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Preparation and Use of Reporter Constructs for Imaging Morphogenesis in *Xenopus* Embryos

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To understand dynamic cell and tissue mechanics during morphogenesis requires the use of live reporters for cell architecture as well as reporters for the location and activity of proteins in cells within a multicellular tissue. Fluorescently tagged proteins are some of the most useful reporters for live-cell work. Fluorescently tagged proteins, either conjugated to small fluorochromes or molecularly engineered chimeric fluorescent proteins require methods to distribute fluorescent reporters evenly across a tissue or localize fluorescent probes within a subset of cells. An evenly distributed reporter is useful when visualizing patterns of protein localization within a field of cells. Alternatively, restricting the expression of a reporter to single cells or patches of cells mosaically can reveal patterns of subcellular localization like the planarly polarized localization of van Gogh (Strabismus; Yin et al., 2008). We will discuss methods to synthesize mRNA encoding fluorescent proteins and methods to express proteins evenly as well as in scattered populations within a multicellular tissue.

a. Synthesis

Capped mRNA encoding fluorescent reporters are transcribed according to standard methods from linearized expression plasmids (Ampliscribe, Epicentre Biotech; Madison WI). Each batch of mRNA must be tested for quality and snap frozen into small aliquots, typically 3 to 5 μ l, at twice the concentration used for microinjection, approximately 0.2 μ g/ μ l, in 0.25 ml RNase-free PCR-style centrifuge tubes. Small aliquots should be stored at -80°C and can be thawed and reused once. Volumes of mRNA delivered by microinjection are measured by injecting bubbles of mRNA into mineral oil. The diameter of the bubble is measured with a calibrated eye-piece reticle. Volumes of mRNA are measured before and after microinjection sessions since it is not uncommon for glass microneedles to break or clog during a session.

b. Evenly distributed expression

To obtain embryos evenly labeled with a fluorescent reporter requires 4 to 6 injections at the one cell stage. Embryos need to be injected between 20 and 60 minutes after fertilization to ensure diffusion of mRNA before the first cell division at 90 minutes. Total mRNA of approximately 0.1 to 1 ng can be injected into four injection sites equally spaced along the

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“equator” and single injection sites each at the animal and vegetal pole for even distribution of mRNA. Subsequent cell divisions occur at 30 minute intervals and reduce the chances of even distribution. Diffusion rates of mRNA may vary at these early stages depending on whether the RNAs encode transmembrane or cytoplasmic proteins. We suggest the use of a confocal microscope to assess distribution rather than a fluorescence equipped stereoscope since the cell-to-cell fluorescence is difficult to judge in the stereoscope.

c. Mosaic expression

To obtain scatter-labeled samples, individual blastomeres can be injected in early cleavage stage embryos. Evenly expressed membrane localized fluorescent signals can make it impossible to distinguish which cell “owns” which structure and which cells are responsible for a particular pattern of localized protein within the packed cellular tissue. *Xenopus* embryos undergo multiple synchronous rounds of cell division allowing relatively easy microinjection into targeted cells at the 2-, 4-, 8-, 16-, 32-cell stages. For instance if one desires a scatter of cells along the midline during gastrulation injecting a single blastomere near the cleavage furrow at the 2-cell stage will produce a limited number of scatter-labeled cells from a more solidly labeled lateral patch. Control over the final mRNA concentration per cell is possible at the earliest cleavage stages but becomes difficult at later stages when asymmetric cell division produces blastomeres of different sizes. The amount of mRNA injected should be adjusted for the size of the targeted blastomere to avoid exceeding toxic levels of mRNA. Mosaic expression can be targeted to specific tissues if blastomeres are properly targeted in regularly cleaving embryos (Lane and Sheets, 2005). Embryos with the mosaic expression in the desired regions need to be selected using a fluorescence-equipped stereoscope.

d. Co-expression of multiple reporters

To visualize multiple structures or proteins within a tissue in a single embryo, different mRNAs can be injected either together or at different stages. To observe two proteins from mRNA within the same cells mRNAs can be mixed prior to the injection. However, tracking the proteins within single cells within a tissue poses a challenge because of expression can overlap in adjacent labeled cells, however, one can adjust timing of injections to produce different patterns of two fluorescent proteins. Even distribution and mosaic expression can be produced in the same tissue by injecting one mRNA at the 1-cell stage for even distribution and returning at a later cleavage stage to inject the second mRNA for mosaic expression. For instance, evenly distributed membrane-localizing mRFP can help identify the “ownership” of F-actin tracked by mosaic expression of moesin-GFP.

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