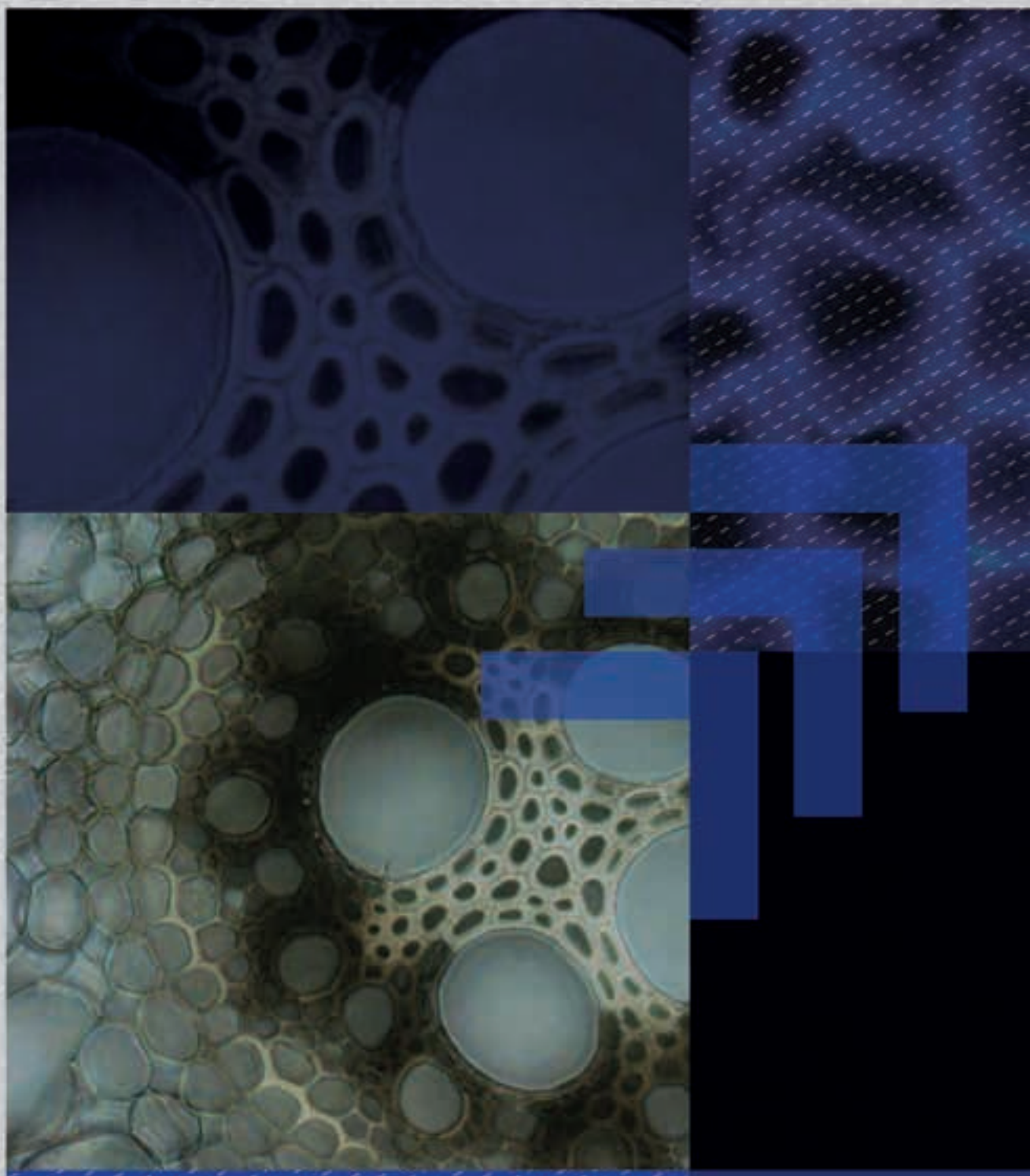

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HOW DO PROTEINS FOLD, UNFOLD AND MISFOLD?

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Jayant B. Udgaonkar

The function of any protein is determined by its three-dimensional structure. We study how a polypeptide chain self-assembles into its correct conformation during folding, how the native structure of a protein dissembles during unfolding, and how a protein forms aggregates when folding or unfolding goes wrong.

The polypeptide chain of a protein must bend, loop, coil, turn and twist itself in a very precise manner while folding into the unique structure that enables the protein to function in the cell. The protein folding problem is to understand how structure develops as a protein folds. It has been a long-standing, unsolved puzzle in biology, whose solution has obvious biotechnological as well as medical implications. In particular, the improper folding of some proteins, and their consequent aggregation into amyloid fibrils, are characteristic features of several neuro-degenerative diseases as well as of the prion diseases. An understanding of the mechanism of protein folding will also lead to a better understanding of the other facet of the protein folding problem, which is: how to predict the functional structure of a protein from the amino-acid sequence that specifies it.

My laboratory uses several small proteins, including monellin, the SH3 domain of PI3-kinase, barstar, tau, α-synuclein, and the mouse prion protein as archetypical model proteins for studying how proteins fold, unfold as well as aggregate. We also study how correct folding is assisted by the chaperone GroEL. We use the tools of protein engineering and physical biochemistry. These include diverse optical spectroscopic methods such as time-resolved fluorescence, as well as mass spectrometry and nuclear magnetic resonance spectroscopy. Our kinetic measurements span the time domain of 40 microseconds to 10 hours.

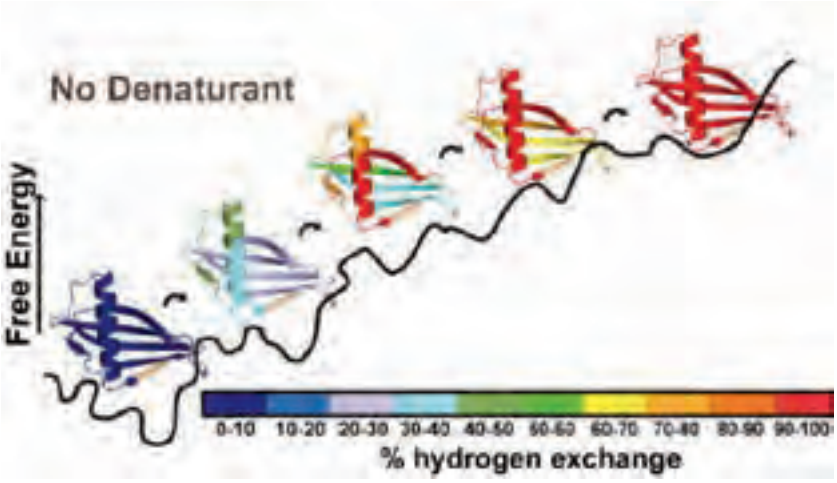

Fig 1: Gradual, diffuse change in secondary structure during the uphill unfolding of monellin in native conditions.

1. Secondary structural change can occur diffusely and not modularly during protein folding and unfolding reactions

POOJA MALHOTRA

An outstanding question in protein folding today is the question of how cooperativity arises in protein folding and unfolding reactions. The structural basis of cooperative transitions is poorly understood. Using hydrogen exchange-mass spectrometry (HX-MS), we have shown that a lack of coupling between interactions in different secondary structural units leads to structurally diffuse folding and unfolding reactions. We have shown that secondary structure formation is dictated by, and occurs proximal to localized tertiary interactions.

2. Microsecond rearrangements of hydrophobic clusters in an initially collapsed globule prime structure formation during the folding of a small protein

RAMA REDDY GOLUGURI

Another outstanding question in protein folding today concerns how initial polypeptide chain collapse primes structure formation. Using a custom-built, continuous flow microsecond mixer in conjunction with multiple structural probes, we have examined the formation of specific and non-specific hydrophobic clusters, and how these clusters evolve before specific structure begins to form.

3. Structural characterisation of a folding/unfolding pathway of a SH3 domain

PRASHANT JETHVA

How different denaturants as well as osmolytes interact with proteins and affect their dynamics and unfolding, is poorly understood. Using HX-MS, we are studying the differential effects of the different denaturants, urea and guanidine hydrochloride, and the counteracting effect of the osmolyte, TMAO on the unfolding pathway of the PI3K SH3 domain.

4. Dissecting the cooperativity of the folding and unfolding reactions of monellin

SANDHYA BHATIA, IN COLLABORATION WITH G. KRISHNAMOORTHY

Delineating the extent of cooperativity and heterogeneity inherent in folding or unfolding reactions is a major challenge. We have used multi-site time-resolved FRET measurements of the equilibrium unfolding of monellin to bring out the segment-wise complexity in unfolding that is masked by ensemble averaging probes.

SELECTED PUBLICATIONS

Malhotra, P. and **Udgaonkar, J.B.** (2016). Secondary structural change can occur diffusely and not modularly during protein folding and unfolding reactions. ***J. Am. Chem. Soc.*** 138, 5866-5878.

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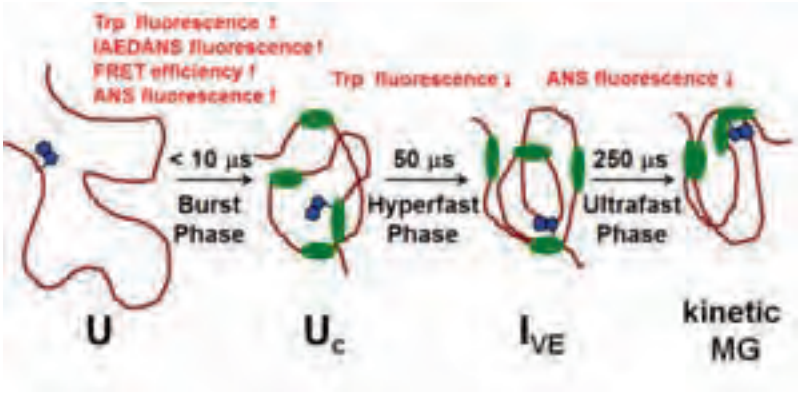

Fig 2: Sequence of polypeptide chain rearrangements that prime structure formation in the kinetic molten globule of monellin.

5. Modulation of the folding pathway of the PI3K SH3 domain by GroEL

NEHA NANDWANI

The mechanism by which the molecular chaperone, GroEL, slows down the folding of the PI3K SH3 domain has been studied. Our data shows that this happens by reversible binding of the chaperone to an early folding intermediate.

6. Folding during an unfolding reaction

SREEMANTEE SEN AND RAMA REDDY GOLUGURI

Microsecond folding experiments have shown that the unfolding of the PI3K SH3 domain commences with a folding reaction in which a tryptophan residue gets transiently buried. We have shown that mutations to hydrophobic residues accelerate this unusual folding reaction.

7. Identification and structural characterisation of the precursor conformation of the prion protein which initiates misfolding and oligomerization

ROUMITA MOULICK

Mutagenesis of the hydrophobic core of the mouse prion protein has been shown to lead to the stabilization of a monomeric intermediate. We have found that the rate at which the protein misfolds to form cytotoxic β-structured oligomers exhibiting partial proteinase K resistance correlates well with the extent to which the intermediate populates, suggesting that it is the direct monomeric precursor initiating misfolding and oligomerization.

8. Pathogenic mutations within the palindromic region of the prion protein induce structure therein and accelerate the formation of misfolded oligomers

A.T. SABAREESAN

Little is understood about how the intrinsically disordered N-terminal region (NTR) of the prion protein modulates its misfolding and aggregation, which lead to prion disease. We have shown that pathogenic mutations in this region cause this region to adopt weak structure as monitored by HX-MS, and thereby to accelerate misfolding and oligomerization. Importantly, this structure formation occurs prior to conformation conversion in the oligomerized protein.

9. Real-time NMR delineates the specific and non-specific effects of salt in inducing the oligomerization and misfolding of the mouse prion protein

ISHITA SENGUPTA, IN COLLABORATION WITH SUHAS BHATE AND RANABIR DAS

The mechanism by which the misfolding of the prion protein at low pH is triggered by the addition of salt, is poorly understood. Our real time NMR measurements have shown that two protein molecules associate in the rate-limiting step of oligomerization, as a likely consequence of general charge screening by the salt. Our experiments also identify a specific salt-bridge interaction that has to be broken for oligomerization and misfolding to proceed.

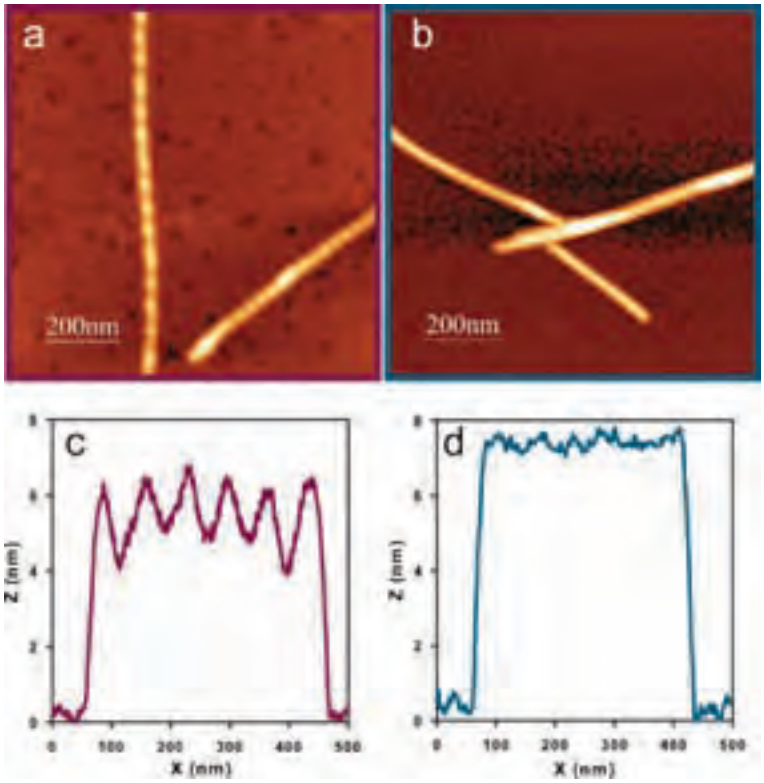

Fig 3: α-synuclein forms fibrils with ribbon and helical morphologies in the presence of thioflavin T, and with only ribbon morphology in its absence.

10. Small molecule-induced reduction in polymorphism in α-synuclein fibrils

HARISH KUMAR

The mechanism of fibril formation by many proteins is difficult to establish because of the heterogeneity of the aggregation reaction. We have shown that in the presence of the small dye molecule, thioflavin T, only ribbon fibrils are formed by α-synuclein, while both ribbon and helix morphologies are seen in its absence. The core of the fibrils is more extended in the ribbon fibrils, and the more structured core results in accelerated fibril formation.

VIEW FROM UNDERGROUND: SALT AND DROUGHT

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M. K. Mathew

Drought and salt are major factors limiting crop productivity. We examine how plant roots handle these stresses. We look at whole root responses as well as cellular adaptations to drought and salt.

Endocytic Mechanisms in Salt Tolerance

ANIRBAN BARAL, IN COLLABORATION WITH PROF. S MAYOR, NCBS

Endocytosis, the internalization of plasma membrane along with embedded proteins and extracellular fluid, is a ubiquitous cellular process in metazoans. Multiple pathways of endocytosis have been identified in animal systems. The *Arabidopsis* root is a well stratified organ composed of distinct cell layers which are clearly demarcated in terms of position, shape (Fig 1. A, B, C), developmental origin and gene expression profiles – and is amenable to imaging in its entirety, thus allowing the study of endocytosis in an intact functioning tissue. Exploiting the optical transparency and physical accessibility of young *Arabidopsis* roots we have explored the full panoply of endocytic mechanisms in the different cell layers. We find that at least three distinct mechanisms of endocytosis operate in the root. A relatively well characterised clathrin-dynamin mediated mechanism operates across all cell layers and serves to take up transmembrane proteins. In addition, a clathrin-independent pathway operates constitutively in the epidermis – the outermost layer of the root. This pathway takes up lipid but excludes transmembrane proteins. Finally, salinity stress induces a clathrin-

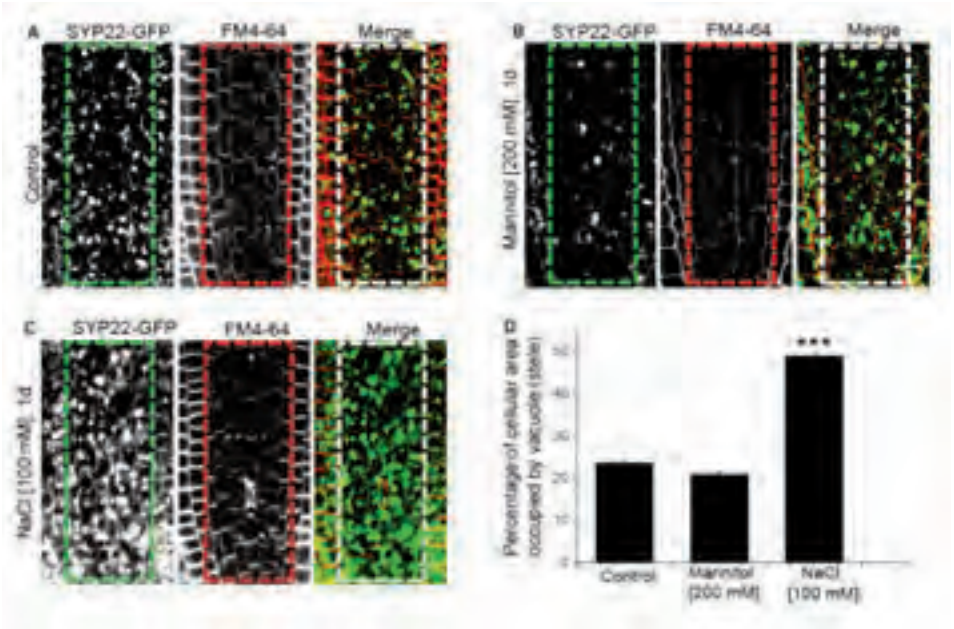

Fig 1: Confocal images of *Arabidopsis* roots labelled with SYP22-GFP, a fluorescent marker of the vacuole membrane, and with FM4-64, which marks the plasma membrane in these images. The box outlines the stele region of the root.

A shows roots of plants under control conditions;

B of plants subject to osmotic stress;

C of plants subjected to NaCl stress.

D shows the fraction of cellular area occupied by the vacuole in each of the conditions A, B and C.

independent pathway in all layers of the root that is catholic in its choice of cargo, and employs molecular components that are not shared with the constitutive clathrin-independent pathway.

Concomitant with the induction of this pathway, we observe the expansion of small acidic compartments into larger vacuole-like structures in inner cell layers. It may be noted that large vacuoles are a feature of the epidermis, but not seen in internal layers. Thus saline stress reprogrammes endocytic pathways and remodels a vital compartment involved in intracellular trafficking.

Mutant plants deficient in the third pathway fail to make large vacuoles in internal cell layers. They are also severely salt sensitive. We speculate that there could be a correlation between construction of mature vacuoles and the operation of the clathrin-independent endocytic pathways. We suggest that the salt-induced pathway of endocytosis contributes to the formation of large vacuoles in internal cell layers and is critical to the mounting of a successful defence against salinity stress.

Root Responses to Drought and Salinity

RUKAYA AMIN, IN COLLABORATION WITH PROF. H.E. SHASHIDHAR, UNIVERSITY OF AGRICULTURAL SCIENCES, GKVK BENGALURU

Salinity and drought adversely affect rice production globally. While rice is very sensitive to salinity, some traditional varieties including Pokkali are relatively salt tolerant. Similarly, the usual practice of growing rice in paddies with submersion consumes close to 5000 liters of water to produce 1 Kg of rice grain. Efforts to breed "aerobic" varieties that can be grown without puddling have been successful, stimulating a major effort to breed varieties that provide good yields with minimal watering. BI-33 is a drought tolerant variety developed by Professor H.E. Shashidhar at the *University of Agricultural Sciences, Bengaluru.*

We have investigated the responses of different rice varieties, including Pokkali and BI-33, to salinity and drought. We find that tolerant varieties grow long roots to mine water from subsurface sources. Deeper layers of soil have limited oxygen, so the tolerant varieties form aerenchyma in the roots to transport oxygen from the shoots to distal portions of the root. Oxygen loss to the surroundings is limited by building a waxy coat to the root. Waxy barriers within the roots are reorganised in a manner to either optimise fluid flow (under drought) or to minimise entry of external fluid directly into the xylem stream (under salinity). These adaptations appear to make a large contribution to the plant's ability to survive the applied stress.

Local vs Global Responses to Salinity and Drought

RUKAYA AMIN, IN COLLABORATION WITH PROF. H.E. SHASHIDHAR, UNIVERSITY OF AGRICULTURAL SCIENCES, GKVK BENGALURU

Drought and salt stress are first sensed by the root system of plants. Many physiological responses, including opening and closing of stomata for gas exchange, are regulated by the stress hormone ABA which is generated in the root, and sent up to the shoot which then synthesises additional ABA to sustain the response. To

SELECTED PUBLICATIONS

Baral, A., Irani, N., Fujimoto, M., Nakano, A., Mayor, S. and **Mathew, M.K.** (2015). Salt induced remodelling of spatially restricted clathrin-independent endocytic pathways in Arabidopsis root. ***The Plant Cell,*** 27, 1297-1315.

Baral, A., Shruthi, K.S. and **Mathew, M.K.** (2015). Vesicular trafficking and salinity responses in plants. ***IUBMB Life*** 67, 677–686.

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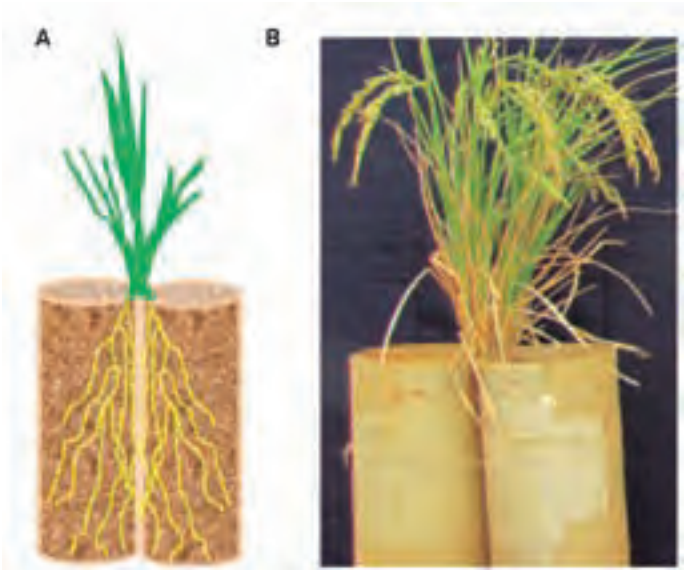

Fig 2:

A. Schematic of the Split Root Experiment. Roots from a single rice plant were separated into two roughly equal portions and each introduced into a separate soil column. Plants were allowed to grow into well watered soil columns before the start of the experiment. During the experiment each column could be either watered regularly or subject to drought or salinity stress.

B. Picture of a plant at the start of the experiment.

address whether responses are systemic or local we have used a split root system (Fig. 2), where the plant roots are divided into two and each half treated independently to water, salt or drought. Four varieties were examined – the salt tolerant Pokkali, the drought tolerant BI-33 and two sensitive varieties Jaya and IR-20. ABA concentrations in the xylem sap increased dramatically after Day 1 in all four cultivars in response to stress on at least one side the of the split root system. The sensitive varieties appeared to derive much of their nutrition and fluid from the watered side when subjected to asymmetric conditions, with roots on the stressed side atrophying. Roots on the stressed side of tolerant varieties, however, underwent anatomical and physiological modifications which favoured fluid uptake and maintenance of xylem sap flow even under these conditions.

COMPUTATIONAL APPROACHES TO PROTEIN SCIENCE

mini@ncbs.res.in

R. Sowdhamini

We employ computational algorithms to enable efficient function annotation of unknown gene products. Ongoing and future science are geared towards modelling protein/ligand interactions with applications in biomedical research and in plant genomics, aided by in-depth and collaborative projects.

Sequence search algorithms for efficient connection of protein families in sequence space

Proteins maybe related to each other at different levels: closely related homologues with adequate sequence similarity or sequentially diverged superfamilies, but with striking similarity in structure and biological function. Several protein superfamilies themselves might share similar folds. While it is a no-brainer-test to show by sequence search algorithms that homologous proteins are indeed related, it can be quite challenging to obtain absolute coverage in sequence searches and to demonstrate that proteins belonging to the same superfamily 'see each other' in sequence searches. We have recently devised computing codes that employ cascaded JACKHMMER searches to connect distantly related proteins which have the potential to be searched within the entire non-redundant sequence database (Kaushik et al. 2015). Sensitive sequence search protocols, using JACKMMER, and other techniques can be quite powerful to perform whole-genome and cross-genome studies of particular classes of protein families – for instance, we had recently employed such a search protocol to search for potential RNA-binding proteins in the entire human genome (Ghosh and Sowdhamini 2016).

Analysis and prediction of protein-protein and protein-ligand interactions

Protein-Protein Interfaces (PPIs) can be good drug design targets and are often required to recognize key residues, namely "hotspots", that are crucial at interfaces. We have devised algorithms to recognize residue hotspots at PPIs, by quantifying the extent of inter-residue interactions. These are coded within PPCheck and PIMA servers, developed in the laboratory (Sukhwal and Sowdhamini 2015; Mathew and Sowdhamini 2016) (Fig 1).

Similarly, protein-ligand interactions can be modelled using docking algorithms and docking can be applied between a protein against large numbers of small molecules in order to recognize those that have drug-like properties. Such 'virtual screening' exercises indeed permit huge reduction in exploration of small molecules that need to be screened using *in-vitro* and *in-vivo* assays. We have recently performed

Fig 1: PIMA results showing the network of interactions amongst protein chains for human hemoglobin. Each protein chain is denoted by a node, where the diameter of each node denotes the size of protein chain. Connected edges confirm physical interactions between chains. The edges denote the strength of interactions at the interface as measured by PPCheck – width denotes the elaboration of residue interface, while the colour coding denotes PPCheck normalised energy values from red (strong) to yellow (weak).

virtual screening on biomedically important proteins such as 5-hydroxytryptamine 2 receptor (Gandhimathi and Sowdhamini 2016), Toll-like receptors (Mahita et al. 2015) (in collaboration with Dr. Mallikarjuna Rao Pichika of International Medical University, Malaysia) and for odourant receptors (Harini and Sowdhamini 2015). The identification of small molecules that can specifically bind to the protein of interest is absolutely crucial in these proteins in order to avoid side effects during drug design. In the case of odourant receptors, such large-scale docking can provide a computational map of the odour affinities for these proteins, which in turn, give glimpses of the combinatorial code in which odour recognition takes place in the olfactory system.

Plant genome analysis and genomics

Plants are interesting model systems whose whole genome information and their response to biotic and abiotic stress conditions offer rich resources of useful data. For instance, our laboratory has participated in the development of computational tools, like STIFAL, to recognize putative binding sites of stress-involved transcription factors, upstream of stress-upregulated genes (Sundar et al. 2008). These data were initially provided for *Arabidopsis* genome (Shameer et al. 2009) and more recently for some rice genomes in the public domain resource, STIFDB2 (Naika et al. 2013). Meta-analysis and data enrichment techniques, using statistical tools and network modelling, reveal popular genes that are upregulated for a variety of tools and frequently used transcription factors. In collaboration with a group in Norway, we recently investigated the choice of transcription factors, when Arabidopsis plants are subjected simultaneously to multiple stresses (Barah et al. 2015). Similarly, in collaboration with Dr. Nataraja Karaba's group of University of Agricultural Sciences, we performed sequence analysis of popular gene products, annotated as hypothetical proteins, which are frequently upregulated in stress conditions in the mulberry plant (Dhanyalakshmi et al. 2016). Such attempts have been streamlined in the form of a webserver for general use in future and validated using RT-PCR experiments as well. Indeed, the laboratory has also been focusing on plant genomics by selecting medicinally important herbal plants, like Tulsi *(Ocimum tenuiflorum)* (Fig 2) and Pirandai *(Cissus quadrangularis)*, for deciphering their draft genome sequences such that it is possible to obtain an early glimpse of the enzymes and chemistry that allow these plants to produce medicinally valuable secondary metabolites (Upadhyay et al. 2015; Gandhimathi et al. 2016).

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Fig 2: Genome sequencing of Tulsi. (a) Different types of Tulsi (b) Metabolic pathway involved in the synthesis of a secondary metabolite, Taxol.

Putative enzymes in the pathway were searched within the assembled genome of Tulsi using sequence information of counterparts in other genomes. The enzyme names show the queries from different organisms, used for the search (names of the plants for the two letter codes: Px-Petunia x hybrid; At-*Arabidopsis thaliana;* Tu-*Triticum urartu;* St-*Solanum tuberosum;* Sl-*Solanum lycopersicum;* Pv-*Panicum virgatum;* Gm-*Glycine max;* Sb-*Sorghum bicolour;* Si-*Setaria italic;* Pt-*Populus trichocarpa;* Cp-*Carica papaya;* Oz-*Oryza sativa;* Vv-*Vitis vinefera;* Zm-*Zea mays;* Br-*Brassica rapa;* Me-*Manihot esculenta;* Bd-*Brachypodium dystachyon;* Hv-*Hordeum vulgare;* Cb-*Clarkia breweri;* Ob-*Ocimum basilicum;* Sp-*Spirodela polyrhiza).

The numbers on the side of each enzyme is the Plantcyc identifier, while there is one EC number for each enzyme at the end.

We will continue to provide computational solutions to biological problems, like connecting distantly related proteins, modeling interactions of proteins with other biomolecules and exploring plant genomes where required in collaboration with other laboratories, in our quest to expand our knowledge of protein science.

ADAPTATION, THE BACTERIAL WAY!

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Bacterial adaptation to their environments is complex and multi-pronged. Not only do they use combinations of regulatory players to determine what molecules to produce when, they adapt often by changing their genetic makeup in small steps. We ask how these phenomena operate using genetics and number crunching with computers.

Silencing horizontally acquired genes

Horizontal gene transfer is a major mode of evolution in bacteria. Genes acquired by horizontal transfer are often not required for growth under comfortable conditions and are kept transcriptionally silent. The central player in such gene silencing is the protein called H-NS. In certain pathogenic bacteria, including Salmonella and the pathovars of *E. coli*, this protein is involved in regulating virulence, and in fact may have been a crucial player in evolving pathogenesis. In laboratory *E. coli*, we have shown that silencing of horizontally-acquired genes by H-NS may be essential for maintaining global gene expression homeostasis. This is a function of combinatorial control, including backup by a homologous protein StpA and a positive feedback loop involving a transcription terminator. An implication of this work for pathogenic bacteria is the hypothesis that over-expression of virulence factors would be a chink in the armour of the bacterium, in part because of collateral damage this would have on 'house-keeping' genes.

Our recent work has revealed a fundamental link indicating the convergence of chromosomal gene organisation and regulatory networks. We have implied that the *E. coli* chromosome encodes evolvability features, which enable the bacterium to undergo structural variations capable of reversing gene expression imbalances caused by regulatory perturbations (Fig 1). Through this work, we have been able to conclude that "mechanisms that ensure evolvability by which gene organisation can be transiently altered to maintain gene expression states might be hardwired into the genome."

Our recent effort to map the supercoiling state of the E. coli chromosome on a genome-wide scale using psoralen crosslinking, DNA microarrays and bioinformatics should be seen as a platform to bring chromosome topology, gene organisation and global regulatory networks together.

Shaping the bacterial chromosome

The organisation of a chromosome can be defined in primary structure by the ordered sequence of genes, and in higher order by its topology. Whereas the functions of local chromosome structure such as DNA bending or wrapping by various DNA-binding proteins are reasonably well established, the selective value of chromosome shapes, and its potential links to gene order, remain nebulous. This is true not only of eukaryotes, but of

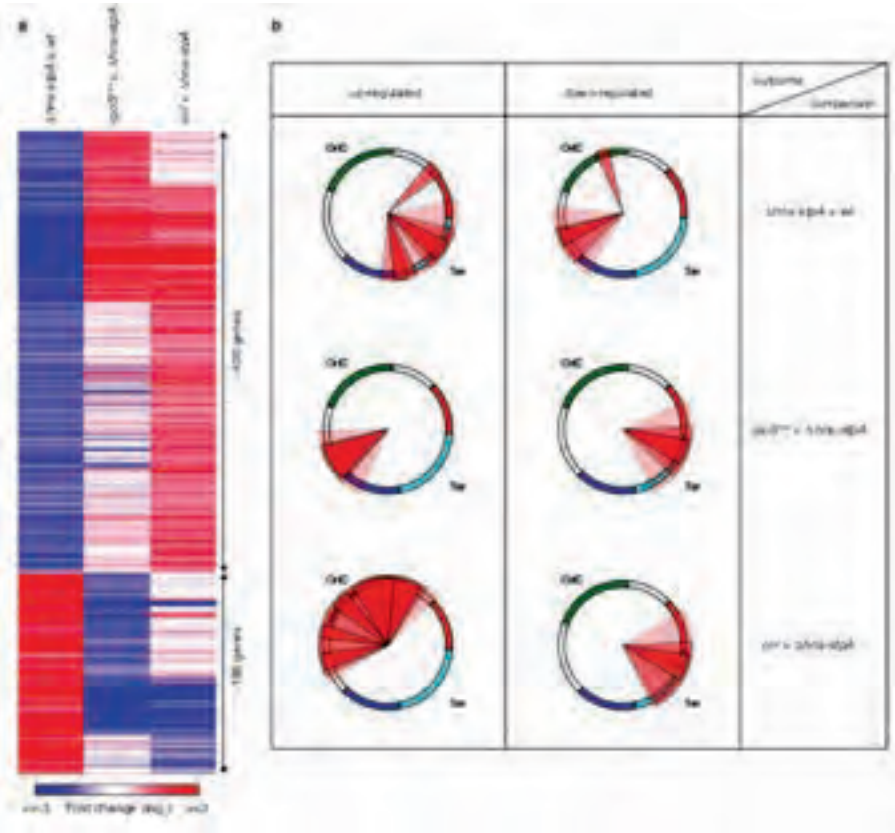

Fig 1: Convergence of structural variation and perturbation of global regulatory networks. The disruption of gene expression homeostasis caused by the loss of the global gene silencing network is partially reversed by a duplication of ~40% of the chromosome centered around the origin of replication (oriC). This figure is reproduced from Srinivasan et al. 2015.

bacteria as well, where chromosomes are compacted to over 1,000 fold, into at least two predominant shapes. One such shape separates the origin (oriC) and the terminus (Ter) of replication, while bringing the two replichores into close contact. These are based on only a few model bacteria, and the present lack of information across many bacteria precludes further analysis of associations between gene order and chromosome topologies. We asked whether we can exploit the wealth of genome sequence data to infer broad chromosome topological features of a bacterial chromosome. Would this enable us to test for associations between chromosome topologies and gene organisation, and to think about the selective value of a given chromosome topology? In summary, our comparative genomic analysis of gene positions across ~300 pairs of closely-related organisms, showed the following: (a) gene order conservation, in terms of the distance of a gene from the oriC, is high; (b) many organisms show a high degree of inter-replichore translocations, which are not merely limited to the inversion-prone Ter or the oriC regions or by phylogenetic distance; (c) translocation maps may reflect chromosome topologies. Thus gene order and chromosome structure may be interlinked.

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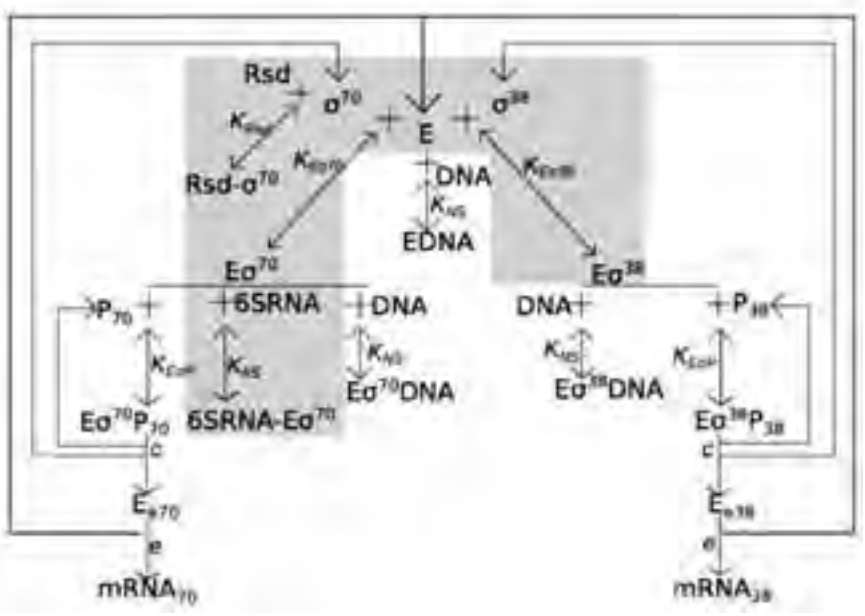

Fig 2: A theoretical model of sigma factor switching in stationary phase. Adapted from Lal et al. BioRxiv. doi: 10.1101/058339

From feast to famine: the switch

In bacteria, the RNA polymerase enzyme carries out transcription of all genes. However, the bacterial RNA polymerase requires a bound sigma factor to recognize promoters and initiate transcription. In the model bacterium E. coli, seven sigma factors compete with each other to bind to RNA polymerase and direct transcription from their target promoters. The most prominent of these are the housekeeping sigma factor S70 and the stationary phase sigma factor S38, which is produced during stationary phase and directs transcription of stress response and starvation-related genes.

The noncoding 6S RNA and the protein Rsd are both abundant during stationary phase and are hypothesized to regulate the competition between these two sigma factors. 6S RNA binds to the RNA polymerase complex containing S70 and prevents it from carrying out transcription, whereas Rsd binds to free S70and prevents it from binding to RNA polymerase. The increased production of both of these regulators in stationary phase is supposed to reduce the activity of S70 and allow S38 to bind to RNA polymerase and direct transcription.

Despite multiple studies, the function of these regulators remains unclear. Why does *E. coli* need two regulators to sequester S70? Which promoters are affected by these regulators and what is their effect on the cell during stationary phase?

We have combined diverse approaches – large-scale gene expression studies, microbiology, biochemistry, and mathematical modeling – to discover the functions of Rsd and 6S RNA during five phases of bacterial growth. Through this work, we have described multiple interactions between Rsd and 6S RNA, and have indicated that these interactions are vital to their function. While previous studies (examining these regulators in isolation) suggested that Rsd has no significant impact on the cell, we show that Rsd and 6S RNA together regulate 40% of the genes in E. coli. We also present the first theoretical model of bacterial stationary phase (Fig. 2), and use this to explain how, by binding to distinct subunits of RNA polymerase, Rsd and 6S RNA have different effects on global transcription.

POST-TRANSLATIONAL MODIFICATIONS IN HOST-PATHOGEN INTERACTIONS

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Viral factors hijack the host machineries to their own advantage. During infection, several host defense mechanisms are destabilized by using the ubiquitin-proteasome pathway. This group studies the interactions between viral factors and host proteins to understand this process.

Post-translational modifications add a completely new dimension to the complexity of cellular processes. For example, covalent attachment of ubiquitin molecules to a protein can change its fate, function or locality. The impact of this modification (known as ubiquitylation or ubiquitination) in diverse cellular mechanisms is a subject of immense interest. The ubiquitylation reaction involves, in general, the sequential action of an activating (E1), a conjugating (E2), and a ligating (E3) enzyme (Fig 1). First, ubiquitin is activated by E1, followed by conjugation of the ubiquitin to E2 via a thioester bond formation. The E2s interact with the class of proteins known as ubiquitin ligases or E3s, which catalyze thioester bond hydrolysis and transfer the ubiquitin to the targeted protein. This process occurs in a highly regulated manner that allows the recognition and targeting of specific proteins. Our lab is interested in studying the role of this modification in the life-cycle of the herpes virus.

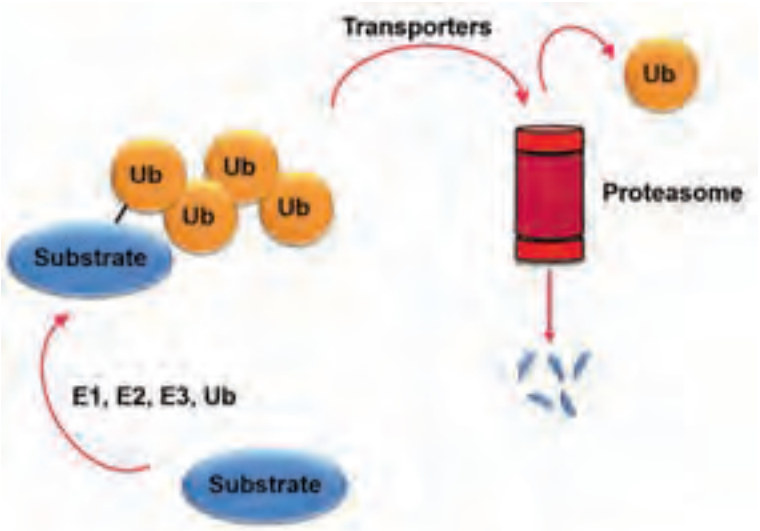

Figure 1: Ubiquitin-Proteasome pathway

Fig 1: The scheme shows the major steps of the Ub-proteasome pathway. Unfolded or tightly regulated substrates are marked for degradation by a process where three classes of enzymes E1, E2 and E3 conjugate the substrate with a chain of Ub molecules. Transporter molecules then recognize the poly-Ub chain and guide the substrate to the proteasome, where the Ub chains are cleaved for recycle but the substrate is degraded into small peptides.

Herpes simplex virus is a member of a large herpes virus family of DNA viruses that cause diseases in animals and humans. There are 8 herpesvirus types that infect humans: herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus, EBV (Epstein-Barr virus), human cytomegalovirus, human herpesvirus 6, human herpesvirus 7, and Kaposi's sarcoma-associated herpesvirus. More than 90% of adults have been infected with at least one of these viruses, and a latent form of the virus remains in most people.

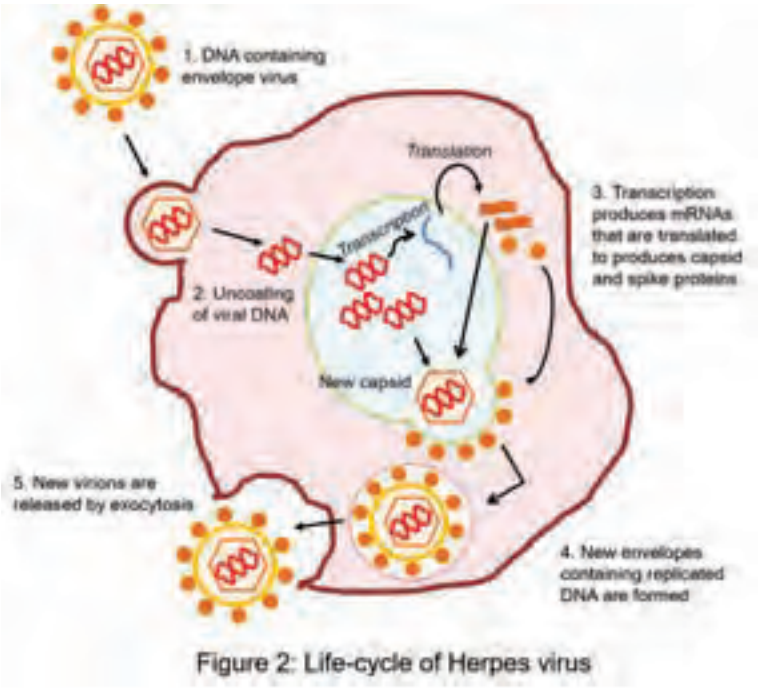

Fig 2: A schematic of the various steps in the life cycle of a herpes virus. Capsids containing the viral DNA fuse with the cell wall to gain entry into the cell and then shed their coats. The viral DNA replicates in the nucleus into multiple copies. Transcription and translation of the DNA produces more envelope capsid and spike proteins to generate new viral capsids that are released by exocytosis.

HSV-1 and HSV-2 cause genital and oral herpes. Lesions due to herpes are painful and lead to physiological morbidity. Neonatal infections of herpes from pregnant mothers to infants are dangerous. According to World Health Organisation (WHO), 80% of infants with disseminated disease die, and those who survive are often brain-damaged. The other serious and systemic manifestations of HSV infection include esophagitis, hepatitis, pneumonitis, meningoencephalitis and retinal necrosis. HSV infection is also associated with a 3-fold increased risk of Human Immunodeficiency Virus (HIV) infection. Indeed, high HSV-2 prevalence areas acquire 40-60% of new HIV infections (Looker et. al. 2008). There is hope that HSV suppressive treatment can both reduce HIV infectiousness and progression.

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All herpes viruses transcribe the viral DNA in the nucleus of the host cell. Entry of the herpes virus into host cells involve complementary interactions between the receptors at cell surface and glycoproteins present at the virus surface, following which the cell membrane fuses with the viral envelope and the viral capsid enters into the cell. Post entry, the viral genome emerges from the capsid and travels to the nucleus. Within the nucleus, replication of viral DNA and transcription of viral genes occurs. Intriguingly, post initial infection, herpes viruses can either exist in a quiescent, symptom-free form known as latent infection, or a productive symptomatic stage known as lytic infection. A small number of viral genes termed latency associated transcripts (LAT) accumulate in some host cells and the virus can persist in the host indefinitely in this fashion. Reactivation from latent to lytic stage is commonly induced by some form of stress. Upon reactivation, cell cycle gets arrested and transcription of viral genes transitions from LAT to multiple lytic genes. Transcription of lytic genes leads to enhanced replication and virus production.

Several important questions are unresolved in the initial stages of lytic infection: By what mechanism does the virus avoid host immune bodies that are designed to repress foreign genome transcription in the nucleus? How does the virus switch between latent and lytic infections? And how does the virus regulate cell cycle arrest to enhance viral replication? Using a combined approach of structural and functional studies, we plan to identify the mechanisms at play during the early stages of infection, by which the herpes virus ensures an effective life-cycle in the host. If identified, these mechanisms could be exciting therapeutic targets for intervention in early stages of herpes infection.

Both, the kinetics of ubiquitin conjugation and the events post conjugation are equally fascinating. For example, in DNA repair, the ubiquitinated substrate goes on to play an important role in recruiting other DNA repair factors to the site of DNA damage. For example, K63 type poly-ubiquitinated chromosome factors recruit BRCA1 associated complexes at the site of a double strand break. BRCA1 is an ubiquitin ligase that is critical for DNA repair by the homologous recombination pathway. Mutations in mammalian genes encoding the breast and ovarian cancer susceptibility proteins like BRCA1 are associated with developmental abnormalities and tumorigenesis. In contrast, during protein regulation by the 26S-proteasome pathway, the poly-ubiquitinated substrates are transported to the proteasome, where they are identified, linearized and degraded into small peptides. Our lab is interested in studying the kinetic events in these pathways involving the ubiquitinated substrate. Using a combination of various techniques involving structural biology, NMR spectroscopy, biophysical measurements, molecular dynamics simulations and biochemical assays, we plan to study these events that are critical for cell survival.

STRUCTURAL STUDIES OF RIBOSWITCHES AND RNA-BINDING PROTEINS IN BACTERIA

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We are currently focused on understanding structural and functional mechanisms by which non-coding RNAs bind intracellular metabolites to control the expression of downstream genes. We are particularly interested in understanding their roles in bacterial pathogenesis with an emphasis on mycobacterial species.

Bacteria respond to their environment through a carefully orchestrated regulation of genes. A major mode of gene-regulation in response to changing environments is via non-coding RNAs wherein metabolite-sensing RNAs and RNA-protein complexes control processes of growth, metabolism, adaptations and responses to changing environments. The overall aim of our research is to identify cellular pathways that are regulated by RNAs, determine how different classes of RNAs create the functional and structural diversity required to bind proteins and metabolites and ultimately understand and exploit interactions that control the ability of some bacteria to cause disease. We are addressing these questions using a combination of X-ray crystallography, RNA-protein biochemistry, biophysical and bioinformatics approaches.

Currently our group is focused on a class of non-coding RNAs called riboswitches, which directly bind cellular metabolites to control the expression of downstream genes. These sensory RNAs adopt complex structures that are fine-tuned to recognize their cognate ligand. We are developing generalized methods by which to pull-down and identify cellular metabolites that interact with predicted riboswitches in bacterial genomes.

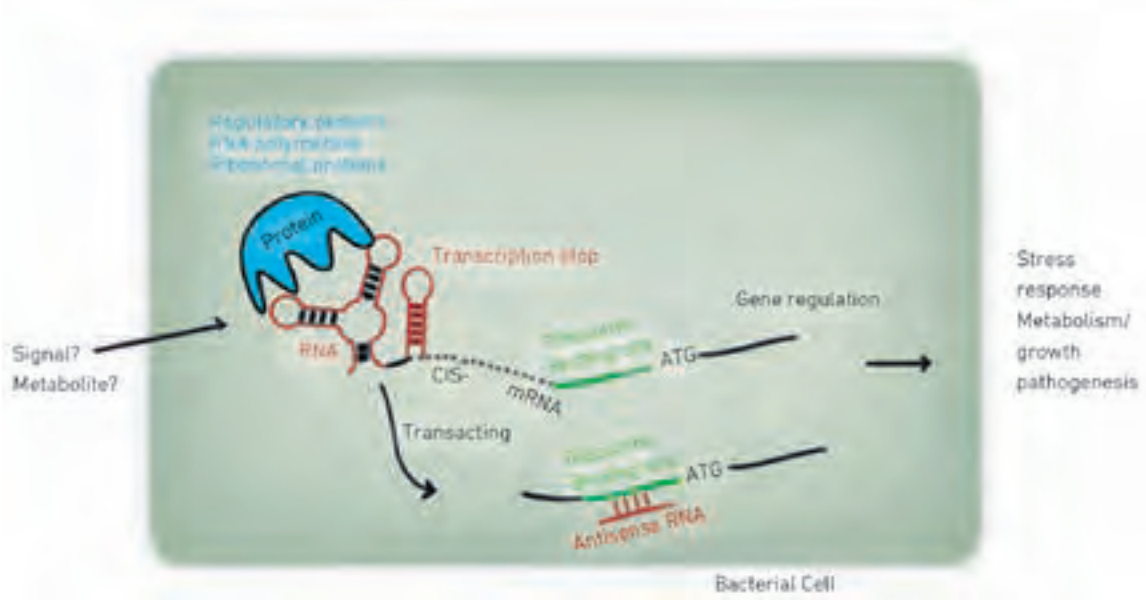

Fig 1: Bacterial gene regulation by ligand sensing RNAs and RNA-protein complexes

Given that riboswitches are highly specific in recognizing their cognate metabolite and that RNAs are easily amenable to *in vitro* evolution, we are also exploring ways to design riboswitch-based fluorescent biosensors for detecting intracellular fluxes in key metabolites.

In a distinct project, we are investigating the role of RNA-protein complexes in controlling bacterial metabolism. Certain classes of bacteria utilize ethanolamine as a nutrient source, and we have discovered that the process of ethanolamine breakdown is tightly regulated by a conserved RNA motif. We find that this RNA motif can adopt two different conformations – one that terminates transcription and the alternate that acts as an antiterminator. The antiterminator specifically recruits proteins containing the RNA-binding ANTAR domain. Ethanolamine induces phospho-activation of the ANTAR protein, leading to structural changes in the protein, resulting in its interaction with the RNA motif. Remarkably, transcription of this RNA motif itself is tied in with a cofactor-sensing riboswitch; thus integrating substrate and cofactor sensing to control a key metabolic process that enables bacteria to utilize ethanolamine as a source of carbon, nitrogen and energy.

The observation that ANTAR proteins are widespread in bacteria led us to investigate the distribution of ANTAR RNAs. For this we used a bioinformatics approach used widely to search for large structured RNAs in sequenced genomes. We have optimised this search method for short RNAs, and have identified over 2400 candidate RNAs across bacterial genomes. We find that ANTAR RNAs occur upstream of diverse genes involved in metabolism, cofactor biosynthesis, nitrate transport, nitrogen homeostasis etc. Importantly, strong candidate RNAs were identified in the genomes of pathogenic strains of mycobacteria. Based on genomic contexts, many of these appear to be linked to oxygen-response and pathogenesis of mycobacteria. Our preliminary results suggest that ANTAR regulation in mycobacteria may be intimately connected to stress-response and likely linked to bacterial dormancy, a phenomenon that poses one of the biggest challenges in targeting mycobacterial infections. We are developing the tools required to investigate the role of these RNAs in pathogenic mycobacteria and are also analysing ANTAR-binding RNAs from these species.

Antitermination mechanisms involving protein-RNA interactions have been described previously. However, ANTAR is not only more widespread than other antitermination systems, but uniquely requires a two stem-loop motif that forms a mutually exclusive structure with a transcription terminator. To understand what determines the selective recognition of conserved RNAs by a diverse set of ANTAR proteins, we are using X-ray crystallography and allied biophysical methods to gain structural insight into ANTAR function and ligand-sensing riboswitches that interface with ANTAR regulation.

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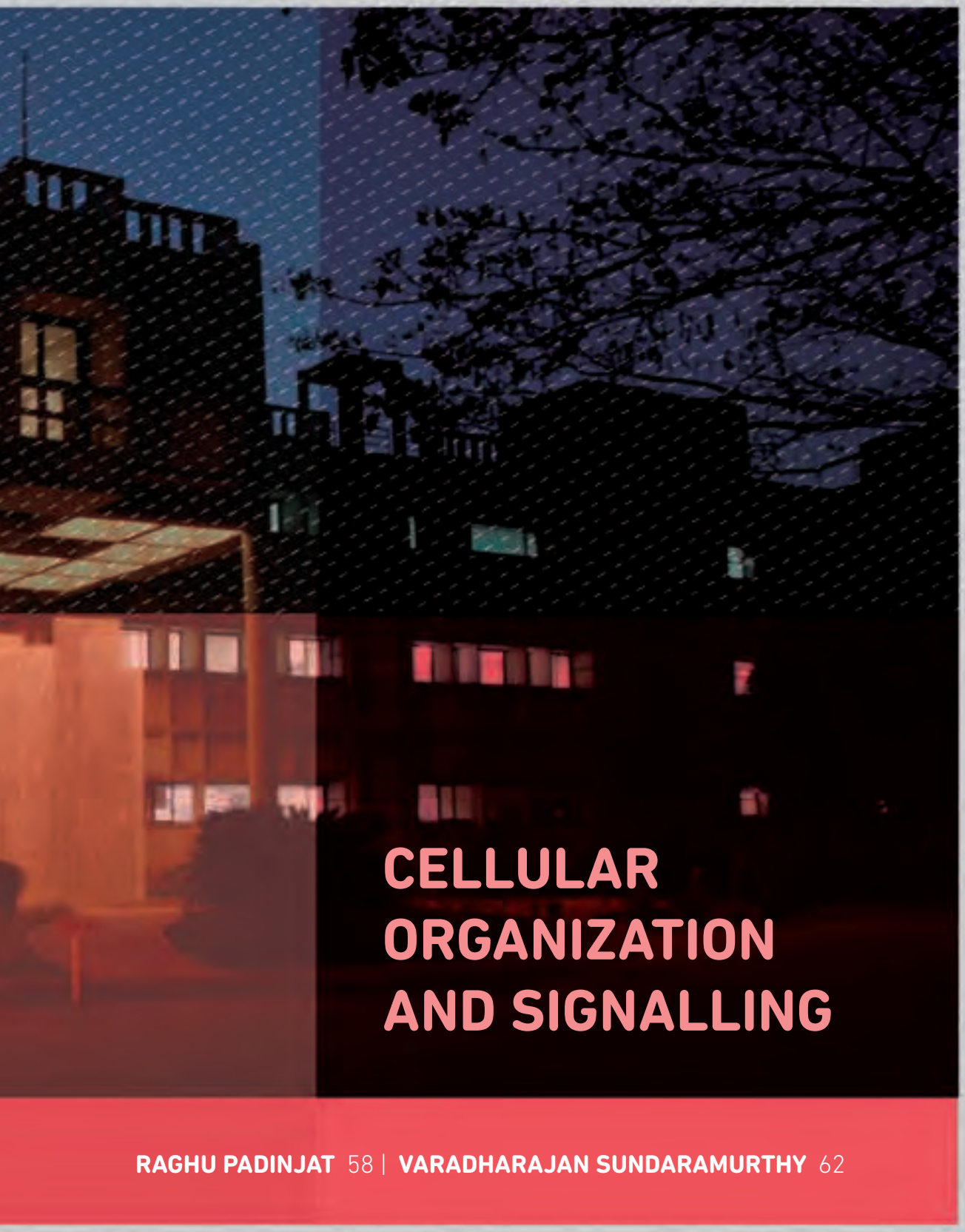

CELLULAR ORGANIZATION AND SIGNALLING

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PATHOBIOLOGY OF CD66+ CELLS IN HUMAN CERVICAL CANCER PROGRESSION

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Our group has two interests, i) understanding the nature of human cervical cancer progression with a particular focus on sub-sets such as CD66+ cells with distinct neoplastic traits and ii) enabling bio-medical efforts such as the "1000 dengue genomes" project.

Human cervical cancers constitute a major burden of female malignancies in our country. In the absence of implementation of effective prevention strategies either through screening and/or vaccination, this burden of cancers will remain a major public health problem for several decades.

The primary causative agent of human cervical cancers are papillomaviruses of the highly oncogenic type. The mechanisms that these viruses use to immortalize and transform cells are rather well understood. However, the additional events in terms of either genetic events and/or signalling mechanisms that sustain these cancers have received much less attention. Given the very low frequency of activated Ras oncogene mutations in human cervical cancers, we were interested in what cellular processes might potentially phencopy this pathway, given the widespread activation of Ras in diverse human solid cancers. Work in the laboratory of Spyros Artavanis-Tsakonas and ours reported in the mid-nineties that human cervical cancers show persistent features of disregulated Notch signalling. We, for the next decade or so focused on asking the question "is there evidence for ligand dependent Notch signalling in human cervical cancers?" Our cumulative data led us to suggest that in the absence of mutations in the Notch pathway genes, a ligand dependent Notch dependent process serves to act as a "second signal" in human cervical cancer progression (reviewed in Maliekal T. et. al., Oncogene 2008).

Meanwhile, work emerging from around the globe in several laboratories, suggested that there is considerable heterogeneity in the pool of human epithelial cells that constitute a given cancer. The emergence, properties and contribution to malignant tumour progression of this heterogeneity has been a matter of considerable vigorous debate. We approached this problem by focusing on growing primary human cervical cancer derived cells under growth conditions that were suggested to optimise the derivation of heterogeneous pools of cells with distinctive tumorigenic properties. These "spheroidal growth cultures" were optimised by our laboratory over a few years and on undertaking a comparative analysis of gene expression of these cells with standard cultures, we identified a sub-set of CD66+ cells that appeared to have potentially unusual tumour promoting properties.

Over the last five years, we have characterised this cell population in three distinct types of complementary experiments. First, we showed that such CD66+ cells generated tumours in mice at a relatively low frequency, are associated with metastasis and dependent on ligand dependent Notch signalling. We followed up this work by looking at a small fraction of CD66+ cells in an established human cervical pre-cancer cell line derived in Laimonis Laimin's laboratory. This cell line harbours episomal copies of the high risk virus HPV 31 and we were able to fractionate consistently a CD66+ sub-set of cells from this cell line with unique migratory properties and a gene expression profile suggestive of a partially differentiated persistent

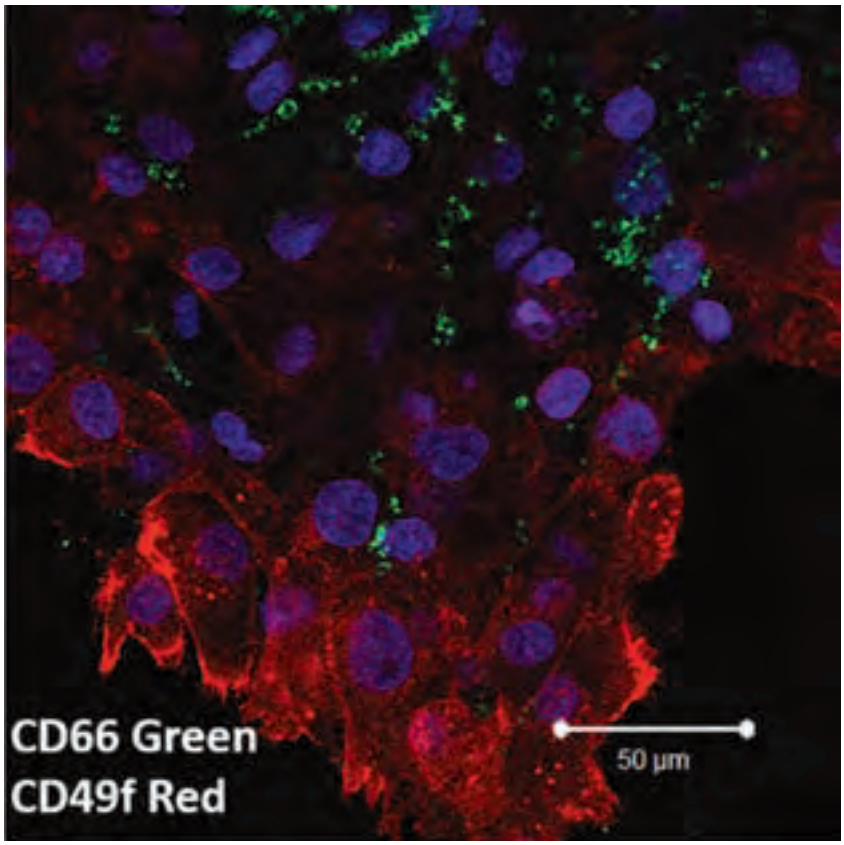

Fig 1: Invading Protrusions from CaSki spheroids in matrigel shows two distinct cellular subsets marked by the expression of CD66 and CD49f.

progenitor. The implication of this data is that CD66+ cells might select for cells with a partial differentiated phenotype in order to upregulate key viral oncogenes and simultaneously retain an element of stemness to maintain the persistence of these cells.

In the third set of experiments, we analysed the relationship of CD66 expression to that of CD49f, a key integrin sub-unit implicated in human cervical cancer stem cells. In a collaborative study undertaken with both the Kidwai Memorial Institute of Oncology and Adyar Cancer Centre implicates CD66 in metastatic progression and CD49f in local recurrence. Given the suggestion that epigenetic regulators might be responsible for driving the progression of CD66+ cells in the absence of specific identifiable metastatic progression mutations, we are currently focusing on the role of Suv39H1. In parallel, we are also beginning to look at the functional role of CD66 and particularly the possible functional role for the src family kinases downstream.

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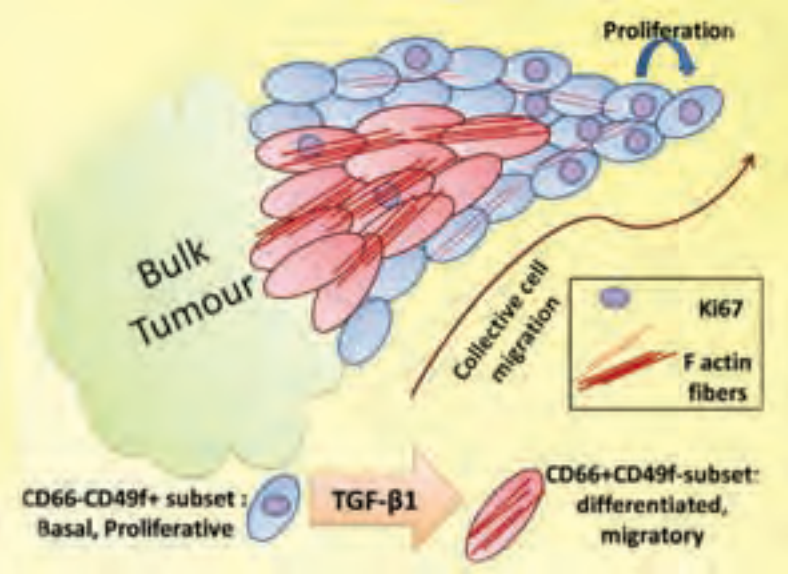

Fig 2: Representative model of how CD66 and CD49f subsets act together in collective invasion and tumour progression.

In parallel, since 2008, we have been engaged in the development of an active engagement with a medical campus (St. John's Medical College and Hospital). From an initial dual teaching effort, we have evolved a laboratory infrastructure in the hospital campus. The hematological genomics and cancer stem-like cells focus has led us to develop genomic platforms. In particular, we have developed a HLA platform which can be used for organ registries. A striking outcome has been the development of a network to study emerging pathogens by next generation sequencing efforts. The current expansion of this particular program is the attempt to sequence a "1000 dengue genomes". Additionally, with a focus on the progression of Chronic Myeloid Leukemia stem-like cells, we have focused on a microRNA-182 and shown an association with tyrosine kinase inhibitor resistance, a mainstay drug in therapies. We have used CRISPR to knock out this microRNA locus in an appropriate cell line and are in the final stages of characterisation of the phenotypes.

SIGNALLING STRATEGIES REGULATING CELL SURVIVAL

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Cell-cell interactions, mediated through cell surface receptor-ligand interactions, are important for the development and functioning of multi-cellular organisms. Notch1 is a cell surface receptor, which regulates many processes including cell survival. Other groups and our own have shown that Notch activity blocks the induction of apoptosis, although the underlying mechanism[s] are not completely understood. Described here, are aspects of Notch anti-apoptotic activity, uncovered by our experiments.

Sirtuin1 regulation of Notch activity

NIMI MARCEL, LAKSHMI PERUMALSAMY, SANJAY K SHUKLA

Notch receptor activity in T-regulatory cells, a cell type in the mammalian immune system, is indispensable for survival in conditions of diminished nutrient availability (Marcel & Sarin 2016). However, the mechanisms regulating receptor activity and ensuing signaling downstream of the receptor remain poorly understood. Notch mediated anti-apoptotic activity, in many contexts, is unusually executed from a cytoplasmic location (Perumalsamy et al., 2009) and thus, is spatially decoupled from its well-characterised transcriptional activities in the nucleus. Despite this distinction, ligand-dependent processing to generate the Notch intracellular domain (NIC), the active signalling intermediate, is common to both aspects of Notch activity. A cell-line based screen for regulators of NIC-mediated anti-apoptotic activity, identified Sirtuin (Sirt)-1, a NAD^+-dependent deacetylase, usually induced in response to energy stress, as a modulator of NIC activity. This was consistent with our earlier observation that Notch activity protected T-cells from apoptosis induced by nutrient deprivation (Perumalsamy et al., 2012).

Experiments in cell lines also established a specific NIC-Sirt1 interaction, as Bcl-xL – a well-characterised antagonist of apoptotic cascades – was not regulated by Sirt1. The loss of activity in an enzymatically deficient form of Sirt1, prompted the identification of putative target (lysine) residues on NIC. Sequential site-directed mutagenesis identified four lysines, which when substituted by charge-conserving arginine, liberated NIC from Sirt1 regulation, albeit without loss of anti-apoptotic activity. The possibility suggested by this experiment, that deacetylation precedes another modification of the same or proximate amino-acid residues, is currently under investigation.

Developing these observations further, we examined Sirt1-Notch interactions in T-regulatory cells (Tregs) derived from mice. The processed Notch1 receptor (NIC) is detected in Tregs in membrane-proximal complexes. This organization is readily visualised in Tregs cultured in conditions that mimic nutrient deprivation, if cells are stained with an antibody that specifically recognizes the processed form of NIC (Fig 1).

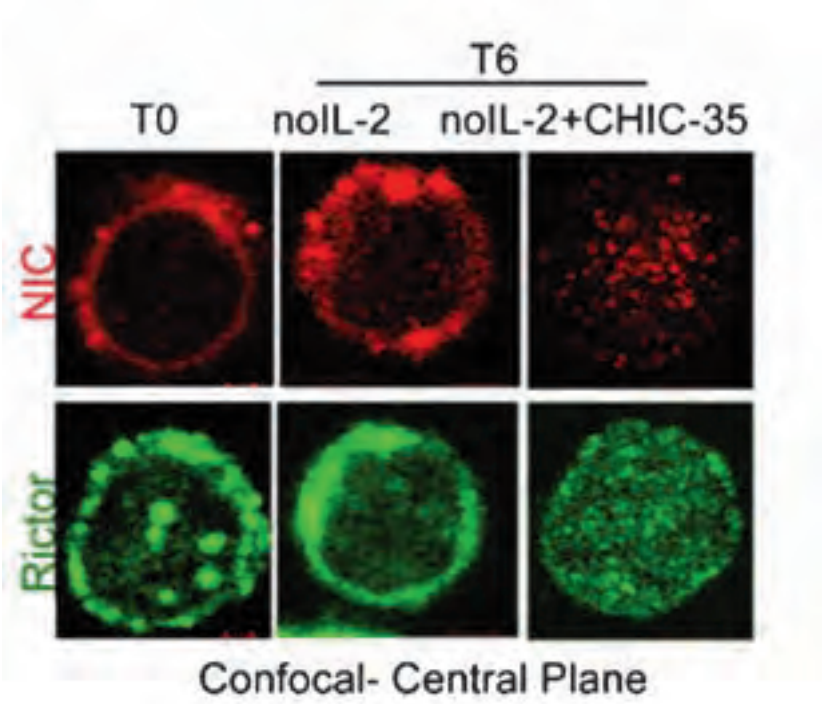

Fig 1: Activated cells (Tregs) cultured in the conditions indicated for 6 hours and stained with antibodies for Notch1 (red) and Rictor (green). CHIC35 is an inhibitor of Sirtuin. Scale Bar: 5µm.

Sirt1 inhibition disrupted this organization and resulted in NIC localizing to the nucleus. Remarkably, this correlated with a functional switch, with nuclear outputs enhanced and a concomitant loss of signalling outputs exemplifying non-nuclear functions of Notch in Tregs treated with the inhibitor. Pursuing this line of experiments, the ablation of Sirt1 by RNA silencing, confirmed the requirement for Sirt1 in the cellular localization of NIC. Not unexpectedly, Sirt1 is present in immune-complexes isolated using NIC as the probe. Functionally, not only survival in assays of nutrient withdrawal, but Notch-dependent suppressor activities *in vivo* were attenuated by the ablation of Sirt1 in Tregs. These interactions were also verified in Notch1 deficient Tregs reconstituted with NIC or the Sirt1-independent NIC recombinant. Thus both the activity and localization of NIC was attenuated by chemical inhibition of Sirt1, whereas, the Sirt1-independent NIC recombinant, was resistant to this perturbation (localization shown in Fig. 2).

While work from other labs (also verified by us) has shown that Sirt1 inhibits NIC transcription, the current experiments in Tregs and cell lines suggest an opposite regulation of Notch activity when signalling from a non-nuclear location. Thus, the Sirt1-Notch interaction may constitute an important checkpoint that tunes non-canonical Notch1 signalling.

Notch1 regulation of calcium homeostasis

SOWMYA LAKSHMINARAYANAN, PRIYANKA KUNDU, NIMI MARCEL

Calcium is a well-known second messenger implicated in a wide array of signalling processes. It is not surprising therefore, that apoptotic signalling frequently correlates with changes in the levels and distribution of intracellular calcium, with uptake in mitochondria, triggering apoptotic cascades. The

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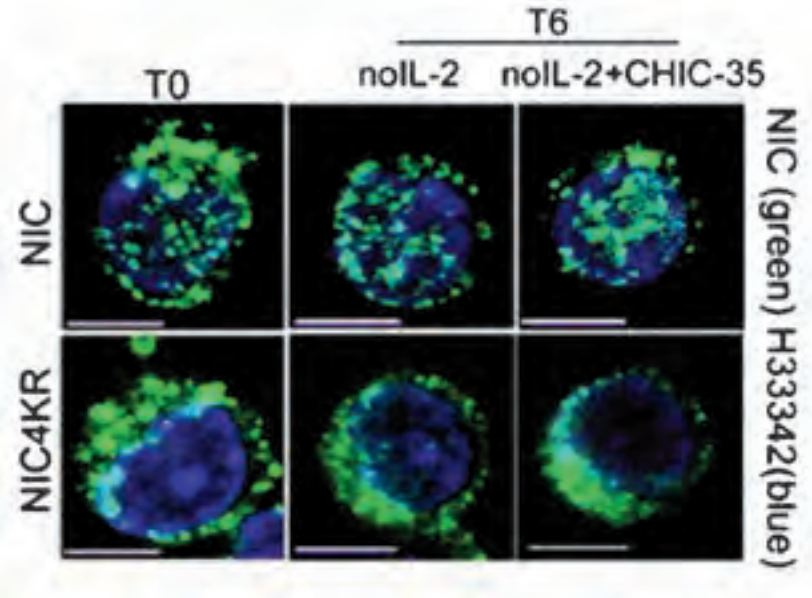

Fig 2: Activated cells (Tregs) expressing recombinant NIC or the modified NIC-4KR construct. Cells were cultured in the conditions indicated for 6 hours and stained with antibodies for processed Notch1 (green) and counterstained with the DNA binding dye Hoechst 33342 (blue). CHIC35 is an inhibitor of Sirtuin. Scale Bar: 5µm.

endoplasmic reticulum (ER) is the major cellular store of cellular calcium and its connections with other organelles and molecular controls underlying the movement of calcium are relatively well characterised. The mitochondria and ER contribute to apoptotic cascades by feed-back loops, which cause calcium release from the ER to activate apoptotic signalling in mitochondria.

Building on an earlier observation of Notch inhibition of apoptosis coordinated by mitochondrial intermediates (Perumalsamy et al., 2010), we investigated if Notch activity influences the distribution and regulation of cellular calcium. Notch integration with the machinery regulating cellular calcium was studied in cellular systems where Notch activity has been demonstrated in earlier work. The experiments revealed that ligand dependent Notch activity lowered Ca^{2+} content in the ER stores, and a concomitant reduction in the amount of Ca^{2+} released in response to stimuli. Further, Notch co-purified with molecular complexes implicated in the regulated movement of calcium from the ER. Together the data suggest, that Notch activity protects cells from damage, by regulating the amount of calcium released from the store.

We are currently exploring the possibility that this underlies Notch regulation of mitochondrial function in cells exposed to nutritional stress, noted in other work (Marcel & Sarin, 2016). Thus, interactions with calcium regulated signalling networks may be an important component of Notch activity in cells.

MECHANISMS OF MEMBRANE ORGANIZATION AND ENDOCYTOSIS

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How does the cell build functional signalling complexes at the plasma membrane? What are the requirements to create a responsive endocytic platform? The principal driving force of our laboratory is to uncover physico-chemical rules that govern local organization of the cell membrane and connect this to cellular and organismal physiology.

Background:

The plasma membrane does not merely separate the outside from the inside of a cell but also mediates bilateral communication. To understand how eukaryotic cells respond and react to their environment, we study how a cell can regulate the local organization of its membrane constituents, while the membrane itself behaves like a fluid matrix. New insights from a variety of studies including that from our laboratory, show that the local chemistry of 2D plasma membranes is finely tuned. It also appears far from an equilibrium mixture. We are developing a new framework wherein the cell membrane behaves as an active composite, with the underlying dynamic cortical actin filaments controlling the local composition of membranes. There are numerous offshoots from such an understanding of membrane organization. Among them, we now seek to explain how cells can construct signalling complexes and sort membrane constituents in response to their environment. The cell membrane is also the site for assembling endocytic machinery, in response to a number of extrinsic and intrinsic cues. To broaden our understanding of membrane homeostasis, we also study endocytic mechanisms, in particular, a class of non-canonical endocytic pathways that function in the absence of both clathrin and dynamin. This pathway is important for maintaining membrane composition and tension in cells and in the control of signaling output activated from morphogens within tissues.

Specifics:

We are now involved in several specific lines of inquiry based on the broad programme articulated above.

1) Tool development for nanoscale imaging

THOMAS VAN ZANTEN AND CHITTASPANDINI KULKARNI

Over the years we have developed microscopy tools and techniques based on Försters Resonance Energy Transfer (homo-FRET) to study the organization of cellular components in living cells at the nanometer scale, well beyond the diffraction limit of light. In collaboration with Anil Kumar (IIT Madras) we are currently developing new lasers for super-resolution STED imaging. Additionally, based on recent findings from our laboratory, we are pursuing a number of avenues to directly observe organization and dynamics of molecules in the cell over the full range of temporal and spatial scales. These include cryo-EM, super resolution and fluorescence fluctuation based methods. These are necessary for drawing any conclusion about the nature of the living actin membrane composite.

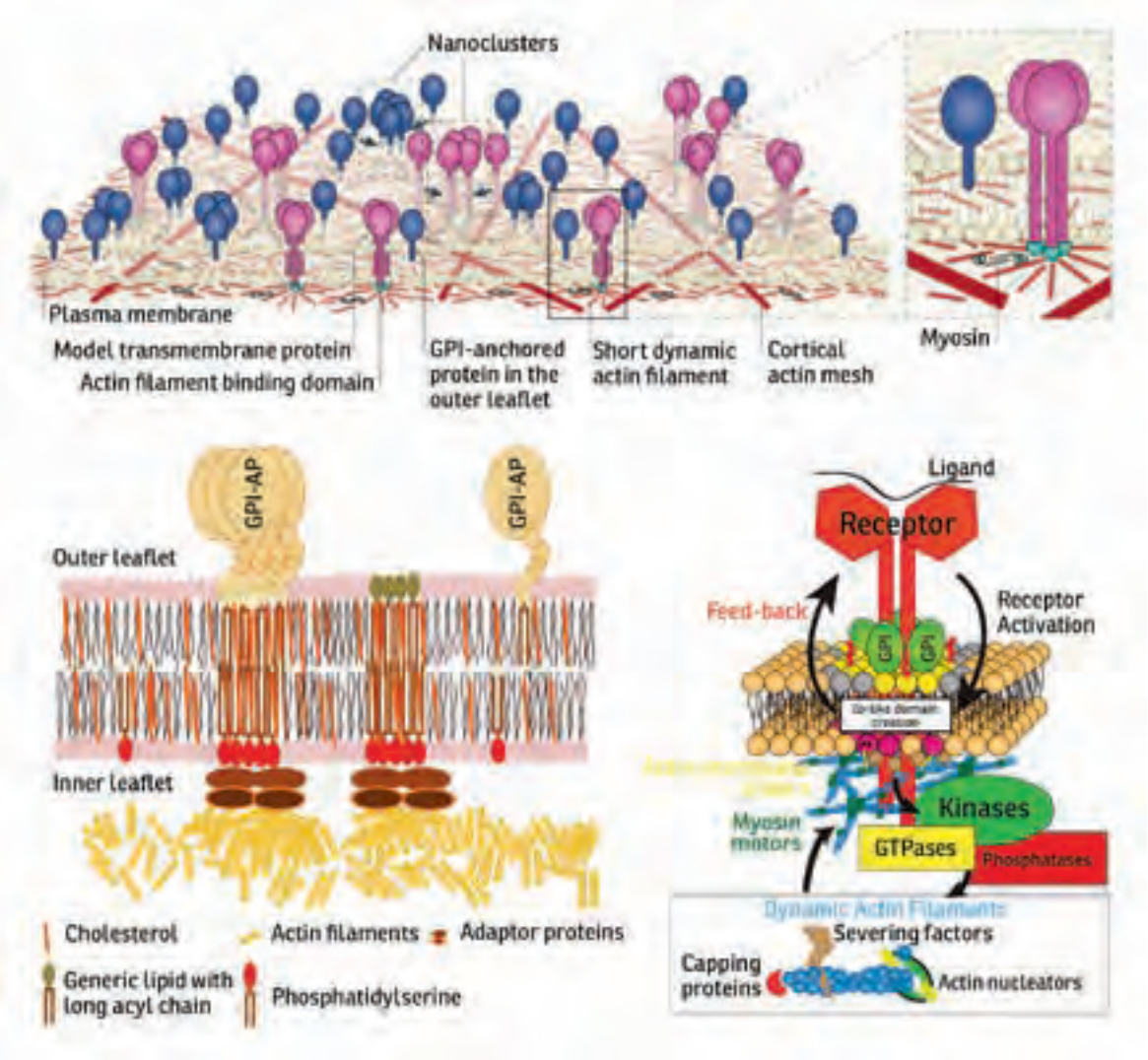

Fig 1: Nanoclusters are built by contractile actin platforms, resembling asters, and transbilayer coupling and mechanisms for nanocluster formation of outerleaflet lipid-anchored proteins.

2. Experimental and theoretical studies on membrane domain formation in living cells

SUVRAJIT SAHA, ANUPAMA AMBIKA ANIKUMAR, THOMAS VAN ZANTEN, SANKARSHAN TALLURI AND CHANDRIMA PATRA

In collaboration with Madan Rao (Simons@NCBS), we have shown that there is an unexpected organization of specific lipids, lipid-tethered proteins and membrane proteins at the cell surface in the form of small dynamic

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nanoclusters. The characteristics of this organization suggested the involvement of non-equilibrium mechanisms in their formation. Our theoretical active-composite framework envisions the presence of dynamic filaments of actin associated with myosin motors, closely juxtaposed to the membrane. This configuration results in small contractile actin platforms, which in turn pattern membrane components associated with actin (Fig. 1). For membrane proteins that bind actin, the connection is of course direct. For other membrane molecules such as outer leaflet lipids or lipid-tethered proteins, we have shown that there is a transbilayer connection with molecules at the inner leaflet to link up with cytoplasmic actin.

To experimentally test this hypothesis and find the rules governing such a transbilayer linkage, we collaborated with Ram Viswakarma (IIIM Jammu) to create variants of GPI-anchors and other lipids.

Visualizing the nanoscale organization of these variants together with molecular dynamic simulations of lipids in asymmetric bilayers by Anirban Polley (working with MR), has highlighted the importance of cholesterol and acyl lipid chain length for transbilayer coupling (Fig. 1). We are now trying to understand how these components locally reorganize to form larger signalling platforms that support information transfer, in collaboration with Amit Das (working with MR).

3. *In vitro* reconstitution of actin and myosin-driven membrane systems

DARIUS KÖSTER, ABRAR AHMED, SANKARSHAN TALLURI AND KABIR HUSAIN

To fully understand the role of dynamic actin filaments and myosin motors in membrane organization, we reconstruct this active composite outside the cell. Using minimal constituents (Fig. 2), we explore the ensuing rich phase space of actin and myosin configurations that drive the arrangement of reconstituted membrane-linked actin filaments advected by myosin. One distinct region of this phase space, of filament concentrations and lengths, supports a remodelling steady state that remarkably recapitulates key features of membrane organization that we had previously only observed at the surface of living cells. Consequently, this reconstitution could provide a link between the *in vivo* experiments and the theoretical framework of an active composite model of the cell surface.

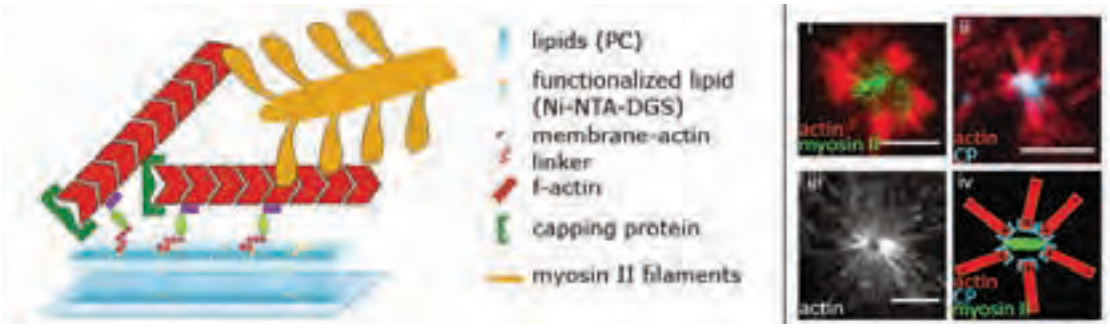

Fig 2: *in vitro* reconstitution of actin and myosin driven membrane systems

4. Dynamics of multi-molecular complex formation during signalling in multiple cell systems

MARCUS TAYLOR, JOSEPH MATHEW, RUMAMOL CHANDRAN, PARIJAT SIL, KABIR HUSSAIN AND THOMAS VAN ZANTEN

But what are the functional consequences of these organizational rules and principles? In this direction, we are exploring the construction of diverse functional signalling systems such as the T-Cell receptor complex (in collaboration with Ron Vale, UCSF, USA), the integrin receptor, an adhesion receptor such as cadherin (in collaboration with Thomas Lecuit, IBDM, France), CD44 and the acetylcholine receptor (in collaboration with

Francisco Barrantes, Buenos Aires, Argentina) (Fig 1). Using membrane bilayer systems, where the ligand is presented in a controlled manner and the creation of receptor assemblies can be followed in time, we monitor changes in signalling output in conjunction with the organization of membrane molecules and determine their relationship(s). Based on the rules and principles uncovered above, we propose to perturb these systems in specific ways and visualize changes. For example: how does the affinity of a TCR-antigen affect TCR complex formation and the resultant manifestation of an output signal? How do integrin receptors react to extracellular chemistry and its physical properties (stiffness)? How does signal initiation change local membrane organization? What is the role of these membrane mechanisms in the context of stem cell fate determination?

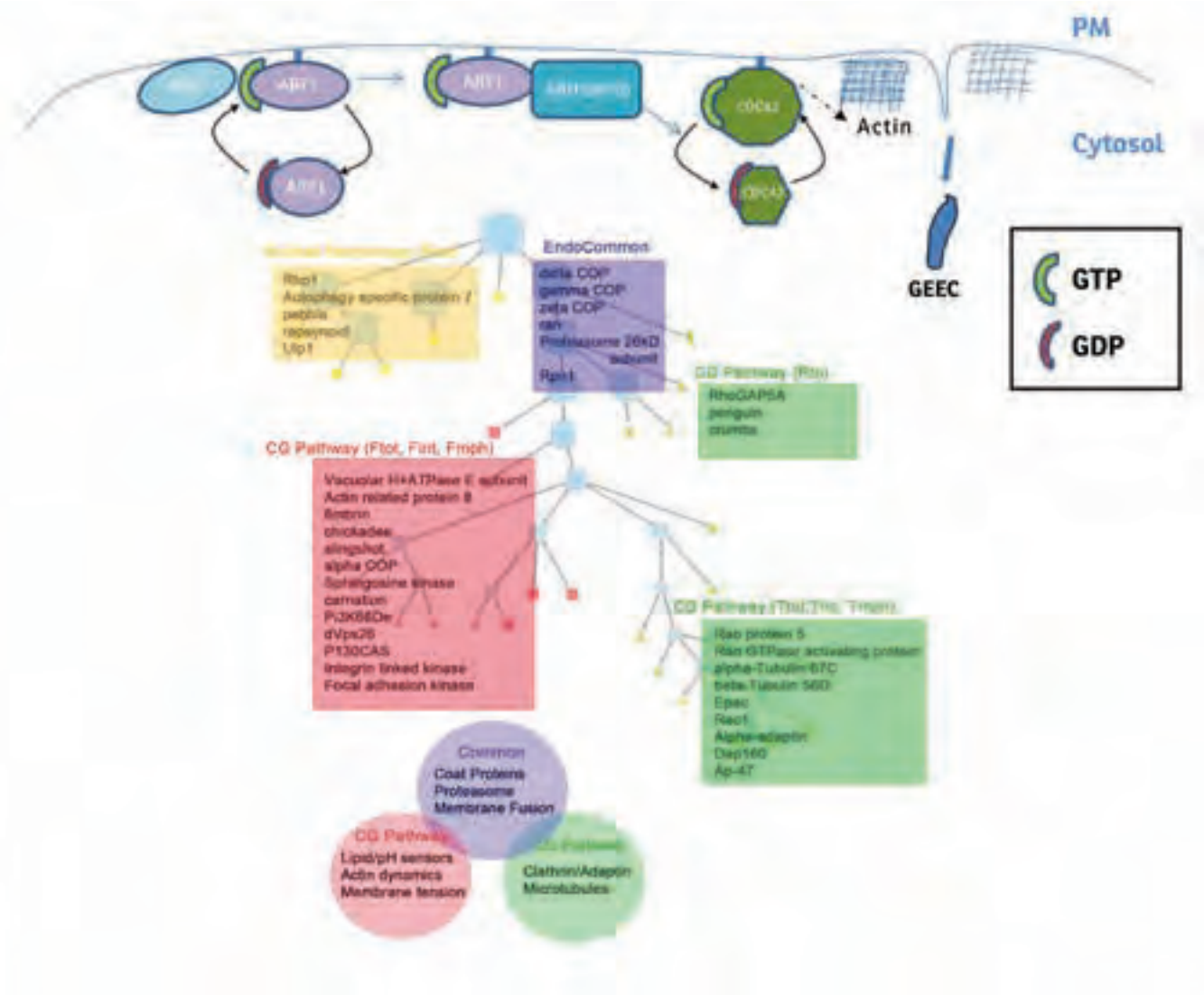

Fig 3: Molecular players involved in CG endocytosis

5. Regulation and evolution of dynamin-independent endocytosis

GAYATRI MUTHUKRISHNAN, MUGDHA SATHE AND NIKITA RAJ

Using cell-based assays at the individual gene scale and genome wide-RNAi screening methods, we have uncovered a rich haul of molecular players that regulate clathrin-dependent (CD) versus clathrin- independent (CLIC/GEEC: CG) endocytosis (Fig. 3). To study the assembly of a CG endocytic platform and its regulation, we have set up a TIRF based assay system that allows the simultaneous detection of single endocytic events with the recruitment of specific molecular players. These studies will contribute to a systematic understanding of the endocytic machinery responsible for this ubiquitous CG pathway. To study the evolutionary antecedents

of this pathway in the context of eukaryotic evolution we are, together with Mukund Thattai (NCBS), exploring phylogenetic signatures of the set of genes that contribute to endocytosis.

6. Functional roles of clathrin and dynamin independent endocytic pathways in cell and animal physiology

JOSEPH JOSE THOTTACHERRY, BINI RAMACHANDRAN, ANUPAMA HL AND CHAITRA PRABHAKARA

The existence of distinct endocytic pathways raises questions about the functional significance of these ubiquitous cellular processes at the level of cells and the context in which cells locate themselves. Given the identity of the molecular players that influence the CG pathway and the nature of the early endocytic carriers that are produced, we propose that the CG pathway acts as a sensitive regulator of membrane tension or area homeostasis. To examine this, we are studying the effect of the physical environment (stiffness and tension) on endocytosis.

We propose that this endocytic pathway is important for controlling the membrane composition of the cell surface since the CG pathway is regulated by cholesterol, and CLICs that are formed by this endocytic pathway carry many molecules known to prefer liquid ordered phases or raft-like regions of the lipid bilayer. This would imply that the CLICs and GEECs have membrane composition distinct from the CD endocytic compartments. We are currently analyzing the membrane composition of these compartments to verify this hypothesis.

Additionally, to understand the functional role of the CG pathway in a metazoan, we are studying its influence in the development of the wing epithelium in Drosophila. We find that the CG pathway plays a major role in regulating the supply of the secreted Wingless protein to its native receptor, dFrizzled2. How does the CG pathway intersect with Wingless signalling? What is its precise mechanism? We are interested in examining the broader implications of these findings on the development of a tissue, considering that the CG pathways may be sensitive to a distinct set of external inputs in comparison to the CD pathway.

7. Remodelling of cell surface co-stimulatory proteins by HIV nef

Understanding how the HIV protein, Nef, remodels the cell surface of infected macrophages, in particular, co-stimulatory proteins, has allowed us to initiate studies to look for small molecule drugs to reverse the effects of this modulation.

Functionally, this could result in activating immune mechanisms against HIV and thus could be an interesting drug target. This work (funded by the Business Innovations Grant from the Department of Biotechnology) is now carried forward in the translational space at CCAMP, spearheaded by Anandi Karumbati to discover new therapeutic targets for altering the fate of HIV infection.

PHOSPHOINSOITIDE SIGNALLING IN CELL BIOLOGY

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Raghu Padinjat

Chemical messengers based on the lipid phosphatidylinositol are part of an evolutionarily conserved mechanism of cell signalling. These molecules regulate key cell biological processes in eukaryotes. We study the logic underlying cellular signalling mediated by these molecules.

Our long term scientific interest is the analysis of cellular signalling mediated by lipid molecules generated during phosphoinositide metabolism. Phosphoinositide signals provide molecular controls for key sub-cellular processes such as membrane remodelling, cytoskeletal function, transcription and translation. Through these processes, this signaling pathway orchestrates basic cellular behaviours such as cell division, shape changes, polarized movement and cell death. Therefore, this pathway plays a key role in a number of physiological processes including early embryogenesis, lymphocyte development and function as well as neuronal activity. The overall goal of our work is to understand how the architecture of this signalling cascade is designed to optimally deliver physiological outputs.

We use *Drosophila* as our model system; the goal is to discover key principles of signal transduction that are likely to be conserved during evolution but are experimentally more tractable in *Drosophila*. It is hoped that in the medium term, our analysis in *Drosophila* will inform studies of equivalent signalling pathways in mammalian models with more immediate biomedical relevance.

Co-ordination and control of the phosphoinositide cycle during cell signalling

Of the seven phosphoinositides, phosphatidylinositol 4,5 bisphosphate (PIP_2) is hydrolyzed in response to receptor activation at the plasma membrane and generates second messengers. Following this process, PIP2 is regenerated by a multi-step reaction that involves membrane bound lipid intermediates distributed across two subcellular compartments, the endoplasmic reticulum and plasma membrane. We are investigating the mechanisms by which the PIP_2 cycle is co-ordinated in space and time so that plasma membrane levels of this lipid are stably maintained during cell signalling.

We study this question in the context of G-protein coupled phospholipase Cβ (PLC) activity using *Drosophila* photoreceptors as a model system that offer a number of specific advantages for this work (Hardie and Raghu, 2001). In addition to being tractable to sophisticated molecular genetics, the sensory transduction cascade in fly photoreceptors uses G-protein coupled PIP_2 turnover to transduce the detection of light and under conditions of bright light these cells experience high levels of PLCβ activity but see limited depletion of plasma membrane PIP_2 (Raghu et al., 2012). Thus photoreceptors offer an excellent system to study the regulation of plasma membrane PIP_2 levels. We are using a combination of molecular genetics, live-cell imaging and electrophysiology and mathematical modeling to study PIP_2 signalling in *Drosophila* photoreceptors.

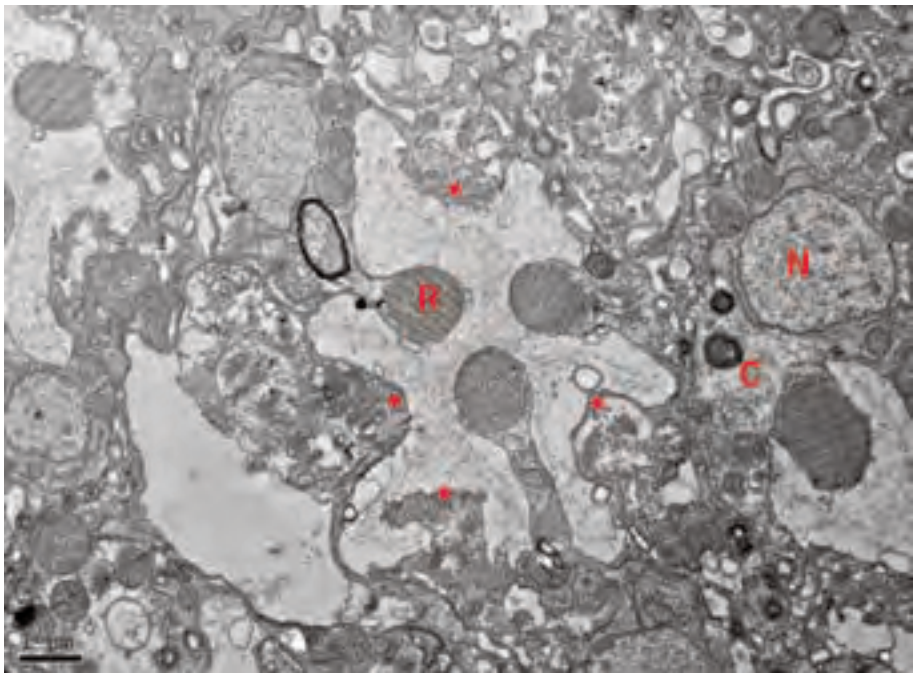

Fig 1: Transmission electron micrograph of adult *Drosophila* photoreceptors. Defects in lipid signaling result in the collapse of the apical domain of this polarized cell. The apical domain membranes form a rhabdomere (R). N is nucleus, C is cell body and * marks the rhabdomere undergoing degeneration.

Our group has identified the phosphoinositide kinases that regulate PIP_2 synthesis in *Drosophila* photoreceptors. These are the enzymes that perform the final step in the synthesis of PIP_2. The completed *Drosophila* genome contains three genes that encode phosphatidylinositol-4-phosphate 5 kinase (PIP5K) activity and one phosphatidylinositol-5-phosphate 4 kinase (PIP4K) activity (Balakrishnan et al., 2015). To date the contributions of these two classes of enzymes in regulating PIP_2 turnover in the context of G-protein coupled signalling *in vivo* is unknown.

We have systematically generated loss-of-function mutants in all of these genes and studied their requirement for phototransduction and other PIP_2 dependent functions in photoreceptors. Using this approach we are building a portrait of the regulation of functional PIP_2 pools in a cellular membrane. Recently we have identified a PIP5K that specifically controls the PIP_2 pool required for phototransduction, but not other PIP_2 dependent functions in photoreceptors (Chakrabarti et al., 2015). This opens the door to address key questions relating to how PIP_2 levels are regulated during signaling. How are PIP2 levels at the plasma membrane sensed and communicated to the PIP kinase in order to regulate its activity? How is the activity of PIP5Ks regulated by lipid metabolites generated during G-protein coupled PIP_2 hydrolysis? These research themes are presently being developed.

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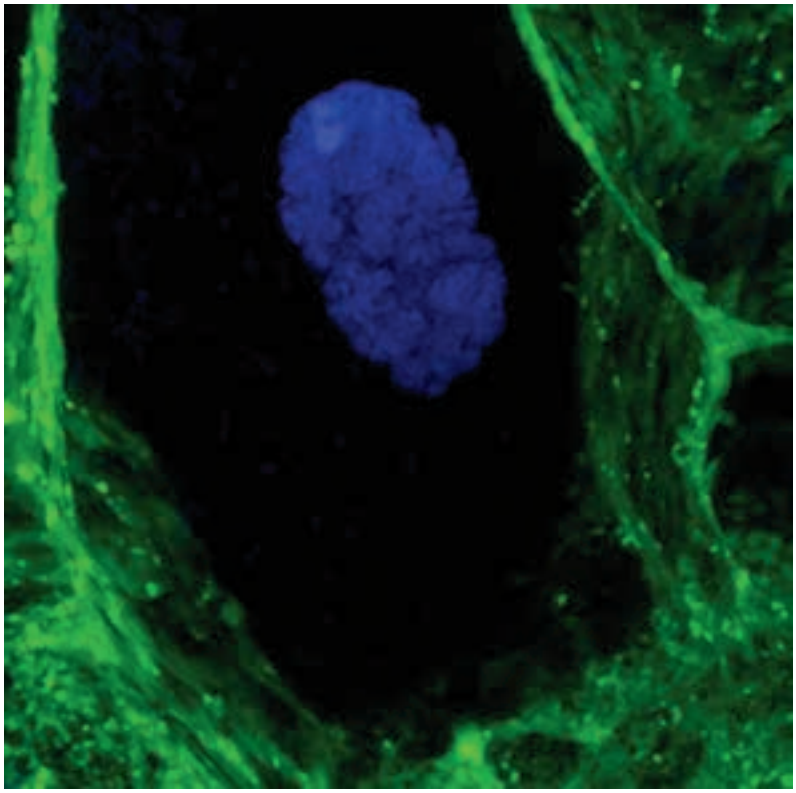


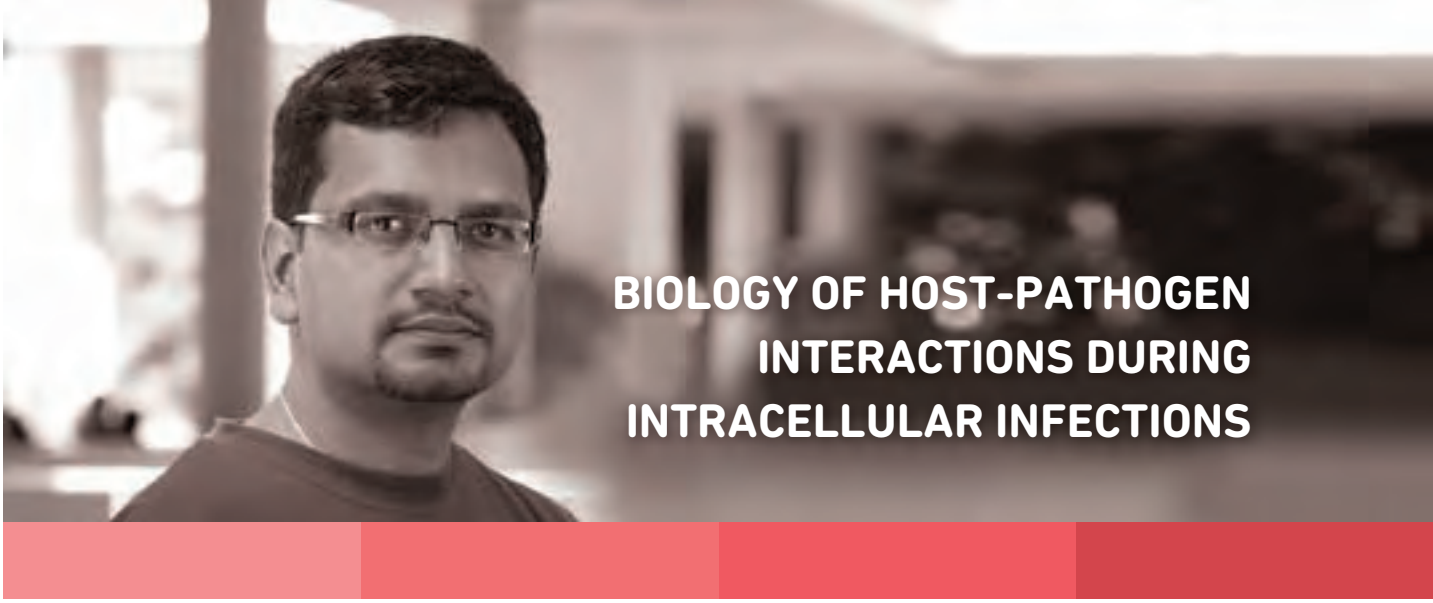
Fig 2: A *Drosophila* larval salivary gland cell expressing a green fluorescent protein tagged protein probe (Green) for phosphoinositide detection. This probe is seen to be present at the plasma membrane. Nucleus is marked in blue.

Membrane contact sites and the regulation of the PIP_2 cycle

The principal site of PI synthesis is the endoplasmic reticulum. In order to generate PIP_2 by sequential phosphorylation of positions 4 and 5, PI needs to be transferred to the Golgi and/or plasma membrane where the relevant kinases are thought to operate. This function is thought to involve PI transfer proteins (PITP). *In vitro*, these proteins transfer PI from liposomes enriched in this lipid to those with low levels of PI. One class of PITPs is *rdgB*, was first isolated as a *Drosophila* mutant with a profound defect in phototransduction [reviewed in (Trivedi and Raghu, 2007)]. However the molecular basis for these defects is unknown although it has previously been shown that *rdgB* mutants have a profound defect in PIP_2 resynthesis during phototransduction. The RDGB protein is localized to a specialized subdomain of the endoplasmic reticulum of photoreceptors, the sub-microvillar cisternae (SMC). The SMC are separated from the microvillar plasma membrane by a cytoplasmic gap of 10 nm. We are using the function of RDGB at this location as a model of membrane contact site function in eukaryotes.

PIP4K and the regulation of cell growth

The enzyme phosphatidylinositol-5 phosphate 4-kinase (PIP4K) can phosphorylate phosphatidylinositol 5 phosphate (PI5P) at position 4 to generate PIP_2. PIP4K enzymes are evolutionarily conserved in multicellular organisms but appear to not be represented in the genomes of unicellular eukaryotes. We have studied the single gene encoding the *Drosophila* ortholog of PIP4K and shown that it is essential for the regulation of cell size and larval development. The molecular mechanism underlying this is mediated by regulation of mTOR, a protein kinase that is a key regulator of protein synthesis and cellular metabolism (Gupta et al., 2013). We are studying the mechanism by which PIP4K regulates mTOR signalling and the cell biological basis of its function.



BIOLOGY OF HOST-PATHOGEN INTERACTIONS DURING INTRACELLULAR INFECTIONS

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Varadharajan Sundaramurthy

The broad goal of our lab is to understand the interactions of intracellular pathogens with host cells, with particular interest in the modulation of host trafficking pathways. We combine cell biological methods, high content imaging and computational approaches to address these questions.

Influence of *M. tuberculosis* infection on macrophage trafficking pathways

Intracellular pathogens interact with their host cells in very intimate ways. Within their host cells, *M. tuberculosis* interacts with and extensively modifies various host cell processes such as trafficking, signalling and metabolism. While it is well known that *M. tuberculosis* inhibits the fusion of phagosomes to lysosomes, and resides within the modified phagosomes, the consequences of its sustained presence on host cellular processes is not clear. In my work, we aim to quantify the influence of intracellular *M. tuberculosis* infection on host trafficking pathways with special emphasis on endocytosis, and to ascertain the importance of this modulation for pathogenesis. We are currently generating quantitative descriptors for the modulation of host endocytic pathway by *M. tuberculosis* infection at single cell resolution. Towards this, we are using quantitative image analysis tools at single cell resolution (Fig. 1). Using these tools, we are currently generating quantitative descriptors for the modulation of host endocytic pathways by *M. tuberculosis* infection.

Morphological profiling to uncover links between endocytosis and autophagy

Coordination of cellular processes is critical for maintaining homeostasis and responding to changing environmental conditions. Yet most biological processes are studied independently of each other and ignore cellular heterogeneity. A part of the work in my lab is aimed at combining cell biology and computational imaging approaches to uncover the links between two key intertwined cellular trafficking pathways, namely autophagy and endocytosis. Several fundamental questions about their interdependence remain outstanding. For example, is there co-regulation of the two pathways via the shared lysosomes? Do autophagosomes and endosomes influence the trafficking of each other? Answering these questions will be critical towards identifying new mechanisms at the intersection of the two pathways.

We use pathway-specific small-molecule modulators to perturb cellular systems, and carry out multiple imaging assays related to endocytosis and autophagy. In collaboration with Dr. Shantanu Singh and Dr. Anne Carpenter (Broad Institute, Cambridge, US), we aim to integrate the resulting cellular phenotypic consequences using image analysis and machine learning. This phenotypic information, available at the

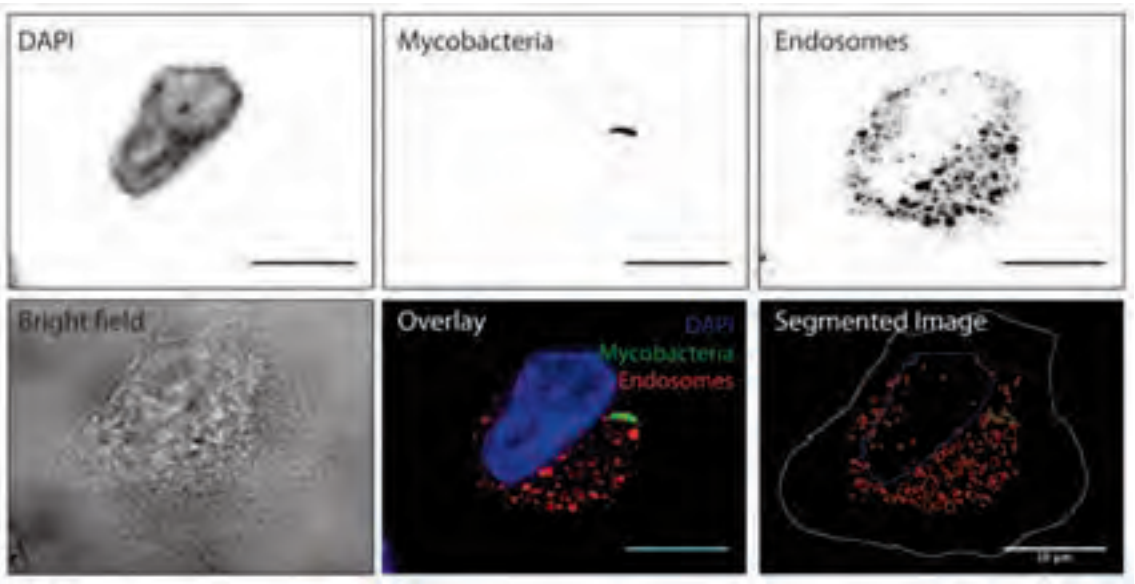

Fig 1: Human macrophages were infected with *Mycobacterium* expressing GFP and stained with antibodies for different endosomal proteins. A representative image of an infected cell stained for early endosomal marker is shown here. The top panels show the individual channels of nuclei, *Mycobacteria* and endosomes. Channels have been inverted to enhance contrast. The bottom panels show the bright field, overlay of all channels and the outlines of segmented objects. Segmentation allows quantification of multiple features from individual objects. Segmentation is performed using CellProfiler. Scale bar = 10 µm.

single-cell level, will allow us to discover and test hypotheses about the interactions between the pathways. This will reveal their role in normal cellular function and in diseases that impinge on these pathways, such as intracellular infections.

Role of hepatocyte polarity in liver stage Plasmodium infections

When a malaria infected mosquito bites a human host, it injects a form of the parasite called sporozoites. The injected sporozoites home in to the liver, where they infect hepatocytes and multiply to hundred thousand fold in a few days. Despite this enormous rate of growth and division, the parasite manages to keep the host immune system largely silent during this phase. Consequently, the liver stage of the disease is asymptomatic and is often termed as 'silent stage'. In my work, we are interested in studying the association of the parasite with its host liver cells in the context of the liver tissue, using advanced imaging and quantitative image analysis methods.

In particular, we aim to explore the potential relationships between the parasite and the host hepatocyte polarity. Given the specific tropism of sporozoites for hepatocytes and the central role of polarity in the functioning of the hepatocyte, we suspect a connection between *Plasmodium* liver stage infection with hepatocyte polarity. In collaboration with Dr. Maria Mota's group (Institute of Molecular Medicine, University of Lisbon, Portugal), we use high-resolution 3D imaging using 2 photon microscopy to study the organization of *Plasmodium* infected liver tissues and the association of the parasites with the hepatocyte apical and basolateral membrane domains.

Tool development for *P. vivax* malaria research

P. vivax is one of the plasmodium species that causes human malaria. Vivax infections have long been considered benign but this incorrect view is changing due to the significant morbidity associated with the

disease. More than half the total cases of malaria world-wide are caused by vivax infections. Several countries of the world are endemic for vivax malaria, including India. The biology of *P. vivax* is fundamentally different from the better studied *P. falciparum.* For example, *P. vivax* preferentially infects immature red blood cells, or reticulocytes, whereas *P. falciparum* infects mature red blood cells. Another very interesting difference is the ability of *P. vivax* to form hypnozoites in the liver stage. The hypnozoite forms can 'hide' in the liver and get reactivated months or even years after the primary infection leading to the relapses of the disease.

Given the high prevalence and morbidity of *P. vivax* infections, there is a desperate need to overcome these challenges and develop a tool box that will allow us to understand the differences between vivax and

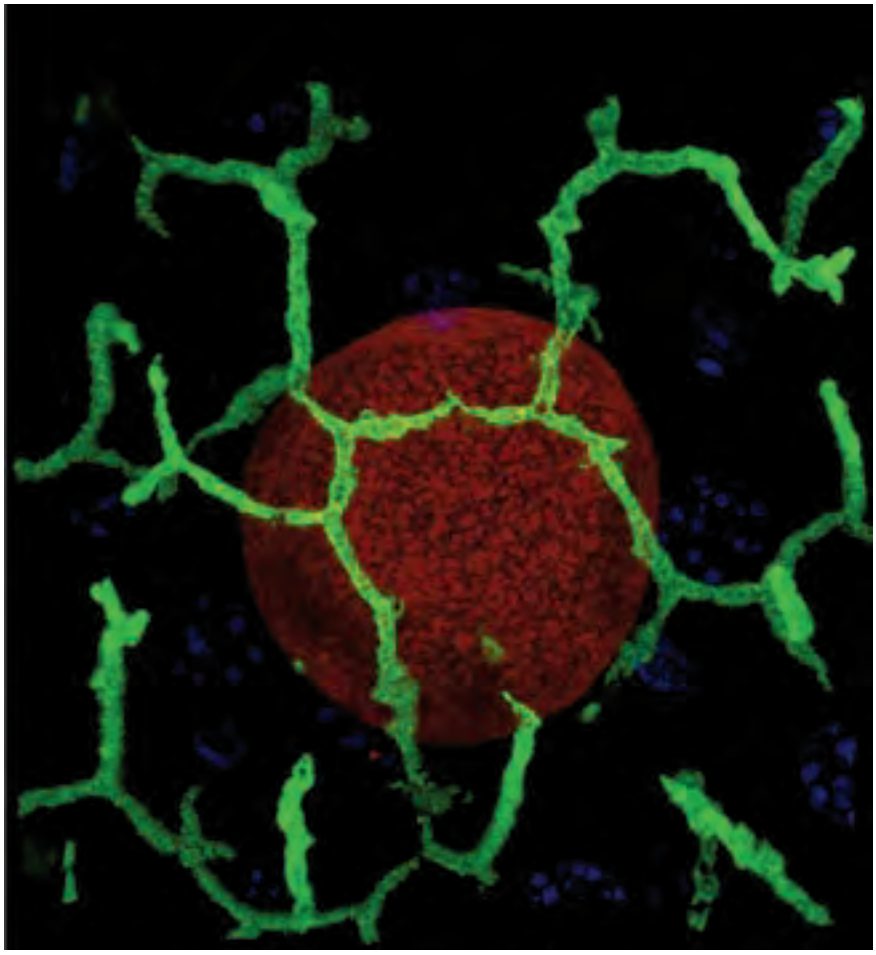

Fig 2: *P. berghei* infected liver tissues were sectioned and immuno-stained for hepatocyte apical membrane marker (green), nuclei (blue) and the parasite membrane marker UIS4 (red). The image is a 2D projection of z-slices covering a volume of ~ 65 µm3.

falciparum malaria at molecular and cellular levels. This could lead to the development of much needed vivax malaria specific drugs. Unlike falciparum malaria, the relapsing nature of vivax malaria necessitates the inclusion of liver-stage acting drugs. Currently only one drug, Primaquine, is approved for this purpose. Using a single drug for infectious diseases is dangerous due to the risk of emergence of drug resistant strains. Moreover, Primaquine is contraindicated in pregnancy, infants and in Glucose-6-phosphatase dehydrogenase deficiency (the most common human genetic disorder) conditions. Thus vivax research offers significant challenges as on one hand, there are several fundamental unanswered questions regarding the biology of the parasite and on the other hand, an urgent need for development of new drugs.

In my lab, we aim to fill this crucial gap by developing assays to quantify the development of *P. vivax* sporozoites in human liver cells. This could serve two purposes. First, establishment of such an assay will

lead to detailed studies on the mechanisms of formation and activation of hypnozoites. Second, it could serve as a platform to screen for anti-vivax compounds that are effective in the liver stages. In collaboration with Dr. Susanta Ghosh (National Institute of Malaria Research, Bengaluru) and Dr. Kouichi Hasegawa (inStem, Bengaluru and Univeristy of Kyoto, Japan), we are trying to derive hepatocytes from iPS cells and infect them with *P. vivax* sporozoites.

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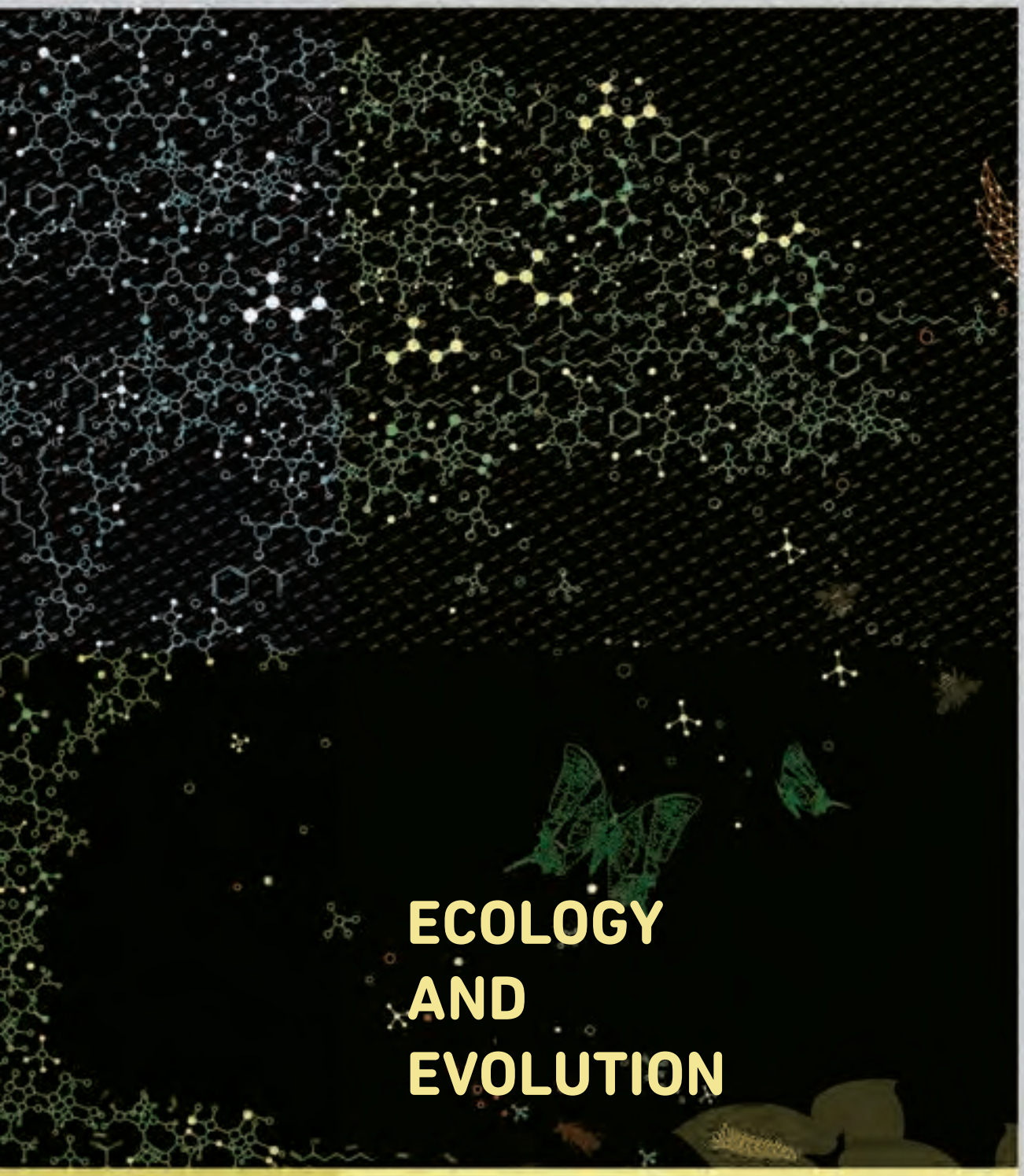

ECOLOGY AND EVOLUTION

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DECONSTRUCTING INDIAN BIODIVERSITY: EVOLUTIONARY ORIGINS AND FUTURE PROSPECTS

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Uma Ramakrishnan

India has a population of over a billion people, with only 4% of its area protected as wildlands. Yet the Indian subcontinent harbours incredible biodiversity. Do we know what this diversity is? How has this diversity come to be? How are we impacting this diversity? My research attempts to address these questions. We conduct fieldwork to sample behavioural, ecological and genomic data from these wild populations. We analyse these data in population genetic and phylogenetic contexts to better understand the evolution, population ecology and conservation of populations.

1) Biogeography of the Indian subcontinent

The Indian subcontinent is a fascinating place to study biogeography because of its dynamic geological history. Little research has been directed at addressing how the current bird and mammal biodiversity in the subcontinent has been assembled. How have the physical features of the subcontinent impacted speciation and biodiversity? Over the past several years we have studied species richness patterns in the Indian subcontinent and tried to investigate patterns of speciation in mountains. In last year's report, I highlighted our results from the Western Ghats. Here, I focus on pan-India patterns and preliminary results from the Himalayas.

At the confluence of three biogeographic zones, India serves as an ideal location to understand community assembly. Because India came to Asian relatively recently (around 40 million years ago), species of different evolutionary origins (and hence environmental preferences) have moved into the Indian subcontinent. But which species are successful here? Does shared biogeographic history lead to these species having similar relationships with the environment in their new home, India? We tested whether species occurred in regions environmentally similar to their historical ranges using a macro-ecological study of the Indian subcontinent. Species were classified as belonging to four biogeographic affinities based on their geographic distributions: Eastern, Northern, Western and endemic. We investigated spatial patterns of species richness for all mammals (over 1° x 1° grid cells) and for each biogeographic group (Fig. 1 a-f). Generalized Additive Models (GAM) were used to investigate environment-diversity relationships for all mammals in different biogeographic groups, and across major mammalian orders in the Indian subcontinent. Species richness of all mammals was found to be highest in the montane regions of the Eastern Himalayas and the Western Ghats. Species richness of each biogeographic group was highest at the border it shared with Asia, in the direction of immigration from Asia. Environment and spatial variables were both correlated with species richness in the Indian subcontinent and each biogeographic group showed a distinct richness-environment relationship (Fig. 1g). Priya Tamma (PhD student, Tamma et al., 2016) showed that biogeographic groups segregated along environmental space, in keeping with our predictions based on their global distributions. We conclude that historical factors such as

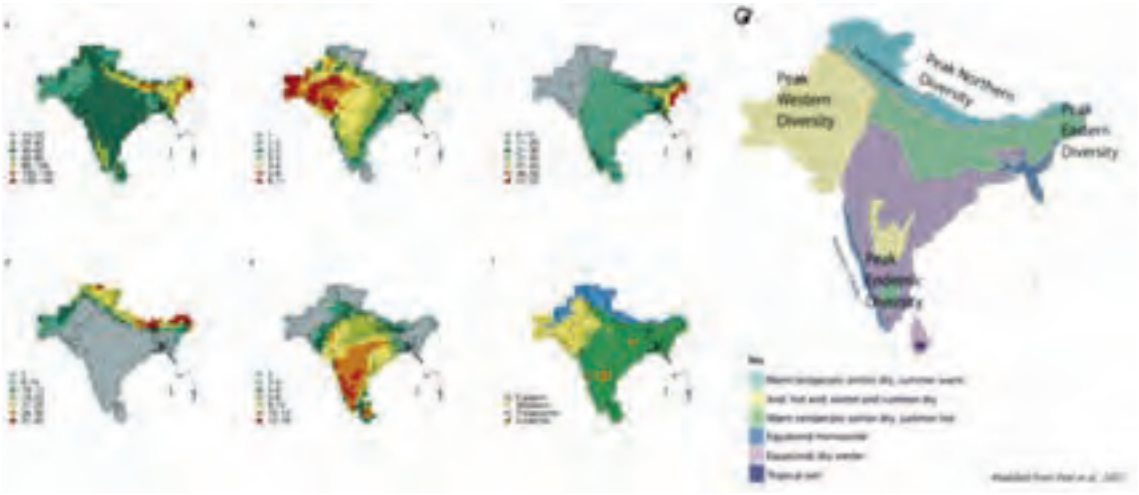

Fig 1: Species richness of mammals in the Indian subcontinent and their climatic preferences. Species Richness of (a) all mammals (b) Eastern, (c) Western, (d) Northern (e) Endemic and (f) Group with the highest proportion of species in each cell (see below). The total species richness for each group are different - this is reflected in the legend for each group. 1a: displays the direction (with respect to the Indian subcontinent) of the three biogeographic groups - Eastern, Western and Northern. 1f: The proportion of each group in each cell was calculated, and the cells are coloured based on the group with the highest proportion. This group dominates the community in each cell. 1e: An illustration of the Koppen climate space in the Indian subcontinent, with the centre of diversity for each group in the subcontinent overlaid on the climate map.

immigration and the distinct evolutionary histories of species impact species richness patterns in the Indian subcontinent. We can now use phylogenetic data to test these patterns at the Indian scale.

For birds, we examined whether phenotypic units or subspecies distributions were impacted by known biogeographic barriers. In particular, we asked whether climatic and/or physical factors might limit phenotype distributions in the Indian subcontinent for endemic species. We identified all physical, vegetation and climatic barriers to identify potential biogeographic units within peninsular India. We collated occurrence of endemic or disjunct distribution species and sub-species using published range maps. We then quantified turnover between potential units, allowing us to identify significant barriers. Three time-step climate data (Last Glacial Maxima, mid-Holocene and present) allowed us to examine differences between these potential biogeographic regions through time. The Palk Straits, followed by the Goa Gap (~16° N) and the Godavari river emerged as the major barriers in this region. The Palk Straits and Godavari are physical barriers characterised by high sub-species turnover. The Goa Gap is a climate-mediated ecological divide, where both nestedness and turnover contribute to community dissimilarity. Climatically intermediate regions appeared unstable in the past and showed inconsistent affinities across families. Vivek Ramachandran (postdoc) and V. V. Robin (NCBS fellow) suggest that the relative climatic stability of wet regions in the southern Western Ghats could be responsible for high subspecies endemism here. Our approach provides hypotheses that can be tested with comparative multi-species phylogeographic data in the future.

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A phylogenetic study of three small mammal groups across several sites in the Himalayas suggests a critical role for dispersal, followed by allopatric speciation in the generation of diversity here (Priya Tamma, PhD student). Transcriptomic studies investigating the impacts of cold and hypoxia on Pikas (collaborative project with Katie Solaris (PhD student, Stanford University) and Elizabeth Hadly) have identified several differentially expressed genes at high elevation. Future research will focus on adaptation in high-altitude communities, especially in the Western Himalayas (postdoctoral researcher, Vivek Ramachandran).

2) Population genomics and uncovering cryptic population structure

Understanding and identifying cryptic diversity is critical to understanding species biology and planning future survival. This is particularly important for species pairs that show geographical as well as morphological overlap, and where taxonomic identification is difficult. Over the past two decades, molecular genetic data in the form of mitochondrial DNA and nuclear microsatellites have provided significant insights into population structure and the partitioning of genetic variation in wild populations. More recently, genome-wide SNPs provide higher statistical power to investigate population structure. However, genomic approaches are difficult to apply in non-model organisms. Over the past few years, we have been working to standardize methods for population genomics for species in the wild.

The Oriental fruit bat genus *Cynopterus*, with several geographically overlapping species, presents an interesting case study to evaluate the evolutionary significance of coexistence versus isolation. We examined the morphological and genetic variability of congeneric fruit bats *Cynopterus sphinx* and *C. brachyotis* using 405 samples from two natural contact zones and 17 allopatric locations in the Indian subcontinent. We then investigated population differentiation patterns, evolutionary history, and the possibility of cryptic diversity in this species-pair.

Analysis of microsatellites, *cytochrome b* gene sequences, and restriction digestion based genome-wide data revealed that *C. sphinx* and *C. brachyotis* do not hybridize in contact zones. However, cytochrome b gene sequences and genome-wide SNP data helped uncover a cryptic, hitherto unrecognized cynopterine lineage in Northeastern India coexisting with *C. sphinx*. This cryptic lineage is not picked up by microsatellite markers (Fig. 2, Chattopadhyay et al., 2016).

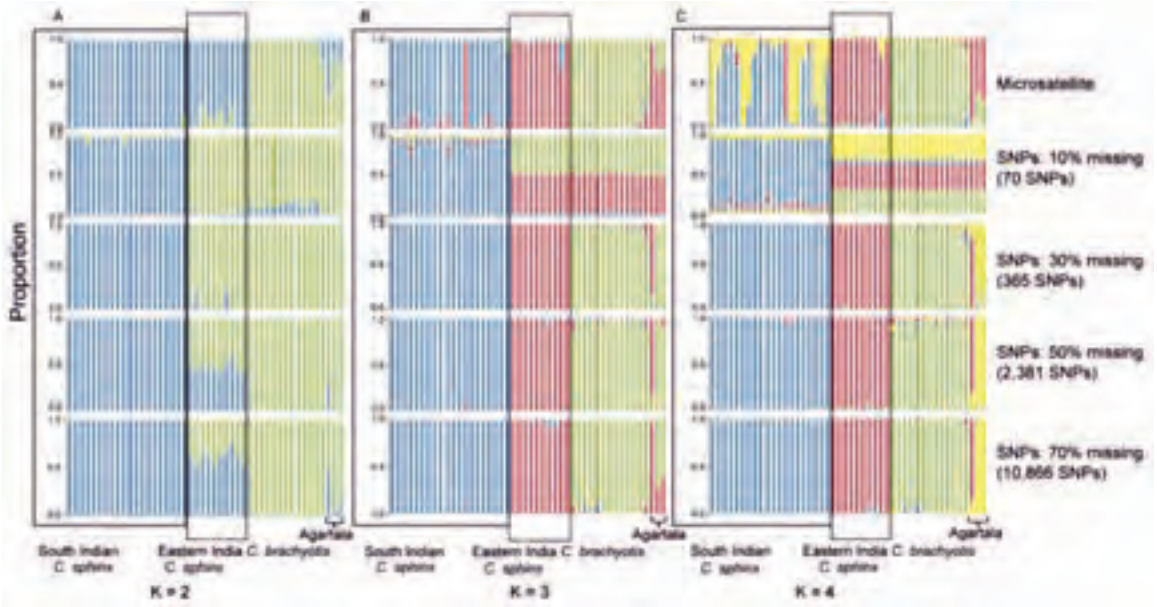

Fig 2: Barplots of the ancestry coefficient q of microsatellite (assignment with all 9 loci) and various SNP datasets at a) K = 2, b) K = 3 and c) K = 4. Intraspecific subdivisions within *C. sphinx* (eastern *C. sphinx* and southern *C. sphinx)* and the Agartala lineage were identified from the comparative analysis of SNP dataset and are specified accordingly.

Our results uncover novel diversity and detect a pattern of genetic introgression in a cryptic radiation of bats. Our results highlight the importance of genome-wide data to study evolutionary processes of morphologically similar species-pairs.

We have just completed analyses of 10,230 SNPs from 39 wild-caught tigers from across the Indian subcontinent. These data also reveal cryptic population structure. In the future, we hope to focus on population genomics, with SNP analyses and whole-genome sequencing of individuals from wild populations in India.

3) Future directions: genomics from degraded DNA?

In order to effectively manage fragmented and small populations, basic parameters about population demography and connectivity must be known. How many individuals are in the protected area? Are numbers increasing or declining? How isolated are protected areas from each other? How are ongoing changes in landscapes impacting connections between protected areas? These are some of the hardest questions to answer because they require tracking individuals over time and space. Yet, current methods are not feasible for many species (including radio-collaring efforts or camera surveys). However, individuals leave genetic material wherever they go — the most abundant and obvious source being feces, but also including hair, saliva and more. So far, we have worked with fecal DNA in the context of microsatellites. Our work and other work with genome-wide SNPs have established that they should be the markers of choice. But how do we get genome-wide data from fecal DNA? We are currently collaborating with the Program in Conservation Genomics at Stanford University to develop new technology to address this important issue. We will start with tigers, but seek to expand these methods to other species of conservation concern in the future.

TERRESTRIAL ECOSYSTEMS AND COMMUNITY ECOLOGY

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Mahesh Sankaran

Can our ecosystems cope with the challenges of ever-expanding human activities? We work on understanding the dynamics of grasslands and mixed tree-grass ecosystems, their responses to changes in climate, and what this means for their future distribution and functioning.

Current research in the lab is grouped around broad themes that examine:

- How interactions and feedbacks between climate, biogeochemistry, fires and herbivory influence the structure, composition and stability of ecosystems and nutrient cycling.
- How global change drivers such as increased temperature, altered precipitation regimes, and nutrient deposition will impact ecosystem function, stability and services.

Most of our research has been carried out in savannah ecosystems in Africa and India. We have now extended this work to encompass a wider range of ecosystems including rainforests and grasslands. Using both field surveys and experiments, we address the above questions across the gamut of natural ecosystem types in India, with the goal of bringing a comprehensive understanding of biome-scale vegetation and nutrient dynamics in the sub-continent.

1. Determinants of savannah structure and function

MAHESH SANKARAN, JAYASHREE RATNAM

Savannah ecosystems cover more than a fifth of the Earth's land surface (~33 million km^2), and support a large proportion of the world's human population, and a majority of its rangeland, livestock and wild-herbivore

biomass. Savannahs can be abstracted into a few components: trees, grasses, grazers and browsers, the interactions among which are mediated by climate, soil, fire and human use. Yet, despite this apparent simplicity, understanding how these different components interact to function as an integrated whole remains a challenge.

Our long-term work in Africa, and our recent initiatives in India, center on mechanistically understanding the individual and interactive effects of 'top-down' (herbivory and fire) and 'bottom-up' forces (resource availability) in regulating savannah structure. Specifically, we are looking at how rainfall, soil nutrients, fire and herbivory interact to influence patterns of woody growth, recruitment and mortality in savannahs. Ultimately, these initiatives will provide us with a more nuanced picture of the role of competition, resource availability and disturbances in regulating savannah dynamics.

2. Savannah-forest transitions in the Indian sub-continent

JAYASHREE RATNAM, CHENGAPPA S. K., SIDDHARTH MACHADO, NANDITA NATARAJ, AROCKIA CATHERIN

Savannahs are mixed tree-grass systems characterised by a discontinuous tree canopy in a continuous grass layer. Within the bounds of this definition, actual tree cover in the world's savannahs is highly variable, ranging from sparsely 'treed' grasslands to heavily 'treed' woodlands, often along a gradient of increasing precipitation. At the mesic end of the savannah biome, where savannahs transition into forests, distinguishing between savannah and true forest based on vegetation structure can be difficult, but we expect major functional differences between the two ecosystems. We are utilizing a predictive framework to characterise savannah and forest species based on differences in functional traits, and are working towards a functional reclassification of savannah regions across India and Asia, with implications for management and conservation of these biomes.

3. Responses of savannah and dry forest ecosystems to global change drivers

VARUN VARMA, LALITHA KRISHNAN, YADUGIRI V.T., CHANDAN PANDEY

Since industrialization, the amount of nitrogen and phosphorous cycling through the biosphere has more than doubled. In addition, rainfall regimes are also changing, with most climate models predicting an increase in rainfall variability and the occurrence of more frequent extreme rainfall events over large areas of the globe, including India. Our research aims to understand the effects of nutrient loading and altered precipitation regimes on the early life history stages of leguminous and non-leguminous savannah and dry forest trees using fully replicated greenhouse and large-scale field experiments. Specifically, we are interested in determining what functional traits of trees predispose them to performing better or worse in the face of such changes. Ultimately, the objective is to develop a framework to predict a priori, which species will have a competitive advantage under these altered conditions. We are also exploring the effects of global change drivers on plant performance via their effects on plant-fungal mutualisms.

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4. Responses of alpine grasslands to warming

DHARMENDRA LAMSAL, JOYSHREE CHANAM, YADUGIRI V. T.

Our work on the impacts of climate change on ecosystem functioning and processes also encompasses alpine grassland ecosystems of the Himalayas. As part of this effort, we have established a warming experiment – which is currently in its third year – using open-top chambers (OTCs), to look at the effects of future temperature rise on high-altitude grasslands in the Sikkim Himalayas. Replicated warming chambers and paired controls have been set up in each of 5 different elevations from 3000 m to 5000 m to investigate the role of warming on plant community shifts and nutrient cycling in grasslands, and how such effects vary across elevation gradients.

5. Land use and carbon sequestration in tropical rainforests

M. O. ANAND, ASMITA SENGUPTA

Earth's tropical forests serve as important repositories of biodiversity and provide critical ecosystem services such as carbon sequestration and climate control. However, tropical forests are highly threatened. While vast extents have been clear felled and logged, many remaining areas are highly fragmented. Furthermore, hunting and habitat degradation have also resulted in dramatic declines in large-bodied frugivore populations in forests, with attendant declines in the abundance of large-seeded tree species that they disperse. Our work in the central Western Ghats investigates the impacts of forest fragmentation and defaunation on tree community structure and composition and the resulting consequences for carbon storage; an important ecosystem service provided by these forests.

6. Ecology for the long haul: long-term vegetation dynamics in the Indian subcontinent

JAYASHREE RATNAM, H. V. RAGHAVENDRA, AMOL KULAVAMODE, M. O. ANAND, KARTHIK TEEGALAPALLI

To date, the most in-depth understanding of forest dynamics has come from long-term monitoring plots that have been established in different forest ecosystems worldwide. However, we have little information on the long-term dynamics of the major forest types in the Indian region. To address this lacuna, we have established of a network of six 1 ha forest monitoring plots in India, spanning a gradient from savannah and dry forests through to wet evergreen forests. We are using these plots to address fundamental questions in vegetation ecology, community dynamics, and the cycling of energy and nutrients in ecosystems. The

network will enable us to better understand the factors regulating forest dynamics, and will provide critical data for both regional and global estimates of tropical vegetation responses to climate change.

7. Shola-grassland mosaics of Southern India

ATUL JOSHI, HARINANDAN P. V.

Forest-grassland mosaics occur on many continents across the earth, including Asia, America and Africa. In India, such mosaics occur in the high-altitude hilltops of the Western Ghats. They are characterised by stunted evergreen montane forests, locally known as 'sholas' in ridge-top depressions or valleys, set in a matrix of montane wet grasslands on surrounding hill slopes. The abrupt transitions between adjacent shola forests and grasslands in the mosaics make them model systems to study state transitions between biome types. We are using an experimental approach to look at the factors that currently limit tree establishment in grasslands, how these are likely to change with changes in rainfall and temperature, and how responses differ between native and exotic tree species. In addition, we are also investigating the impacts of land-use change on the carbon dynamics of these ecosystems.

8. Current and historical ecology of the grasses of the Indian subcontinent

HARINANDAN P.V., ATUL JOSHI, ANUSREE A.S.

The rise and spread of C_4 grasses and tropical savannah ecosystems during the Miocene ranks amongst the most dramatic events of biome evolution in geological history. The causes underlying the rapid expansion of C_4 grasslands, however, remain unclear. Ultimately, a resolution of this debate will require a synthesis of both current and historical data. To this end, we have used sediment cores to reconstruct past climate and vegetation in an arid grassland ecosystem in Northwest India. We have also compiled a nationwide spatially explicit database of grass species distribution for India that we are using to address a range of different questions from the climatic and phylogenetic controls of grass species dominance to macro-ecological patterns of diversity and distribution in Indian grasses.

SPECIATION, ADAPTATION AND MORPHOLOGICAL DIVERSIFICATION IN TROPICAL REGIONS

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Krushnamegh Kunte

Diversity is the cornerstone of life on earth. We are evolutionary biologists who study biodiversity, its organization and complexity, the selective processes that shape it, and the means to preserve it in tropical regions such as India.

Our lab has a broad interest in biology encompassing the fields of natural selection theory, genetics, population and community ecology, and conservation biology. However, we use two systems as microcosms to study a range of phenomena that fascinate us, such as morphological evolution, sexual dimorphism, geographical distribution of animals, and speciation. The first is Batesian mimicry, which is a phenomenon whereby unprotected prey species (called "mimics") gain protection from predators by mimicking toxic or otherwise protected species (called "models"). Predators learn to avoid models based on prior experience, and subsequently avoid eating mimics due to misidentification. Batesian mimicry has been at the forefront of evolutionary research since the beginning, and we study this phenomenon from ecological dynamics to molecular genetics using the latest tools and technologies. Our second research system is Indian butterflies, which, with ca 1,800 species and subspecies distributed in an interesting geographical mosaic, offer virtually unlimited opportunities to study biodiversity, biogeography, community ecology, population biology and conservation issues. Summaries of our major research projects are given below. Further information is given on our lab website (http://biodiversitylab.org/research).

Diversity and evolution of Batesian mimicry

The magnificent diversity of Batesian mimicry is manifested in hundreds of mimetic butterfly species in tropical forests. There is tremendous variation in the nature of Batesian mimicry: mimicry can be sexually monomorphic, polymorphic or sex-limited within and across species. This offers an excellent system to study natural, sexual and frequency-dependent selection that shapes the evolution of sex-limited traits and polymorphism. Early evolutionists such as Charles Darwin, Alfred Russel Wallace, Henry Bates (who first proposed the theory of mimicry and in whose honour it is named) and Edward Poulton hailed Batesian mimicry as a fine example of natural selection, pointing to the often perfect resemblance between the mimics and their models. They saw this as one of the strongest demonstrations of evolution by natural selection, in this case brought about through the agency of predators such as birds. We now know many other good examples of natural selection, but interest in Batesian mimicry has persisted to this day among evolutionary biologists. This is because we have a very strong theoretical framework for Batesian mimicry. Furthermore, extensive field observations as well as numerous laboratory experiments have provided rich details of the phenomenon under a variety of ecological conditions and in a number of organisms.

Building on this extensive foundation, we study: (a) how natural and sexual selection influence speciation and morphological diversification in mimetic butterflies, and (b) how different mimicry types have evolved

in relation to each other. To answer these questions, we have developed: (a) a mathematical model of the selection regimes under which various mimicry types may be favoured, (b) a graphical model of the evolution of mimicry types to study character state changes within a phylogenetic framework, and (c) a set of ecological methods to address how selection shapes mimicry in the field. We are testing these models with data on communities of mimetic butterflies in the Eastern Himalaya and Western Ghats, and by analysing evolution of mimicry types on a molecular phylogeny of Papilio swallowtail butterflies.

Fig 1: A Batesian community involving predators, models and mimics.

Molecular genetics and evolution of mimicry in the *Papilio polytes* butterfly

Papilio polytes, a widely ranging Asian swallowtail, has a single non-mimetic male form and several female forms, most of which mimic locally abundant toxic *Pachliopta* butterflies. We are studying this female-limited mimetic polymorphism in *P. polytes* to understand the molecular genetic basis of sexual dimorphism and polymorphism. We are also aiming to understand what kind of genetic changes enable major switches between wing colour patterns in butterflies, and what selective pressures favour their evolution. We are studying these factors at the continental scale, covering the entire Oriental Region. This is interesting because *P. polytes* is a regionally variable species in which some female forms occur in some populations but not others. This offers an opportunity to study local adaptation and compare genomic backgrounds on which wing patterns and their genetic bases have evolved. Furthermore, there is a genetic dominance hierarchy between the female forms, the non-mimetic female form being recessive to all the mimetic female forms. Thus, we also study the basis and nature of genetic dominance.

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Fig 2: Female-limited mimetic polymorphism in the *Papilio polytes* swallowtail butterfly.

Population biology of butterfly migrations, and its evolutionary and genetic consequences:

A spectacular natural event takes place across Southern India every year: millions of butterflies migrate from the Western Ghats to the eastern plains around May, and a reverse migration takes place in October or November. These migratory swarms may contain half a dozen species, but are overwhelmingly dominated by two: Tirumala septentrionis *dravidarum* (Dakhan Dark Blue Tiger) and *Euploea sylvester coreta* (Double-branded

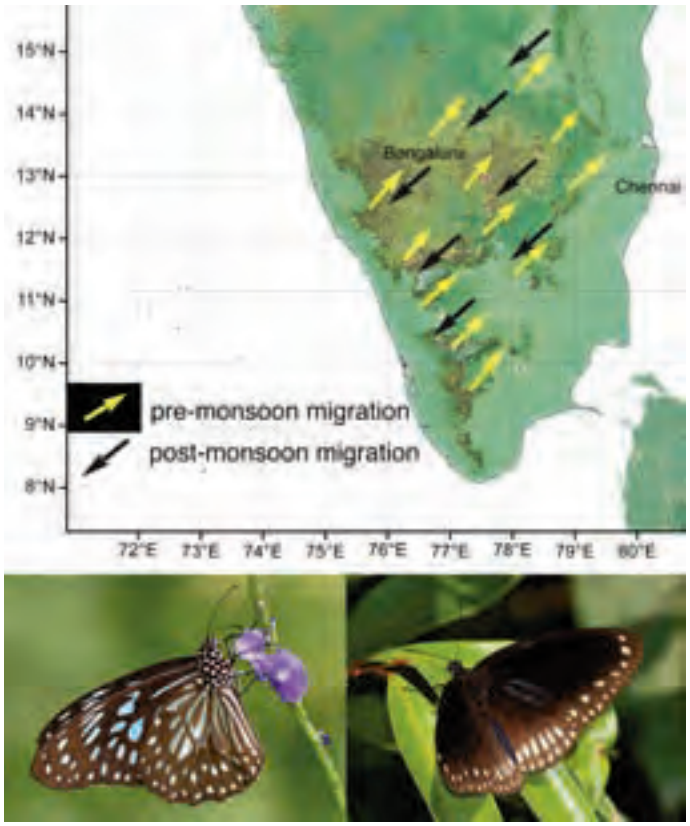

Fig 3: Butterfly migration in peninsular India.

Black Crow) (Nymphalidae: Danainae). This migration is quite fascinating because: (a) it is longitudinal (east-west), not latitudinal (north-south) or altitudinal like most other well-known migrations, and (b) it seems to be driven by the Indian monsoon, not by cold or drought, as is the case in many other migrations. We are currently investigating how the Indian monsoon influences the population biology of these butterflies, and what are the evolutionary and genetic consequences of migratory behaviour.

Ecology, biogeography, phylogenetics and conservation of Indian butterflies

One of the long-term goals of our lab is to study the ecology and patterns of diversification, endemism and evolution of Indian butterflies. Migration, seasonal population dynamics, biogeography, phylogeography, community structure and mimicry are some of the areas in which we have several ongoing projects. We have started a modern research collection of Indian butterflies, with associated geo-referenced data and DNA library, which we are using for our taxonomic, phylogenetic, phylogeographic, conservation genetics and conservation prioritization work on Indian butterflies. We are also spear-heading the development of a continually updated and expanding website on Indian butterflies (http://ifoundbutterflies.org).

GENETIC AND ECOLOGICAL FACTORS UNDERLYING ADAPTIVE EVOLUTION

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Our lab combines diverse approaches to understand the evolutionary and ecological processes underlying adaptive evolution. We often use experimental evolution of insects and bacterial systems to determine the dynamics of adaptation under new genetic and ecological selective pressures.

A central goal of modern biological research is to understand the ecological drivers and genetic basis of evolutionary change in organisms, which is important to address a number of fundamental questions in biology. For instance, how many mutations are responsible for rapid adaptation to novel environments? What are the mechanisms responsible for major behavioural and phenotypic changes in animals, and can we predict how species will adapt to global climate change? My major goal is to understand the evolutionary and ecological processes underlying adaptive evolution. My lab addresses two broad questions:

1) What evolutionary and ecological factors govern the evolution of bacterial genomes, and how often do the observed patterns reflect adaptive (vs. stochastic) evolution?

Specifically, how do correlated genome-level features such as codon bias, tRNA gene copy number and genomic GC content influence each other's evolution? We recently showed that the effects of highly deleterious codon changes in a key gene (fae, encoding the formaldehyde activating enzyme) of *Methylobacterium extorquens* could be rapidly overcome via parallel, single synonymous mutations (Fig. 1). This is surprising since synonymous mutations are typically assumed to have negligible adaptive significance. Contrary to current understanding, our work also suggests that selection on codon use acts via their multiple indirect effects on local sequence characteristics, rather than translation rate or accuracy.

In another project, we analysed 284 prokaryotic genome sequences to test for selection acting on Shine-Dalgarno (SD)-like sequences in coding regions. The SD motif typically occurs in the 5' untranslated region of genes, and is used for translation initiation. We found evidence consistent with both purifying and positive selection on internal SD-like motifs depending on the location within the gene, and showed that controlling for genomic GC content is critical to this inference (Fig. 2). These results demonstrate the complicated nature of selection acting on specific sequence elements in bacterial genomes. Our current and future efforts are aimed at testing multiple hypotheses to explain the evolution of codon use in bacteria, focusing on selection on tRNA genes and genome GC content.

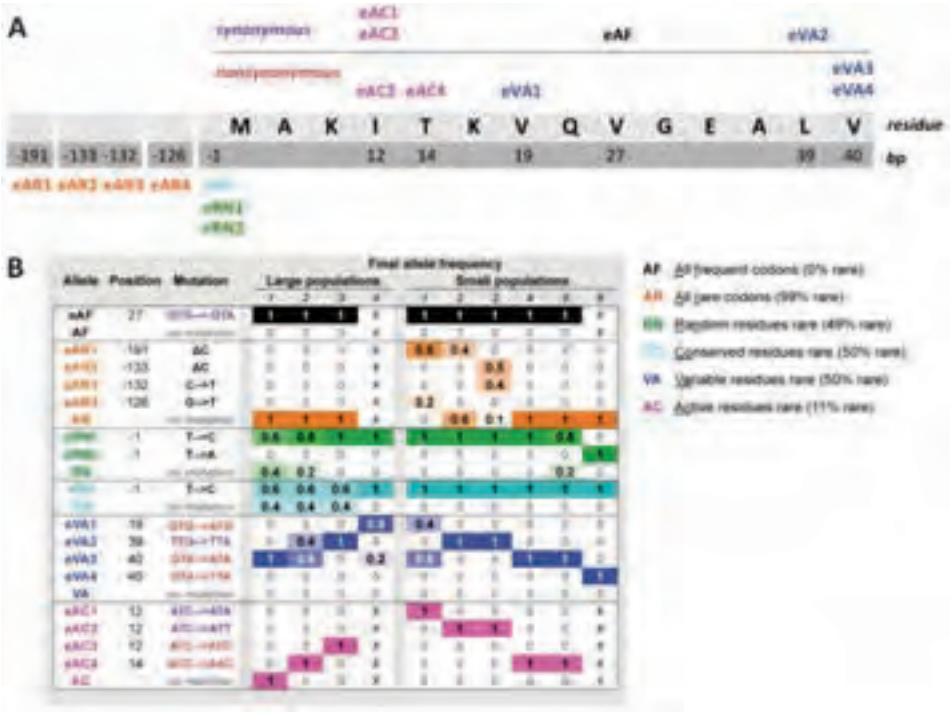

Fig 1: (from Agashe et al 2016, *MBE)*: Evolved coding and noncoding mutations observed in strains carrying synonymous variants of the *fae* gene.

(A) The first 14 amino acid residues of the *fae* coding and relevant upstream sequence are shown, indicating nucleotide positions that acquired mutations during experimental evolution. Coding mutations are shown above the gene sequence and noncoding mutations are shown below. No mutations were observed in the rest of the *fae* gene, and each tested clone had a maximum of one mutation. Alleles are named as follows: "e" indicates evolved allele, letters identify the ancestral fae strain, and the number differentiates multiple evolved alleles for each ancestor.

(B) Each evolved mutation is described, along with its final frequency in replicate evolved populations (n = 5 isolated clones per large population; n = 10 clones for small populations). The frequency of clones without a *fae*-associated mutation is also indicated for each ancestor. Each column represents an independent population, with the cell value and colour intensity indicating the final frequency of the corresponding allele in that population (maximum colour intensity indicates a fixed mutation with frequency 1). In some cases, there were fewer replicate populations, and these "missing" populations are marked with an X.

2) What evolutionary and ecological factors govern insect adaptation to new resources?

Specifically, what are the roles of migration, behavioural choice, and gut bacterial community composition during such adaptation? To address these questions, we allow flour beetle *(Tribolium castaneum)* populations to adapt to new suboptimal resources in the lab and will soon begin to map phenotypic and

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***Equal contribution**

genetic changes in the evolving populations. We are also testing whether behavioural preferences for alternate resources evolve in conjunction with physiological adaptation. Finally, we recently found that beetle gut microbes likely mediate adaptation to novel resources by altering female oviposition and larval development, and we are following up this exciting opportunity to understand the mechanistic basis of the association.

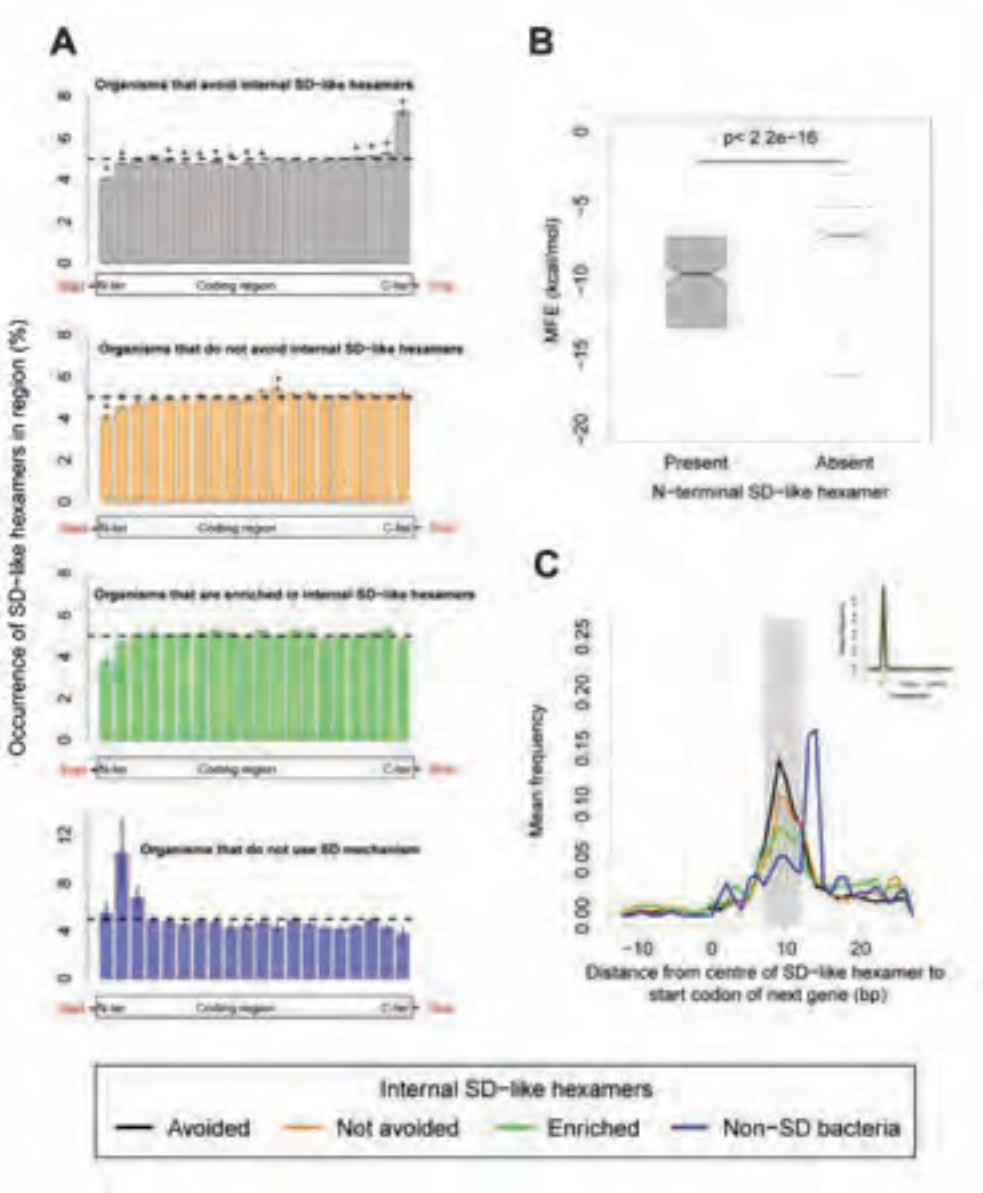

Fig 2: (from Diwan & Agashe 2016, GBE): Functional and positional analysis of genes containing internal SD-like hexamers.

(A) The distribution of SD-like hexamer occurrence (mean±SEM) across the relative length of all genes in 284 organisms, for genomes that avoid, do not avoid, are enriched in internal SD-like hexamers and organisms that do not use the SD mechanism of translation initiation. Dashed lines indicate the random expectation for occurrence in each region (gene length / 20 regions = 5%). In each panel, asterisks indicate gene regions where the occurrence was significantly different from this expectation (one-sample Wilcoxon signed rank test with Benjamini-Hochberg correction for multiple comparisons, $p < 0.05$)

(B) N-terminal Minimum Folding Energy (MFE) of genes with an N-terminal SD-like hexamer (grey bars) vs. those without (white bars), for 277 organisms that use the SD mechanism of translation initiation. The two distributions were significantly different from each other (paired Wilcoxon test, $p < 2.2e-16$).

(C) The mean frequency distribution (±SEM) of the distance from the centre of a C-terminal SD-like hexamer to the start codon of the next gene (inset plot shows the entire distribution). The grey rectangle indicates the most common distances between the ribosome binding site and the start codon of genes in *E. coli* (7 – 12 bp).

We primarily use laboratory experimental evolution, next-generation sequencing, and bioinformatics and molecular biological approaches in the lab. However, to place our findings in the appropriate ecological context, we are increasingly including analyses of natural populations. For instance, we have extensively characterised 20 wild-collected populations of flour beetles and recently sequenced the genomes of those showing extreme life history or behavioural phenotypes. We are now beginning to elucidate the mechanisms responsible for the observed trait variation. We also aim to explore microbial evolution in natural populations, focusing on microbial communities associated with insect guts. In summary, we will continue to address various aspects of adaptive evolution using bacterial and insect populations.

TRACKING THE OBJECTS OF INSECT AFFECTIONS ACROSS SPECIES AND CONTINENTS

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The naturalist-inspired chemical ecology group studies how animals, and especially insects, identify objects in nature. They take field trips, record neurons, generate models, and even build virtual worlds, to understand how insects have evolved to detect cues and make decisions.

"A multitude of animal people, intimately related to us, but of whose lives we know almost nothing, are as busy about their own affairs as we are about ours." - John Muir

The fundamental task for any living organism is to identify objects in the world around them. All organisms must, for example, discriminate what to eat from what might eat them. As humans, we learn to identify most objects through learning. Yet most insects are solitary, which means that once they emerge from eggs or pupal cases, they must immediately identify some objects, such as food or enemies, without prior experience. Our group has found that hoverflies are able to identify flowers from tropical Bangalore, alpine Sikkim, temperate Germany, and subarctic Sweden despite massive changes in climate and geography (Fig. 1). And they do this with a brain containing nearly one million times fewer neurons than our own. How is this possible?

Understanding how small networks like insect brains perform object identification can uncover basic principles of sensory processing as well as provide opportunities to generate better pest and pollination management strategies. To understand insect object identification, The NICE group performs research across species, mountains, and continents. This past year witnessed the first full year of the lab. During this period, we began preliminary analyses based on five factors impacting object identification:

Fig 1: Hoverflies *(Episyrphus sp.)* across latitudes, altitudes, and continents.

Physiology

The "Information Processing Hypothesis" for host choice implies that new objects, such as invasive species, are more likely to be accepted by native animals if they require little to no change in neural wiring for recognition. The *Rhagoletis* complex is a major evolutionary model for speciation, with closely related populations specific to several different introduced and native fruits. In addition, the visual and odour cues they use for fruit identification are well characterised. Our preliminary calcium imaging analyses in the *Rhagoletis* brain suggest a reversal in the pattern of host volatile processing between introduced and native hosts. With our Notre Dame University collaborators, we are currently pursuing both developmentally regulated changes in central neurons as well as changes in global neuromodulator levels as potential sources for this switch in processing of new objects.

Ecology

Models of optimal diet breadth in insects suggest that even suboptimal hosts could become preferred objects simply from abundance of objects present, as in agricultural monocultures. We are testing this hypothesis using a current Indian agricultural pest. The Coffee White Stem Borer is one of the most economically devastating pests in India today. However, while CWSB is indigenous to India, coffee is not. As part of a project funded by the Indian Coffee Board, we have performed a series of field-to-lab analyses to uncover the mechanisms underlying CWSB preference for coffee. We are currently comparing preference with potential native hosts to determine how such host preference for coffee objects could have evolved.

Evolution

Evolutionary constraints in phenotypic plasticity imply that object identification could be constrained simply because it is too costly (genotypically or phenotypically) not to identify the object. As part of the recently funded NER-DBT Chemical Ecology grant, we are currently testing this hypothesis with Dr. Y. Rajashekar at IBSD Imphal. We report the isolation of a biofumigant molecule from aerial parts of a native Indian plant broadly toxic and repellent for all tested insect orders, but inactive on other arthropods or animal groups. The remarkable conservation of identification across millions of years suggests genotypic or phenotypic constraints on the perception and/or toxicity of the molecule. We are currently using various approaches such as toxicology and behavioural bioassays of several species, neurogenetics, and electrophysiology to confirm the molecular mode of action, and identify potential constraints on insect response.

Environment

Environmental factors can have drastic effects on organisms and the cues they detect. Pollination provides an excellent template to examine how objects (i.e. flowers) are identified across continents, elevations, and climates. Along with Karin Nordström of Uppsala and Flinders Universities, we have assessed a range of parameters using multivariate analysis to predict the optimal floral "signature" for hoverflies searching for a pollination site in several climates (Fig. 1). Artificial "flowers" derived from these analyses suggest that

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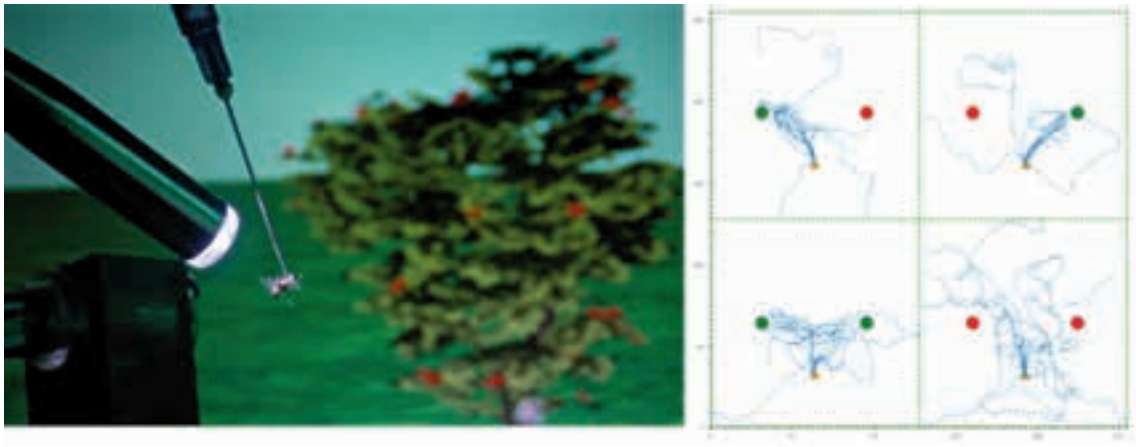

Fig 2: Left: Insect virtual reality setup.
Right: Continuous flight path of a tethered male *Rhagoletis* when presented randomly with 2 spherical, coloured virtual objects. Flight trajectories start in each quadrant at lower middle between the two spheres. Trajectories were automatically reset once fly reached an object or flew past world boundary in any direction.

our models have successfully predicted cues attractive to hoverflies. Once our field experiments have identified the floral signature for each site, we will quantify how the insect brain codes these multimodal cues across continents and climates.

Identification

Finding important objects also leads to a decision problem. When and how much to use each sensory cue, and how to incorporate these cues in the noisy natural environment? To test this, we have established a unique visual-olfactory virtual reality for insects. Our preliminary analyses have already shown that apple flies exhibit differential behaviours to virtual objects (Figure 2). We will extend these analyses to examine the context-aware sensory fusion principles underlying object-oriented search in the apple fly.

CHEMICAL ECOLOGY OF PLANT-INSECT-MICROBE INTERACTIONS

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Chemical ecology is the study of chemically mediated interactions in nature. We study such interactions spanning from biochemistry to ecosystems addressing plant defence responses and their regulation by phytohormones, insect detoxification mechanisms and evolutionary origins of plant defence responses.

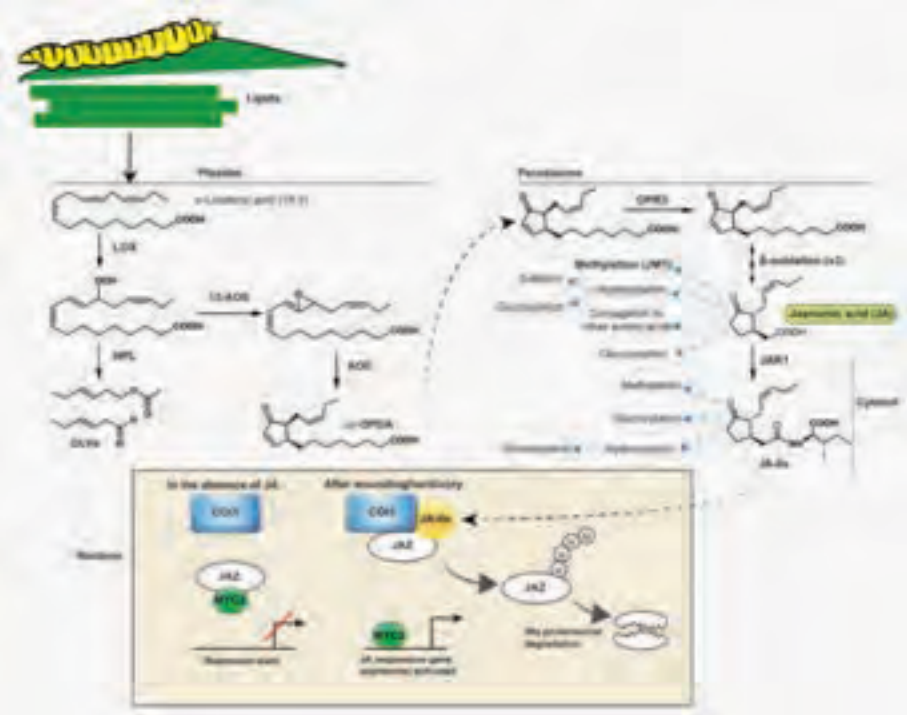
Plant-insect interactions are dynamic systems with constant variations. When attacked by herbivorous insects, plants exhibit biochemical changes resulting ultimately in direct and/or indirect defence responses. Being sessile and exposed to unpredictable environmental conditions has impacted the evolution of direct and indirect defence responses in plants. Direct plant defences range from waxes, thorns and toxins (eg. alkaloids, tannins, flavonoids) to defensive proteins. Indirect plant defences are those which help in recruiting carnivorous predators. By emitting volatile organic compounds (VOCs) or by secreting extrafloral nectar (EFN) upon herbivore damage, plants signal or 'cry for help' to predatory insects which prey upon the damaging herbivore. This 'top-down' strategy is an inducible defence response.

VOCs are mainly composed of terpenoids and green-leaf volatiles (GLV) while EFN consists of aqueous solutions of sugars and amino acids. Both these indirect defences are regulated by the phytohormone, jasmonate (JA), synthesized via oxylipin pathway. Generally, plants express several defences ('defence syndromes') and rarely rely on one defence strategy (Walling 2000; Agrawal & Fishbein 2006). There could be synergism/trade-offs among various plant defences particularly depending on the environmental conditions in which the plant is growing.

Synergistic interactions between various defences could be potentially useful to provide a greater level of defence than a single defence acting independently. On the other hand, expressing multiple defences incurs metabolic cost. Hence constraints on multiple defence allocation could result in trade-offs among different types of defences (Kempel et al 2011).

In our group, various plant defence responses ranging from direct to indirect strategies are investigated. The spatiotemporal variation and interactions between plant defences are assessed when the plant is challenged with different herbivores. Although there has been lot of interest in understanding plant defence mechanisms, most of the studies in the literature are on higher flowering plants of economic importance. Rarely have there been studies on plants that are lower in the phylogeny such as ferns
and mosses.

Ferns represent the first vascular plants and studying their defence responses would help us gain insights into the evolutionary origin of plant defences. To understand the evolutionary origins of plant defences such as volatile emission, the ancient fern *Pteridium aquilinum* (bracken fern) is examined in detail. Bracken fern is one of the most widespread plant species on Earth. It harbours many toxic chemicals ranging from lipids,



terpenoids, phenolics and cyanogenic glycosides to the insect moulting hormone mimic ecdysone and the enzyme thiaminase which causes Vitamin B1 deficiency. In addition, ferns possess a unique sesquiterpene ptaquiloside, that is a known carcinogen. The role of these glucosides in the fern is not clear. We utilize several chemical, analytical and ecological methods to understand plant defence mechanisms.

Direct and indirect defences in a given plant species can vary dramatically within the plant tissues and also between plants growing in different regions (Rasmann & Agrawal 2011). This could generate a patchy distribution of insect populations in these areas. Despite recognition of these variations, there is almost no data available on population level variation in plant defences and its influence on the interactions with insects. Until recently, it has been assumed commonly that climate exerts major constraints on species distribution, however, recent evidence points to the importance of variation in biotic interactions in predicting species distributions and responses to changing environment (Voigt et al 2003, Menéndez et al 2008). We study variation of plant defences across latitudinal gradients in the Western Ghats. This stretch of Ghats in India is biological rich and diverse, making it an ideal place to answer these research questions.

Feeding insects, on the other hand, are not passive targets but can often adapt to dietary challenges by various mechanisms like detoxification of plant defensive compounds, sequestration of plant toxins to avoid predation or alteration of gene expression patterns. In parallel, we will also investigate how insects cope

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with plant metabolites focusing on castor and fern metabolites. We hypothesize that insect gut microorganisms could play a crucial role in insect adaptation against toxic plant metabolites. A comparison between the gut flora of generalist and specialized insects will be made to understand their role in the adaptation process.

Herbivore recognition by plants is crucial to activate appropriate defence responses. Simple tissue disruption is not a reliable indicator of herbivore attack. Insect oral secretions (IOS) form early recognition cues for plants to mount appropriate responses. Compared with the diversity of insect herbivores, very few elicitors have been identified to date from IOS. Screening for IOS for herbivore-associated molecules (HAMs) that trigger calcium and phytohormone signalling is studied as part of this project.

In summary, there is a need to understand plant-insect interactions from the viewpoint of chemical ecology as this provides an ideal framework to address several important, yet unanswered questions such as why plants have so many defences and how these defences are distributed within the plant; or how do insects adapt to toxic food sources? So far, research in this context has focused on characterisation of novel metabolites and asking whether these metabolites could be used for human application. However, the future of discovering novel metabolites for human use depends on how well we understand nature's chemical library and to do this, we not only have to identify but also evaluate the role of these chemicals in ecological interactions. Only by doing this, can we expect to derive useful products from the subtle chemical cocktails in nature and progress towards sustainable agriculture.

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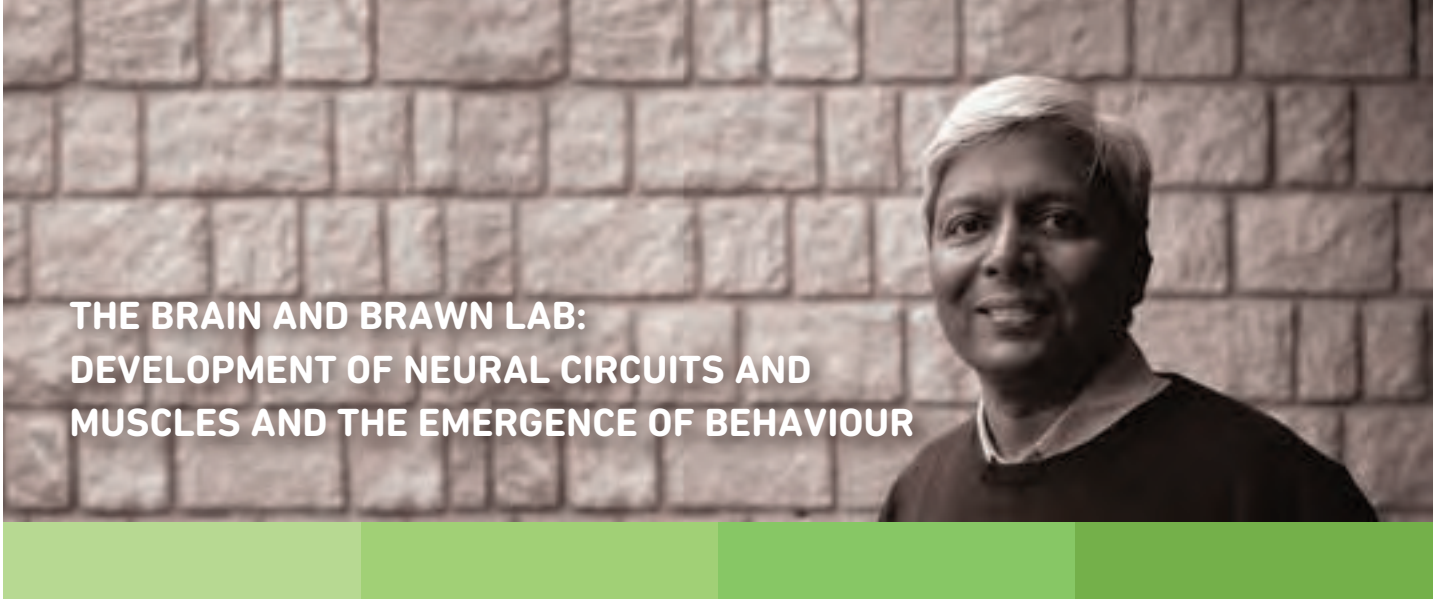
GENETICS AND DEVELOPMENT



THE BRAIN AND BRAWN LAB: DEVELOPMENT OF NEURAL CIRCUITS AND MUSCLES AND THE EMERGENCE OF BEHAVIOUR

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K. VijayRaghavan

Our laboratory studies how the birth, morphogenesis and connectivity of neurons and muscles translate into behaviour. We pare this complex problem to tractability by focusing on the olfactory and motor system of *Drosophila melanogaster.*

Learning to Ignore and Ignoring to Learn: A circuit mechanism for habituation in *Drosophila*

ANKITA CHODANKAR, BASKAR BAKTHAVACHALU, INDULEKHA P SUDHAKARAN, MADHUMALA K SADANANDAPPA, SWATI TRISAL AND MANI RAMASWAMI

Behavioural habituation is an ability of an organism to selectively ignore a familiar stimulus. We hypothesized that neuronal excitation patterns, if repeated without consequence, create inhibitory replicas (negative images) of themselves that filter and attenuate responses to familiar stimuli (Ramaswami, Neuron 2014). In the lab, we study molecular and neural circuit mechanism of long- and short-term habituation in fruit flies. We uncovered that olfactory habituation in flies arises from a selective potentiation of inhibitory (iLN-PN) synapses onto odour-activated excitatory neurons in the antennal lobe (AL). In this genetically accessible neural circuit, we study memory-associated molecular, cellular, and higher order processes that regulate the encoding, storage and override of behavioural habituation.

There are two key lines of ongoing work in the lab. The first is focused on circuit mechanisms of habituation with genetic and behavioural experiments in *Drosophila.* Specific mutant flies show greatly extended retention periods for long-term habituation. To understand the underlying neural mechanisms we are interested in the identity and mechanism of neuromodulators and neuromodulatory cells involved. We are also exploring the connectivity and role of cell types in which specific gene products must function for "forgetting" of habituation memory.

The second aspect of our work is focused on molecular mechanisms of long-term habituation. The formation and persistence of memory associated plasticity depends on spaciotemporal regulation of mRNA translation by RiboNucleoProtein (RNP) particles. Our current study focuses on understanding the role of LC domains of candidate RNA binding protein Ataxin2 in neuronal translational regulation underlying memory associated with olfactory habituation. Flies carrying CRISPR engineered mutations in the LC domains and transgenome flies expressing mutated tagged proteins are tested for effects in in-vivo RNP assembly, regulation of the memory associated CaMKII mRNA and in olfactory habituation. We are currently screening additional neuronal RNP granule proteins using the available transgenome resource.

Fig 1: A) Schematic of an adult *Drosophila* showing the major muscles of the thorax.
B) Sagittal section through the thorax showing the DLMs and their innervation.
C & D) Localisation of major sarcomeric proteins during DLM development.
E) Sagittal section of the adult DLMs showing unfused stem cells.

Muscle development and maintenance

RAJESH GUNAGE, DHANANJAY CHATURVEDI, NAGARAJU DHANYASI, M UMASHANKAR, KRISHAN BADRINATH, KUNAL CHAKRABORTY AND HEINRICH REICHERT

We study muscle development using the Dorsal Longitudinal Muscles (DLMs) of *Drosophila* (Fig. 1A). The ease of genetic manipulations in *Drosophila*, and the similarities between vertebrate skeletal muscles and the DLMs make them a versatile model system to investigate muscle development and regeneration.

During pupal development, the DLMs are innervated by motor neurons (Fig. 1B), which secrete instructive cues that regulate development of the muscles. Our experiments show that manipulating the identity of the motor neurons changes muscle architecture and leads to the mis-localization of sarcomeric proteins.

The sarcomere, the contractile unit of mature muscles is comprised of around thirty different proteins. We have characterised different stages of sarcomerogenesis by light microscopy (Fig. 1C & D). We are presently using transgenomic lines that express candidate proteins fused to GFP to establish spatio-temporal protein expression patterns within developing DLM fibers.

The DLMs are formed by the fusion of muscle precursor cells (myoblasts), some of which persist as stem cells in the adult (Fig. 1E). We show that upon injury these cells undergo clonal amplification followed by fusion to damaged muscles. This process is mediated by muscle-derived Delta signalling via canonical Notch activation, and the ubiquitin ligase Neuralized is used in this process. Our work establishes *Drosophila* as a model for the general understanding of muscle regeneration.

We have also found that deprivation of nutrition specifically in the third larval instar stage stunts systemic growth and arrests the division of DLM stem cells. Further, simulating starvation exclusively in muscles by taking away insulin receptor function, mimics the stunting phenotype. This strongly suggests that muscles mediate the effects of nutrition in specific developmental stages and do so in a muscle stem cell dependent fashion.

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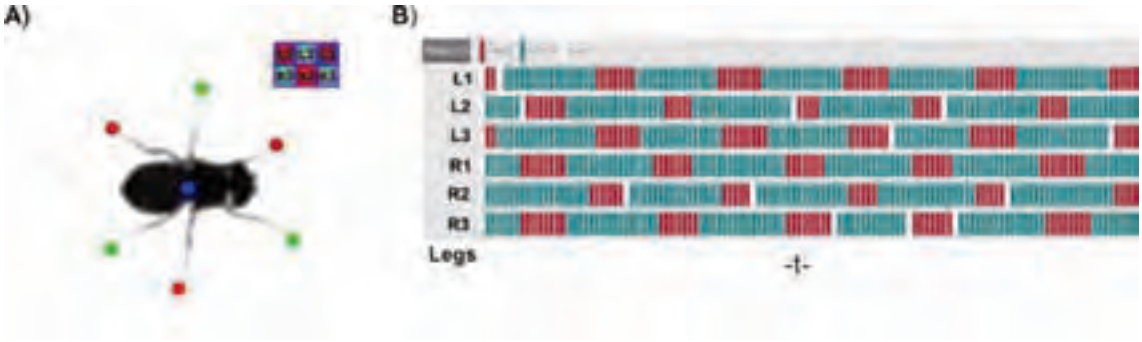

Fig 2: A) Auto-detection of leg movements denoting the leg co-ordinates; leg activity matrix summarizes the inter-leg swing co-ordination state.
B) A "gait diagram" summarizes a single walking bout of a fly; time frames are arranged as columns, red ticks represent swing phase and green ticks stance phase (tick interval 5ms).

Deciphering neuronal circuits for walking and feeding

SWETHA BM GOWDA, PUSHKAR D PARANJAPE, DURAFSHAN SAKEENA SYED, AMAN AGGARWAL, ALI ASGAR BOHRA, O.VENKATESHWARA REDDY, HEINRICH REICHERT

We study the development of motor circuits that drive the locomotion and feeding behaviour in *Drosophila*. Motor circuit comprises of the motoneurons that receive motor commands from the interneurons and sensory neurons. We have identified the lineage and architecture of leg and proboscis motoneurons. We have also elucidated the role of glial and neuronal Semaphorin signalling in the development of leg motoneuron architecture, required for the functioning of walking circuit in adult *Drosophila* (Syed et al., 2016). Next, we are deciphering the role of interneurons in the regulation of walking and feeding behaviour.

To study the walking circuit, we have developed techniques for tracking leg movements in pupae and adult *Drosophila* (Fig. 2A). During development, wild-type pupae move their legs in periodic burst patterns (Fig. 2B); and our results indicate that this episodic activity depends on both motoneuron inputs and sensory feedback. In adult *Drosophila*, we have characterised wild-type walking behaviour using automated kinematic analysis of leg movements, and have found that the pre-motor inhibitory input to motoneurons is an important determinant of leg movement and regulates the speed of walking in adult *Drosophila*. Similarly, in the *Drosophila* feeding circuit, we are now deciphering role of interneurons in taste processing. A behavioural gustatory screen identified the role of a pair of novel interneurons that receive inputs from bitter sensitive sensory neurons resulting in inhibition of proboscis extension and feeding. Taken together, we have identified components of the neural cicuitry required for walking and feeding in adult *Drosophila*. Further experiments are undereway to understand the neural basis of these behaviours in detail.

MOTOR CIRCUIT DEVELOPMENT AND FUNCTION

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In *Drosophila*, intracellular Ca^{2+} signalling by the inositol 1,4,5-trisphosphate receptor and the store-operated channel (dOrai) regulates the formation and function of motor circuits that control flight. Recently we have identified Septin 7, a cytoskeletal protein, as a negative regulator of the Orai channel in neurons. These findings suggest that agonists of store operated calcium entry might serve as a therapeutic intervention for certain neurodegenerative disorders.

1. Loss of SOCE affects flight circuit maturation

TRAYAMBAK PATHAK, TARJANI AGRAWAL, SUFIA SADAF AND SHLESHA RICHHARIYA

Store Operated Calcium Entry (SOCE) is thought to primarily regulate calcium homeostasis in neurons. Subsequent to identification of Orai as the SOCE channel in non-excitable cells, investigation of Orai function in

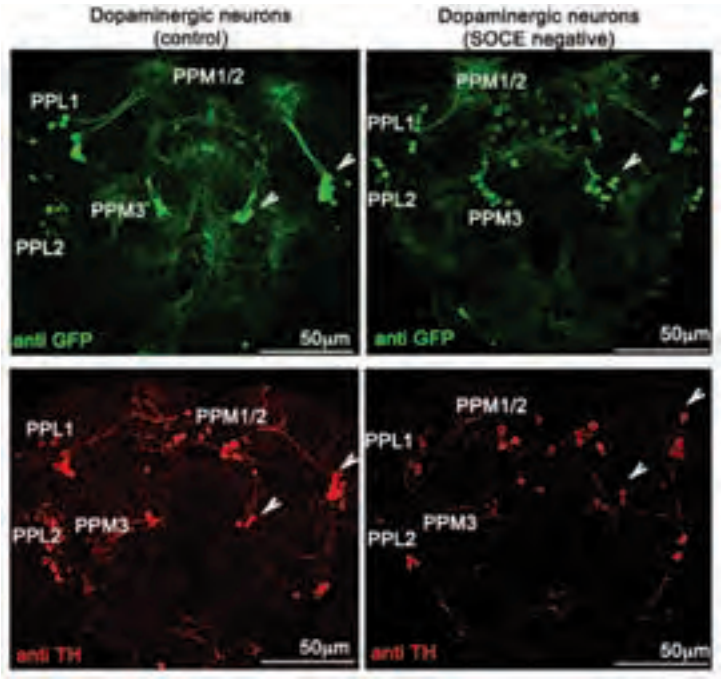

Fig 1: Tyrosine Hydroxylase immunostaining is reduced when SOCE is inhibited by expression of an *Orai* mutant transgene (*Orai* E180A) in specific dopaminergic neurons of the central brain like PPL1, PPL2 and PPM3.

neurons demonstrated a requirement for SOCE in *Drosophila* flight. By analysis of an Orai mutant and by controlled expression of a dominant-negative *Drosophila Orai* transgene, we showed that Orai-mediated SOCE is required in dopaminergic interneurons of the flight circuit during pupal development. Expression of dominant-negative *Orai* in dopaminergic neurons of pupae abolished flight.

The loss of Orai-mediated SOCE alters transcriptional regulation of dopaminergic neurons leading to down-regulation of the enzyme Tyrosine Hydroxylase, which is essential for dopamine synthesis, and the Dopamine Transporter which is required for dopamine uptake after synaptic release. These studies suggest that modulation of SOCE could serve as a novel mechanism for restoring dopamine levels in dopaminergic neurons.

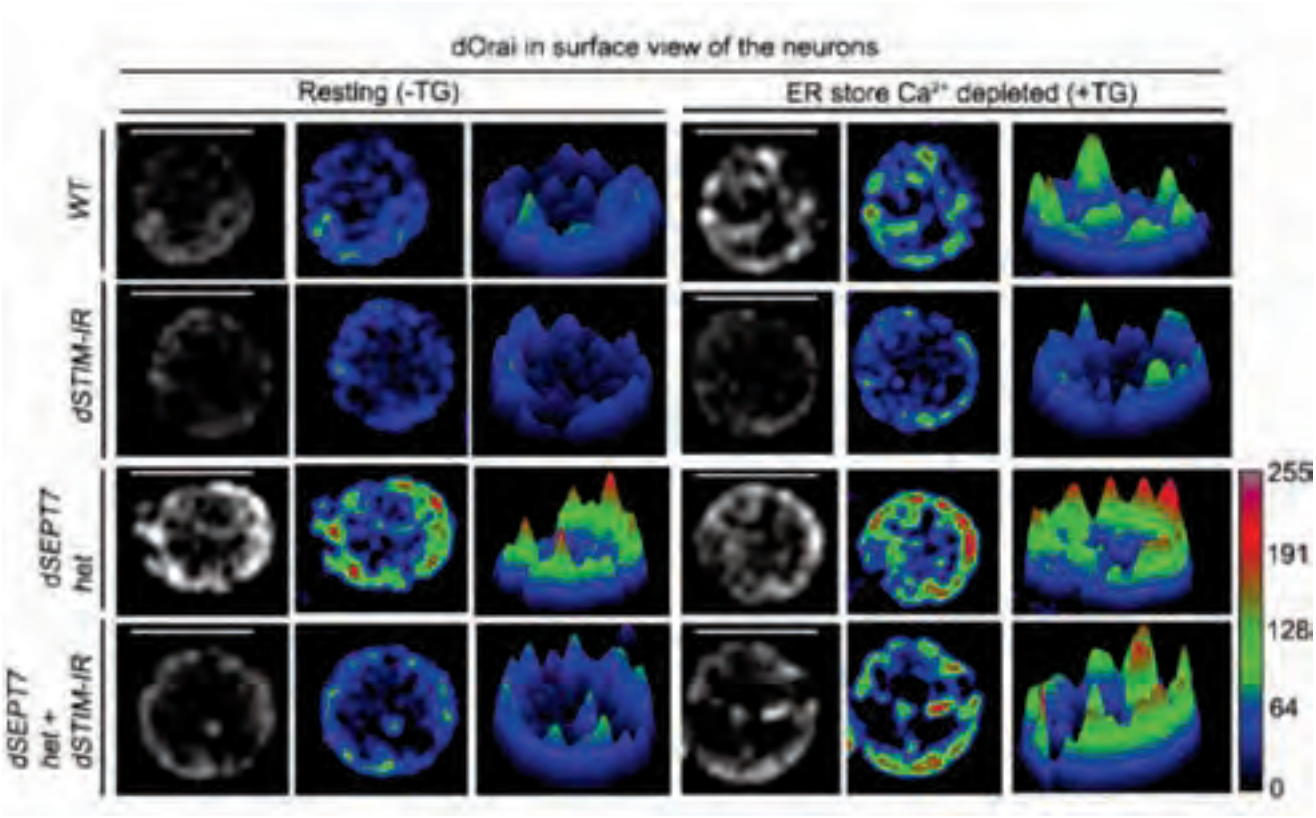

Fig 2: *Orai* clusters indicating open calcium channels form in the absence of SOCE in neurons with reduced Septin 7. Distribution of dOrai proteins on the surface of resting cells and cells after depletion of ER-Ca2+ stores are shown. Neurons with reduced dSEPT7 have more *Orai* clusters even in resting conditions. Grey scale images have been pseudocoloured to depict *Orai* clusters of different intensities. The calibration bar shows the grey scale intensities corresponding to each colour.

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2. Store-independent modulation of Ca^{2+} entry through Orai by Septin 7

BIPAN KUMAR DEB AND TRAYAMBAK PATHAK

Orai channels are required for store-operated Ca^{2+} entry (SOCE) in multiple cell types. Septins are a class of GTP binding proteins that function as diffusion barriers in cells. Here we show that Septin 7 acts as a 'molecular brake' on the activation of Orai channels in *Drosophila* neurons. Lowering Septin 7 levels results in dOrai mediated Ca^{2+} entry and higher cytosolic Ca^{2+} in resting neurons. This Ca^{2+} entry is independent of depletion of endoplasmic reticulum Ca^{2+} stores and Ca^{2+}-release through the inositol-1, 4, 5-trisphosphate receptor (IP3R). Importantly, store-independent Ca^{2+} entry through Orai compensates for reduced SOCE in the *Drosophila* flight circuit. Moreover, over-expression of Septin 7 reduces both SOCE and flight duration, supporting its role as a negative regulator of Orai channel function *in vivo.* Septin 7 levels in neurons can therefore alter neural circuit function by modulating Orai function and Ca^{2+} homeostasis. This finding is significant in the context of identifying Septin 7 as a therapeutic target in human diseases where SOCE is required.

UNDERSTANDING THE SIGNIFICANCE OF EPIGENETICS AND SMALL SILENCING RNAs

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Epigenetic marks superimpose underlying DNA sequences of eukaryotes to provide considerable plasticity in modulating gene expression. Small RNA molecules play a major role in the establishment and maintenance of epigenetic marks in plants. We are interested in understanding the mechanism of these processes.

Small RNAs are key molecules resulting from RNA silencing pathways which regulate the transcription and translation of their targets with the help of their protein partners. Small RNAs are important factors in initiating and maintaining heritable changes in gene expression without changes in DNA sequence ('epigenetics'). Small RNAs and epigenome modifications impact every aspect of eukaryotic development and disease. We are interested in understanding the pathways and mechanisms that generate small RNAs and epigenome modifications. We use various biochemical, genetic, bioinformatic and whole-genome approaches in a wide variety of model plants.

A. Natural variation in miRNA expression among rice species/ varieties

SWETHA CHENNA

MicroRNAs (miRNAs) are a class of endogenously expressed 21nt non-coding small (s)RNAs produced from MIR genes. We are interested in identifying key miRNAs that are differentially expressed among wild and cultivated rice species that have huge phenotypic variation (Fig. 1).

The Indica variety of rice originated from *O. nivara*, a wild plant growing in India and South East Asia. Analysis of sRNA datasets from two wild species (*O. nivara* and *O. rufipogon*) and one cultivated species of rice (*O. sativa var. indica* Pusa Basmati-1), revealed a surprisingly higher abundance of small RNAs originating from Chromosome 2 in wild rice species. This locus coded for a 22 nt miRNA named miR397. The abundance of this miRNA peaked in flag leaf, a tissue that usually provides 70% of energy required for grain filling. In the model dicot plant *Arabidopsis thaliana*, miR397 targets laccase mRNAs, although a functional significance of this interaction has not been understood. Laccases are a key group of proteins with 17 (*Arabidopsis*) to 25 copies (as in rice) in plant genomes, predicted to be involved in lignin polymerization. Lignin polymerization is a key step in a range of plant processes such as lignification of vascular bundles (xylem), formation of secondary cell wall, architecture and height. We found that *O. nivara* miR397 targets 13 laccase mRNAs in rice. Typical of 22 nt miRNAs, miR397 from rice triggers secondary siRNAs from laccase mRNAs in a stepwise cascade silencing. We use a range of genetic, biochemical and molecular techniques to understand role of miR397 in rice domestication.

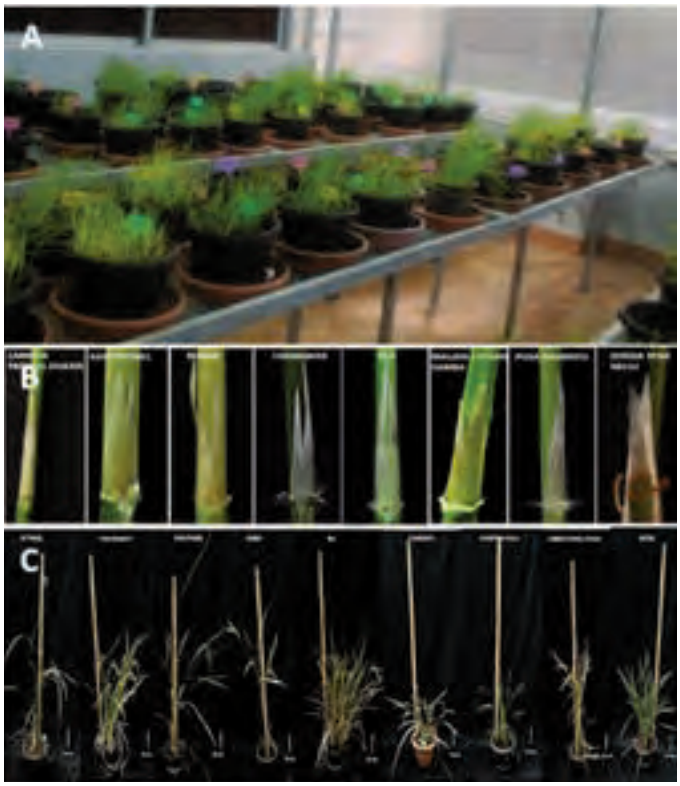

Fig 1: Natural variation in phenotypes of rice landraces. Our laboratory studies role of small RNAs and epigenetics in such changes.

A. Rice landraces grown in the greenhouses.

B. Variation in ligule structure among selected lines.

C. Variation in habit.

B. Contribution of a dsRNA binding partner of Dicer1 in plant miRNA biogenesis

ANUSHREE, COLLABORATION WITH ARATI RAMESH, NCBS

Plant miRNAs are processed from stem-loop structure containing precursors that are recognized and cleaved by Dicer like 1 (DCL1) with the help of dsRNA binding protein Hyponastic leaves 1 (HYL1) and a zinc finger protein named Serrate. We have recently shown that miRNA-miRNA* loop sequences determine abundance of miRNAs by using sequences of miR168, a well-conserved miRNA with almost similar sequences. We have shown that pre-miRNAs with smaller loops led to higher expression of miRNAs (Jagtap and Shivaprasad, 2014).

Unlike animal miRNAs, plant miRNAs have unusually high GC content when compared to the average genome. We find that there is high GC content in mature miRNA and miRNA* sequences compared to other regions of miRNA precursors, suggesting a likely selection for GC richness to make a strong stem. Although genome GC of plants vary across diverse groups, miRNA GC content is almost identical, suggesting a conserved mechanism that selects a specific signature among miRNAs.

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Although miRNAs have unique sequences, their GC signatures are maintained by having position specificity for G or C. We hypothesized that HYL1, a dsRNA partner of DCL1 could be responsible for observed GC signature in miRNAs. Hyl1 mutants lack precise processing ability and accumulate miRNAs at much lower levels. We have identified amino acid residues in the RNA binding domain 1 (RBD1) of HYL1 that are likely to mediate selections of specific GC signatures in miRNA precursors so that DCL1 can cleave precisely. As expected, these residues are present only in HYL1, but not among other RNA binding protein partners of other Dicers that produce small RNAs without a specific GC signature. Our results indicate a previously unknown determinant of plant miRNA biogenesis.

C. miRNA mediated regulation of anthocyanin development in plants.

VARSHA TIRUMALAI

We have characterised the role of three less-conserved miRNAs in anthocyanin development. All three miRNAs regulate expression MYB transcription factors. MYB transcription factors regulate multiple steps of phenylpropanoid pathway and fine tune the pathway to produce lignin, flavonoids, proanthocyanins, and anthocyanins (Fig. 2). Using small RNA, mRNA-seq and proteome data, we show that miRNAs regulate specific MYBs that we have named MYB repressors to regulate the pathway in such a way that anthocyanins are made in specific tissues. This miRNA:MYB interaction seems to be regulating anthocyanin development across eudicots.

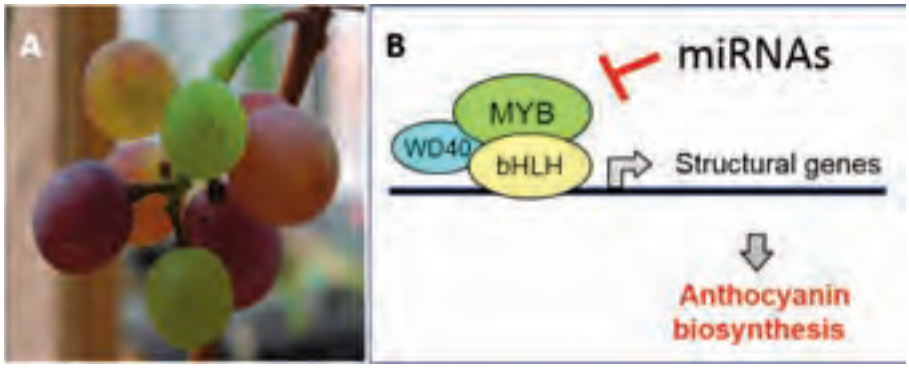
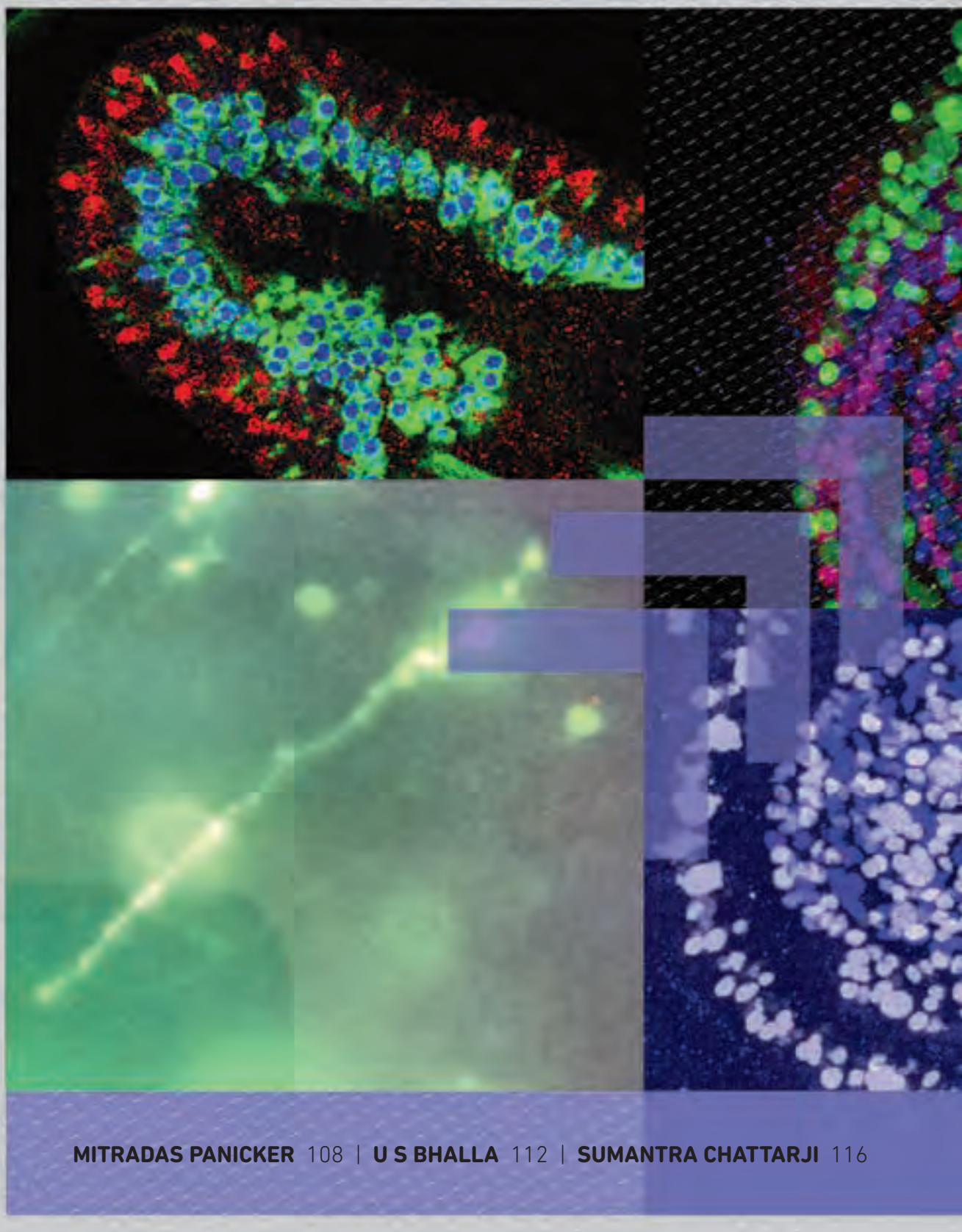


Fig 2: Regulation of phenylpropanaoid pathway by plant miRNAs.
A. Grape variety 'Bangalore blue'.
B. miRNAs regulate MYB transcription factors to control anthocyanin biosynthesis.

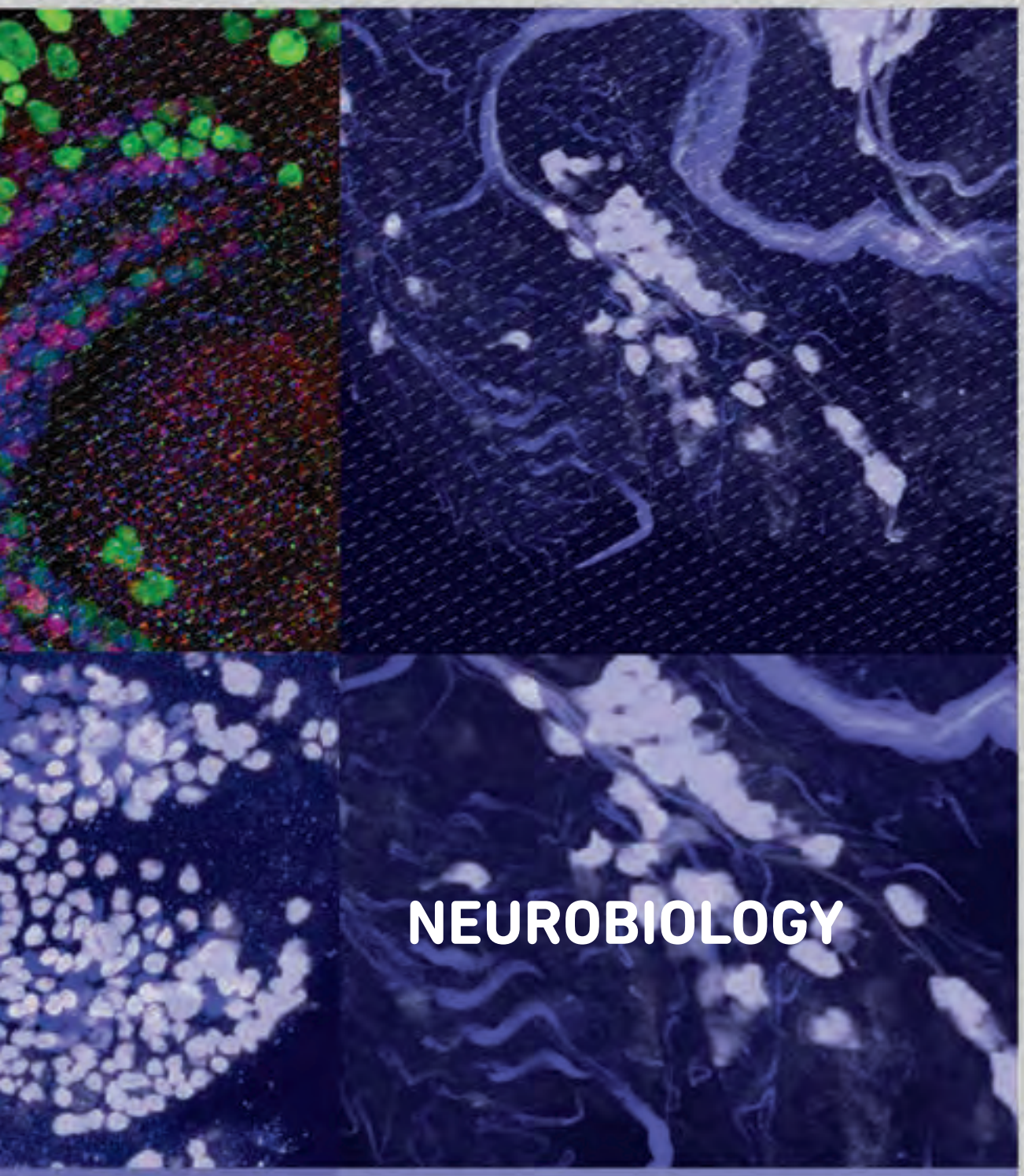
D. Geminiviral silencing suppressor protein βC1 has an unusual DNA binding ability.

ASHWIN NAIR

We have isolated a new virus infecting *Synedrella nodiflora* that holds promise as a vector for epigenetic modification of host promoters. It has a typical DNA A and a DNA β molecule half the size of DNA B. The DNA A is less than 90% similar to any known *Begomovirus*, thus it qualifies as a new species. The sole protein encoded by DNA β (βC1) is a silencing suppressor. We find that it has sequence-independent but structure-dependent DNA binding activity. We are currently studying the functional significance of this observation.



MITRADAS PANICKER 108 | **U S BHALLA** 112 | **SUMANTRA CHATTARJI** 116



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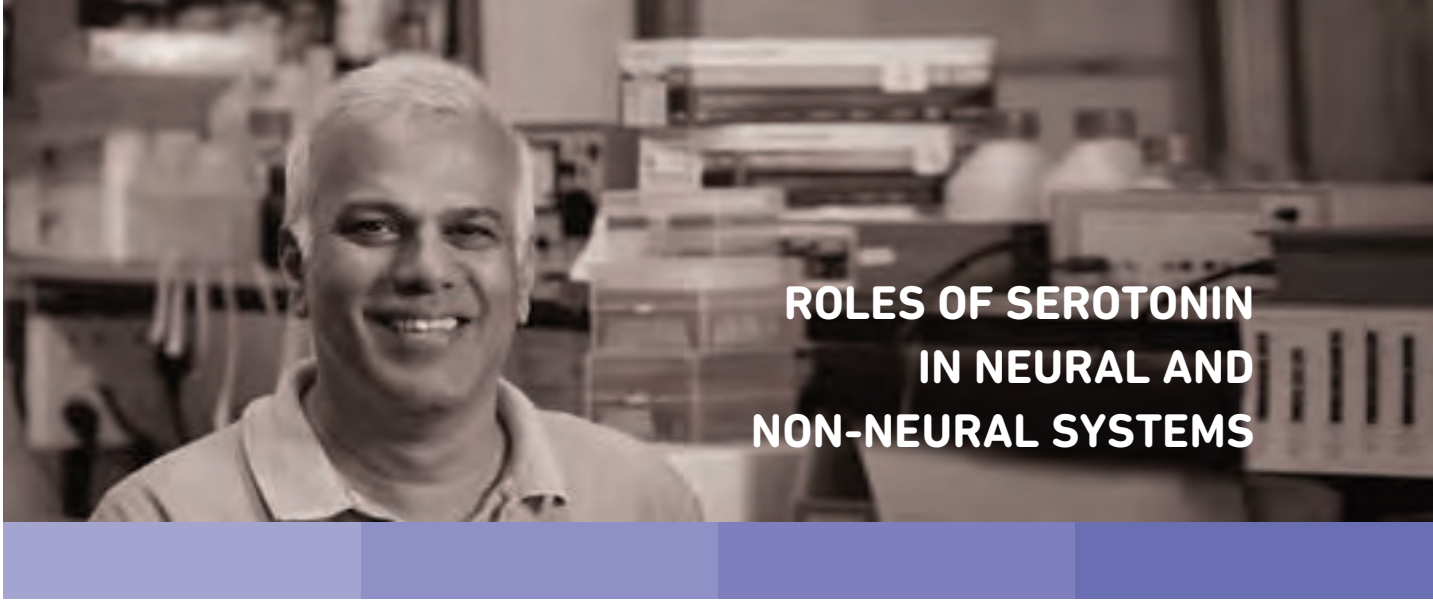

ROLES OF SEROTONIN IN NEURAL AND NON-NEURAL SYSTEMS

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Mitradas Panicker

The role of serotonin in the nervous system and in pluripotent stem cells is being explored using in vitro cellular models and transgenic mice. Cellular models from individual-specific pluripotent stem cells are being used to study Alzheimer's disease.

Serotonin (5-Hydroxytryptamine – 5-HT), widely recognized as a neurotransmitter, plays many important roles outside the nervous system too. Most of this activity is driven through multiple receptor subtypes. We have modified human and rat 5-HT_{2A} receptors to allow for easy visualisation of the receptors within cells and their interactions with various signalling components. These studies have been extended in the last year to a transgenic mouse model that lacks the 5-HT_{2A}. These studies have led to interesting observations regarding animal behaviour in the presence of serotonin, dopamine and antipsychotics – the latter being in wide clinical use, and should help us dissect some of the details of how these receptors are regulated by endogenous and exogenous ligands. Interesting behavioural variations have been observed with these animals when given antipsychotics.

Our studies have revealed that serotonin is also present in mouse and human embryonic stem cells and induced pluripotent stem cells, where a significant portion of it is localized to the mitochondria. Exogenous application of serotonin increases mitochondrial potential in mouse ES cells and also decreases the levels of reactive oxygen species. Mitochondrial potential is also known to have a significant effect on development.

Functional selectivity at Serotonin Receptors

SHISHUPAL SINGH, SHUCHITA SOMAN, ISHIER RAOTE

A number of endogenous amines have been found to interact with the 5-HT_{2A} receptor. The list includes dopamine, tryptamine, tyramine, norepinephrine, epinephrine, β-phenylethylamine (PEA). These amines act as agonists at the 5-HT_{2A} receptor, with different potencies and efficacies. Different ligands acting at the same receptor but modulating intracellular transduction cascades to different extents is referred to as 'functional selectivity'.

We are currently exploring the differences in signalling that result when dopamine activates the human and rat 5-HT_{2A}. These are being explored at the cellular level. While it is clear that extracellular dopamine at high concentrations can activate the 5-HT_{2A}, the efficacy at which it activates 5-HT2C and 5-HT2B remains to be studied. Our present efforts are also directed to establishing 'synapses' *in vitro* to determine if dopamine can activate these receptors, when released at a synapse.

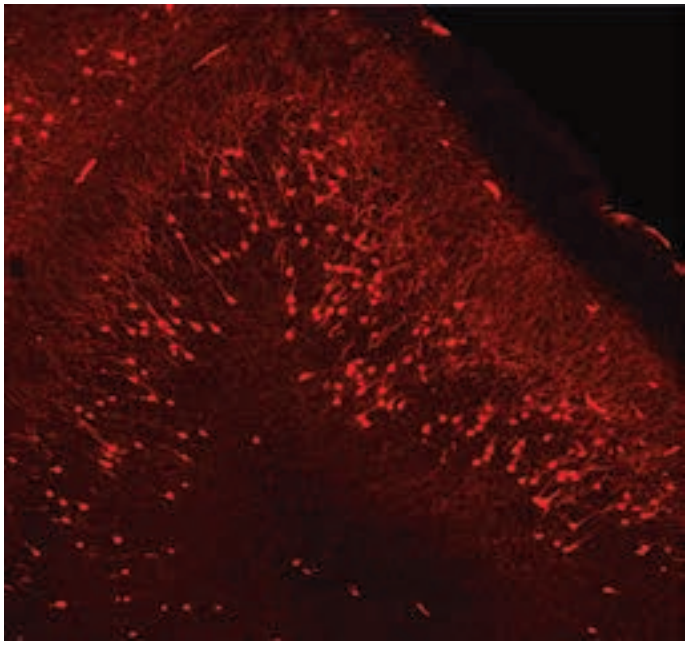

Fig 1: Antipsychotic-dependent labelling of neurons in the mice olfactory bulb using c-fos and td-tomato fluorescent protein.

Antipsychotics and the 5-HT_{2A} knockout mouse

RADHIKA JOSHI

A 5-HT_{2A} global 'knockout' mouse had been generated in collaboration with Dr. Rupasri Ain. The 'knockout' strain was used to study the role of the receptor in antipsychotic-mediated side effects. Clozapine - a representative 'atypical' antipsychotic – was studied in detail. Clozapine, like many antipsychotics, is known to induce sedation but the 'knockout' strain was resistant to sedation, which was quantified by measuring their spontaneous locomotor activity. While the 'knockout' mice were resistant to clozapine-induced sedation in a dose-dependent manner, consistent with what has been reported in the literature, altering the environmental conditions affected the level of sedation. For e.g. in a novel cage the 'knockout' strain was resistant to clozapine-induced sedation but got sedated in its 'home' cage. This effect was not observed with 'typical' antipsychotics.

This result allows us to dissect the effects of 'atypical' antipsychotics such as clozapine. Using an inducible c-Fos reporter construct into the 5-HT_{2A} knockout and wild type strains, we have been able to mark cells that are activated by antipsychotics and other drugs under various conditions. This has enabled us to define specific regions of the brain that are activated by antipsychotics as well as other clinically used drugs and potentially define the circuits involved.

Collaborator: Rupasri Ain

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Role of serotonin in embryonic and induced pluripotent stem cells

MEGHA PB

We had reported some years ago that serotonin is localized to the mitochondria of pre-implantation mouse embryos and that addition of extracellular serotonin increases the mitochondrial potential. The same is true for mammalian pluripotent stem cells. Extracellular serotonin also decreases the levels of Reactive Oxygen Species (ROS) in pluripotent stem cells.

When mouse or human somatic cells are induced to pluripotency, serotonin is synthesized and gets partially localized to the mitochondria. The increase in mitochondrial potential is at least partially mediated through the 5-HT_{2A} and the 5-HT transporter also seems to play a role. We have also observed that the effects of 5-HT on mitochondrial potential is characteristic of the 'naïve' state of mammalian pluripotent cells. We are presently deciphering the mechanisms involved. In the process, we have also established that some of the very widely used xeno-free and feeder-free media for human pluripotent cells cause high levels of ROS to be generated in pluripotent stem cells in culture. This causes nucleic acid damage to stem cells and suggests that ROS levels have to be ascertained in the design of media.

Collaborators: Michael Bader (Max Delbruck Center for Molecular Medicine, Germany), Natasha Alenina (Max Delbruck Center for Molecular Medicine, Germany), Valentina Mosienko (Max Delbruck Center for Molecular Medicine, Germany.

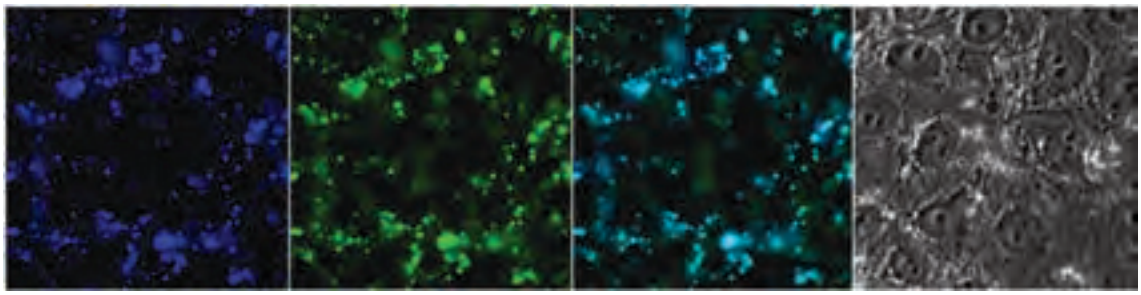

Fig 2: Fluorescent lipid droplets characterise 'primed' human pluripotent stem cells. Fluorescent lipid droplets are present in 'primed' pluripotent stem cells due to the sequestration of retinyl esters (Vitamin A). Lipid droplets can also be identified by staining with BODIPY 493/503.

Lipid metabolism, 'primed' pluripotent stem cells and disease models

MUTHUSWAMY THANGASELVAM, APARNA ASHOK, ASHAQ HUSSAIN, GOPAL DAS

We had reported last year that human pluripotent stem cells in the 'primed' state have fluorescent cytoplasmic lipid droplets due to the presence of retinyl esters. These can serve as a novel endogenous marker for the pluripotent state in the right media, and can be used to isolate pluripotent cells away from their differentiated derivatives. It also serves as an early marker during reprogramming of somatic cells. We have also determined that the lipid droplets are specific to the 'primed' pluripotent state and not to the 'naïve' state. The role of retinol and lipids in the presence of lipid droplets in 'primed' pluripotent stem cells is being explored along with its effects on cellular metabolism, glycolysis and oxidative phosphorylation.

We have also generated iPS cells from individuals with Late Onset Alzheimer's Disease (LOAD). We used lymphoblastoid cell lines from these individuals, with identified alleles at the ApoE locus, which are associated with LOAD. CRISPR-based gene editing was used to switch ApoE alleles to generate isogenic iPS cell lines. Transcriptome analysis of these lines and their differentiated cells are in progress. Neural stem cells from the iPS cell lines have been established and are also being studied to establish *in vitro* phenotypes. Novel SNPs related to Alzheimer's have also been identified.

Collaborators: Sanjeev Jain (NIMHANS), Odity Mukherjee (InStem).

Cell substrate adhesion and 5-HT_{2A}

JOE ANAND KUMAR, BASUDHA BASU

The 5-HT_{2A}, a target for antipsychotics, is also expressed extensively outside the nervous system as well. Over-expression of the 5-HT_{2A} receptor in a weakly adherent non-neural cell line i.e. HEK293 increased cell substrate adhesion significantly, which was blocked by antipsychotics or antagonists. Agonists increased cell substrate adhesion. The mechanism of adhesion was dependent on the cell type and was species-specific. Preliminary results indicate it to be integrin-dependent and that 5-HT_{2A} antagonists affect actin stress-fibre formation. The detailed mechanisms are being explored.

MOLECULAR AND NETWORK MECHANISMS FOR MEMORY AND SEQUENCE LEARNING

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U. S. Bhalla

Speech, music, dance, and thought all involve sequential activity of ensembles of neurons, and the capability for recursion. We are working on how the brain recognizes and remembers sequences *in vivo*, *in vitro*, and in computer models.

Our research utilizes new experimental and computational tools to examine plasticity with a particular interest in sequences and stability of information storage.

Air-borne odour-guided navigation

URVASHI RAHEJA

We have concluded many years of our experimental research program into the olfactory system with an analysis of how rats use air-borne odours to navigate. We find that they utilize a very efficient strategy in familiar environments, which is quite distinct from and much faster than the classic side-to-side 'casting' behaviour of animals locating an object in an open field. If rats know that the odour is coming from one of a few likely sources, they adopt a run-and-scan strategy. Here, they quickly pick one likely target, and run to it, and are successful about half the time. If they make a mistake, they now successively scan through the other possible targets till they find the odour source. This is a very robust behaviour and works well in the presence of novel odours, interfering background odours, loss of stereo odour discrimination, and even turbulence. We modeled the time to find an odour using this strategy as compared to the classic 'casting' behaviour and showed that run-and-scan is faster if there are a small (1-10) number of sources, and if distance to the sources is more than a metre.

Fig 1: Trajectories followed by a rat tracking an air-borne odour in a familiar environment. Left: Direct target track. Middle: Serial scanning, where the first selected target was incorrect. Right: Serial scanning, where the correct target was only one place offset from the first selection.

Hippocampal activity sequences in behaving mice

KAMBADUR ANANTHAMURTHY, SOUMYA BHATTACHARJEE, SHRIYA PALCHAUDHURI

How does brain activity change during the various phases of learning? To address this question, we train mice to associate two events separated by a brief interval. Simultaneously, we monitor neuronal activity using 2-photon calcium imaging of activity. In previous work, we have found that such associations map to a sequence of activity of brain cells in the hippocampus that spans the interval between the first and second stimulus. We have now standardized recordings from the same field of cells over multiple days. This development is based on the use of careful surgical implants of glass windows into the skulls of genetically-modified animals in which the brain cells express a calcium-sensitive reporter protein. The reporter protein expresses stably and eliminates the need to inject reporter dye prior to recordings. These longer recordings open up questions of stability of memory representations in different behavioural contexts.

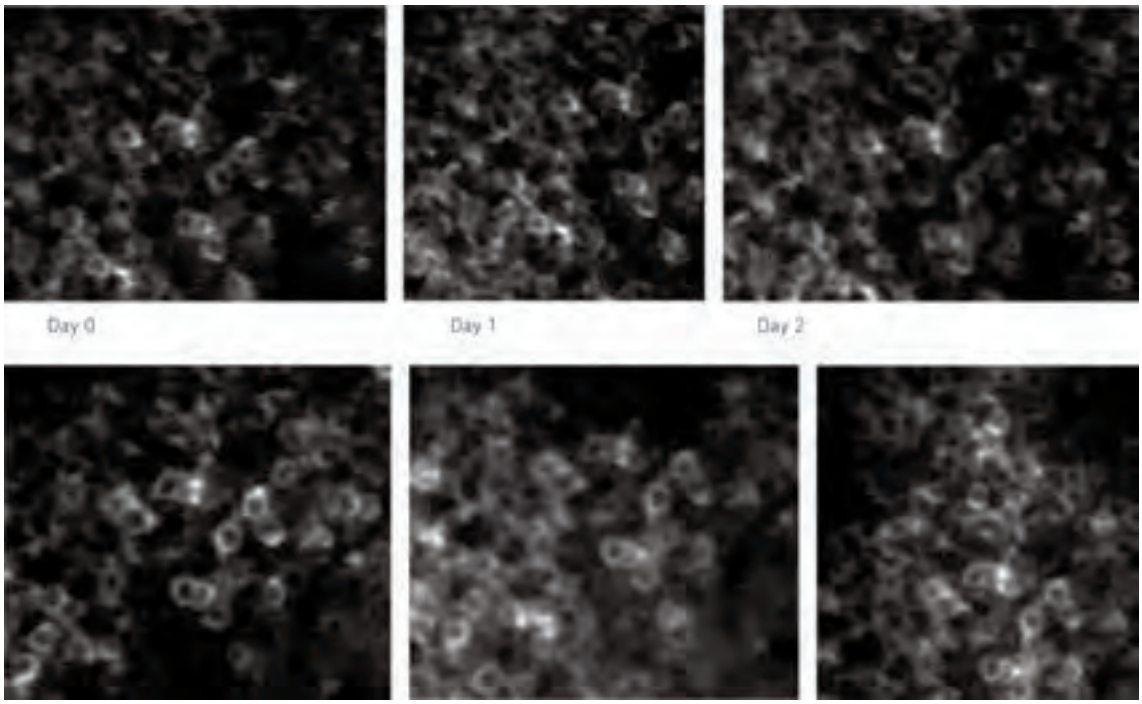

Fig 2: Multi-day recordings. Top row: Day 0,1,2 after implant. Bottom row: Day 14, 15, 16 after implant. Note the consistent neuronal groupings visible in the images from day to day.

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Hippocampal physiology with background activity

AANCHAL BHATIA, DEEPANALI DWIVEDI, ADITA ASOPA, OLIVER MUTHMANN

The brain is never silent, yet most studies of plasticity are done in brain slices where background activity is minimal. We use optogenetic stimulation of CA3 neurons in brain slices from mouse hippocampus to simulate *in-vivo* like background activity. We find that plasticity in CA3-CA1 synapses is modulated by this background. We are developing methods to examine precision of neuronal responses to noise as well as steady input patterns, to compare between wild-type and mutant animals that model human neural developmental disorders. We have also developed analysis methods for handling the deluge of data from high-density microelectrode arrays, to be able to pick out neuronal spikes and relate activity in time and space.

Models of memory stability

DILAWAR SINGH, SAHIL MOZA

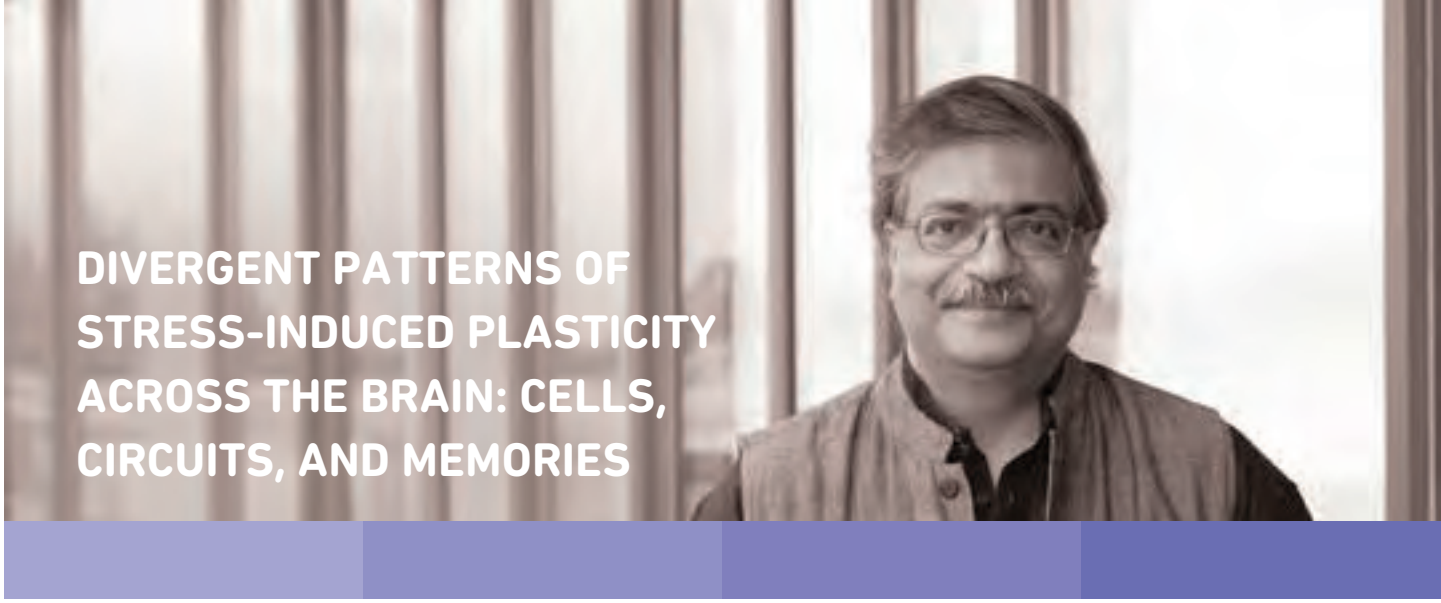
The brain is a noisy place, yet synapses are noisier still. At the very small volumes of a typical synapse, simple thermal noise leads to stochasticity in chemical events on the level of single molecules. If synapses are to store information reliably through chemical signals, they must have mechanisms to overcome this noise. We examine how stability of memory storage is affected by different kinds of diffusive exchange between CaMKII switching modules in the synapse. We compare cases where such modules are independent, or exchange subunits within a synapse, or even between synaptic spines. All these have different outcomes on the stability of storage.

We also ask what factors contribute to long-term stability of memory storage by an exhaustive examination of chemical switches (bistable systems) in the face of stochasticity, mutations, and metabolic context. We are examining how different reaction topologies may exhibit robustness with respect to these different factors.

MOOSE and modelling resources

HARSHA RANI, PRITISH PATIL, DHARMA TEJA VOOTURI, RAHUL GAYATRI*, AVIRAL GOEL, SUBHASIS RAY, UPINDER S. BHALLA

For many years we have been developing the Multiscale Object-Oriented Simulation Environment to carry out simulations that span molecular, electrical, cellular, and network-level phenomena in brain function. MOOSE is now at release 3.02 and has powerful new features for model composition from different sources, such as morphology files, channel definitions in NEUROML, chemical systems from SBML or kkit formats, and so on. We have been extending its capabilities to support GPU and MPI-based parallel computations.



DIVERGENT PATTERNS OF STRESS-INDUCED PLASTICITY ACROSS THE BRAIN: CELLS, CIRCUITS, AND MEMORIES

shona@ncbs.res.in

Sumantra Chattarji

Debilitating emotional problems are a hallmark of stress-related psychiatric disorders. We use animal models to explore the neural basis of these phenomena in the brain's emotional hub – the amygdala – from molecular and synaptic mechanisms at one end to their behavioral consequences at the other.

All memories are not equal – some are more equal than others. For instance, emotionally salient experiences tend to be well remembered, and the amygdala plays a central role in this process. But the rapid and robust encoding of emotional experiences, such as aversive memories, can become maladaptive – traumatic or prolonged stress often turns them into a source of debilitating anxiety. What are the neural mechanisms underlying these powerful emotional symptoms? To answer this question, we combine a range of behavioral, morphometric, biochemical and electrophysiological techniques to analyze stress-induced modulation of neuronal structure and function in the amygdala. We have identified unique features of stress-induced plasticity in the amygdala, which are strikingly different from those seen in the hippocampus, and could have long-term consequences for behavioral symptoms seen in affective disorders.

In earlier studies, stress-induced plasticity in different brain regions was viewed as stand-alone effects manifested as properties intrinsic to individual structures. Further, function was inferred from analysis at the cellular and behavioral levels without any online readout of dynamic changes in neuronal activity in the intact animal. However, neuroanatomical data also points to extensive interconnections between the hippocampus and amygdala. This raises the intriguing possibility that some of the structural and physiological changes triggered by stress in one brain area may, at least in part, influence changes in other areas. Therefore, we are using in vivo recordings in freely behaving animals to investigate the potential interdependence and interactions between brain areas differentially affected by stress.

1. Preventing the delayed impact of acute stress on the amygdala: from animal models to therapeutic strategies

FARHANA YASMIN, KAPIL SAXENA, AND PRABAHAN CHAKRABORTY

Post-traumatic stress disorder (PTSD) is triggered by a single overwhelmingly traumatic event. Moreover, some components of the fear response in PTSD persist well beyond the original event. We aimed to capture some of the defining temporal features of PTSD by building upon our earlier findings wherein a single episode of acute immobilization stress triggered a delayed onset of anxiety-like behavior and spinogenesis in the basolateral amygdala of rats. Using whole-cell recordings from excitatory neurons in lateral amygdalar slices, we find that acute stress causes a significant increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs) 10 days later (Fig. 1). Enhanced pre-synaptic release

of glutamate at thalamic inputs to lateral amygdala neurons contributes to this increase. Further, targeted infusion of an NMDA-receptor antagonist into the basolateral amygdala during acute stress prevents both the increase in mEPSC frequency and spine-density 10 days later (Fig. 1). More recently, we have identified a novel role for the endocannabinoid (eCB) system, which modulates pre-synaptic neurotransmitter release, in stress-induced plasticity in the amygdala. Oral administration of a pharmacological inhibitor of fatty acid amide hydrolase (FAAH), an enzyme involved in hydrolyzing eCB, before acute stress prevents the delayed strengthening of structural and physiological connectivity in the amygdala. These findings provide new insights into the accumulating clinical evidence for an important role played by the eCB system in protecting against PTSD and suggest pharmacological modulation of the eCB system as a possible therapeutic target.
We are also making use of this stress model to further examine its relevance to, as well as treatment against, PTSD in humans. In particular, we are now exploring the intriguing possibility of a post-stress window of intervention. For instance, administering a single oral dose of the anxiolytic drug diazepam after acute stress is effective in reversing the morphological and behavioral effects of acute stress 10 days later. In other words, it may be possible to prevent some of these delayed effects on the amygdala even after the stressful experience has taken place.

Collaborator: Bruce McEwen, Rockefeller University, USA

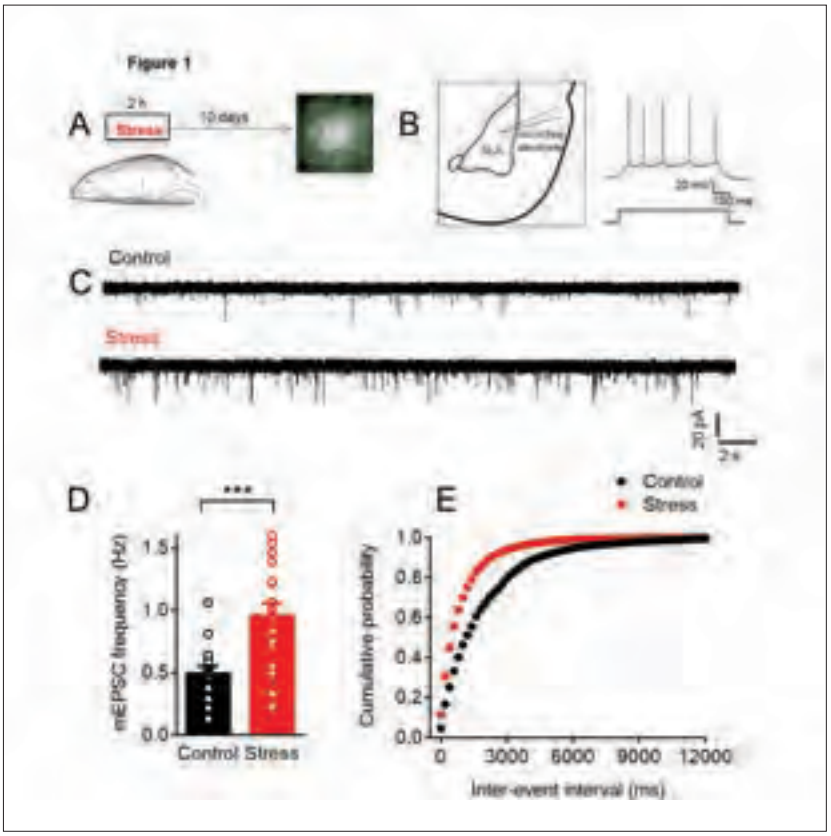


Fig 1: Acute stress causes a delayed increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs) in basolateral amygdala (BLA) principal neurons 10 days later.

(A) Schematic of experimental protocol: rats were subjected to 2 h of immobilization and 10 days later, animals were sacrificed for slice electrophysiology. (B) (left) Placement of recording electrode in a coronal slice of the amygdala. (right) Current-clamp recordings of accommodating action potential firing (top) from a typical BLA principal neuron in response to depolarizing current injection (bottom). (C) Sample mEPSC traces from control and stress groups. (Scale bar: 20pA, 2s). (D) Summary graph showing significant increase in frequency of mEPSCs in stress neurons compared with controls; ***p<0.001. (E) Cumulative distribution plots of inter-event interval for all events show a leftward shift with stress.

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2. Early hippocampal volume loss as a marker of eventual memory deficits caused by repeated stress

MOHAMMED MOSTAFIZUR RAHMAN

Exposure to severe and prolonged stress has detrimental effects on the hippocampus. However, relatively little is known about the gradual changes in hippocampal structure, and its behavioral consequences, over the course of repeated stress. Behavioral analyses during 10 days of chronic stress pointed to a delayed decline in spatial memory, the full impact of which is evident only after the end of stress. In contrast, concurrent volumetric measurements using a 7T MRI scanner in the same animals revealed significant reduction in hippocampal volumes in stressed animals relative to their unstressed counterparts, as early as the third day of stress (Fig. 2). Notably, animals that were behaviorally the worst affected at the end of chronic stress suffered the most pronounced early loss in hippocampal volume. Together, these findings support the view that not only is smaller hippocampal volume linked to stress-induced memory deficits, but it may also act as an early risk factor for the eventual development of cognitive impairments seen in stress-related psychiatric disorders.

Collaborator: Shane O'Mara, Trinity College, Ireland

A

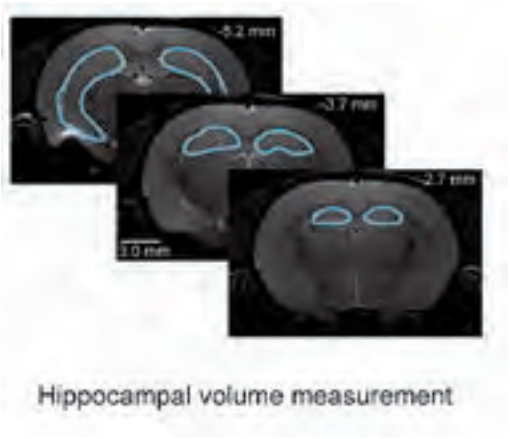


Fig 2: Combined volumetric imaging and behavioral analyses over the course of repeated stress identify early loss of hippocampal volume as an indicator of the eventual decline in spatial memory in the same animal.

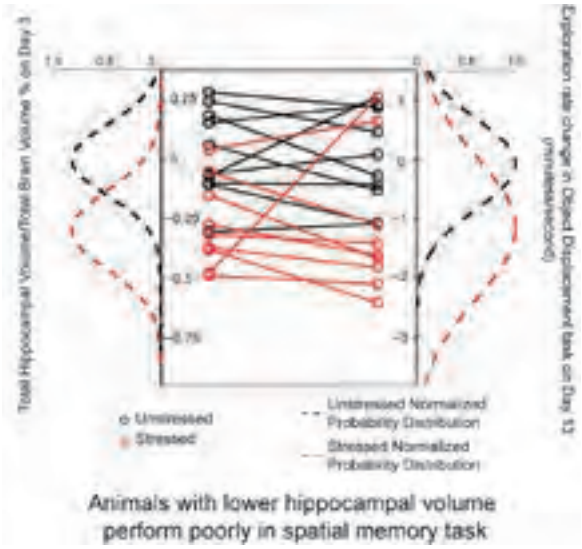
(A) Representative coronal images of the rat hippocampus (blue lines) using a 7T MRI scanner.

(B) The reduction in hippocampal volume on the third day of 10-day chronic stress exhibited a strong correlation with impairment in spatial memory after the end of stress. Stressed animals with the smaller hippocampal volumes (red circles) on day 3 were more deficient in the object displacement task on day 13

(C). Dotted lines: probability distribution for unstressed (black) and stressed (red) animals.

B

C



3. mGluR5-induced modulation of amygdalar plasticity causes distinct effects on specific versus generalized fear

MOHAMMED MOSTAFIZUR RAHMAN, GISELLE FERNANDES, AND SONAL KEDIA

The group I metabotropic glutamate receptor subtype mGluR5 plays an important role in synaptic plasticity as well as learning and memory. In the hippocampus, mGluR5 mediates long-term depression (LTD) of synaptic strength. In the amygdala, by contrast, mGluR5 mediates long-term potentiation (LTP). However, little is known about these divergent forms mGluR-plasticity across the two brain areas because much of our current understanding is based primarily on findings from the hippocampus. While earlier work reported that blocking mGluR5 prevents both anxiety and fear, little is known about how the same receptor in the amygdala gives rise to both. Combining behavioral and electrophysiological analyses with chemical activation of mGluR5, we have identified changes in intrinsic excitability and synaptic plasticity in lateral amygdala neurons that give rise to distinct, mutually exclusive facilitation of generalized versus specific fear (Fig. 3). These results provide a new framework across biological scales for examining mGluR-plasticity in the amygdala, and its behavioral consequences for emotional function in health and disease.

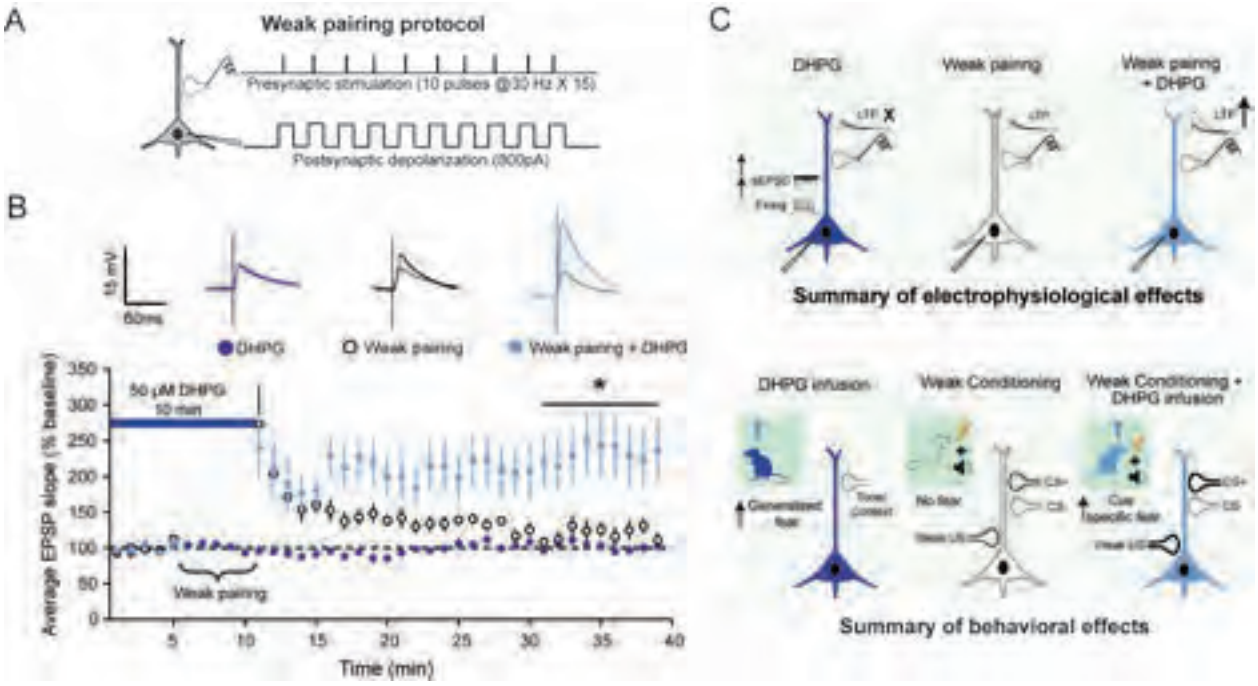


Fig 3: Although mGluR5-activation and a weak associative pairing protocol on their own did not elicit LTP, the two together caused a robust facilitation of LTP.

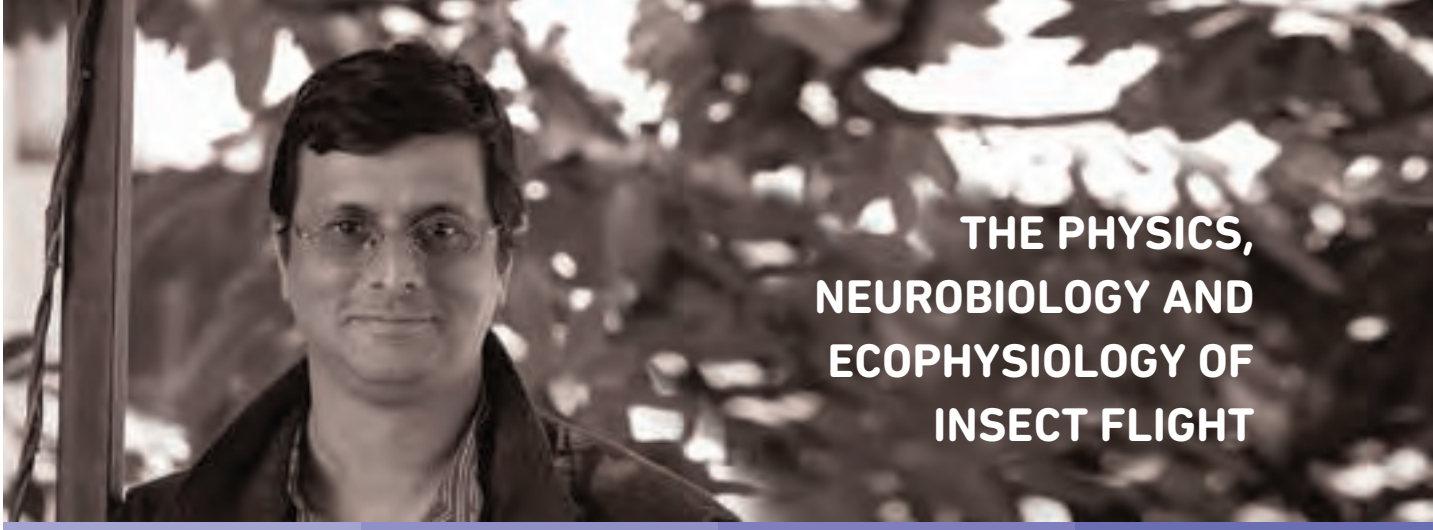
(A) Schematic of the weak associative pairing protocol that involved pairing EPSPs evoked by presynaptic stimulation of thalamic afferents with weak postsynaptic depolarization of LA neurons timed to coincide with the peak of EPSPs evoked by presynaptic stimuli. (B) 10 min bath application of 50 µM of the mGluR5 agonist DHPG (blue bar) alone did not induce LTP (●, n= 11 neurons, *p>0.05*). The weak pairing protocol (brackets) caused only a transient enhancement in the EPSP slope, but no LTP (○, n= 11 neurons, *p>0.05*). However, in the presence of DHPG, the same weak pairing protocol induced robust LTP (●, n=12 neurons, *, *p<0.05*). Superimposed representative EPSP traces before (grey) and 30 minutes after the three manipulations – DHPG alone (dark blue), weak pairing alone (black), and weak pairing + DHPG (light blue). (C) Summary of the electrophysiological effects of *in vitro* activation of mGluR5 in LA slices and how they relate to the behavioral effects of *in vivo* activation of mGluR5.

4. "Socialist" models of stress and emotion

SHOBHA ANILKUMAR, DEEPIKA PATEL, AND ASHUTOSH SHUKLA

While our earlier research using models of physical stressors, such as immobilization, provided valuable insights into the effects of stress at multiple levels of neural organization, it has certain limitations. For instance, these models do not use ecologically natural stressors. To address these gaps, we are now studying the cellular effects of psychosocial stressors like social defeat in the resident-intruder paradigm using Wildtype Groningen rats. Further, earlier behavioral studies focused primarily on aversive learning paradigms such as fear conditioning, and do not measure innate responses to naturally positive and negative emotional experiences. Ultrasonic vocalization is an important part of social communication between rodents. Adult rats use two types of social calls that convey innate emotional valence – 22 kHz aversive calls and 50 kHz appetitive calls. We are now examining the effects of these calls on behavior and amygdalar neural activity, and how these are affected by stress.

Collaborators: Bauke Buwalda, University of Groningen, Holland



THE PHYSICS, NEUROBIOLOGY AND ECOPHYSIOLOGY OF INSECT FLIGHT

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Sanjay Sane

My laboratory explores the mechanisms of how insects fly. We study the biomechanical, neurobiological, physiological and ecological underpinnings of various flight-related behaviours. These include fast aerial manoeuvres, territorial chases, short-distance navigating tasks such as foraging or odour-source localization, and long-distance navigating tasks such as migration

When an insect gets ready to fly, several sensory-motor systems must be simultaneously deployed to ensure proper behavioural output. These behaviours – as varied as antennal positioning, head stabilization, wing and haltere coordination and abdominal reflexes – operate in exquisite synchrony to enable stable flight. Yet, this coordination may occur in time-scales that challenges the ability of their nervous systems to sense and respond. How are these fast reflexes coordinated in timescales under a human eye blink?

Our recent work demonstrates that the answer lies not only in nervous control of flight, but also in the physical architecture of the thorax. To address such questions, my laboratory employs a combination of neuroanatomy, neurophysiology, behavioural analysis, and computational modelling tools to investigate topics ranging from the physics and neurobiology to eco-physiology of insect flight.

1. Antennal Control of flight

Antennal mechanosensory feedback is crucial for flight control in most insects. This feedback is provided by the mechanosensory units of the Johnston's organ, located in the basal antennal segments. These mechanosensors encode a vast range of antennal movements from low to high frequencies with extreme sensitivity, making the Johnston's organ remarkably versatile in its function. For instance, it is known to mediate diverse functions such as audition, tactile sensing, inertial and gravity sensing and even flowsensing (Krishnan and Sane, 2015).

Recently, we showed that antennal positioning during flight is mediated by another set of antennal mechanosensors, the Bohm's bristles, located on the surface of the basal antennal segments. The neurons in these sensors are activated when the antenna undergoes large movements and their sensory arbors directly project on the dendrites of antennal motor neurons. This mechanosensory-motor reflex loop helps maintain antennal position during flight (Krishnan et al, 2012).

Antennal position is also modulated by visual and airflow stimuli, making the antennomotor system ideal for studying multi-sensory integration (Krishnan and Sane, 2014). The mechanisms underlying sensory

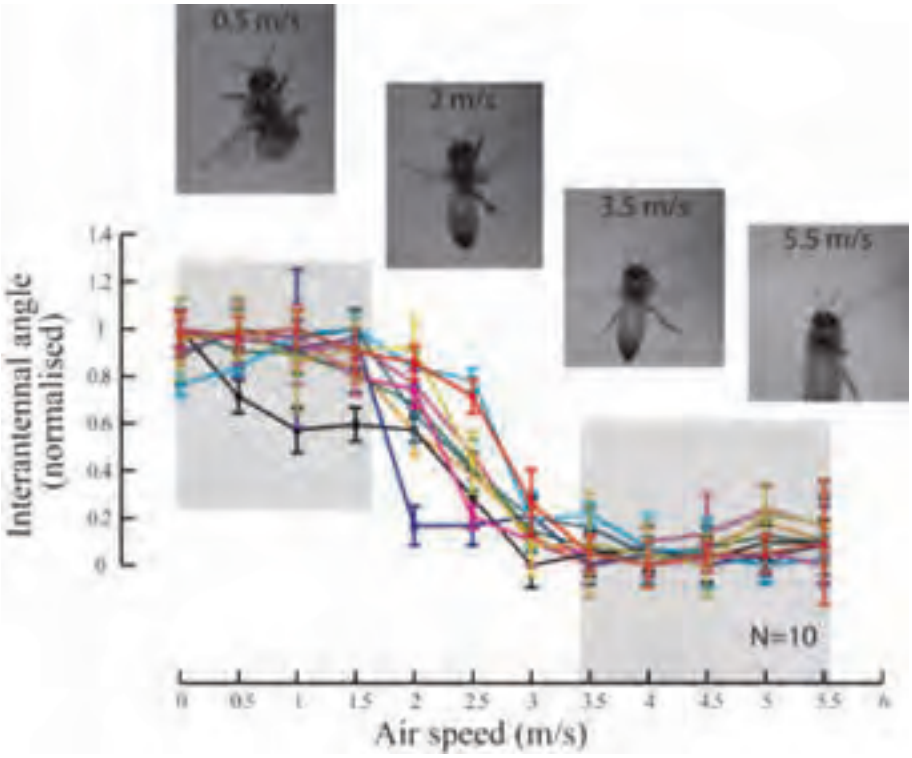

Fig 1: Antennal responses of honeybees to changes in ambient airflow follow a precise sigmoidal relationship.

integration are under investigation. We are studying how sensory information from the brain is transmitted, via the ventral nerve cord, to the flight motor centres. Moreover, from an evolutionary perspective, we are also exploring the effects of size or phylogenetic distance on how these circuits operate in insects with diverse antennal morphology and function.

2. Wing-Haltere coordination in flies

The ancestral insect had two pair of wings. However, in insects of the Order Diptera (flies, mosquitoes, midges etc.), the hind wings evolved into exquisite gyrosensory structures called halteres. They inform the nervous system about changes in body orientation during flight. Flies flap their wings at frequencies of hundreds of strokes per second. Remarkably, the input from the mechanosensory halteres is sufficiently rapid to be able to control the wing movement on a stroke-by-stroke basis. When we reduce or eliminate this input by ablating the halteres through physical or genetic means, the flies are unable to control their flight. Halteres move with a very precise phase-relationship relative to wings in timescales that are often too fast for the nervous system to handle. The phase relationship between wing and haltere motion is set not by the nervous system, but by thoracic mechanics (Deora et al, 2015). We identified specific mechanical connections within the thorax and

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Krishnan, A.* and **Sane, S.P.*** (2015). Antennal mechanosensors and their evolutionary antecedents. ***Advances in Insect Physiology*** 49, 59-99.

showed that the insect wing hinge must contain a clutch mechanism in addition to a previously described gearbox, much like a car transmission. Collaborative research with Dr. Gaiti Hasan's laboratory has helped identify the neural mechanisms which help synchronize the clutch under each wing (Sadaf et al, 2015). These studies show that mechanical means of coordination play an important part in rapid behaviours, and may be especially relevant in miniature insects which require faster movements. We are currently collaborating with Dr. Namrata Gundiah's laboratory at the Indian Institute of Science on various aspects of this project.

3. Odour source localization in flying insects:

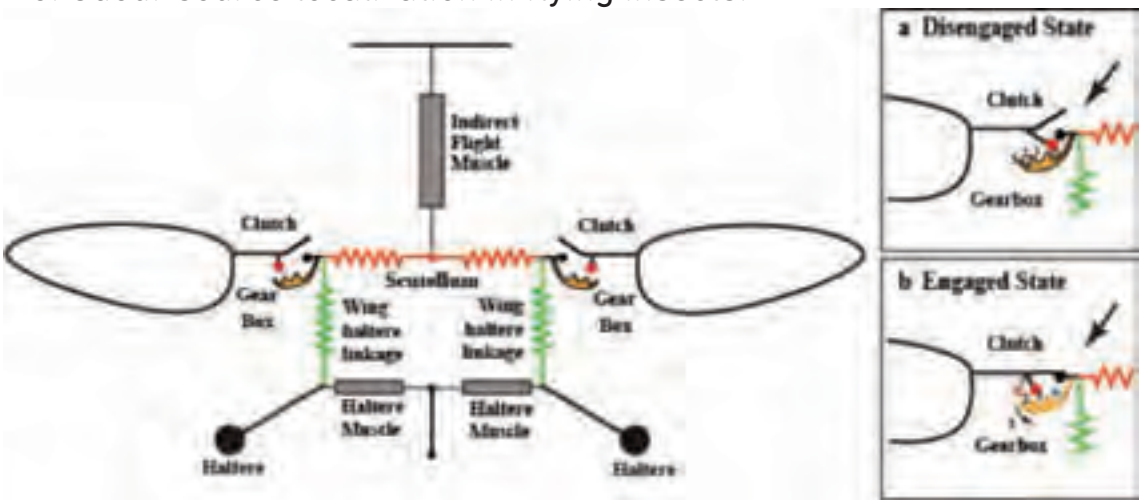

Fig 2: The mechanical model of an insect thorax depicting the various thoracic linkages between wings and halteres, as well as the clutch and gearbox.

through a complex olfactory-visual landscape to locate the sources of odour. Our studies on the fruit fly *Drosophila melanogaster* and Oleander hawk moth *Daphnis nerii* show how insects use multiple sensory modalities to achieve this task. Specifically, we have investigated how insects use their visual landscape to pinpoint the location of odour objects. These studies involve filming insects with high-speed video cameras as they fly in wind tunnels, or semi-natural environments such as the greenhouse.

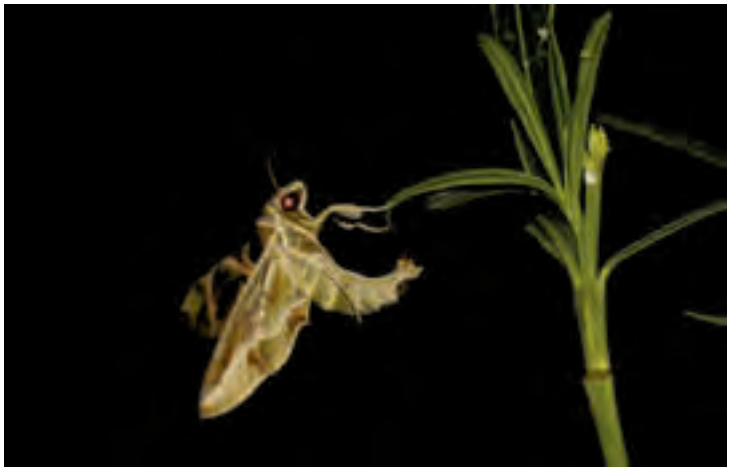

Fig 3: The Oleander hawkmoth, *Daphnis nerii* laying eggs on a fresh Nerium leaf.

4. Eco-physiological study of insect migrations

Bangalore is the site of several spectacular Lepidopteran migrations. These offer us a unique chance to study how flight is sustained over distances often extending to hundreds or thousands of kilometres. To address this question, we use an array of approaches including neurophysiology, eco-physiology, energetics, and natural history. As part of this project, we have been cataloguing moth diversity and studying their interactions with host plants in the Western Ghats and other parts of India at the various field stations engaged by NCBS. Part of this work is also carried out at the Smithsonian Tropical Research Institute in Panama in collaboration with Dr. Robert Dudley of the University of California, Berkeley.

5. Aerodynamics of insect flight

Flapping wings interact with ambient air to generate flight forces and torques. Our investigation of the mechanistic details of these phenomena over several years revealed fundamental differences between flapping and fixed wing flight. These insights led us to create mathematical models to predict the forces on rigid, flapping wings. In collaboration with Dr. Xinyan Deng's laboratory at Purdue University, we have helped develop experimental methods to image 3D flows and have studied solid-fluid interactions in flexible wings. From a biological standpoint, such studies allow us to estimate the self-generated flows in the immediate vicinity of flying insects influencing key biological processes such as thermoregulation, water loss etc.

6. Mound building behaviour in termites

Mounds of the termite, *Odontotermes obsesus* are closed but porous structures often towering over a few metres, and equally deep underground. They contain a queen surrounded by a large colony of soldiers and workers, which farm a fungus that grows only within the mound. The function of the mound structure remains a mystery. We study this problem from the perspective of a single termite, specifically asking how individuals make decisions about where and when to build, how they handle soils of various water content, or know when to build vs. excavate. We are also studying how termite traffic is regulated within the constrained confines with the mound.

DEVELOPMENT, MODULATION AND FUNCTION OF MOTOR SYSTEMS

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Vatsala Thirumalai

In vertebrates, locomotion is generated by multiple circuits in the brain and spinal cord acting in a co-ordinated fashion. We study how these circuits assemble and how they function at all stages of life.

My group uses a multi-pronged approach to understand how the circuits that generate locomotory command assemble, and how they function in early development and later in life. We use zebrafish *(Danio rerio)*, a small teleostean fish inhabiting the shallow water basins of the Ganga and the Brahmaputra, as our model system. The brain of the zebrafish larva contains only about 100,000 neurons but is highly homologous to the mammalian brain, with conserved anatomy and physiology. At 5 days post fertilization (dpf), the larva is an independent organism capable of navigation, foraging and escape, and shows a rich locomotory repertoire. Circuits in the spinal cord, hindbrain and cerebellum are also functional by this stage. We have begun working on the cerebellum and the hindbrain reticulospinal network as candidate motor circuits. We seek to understand how locomotory control emerges in these circuits and what roles neuromodulators play in their function.

1. Single cell and population dynamics of Purkinje neurons

MOHINI SENGUPTA, SRIRAM NARAYANAN, KSHITIJ DWIVEDI

Purkinje neurons are the sole output cells of the cerebellar cortex and integrate information from sensory and motor related circuits. Principally, they are excited by climbing fibers (CF) from the inferior olive and parallel fibers from granule cells. We have recorded from Purkinje neurons in larval zebrafish at 7dpf and found that they exhibit bistability (Sengupta and Thirumalai, eLife, 2015). We found Purkinje neurons in either depolarized or hyperpolarized states. In the depolarized state, Purkinje neurons generate tonic sodium spikes and less frequent calcium spikes. In the hyperpolarized state, they generate bursts of action potentials. We discovered that CF inputs trigger bursts in the hyperpolarized state and calcium spikes in the depolarized state. Further, bursts are timed to co-occur with motor episodes. To determine correlations between population activity of Purkinje neurons and motor episodes, we performed calcium imaging in these neurons. We discovered that each motor episode leads to spatio-temporally unique activation patterns in the Purkinje cell layer (Fig. 1).

2. Dopaminergic neuromodulation of cerebellar circuits and motor function

LENA ROBRA, URVASHI JHA, SRIRAM NARAYANAN, MOHINI SENGUPTA

Dopamine is a potent neuromodulator of motor behaviours in almost all animal models studied. Dopaminergic fibers are found throughout the larval zebrafish CNS, particularly in close proximity to Purkinje neurons. Neurons that receive dopaminergic inputs express the signalling molecule DARPP-32. We developed a polyclonal antibody that binds specifically to the zebrafish Darpp-32 protein. Using fluorescence

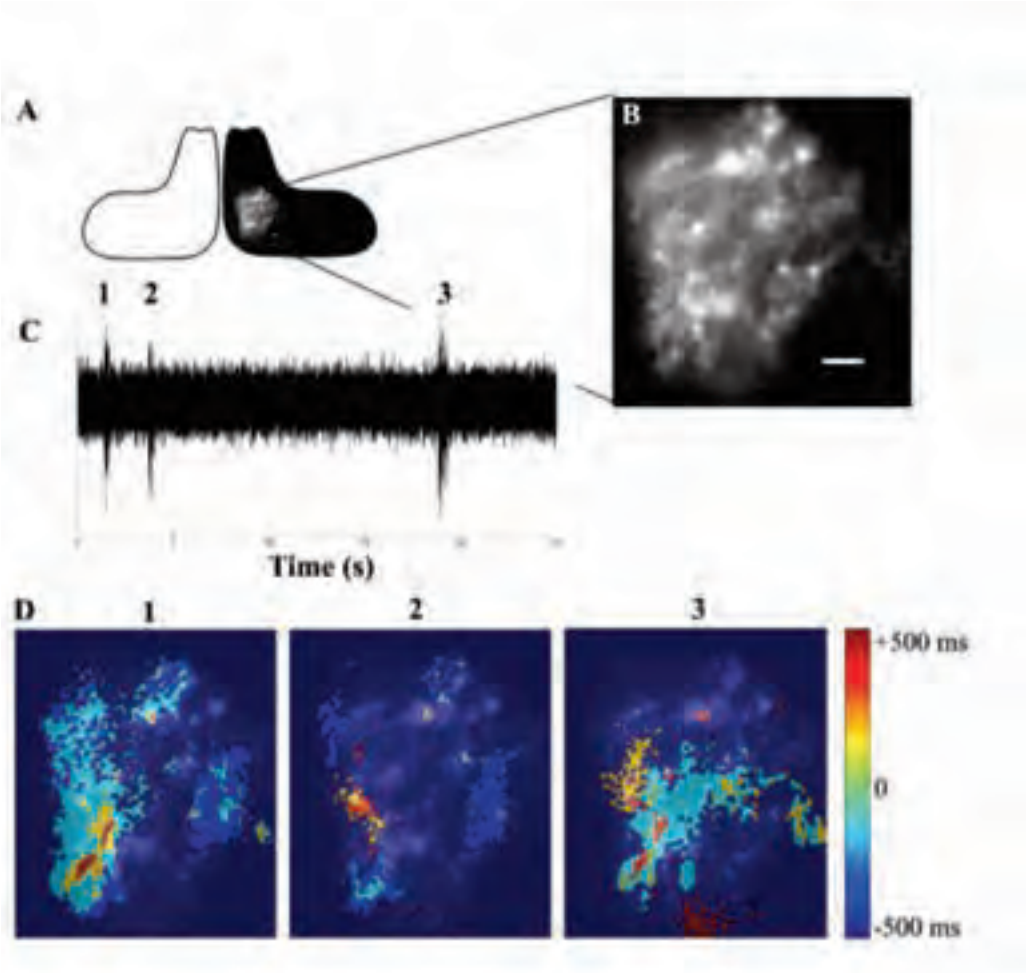

Fig 1: Purkinje neuron dendrites show distributed activity in time and space during motor bouts.

(A) Schematic diagram showing position of Purkinje neuron cluster labelled with GCaMP expression in one cerebellar hemisphere.

(B) Zoomed in image showing Purkinje neuron somata and dendrites expressing GCaMP5G. Scale bar: 10 µm.

(C) Motor recording for the same larva showing three motor bouts 1, 2 and 3 simultaneous calcium activity.

(D) The corresponding calcium activity showing heat map representing a 1s window around the start of the motor bout plotted for each of the three motor bouts.

immunohistochemistry, we showed that Darpp-32 is intensely expressed in Purkinje neurons from a very early stage of development (Figure 2). Next, we wanted to determine whether darpp-32 is required for the normal development and functioning of Purkinje neurons. In collaboration with Daniele Soroldoni and Elisabeth Knust (MPI-CBG, Dresden), we designed guide RNAs to target the genomic darpp-32 locus for CRISPR-Cas9 mediated mutagenesis. We successfully created specific mutations of this gene locus and are raising the F0 generation.

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Sengupta, M. and **Thirumalai, V.** (2015). AMPA receptor mediated synaptic excitation drives state-dependent bursting in Purkinje neurons of zebrafish larvae. ***Elife*** ;4. doi: 10.7554/eLife.09158.

Purkinje neurons are activated during optomotor behaviour – swimming to compensate for perceived optic flow. We designed a closed loop behavioural apparatus where the fish's swimming was used to adjust grating movements at various gains. We showed that pharmacological intervention of dopaminergic signalling via both D1 and D2 receptors leads to altered swim kinematics during open and closed loop optomotor behaviour and altered physiology of Purkinje neurons. We plan to repeat these experiments in fish lacking Darpp-32.

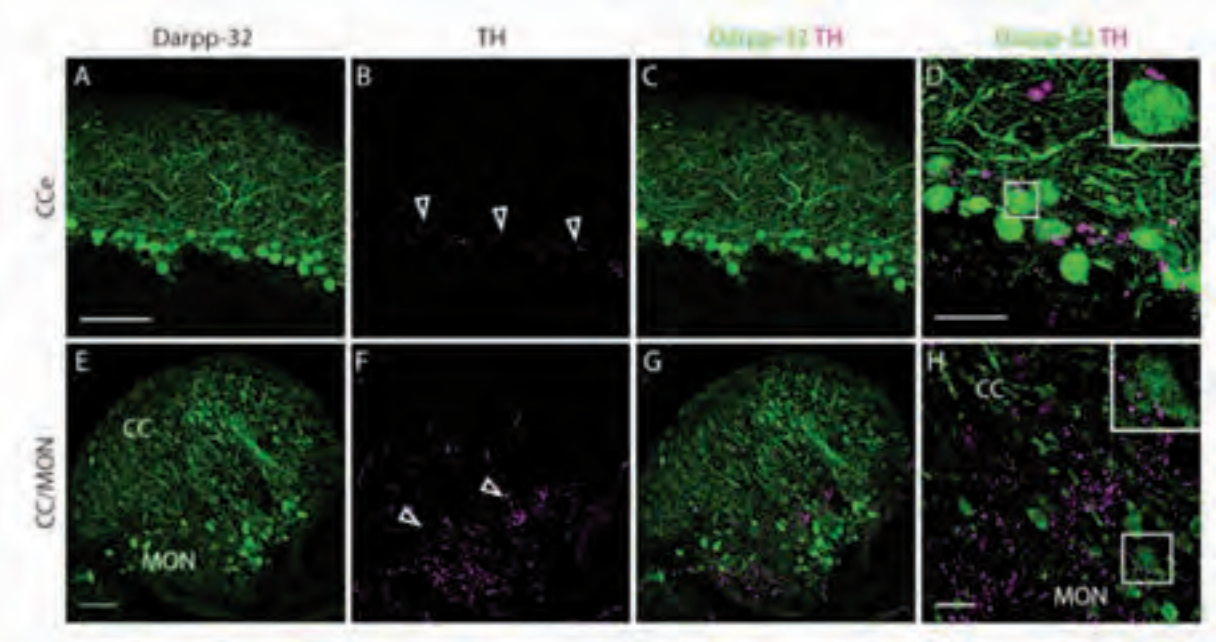

Fig 2: Darpp-32 and Tyrosine hydroxylase (TH) expression in the adult zebra fish brain.

(A-C) TH-positive fibres (open arrow heads in B) run along the Purkinje cell bodies in the CCe. Collapsed z-stacks of confocal images. Scale bar in A-C: 50 µm.

(D) Higher magnification image showing proximity of TH puncta to Purkinje neuron somata. Scale bar in D: 20 µm.

(E-G): Crest cells in the MON express Darpp-32. Their dendrites extend into the cerebellar crest (CC), Darpp-32 positive cell bodies are restricted to the MON. TH positive fibres densely innervate the MON. Mild staining in the TH channel in the CC is unspecific staining of blood vessels. Scale bar in E-G is 50 µm.

(H) Higher magnification image showing TH immunoreactive puncta in close proximity to MON crest cells. Scale bar in I: 20 µm.

3. The role of gap junctions in sculpting motor circuits

SHAISTA JABEEN, SAHANA SITARAMAN, VANDANA AGARWAL AND GNANESHWAR YADAV

Gap junctions are intercellular communication channels through which ions, small molecules and metabolites can be exchanged. They are assembled from connexin proteins. Connexin 35 (Cx35) is a zebrafish ortholog of the mammalian Connexin 36 and is enriched in the nervous system. Cx35 expression appears in the cerebellum after 4dpf, a time when the cerebellar circuit is being assembled (Jabeen and Thirumalai, 2013). Cx35 is present in punctate form on the membranes of Purkinje neurons.

To determine the role of Cx35 in synaptogenesis, we designed a splice blocking morpholino antisense oligonucleotide and demonstrated that it reduced Cx35 protein levels in the cerebellum of morphant larvae. We found that in morphant larvae, Purkinje neurons receive fewer miniature excitatory post-synaptic currents, suggesting a decrease in the number of excitatory contacts received. We are currently testing this hypothesis using direct *in vivo* labelling of chemical synapses and transmission electron microscopy. We are also validating these results in Cx35-/- fish that we generated using TALEN-mediated genome editing. Using time lapse imaging, we are observing the process of dendritic growth and synapse formation in Purkinje neurons of wild type and mutant zebrafish.

4. Auts2 family genes and social behaviour

IGOR KONDRYCHYN, AALOK VARMA

Auts2 is an autism susceptibility candidate gene identified from monozygotic twins, concordant for autism. Recent studies have shown that Auts2 is present in transcription complexes but its function during neural development is poorly explored. We identified auts2 family genes in zebrafish. These are auts2a, auts2b, fibrosin and fibrosin-like 1. These genes have complex regulation of expression, with multiple transcription start sites and splice-site variation. While some isoforms are expressed ubiquitously, others are restricted to the central nervous system. Using TALEN-mediated genome editing we have generated auts2a and auts2b null zebrfaish. Homozygotes appear normal and show normal gross development. Zebrafish are schooling fish and show attraction to conspecifics. We are currently testing whether auts2 mutants show abnormal social behaviour using a two-choice social preference assay.

MECHANISMS OF ANIMAL BEHAVIOUR

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Axel Brockmann

We are interested in the organization and mechanisms of animal behaviour. How do animals do what they do and what are the underlying neural and molecular mechanisms? Our primary experimental paradigm is daily foraging activity, which involves all the higher behavioural capabilities demonstrated for honeybees.

The European-African honeybee, *Apis mellifera*, is one of the most valuable animal models to study sensory and behavioural capabilities in insects and animals in general. Colour vision, spatial navigation and circadian regulation of behaviour were first identified in *A. mellifera*.

Honeybees surpass the common neurobiological invertebrate model organisms with respect to the degree of behavioural complexity and the ease to perform behavioural experiments under natural and laboratory conditions. Although honeybee research currently lacks the sophisticated manipulative tools available for genetic model organisms, in the long run honeybees are the most promising insect species to study neural and molecular mechanisms of complex behaviours in natural conditions.

Research in my lab pursues four general goals: (a) developing assays and procedures to study behaviour at the level of the individual, (b) establishing molecular techniques to detect and measure behaviourally induced molecular changes in single brains, brain parts and individually identifiable neurons, (c) performing comparative behavioural and molecular studies with Asian honeybee species, and finally, (d) using Drosophila to identify candidate neural circuitry and molecular processes involved in honeybee behaviour.

1. Molecular mechanisms involved in honeybee daily foraging behaviour

Honeybee foragers continuously fly back and forth between the nest and a food source. Most, if not all, of the famous sensory and behavioural capabilities of honeybees have been demonstrated using the feeder-training assay. However, so far no one has used this behavioural paradigm to identify molecular mechanisms underlying foraging behaviour, navigation and learning and memory. In pilot experiments testing the expression of different immediate early genes (IEGs), we could show that successful foraging induced the expression of at least three (IEGs): *Egr-1*, *HR 38*, and *kakusei* (see Fig. 1). In addition, we demonstrated that the *Egr-1* response was accompanied by an up-regulation of several candidate Egr-1 downstream genes, e.g. *EcR*, and *Dop2* receptor. These results are interesting because there is accumulating evidence that dopamine and ecdysone signalling are likely involved in learning and memory processes in insects. Honeybee daily foraging appears to be a promising new assay to study molecular mechanisms underlying learning and memory.

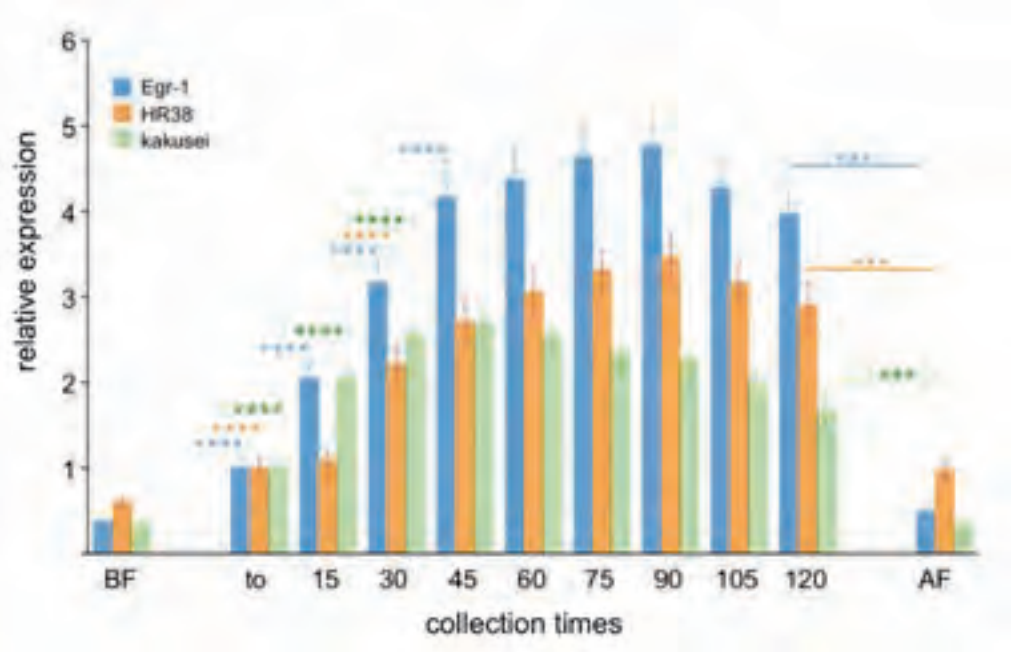

Fig 1: Daily foraging elicits a genomic response in the brain of honeybee foragers that involves up-regulation of at least three immediate early genes **Egr-1, Hr38, and kakusei. In the experiments the feeder was presented from 1400 to 1600hrs (t0 – t120).**

Firstly, it allows studying learning and memory mechanisms under natural conditions. Secondly, it allows studying mechanisms of learning and memory over much longer periods than in any of the established lab assays, since a forager will visit the feeder for as long as it lives (i.e. in a range of weeks).

2. Mechanisms of honeybee dance communication

We are interested in two different aspects of honeybee dance communication: (a) do individual foragers differ in their dance activity and if so, which behavioural and molecular mechanisms regulate individual dance activity; (b) how do individual foragers update waggle run duration after a change in feeder distance. The first research project aims at identifying neuromodulator systems that regulate dance activity. First experiments suggest that glutamate and dopamine might play a role. The second project aims at establishing a behavioural paradigm that allows studying molecular mechanisms involved in the processing of dance information. Our feeder shifting experiments suggest that the majority of foragers need at least two foraging trips to update waggle run information. This period might allow identifying brain processes specific to processing dance information.

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3. Comparative Behaviour of Asian honeybees

Traditionally, behavioural and neurobiological research in honeybees have focused on the European-African species *A. mellifera*, neglecting the variability in social organization and behaviour amongst honeybee species. Worldwide there are twelve species of honeybees and five of them are native to India: *A. florea*, *A. andreniformis*, *A. dorsata*, *A. laboriosa* and *A. cerana*. We have started comparative research projects on the visual and olfactory systems, as well as on colony organization and division of labour. For example, first results indicate that *A. florea* workers become foragers at an age of about 40 days (see Fig. 2), whereas *A. mellifera* and *A. cerana* workers become foragers around the age of 21 days. Current experiments focus on the question whether this difference is species-specific or just a consequence of nesting behaviour. In the long run we are interested in identifying characteristic behavioural differences and identifying the molecular changes underlying these behavioural differences.

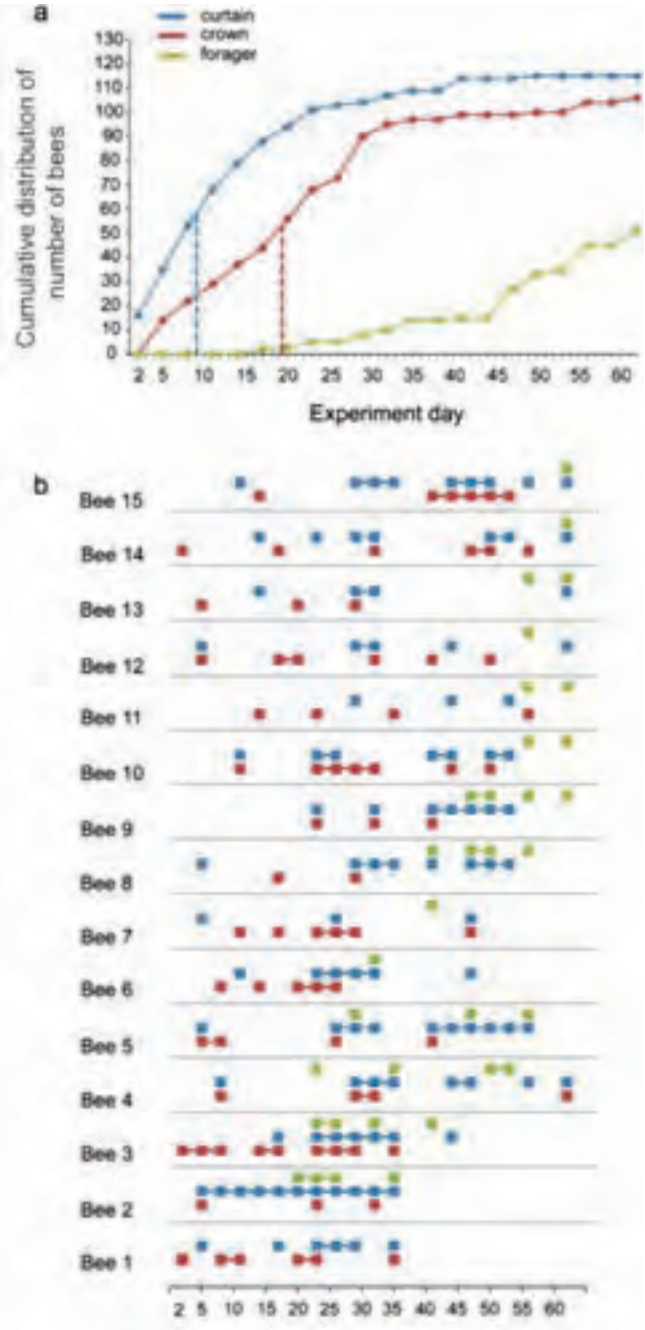


Fig 2: Workers of the open-nesting honeybee species Apis florea show a (2 weeks) delayed onset of flying and foraging compared to cavity-nesting species.

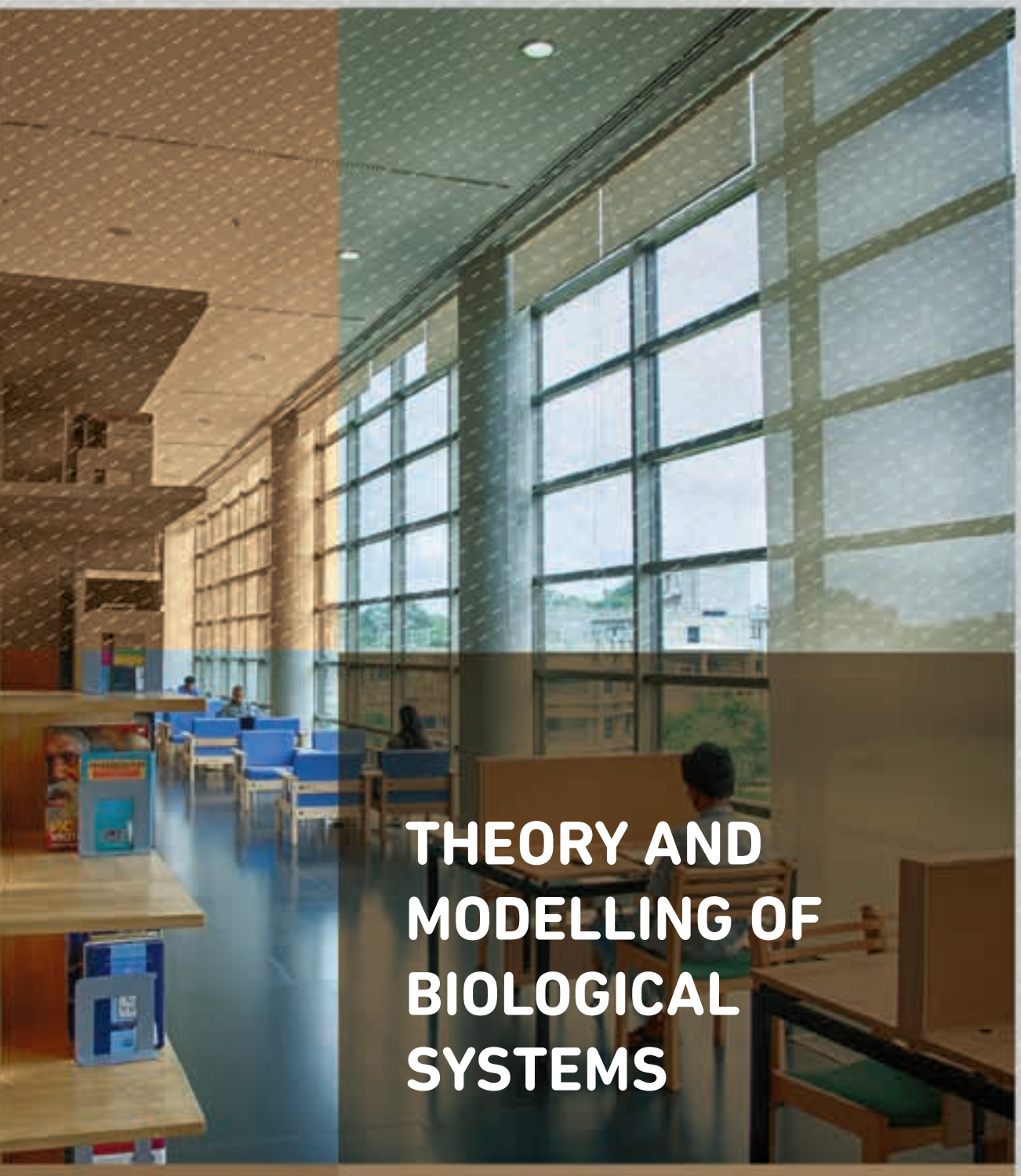
(A) Cumulative numbers of workers observed in curtain, crown and flying.

(B) Observation of behavioural tasks.

4. Sugar-elicited search behaviour in *Drosophila*

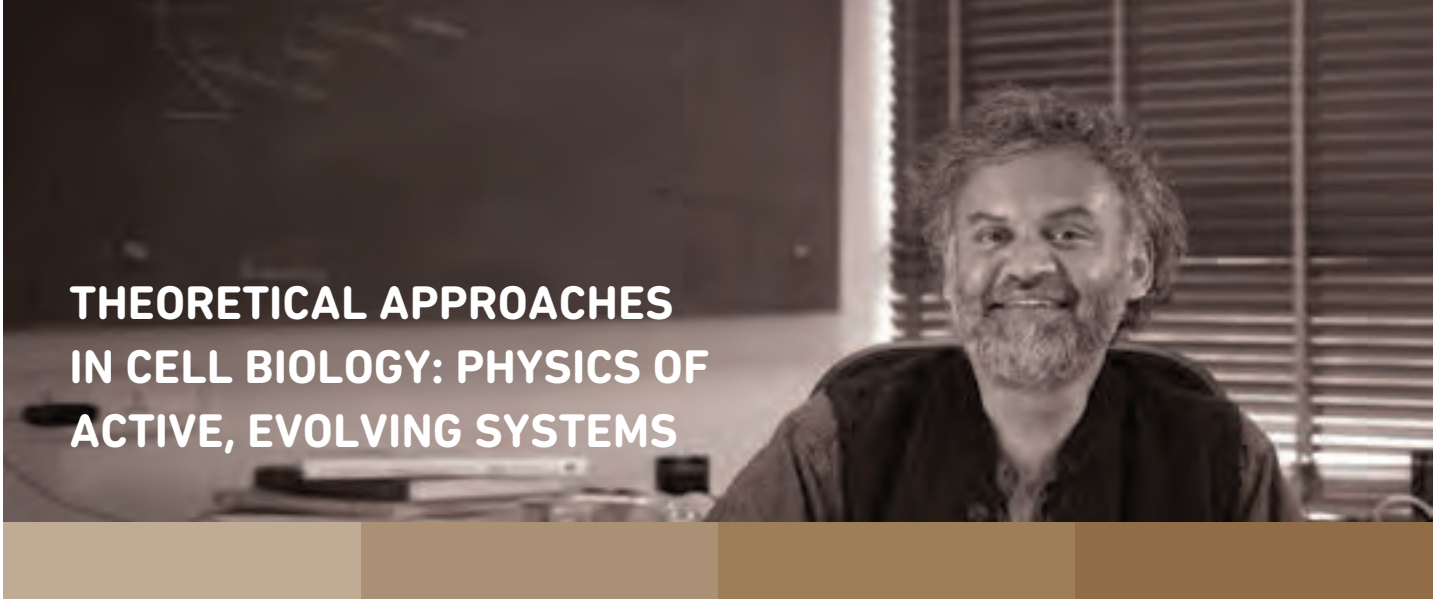
Honeybees and flies show a sugar-elicited search behaviour that is initiated after the intake of food and includes a stereotypic turning behaviour. Similar to dance behaviour, the probability and intensity of search behaviour depends on the reward value of the food source and the hunger state of the individual. In the last few years we have started studying the sugar-elicited search behaviour in *Apis mellifera* and *Drosophila melanogaster*. Our experiments indicated that the search behaviour is more complex than previously assumed. For example, it includes learning of landmarks and mechanisms of path integration. In the future, we will use this assay to identify neural circuits involved in reward-dependent locomotor behaviour as well as path integration.

MADAN RAO 136 | **MUKUND THATTAI** 140 | **SANDEEP KRISHNA** 144



THEORY AND MODELLING OF BIOLOGICAL SYSTEMS

SHACHI GOSAVI 148



THEORETICAL APPROACHES IN CELL BIOLOGY: PHYSICS OF ACTIVE, EVOLVING SYSTEMS

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Madan Rao

Our group studies the interplay between active mechanics, molecular organization, geometry and information processing in a variety of cellular contexts such as cell surface signalling and endocytosis, packing of chromatin within the nucleus, organelle biogenesis and tissue morphogenesis.

The living cell is an active, self-organized medium comprising molecular processes fuelled by a steady throughput of energy. We explore new physical and chemical principles underlying biological organization across scales, from functional biomolecules, to subcellular organelles, to the scale of the living cell and tissue, and their implications to fundamental biological processes. Our group is broadly interested in the organization, flow, processing and control of chemical signals, mechanical stress and information in living cells and tissues.

In particular, we study the folding and packaging principles that govern the three dimensional functional organization of large biomolecular assemblies, such as proteins and chromatin, and their interactions with other cellular components. At a larger scale, at the subcellular, cellular and tissue level, organization can be driven by active mechanisms fuelled by energy. In many cases, such actively driven organization enables the efficient processing of information, computation and control – tasks that a self-organized living machine has evolved to perform. In our studies we use the analytical and numerical tools of nonequilibrium statistical physics, soft matter physics, information theory and stochastic control theory.

1. Active Composite Cell Surface: Composition, shape, information processing & control

KABIR HUSAIN, AMIT DAS, RAJ HOSSEIN, SUMAN DAS, AMIT KUMAR, DEEPIKA JANAKIRAMAN, RITUPARNO MANDAL

Based on years of theoretical and experimental study, we, in collaboration with Satyajit Mayor's group, have proposed a new model for the organization of the cell surface called the Active Composite Cell Surface Model, wherein the multicomponent, asymmetric cell membrane is juxtaposed with a thin cortical actomyosin layer. We have been studying the novel dynamics of clustering and segregation of cell surface molecules driven by active stresses generated by this composite at different scales (Fig.1a, b). Such local control of composition has fundamental implications for information processing (in collaboration with Garud Iyengar) and cell surface computation (Fig.1c). These active stresses can also regulate cell membrane shape changes – a generic model for active membranes (in collaboration with Sriram Ramaswamy) describes the emergence of ruffles, waves and protrusions, and is relevant to the study of actin-mediated endocytosis.

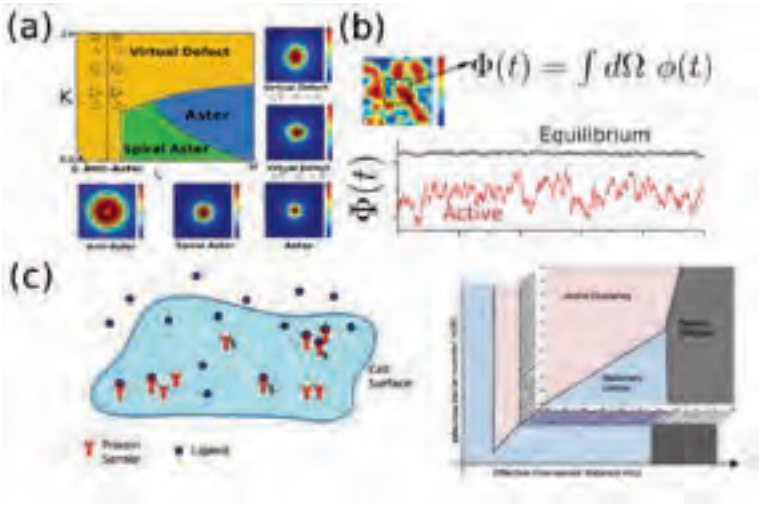

Fig 1: (a) Phase diagram of emergent structures in a thin active contractile fluid comprising actin filaments and myosin motors, as a function of the filament alignment strength K and active stress ζ. The emergent structures are typically finite size asters which can be stationary or moving (virtual defects). The active composite cell surface model posits that the cell surface is a composite of this thin cortical active fluid and the cell membrane. Cell surface molecules that bind to actin are driven to cluster or segregate by the dynamics of these emergent structures.

(b) The dynamics of active segregation of such cell surface molecules is expected to be anomalous, exhibiting strong macroscopic fluctuations and intermittency.

(c) We pose the generic information-processing problem of identifying the optimal strategy for mobile, noisy protein receptors to faithfully "read" an incoming signal (concentration of an extracellular ligand) that varies in space-time. This involves balancing two opposing requirements: clustering noisy sensors to reduce statistical error and spreading sensors to enhance spatial coverage, resulting in a phase transition that explains the frequent re-emergence of a set of architectures. The phase diagram as a function of inter-sensor distance and Peclet number shows that the dynamical and statistical features of active clustering appear to provide such an optimal strategy.

2. Active mechanics of the nucleus and chromatin organization

AMIT SINGH, KRISHNAN IYER, N. RAMAKRISHNAN

In collaboration with G. V. Shivashankar, we have shown that mechanically, the cell nucleus is described as an active polymeric gel. We find that this has implications for the spatiotemporal patterning of chromatin within the nucleus and in-vivo interphase chromatin folding at large scales, which can be quantitatively compared with recent high resolution experiments. Our results (in collaboration with Sunil Kumar) lead us to conjecture that the chromatin conformation resulting from this active folding optimises information storage by co-locating gene loci which share transcription resources. In addition, we are interested in understanding how mechanical and chemical signals propagate from the cell surface to the nucleus to affect chromatin organization and gene expression, using an active hydrodynamics formalism. We have been studying (in collaboration with G. V. Shivashankar) how active stress fluctuations, arising from ATP-dependent chromatin remodelling proteins and the active cytoskeleton, affect the positioning and shape fluctuations of the cell nucleus.

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3. Organelle biogenesis and remodelling

ALKESH YADAV, HIMANI SACHDEVA, N. RAMAKRISHNAN

We are interested in the active dynamics of intracellular trafficking in the endosomal and secretory pathways, which involve the interplay between organelle (membrane) shape, composition and activity of fission, fusion and transport. We have analysed (in collaboration with Pierre Sens) the transport mechanisms of proteins fluxing through the Golgi organelle, as a way to arrive at the sub-structure and dynamics of the organelle itself. We (in collaboration with Mustansir Barma) have formulated analytically tractable, minimalist models, with many-body interactions, that incorporate this interplay between transport and chemical progression, and explore the conditions for de novo biogenesis of distinct cisternae (Fig. 2a). We propose new quantitative measures that can discriminate between the various models of transport in a qualitative manner – this includes measures of the dynamics in steady state and the dynamical response to perturbations of the kind amenable to experiments.

We study the dynamics and morphology of membrane bound organelles (such as Golgi cisternae) subject to active processes of fission-fusion, using both simulational (in collaboration with Sunil Kumar) and analytical methods (Fig. 2b). More recently (in collaboration with Garud Iyengar) we have formulated an optimisation strategy associated with cell-type identification and discrimination, involving the interplay between sequential enzymatic processing of glycans and the dynamics of trafficking across cisternae, that appears to set constraints on the number of Golgi cisternae.

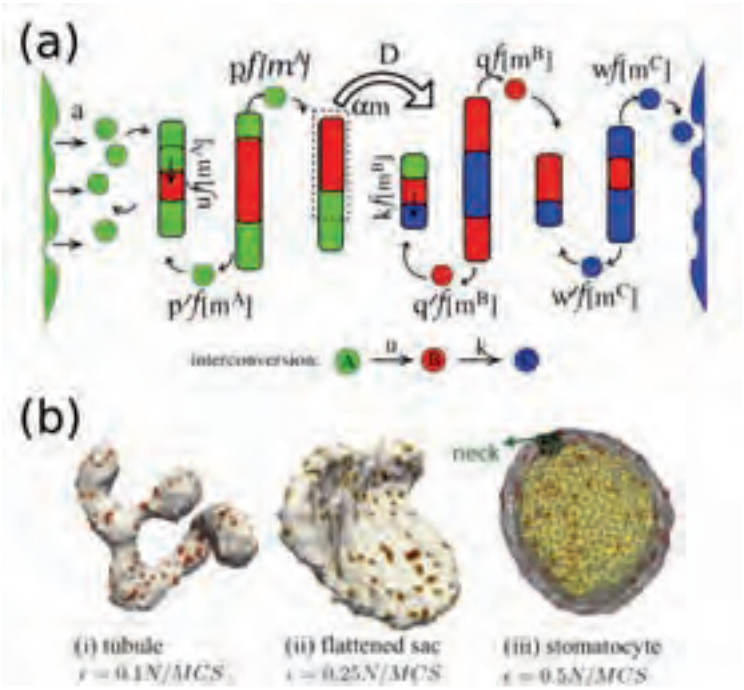

Fig 2: (a) Schematic of the effective transport model describing the secretory pathway with three-species – A, B and C of particles (vesicles). The stochastic model includes injection from the ER (left) with rate a, fission-fusion (with rates w and w') of vesicles, aggregate breakage and movement (rate D), chemical interconversion (rates u, v) and exit from the PM (right boundary) or recycling back to the ER (left boundary). The rates of transport, fission and interconversion depend on the amount or "mass" of the chemical species at the donor and acceptor sites through the flux-kernel f.

(b) Steady state morphology of membranes subject to active fission-fusion using Dynamical Triangulation Monte Carlo simulations. Tubular and flattened shapes appear generically as one increases the rates of active fission-fusion.

4. Active tissue morphogenesis and patterning

DEBSANKAR BANERJEE, RICHARD MORRIS

The patterning and remodelling dynamics in tissues is driven by an interplay between active mechanical stresses from actomyosin, cell adhesion forces from cadherin and a multiplicity of signalling pathways. We have been studying the dynamics of cell intercalation during germ band elongation in the Drosophila wing imaginal disc (in collaboration with Thomas Lecuit), driven by actomyosin pulsation and flow using an active elastomer model (Fig.3). More recently (in collaboration with K. VijayKumar) we have been studying a variety of problems in tissue mechanics, including the scale invariance of developmental patterning in tissues.

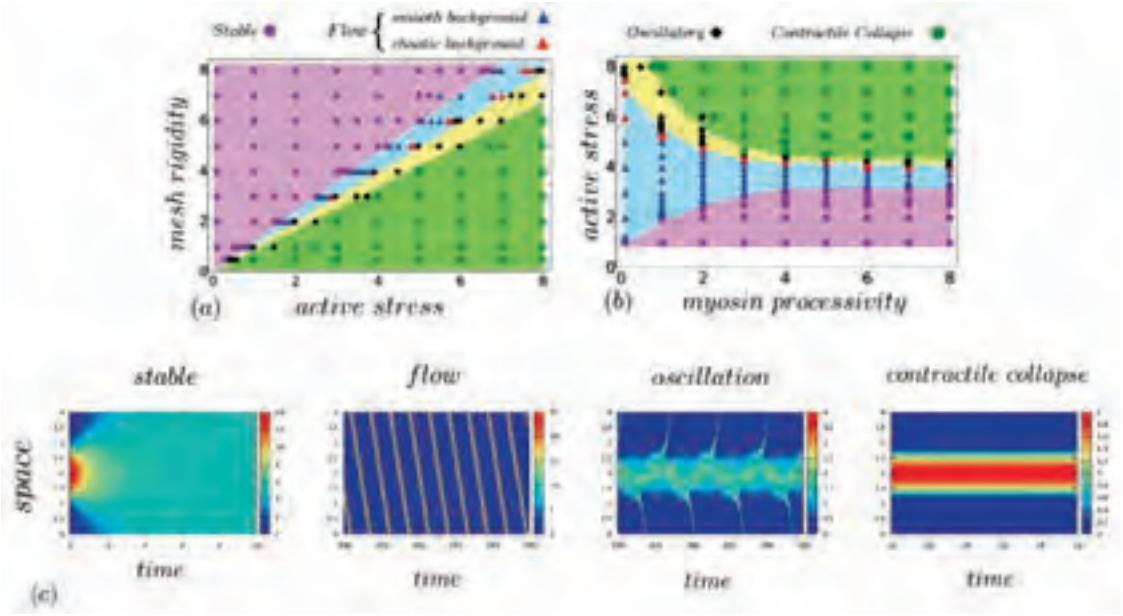

Fig 3: The actomyosin cytoskeletal meshwork within each cell belonging to the tissue, undergoes pulsation and flows. Myosin minifilaments bind and apply contractile stresses on the actin filament meshwork. Both the actin filaments and the myosin minifilaments undergo turnover. The resulting phase diagram in

a. Effective elastic stress density vs. contractile stress density, and

b. Effective contractile stress density vs. processivity, shows a variety of steady states (described in the legend)

c. (i) Stable, (ii) Spontaneous flow, (iii) Oscillations and (iv) Contractile collapse. The corresponding kymographs of the bound myosin density are shown in (c). The red (blue) triangle corresponds to a localised traveling front with a background that is spatiotemporally chaotic (smooth). Symbols are points at which numerical solutions have been obtained.

5. Stochastic control mechanisms within eukaryotic cells

AMIT KUMAR

It has long been recognized that living cells have evolved a variety of homeostatic control mechanisms to maintain physical and chemical quantities at their set point. However, except for a few cases, the physico-chemical basis of homeostatic control in eukaryotic cells is largely unknown. We are developing a mathematical framework to understand the active stochastic control of cell surface area or tension, in terms of the dynamics of active reservoirs (e.g., endocytosis, caveolae, etc.), characterised by different response times and capacities. We use this formalism to study the interplay between endocytosis rate and cell spread area upon detachment from a substrate and upon subjecting the cell to osmotic shock. In this we are collaborating with the group of Satyajit Mayor.

THE ORIGINS OF COMPLEX CELLS

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We use the eukaryotic membrane traffic system as a window to probe the emergence of complex cells two billion years ago. This effort combines population genetics, dynamical systems and graph theory, with genomics data and quantitative experiments.

This is an exciting time to study life's origins. New voyages of exploration across continents and oceans have uncovered an incredible diversity of cellular forms, while submarines probing deep-sea vents have found hints of primordial cells seemingly unchanged for billions of years. What drove those earliest cells to make the leap from a streamlined prokaryotic cell plan to the complex and versatile eukaryotic design?

Eukaryotic cells are defined by their membrane traffic systems. The apparatus of nuclei, mitochondria and other organelles connected by vesicular transport is completely absent in prokaryotes. This system allows eukaryotes to sample their environment, change shape, and communicate by contact, traits that are essential for organised sexual reproduction and multicellularity. Understanding the origins of compartmentalised membrane traffic is therefore key to understanding eukaryote evolution.

How is the membrane traffic system assembled through dynamic protein interactions and information flow? How did it get this way over billions of years of evolution? How does it benefit the cell to have such a system? These questions are connected. If we know the molecules that drive membrane traffic, we can use them to probe evolutionary history using genomics and phylogenetics. Conversely, evolutionary mechanisms such as gene duplication which expand the molecular repertoire of a cell can drive increases in traffic complexity, if it confers an adaptive advantage.

This interplay between the informational genomic level and the mechanistic cellular level generates a rich set of biological and mathematical questions. We work in close collaboration with computer scientists (Arnab Bhattacharyya at the Indian Institute of Science, and Madhavan Mukund at the Chennai Mathematical Institute) to formalize our hypotheses using the tools of dynamical systems, graph theory, and formal verification. We bring together threads from biology, physics and computer science to weave the story of the past, present and future of cellular life.

The past: What did cells look like two billion years ago?

All cells belong to three ancient groups: the archaea and bacteria, both prokaryotes; and the eukaryotes, including plants, animals, and innumerable unicellular organisms. We now know that eukaryotic genomes are a mix of bacterial and archaeal genes. This supports the idea that eukaryotes arose over two billion years ago through a partnership between an archaeal host cell, and a bacterial endosymbiont which eventually became the mitochondrion. What did the early stages of this process look like? Cells rarely leave physical fossils, but

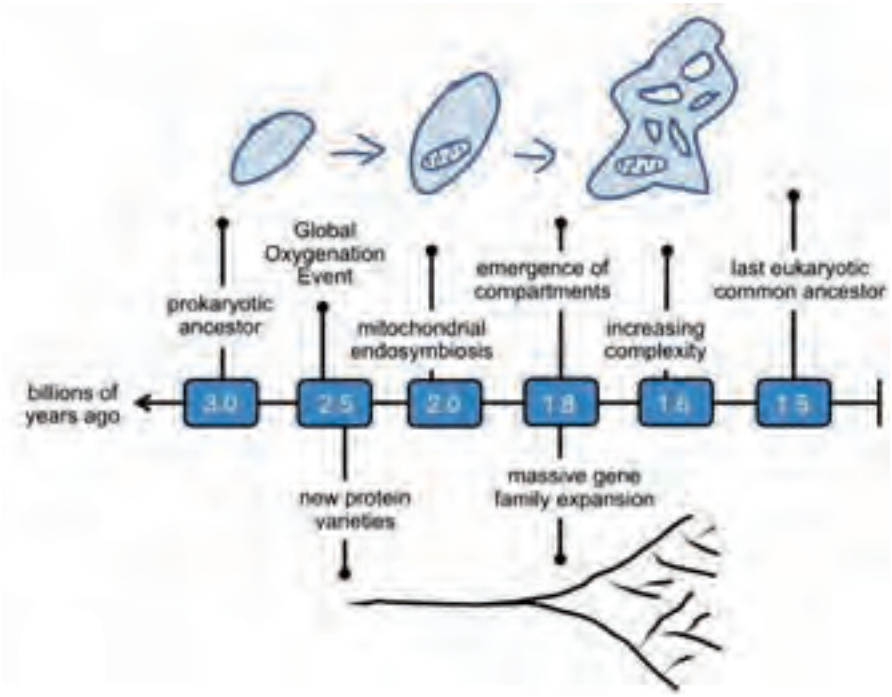

Fig 1: The acquisition of mitochondria was a watershed event, triggering massive gene family expansions and the emergence of the eukaryotic compartmentalized cell plan. Our goal is to rigorously understand how changes at the genomic level drove changes at the cellular level.

there are fossils of other kinds: stretches of DNA sequences which have retained their ancestral state by chance. We have reconstructed the earliest mitochondrial division apparatus by studying the ancestral state of dynamin, a protein which drives the scission of mitochondria in all extant eukaryotes. Remarkably, we find that the ancient division machinery of free-living bacteria, lost in most eukaryotes, persists in the mitochondria of scattered present-day unicellular eukaryotes. By studying these "living fossils" in the laboratory, we might get a glimpse of the biology of the 1.8-billion-year-old last eukaryotic common ancestor.

The present: The mechanism and function of membrane traffic

The membrane traffic system exists in a dynamic equilibrium. At each compartment, the budding and fusion of transport vesicles causes the gain and loss of molecular cargo. The act of budding or fusing itself perturbs the source and target compartments, since the molecules which drive these processes are themselves a form of cargo. We are interested in the global balance conditions by which a system retains its identity against these local perturbations. We can frame these questions in the language of dynamical systems and graph theory. We have previously used dynamical systems models to show that new compartment types can be generated by the duplication and divergence proteins responsible for vesicle budding and fusion. We are presently studying membrane traffic systems as flow networks, using the idea of graph cycles to find rigorous lower bounds on the number of molecular varieties needed to set up traffic systems of a given complexity.

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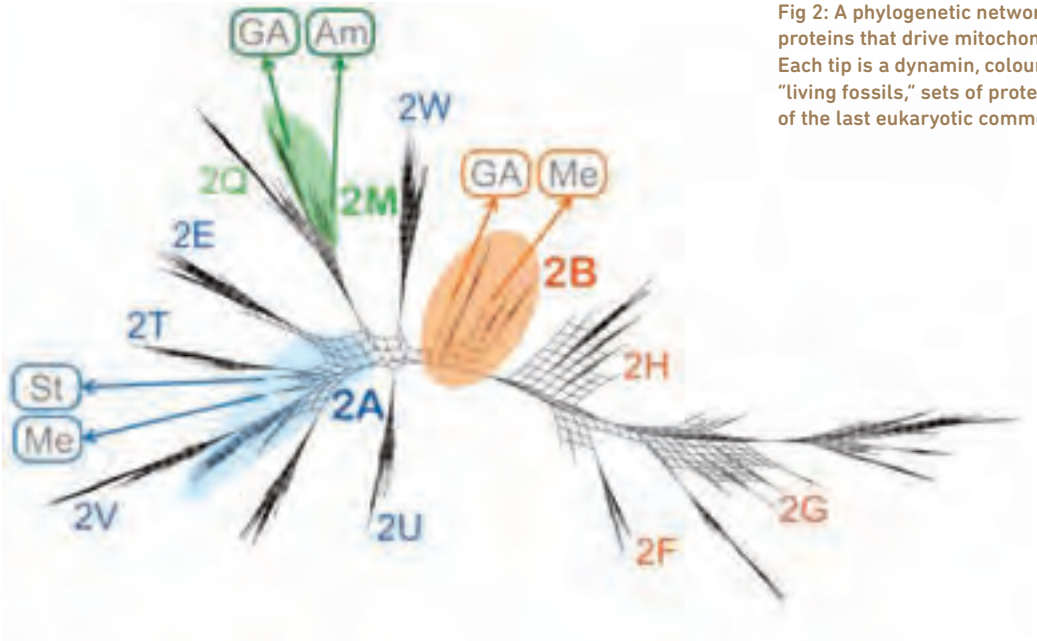

Fig 2: A phylogenetic network of dynamins, proteins that drive mitochondrial division. Each tip is a dynamin, coloured galls represent "living fossils," sets of proteins similar to those of the last eukaryotic common ancestor.

We have recently started to explore the role of membrane-bound compartments in the synthesis of glycans – information-rich branched sugar polymers attached to proteins. The surface of every cell is decorated with glycans which advertise its identity during inter-cell interactions, such as those that mediate sexual reproduction or multicellularity. Glycans are constructed via the sequential movement of substrate proteins though the compartments of the Golgi apparatus; within each compartment, sugar monomers are added to specific branches of a growing tree by glycosyltransferase enzymes. Borrowing ideas from algorithmic self-assembly and comparing with databases of glycan structures, we find that most known glycans can be synthesized by locally acting enzymes with no knowledge of the desired global structure. The final structure of the glycan is determined by precisely which enzymes are present in which compartment of the Golgi apparatus. The connection between glycans and cell identity suggests that two quintessential eukaryotic traits, membrane traffic and sexual reproduction, might be related.

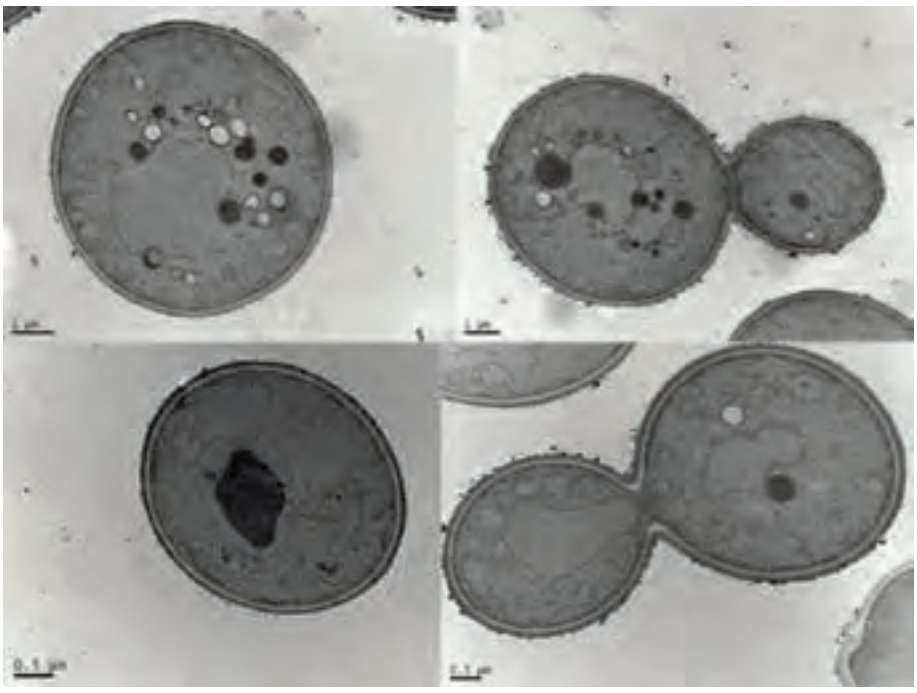

Fig 3: Electron micrographs of the lager-brewing yeast *Saccharomyces pastorianus* (top left, right) which is a hybrid of *S. eubayanus* and *S. cerevisiae* (bottom left, right). The hybrid has a vacuolar system more complex than either parent.

The future: How do eukaryotic cells continue to evolve?

Our abstract models suggest that the simultaneous duplication of a large number of protein complexes can trigger the development of a new membrane compartment. This duplication scenario arises naturally during the hybridization of moderately diverged species. As an experimental model to study this process we use the lager-brewing yeast *Saccharomyces pastorianus*, which is a five-hundred-year-old hybrid between two yeast species (*Saccharomyces cerevisiae* and *Saccharomyces eubayanus*) separated by twenty million years. Our transcriptome analyses show that the hybrid expresses the bulk of the traffic-related machinery from both parents, and is therefore expected to have a more complex traffic system than either parent. Indeed, ultrastructural studies and protein localization studies support this idea: *S. pastorianus* appears to have a highly embellished system of vacuoles. The evolution of plants and animals typically occurs by the gradual accumulation of small changes. In contrast, unicellular organisms can tolerate large changes, even if they are deleterious. By studying the dynamics of hybridization we might understand the role played by rare but large jumps in genomic complexity during cellular evolution.

THE CHOICES OF A CELL*

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I'm interested in understanding the strategies cells use to make decisions based on incomplete and unreliable information about their environment.

Cells are quintessential examples of complex systems, consisting of numerous non-identical components, interacting non-linearly and operating far from equilibrium. They are adaptive, both on shorter timescales where they respond to changes in their surroundings, and on longer timescales by evolution via natural selection.

In order to thrive and reproduce, biological cells - all the way from free-living single cell prokaryotes to eukaryotic cells in a multicellular organism - must constantly make "decisions" about how to respond to changes in their surroundings. For example, when certain viruses infect bacteria, their DNA, inserted into the bacterium, either replicates rapidly and kills the bacterium releasing a burst of hundreds of offspring, or goes into a dormant mode allowing the bacterium to live and replicate normally (Fig 1). This is perhaps the simplest example of a living system making a decision. Evidently, many factors could make one strategy better than the other: bacterial numbers, how fast they're replicating, the ratio of viruses to bacteria, etc.

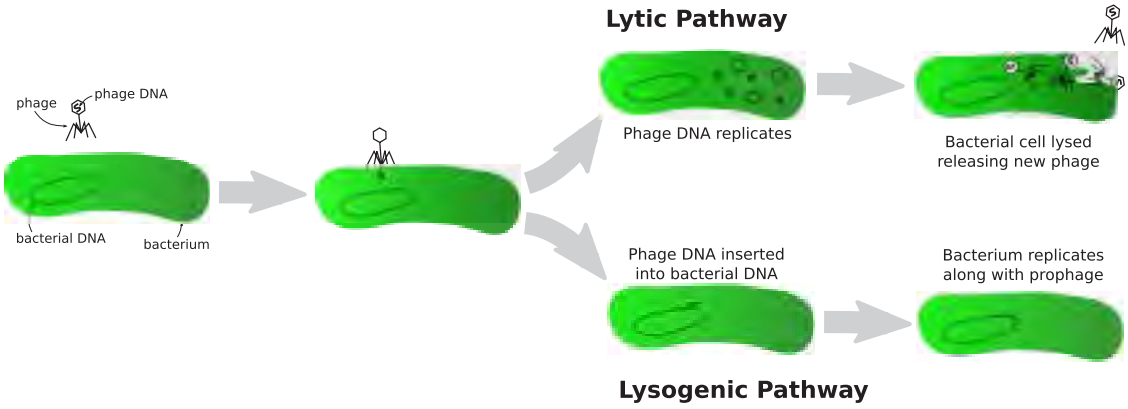

Fig 1: Lysis-lysogeny decision in temperate bacteriophage. One of the simplest examples of a cellular decision occurs when a temperate bacteriophage, a type of bacterial virus, infects a bacterium. After it is inserted into the cell, the phage DNA hijacks the cell's machinery to express its own genes. It may enter the "lytic pathway", where the phage DNA replicates, produces the proteins needed to package the DNA into new phage particles, and finally bursts the bacterial cell open thus killing it and releasing a large number of offspring phage. Alternatively, it may enter the "lysogenic pathway", where the phage DNA inserts itself into the DNA of the bacterium and remains dormant (Avlund et al. J. Mol. Biol. 2009; Avlund et al. J. Virol. 2009). Most studied bacteria contain at least one such dormant 'prophage'. In this state the bacterium replicates normally, along with the embedded prophage, until some later time when a signal, such as DNA damage, switches the phage back to the lytic pathway.

* With apologies to Lewis Thomas

So it is not surprising that this decision is not made entirely randomly. For instance, when two or more lambda viruses simultaneously infect an E. coli bacterium it is more likely to go into the dormant mode, whereas a single infection goes more often into the killing mode (Avlund et al., J. Mol. Biol. 2009). A possible reason could be that multiple infections provide information that viruses outnumber bacteria in the environment (Avlund et al. J. Virol. 2009). Although such viruses have been studied extensively (the lambda virus has been studied for over 60 years, right from the beginnings of molecular biology) we do not completely understand when and why a virus chooses one strategy over the other. Similarly, we lack a full explanation of many other cellular decisions. An example of such a binary decision in higher organisms is when mammalian cells choose between committing suicide or turning on repair mechanisms when they are damaged by UV radiation. Discrete non-binary decisions occur often in the development of an embryo, where initially identical cells, with the same DNA, switch to different states; some become muscle cells, some neurons, some skin cells, and so on. More continuous decisions underlie the response of bacteria to changing environments with fluctuating food sources, physical stresses, predation and competition, etc.

A comprehensive understanding of such decisions requires investigation at many scales:

At the molecular level, one can ask what kind of information the signalling network of a cell can obtain about the environment. Precisely what does having one, or two, or three, infections tell a virus about the ratio of virus to bacteria numbers, and how reliable can this information be? How does the concentration of sugar inside a bacterium correlate with the amount of sugar remaining in the surroundings?

These are inference problems and I use Bayesian approaches to determine the kind of information that can be reliably inferred from cellular signalling pathways. At the population/ecosystem level one can investigate the optimality of different strategies by combining tools from population dynamics and game theory. For example, I am studying a game of two competing viruses that can choose between a variety of strategies, some of which use information about the virus:bacteria ratio to bias the decision, and some which don't. The Nash equilibria of this game, played under different conditions, show when this information helps or hinders a virus. Similar games can be constructed for bacterial strains competing for resources, stem cells ungergoing differentiation, damaged cells committing suicide, etc.

In between, is the cellular level, where lies the decision-making apparatus: a feedback control system that produces/degrades proteins and other cellular components depending on the environmental information it receives from the signalling network (Krishna et al., PNAS 2007; Mengel et al., Curr. Opin. Gen. Devel. 2010) (Figure 2). In combination with the studies at the population and molecular levels, I use tools from control theory and nonlinear dynamics to understand how feedback loops and other control structures are designed to choose better strategies over worse ones.

This kind of multi-scale study of a decision (Figure 3) is likely to open up new experimental directions. For instance, my work with competing virus games indicates the importance of information about bacterial numbers in choosing the optimal strategy. This raises the experimentally testable question of whether viruses hook into the quorum sensing systems of bacteria (almost all bacteria secrete small molecules, whose net concentration in their vicinity they then measure and use to estimate bacterial numbers). Similarly, work on the control of galactose metabolism in bacteria indicates that it needs two regulators, one to take care of steady-states and one to control transients (Semsey et al., J. Bacteriol. 2010), which suggests several experiments that can test and exploit this design.

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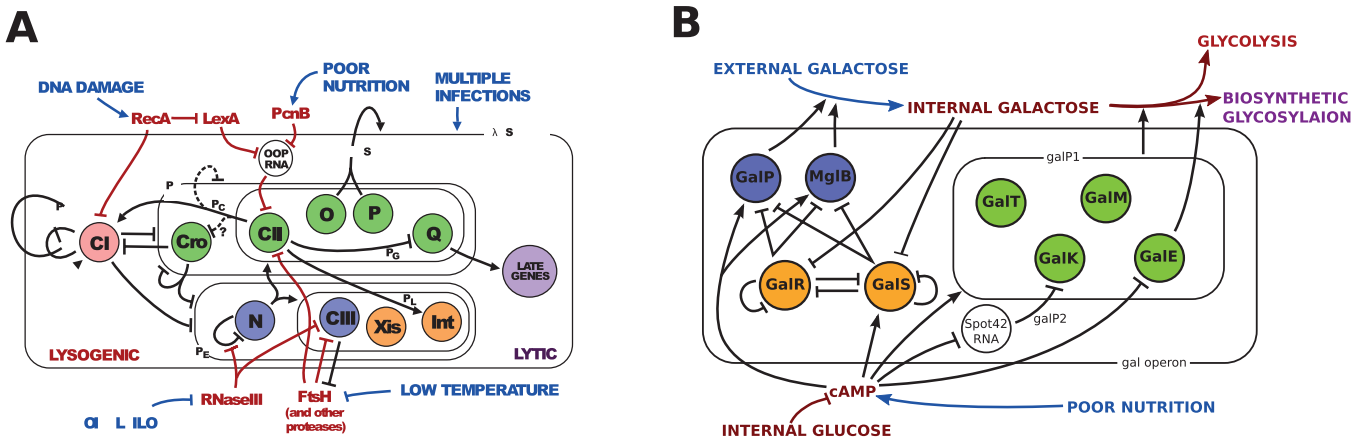

Fig 2: The complex networks that control cellular decisions. Regulatory networks that control (A) the lysis-lysogeny decision in phage lambda (Avlund et al., J. Mol. Biol. 2009; Avlund et al., J. Virol. 2009) (courtesy Ian Dodd and Keith Shearwin, Adelaide Univ.), and (B) the production of galactose transporters and metabolic enzymes in *E. Coli* (Semsey et al., J. Bacteriol. 2007; Horvath et al., J. Biol. Chem. 2010). The significance of this figure is not the details of the network interactions, but to show that these networks are complex and integrate diverse pieces of information about the surroundings. Our understanding of the components and their interactions is still far from complete.

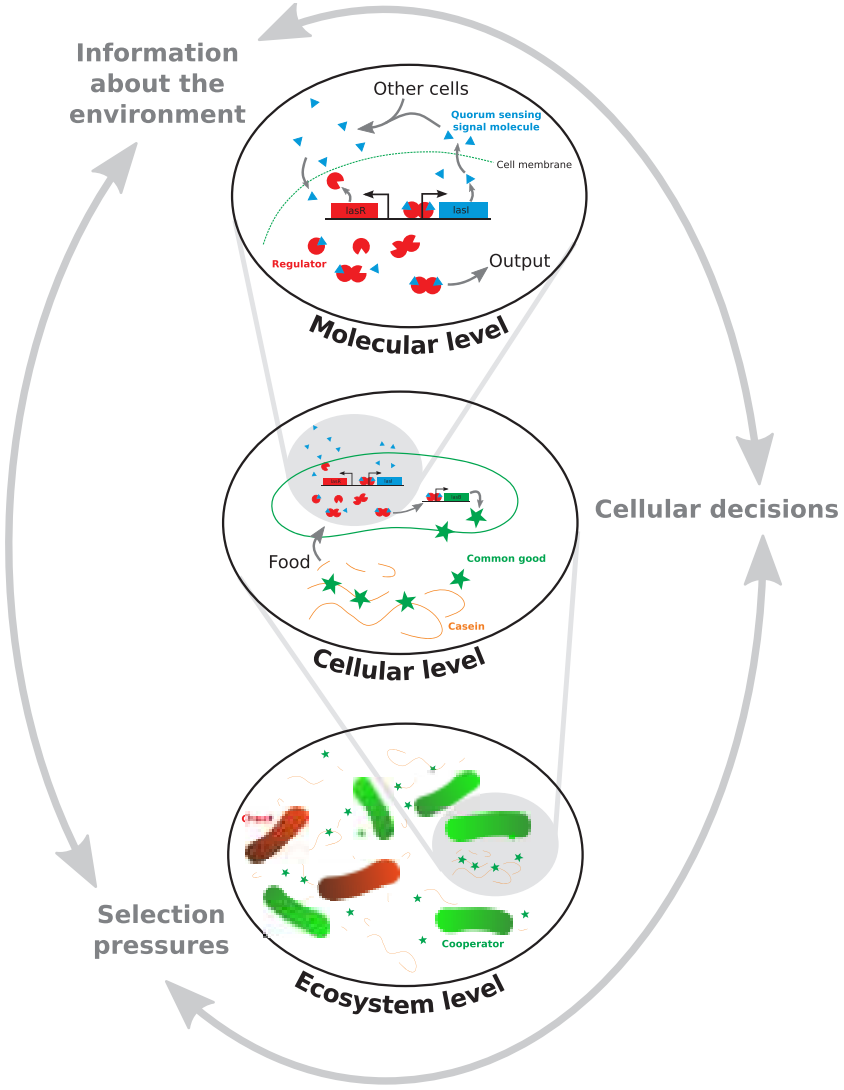

Fig 3: Towards a multi-scale understanding of biological decisions. This figure summarizes my approach to studying cellular decisions. Consider a population of *P. aeruginosa* bacteria which can produce a common good, the protease LasB (Cowell et al., Microbiology 2003), that breaks casein proteins into smaller digestible polypeptides. It is important for the bacteria producing the common good (cooperators, green) to carefully regulate the production so they are not out-competed by mutant bacteria (cheats, red) that obtain the benefit of the good but avoid the cost by not producing it themselves (Sandoz et al., PNAS 2007). At the cellular and molecular level, the production decision uses a quorum sensing system (Cowell et al., Microbiology 2003), consisting of small signal molecules (blue triangles) that are secreted and diffuse around. Selection pressures at the population level (presence of cheats, casein availability, environmental conditions, etc.) determine the best physiological responses of individual cells and the kinds of information about the surroundings the molecular signalling pathways need to collect. The unavoidable uncertainties in the molecular information, in turn, constrain the possible physiological responses of the cell. We can only begin to understand such cellular decisions once we understand the connections between processes at these different length- and time-scales.

I have long-running collaborations with biologists at the Niels Bohr Institute (Horvath et al., J. Biol. Chem. 2010; Hunziker et al., PNAS 2010) and Adelaide University (Avlund et al., J. Mol. Biol. 2009; Avlund et al., J. Virol. 2009), as well as some more recent collaborations with laboratories studying genetic and signalling networks and bacterial quorum sensing at NCBS, the University of Copenhagen and the University of Washington, Seattle. With these collaborators, I design experiments to test questions that arise from the theoretical investigation of biological decisions and, in turn, use data from experiments to design further theoretical studies.

COMPUTATIONAL PROTEIN FOLDING AND FUNCTIONAL DYNAMICS

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Shachi Gosavi

My group uses computational methods to understand the architecture of proteins. We are specifically interested in understanding how protein function and conformational dynamics affect the folding of proteins and how folding simulations can by themselves inform on protein function.

Natural proteins fold robustly because of a funnel-shaped energy landscape. This funnel shape arises because native interactions dominate the folding landscape while interactions not present in the native state (i.e., non-native interactions) contribute only in an average way. Structure based models (SBMs) of proteins ignore non-native interactions by encoding only the folded structure of the protein into the energy function. This energy function can then be used to perform molecular dynamics (MD) simulations. SBMs have been successfully used to understand the folding routes and the folding rates of several proteins. The advantage of SBMs is that they simplify the energy function such that large proteins can be folded and unfolded. In my group, we use and develop SBMs and variants to understand the folding and the conformational dynamics of natural and designed proteins.

Natural proteins have evolved to fold on a biologically reasonable timescale and to be as stable as is necessary to perform their function. However, selection directly acts only on the functional residues (where function could be binding, catalysis, cellular localization, etc.). These, functional residues cannot be mutated to make protein folding more efficient or protein stability greater. Given the choice of only twenty amino acids at each position, it has become apparent that parts of the protein which function are likely to be the least foldable or stable. Functional regions thus perturb folding from the "ideal" and we use SBMs to understand both what ideal folding is and how functional regions perturb it.

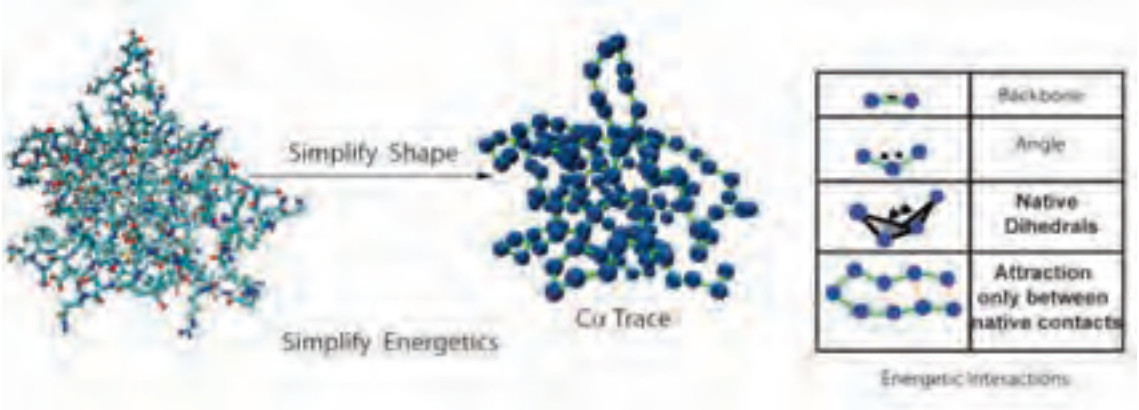

Fig 1: Cartoon of a coarse-grained structure based model. The protein shape is simplified by coarse-graining it to a Cα level. The energetic terms that contribute to the potential energy function are listed in the table. The parameters for these terms are all derived from the folded state of the protein. All Cα atoms not in contact in the folded state of the protein interact through a purely repulsive interaction.

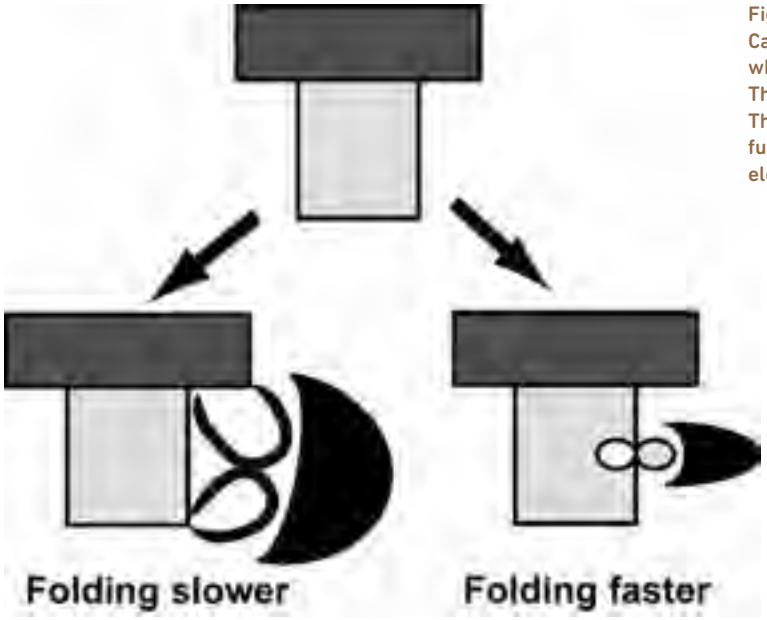

Fig 2: The folding-function trade-off. Cartoon of an ideal fold and two ways in which function can be introduced into it. The binding partners are shown in black. The effect of folding depends on whether function is added through extra structural elements or by reassigning fold residues.

Understanding the role of function in the folding landscapes of natural proteins

S. YADAHALLI, V. V. HEMANTH G. R., N. M. MASCARENHAS, V. TERSE, HITESH R.

We have discovered that studying the folding of proteins in the context of their function, helps better understand both the folding and the function of the protein.

A case in point is the *E. coli* protein RNase-H. The RNase-H domains are present in diverse species from viruses to humans. They degrade the RNA strands in DNA-RNA hybrids and have been shown to aid genome stability. In trying to understand the folding of *E. coli* RNase-H, we found that its choice of folding pathway is governed by the structure of its substrate binding site and that we could use choice of pathway as a functional assay to predict residues which take part either directly or indirectly (by helping preserve the correct binding conformation) in substrate binding. Previous experiments have shown that mutation of several of these residues reduces the activity of *E. coli* RNase-H.

Another example is that of the protease inhibitor stefin-B. Stefin-B and the sweet protein monellin both have the same overall structure (topology). However, stefin-B readily domain-swaps while monellin does not. Domain-swapping is a process by which two identical protein monomers exchange structural units and convert to interlinked dimers whose subunits have the same folded structure as the original monomers. Domain-swapping is biologically relevant because it is the first step in the mechanism of disease-causing fibrillar aggregation in some proteins including cystatin-C which is structurally and functionally homologous to stefin-B.

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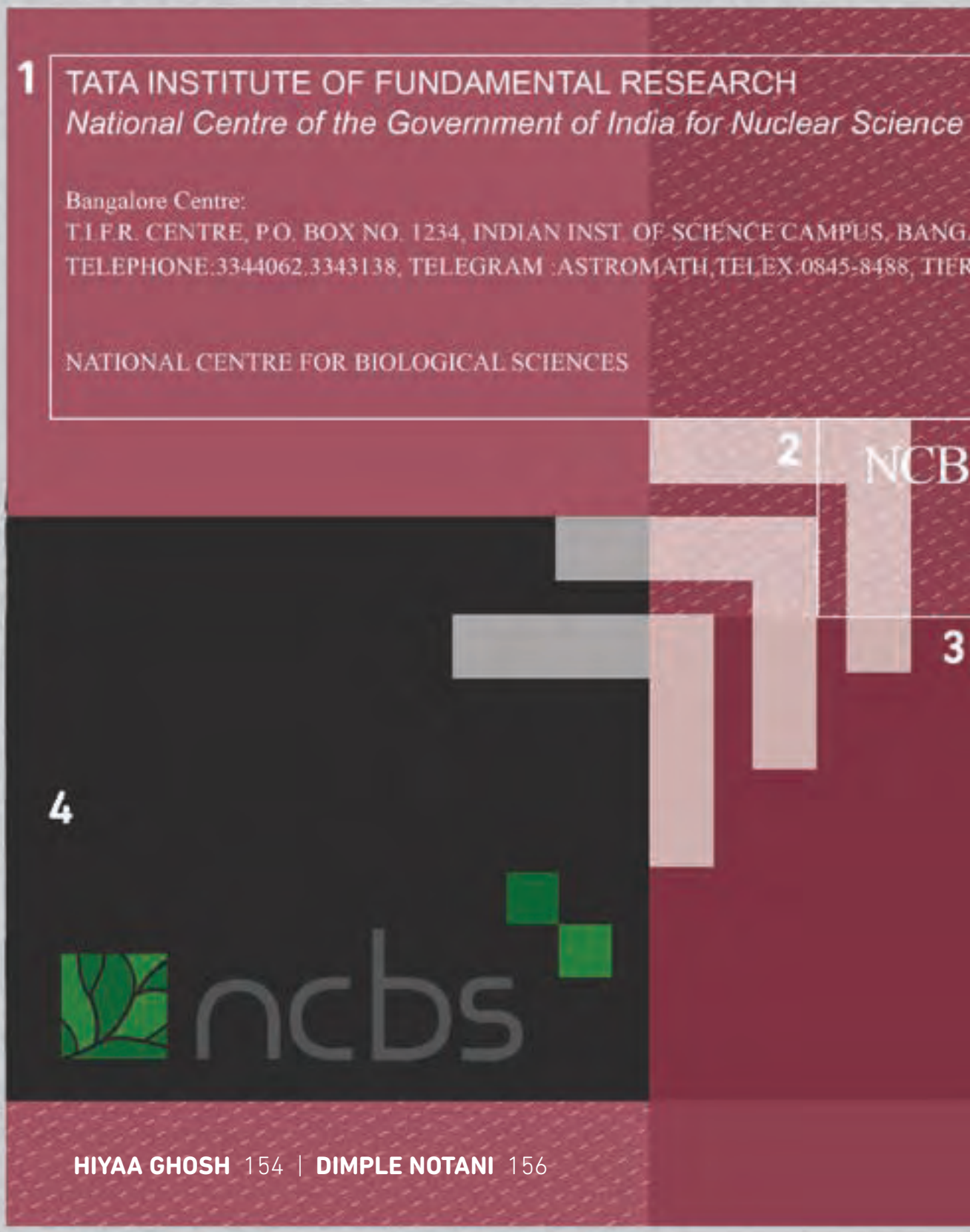
We computationally studied the folding of stefin-B and monellin in order to understand the molecular basis for the difference in their domain-swapping propensities. Our simulations showed, in agreement with experiments, that monellin folds cooperatively without the population of an intermediate while stefin-B populates an equilibrium intermediate. The structure of this simulation intermediate is such that it can directly lead to domain-swapping. Further, the population of this intermediate is caused by two localized regions of stefin-B that have been implicated in protease inhibition. In contrast, the functionally important residues of monellin are not localized and are distributed over the protein fold. We conclude that the localized protease-binding regions of stefin-B promote the formation of a folding-intermediate which can lead to domain-swapping. Thus, domain-swapping and in turn aggregation can be a by-product of the constraints that function imposes on the protein structure.

By computationally studying the folding of several proteins (the cystatin family of proteins, the serpins, the β-trefoil fold family, the OB fold proteins, etc.) whose experimental folding has already been quantified, we hope to further understand how proteins accommodate both their function and their folding in a single amino acid sequence.



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NEW INVESTIGATORS

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1, 2, 3, 4 – Evolution of the NCBS logo

MOLECULAR-CELLULAR REGULATIONS IN THE ADULT BRAIN

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My lab seeks to understand specific molecular actions that underlie cell-specific processes and inter-cellular interactions influencing normal functionality of the brain. Broadly, we are interested in adult neurogenesis, neuronal maintenance and neuro-immune interactions in the adult brain.

The various cell types in the brain present an interestingly diverse set of structural and functional regulations that remain poorly understood. As we learn more about brain pathologies – degenerative, psychiatric or inflammatory – the need for deeper insights into the molecular regulations of distinct cellular processes pertaining to neuronal and glial biology has become increasingly clear. My lab aims to understand specific molecular actions that underlie cell-specific processes and inter-cellular interactions influencing normal functionality of the adult brain. Seeking the basic biology and its potential implications during pathology, we are currently investigating three cellular processes in the adult brain: a) maintenance of mature neurons, b) adult neurogenesis, and c) neuro-immune interactions.

In a bottom-up approach, we are investigating a transcription factor, E2-2 (Tcf4), that is abundantly and continually expressed in the adult brain, and is genetically associated with neurodevelopmental and psychiatric disorders. Interestingly, we have discovered that E2-2 is expressed in multiple cell types in the adult brain, and potentially plays distinct roles in mature neurons, neural progenitors and through immune functions. Further investigating the mechanistic details, we aim to understand:

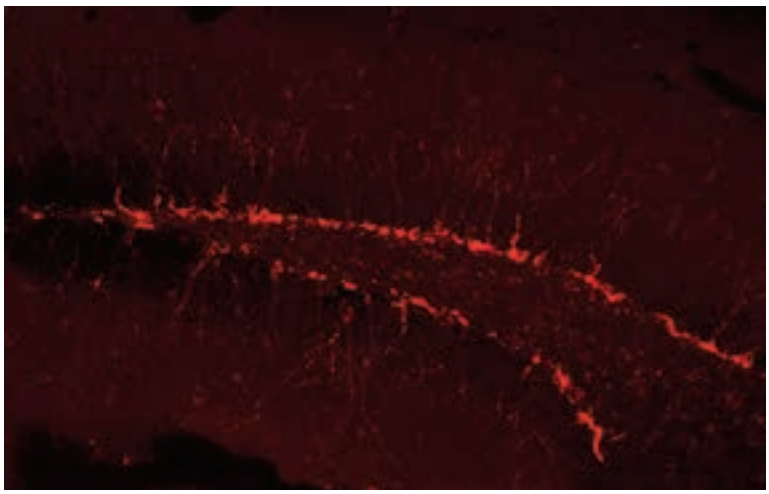

Fig 1: New born neurons in the Subgranular zone of adult brain hippocampus

- how long-living neurons are maintained throughout a lifespan

- what regulates fate decisions during adult hippocampal neurogenesis, and the functional role(s) of newborn neurons in the adult brain

- how immune processes, both inside and outside the nervous system, influence neuronal functions and homeostasis in the adult brain.

Using mouse as our model organism, we carry out cell-specific deletions in adult animals and study its effects at a molecular and systems level. The bottom-up approach of investigating a transcription factor gives us the scope to ask pointed questions and follow a complete molecular program that governs specific cellular processes. Furthermore, association with psychiatric and developmental diseases provides an additional opportunity of mapping specific functions to abnormalities at the systems level. The overall goal is to gain insights into the homeostatic processes in the healthy brain, in order to be able to better correlate conditions of impaired functionalities to specific cellular processes, potentially enabling enhanced therapeutic interventions.

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Interplay between regulatory elements, non-coding RNAS and chromatin-architecture in gene regulation.

Eukaryotic gene expression is tightly controlled in a spatio-temporal manner by distal regulatory elements known as enhancers. Enhancers being highly cell type-specific, deliver crucial transcriptional machinery to the target genes by virtue of looping. Although discovered 35 years ago, enhancers' functions and their mechanisms of action still remain poorly understood. Yet another layer of enhancer-mediated transcriptional regulation has been uncovered by the recent discovery of ncRNA (eRNA) transcription from active enhancers, further widening the gap between the knowns and unknowns of enhancer-mediated gene regulation. eRNAs bear several common and unique features with lncRNAs and their expression levels are highly correlated with the activity of the functional enhancers both in developmental and signal-regulated transcription programs. Since enhancer alterations have been linked with defects in development and disease outcomes, understanding enhancer functions is crucial in developing therapeutic strategies that target enhancers and eRNAs.

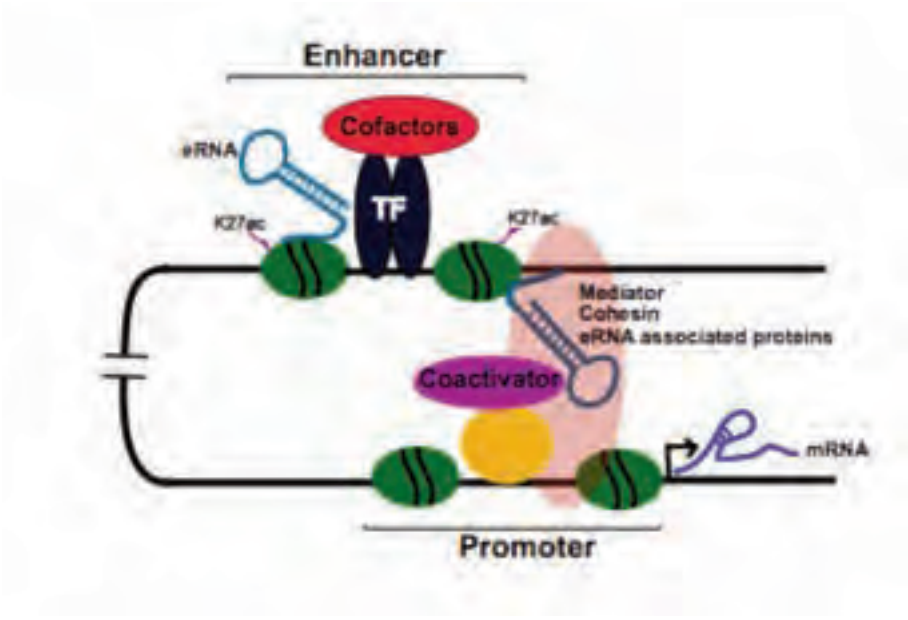

Fig 1: The schematic depicts the functional anatomy of an enhancer-promoter unit: Functional enhancers, recruit lineage and tissue-type specific transcription machinery, triggering eRNA transcription leading to target gene activation via looping.

Using locus-specific as well as genome-wide approaches, we strive to uncover the following aspects of this conundrum: (i) How is the specificity between an enhancer and the corresponding promoter defined? (ii) Role of enhancers in the three-dimensional chromatin architecture, its alterations during signalling cascades. (iii) Function of eRNAs-associated distinct protein cargos in the enhancer-mediated activation vs. repression events.

COLLECTIVE DYNAMICS IN LIVING MATTER

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Our research program aims to provide an understanding of the collective outcomes (mechanical and evolutionary) in living systems using quantitative approaches rooted in the framework of disordered, non-equilibrium matter. At the heart of our approach are experimental probes of theory driven ideas.
Cell populations, biological tissues, highly coordinated animal groups and interacting populations are a form of complex material with emergent coherent properties that arise from mechanical, biochemical and socially-mediated interactions between individuals. Unlike in purely physical processes, the individual in a biological ensemble is capable of transmitting, integrating and processing information, as well as tuning dynamically adaptive responses. These augmented capabilities of individual units allow the population to exhibit collective organization and behaviour beyond what is found in traditional materials. While on the one hand, biological systems represent a form of self-organizing condensed matter, the non-linear spatio-temporal interactions and hierarchies also make them complex dynamical networks.

Such a perspective naturally suggests two complementary approaches: (i) to distill unifying physical principles that govern biological systems by probing them as a unique state of matter or as a unique class of dynamical systems and (ii) to construct *de novo*, using traditional materials, systems with functions that are defining features of living matter such as self-propulsion, replication etc. and study the resulting emergent collective behaviour.

Of particular interest to our research program is one of the defining features of such matter namely disorder – right from the proteins and macromolecules in a cell to neural networks in the brain to the interactions between ecological communities, both the microscopic mechanical structures and the interactions between

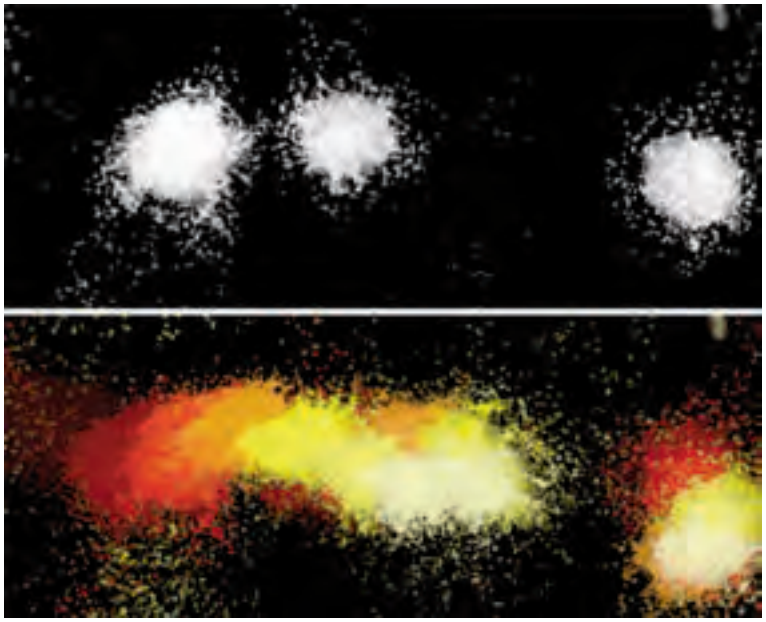


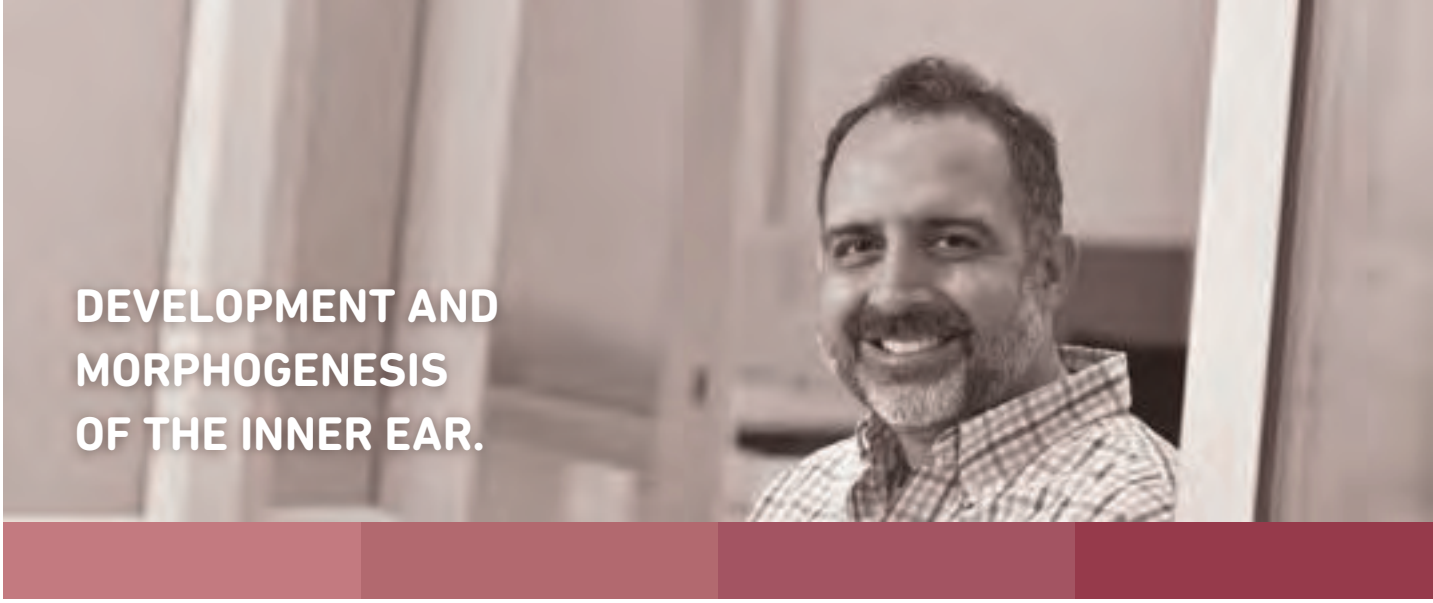
Fig 1: Collective dynamics in active matter.

Top: A snapshot of cluster formation in a large population of self-propelled particles (each white dot is an active emulsion droplet which is 50 microns in diameter).

Bottom: Dynamics of the collision and merging of the active clusters with time encoded in color.

constituents are disordered. Disordered systems often display a complicated type of phase transition, labelled the freezing transition, where the system configuration gets "frozen" into a particular state such as a "glass". It might be expected that activity and information fluxes "fluidize" biological systems (of which there is a growing body of evidence) leading to their rich dynamics and perhaps life itself.

Our research endeavour is to study the complex transitions defining this unique biological matter and distill the underlying organizational and functional principles. We use the tools and perspectives of statistical mechanics, non-equilibrium condensed matter physics and non-linear dynamical systems to understand communities and collective behaviour. Research subjects include model biological systems (bacteria, nematode worms) as well as artificial biomimetic systems (active emulsions, lipid bilayers, mechanical oscillators) -- in revealing the differences between the various systems, artificial and biological, as well as the underlying commonalities in mathematical features, we hope to better understand the relationship between microscopic and macroscopic emergent collective behaviour not only in biology but also in other complex systems far from equilibrium.



DEVELOPMENT AND MORPHOGENESIS OF THE INNER EAR.

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Raj Ladher

We are interested in how the inner ear develops, focusing on the genetic and epigenetic factors that generate specific functional cell types that enable hearing and balance sensation.

The specialisation and organisation of cells to form organs that effectively carry out functions vital to life, is a fascinating problem. We investigate the formation of the inner ear as a model for cellular and tissue level differentiation.

The inner ear is a complex structure that is actually generated from a relatively simple group of cells. These cells should have become skin, yet receive a series of instructions that change their potential and their shape. Over time, a subset of these cells form inner ear hair cells. These are the sensors of the vertebrate inner ear, converting the mechanical vibrations associated with sound and balance into electrochemical impulses that are sent to the brain and possess sub-cellular adaptations in the form of fine hair-like protrusions from the top of the cell, that enable the sensitive and precise detection of these vibrations. The formation of these cells is also a consequence of instructions. How do inner ear cells receive these instructions and then decode and implement them? What are the physical and molecular responses of cells to these dynamic genetic and epigenetic cues? How can variation be introduced into the development of cells and tissues to enable fine-level functional tuning?

Using a variety of molecular, cellular, imaging, and computational techniques, our aim is to generate a blueprint of the inner ear, that we can interrogate to understand congenital hearing impairment in particular and developmental morphogenesis in general.

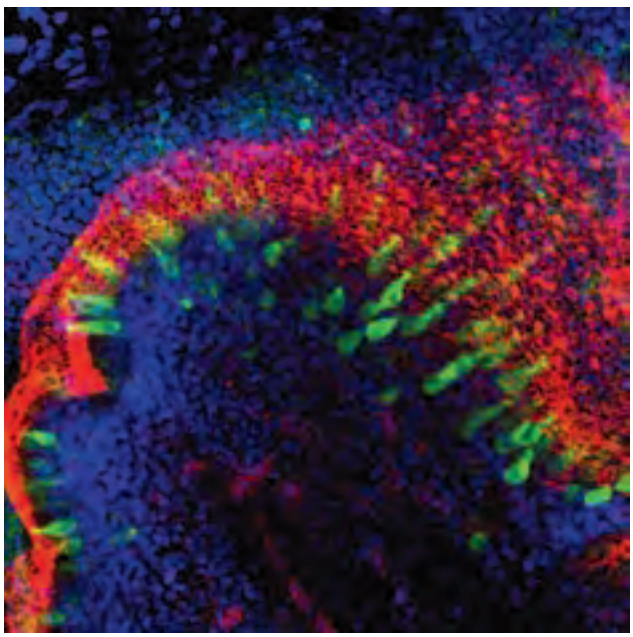

Fig 1: Cells of the avian cochlea, with cells outlined in red (actin) and hair cells shown in green (myosin 7a) with nuclei highlighted in blue.

PUBLICATIONS

NCBS PUBLICATIONS (2016-2015)

BIOCHEMISTRY, BIOPHYSICS & BIOINFORMATICS

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CELLULAR ORGANISATION & SIGNALLING

SUDHIR KRISHNA

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Gowda, M., Ambardar, S., Dighe, N., Manjunath, A., Shankaralingu, C., Hallappa, P., Harting, J., Ranade, S., Jagannathan, L. and **Krishna, S.** (2016). Comparative analyses of low, medium and high-resolution HLA typing technologies for human populations. ***Journal of Clinical and Cellular Immunology,*** 7, 399-406.

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SATYAJIT MAYOR

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ECOLOGY AND EVOLUTION

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THEORY AND MODELLING OF BIOLOGICAL SYSTEMS

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MEETINGS AND WORKSHOPS 2016

Science cannot grow in isolation. The meetings and workshops programme has its genesis in our realisation that students at NCBS and other scientific institutes in India, benefit tremendously from exposure to the best international science. The meetings and workshops programme also plays a role in showcasing Indian science at its best to visitors from outside the country.

NCBS Annual Talks

Coming of Age: Transitions in Biological Systems

11th-13th JAN 2016

CANCER BIOLOGY

Cancer Biology

Lecture Series in Advanced Cell Biology 2016

07th FEBRUARY 2016 - **27th** MARCH 2016

Alumni Day & Commemorative Event for Prof.Obaid Siddiqi

26th MARCH 2016

6th Annual Science Journalism Workshop

The workshop's main objective was to impart the basic skills necessary for communicating science to the lay person via the written word.

13th-25th JUNE 2016

Physics of Life 2016, 4th NCBS-Simons Annual Monsoon School

A workshop aimed at exposing undergraduate students from mathematics, physical sciences and engineering backgrounds to exciting problems in biology through research talks by biologists and physicists.

15th-24th JUNE 2016

For full listing
scan the QR code

Computational Approaches to Memory and Plasticity

A 16-day summer school on the theory and simulation of learning, memory and plasticity in the brain.

01st-16th
JULY 2016

VIIIth Bangalore Microscopy Course 2016

A course including didactic and hands - on training in state-of-the-art optical microscopy techniques.

18th-26th
SEPTEMBER 2016

Mechanical Forces in Cell Biology

A discussion meeting on mechanical forces in the function of animal cells

04th- 06th
OCTOBER 2016

ncbs@25

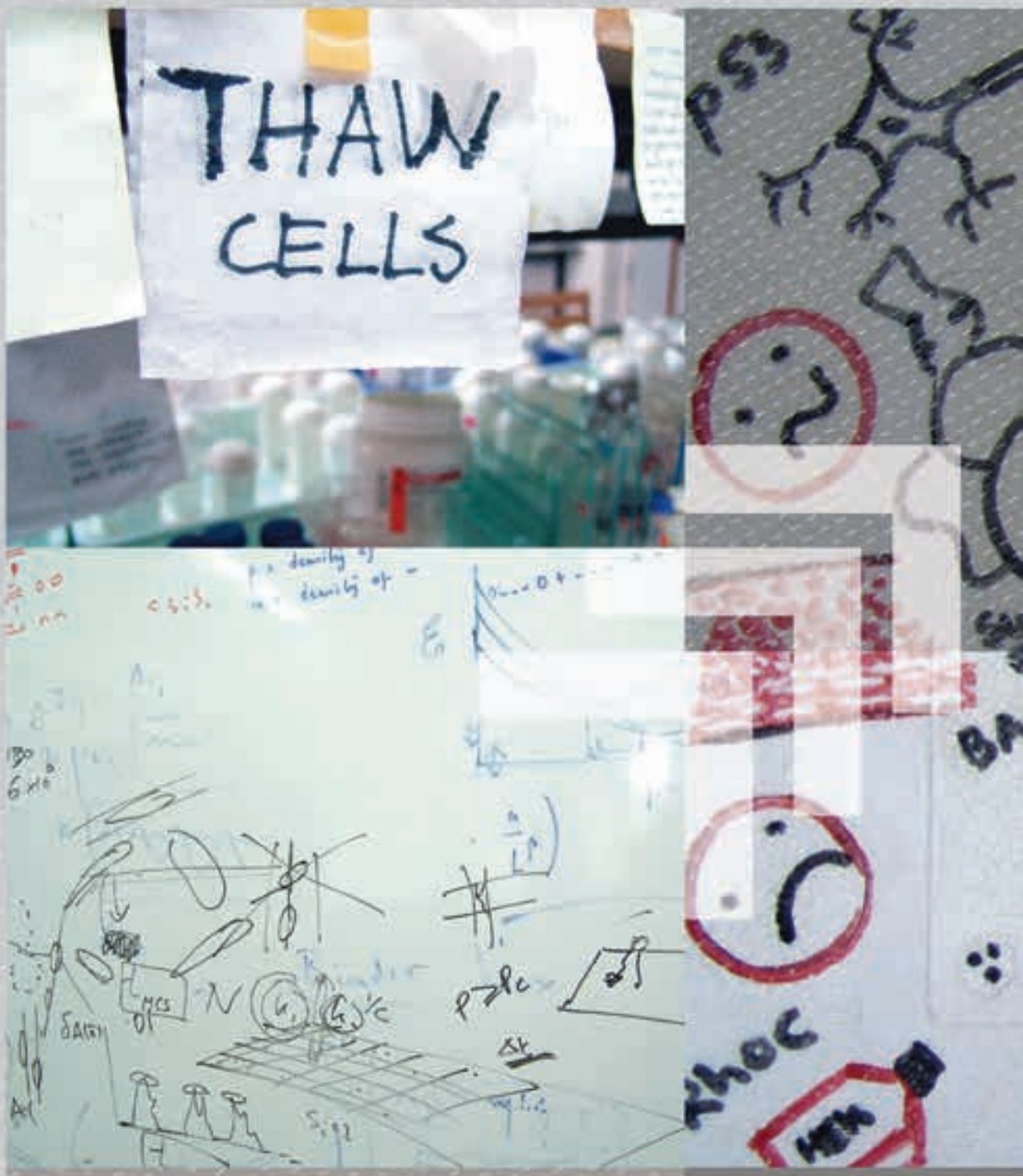
NCBS Celebrates 25 years

22nd
OCTOBER 2016

NCBS Hosts Science Day

An outreach event where schools learnt about how science can be applied in every-day life.

12th
NOVEMBER 2016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CELLS

ACADEMIC ACTIVITIES

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ACADEMIC PROGRAMMES AT NCBS

Freedom of academic inquiry is central to the success of a research environment. Young researchers-in-training, whether undergraduates, graduate students, or post-doctoral researchers, absorb the scientific culture of their host institution: the values of scientific excellence, broad exploration, academic integrity, and social responsibility. When these people leave our campus to start their independent careers, they become our ambassadors, and help to spread these fundamental values. In our 25th year, we can proudly look back at our role in training many generations of young scientists.

Our academic programmes continue to grow and strengthen, reflecting the diversity of research areas. Our PhD and Integrated-PhD Programmes are based on a structure that combines close mentorship with rigorous training. Today we offer courses and hands-on workshops in more areas than ever before: from core topics in fundamental biology, to applied areas linking science and health; from technological tools to mathematical and statistical methods; from the study of model organisms to the analysis of ecosystems and climate change. These programmes prepare our students to tackle research across the rapidly changing spectrum of the life sciences.

Our MSc Programme in Wildlife Biology and Conservation, jointly conducted by NCBS and the Centre for Wildlife Studies, continues to attract students with a passion for fieldwork and ecology. Uniquely, it combines on-campus coursework with off-campus research at field stations across the country, from the Andamans to the Western Ghats to the Himalayan foothills at Sikkim. Each student in this programme produces an original thesis, and most also publish their work as peer-reviewed papers. The alumni of this programme have gone on to careers in research, in NGOs and policy think tanks, and in Government departments.

Our post-doctoral programmes are flourishing. The Campus Fellows Programme is our flagship initiative, recruiting the best post-doctoral researchers internationally and allowing them complete freedom to design and pursue innovative research. The Simons-NCBS Fellows Programme hosts researchers with basic training in areas such as mathematics and physics, who apply their skills to tackle fundamental problems in biology. In parallel, joint post-doctoral programmes have added a new collaborative dimension. We have recruited our second batch of NCBS-inStem-Cambridge Fellows, and have instituted a similar joint programme with Institut Curie. Fellows recruited under these joint programmes combine the strengths of multiple campuses and often nucleate new lines of inquiry in their host laboratories. Our core post-doctoral programmes are growing at an unprecedented rate. Many of our post-doctoral researchers are supported by competitive extramural fellowships from sources such as AXA, EMBO, and the Wellcome Trust-DBT India Alliance. The 99 Campus Post-doctoral Association now has over a hundred members. It plays a key role in representing our post-doc community, and also coordinates activities such as the annual Post-doctoral Research Symposium.

We are aware that our students are no longer restricted to a traditional academic path leading from a PhD to a post-doc to a faculty position. This is why we offer broad exposure to the modern career options that rigorous scientific training affords: science journalism and scientific writing; research opportunities in industry; the world of innovation and entrepreneurship. Students and post-docs have the opportunity to meet and interact with mentors in targeted workshops or during our annual campus-wide Career Day, so they are better prepared to make decisions about how to launch their independent careers.

Apart from our graduate students and post-doctoral researchers, large numbers of undergraduate students visit our campus for periods ranging from weeks to months, to carry out research projects. We have formal internship programmes with BITS Pilani, IISER Mohali, MSU Baroda, Manipal University, IFOM Milan, and the University of Wurzburg. We continue to host students from the Tibetan community each year, who come to us directly after graduating from high school. Our faculty members also host students from programmes such as the KVPY or the Academies' SRFP. We run workshops for undergraduates, such as the annual Monsoon School on the Physics of Life. Many undergraduate students who have spent time on our campus through these various activities often return to pursue their PhDs guided by our faculty.

Finally, our campus now operates a calendar of outreach activities throughout the year. Science Day invites school students from around the city, to interact with our graduate students and postdocs, play with hands-on exhibits, and visit our research facilities. Our Campus Post-doctoral Association runs an annual summer workshop for undergraduate students, teaching advanced topics in areas such as cell and developmental biology. The Science and Society Programme enables engagements between science and art, theatre, history, and sociology. We invite eminent scholars to speak about the implications of scientific practice, and the role of a scientist in an increasingly technological world.

Mukund Thattai
Head, Academic Activities

POST DOC ASSOCIATION

The Postdoctoral Fellows Association (PDFA) is an active body that fosters networking amongst postdocs in the campus. This year PDFA is organizing the first National PostDoc Symposium in November bringing together postdocs from across India, as an initiative to showcase the talent of Indian trained postdocs in national forum. Faculties from different institutions will be the mentors of the participating postdocs for guidance in their work and career.

In the previous year, The PDFA has been involved in organizing a multiple activities. Postdocs interested in teaching had organized an eight-week lecture series themed "Cancer biology" for undergraduate students from local colleges. In these series,each lecture was followed by an activity to design novel experiments and discuss contemporary scientific research paper. The series was deeply appreciated by the undergraduates. To increase interaction amongst postdocs "Chalk Talk" series happens bimonthly for informal discussions on research work with fellow postdocs.

Fig 1: Great Indian Hornbill, Valparai Plateau
Photo Credit – Kalyan Varma

MSc WILDLIFE PROGRAMME

The Masters Program in Wildlife Biology and Conservation is offered by National Centre for Biological Sciences in partnership with Centre for Wildlife Studies and Wildlife Conservation Society – India.

Started in 2004 with the goal of training India's future conservation leaders, the Masters program in Wildlife Biology and Conservation is now in its 12th year. We are proud to say that the 74 alumni from our first 5 cohorts have done exceptionally well, both in scientific research (with over 50 scientific publications and more than 100 popular articles) and in engaging with wildlife conservation across nearly 25 Indian states. They have also won several awards in recognition of their achievements in conservation.

2015–16 has been another event-filled year in the program. In October-November 2016, after completing their program coursework, the students of the 6th cohort of the program (2014–16) left for field sites spread across the country to conduct field work for their thesis projects. Returning to NCBS in April-May 2016, they set about analysing their data and submitted their theses in July 2016. As of this writing, they are waiting in the wings, preparing to defend their work and graduate by mid-October 2016.

Spread across more than 10 states in the country, these projects have addressed a wide-spread breadth of topics including the assessment of wetland bird communities in human-managed wetlands, the use of modified production landscapes by bird, bat and mammal communities, the biology of endangered primates and hornbills outside their rainforest habitats, the impacts of mini-hydel projects on downstream fish communities, the impacts of forest logging on bird communities, carbon services from fragmented forests and the transmission of disease in coral reef communities in waters polluted by anthropogenic activities.

An emergent theme in the thesis projects of the 2014-16 cohort has been their focus on documenting and understanding wildlife outside of protected areas, in landscapes that are facing rapid changes from anthropogenic activities such as land-use transformation and development projects. These projects acknowledge that a major challenge for wildlife conservation in the future will be to conserve wildlife outside the traditional framework of the protected area network. We believe that our students will be a part of addressing this challenge and we wish them every success as they leave us to pursue these ideas in the real world.

Fig 2: Field trip to Velavadar National Park, Gujarat, August 2015. a) Black-buck foraging in the grassland b) A predatory Indian wolf in search of blackbuck fawn c) Students interacting with Forest Department Officials on issues relating to park management and d) Students sampling vegetation to understand the effects of Invasive Prosopis juliflora on grassland composition

Fig 3: Collecting data for their dissertations in far-flung field sites a) Shishir Rao preparing to sample water quality in streams of the Western Ghats in Karnataka b) A quick breakfast in the wee hours of the morning before Binod Borah (on the right in the image) heads out to record mixed foraging flocks of birds in EagleNest Wildlife Sanctuary in Arunachal Pradesh c) Pooja Pawar identifying tree species that are part of the diet of the Great Indian hornbills in Valparai, Tamil Nadu and d) Aditi Pophale sampling pink-spot disease in coral reefs off the Lakshadweep Islands

Our *alumni* continue on their upward trajectory, bringing high quality to their science and an abiding passion to their work. From publications in high-impact journals including Nature Communications and BioScience, to on-the–ground engagement including training of forest department rangers, participatory conservation management in multiple projects across the country, conservation education and outreach through film-making, memory projects and children's activity programs, they have continued to do us proud in 2015-16.

2015-16 has also seen an expansion in the scope of our program activities. Recognizing that we will only be as effective in conservation as the strength of our networks and collaborations, we have begun to actively engage with stakeholders such as the Forest Department. In October 2015, our faculty engaged in a knowledge exchange program with 68 trainee range officers from the forest departments of the four southern states of Karnataka, Andhra Pradesh, Kerala and Tamil Nadu. Likewise, realizing that our access to some of the best conservation scientists in India and abroad places our program in a unique position to facilitate teaching in advanced conservation science, we have begun organizing workshops in specialized topics that are open to students across the country. In 2015-16, these have included workshops in "Niche Modelling" and "Data Analyses with Hierarchical Models", that were conducted by colleagues from Colorado State University and the United States Geological Service, and attended by more than 50 students and practitioners from across the country. In these activities, we have been funded by The Tata Trusts (Mumbai) and the Indo-US 21st Century Knowledge Initiative grant (National Science Foundation, USA). We are very grateful for this support that is helping us to sustain and expand our activities, and look forward to another productive year in the program.

LECTURES AND VISITS

APURVA SARIN

2015

- Notch activity in T-cells and consequences to immune homeostasis. December 6-8, 2015. All India Cell Biology Conference, Thiruvanthapuram. India

AXEL BROCKMANN

2016

- 26th National Conference on Chronobiology, University of Mysore, Mysore.
  Talk: Honey bee foraging activity and the circadian clock.

- IBRO-APRC-NBRC School "Development and Functions of Brain Circuits: From Molecules to Behaviour.
  Talk: Honeybees and the mechanisms of animal behaviour.

- 3rd M K Chandrashekaran Memorial Meeting, JNCASR, Bangalore
  Talk: Studies on the dance communication in honeybees.

2015

- Second Meeting of the Indian Subcontinent branch of the International Neuropeptide Society / Society for Biologically Active Peptides NISER, Bhubaneswar.
  Talk: Studies on the behavioural function of neuropeptides in honeybees.

- Institute of Advanced Study in Science and Technology, Guwahati, India
  Talk: Honeybees and the mechanisms of animal behaviour.

- Indian Bee Course, Department of Animal Science, Central University of Kerala, Nileshwar, Kerala
  Talk: Neuro- and molecular biology of honeybee behaviour.

- International Conference on Biotechnological Advances in Environmental Health and Biodiversity Conservation (EHBC), Imphal Manipur, India
  Talk: Honeybees and the mechanisms of animal behaviour.

DEEPA AGASHE

2016

- National Seminar on Frontiers in Biotechnology, Bharathiyar University, Coimbatore, India
- Undergraduate Lab research talk, Indian Institute of Science (IISc), Bangalore, India
- ICTS School on Population Genetics and Evolution, ICTS, Bangalore, India

2015

- Conference on Bacterial Expressions, NCBS, Bangalore, India
- Jawaharlal Nehru University (JNU), New Delhi, India
- National Conference on Ethology & Evolution, IISER Mohali, India
- J Craig Venter Institute, La Jolla, USA
- Department of Ecology and Evolutionary Biology, University of Minneapolis, USA
- Department of Microbiology, Michigan State University, USA
- Department of Organismic and Evolutionary Biology, Harvard University, USA

GAITI HASAN

2015

- Drosophila flight: an invertebrate paradigm of motor coordination. Jan 2015. Annual Biology meeting, IISER, Bhopal.
- Drosophila flight: an invertebrate paradigm of motor coordination. Jan 2015. Presidency University, Kolkata.
- Encouraging flight: a calcium-dopamine nexus. July 2015. Brandeis University, Waltham, USA.
- Encouraging flight: a calcium-dopamine nexus. October 2015, Dept of Biological Sciences, TIFR, Mumbai.
- Invertebrate models of human disease. November 2015. Annual meeting of the Current Science Editorial Board, Bangalore.
- A neural circuit for adaptation to nutritional stress. Dec 2015 International Neuropeptide Society meeting, NISER, Bhubhaneshwar.
- A neural circuit for adaptation to nutritional stress. Dec 2015 Biennial meeting of Indian Drosophila Biologists, IIT, Kanpur.

KRUSHNAMEGH KUNTE

2015

- University of Würzburg, Germany.
- Ludwig-Maximilians-Universität, Munich, Germany.
- Imperial College London, Silwood Park campus, UK.

M. K. MATHEW

2016

- St. Joseph's College, Bangalore. India
- Arabidopsis Meeting, IISER Mohali, 20 -22 March 2016. India

2015

- University of Guwahati: Institute for Advanced Studies in Science and Technology, Guwahati. India
- NUS – IISER Thiruvananthapuram Symposium September 21 -22, 2015. India

MITRADAS M. PANICKER

2015

- Lipid Bodies associated with the Primed Pluripotent Stem Cell State - A novel endogenous marker. At the Achucarro - Basque Center for Neuroscience, Bilbao, Spain. July 27, 2015
- Pluripotency sings the Blues. At the Bader Laboratory, Max Delbruck Center for Molecular Medicine, Berlin, Germany. July 8, 2015
- Human Induced Pluripotent Stem Cells based modelling of Late Onset Alzheimer's Disease (LOAD) genetics in an Indian cohort. Conference on "Neurodegenerative Diseases: Pathogenesis to Therapy" Centre for Brain Research, Indian Institute of Science, Bangalore. November 18, 2015

R. SOWDHAMINI

2016

- "Genome sequencing of Tulsi - a herbal plant" in International Symposium 'Bioinformatica Indica' in University of Kerala during 6th to 8th January 2016
- "Computational Analysis of Proteins that undergo domain swapping and prediction in the human genome" in 'Coming of Age: Transitions in Biological Systems' in NCBS Annual talks from January 11-13, 2016
- "Analysis and Prediction of Domain Swapping in Proteins" in SBCADD'2016 in Alagappa University, Karaikudi from February 13th to 17th, 2016
- "Genome Sequencing of Tulsi" in Indo-French workshop on 'Exploring the scope of collaborations in marine biology and biotechnology between France and India' in Indian Institute of Science during 7th to 9th March, 2016
- "Protein domain swapping" in workshop on 'Advanced Techniques in Protein Design and Engineering' in IISER Mohali during 15th to 19th 2016
- "Three-dimensional modeling of protein-ligand interactions: implications in drug design" in 'Bioinformatics - recent advances in genomics' which is scheduled to be organized at RMRC, Port Blair on 28th & 29th March 2016
- "Women in Science" during Science Week on 26th May 2016 in University of Agricultural Sciences, Bangalore held during 23rd to 27th May 2016

2015

- "Bioinformatics genome-wide survey of myosins" in "Genomics and Proteomics Research" as a part of SELECTBIO conference in Sheraton Bangalore Hotel during May 28-29, 2015
- "Computational Analysis and Prediction of Proteins that undergo domain swapping" in Albany 2015: The 19th Conversation held in Albany University between 9th and 13th June 2015
- "Cardiomyopathies and energetics calculations of myosin coiled-coils" in JNCASR-NNMCB `Program on Mathematical Biology' held on September 11 2015 at JNCASR
- "Sequence and Structural Analysis of Odorant Binding Proteins in three Mosquito Genomes" in *Genomics & Proteomics of Disease Vectors and Pathogens*â€ at VCRC, ICMR Puducherry on 3rd November 2015

RANABIR DAS

2016

- NMRS Symposium, IIT Kharagpur, Feb 2016
- Meeting on "Ubiquitin and Ubiquitin like Modifications: Mechanisms and Implications for Human Diseases", NCBS-TIFR, Jan 27-28, 2016

RAGHU PADINJAT

2016

- 6th International Lipid Meeting, Singapore, December 2016
- Indian Society for Developmental Biology Meeting, Hyderabad, India. September 2016

2015

- 3rd Asia-Pacific Drosophila Research Conference, Beijing, May 2015
- Indian Society for Cell Biology, Trivandrum, India, December 2015

SANDEEP KRISHNA

2016

- Models of Life conference, Krogerup, Denmark.
- Excursions in Complexity meeting, Niels Bohr Institute, Copenhagen.

SATYAJIT MAYOR

2016

- Wellcome Trust's Board of Governors and Executive Board Visit to Delhi – February 2016. Title : "The shifting geography and language of basic research in biological sciences."
- Talk at St. Stephen's College, Delhi – February 2016. Title : "Shifting Geography and language of Biosciences."
- YIM 2016, Delhi - February 2016. Title : The Bangalore Life Science Cluster
- Talk at the International Society on Optics Within Life Sciences (OWLS) Organized by TIFR - March 2016. Title: "Fluorescence Spectroscopy in the pursuit of living cell membrane structure"
- Key Note Lecture at the Affiliates Meeting at Manipal University, Manipal – April 2016. Title : "A New Fabric for the Biological Sciences in the 21st century: opportunities and challenges for the Indian Biologist"
- National Academy of Sciences - 153rd Annual Meeting in Washington, DC – April 2016. Compositional regulation at the local and cell-wide scale in membranes of living cells
- Talk at the Teaching of the Sciences in Higher Education in Bangalore University, Bangalore - May 2016. Title: Interdisciplinarity in Life Sciences in the context of curricula design and teaching
- Talk at the IISc, Organized by Indian Academy of Science, Bangalore – June 2016. Title :" The shifting geography and language of biology in the 21st century: a cell biologist's perspective."
- Signaling by Adhesion Receptors, Organized by Gordon Research Conferences – June 2016. Title : Local Membrane Organization and Integrin Signalling- An Active Actin-Membrane Composite

- Talk at the B S F Science Festival 2016, Bangalore - July 2016. Title : "A new language for biology in the 21st century- Are we equipped to speak it?"
- Talk at Limit Infinity- a school fest co-organized by Infosys Science Foundation – August 2016. Title : Is Biology Unique? Can we ever understand how a cell works?
- Talk in Heidelberg, 'Molecular Architecture and Cellular Functions of Lipid/Protein Assemblies. Transregio 83 Symposium. Title : Regulated organization and structure of living cell membranes
- Mechanical Forces in Cell Biology, NCBS Bangalore – Oct 2016. Title: Rafts come alive: actively driven organization of membrane components in living cells triggered by Integrin signaling
- Talk at Cell Biology of Infections Meeting, NCBS Bangalore- Oct 2016. Title : Molecular Machinery and Function of a Clathrin and Dynamin Independent Endocytic Pathway

2015

- The 15th HFSP Awardees Meeting – July 2015. Title : "Spatiotemporal remodeling of membrane nanoplatforms under mechanical forces"
- Prof. V Ramalingaswami Memorial Lecture. Organized by Jawaharalal Nehru Centre for Advanced Scientific Research – July 2015. Title : "A new fabric for the Biological Sciences in the 21st Century: The shifting geography and language of biology"
- The Bangalore Science Forum at The National college Basavanagudi, Bangalore – July 2015. Title : "A new fabric for the Biological Sciences in the 21st Century: The shifting geography and language of biology"
- The Evolutionary Cell Biology conference - Organized by Kavli Institute for Theoretical Physics at Santa Barbara, USA - August 2015. Title : The Active Actin Membrane Composite
- The multidisciplinary era of endocytic mechanics and functions - Organized by INSERM at Mandelieu-la-Napoule, France - September 2015. Title : Mechanism and functions of a clathrin and dynamin independent endocytic process
- TWAS 13th General Conference & 26th General Meeting, Vienna, Austria – November 2015. Title : "Imaging at the nanoscale reveals 'life at the cell's edge'."
- ASCB - Annual Meetings - December 2015. Title : Local control of membrane composition by Integrin receptors

SUDHIR KRISHNA

2016

- DAPCON- 2016, University of Delhi, February 2016
- International cancer workshop for SAARC nations, Delhi, March 2016
- ACOS & IACR meeting, Delhi, April 2016
- Bits Pilani, Goa Campus, April 2016
- CDFD, Hyderabad, May 2016

2015

- IFOM, Milan, Italy, July 2015
- AOGIN India, CMC, Vellore, August 2015
- 11th Indo-Australian biotechnology conference, Sydney, Australia, September 2105

SUMANTRA CHATTARJI

2016

- The Herrenhausen Symposium on Psychiatric Disorders (organized by Nature Medicine, Nature Neuroscience and the Volkswagen Foundation), Hannover, Germany

2015

- Invited talk, 54th Annual Meeting of the American College of Neuropsychopharmacology (ACNP), Hollywood, FL, USA
- Invited talk, Gordon Research Conference on "The Amygdala in Health and Disease", Easton, MA, USA
- Invited talk, International Brain Research Organization, 9th World Congress, Rio De Janeiro, Brazil

UPINDER S. BHALLA

2016

- 4-7 Jan, Bangalore, ICTS, Information Processing in Biological Systems: Many ways to lose your mind: Dimensions of robustness in noisy chemical bistable circuits
- 8 Jan, Chennai, Workshop on Computational Brain Research at IIT Madras: Neuroscience at The Bangalore Biocluster
- 1 February, Hyderabad, L.V. Prasad Eye Institute. Joint Academy Seminar on the Brain and the Eye. Lighting up brain connections and plasticity
- 21-22 April, Dublin, Trinity College, Indo-Irish Neuroscience Conference: Learning and decoding of neural activity sequences by chemical signals in single neurons
- 2-5 May, HHMI Janelia Research Campus, Molecular Mechanisms in the Synapse: Experiments and Modeling: Sequence recognition through multiscale signaling in morphologically detailed models of pyramidal neurons
- 11 June, Cluj, Romania, TENSS Course: Models and cellular biophysics
- 30 June, Bangalore Centre for Neural Systems, Indian Institution of Science. Bangalore Cognition workshop Learning and recognizing activity sequences in the brain

2015

- 10-12 June, Cluj, Romania, TENSS Course: Molecular and multiscale computation in neurons
- 18 June, Bangalore, CNS/IISc Spikes Seminar Series: Olfactory bulb coding of odors, mixtures and sniffs is a linear sum of odor time profiles.
- 21 Aug, Bangalore, Deeksha High School Annual Day: Research in Neuroscience
- 6-8 Sep, Gdansk, Poland, 12th International Congress of Polish Neuroscience Society: Optical control of network context in hippocampal synaptic plasticity
- 9 Sep, Gdansk, Poland, NENSKI Onstitute, NAMASEN Meeting: Space and plasticity: a multiscale modeling study
- 9-10 Nov, Maryland, HHMI/eLife: eLife Editors General Assembly
- 12 Nov, Cambridge/Mass, MIT, Seminar: Where, what and how much: The code in your nose

VARADHARAJAN SUNDARAMURTHY

2015

- NCBS-Max Planck Lipid Center, NCBS, Bangalore, India
- Invited speaker for Confluence 2015, Symposium of Systems Biology, IADC college, Bangalore, India
- National Institute of Research in Tuberculosis, Chennai, India

VATSALA THIRUMALAI

2016

- Understanding the Cerebellum: A window through the optomotor response. SPIKES students seminar, Centre for Neuroscience, IISc, Bangalore. 22nd January 2016
- Understanding the Cerebellum: One Purkinje Neuron at a time, Indo-Irish Neuroscience Meeting, Trinity College, Dublin. 21st April 2016
- Understanding the Cerebellum: One Purkinje Neuron at a time, Institut du Cerveau Et De La Moelle Epiniere (ICM), Paris. 26th April 2016
- Mechanisms and functional implications of bistability in Purkinje neurons: Lessons from zebrafish, Neuro-Evo: A comparative approach to cracking circuit function, Janelia Research Campus, Ashburn, VA. 16th May 2016.
- Understanding the Cerebellum: One Purkinje Neuron at a time, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore. 24th June 2016
- Descending motor control: Representations of efference copy in cerebellum, 4th Bangalore Cognition Workshop, Indian Institute of Science, Bangalore. 28th June 2016
- Understanding the Cerebellum: One Purkinje Neuron at a time, EuroSPIN PhD student meeting 2016, NCBS, Bangalore. 29th June 2016
- Assembly and function of neural circuits, Workshop on Stem cells and circuits, University of Kashmir, Srinagar. 16th July 2016

2015

- Understanding the Cerebellum: One Purkinje Neuron at a time, IBRO-APRC School on Molecular Advancements in Neurobiology, Benares Hindu University, Varanasi. 18th September 2015

HONORS AND AWARDS 2015 - 2016

ASWIN SAI NARAIN SESHASAYEE

2015

- Associate of the Indian Academy of Sciences recognition to talented scientists below the age of 35 (2013-2018)
- Ramanujan Fellowship - From the Department of Science and Technology, Government of India (2011-2016)

DEEPA AGASHE

- DST INSPIRE Fellowship

EDITORSHIP

- Associate Editor, Molecular Biology and Evolution (Oct 2015 present)
- Guest Editor, Current Science Special Section on Evolutionary Biology (2015)

DIMPLE NOTANI

2016

- WT DBT IA Intermediate Fellowship (2016-2021)

GAITI HASAN

2015

- Elected fellow of The World Academy of Sciences (November 2015)

HIYAA GHOSH

2016

- Ramanujan Fellowship (2016-2021)

JAYANT B. UDGAONKAR

EDITORSHIP

- Member, Editorial Advisory Board of Biochemistry (2013-2015)
- Associate Editor of Biochemistry (2015)
- Guest Editor, Section on Folding and Binding, Curr. Opin. Struct Biol. (2013, 2016)

KRUSHNAMEGH KUNTE

2016
- Ramanujan Fellowship, Dept. of Science and Technology, Govt. of India (2012 – 2017)

M. K. MATHEW

2015
- TAA Excellence in Teaching Award for Biology

MADAN RAO

2015
- Labex CelTisPhysBiol Chair 2016, Institute Curie, Paris
- Distinguished Alumnus Award 2016 of IIT Bombay

2016
- Membership of INSA

MAHESH SANKARAN

EDITORSHIP
- Subject Editor, Biorotpica (2014 - present)

MUKUND THATTAI

EDITORSHIP
- Editorial Board Member, Journal of Experimental Zoology B (2013 - present)
- Academic Editor, PLoS ONE (2007 - present)

P. V. SHIVAPRASAD

2015
- Ramanujan Fellowship, DST-SERB, Government of India (2013-2017)

EDITORSHIP
- Member, Editorial Board of 'Matters' (2016 onwards)

R. SOWDHAMINI

2016
- J.C. Bose National Fellowship

RADHIKA VENKATESAN

2015
- Ramanujan Fellowship (SERB, Govt. of India), (2015-2020)

2016
- Head, Max Planck- India (DST) Partner group in Chemical Ecology with Max Planck Institute for Chemical Ecology, Jena, Germany
- Early Career Research Award (SERB, Govt. of India)

RAGHU PADINJAT

2015
- Wellcome Trust-DBT India Alliance, Senior Fellowship for Basic Research (2015-2020)

SATYAJIT MAYOR

2015
- Foreign Associate, US National Academy of Science (2015)

2016
- Margadarshi Fellowship, DBT-Wellcome Trust Alliance (2016 - 2021)

EDITORSHIP
- BiochemicaBiophysicaActa (2008 - present)
- The Biochemical Journal (2011 - present)
- Molecular Biology of the Cell (2004 - present)

MEMBERSHIP OF SOCIETIES AND DECISION MAKING BODIES
- President, Asia Pacific Organization of Cell Biology (APOCB), (2014 - 2015)
- Editor, Biochemical and Biophysical Research Communications (2014 - present)
- Editorial Board, Cell (2008 - present)
- Editorial Board, Journal of Cell Science (2011 - present)

SHANNON OLSSON

2015
- Ramanujan Fellowship (2015 - 2020)

EDITORSHIP

- Review editor for Frontiers in Integrative Neuroscience (2014 - date)
- Frontiers in Physiology (2014 - date)

SUMANTRA CHATTARJI

EDITORSHIP

- Current Opinion in Physiology (2016)
- Journal of Physiology (2016)
- IBRO Reports (2016), Science Matters (2016)

UMA RAMAKRISHNAN

2015

- Speaker, INK Asia, Singapore
- Bass Fellow, Field Museum of Natural History, Chicago

2016

- Fulbright Nehru Academic Fellow
- Parker Gentry Conservation Award

UPINDER S. BHALLA

2016

- J.C. Bose National Fellowship

MEMBERSHIP OF SOCIETIES AND DECISION MAKING BODIES

- Member, Governing Board, International Neuroinformatics Coordination Facility (2015)
- Journal of Computational Neuroscience (2000 - present)
- Neuroinformatics (2010 - present)
- Frontiers in Neuroscience (2010 - present)
- eNeuro- Board of Reviewing Editors (2015 - present)
- eLife- Board of Reviewing Editors (2015 - present)

VARADHARAJAN SUNDARAMURTHY

2015

- Ramalingaswamy Re-entry Fellowship (2015 - 2019)
- DST-Max Planck Partner Group (2015 - 2017)

VATSALA THIRUMALAI

2015

- Wellcome Trust DBT India Alliance Intermediate Fellow (2010 - 2015)

EDITORSHIP

- Editorial Board, Journal of Neurophysiology
- Reviewing Editor, Frontiers in Neural Circuits

SUPPORTING OUR SCIENCE

NCBS is now 25 – neither an infant nor a mellow, settled fixture. At 25 years we are still young and yet can lay claim to a modicum of history. A generation of scientists have now passed through NCBS, with the first retirements on campus in the person of our colleague Prof. M.M. Panicker. He has been informal mentor to more students than I can remember, and his enthusiasm in steering many of us to new ideas is now a deeply ingrained part of the NCBS willingness to suprise. Even this small dose of history is a challenge. A preview of NCBS at 25 would have astonished (and I would like to believe, gladdened) the people in NCBS at time zero. Will our new colleague Anjana Badrinarayanan be as postively astonished by a glimpse of us at 50? We must sustain our capacity to surprise, otherwise something vital will have been lost.

One of the wellsprings of youth, surely, is to tap into the wider scientific world for inspiration. We have long had lively collaborations with individual colleagues next door and also around the world. The shape of such ties have broadened, and there is a looming new form of collaborations that we must master. This is the establishment of multi-institutional consortia, pulling together many people and places, to take on problems on entirely different – maybe even astonishing – scales. We see seeds of such consortia in the last couple

of reports, in the form of the chemical ecology programme and the multi-institutional programs on brain disorders. Our horizons must expand to participation, and leadership of such ambitious efforts on much greater scales, and across many countries. It is precisely at such junctures that the operational burdens seem to outweigh scientific ambition. There is indeed a legacy and accumulating drag of institutional strictures as we cross 25 years. Possibly this is our most immediate 'sound barrier': to find ways to leap ahead into uncharted zones of scientific phase space, while negotiating and managing a viscous environment.

A major and ongoing transition over the past year is the reconfiguration of our 25 scientific facilities. By happy coincidence these share the number of our years. These facilities have the potential, in a catalytic way, to be part of the broadening of our ties and scientific outreach. By dint of enormous work over many years, we have established a formula of close faculty involvement with outstanding facility personnel and a rigorously trained student user community. The exemplar of this is the Central Imaging and Flow Facility, led by Dr. H. Krishnamurthy, which has developed not just a world-class, but a world-leading facility for imaging, training, and technology development. This is the model for all our facilities, and we welcome Krishna to his new role of heading the entire set of Facilities. Raghu Padinjat has closely steered this major transition, with the ground work done by dozens of NCBS and inStem faculty who constitute the Facility Advisory Committees. These facilities would hardly be catalytic, and be sadly underused if we were not to throw them open to use for the national scientific community. In the past year we have evolved a transparent and scalable framework for running facilities for effective internal and external use, and C-CAMP remains the gateway for hundreds of external institutional users.

If the facilities are one of our engines in leaping ahead, the other must surely be our staff. They propel us, and also are our first line of defense against the entropy in the system. Much of the fuel for our work is provided by grants. We welcome Mr. Srinidhi in the Grants section. He has been quite stellar in his transformation and resolution of an enormous backlog of grants in diverse paper formats into the consolidated Tata Institute Information System package. The TIIS transition has challenged all our administrative sections: Establishment, Purchase, Accounts, and Grants, all of whom have responded heroically. With this enormous energy barrier soon to be behind us, we look forward to vastly streamlined operations.

Our technical staff, who work as a unit across all campus infrastructure, have their own huge challenge literally looming, with the impending and much anticipated opening of the vast new inStem building. Integration of this with the existing campus infrastructure promises to keep the pace of change on track, and will bring new scientific opportunities.

To circle back to our sources of scientific inspiration, we must acknowledge two special groups of people who keep us connected with the best of the wider world of science. First of these are our Adjunct faculty. Prof. Barrantes of the Institute of Biomedical Research, Catholic University of Argentina, has been visiting us for almost 20 of our 25 years, and has recently been on campus again. Prof. Mani Ramaswami of Trinity College Dublin is another long-standing collaborator who has visited multiple times, and this year hosted a very lively neurobiology meeting involving NCBS and collaborators at Trinity. We have lost one of our other long-standing adjunct faculty members, but in a happy way: Madan Rao has finally taken the leap and is now fully with us as Senior Professor at NCBS. We are delighted to have Dr. Ullas Karanth frequently here with many interactions around the stimulating WCS-NCBS Master's programme. Sanjeev Jain is another frequent visitor with the flourishing of the ASHD programme, in which he is deeply engaged. Finally Jim Spudich from Stanford has also visited us and continues to inspire our long-term plans.

The second key group of people are our Advisors: our Scientific Advisory Board and the scientific members of our Management Board. We ask a lot of them, this year more than most. Every five years we conduct intensive reviews of the research plans of each of our faculty members, and we are enormously indebted to the members of these Boards for keeping us on track and in inspiring us.

So, at 25, are we yet grown up? Possibly our founder Obaid Siddiqi would be glad to see where we are at this milestone, but he would surely look, as we must, to the next 25 years and beyond.

Upinder S. Bhalla
Dean, NCBS

FACILITIES AND RESOURCES

RESEARCH FACILITIES

- 1a, 1b Central Imaging & Flow Cytometry Facility (CIFF)
- 2 Radioactivity Lab
- 3a, 3b Mass Spectrometry Facility
- 4 Biosafety
- 5 Chemcore
- 6 High-Throughput & High Content Screening Facility
- 7 Next Generation Genomics Facility (NGGF)
- 8 Spectrometry (Optical spectrometers / NMRs / X-ray facility)
- 9 Structural Biology (Biophysics/Crystallography)
- 10 Mouse Genome Engineering Facility
- 11 Drosophila Facility
- 12 Greenhouse
- 13a, 13b Electronic & Mechanical Workshops

TECHNICAL SERVICES

- 14 IT Section
- 15 Architect
- 16 AC
- 17 Instrumentation
- 18 Civil
- 19 Electrical

HEALTH AND WELL-BEING

- 20 Creche
- 21 Medical Facilities
- 22 Sports
- 23 Library

FACILITIES AND RESOURCES

ADMINISTRATION

The NCBS administration is the foundation layer upon which much of the science on the campus is placed and forms several links that help the campus function efficiently. Staff from the administrative teams are also the points of connect to external organizations that are invaluable for campus activities. Diverse teams support campus research via administration including accounts, establishment, procurement and stores.

Deans office, Academic Office. Directors Office. Travel and others. The NCBS front desk team coordinate myriad matters ranging from taxis to guests to ambulances and more. The security team ensure that the extended NCBS campus, laboratories, offices, housing sites and other locations are safe and monitored round the clock. A dispatch team coordinate a large number of on-time deliveries to a range of locations in India and overseas.

HEALTH AND WELLBEING

MEDICAL FACILITIES

The NCBS campus is equipped with a state of the art medical centre as well as a medical room at the off-site housing centre. Both of these facilities function as primary health centres. Doctors as well as a physiotherapist staff the centre. The medical centre offers outpatient consultation services as well as prophylactic services such as vaccinations.

When required specialist consultations and inpatient services are catered for by designated tertiary care hospitals in the vicinity of the NCBS campus. There is an on-site ambulance 24*7 that is able to transport those needing inpatient care to nearby hospitals.

COUNSELLING SERVICE

A free and confidential counselling service is available for all campus members. It is provided by an independent support group- Parivarthan.

SPORTS FACILITIES

There is a wide range of excellent sports facilities on campus. The sports complex includes a well-equipped gymnasium as well as facilities for indoor sports such as badminton, squash and table tennis along with prov1sions for outdoor sports including cricket, football, tennis and basketball. The Swimming pool is the pride and joy of this campus, supervised by lifeguards and treated to maintain the very highest standards of cleanliness and safety. A climbing wall adorns the east side of the Southern lab complex.

CRECHE

An on-site crèche caters to the childcare needs of on campus colleagues. The crèche 'Dolna' operates from both the main campus as well as the housing site and offers childcare during working hours to children of staff

and students who work on campus. Children engage in activities include nature walks around campus and well-planned activities that promote learning. They also benefit from science related activities such as bird watching or campus walks with students and faculty of NCBS and other advantages that typically come with being part of a growing campus.

HOSPITALITY

The hospitality services see to our comfort in food, living, and cleanliness. Two canteens, four cafeterias and an upcoming food court are situated over the different blocks on campus. The service is available between 7.30am - 11.00 pm. The staff endeavour to cater to a diverse mix of palates. And visitors can choose between a variety of ethnic and intercontinental cuisines. Services that also include House keeping are subject to stringent checks by select faculty and student who form the hospitality committee. NCBS also manages a selection of guest houses which provide comfortable accommodation for our visitors.

LABORATORY SUPPORT AND SAFETY

A range of services comprise the support offered to our researchers within their laboratory environments. These include laboratory kitchen services, order consolidation, primer orders, media preparation, supply of gases, laboratory waste disposal, greenhouse and tissue culture facility maintenance etc. In addition, safety in the laboratory is a priority and support is provided via regular safety audits and recommendation.

MEETINGS AND WORKSHOPS

In a rapidly changing scientific world. It is vital for NCBS to develop national and global connectivity, bringing the best lecture courses, workshops and scientific meetings to the Indian life science community at our campus. The Meetings Office at NCBS has overall responsibility for organizing and managing a thriving programme of meetings, workshops, symposia, public talks, courses, colloquia and the NCBS Annual talks. Each year the demands on this office are increasing as we organize more meetings workshops aimed at increasing our critical mass for the breadth of research pursued on our campus. These events serve to provide vital networking opportunities for our researchers and also result in raising our scientific profile within the research community.

RESEARCH FACILITIES

ANIMAL CARE AND RESOURCE CENTRE (ACRC)

The ACRC is dedicated to providing quality laboratory animals for research and 24/7 care of animals. All projects run in the ACRC are cleared by the Institutional Animal Ethics Committee (IAEC). The ACRC currently houses four species of laboratory animals: Mice (around 180 strains, which include inbred, outbred and transgenic lines), Rats (around 10 stocks), Zebrafish (around 12 lines) and Xenopus laevis frogs. All the animal rooms have a controlled macro environment with 22+/-1oC room temperature, 50-60 RH, once-through air-condition system with 0.3 micron HEPA filtered air supply at 15-20 ACPH and 14/10 light-dark cycle maintained. All mouse colonies are housed in Individually Ventilated Caging (IVC) systems. The ACF maintains all mice and rat colonies under Specific Pathogen Free (SPF) conditions. Health monitoring of the SPF animal colonies is done every quarter. Depending on the requirements of the approved research protocol, the ACRC imports new strains of animals from commercial suppliers and/or collaborators from abroad. The ACRC handles breeding, maintenance and supply of healthy laboratory animals to support the ongoing research at the centre.

In addition, the ACRC provides orientation and training to all authorized animal facility users to assure high standards of humane, ethical and responsible use of animals in their research. It also provides veterinary assistance with animals including anaesthesia, surgical procedures, peri-operative care, collection of biological samples, administration of medications and treatments, and record keeping as per CPCSEA guidelines.
The ACRC utilizes the Animal Colony Management Software (ACoMaS) system, an integrated rodent cage barcoding and tracking system designed to best assist NCBS animal facility users and staff in the most humane & ethical usage of animals required for their bio-medical IAEC approved research projects.
Find more details on ACF at https://www.ncbs.res.in/research-facilities/acrc

BIOSAFETY FACILITY

The Bio safety facility in NCBS provides class 2 Biological safety cabinets which allow the users propagation of viruses as well as human PBMC/tissue samples. The facility is equipped with C02 incubators, centrifuges, fluorescent microscope and fridge/freezer spaces for storage of samples. The basic consumables required are also stocked within the facility for easy access. There is also a separate autoclave system for waste management and users are asked to follow strict rules in disposal of any hazardous wastes generated during the experiment. To be an authorized user, a detailed orientation is provided to users w.r.t the working and functioning of the facility and monitored for initial experiments by facility incharge. Any research work which is cleared by the NCBS Bioethical committee and institute human ethics committee (IHEC) can be conducted in the facility. A detailed medical record is maintained for all active users with the help of the campus medical facility.

CENTRAL IMAGING AND FLOW CYTOMETRY FACILITY (CIFF)

Most research projects at NCBS rely heavily on the latest methods in bio-imaging and flow cytometry. State-of-the-art equipment at CIFF caters to meet this popular demand of biologists. CIFF is used by researchers from all over India and abroad. CIFF is an operator-free facility and offers regular training for new users and it has over 400 well-trained internal users. We train approximately 100 external students every year through various

imaging and flow cytometry courses/workshops organized at NCBS. External researchers use CIFF through C-CAMP. CIFF is used for over eighteen thousand hours an year. The open access system at CIFF coupled with its strong training programs has attracted several reputed manufactures of high-end microscopes and flow cytometers to station their systems at CIFF to demonstrate the versatility and performance of the instruments to the potential buyers, while they serve the needs of NCBS. The mandate of the CIFF's R&D centre is to establish infrastructure and develop human resource in electrical, optical and software engineering and to carryout R&D activities in biomedical related optical instrumentation and software. In 2009, CIFF started the Bangalore Microscopy Course, which is the only international microscopy course offered in Asia every year.

LIGHT MICROSCOPY

CIFF has ten confocal microscopes of various configurations and imaging capabilities. Point/Line scanning confocals are equipped with single/multiphoton lasers, FRET (homo and hetero) and FRAP, FLIM, FCS and Anisotropy modules. STED, TIRF, Laser dissection, AFM, NSOM and dual excitation scopes are also available. We have developed anisotropy module on a point as well as on a line scanning confocal microscope in collaboration with Carl ZEISS. Similar anisotropy module was developed using two-camera system on a spinning disc confocal microscope in collaboration with Andor Technologies.

FLOW CYTOMETRY

CIFF is equipped with ten flow cytometers. Six of them are analysers and four are sorters. The analysers are equipped with multiple lasers and some of the systems have IVD approval for clinical diagnostics and CIFF offers regular training on basic and clinical cytometry. CIFF has facilitated biotech companies in developing diagnostic kit for AIDS monitoring using wet and dry reagents. CIFF offers service to plant breeders on ploidy analysis and genome estimation. The flow sorters are equipped with four lasers and they mainly used for sorting cells as well as organelles.

ELECTRON MICROSCOPY (EM)

The electron microscopy facility enables the imaging of biological samples at a resolution currently not possible with light microscopy based approaches. The facility is equipped with instrumentation and laboratory infrastructure that facilitates a range of biological sample preparation. These include chemical fixation, ultrathin sectioning of samples at both room temperature and low temperature as well as a Vitrobot with cryo transfer unit. Transmission electron microscopy is performed on a 120 kV Tecnai G2

Spirit Bio-TWIN that is enabled for both tomography and cryoelectron microscopy. The facility also provides dedicated computers and software for the analysis of the micrographs and software for visualization and reconstruction the 3D structures.

CHEMISTRY CORE FACILITY

The Bangalore Life Sciences Cluster has established a Chemistry Core Facility to facilitate the synthesis of small molecules. It is equipped with state-of-the-art fume hoods, rotary evaporator, and the basic chemistry wet-lab equipment. This facility enables researchers of Bangalore Life Sciences Cluster to perform chemical synthesis of desired molecules. In addition to small molecular synthesis, some of the key chemical reactions on biomolecules can be done in this facility. The facility offers users training in the area of chemical synthesis and purification characterisation techniques.

At present this core is actively supporting chemistry needs of 15 labs in the campus. Multiple labs across the chemistry, chemical biology, and chemical ecology disciplines are being benefitted by this core facility.

DROSOPHILA FACILITY

The use of genetically tractable model organisms such as fruitfly Drosophila offers a potential solution to the problem of interpreting the function of proteins encoded in the eukaryotic genomes. In addition to being tractable to classical genetics analysis, Drosophila research is also enabled by the ability to manipulate its genome with increasing levels of sophistication.

At NCBS Drosophila is used as a model organism to address a range of fundamental questions in biology. In order to facilitate this, the Drosophila Facility at NCBS offers researchers several services:

1. Centralized support for Drosophila culture
2. Maintain a collection of commonly used, genetically defined Drosophila stocks.
3. Generate transgenic Drosophila strains using state-of-the-art technology for both for internal projects and as part of global collaborative initiatives.

The facility also carries out developmental work towards enabling modern genome editing technologies in Drosophila. The facility also supports the work of the wider Drosophila community both in India and overseas; research groups can use the services of the transgenic facility via C-CAMP.

FIELD STATIONS

We are living in a rapidly changing world! Just as technological change in our generation has been tremendous, the environment around us is changing drastically. An understanding of ecology and evolution might allow us to predict how populations and species may change in response to environmental change. Such knowledge requires acquiring data on individuals and species over large spatial and temporal scales, and the ability to conduct field-based ecological/evolutionary research (1) over long periods of time and (2) at multiple locations. Nurturing such efforts requires multiple dedicated sites or field stations, which foster innovative/integrative/collaborative research, long-term ecosystem monitoring and venues for high quality, hand-on education.

NCBS made a commitment to engage in such long-term studies through particular natural labortaories, or field stations. Since 2010, NCBS has been engaging with two separate field stations run by MCBT (Madras Crocodile Bank and Trust), the Andaman and Nicobar Environmental Team (ANET) field station on South Andaman Island; and the Agumbe Rainforest Research Station (ARRS) in Shimoga district, Karnataka. Both of these field stations possess land and essential infrastructure, and are located in areas of rich biodiversity and great ecological interest. Broadly, the expectations for NCBS field stations include the following: (1) increased access for NCBS faculty and scientists from other institutions (especially those who are not full-time field biologists) to wild lands (2) enhancement of interdisciplinary, international and paradigm-changing research at these locations (3) establishment of long-term monitoring sites at these locations and (4) providing a base for field courses at the basic and advanced level. In collaboration with IISER Pune, we recently initiated a long-term field research station at the Eaglenest wildlife sanctuary in Arunachal Pradesh, in the Eastern Himalaya biodiversity hotspot. NCBS researchers have also been engaged in long-term monitoring and research in Nagarjunasagar Tiger Reserve and the Sikkim Himalaya. We hope that several of these efforts will crystalize in the coming years.

GREENHOUSES

The greenhouse facility is indispensable for all those who work on plants or plant animal interactions. They also help to maintain pure or modified plant/animal lines.

Greenhouses at the campus are located on the Eastern Laboratory terraces with a total built area of 615 sqm. There are six set of greenhouses: Plant house (for an area 150 sqm). Butterfly house (75 sqm). Ecology house [130sqml. Moth house (180 sqm) and two Transgenic greenhouses (80 sqm). These greenhouses are equipped with fully automated climatic control system to control light, temperature and humidity using special lights, shading screens (UV stabilized cool net), evaporative pad (cellulose) and fan cooling system, heaters (Biotech

heat converter of 2.4KW to circulate heat), humidifiers (overhead misting system with foggers) and dehumidifiers. The greenhouses can be set to a range of 0.1 to 59.90 C with ±10 accuracy. Photo-synthetically active radiation lamps having wavelengths of 400 to 700 mm have been installed to provide perfect light conditions. Monitoring and precise regulation of conditions through a microprocessor based control system that creates a sustainable growing environment through the conservation of heat, electricity and water is in place.

HIGH-THROUGHPUT AND HIGH CONTENT SCREENING (HTS/ HCS) FACILITY

The HTS and HCS facility has evolved to assist academic and industrial researchers in developing and performing high-throughput and high content screens. The facility assist's in the adaptation of both biochemical and cell based assays into the HTS/HCI formats through miniaturization and automation.

The centre is equipped with state-of-the-art automated liquid handlers, multi-mode plate readers, automated imaging platforms, multiplexing system to quantify multiple analytes from single sample while housing a collection of RNAi librariess, small molecules and cell lines.

The facility supports biochemical and cellular assays through different readouts such as absorbance, fluorescence kinetics, fluorescence polarization, TR-FRET. Alpha Screen, Alpha Lisa, bioluminescence and chemiluminescence.

The automated imaging platforms within the centre facilitates high content live and fixed cell imaging at better throughput owing to robotics and on-the-fly analysis. Some of the in-built analysis parameters include cell cycle analysis, cell spreading, cell motility, spot detection, etc.

In addition, the facility also hosts a BSL-2 Tissue Culture laboratory for mammalian and insect cell culture. In near future, the facility will add more instruments on automation and screening. Facility is developing new screening platforms such as small organism based screens, ADME assays and 3D and Co culture systems.

Facility has a dsRNA library against drosophila genome, shRNA library against human genome and a chemical library of 19000 compounds which can be used as starting points for screening. Facility did completed couple of projects in both screening and imaging modes. Now we have advanced to a stage where we can take up more work and contribute to campus science.

MASS SPECTROMETRY FACILITY (MS)

The mass spectrometry (MS) resources on campus aim to provide researchers with state-of-the-art techniques and equipment to characterize biomolecules including proteins and peptides (proteomics, sugars including glycoproteins (glycomics), metabolites (metabolomics) and lipids (lipidomics). A number of state of the art instruments for the separation of biomolecules and their analysis by mass spectrometry based approaches are available. In addition to providing analytical services, the mass spectrometry facility on campus provides training to on-campus scientific staff on the use of this technology as well as develop new analytical methods required to facilitate on campus research.

NEXT GENERATION GENOMICS FACILITY (NGGF)

DNA is the instruction manual for life. Reading DNA sequence encoded in our genomes (genomics) was first developed by Sanger using the "dideoxy" method over 40 years ago. Next generation sequencing (NGS) allows parallel sequencing, resulting in the generation of Giga bases of data in a short amount of time. This recent technological advance (NGS) has reduced the cost per genome significantly, making genomic data more

accessible to individual researchers. Decreasing costs are making genomics an attractive and integral part of any successful bioscience research project. The NGGF providing genomics services to scientists, train researchers and also to work on national and international genome projects. NGGF has helped scientists across the campus and India to sequence genomes from a range of organisms as well as facilitate transcriptome analysis. At our facility, we have sequenced different organisms comprising of more than 600 genomes from prokaryotes (bacteria and metagenomes) and eukaryotes (fungi, plants and animals), which are important to the Indian subcontinent. The services have been utilized by both academia and industry, several peer-reviewed papers have been published using the facility and many genomes/transcriptomes have been deposited in NCBI.

RADIOACTIVITY LAB

The radioactive lab facility has been classified as a TYPE 1 radioactive laboratory. The radioactive nuclei that the Rad Lab is equipped to handle are 3H, 32P. 14C, 55 Fe and 45Ca. It has a floor area of 20 sq.m, and therefore the number of users is accordingly limited. The facility offers a rigorous training program for new users under the supervision of the Campus Radiation Safety Officer. In addition to the use of radionuclides, the training program includes modules on the safe disposal of radionuclides in line with regulations.

SPECTROSCOPY LAB

Located on the ground floor of the SLC building, the spectroscopy laboratory is currently equipped with two NMR spectrometers and a home laboratory X-ray source for protein crystallography. The two NMR spectrometers are (i) Bruker Ascend™ 800 MHz and (ii) Bruker Ascend™ 600 MHz. Both the spectrometers are equipped with Z-gradient and full temperature control capability for liquids. Probes currently available for these instruments are: (i) An Inverse Triple Resonance (TXI) 5 mm probe for 1H observation and 13C and 15N decoupling, (ii) A Triple Resonance (TCI) 13C-enhanced 5 mm CryoProbe for 1H observation and 13C with 15N decoupling. The home laboratory X-ray source is Rigaku R-AXIS IV++ with imaging plates as detectors. The X-ray source is also equipped with a Rigaku BioSAXS-1000 and a Pilatus 100K/R detector for Solution X-Ray Scattering (SAXS) measurements. The laboratory is dedicated to assist the NCBS researchers to provide instrumentation for, and aid in collecting data on the structure and dynamics of biological macromolecules. The lab is managed by two facility managers: (i) Dr. Purushotham Reddy supervises the NMR spectrometers and (ii) Dr. Vinod Nayak supervises the X-ray source.

LIBRARY

The primary aim of the Scientific Information Resource Centre (SIRC) - library is to develop, organize, preserve and deliver information and scholarly resources for the NCBS community.

To these ends, the SIRC explores and implements new technologies to provide effective information services, expand the library's resource collection, and develop a librarian-user partnership.

The library has extensive print and electronic collections including about 6500 books, 11000 bound journals, and a CD/DVD collection of other educational resources. The SIRC subscribes to 95 print journals and multiple electronic resources, participating in consortiums such as TIFR, DAE and UGC-Infonet for expanded access. The SIRC also subscribes to magazines and newspapers of general interest and offers additional services including referencing, scanning, off-campus access, inter-library loan and document delivery.

TECHNICAL SERVICES

The campus Technical Services provide an uninterrupted and seamless physical infrastructure for running world-class science in a changing and sometimes challenging external context. The primary responsibilities of the technical services are two-fold. First, to maintain and upgrade existing facilities.

Second, to develop new facilities, laboratory spaces and common areas.

The technical services provide a very responsive digital (e-mail based) helpdesk system for users to report and track a wide variety of technical and other problems and requests to facilities. Tech teams closely monitor the helpdesk requests / complaints and attend to them with at most care and professionalism. Indicatively, tech services team attends to about 1000 helpdesk complaints a month. Many of which may be tiny problems to attend, but, they do help us to maintain the labs, the facilities and the entire campus as it exists now.

ARCHITECTS

The Bangalore Biocluster aims to set up a unique hub for highly interactive research for basic scientists and clinical researchers. To develop this space, a dedicated Architectural team at NCBS helps design, plan and coordinate the activity of setting up labs, facilities, and common spaces throughout the campus. Their role is key to ensuring that the Biocluster can accommodate the expanding infrastructural needs of scientists, while maintaining the natural beauty and aesthetics of the campus. In this task, they work in close coordination with a team of engineers who develop and maintain these facilities for the campus scientists.

THE ENGINEERING TEAM CONSISTS OF THE FOLLOWING SECTIONS:

CIVIL SECTION

Civil Engineering team maintains the buildings, roads, pathways, landscape & greenery, water management system, fire hydrant system and green house facilities.
The team also manages the major / minor civil renovation and space modification works as and when required. New or construction works are also executed by the team.
The civil team also helps other service teams in day to day activities for smooth and optimal functioning of campus facilities.

ELECTRICAL SECTION

The Electrical team manages all Electrical installations (HT and LT) in the campus. Mainly, the team is into the operation and maintenance (24/7 X 365 days) of HT and LT electrical installations & DG sets to provide electric power on 24/7X365 day basis for the entire campus. To indicate, there have been no major power outages of more than 5 minutes in the campus for the entire past.

The team maintains the UPS systems feeding power to important facilities such as Imaging facility (CIFF), Cluster, Mass spec, NMR and all the lab / office equipment.

Apart from O&M, Electrical team also undertakes all major / minor renovation and modification works in the campus.

Along with the above, the team also manages Fire alarm systems and Lifts in the campus.

IT SECTION

The IT section provides support, design, technology selection, systems management, administration, review and development of IT policies and standards to help the scientific community on campus.

NCBS is connected to the outside world via both NKN link and TTSL with BGP configured for fail-over and node

isolation from the Internet. The campus backbone is connected in ring with 10G uplinks from the core to the edges with a capability to expand to 40G in the future. Campus wireless deployment is focused on providing as much coverage as possible with seamless transfer across devices. In addition to this, NCBS also provides free wireless connectivity (named Hotspot) for all guests to the campus.

NCBS runs its own mail server, web server, firewall, backup server etc., all of which are serviced by the IT team. Most of the servers being deployed use open source solutions to meet current requirements. The IT team also uses open source solutions to design with new applications such as schedulers for campus services, and bar code systems to monitor animals in the animal and fly facilities. Virtualization is also being carried out by the group for critical servers to avoid blackouts.

To facilitate intensive computations, the NCBS IT Services has set up a high performance computing infrastructure amounting to around 300 TFlops of computing power, hosted at the data centre. The high performance clusters are used for computation intensive simulations, model calculations, NGS Data Analysis. In an effort to meet the ever growing requirement for data storage for scientific and computational raw data, a centralized data storage system has been implemented. The system has a capacity of 750 TB with redundancy and high availability which can scale beyond a petabyte for future expansions.

The IT team also manages the campus CCTV surveillance infrastructure, and is also involved in the maintenance of the automated Gas based fire extinguisher system installed at the data centre and the CIFF facility.

HVAC SECTION

The AC Maintenance team manages the heating, ventilating and air conditioning (HVAC) needs of the campus. HVAC is a critical requirement for all the core scientific activities on the campus (about 20,000 m2 of conditioned space). There are 3 independent centralized chiller plants (with collective capacity of 1100Ton) and Air Handling systems (about 200) to meet the load requirements. Many of the AC systems are designed with sophisticated and accurate feedback control systems (using BMS technologies) to meet the critical and fail safe requirements of the important facilities. Several package units, split units and coldrooms are also installed in few places to meet the requirements.

The AC team oversees the round-the-clock operation, monitoring and maintenance of chiller plant & HVAC in critical facilities like Animal house, Bio-safety lab, Computer cluster, Mass spectrometry lab, cold rooms etc. Along with regular scheduled maintenance activities, the team also manages help desk calls / complaints. The team also manages modification and up gradation of HVAC works in the campus.

INSTRUMENTATION SECTION

The instrumentation team has the huge task of maintaining the lab and facility equipments. The team manages to maintain about 10000 equipments across the campus. Further, the team also imparts regular training for students / users on safe and optimum usage of several equipments.

The Instrumentation team provides support to laboratories in selection, procurement, installation and testing of equipments specially meant for scientific activities. In co ordination with other teams, pre-installation requirements for equipments are also managed by the team.

Along with the above, the team also manages the Audio-visual systems of Lecture Halls, seminar halls and meeting rooms. The Telephone systems, Access control system, PA systems, Video/ Audio conferencing, RO water plants, laboratory Gas & vacuum systems are also managed by the instrumentation team.

MECHANICAL AND ELECTRONICS WORKSHOPS

NCBS has a well-equipped in-house mechanical workshop that helps researchers in fabricating custom-made experimental equipment. Currently, three skilled and experienced machinists help researchers with the design

and assembly of diverse scientific equipment such as Faraday cages for electrophysiology rigs, custom platforms to hold tissue samples, wind tunnels, micro-fluidic chambers etc.

The recent addition of a CNC vertical milling machine now allows the fabrication of intricately designed experimental products such as critical moulds with the capacity for multiple productions and a constant manufacturing accuracy. The Mechanical workshop also runs short courses to train students in using i ts facilities.

The electronics workshop has an experienced electronic engineer for the designing and assembly of electronic circuits, preparation of printed circuit boards, and programming for ARDUINO digital drive cards required for research purposes. The workshop also manages stocking of basic electronic components needed for testing and assembly of electronic circuits for the research as well as the occasional maintenance of existing equipment. With the addition of a 3D printer, the work shop is able to generate 3D scientific models for scientific requirements.

QA/QC UNIT

The QA/QC unit provides a quality and safety coverage to the activities of engineering construction and maintenance in the campus, by providing suitable inputs in the process, from concept to commissioning. Currently the QA/QC unit gives insights majorly into civil & electrical activities in the campus. QA/QC unit works under the guidelines similar to that of CPWD / ISRO (DOS). Team also provides assistance in technical evaluation of tenders, field tests, and material inspections as required.

INFRASTRUCTURE & CONSTRUCTION (PROJECTS) GROUP

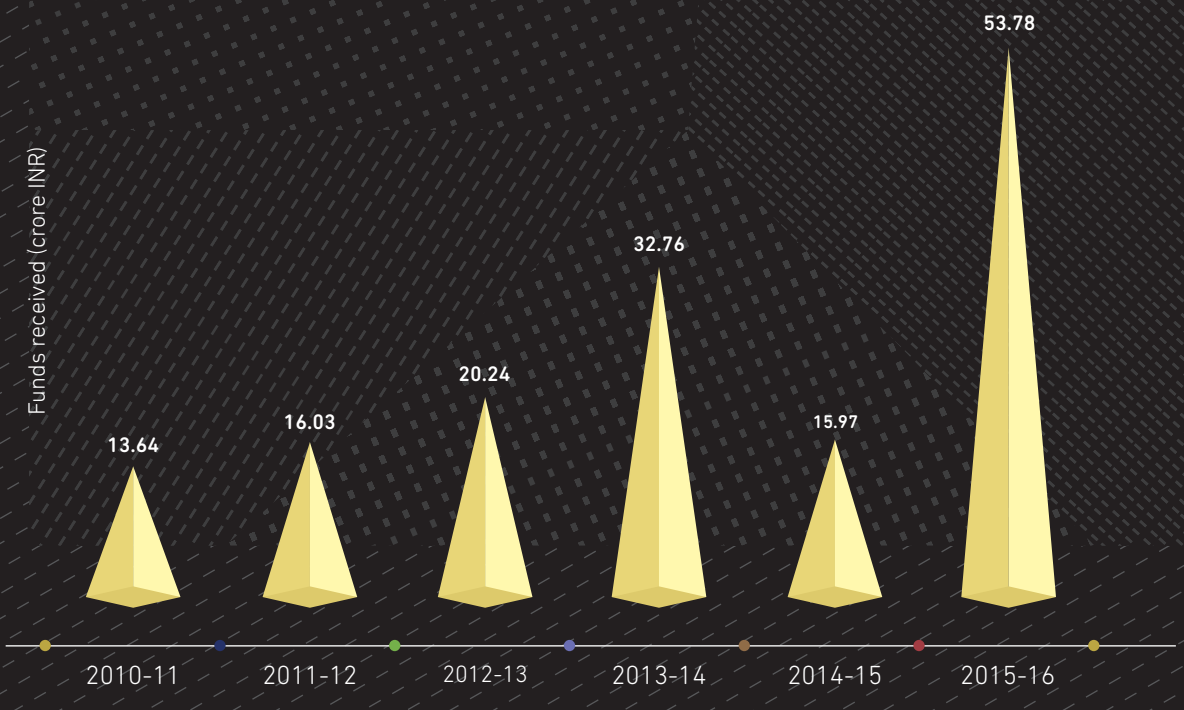
The group is responsible for all the major construction projects in the campus. Recently, the team has completed the construction of a laboratory building and campus. And, currently, have initiated for construction of a hostel building. In the past, the team has completed the construction of the following facilities: a dining-cum-recreation hub, housing and hostel complex at mandara campus, the clinic building, and, a vertical extension to the current animal house.

RDO@NCBS

Research at the Bangalore Life Science Cluster, which includes NCBS, inStem and CCAMP, spans a diverse range of questions and approaches in the broad area of life sciences. The Research Development Office (RDO) was created to facilitate research and training at the Cluster, via research funding and communications. The office continues to offer a concerted mechanism for managing these activities across the three member institutes of the Bangalore Life Science Cluster.

Over the course of the last six years, the Sponsored Research team within the RDO has continued supporting the diverse needs of the campus in fundraising, grants management and contract negotiation for research funding from funding agencies, corporate sources, charitable organizations and philanthropic donors. The Communications Office team within the RDO supports matters relating to print communications, graphic design, external liaison, news, press engagements and other communications. Over the course of this year, the team has created and sustained social media channels for NCBS and inStem and also for three major

EXTRAMURAL FUNDS RECEIVED AT NCBS



research programs on the campus- the Chemical Ecology program, the Simons Centre for the Study of Living Machines and the newly launched Accelerator program for Discovery in Brain Disorders using Stem cells (ADBS). More recently, the Developmental Activities team at the RDO has started working across with campus colleagues and external individuals and organizations to identify fundraising priorities, facilitate campus funding, manage donor engagement events, communications matters and build sustainable relationships with donors.

Building on previous philanthropic support from organizations such as the Wadhwani Foundation, Simons Foundation and the Tata Trust, the campus has recently invested considerable effort into developing new links to other sources of charitable funding. Earlier this year, the campus launched a bold new initiative centred around the use of stem cell technology in research, diagnostics and therapeutics. Titled "Accelerating the application of Stem cell technology in Human Disease" or ASHD, the new program is jointly supported by the Department of Biotechnology (DBT), Government of India and the Pratiksha Trust, a charitable trust setup by Infosys co-founder Mr. Kris Gopalakrishnan and his family. In parallel, the campus has now also launched an Endowment Fund, with generous corpus support from the Infosys Foundation for the creation of student travel awards.

An Endowment Fund, in addition to other existing funding support for the campus, is now essential for the Cluster to build, develop and sustain an outstanding, internationally competitive scientific hub in India, at par with the best institutions worldwide. An Endowment fund would be invaluable for us to catalyse nascent ideas, support unanticipated expenses, further the exchange of talented research investigators to and from our campus and help us engage effectively with the broader social context we work in. This is the 25th year of NCBS and we plan to initiate the campus Endowment Fund with a corpus of 25 Crores in 2016 and will strive to add 25 Crores each year until we reach our target of 100 Crores in 2020. It is our hope that if philanthropists support our mission and value our work, the cumulative impact will be immense.

Developing the portfolio of philanthropic and other support to the campus has required sustained work from the team on all fronts, including our outreach activities. Work at the RDO is made possible by a well-knit group of dynamic and professional individuals, entrepreneurial in spirit and firmly committed to offering several key services to the campus at the boundaries of science, management and outreach. With a vibrant team, emerging opportunities on the campus and new connections on the outside, we look forward to a rewarding journey further ahead for the RDO, supporting campus research funding, communications and the Endowment Fund.

Savita Ayyar
Head, RDO

SCIENCE AND SOCIETY PROGRAMME

The Science and Society Programme at NCBS seeks to understand and engage with themes and perspectives of the intellectual foundations, human dimensions and impacts of scientific and technological development, with a particular focus on biology. Promoting public understanding of science not only in terms of scientific literacy but also framed with the larger historical, sociological and philosophical perspectives is an important need. Such an attempt we believe will contribute to bring in to sharper focus, the questions and relationships between the process and practice of science and the rest of society. We also believe that creating spaces for such dialogue will contribute to greater public engagement in making decisions about science related issues in a democratic society.

In parallel, there is a pressing need for our students to be well informed about the historical foundations of contemporary science and technology, the moral, ethical and philosophical implications of modern technologies, and the broader social, economic, cultural and political realities of the 21st century innovations in science.

In 2016, our programme organized:

- 7 public lectures on themes ranging from the neuroaesthetics to philosophy of biology
- A theatre based art/science project for the public understanding of science, which lead to a theatre production "The Vaidya's Oath" and an extensive school outreach

- An exhibition exploring the history of NCBS looking back to the early beginnings of biology research at NCBS
- A reflective session on Obaid Siddiqi
- Future of Nature – a lecture series exploring disciplinary intersections in ecology, history, politics, economics and other approaches

The programme has also initiated the exercise of setting up an institutional archive. The preliminary stages of the collection can now be accessed through a digital story curated by Venkat Srinivasan.

THE SIMONS CENTRE FOR THE STUDY OF LIVING MACHINES

NCBS and the Simons Foundation partnered in 2013 to establish the Simons Centre for the Study of Living Machines. This Centre brings together five research groups who use theoretical methods and quantitative experiments to address complex biological problems. The current Centre members and their research interests are: Shachi Gosavi, protein dynamics; Sandeep Krishna, cellular networks and decision making; Madan Rao, cellular biophysics; Mukund Thattai, computational and evolutionary cell biology; and Shashi Thutupalli, experimental studies of collective behaviour. The Centre's research is organised around two major themes: microscopic: molecules, and mechanisms; macroscopic: cells and cooperation. These themes are integrated with theoretical approaches from physics and computer science. Our activities include an active international visitors program, a competitive post-doctoral program, and an annual schedule of meetings and workshops.

Scan QR code for the website

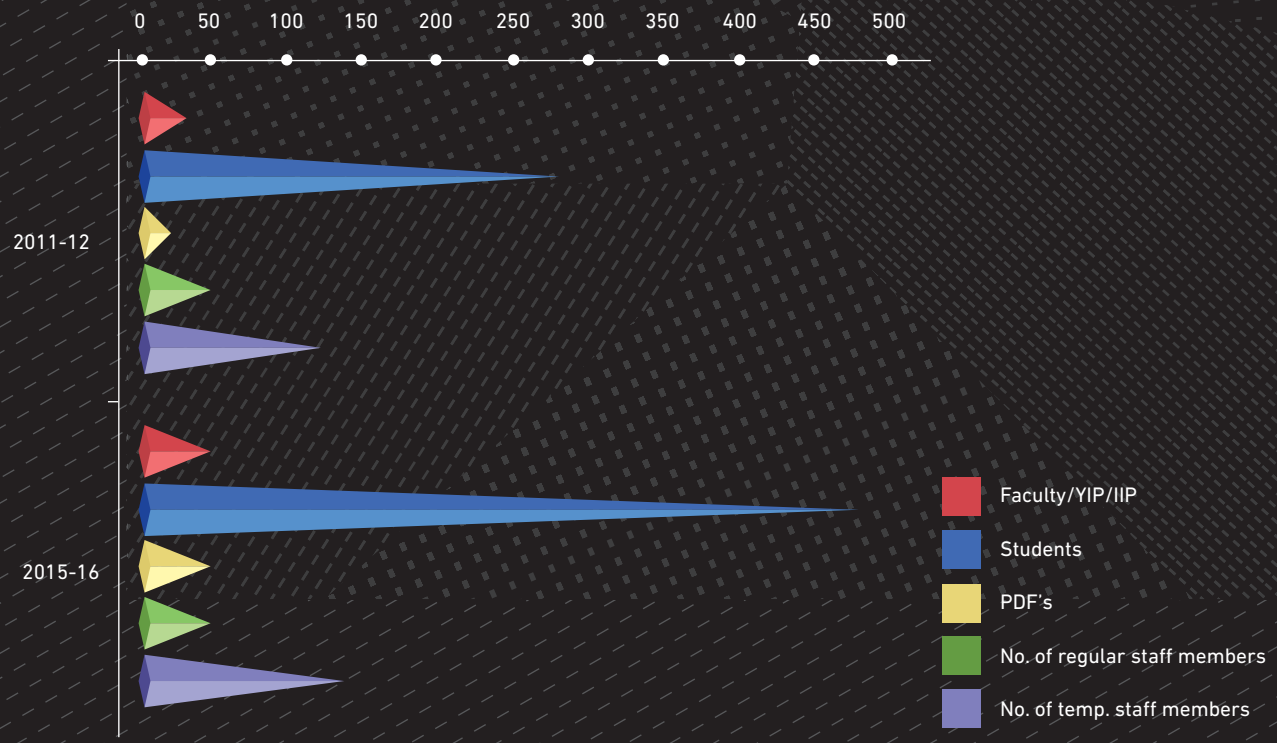
ADMINISTRATION AND FINANCE

What is loosely called 'administration' at NCBS is really the slew of services that range from engineering and architecture, laboratory maintenance, campus services and the core finance and general administration required for all of these. Efficient functioning of the Centre is the outcome of a remarkable team work. Each major area has its head, who reports to the Centre Director. This report touches on the salient aspects of areas of non-academic functions and is intended as on overview.

NCBS reached an important milestone having celebrated the Silver Jubilee of its establishment during 2016-17. The centre continues to strive to enhance its excellent reputation in research, teaching, mentoring and its international presence. It can be confidently affirmed that the curve of growth has maintained its upward trend, both in terms of quantity and quality.

Indeed, during the last few years, NCBS has grown in size and stature. The table below shows our growth, both in terms of manpower and finances, during the last five years (2011-12 to 2015-16).

MANPOWER GROWTH



FINANCIAL PROGRESS

EXPENDITURE (Rupees in Millions)			
Sl. No	**Particulars**	**2011-12**	**2015-16**
1	Research & Development	311.80	350.92
2	Extra Mural Grants	306.84	358.26
3	Salaries & Fellowships	139.57	141.33
4	Operational Expenditure	210.22	317.44
5	Construction	184.73	63.67
	Total	**1153.16**	**1231.62**

It will be seen from the figures relating to 'Financial Progress' that expenditure on Research & Development and Extra Mural Grants has shown 14.63% increase (from Rs.618.64 million to Rs.709.18 million) while the corresponding increase for Salaries & Fellowships is a meagre 1.26% (from Rs.139.57 million to Rs.141.33 million). The rate of growth of expenditure on research activities outstripping the corresponding growth on salaries & fellowships is indication both of a vibrant research environment and high level of efficiency of the administrative support services. The expenditure on construction activities has tapered off consequent to the completion and commissioning of the Southern Lab Complex.

The remarkable model of cooperation between NCBS-TIFR, Institute for Stem Cell Biology & Regenerative Medicine (inStem) and Centre for Cellular & Molecular Platforms (C-CAMP) under the institutional mechanism of Bangalore Life Sciences Cluster (BLiSc) was reported in our Annual Report of last year. This model of synergistic collaboration has had a fair degree of success for mutual benefit of the constituent institutions of the Cluster. As the Cluster evolves as a functional entity, it is essential that it aims to align the 'scientific goals' of the Cluster constituents. The oversight must focus on the larger canvas and emphasize on value creation for the scientific community of the 'Cluster'. It is important to have the 'big picture' of the Cluster in perspective at each stage.

A major development that has come into effect from April 1, 2016 is the operation of 24 identified facilities as cost centers that would manage their own allocated budget. This calls for budgetary and other financial information/data to be made available on real time basis for appropriate analysis, communication and presentation for strategic decision-making. There has to be constant oversight of the cost centers. The need for oversight emerges from the fact that the Cluster institutions which are predominantly funded by Government of India will have to necessarily adhere to the requisite financial discipline, in the context where funding has become more complex in consequence of a rigorous regulatory environment constraining allocations and release of resources.

The growth in infrastructure and facilities on our campus has posed new challenges with increased complexity of handling multiple tasks, by individuals and operational units, across institutions. The strategic way forward is to sustain the growth path with appropriate alignment of all resources, most importantly the human and financial resources. The challenge, therefore, is to devise appropriate strategies to harness the available resources, and deploy them optimally in a manner that facilitates our researchers to achieve scientific excellence.

During the coming years we are headed for a phase of acceleration of cooperative endeavors that, we hope, will create a lasting impact in the scientific world. We need to create capacity to respond to challenges along the way as we are headed on a journey committed to create value for the scientific community.

I would like to gratefully acknowledge the continuing support of Tata Institute of Fundamental Research and the Department of Atomic Energy (DAE) in our endeavors. I would like to sincerely thank all my colleagues for their support. The support of my colleagues has helped us to work as a dynamic team without barriers of hierarchy that tend to impede collective team effect that forms the foundational prerequisite for greater efficiency and qualitative output.

K P Pandian
Head, Strategy
(since 01.04.2015)

Sunil Kumar
Head Administration & Finance
(30.03.2016 to 16.09.2016)

NCBS INTERNATIONAL COLLABORATIONS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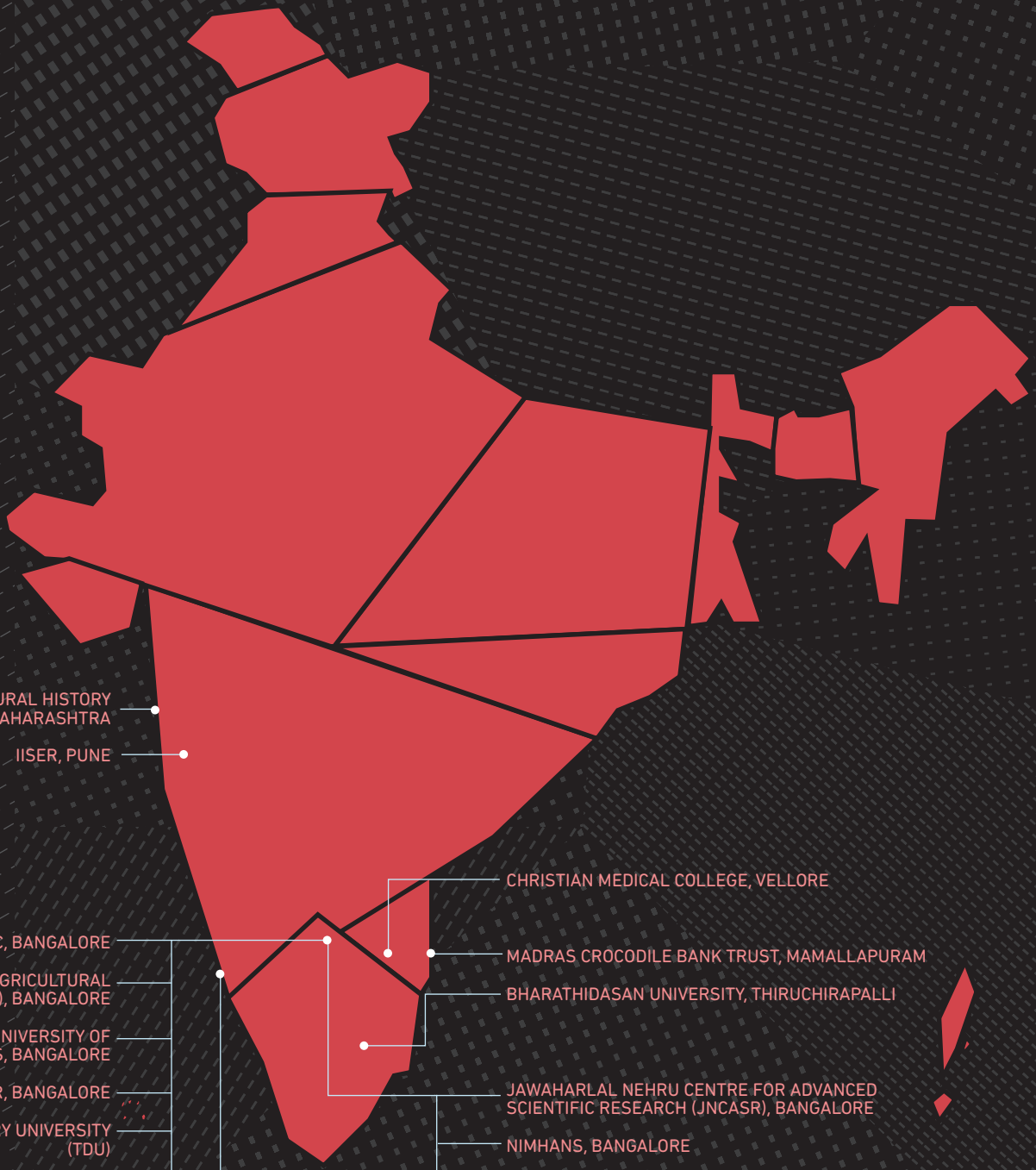

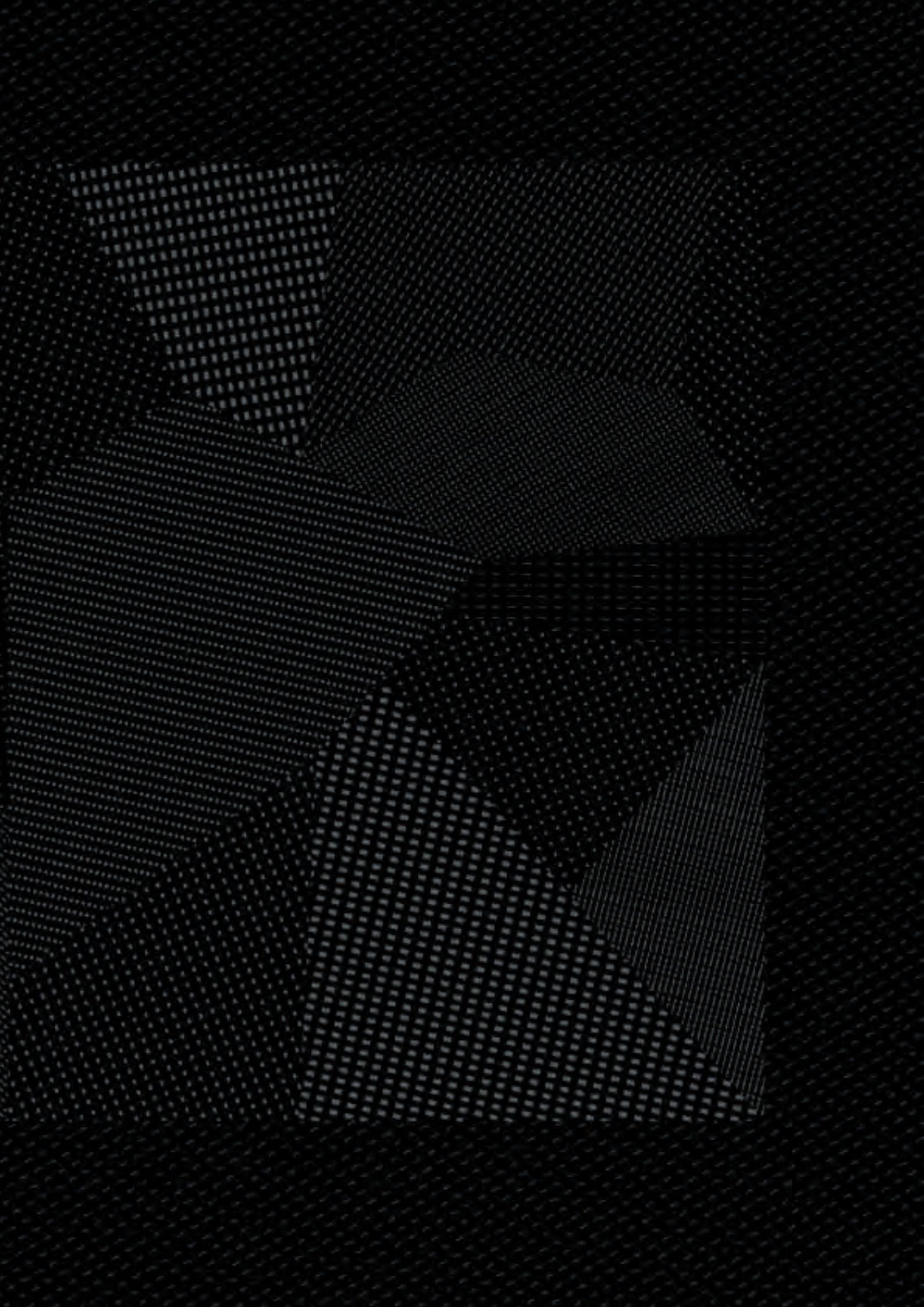

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