Published in final edited form as:

Cold Spring Harb Protoc. ; 2013(4): 362–365. doi:10.1101/pdb.prot073874.

Microsurgical Approaches to Isolate Tissues from *Xenopus* Embryos for Imaging Morphogenesis

Hye Young Kim¹ and Lance A. Davidson^{1,2,*}

¹Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15213

²Developmental Biology, University of Pittsburgh, Pittsburgh, PA 15213

To understand dynamic cell and tissue mechanics during *Xenopus laevis* morphogenesis requires the use of live reporters of 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. Once the desired expression pattern of fluorescent reporters has been found, the next step is preparing samples of tissue explants to bring subcellular structures and protein dynamics within the range of high-numerical aperture (NA) objectives. Visualization of dynamic living structures like the cytoskeleton requires the use of high-resolution confocal microscopes outfitted with oil-immersion, high-NA objectives. These objectives, typically 40×, 63×, and 100×, have very short working distances (i.e., samples must be brought within 150 μm of the coverslip, but perform optimally only when samples are <25 μm from the coverslip). Thus, to visualize protein dynamics and follow cell behaviors during morphogenetic events, tissues must be isolated by microsurgery and cultured on the glass surface of custom-made culture chambers. To visualize multicellular tissues from *Xenopus* embryos, we microscopically dissect tissues from the desired part of the embryo using fine tools made from human hair. In this protocol, we discuss tools, microsurgery technique, and specific surgical manipulations to isolate important tissues from the gastrulating embryo.

a. General tools and approaches

The first step to isolate tissues from embryo is preparing tools for microsurgery. Sharp forceps are used to remove the vitelline membrane and hair loop and knives are used to excise tissues from the embryo (Sive et al., 2000). To reduce bacterial contamination of explants, microsurgical tools are routinely cleaned with 70% ethanol and culture media is supplemented with antibiotics. Microsurgery should be done in a media formulated to match the ionic composition of embryonic interstitial media (Danilchik's For Amy or DFA; (Sater et al., 1993)). DFA consists of: 53 mM NaCl, 5 mM Na₂CO₃, 4.5 mM Potassium Gluconate, 32 mM Sodium Gluconate, 1 mM CaCl₂, 1 mM MgSO₄, 1 gram of bovine serum albumin per liter, buffered to pH 8.3 with 1 M bicine. This media is optimal for mesodermal cells and reduces dissociation of tissue that is common with isotonic MBS or Steinberg's Solution. Providing a small “backstop”, or supporting wall with a glass fragment can aid microsurgical procedures by preventing tissue movements. After eggs have been de-jellied

* Author for correspondence: lad43@pitt.edu (phone) 412-383-5820.

the vitelline membrane must be removed manually using sharpened forceps. After microsurgery explants should be transferred to a clean dish to remove lysed cell debris and then moved to the long term culture chamber with a plastic disposable Pasteur pipette. Whole embryos from the same clutch should be transferred with the explants to help identify the “equivalent” stage of the isolated tissue. Based on the morphogenetic movement or cell behavior of interest, specific embryonic tissues can be microsurgically isolated from the desired region at the desired developmental stage. Below we introduce microsurgical procedures for isolating different types of explants to observe gastrulation movements. These explants are presented in terms of their microsurgical difficulty; animal cap explants are the easiest and “windowed” embryos the most difficult of the three presented.

b. Animal cap explant

Ubiquitous cellular movements common to many cells in the developing embryo can be observed within animal cap explants. Cells movements in animal cap explants are relatively simple and do not involve either directed migrations or polarized rearrangements found in other tissues. To isolate animal cap tissue from the early gastrula, insert a fine hair-knife into the blastocoel at initiation site (dotted line; fig 1A), then raise the knife to cut the tissue from inside out. Repeated cuts along the margin of the animal ectoderm will separate animal cap tissue from the vegetal half of the embryo. If the cuts are too far vegetal the animal cap may contain neural or mesodermal cells, these can be removed by trimming the edge of the explant to the desired size using the hair-knife in combination with the hair-loop.

c. Marginal zone explant

Mediolateral cell intercalation and mesendodermal monopolar migration behaviors during gastrulation can be observed within marginal zone explants. To isolate marginal zone tissues (shaded region; fig 1A), first identify the upper, or primary blastopore lip of the embryo, the site where bottle cells first contract, and make deep cuts perpendicular to the equator (e.g. like lines of longitude) at $+90^\circ$ and -90° from the midline with eyebrow knife. Flip the embryo to face the animal pole and make a cut connecting the two longitudinal incisions across the animal pole. Use the hair-loop and eyebrow knife to gently separate mesendoderm from the overlying ectodermal cell layers. Flip the embryo to again face the blastopore, and make an incision just vegetal to the line of bottle cells. Remove the explant from the embryo, trim large endoderm cells from the explant, and trim torn fragments from the edges of the explant. The MZ explant should be rectangular shaped and somewhat thinner in the animal half than the vegetal half.

d. “Windowed” Embryo

Stereotypical mediolateral cell intercalation behaviors in mesoderm can be viewed in the “windowed” mid- or late-gastrula stage embryo (stage 11.5 to 13) after removing a small piece of neural ectoderm from the dorsal side of embryo. To prepare a windowed embryo (fig 1B), insert a fine hair knife under the neural ectoderm parallel to the surface of the embryo and perpendicular to the anterior posterior axis above the blastopore lip (arrow; fig. 1B). Using the hair-loop to hold the embryo then lift the hair-knife to cut through the two layers of the neural ectoderm. Make two additional incisions at the boundary of the neural

plate parallel to the anterior posterior axis. Peel back the neural ectoderm from posterior to anterior until the neural ectoderm appears like the tongue of a shoe. (Note: At stages later than 12.5 it can be difficult to remove notoplate cells from the notochord, however, these cells do not interfere with observations of the presomitic mesoderm.) Once sufficient mesoderm is exposed remove, the neural ectoderm “tongue” with an incision that is perpendicular to the anterior-posterior axis. To attach mesodermal cells to the fibronectin coated coverslip at the bottom of the chamber may require a larger coverslip fragment and large dollop of silicone grease. Mesodermal cells exposed in the window remain viable within a live embryo and their behaviors can be viewed directly. Windowed embryos can develop for many days with their dorsal surface firmly attached to the culture chamber.

References

- Sater AK, Steinhardt RA, Keller R. Induction of neuronal differentiation by planar signals in *Xenopus* embryos. *Dev Dyn*. 1993; 197:268–280. [PubMed: 8292824]
- Sive, HL.; Grainger, RM.; Harland, RM. Early development of *Xenopus laevis*: A laboratory manual. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY.: 2000. p. 338

Figure 1. Explant preparation

(A) Schematic of a sagittal cross-section of an early gastrulation stage embryo (left hand panel). The animal cap explant is isolated through incisions from grey dotted line of animal side of embryo. Marginal zone explant isolated from the grey filled area along the dorsal side of embryo. The right hand panel shows individual microsurgical steps to isolate a marginal zone explant. The explant is allowed to adhere to the substrate after being held in place by a coverslip fragment bridged with silicone grease. (B) Schematic of the enface view of the prospective dorsal tissues of a mid-gastrula embryo (left hand panel). The 'windowed' embryo is prepared by removing grey area of ectoderm tissue from the whole embryo. The right hand panel illustrates microsurgical steps to make the windowed embryo. The window is placed face-down on large coverslip and the entire embryo is fixed in place by a coverslip fragment held in place with silicone grease. (1°, primary blastopore lip; AC, animal cap; ap, animal pole; bp, blastopore lip; cs, glass coverslip; g, silicone grease; me, mesendoderm; MZ, marginal zone; ve, vegetal endoderm; large endoderm cells).