The Fifth Bangalore Benny Shilo Course in Developmental Biology

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National Centre for Biological Sciences, TIFR, Bangalore

Teachers

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Grant project topics

Benny Shilo (Weizmann Institute of Science, Israel)

Project grant title: Apical localization of the Diaphanous/Formin actin-polymerization machinery in tubular epithelia

The Diaphanous (Dia)/Formin protein mediates the formation of linear actin cables. It is activated by GTP-bound Rho, and operates as a homodimer which promotes the addition of actin monomers to a growing cable, in a polarized fashion. Our studies on the Dia pathway and apical secretion in the Drosophila embryo, highlighted the seminal role that preexisting polarity in the tubular cells plays in directing the components of the Dia pathway to the apical side (Massarwa et al., 2009). Since this targeting is restricted to tubular organs, and is common to all tubular organs of the embryo, we will use these features to define and study this novel apical localization machinery. The universality of the regulation of a highly conserved cellular machinery, and the similar phenotypic consequences to secretion in all tubular organs, strongly suggests that this machinery will execute the same processes in vertebrate tubular organs.

Eyal Schetjer (Weizmann, Israel)

A transient actin-based structure, commonly refereed to as an actin "focus", has been repeatedly described in association with myoblast fusion in Drosophila embryos. Although present at "the right time and place" to influence fusion, various structural aspects of the actin foci are not fully resolved, and their functional relevance to the fusion process remains unclear. Open questions include:

1. What kind of actin array does this structure represent ?
2. What is the identity of the actin-nucleating system involved ?
3. Does the focus form in both fusing cells, or is there a preference for formation on the myotube/founder or myoblast side ? Does the focus show a particular sub-cellular localization ?
4. Genetic approaches have implicated a variety of actin-associated elements in the myoblast fusion process. Which (if any) of these is connected to the actin foci, and what is the nature of this connection?
5. Most importantly- are the actin foci relevant to the fusion process ? Do they contribute to fusion- or perhaps hinder it in some fashion?

References

Gildor, B., Massarwa, R., Shilo, B. Z. and Schejter, E. D. (2009). The SCAR and WASp nucleation-promoting factors act sequentially to mediate Drosophila myoblast fusion. EMBO Rep 10, 1043-50.
Kesper, D. A., Stute, C., Buttgereit, D., Kreiskother, N., Vishnu, S., Fischbach, K. F. and Renkawitz-Pohl, R. (2007). Myoblast fusion in Drosophila melanogaster is mediated through a fusion-restricted myogenic-adhesive structure (FuRMAS). Dev Dyn 236, 404-15.
Kim, S., Shilagardi, K., Zhang, S., Hong, S. N., Sens, K. L., Bo, J., Gonzalez, G. A. and Chen, E. H. (2007). A critical function for the actin cytoskeleton in targeted exocytosis of prefusion vesicles during myoblast fusion. Dev Cell 12, 571-86.
Onel, S. F. and Renkawitz-Pohl, R. (2009). FuRMAS: triggering myoblast fusion in Drosophila. Dev Dyn 238, 1513-25.
Richardson, B. E., Beckett, K., Nowak, S. J. and Baylies, M. K. (2007). SCAR/WAVE and Arp2/3 are crucial for cytoskeletal remodeling at the site of myoblast fusion. Development 134, 4357-67.
Richardson, B., Beckett, K. and Baylies, M. (2008a). Visualizing new dimensions in Drosophila myoblast fusion. Bioessays 30, 423-31.
Richardson, B. E., Nowak, S. J. and Baylies, M. K. (2008b). Myoblast fusion in fly and vertebrates: new genes, new processes and new perspectives. Traffic 9, 1050-9.
Rochlin, K., Yu, S., Roy, S. and Baylies, M. K. (2009). Myoblast fusion: When it takes more to make one. Dev Biol.

Ze'ev Paroush (Hebrew University, Jerusalem, Israel)

Do we really understand the structure and function of developmental enhancers?

Consider the following paragraph, which opens the paper by Swanson and colleagues Enhancers, or cis-regulatory elements, are the primary determinants of spatiotemporal patterns of gene expression. In order to properly regulate their target genes, enhancers must perform a number of functions, such as identifying and communicating with the promoter, sometimes over great distances, and triggering transcription in certain cells, but not in others. Many enhancers are capable of driving a heterologous promoter in the proper pattern when removed from their normal genomic context. This autonomy implies that enhancers can assemble a complete set of biochemical activities that together are sufficient for robust, patterned transcriptional activation at a remote promoter. Do different DNA-binding factors recruit distinct types of activation activities, or must the enhancer merely accumulate enough of a single limiting activity to exceed a threshold for activation?

Andrea Brand (University of Cambridge, UK)

Discovering how stem cells are maintained in a multipotent state and how their progeny differentiate into distinct cellular fates is a key step in the therapeutic use of stem cells to repair tissues after damage or disease. Human neural stem cells have been isolated, raising the prospect of treatment for neurodegenerative disorders such as Parkinson's Disease, Huntington's Disease and spinal cord injury. In addition, recent results suggest that tumours can arise from cancer stem cells in which the normal control of self-renewing divisions is overridden. Therefore, a better understanding of the molecular mechanisms underlying the behaviour of stem cells and their progeny may also identify novel targets for cancer treatment. We are investigating the genetic networks that regulate neural stem cells in Drosophila. By identifying neural stem cell-specific genes, and genes specific for differentiating daughters, we can begin to assess the potential for redirecting post-mitotic cells to divide in a regenerative manner, or for inducing stem cells to differentiate into specific neuronal subtypes.

Stem cells can divide symmetrically to expand the stem cell pool, or asymmetrically to self-renew and generate a daughter cell destined for differentiation. Symmetrically dividing stem cells exist in the optic lobe of the brain, where they convert to asymmetrically dividing neuroblasts. By comparing the transcriptional profiles of symmetrically and asymmetrically dividing stem cells, we identified Notch as a key regulator of the switch from symmetric to asymmetric division. The balance between symmetric and asymmetric division is critical for the generation and repair of tissues, as unregulated stem cell division results in tumourous overgrowth.

During asymmetric division cell fate determinants, such as the transcription factor Prospero, are partitioned from the neural stem cell to its daughter. We showed that Prospero, acts as a binary switch between self-renewal and differentiation. By identifying Prospero's targets throughout the genome we showed that Prospero represses genes for self-renewal and activates differentiation genes. In prospero mutants, differentiating daughters revert to a stem cell-like fate: they express markers of self-renewal, continue to proliferate, fail to differentiate and generate tumours.

Neural stem cells transit through a period of quiescence at the end of embryogenesis. We showed that insulin signalling is necessary for these stem cells to exit quiescence and reinitiate cell proliferation, and have identified the cell type that secretes the insulin-like peptides that reactivate these neural stem cells in vivo.

Please see Brand lab home page: http://www.gurdon.cam.ac.uk/brand.html

References-

Egger, B., Gold, K.S. and Brand, A.H. (2010). Notch regulates the switch between symmetric and asymmetric neural stem cell division in the Drosophila optic lobe. Development, 137, 2981-2987.

Bardin, A.J., Perdigoto, C.N., Southall, T.D., Brand, A.H., and Schweisguth, F.(2010). Transcriptional control of stem cell maintenance in the Drosophila intestine. Development137, 705-714.

Monier, B., Pelissier, A., Brand, A.H. and Sanson, B. (2010). An actomyosin-based barrier inhibits cell mixing at compartmental boundaries in Drosophila embryos. Nature Cell Biology12, 60-65.

Southall, T.D and Brand, A.H. (2009). Multiple transcription factor binding identifies neural stem cell gene regulatory networks. EMBO J. 28, 3799-807.

Choksi, S.P., Southall, T., Bossing, T., Edoff, K., de Wit, E., van Steensel, B., Micklem, G. and Brand, A.H. (2006). Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. Developmental Cell 11, 775-789.

Thomas Lecuit (IBDML, Marseille, France)

Cells and tissues display remarkable robustness, i.e. the ability to keep a polarized or vectorial organization, important for their physiological role. Meanwhile epithelia show tremendous plasticity, during embryonic development and organ regeneration, i.e. the capacity to adapt to intrinsic or extrinsic signals or perturbations. This course will address the basic principles of cell and tissue organization and dynamics with a special focus on the problem of how robustness and plasticity are jointly regulated.
We want to understand how molecular machineries control cell polarity and cell shape changes and how these in turn affect tissue cohesion and plasticity. We will cover basic cell biological mechanisms, how developmental signals control these processes in space and time and how physical properties constrain their dynamics in vivo. Understanding how tissue level dynamics emerges from subcellular mechanics is the major challenge of studying morphogenesis. This requires the integration of physical and biochemical description of the processes.

Erez Raz (University of Muenster, Germany)

Germ cells, the progenitors of sperm and egg, are typically specified early in development at a specific position in the embryo and then migrate to the site where the gonad develops, the location where they give rise to the gametes. Germ cells thus serve as an excellent model for studying medically relevant issues in cell and developmental biology, such as the molecular mechanisms controlling cell fate decisions and invasive cell migration. The mechanisms ensuring proper germ-cell specification and behavior will be described, in particular the special feature of these cells that facilitates their motility. In the second lecture, the mechanisms promoting directed migration towards the gonad will be describe, focusing on the role of the chemokine SDF-1 and its receptors CXCR4 and CXCR7.

Benjamin Podbilewicz (Technion- Israel Institute of Technology, Haifa)

HOW CELLS FORM ORGANS

Fundamental basic biological questions include: how cells interact with one another and rearrange themselves to produce organs, how complex organisms develop from a single cell, and how organisms evolve.

We are using the nematode Caenorhabditis elegans to investigate cell fusion, organ formation, and nerve cell development. The wealth of anatomical, genetic, developmental, and molecular information available for C. elegans provides a multidisciplinary and powerful approach for these studies. Our work has focused on the study of one fundamental biological question: How do cells fuse? Cells fuse during fertilization and formation of organs (1-3). For example, macrophages, eye lens cells, placental cells, and muscle human cells fuse (4).

We also work on how cells migrate, change shapes, and sculpt organs and how cell fusion and organ formation evolve (5). We initially approached cell fusion by mutational analysis, obtaining many mutations in two genes that we found are critical for the cell fusion process. We identified EFF-1 and AFF-1, two membrane proteins essential and sufficient for cell fusion in C. elegans (6, 7). EFF-1 and AFF-1 are the founders of the first family of eukaryotic cell fusion proteins (fusogens). EFF-1 and AFF-1 from nematodes can fuse heterologous insect cells. EFF-1 is required in both fusing cells. Our ultimate goal is to understand the molecular and physicochemical mechanisms of cell membrane fusion. We have accomplished a complete description of the cellular events leading to the formation of an organ (8). Using genetic analyses we identify genes that function to control different cell fusion events in C. elegans and in other organisms and how this process is regulated in development. Cell-cell fusion may control the size of giant cells by preventing fusion with neighboring cells. We now focus on fertilization (sperm-egg fusion) and the development of four organs: vulva, skin, muscles and pharynx.

Pruning of neuronal trees. We recently discovered that EFF-1 is also required to sculpt complex neuronal trees required for sensing strong mechanical stimuli (pain receptors). We found that EFF-1 trims abnormal or excessive neuronal branches as a novel quality control mechanism. EFF-1 works in specific neurons by fusing excess and abnormal branches. In additional EFF-1 retracts branches (9). We have identified other genes that participate in the generation and maintenance of complex neuronal trees and we hope that our discoveries in C. elegans may help to understand and repair degenerative diseases of the nervous system, mental disorders and accidental breaking of neurons.

Evolution of organ formation. We study cellular events during vulva formation across species. We found that changes in the direction of cell divisions can result in differences in size and shape of the vulva (10, 11). We found that evolution of most vulval characters are biased and proposed that evolution of the vulva in nematodes is governed by selection and/or selection-independent constraints and not by stochastic processes (12).

References-

1. B. Podbilewicz, J. G. White, Dev Biol 161, 408 (1994).
2. B. Podbilewicz, Cell fusion WormBook, http://www.wormbook.org. (2006)
3. A. Sapir, O. Avinoam, B. Podbilewicz, L. V. Chernomordik, Dev Cell 14, 11 (2008).
4. M. Oren-Suissa, B. Podbilewicz, Developmental Dynamics 239, 1515 (2010).
5. M. Oren-Suissa, B. Podbilewicz, Trends Cell Biol 17, 537 (2007).
6. W. A. Mohler et al., Dev. Cell 2, 355 (2002).
7. A. Sapir et al., Dev Cell 12, 683 (2007).
8. R. Sharma-Kishore, J. G. White, E. Southgate, B. Podbilewicz, Development 126, 691 (1999).
9. M. Oren-Suissa, D. Hall, M. Treinin, G. Shemer, B. Podbilewicz, Science 328, 1285 (2010).
10. B. Podbilewicz, Nematology 2, 99 (2000).
11. I. Kolotuev, B. Podbilewicz, Dev Biol 313, 142 (2008).
12. K. Kiontke et al., Curr Biol 17, 1925 (2007).

Heinrich Reichert (University of Basel, Switzerland)

The brain is characterized by an enormous diversity and specificity of neural cell types that are interconnected to form highly complex neural circuits for information processing. The astonishing cellular diversity in the brain arises from neural stem cells which undergo different modes of symmetric and asymmetric divisions to self-renew as well as produce differentiated neuronal and glial cell types. Understanding the cellular and molecular mechanisms that underlie this process and give rise to the vast array of different neural cell types in the brain remains one of the most challenging problems of modern biology. A neurogenetic model system that is ideal to analyse the mechanisms by which neural stem cells generate and specify the many neural cell types of the brain is Drosophila, an organism in which powerful classical genetic, molecular genetic, genomic and transgenic technologies are available. Drosophila neural stem cells, called neuroblasts, are similar to vertebrate neural stem cells in their ability to self-renew and to produce many different types of neurons and glial cells. In the past two decades, great advances have been made in understanding the molecular mechanisms underlying initial formation, establishment of cell polarity, and regulation of asymmetric cell divisions in Drosophila neuroblasts. However, we currently have only a very poor understanding of how a limited number of neuroblasts can generate the vast number of cell types found in the brain. It is now a fundamental challenge to interconnect at the molecular level the sequence of developmental events by which the diversity of differentiated cell types that make up the adult brain derives from these neural stem cells.

Special Lecture

Amos Arieli (Weizmann Institute of Science, Israel)

IN SEARCH OF THE REAL: Some Observations on the Lack of Objectivity of Sensory Perception

ABSTRACT

We acquire most of our knowledge through observation and direct experience via the senses. Sensory information is partial; nevertheless, since we make correct assumptions about the world, we have the intuitive feeling that our sensory organs are reliable. All the time we make assumptions about the world. Sensory perception is an active and continuous examination of our early assumptions.

In all sense organs we face problems of subjective and objective aspects. We can investigate and analyze the performance of the senses to the strength of the stimuli, the ability to distinguish between similar stimuli, etc - this is the objective aspect of the senses. On the other hand, active sensing is an essential component in the development of sensory perception and its relation to behavior. Therefore, we cannot relate to sensory perception as being objective: it is the integration of information collected by the senses in a specific internal and external context, together with the brain's knowledge about the control of movement – that brings us to what we feel as the subjective response of the sensory system. One of the central aspects in the research of the senses is the investigation of causality in the reciprocal relations between the objective and subjective phenomena. In this sense, visual illusions are a powerful tool to dissociate the subjective perception from the physical world and to illuminate the inner work of the brain. In my talk I will discuss the implications of this basic approach on some necessary components for normal development of sensory perception, and on both our construction and experience of the reality in which we live.