Real-Time Imaging of Morphogenetic Movements in
*Drosophila* Using Gal4-UAS-Driven Expression of GFP
Fused to the Actin-Binding Domain of Moesin

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During metazoan development the ongoing process of
cell-fate specification channels cells to distinct fates. Once committed, cellular ensembles exhibit tightly co-
dordinated sequences of morphogenetic activities that
collectively contribute towards forming the final struc-
ture of the organism. These activities involve a wide
variety of cell behaviors: individual cells may change
shape, divide, or migrate to different areas, entire tissue
layers can spread across a surface or fold up to form a
three-dimensional structure. A better understanding of
such cellular behaviors can be obtained by simply watch-
ting them as they occur in a living, unperturbed organ-

ism. This forms the basis of the real-time imaging study
that is fast becoming a powerful technique for compre-
hending cellular dynamics.

The advent of green fluorescent protein (GFP) and its
engineered and mutagenized variants as viable cellular
markers has greatly facilitated non–invasive imaging in
different biological systems (Chalfie and Kain, 1998;
Tsien and Prasher, 1998). In *Drosophila*, GFP expressed
by fusing it downstream of promoter elements has been
used to study tissue morphology (e.g., Hazelrigg, 1998;
von Roessel and Brand, 2002). However, GFP driven by
a ubiquitously expressed promoter/enhancer is not use-
ful for imaging tissues buried deep within the organism
and use of a tissue-specific promoter/enhancer restricts
study to a particular tissue type. The Gal4-UAS system
circumvents these problems and enables fluorescence
labeling of desired tissue types by crossing UAS-GFP to
appropriate Gal4 drivers (Brand and Perrimon, 1993;
Brand et al., 1994). Despite this, when expressed alone
GFP diffuses within the cell cytoplasm and fails to out-
line cell boundaries, making its use as a reporter of cell
shape changes in live images marginal.

Here we describe the use of UAS-GMA, a chimeric con-
struct that fuses the actin binding region of *Drosophila*
moesin to the C-terminus of GFP (Bloor and Kiehart, 2001;
and see below). This UAS-GMA construct serves as an
excellent marker for tracking live cell morphology because
it binds tightly to the cortical F-actin that comprises the
majority of cellular F-actin in most cells (see below). More-
over, in our hands this marker outperforms GFP fused
directly to actin (Verkhusha et al., 1999), apparently be-
cause the signal-to-noise ratio is better: less GMA remains
free in the cytoplasm compared to globular, GFP-actin. This
empirical observation is supported by what is known about
the biochemistry of actin assembly and the interaction of
moesin with Factin. The Kd of actin-binding of vertebrate
moesin for F-actin is in the nM range (Nakamura et al.,
1999), whereas cellular pools of polymerized Factin (and,
therefore, we assume GFP-actin) constitute from 25–60%
of the total actin, depending on metazoan cell type (Pollard
et al., 2000). Because we expect actin to be in significant
molar excess over expressed GMA, significantly less than
0.01% of the GMA should be free in the cytoplasm, not
bound to Factin. In contrast, 40–75% of the GFP-actin will
be free in the cytoplasm, depending on cell type. In muscle
cells, where actin is present in very high concentrations
throughout the cytoplasm (sarcoplasm), it also serves as an
excellent marker for cell shape.

Moesin is the *Drosophila* homolog of the moesin,
ezrin and radixin (MER) group of vertebrate proteins
that function as membrane–cytoskeleton linkers (Furth-
mayr et al., 1992). The conserved N terminal "head"
domain of the MER family attaches to the membrane
proteins, while the C terminal tail binds to Factin. The
DNA sequence that encodes the C terminal 140 amino
acids of *Drosophila* moesin and includes the entire actin-
binding domain (Edwards et al., 1994; see accession NM
080343 for the complete sequence of *Drosophila* moe-
sin) was fused to the human codon bias S65T version of
GFP protein to generate the chimeric GMA construct
(accession U50963, CLONTECH Laboratories, Palo Alto,
CA). The S65T mutation speeds protein folding and in-

crease the quantum efficiency of GFP and fly codon bias is very similar to human codon bias, so the chimeric protein is expected to and does express well in fly tissues. In the chimeric GMA construct the GFP stop codon is replaced with a codon that encodes leucine (TGA to CTT). The very next codon is the naturally occurring one that encodes the leucine (CTG) that is 140 amino acids upstream of the C-terminus of Drosophila moesin (the protein junction is LYKLLQD, where LYK is from GFP, L is added in construction of the chimera, and LQD is from moesin). This chimeric construct was cloned directly into the pUAST vector using the EcoRI-NotI restriction sites, transgenics were generated by standard methods, and independent lines bearing insertions in all three chromosomes were obtained (Bloor and Kiehart, 2001). Insertion of the construct had no harmful effect on fly development or behavior even when expressed ubiquitously (unpublished data on UAS-GMA; see Kiehart et al. [2000] for ubiquitous expression of a related construct that includes the extended alpha helical region of moesin). When GMA protein was expressed in specific tissue types, by crossing the transgenics to different Gal4 drivers, both portions of the chimeric protein were found to retain their normal in vivo functions: the GFP portion yielded bright fluorescence in living cells while the moesin tail associated with actin-rich cell cytoskeleton, thereby accentuating cell shape and cell surface projections (Fig. 1). To evaluate the effect of expression of GMA on the pattern of actin in cells, we expressed the construct in a striped pattern in Drosophila embryos using paired or engrailed Gal4 as a driver. Specimens were then fixed and stained with rhodamine phalloidin. The distribution of actin in epithelial cells that were expressing GMA is indistinguishable from the distribution of actin in cells in adjacent stripes that are not expressing the construct (Fig. 2), demonstrating that this construct has no effect on the overall distribution of actin in living cells. All the transgenic lines produced indistinguishable results.

We next used UAS-GMA to visualize live cell movements and tissue interactions in living embryos and pupae. For our studies, we used a transgenic line bearing the insert in the third chromosome and concentrated on two developmental events: the formation of the somatic muscles in the embryo and the histolysis of larval muscles during pupal myogenesis.

In embryos, the regularly arrayed somatic muscles constitute the most well-studied derivative of mesoderm. Each somatic fiber is seeded by a special myoblast, the “founder” myoblast (Bate, 1993). Once the founders are specified at the correct position, subsequent events involving myoblast migration, aggregation, and fusion produce the syncytial fibers. The entire process spans from embryonic stage 12 to stage 15, which corresponds to ~7.20 h to 13 h AEL (after egg lay), respectively, at 25°C.

FIG. 1. GMA is an effective marker to follow morphogenetic movements in vivo. a: Confocal images of a 23-h APF 1151Gal4, UAS-GMA live pupa showing clusters of nerve-associated myoblasts in the dorsal abdominal hemisegments A3 and A4. GMA accentuates the shape of the myoblast clusters and reveals fine, actin-rich extensions protruding from the myoblasts (indicated by arrows). b: A 12-h APF duf-Gal4, UAS-GMA live pupa showing the three larval templates in the mesothorax. Both images were collected with a 0.8 NA, 25× multi-immersion objective on a Zeiss LSM 510 confocal microscope. Anterior is to the top and dorsal midline is to the left. A 40-μm scale bar is shown for a and b in a.
To image embryonic somatic myogenesis, we crossed UAS-GMA to *dumbfounded (duf)*-Gal4, a founder specific Gal4 driver (M. Ruiz-Gomez and M. Bate, pers. commun.; see also Ruiz-Gomez et al., 2000). *duf*-Gal4 expression is initiated in the founder cells and continues within the growing myotubes. Embryos from the above cross were aged for 7–8 h at 25°C and imaged live for 10 h using confocal microscopy at 20–22°C. Time-lapse images demonstrated the overall development of the somatic muscles. At the magnification at which we acquired the images, single founder cells were not observed. But progressively the growing myotubes appear and their transformation into fully-formed muscle fibers was recorded (Fig. 3). Particularly prominent in the images is the active movement of the growth-cone-like processes put out by muscle primordia (Fig. 4). The growing tip can be seen exploring the surface of the epidermis for its attachment site (Figs. 3d,e, 4) and subsequently forming stable attachment (Fig. 3h).

With the onset of metamorphosis, the larval muscles begin to histolyse and their remains are phagocytosed. Concurrently, the adult myoblasts, which had remained associated with imaginal discs (in thoracic segments) or with peripheral nerves (in thorax and abdomen) during the larval life, migrate out to precise spatial locations and fuse to form the new set of adult muscles (Bate, 1993). Histolysis of muscles in the thoracic and abdominal segments occurs in two distinct phases. The first phase, occurring very early during metamorphosis, involves the degeneration of most of the thoracic muscles. One set of muscles that escape histolysis in this phase are the larval internal dorsal oblique muscles (DA1, 2,3) which are...
FIG. 3. Somatic myogenesis in a developing embryo. a–h: Selected images from a time-lapse confocal sequence of somatic muscle biogenesis in a duf-Gal4, UAS-GMA embryo. Time in hours: minutes is given in the lower right, with 00.00 representing the start time of imaging. a: The age of the embryo corresponding to this image is 8.5 h AEL. At this stage, single duf-expressing founder myoblasts appear in the somatic mesoderm but is not resolvable under these conditions. b,c: With increasing accumulation of GMA, the developing myotubes become clearly visible. d,e,f: Growing tips of myotubes send out filopodial extensions as they migrate toward their insertion sites on the epidermis. Extensions projecting from two syncytial myofibers are indicated by arrowheads. h: Fully-formed set of somatic muscles in the embryo (see also Movie 1). The flies for the duf-Gal4 × UAS-GMA cross were cultured in small population cages. Embryos were collected in grape juice plates for 1 h at 25°C and further aged for 7 h. Next, embryos were dechorionated by gently teasing with forceps, immersed in a 1:1 mixture of halocarbon 27 and 700 (Halocarbon Products Corp., N. Augusta, SC) and mounted in an oxygen-permeable Teflon window chamber which allowed development to proceed. A single embryo was imaged once every 9 min for a period of 10 h using a Zeiss LSM 510 confocal microscope with 25× multi-immersion 0.8 NA Zeiss objective. For each timepoint a stack of Z-sections were taken which were reconstructed into a single 3D image. These series of images can be played back as QuickTime movies (Movie 1). Scale bar in a is 50 μm and is for all panels.

FIG. 4. Dynamic movement of filopodia in the growing myotubes. a–f: Series of successive time point images (corresponding to frames 46–51 of Movie 2) during embryonic myogenesis, showing the active movement of muscle filopodia. The growing myotubes are involved in the continuous process of extending and retracting filopodial extensions in search of correct attachment site. The white arrows in a,b,c trace one such filopodial extension which is in the process of being withdrawn. A new filopodia being sent out by an adjacent muscle fiber is indicated by arrowhead in d,e,f. Time in hours:minutes (from the start of the time-lapsed sequence) is given in the bottom right-hand side. A single embryo was imaged once every 9 min for a period of 10 h using a Zeiss LSM 510 confocal microscope with 25× multi-immersion 0.8 NA Zeiss objective. For each timepoint a stack of Z-sections were taken which were reconstructed into a single 3D image. These series of images are included in QuickTime Movie 2. Scale bar in a is 30 μm and is for all panels.

FIG. 5. Larval muscle degeneration viewed live in a metamorphosing pupa. a–i: Images from selected time points of a time-lapse imaging study of UAS-GMA in larval muscle degeneration during early metamorphosis. a: Intact larval muscles present in a 0-h APF pupa. The denticles in the pupal case (arrow) are autofluorescing. b,c,d: Progressive degeneration of the muscles in the thoracic segments. The small arrowhead follows the gradual histolysis of one dorsal muscle fiber. e: Thoracic muscle histolysis is 100% complete. Note the intact abdominal muscles (big arrowhead) whose histolysis begins at a later time period (at around 24 h APF). Surprisingly, the larval templates are not observed here. f,g,h,i: The larval templates start becoming visible. In f and i the templates are marked by white asterisks (see also Movie 3). The Mhc-GAL4 × UAS-GMA cross was maintained in normal fly food media. 0-h APF pupae were collected, glued to the membrane of the Teflon windowed chamber with the dorsal side up, and imaged once every 8 min for 11 h with a Zeiss LSM 510 confocal microscope using a 10× dry, 0.50 NA Zeiss objective. In all images anterior is to the top and dorsal side is facing up. Scale bar in a is 200 μm and is for all panels.
subsequently remodeled to function as “templates” for the dorsal longitudinal muscles (DLMs) in the mesothorax (Fernandes et al., 1991). The abdominal muscles degenerate at a later time point, beginning around 20 h APF (after puparium formation).

Using UAS-GMA, we imaged the histolysis of thoracic muscles in the metamorphosing pupa. For labeling the muscles we used the muscle myosin heavy chain (Mhc)-Gal4 driver, a homozygous viable line on the third chromosome containing a muscle mhc promoter fused to the open reading frame of Gal4 (Davis et al., 1997). Mhc-Gal4 drives expression exclusively in muscles beginning in the early first larval instar. At the beginning of metamorphosis mhc expression is shut off but both the Gal4 and the GMA proteins perdure. Imaging the pupa from 0 h APF to 11 h APF captured the progressive degradation of the thoracic proteins. Furthermore, expression of the Gal4 reporter remained intact throughout the period of imaging. The abdominal muscles remained intact throughout the period of imaging.

Real-time imaging of thoracic muscle histolysis produced a tantalizing observation. There was a brief period, between 4.5 h APF to 5 h APF, when the DLM templates were totally absent (see Fig. 5d). The templates appeared only after 5 h APF. This observation is in apparent contradiction to our present understanding of the templates. Because of the long perdurance of both mhc Gal4 and the GMA actin marker, we are convinced that this is not because the markers are absent. The unhistolysed fibers undergo dramatic morphological re-arrangements, including vacoulation and changes in shape, prior to their transformation into “templates” (Fernandes et al., 1991). There have also been suggestions of extensive cytoskeletal reorganization occurring in the very early stages in these fibers (Fernandes et al., 1991; Tiets, 1955). Our data probably hint to such reorganizations and studies to explain it are being pressed presently. A point that needs to be kept in mind is that our imaging was done at ~20–22°C. At this temperature, development of the animal is slower compared to conditions normally cited in the literature (25°C). Thus, the duration and timing of various developmental events may appear to be longer and retarded compared to “standards” established by previous studies (e.g., Fernandes et al., 1991). Nevertheless, these data establish a spatial-temporal reference frame which, aided with genetic and molecular data, should be helpful in understanding the orchestrated process of pupal muscle histolysis.

High fluorescence signal, high-affinity actin-binding activity, and innocuous behavior within the cell makes UAS-GMA an invaluable tool for live imaging of morphogenetic events over long hours and at high spatial resolution. Imaging of wild-type behavior can further form the basis for large-scale screening for mutants that are defective in these events. Additionally, the ability of GMA and the construction of new spectral variants (YMA, CMA, etc.) to illuminate actin cytoskeletal dynamics suggests important applications in studies concerning cell cytoskeletal architecture (Edwards et al., 1997; Kiehart et al., 2000; Bloor and Kiehart, 2001, 2002).

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LITERATURE CITED


