

Chapter 2

Manipulating and Imaging the Early *Xenopus laevis* Embryo

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Abstract

Over the past half century, the *Xenopus laevis* embryo has become a popular model system for studying vertebrate early development at molecular, cellular, and multicellular levels. The year-round availability of easily fertilized eggs, the embryo's large size and rapid development, and the hardness of both adults and offspring against a wide range of laboratory conditions provide unmatched advantages for a variety of approaches, particularly "cutting and pasting" experiments, to explore embryogenesis. There is, however, a common perception that the *Xenopus* embryo is intractable for microscope work, due to its store of large, refractile yolk platelets and abundant cortical pigmentation. This chapter presents easily adapted protocols to surmount, and in some cases take advantage of, these optical properties to facilitate live-cell microscopic analysis of commonly used experimental manipulations of early *Xenopus* embryos.

Key words: *Xenopus laevis*, embryo, dorsal–ventral axis, cytoskeleton, time-lapse microscopy, live-cell confocal imaging.

1. Introduction

Xenopus laevis offers numerous attractions as an experimental model for early vertebrate development (1). The adults are easy to maintain in the laboratory. Unlike many amphibians, gravid *Xenopus* females can be induced by hormone injection to spawn at any time of year, and they produce several thousands of eggs at each spawning. Eggs are easily fertilized, and embryos can be cultured under non-sterile, table-top, room-temperature conditions to feeding-stage tadpoles in less than a week. With a little care, tadpoles can be reared through metamorphosis to produce froglets in 6 or 7 weeks. A well-documented normal table provides an anatomic description of the entire range of embryonic

stages from fertilization through metamorphosis (2) (*see also* <http://www.xenbase.org/anatomy/>).

This chapter describes basic methods and tools used for culturing, experimentally manipulating, and imaging early *Xenopus* embryos. Several methods are presented for live-embryo analysis of experimental manipulations, emphasizing some of the main experimental advantages of the early *Xenopus* embryo: its great size, relatively consistent cleavage pattern, and tolerance to a wide variety of experimental perturbations. These properties facilitate the microdissection and explantation of specific cells or tissue layers and make possible the introduction—via external exposure or targeted microinjection—of various reagents, including small molecules, antibodies, mRNAs, and morpholinos, to interfere with various signaling pathways important for tissue specification and body axis formation. Because tissue specification, early morphogenesis, and body axis formation are integrated within a brief developmental window, perturbations of many of the relevant pathways result in a characteristic array of axial defects that become evident by early tadpole stage (3). Thus, the impact of a perturbation—or the effectiveness of its rescue—can be determined by visual assay within 2 days.

Although the size and opacity of the *Xenopus* embryo present some unique optical challenges, the results of many experimental perturbations are easiest to analyze via direct observation using a stereomicroscope. Incipient body axes can be recognized from very early stages because of consistent regional pigmentation differences that develop shortly after fertilization. This natural marking has facilitated the development of comprehensive fate maps (4–6) as well as maps of the prospective movements of both deep and superficial tissues during gastrulation (7, 8) that make possible lineage-specific perturbations. The cytoplasm of *Xenopus* eggs is filled with yolk platelets—large (2–15 μm) membrane-bound inclusions that provide each cell with its own nutrient and energy store that can sustain isolated blastomeres and tissue explants in culture for days. Yolk platelets are highly refractile, and their collective light scattering render the cytoplasm essentially opaque. While this property—as well as the embryo's great size—makes it difficult to view the deep contents of most cells, the opacity itself makes possible direct visualization of live cells without the need for fluorochromes or vital dyes (9, 10). Autofluorescence of yolk in fixed embryos provides an excellent fluorescent-cytoplasm background for confocal analysis of embryos subjected to axis-perturbing treatments (11, 12).

Despite their opacity, early *Xenopus* cells and tissues make excellent subjects for live-cell imaging, particularly via confocal microscopy. Many of the morphogenetically dynamic events of cleavage, gastrulation, and neurulation, including membrane protrusive activity (10, 13–15); cytoskeletal rearrangements during

wound healing (16); cortical rotation (17) and cleavage furrow formation (18–20); and microtubule-dependent localization of cortical determinants (21, 22), happen to occur near the cell surface. These events are easily captured via confocal time lapse in embryos expressing GFP constructs or labeled with fluorescent lipid dyes.

2. Materials

2.1. Experimental Tools and Equipment

1. Stereomicroscope (*see Note 1*).
2. Fiber-optic illuminator (*see Note 2*).
3. Temperature control (*see Note 3*).
4. Watchmaker's forceps (e.g., Dumont #5, Fine Science Tools) (*see Note 4*).
5. X-Y-Z micromanipulator (Narishige M-152 or equivalent) on magnetic stand.
6. Borosilicate glass Pasteur pipets and latex rubber bulbs.
7. Wax: a small block of any food-grade wax, such as dental wax, Gouda cheese covering, or beeswax.
8. A few thin, straight-shafted human hairs and full-length eyebrow hairs for hair loops and eyebrow knives.
9. Alcohol lamp and lighter.
10. Diamond-tipped pencil (e.g., Ted Pella).
11. Agarose, high gelling temperature Agarose Type V (Sigma): 1% w/v in MMR/3.
12. Polystyrene dishes, 60 mm × 15 mm and 35 mm × 10 mm.
13. Nylon snap caps from 2 dram shell vials.
14. Glass coverslips, 22 mm × 22 mm #1 and 24 mm × 40 mm #1.5.
15. Glass depression slides, 3.2 mm thick (Ward's).
16. Fraction collector tube rack (Gilson "Code 1" rack).

2.2. Fertilization and Embryo Culture

1. MMR (Marc's modified Ringers): 100 mM NaCl, 2 mM KCl, 1 mM MgSO₄, 2 mM CaCl₂, and 5 mM HEPES (pH 7.4). The original recipe for this medium (23) included 0.1 mM EDTA, now usually omitted. MMR is used at full strength (1× MMR) as a tissue isotonic medium for organ (e.g., testis or oocyte) culture and in diluted form (e.g., MMR/3) as a pondwater substitute for fertilizing eggs, culturing early embryos, and rearing early tadpoles.

2. Testis solution: 10% fetal bovine serum and 0.25 $\mu\text{g}/\text{mL}$ gentamycin in $1 \times \text{MMR}$.
3. Dejelling solution: 2.5% cysteine, pH 8.0–8.5 in $\text{MMR}/3$. In a 50 mL beaker with a magnetic stir bar, dissolve 1 g of L-cysteine in 40 mL $\text{MMR}/3$. Add 6 drops of 10 N NaOH. 40 mL dejelling solution should be sufficient to dejelly as many as 5 or 6 large spawnings. Cover tightly with ParafilmTM and use within ~ 8 h. Discard any remaining cysteine solution at the end of the day, as prolonged exposure to atmospheric oxygen produces a tough-to-remove precipitate.
4. DeBoer's solution: 110 mM NaCl, 1.3 mM KCl, and 0.44 mM CaCl_2 , pH 7.2–7.4, adjusted with NaHCO_3 . Diluted to $1/20\times$ for rinsing away cysteine at the end of the dejelling procedure.
5. Blastocoel buffer (24): 53 mM NaCl, 15 mM NaHCO_3 , 1 mM CaCl_2 , 1 mM MgSO_4 , 3.6 mM Na_2CO_3 , 4.5 mM potassium gluconate, 23.4 mM sodium isethionate, 1 mg/mL bovine serum albumin, and 5 mM bicine, pH 8.3. Filter sterilize and store in 50 mL aliquots.
6. Cell dissociation medium (calcium- and magnesium-free medium (25)): 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO_3 , and 7.5 mM Tris-HCl, pH 7.6.
7. Human chorionic gonadotropin (hCG; Sigma). Lyophilized hCG is reconstituted to 4,000 U/mL with sterile water and stored at 4°C for up to 1 month.
8. Pyrex petri dishes, 60 mm \times 15 mm.
9. Stopwatch.
10. Pasteur pipets.
11. Sample pestle and matching 1.5 mL Eppendorf tube.

2.3. Experimental Manipulations

In addition to embryos, general materials, tools, and equipment listed above, specific items required for particular experimental procedures are listed below.

2.3.1. Ventralizing Embryos with UV Irradiation

1. UV lamp (UVP, Inc. Mineralite; or Cole-Parmer 254/365 nm 4 watt; or equivalent).
2. Eye protection goggles (rated for short-wave UV).

2.3.2. Dorsalizing Embryos by Exposure to LiCl

1. Lithium chloride solution for external exposure: 300 mM LiCl in $\text{MMR}/3$.
2. Lithium chloride solution for microinjection: 300 mM LiCl in water.

2.3.3. Vitelline Envelope Removal

1. Two good pairs of watchmaker's forceps.
2. Agarose-coated polystyrene dishes with melted 1 mm wells, constructed as described in **Section 3.3.3**.

2.3.4. Microinjection

1. Microinjection gas pressure delivery system (Medical Systems PL1-100 Pico-injector[®] or equivalent).
2. X-Y-Z micromanipulator (Narishige M-152 or equivalent) on magnetic stand.
3. Micropipet puller (Sutter P-97 or equivalent).
4. Pulled micropipets from ~1 mm capillary tubing (Narishige, Drummond, and Sutter all supply good-quality borosilicate glass). Pulled micropipets should taper gradually over about 1 cm length to a tip whose outer diameter is about 10 μm . Tips will be clipped off during injection calibration, so it is not important whether they are initially open or closed.
5. A good pair of watchmaker's forceps.
6. Polystyrene dish.
7. 2 cm \times 4 mm strip of glass cut from a microscope slide.
8. Vacuum grease (e.g., Dow-Corning, high vacuum).
9. Ficoll solution: Ficoll 400-L, MW 40,000 (Sigma): 6% w/v in MMR/3.
10. Stage micrometer, ruled with 10 μm intervals (Graticules Ltd. PS8, 100 \times 0.01 1 mm, or equivalent).

2.3.5. Dorsal Marginal Zone ("Keller") Explants

1. Agarose-coated polystyrene dishes with melted 1 mm wells, constructed as described in **Section 3.1**, Step 5.
2. Watchmaker's forceps.
3. Braking pipet (**Section 3.1**, Step 1).
4. Hair loop (**Section 3.1**, Step 2).
5. Eyebrow knife (**Section 3.1**, Step 3).
6. Blastocoel buffer (**Section 2.2**).
7. Pasteur pipet.
8. Depression slide.
9. Modeling clay.
10. 2 mm \times 4 mm strip cut from #1 coverslip.
11. Vacuum grease.
12. Stereomicroscope or confocal microscope (10, 26).

2.4. Time-Lapse Stereomicroscopy

1. Time-lapse image capture system (*see* **Note 5**).
2. Depression slides.

3. Coverslips.
4. Modeling clay.
5. ImageJ software (available gratis at <http://rsbweb.nih.gov/ij/>).
6. Quicktime Pro (v. 7.x.x) or equivalent authoring software (upgrade available for both Mac and PC). Note that Quicktime X (bundled with Mac OS Snow Leopard) cannot presently be upgraded to Pro to enable authoring/editing functions: reinstall v. 7.x, which can be registered and upgraded.

2.5. Whole-Mount Confocal Microscopy

2.5.1. Fixation and Staining for Microtubules

1. Formaldehyde/glutaraldehyde fixative ("FG fix") buffer: 80 mM K-PIPES, pH 6.8, 5 mM EGTA, and 1 mM MgCl₂. Avoid sodium: use KOH to adjust pH (adapted from (27)).
2. Formaldehyde (37% stock; commercial formalin).
3. Glutaraldehyde (sealed ampules of 25% stock, EM grade; Ted Pella).
4. Tris-buffered saline (1× NTBS): 155 mM NaCl, 10 mM Tris-HCl, pH 7.4, and 0.1% Nonidet P-40.
5. Phosphate-buffered saline (1× PBS): 128 mM NaCl, 2 mM KCl, 8 mM Na₂HPO₄, and 2 mM KH₂PO₄, pH 7.2.
6. Bleaching solution: 10% HOOH in 67% MeOH. Add 1 vol fresh 30% HOOH to 2 vol MeOH.
7. Borohydride solution: 100 mM NaBH₄.
8. Primary antibody working solution: 1× NTBS, 10% fetal bovine serum (FBS), 5% DMSO, and mouse anti- α -tubulin (Sigma; DM1A), diluted 1:1,000.
9. Secondary antibody working solution: 1× NTBS, 10% fetal bovine serum, 5% DMSO, and Alexa-conjugated anti-mouse IgG (Sigma), diluted 1:100 in FBS solution.
10. Rocking platform or nutator.
11. Absolute methanol.
12. Murray's Clear (benzyl benzoate:benzyl alcohol::2:1 v/v).
13. Upright or inverted confocal microscope.
14. Observation chambers, constructed as described in **Section 3.1**, Steps 6–7.

2.5.2. Fixation and Staining for Microfilaments

1. FG fix buffer. *See Section 2.5.1*, Step 1 (Adapted from (27)).
2. Formaldehyde (8% stock solution). Commercial formalin is not used for this solution; better results are obtained with freshly made paraformaldehyde. For 200 mL of stock, heat

180 mL distilled H₂O to 55°C—do not exceed 60°C! Using a fume hood, weigh 16.84 g paraformaldehyde (EMS). Add to the heated water, cover beaker with Parafilm, and stir for 10 min. Add drops of 1 M NaOH until the solution clears. Adjust pH to 7.40 with HCl. Filter through Whatman #2 filter paper. Add dH₂O to 200 mL. Store at –80°C in 50 mL tubes—25 mL per tube.

3. Glutaraldehyde (sealed ampules of 25% stock, EM grade; Ted Pella).
4. Tris-buffered saline (1× NTBS): 155 mM NaCl, 10 mM Tris–HCl, pH 7.4, and 0.1% Nonidet P-40.
5. Phalloidin working solution: NTBS containing 5% DMSO and 2 units/mL Alexa-543 phalloidin (Invitrogen). Because Alexa-phalloidin is supplied as a methanol stock, it must be dried under vacuum in a spin-vac and then reconstituted at desired concentrations in NTBS/DMSO.
6. Rocking platform or nutator.
7. Upright or inverted confocal microscope.
8. Observation chambers, constructed as described in **Section 3.1**, Steps 6–7.

2.6. Live-Cell Confocal Microscopy

2.6.1. Time Lapse of Aggregating Germ Plasm

1. Inverted confocal microscope.
2. DiOC₆(3) (3,3'-dihexyloxacarbocyanine iodide; Invitrogen or Kodak): 1 mg/mL in anhydrous ethanol, frozen at –20°C in 10 µL aliquots.
3. Coverslip-bottomed observation chamber (**Section 3.1**, Step 6).

2.6.2. Time Lapse of Cortical Vesicle Translocation at the Embryo's Equator

1. Upright confocal microscope.
2. DiOC₆(3): 1 mg/mL in anhydrous ethanol, frozen at –20°C in 10 µL aliquots.
3. Polystyrene dish.
4. Right-angle prism (Newport or Melles Griot right-angle prism, A = B = C = 5.0 mm with aluminized hypotenuse).
5. Modeling clay.

2.6.3. Imaging Filopodia in the Blastocoel of Embryos Expressing GFP-Mem

1. GFP construct mRNA (~1 µg/µL), frozen at –20°C in 3–5 µL aliquots. Protocols for transcribing and capping synthetic mRNAs from pCS2 plasmids via mMessage mMachine (Ambion) are provided in **Chapter 3**, this volume.
2. Microinjection gear, as listed in **Section 2.3.4**.
3. Watchmaker's forceps.
4. Braking pipet (**Section 3.1**, Step 1).
5. Hair loop (**Section 3.1**, Step 2).

6. Blastocoel buffer (**Section 2.2**).
7. Depression slide.
8. 22 mm × 22 mm coverslip.
9. Modeling clay.
10. Upright confocal microscope.

*2.6.4. Time Lapse of
FM1-43-Stained
Filopodia Within
Blastocoel*

1. Polystyrene petri dish with agarose bottom: 1% agarose is dissolved in calcium-free cell dissociation medium.
2. Cell dissociation medium.
3. Watchmaker's forceps.
4. Braking pipet (**Section 3.1**, Step 1).
5. Hair loop (**Section 3.1**, Step 2).
6. Blastocoel buffer (**Section 2.2**).
7. FM1-43 (Invitrogen): 40 mM in water, frozen at -20°C in 10 μL aliquots.
8. Depression slide.
9. 22 mm × 22 mm coverslip.
10. Modeling clay.
11. Upright confocal microscope.

3. Methods

3.1. Experimental Tools and Equipment

In addition to a stereomicroscope and accessory gear for illumination, temperature control, microinjection, and image capture, some handbuilt tools are needed for a wide range of experimental protocols. These items include (a) tools for pipetting, nudging, and cutting embryo or tissue explants and (b) various kinds of observation chambers to accommodate different kinds of microscopes. A collection of these easily built tools and chambers should be on hand in advance of most experiments.

1. Braking pipet. A braking pipet is a transfer pipet with a narrow aperture or constriction to permit slow-volume fluid transfer in the vicinity of easily damaged explants or devitelinated embryos. Melt a Pasteur pipet near its tip with an alcohol lamp to provide an ergonomic 20 or 30° bend (**Fig. 2.1a**). The bent tip is then remelted, drawn out with a pair of blunt forceps, and cut with a diamond pencil to produce a taper (**Fig. 2.1b**) which can be fire-polished to close down its aperture to desired size.
2. Hair loop. Prepare a tapered, bent Pasteur pipet handle as above. Heat the tip again and insert it briefly into a block of wax to melt and draw up a few microliters of molten

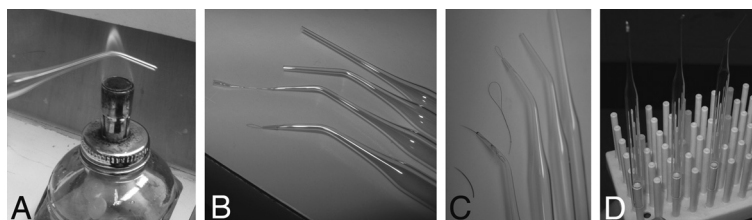


Fig. 2.1. Preparing and storing hair loops and eyebrow knives. (a) Bending the pipet shaft; (b) shaft is pulled out and cut with a diamond pencil to produce a tapered end; (c) hair loop or eyebrow hair is inserted into tapered end and immobilized with molten wax; (d) tools are stored upright on a pegged tube rack.

wax. Prepare a hair loop (**Fig. 2.1c**) by folding a hair in half and then twisting the ends between thumb and forefinger. Finally, remelt the wax and insert the twisted ends of the hair into the tapered tip and hold it in place a few seconds until the wax hardens.

3. Eyebrow knife. Prepare a tapered, bent Pasteur pipet handle as above and insert into it a long, relatively straight, uncut eyebrow hair. Leave about 3 mm of its length protruding from the end of the pipet.
4. Tool rack. Tools are stored upright on a pegged fraction-collector rack (**Fig. 2.1d**).
5. Agarose-coated culture dishes. Clean polystyrene is too sticky for culturing small tissue explants or dissociated cells. A nearly frictionless surface suitable for explant culture is generated by pouring 1 or 2 mL of molten 1% (w/v) agarose in MMR/3 into polystyrene dishes. Round-bottomed depressions of approximately 1 mm diameter are then melted into the agarose surface with the fire-polished tip of a 100 μ L glass capillary tube to provide support for explants and devitelinated embryos (**Fig. 2.2**). Agarose dishes can be stored at 4°C for up to several weeks if covered and wrapped tightly with a strip of Parafilm.
6. Observation chambers for inverted compound microscopy. An inexpensive, disposable observation chamber is quickly constructed from a nylon shell-vial snap cap and coverslip (**Fig. 2.3**). Cap is carefully cut with a fresh razor blade to produce a thin-walled cylinder. The cylinder is dipped briefly in molten wax and placed on a clean #1.5 coverslip. The coverslip is then quickly passed through an alcohol lamp flame to reheat the wax, which flows around the lip of the cylinder, sealing it to the glass. The chamber is deep enough to accommodate forceps and hair loops, so last minute manipulations of live specimens can be performed in situ. The wax seal resists Murray Clear for several hours; this disposable chamber is therefore ideal for observing cleared



Fig. 2.2. Melting rounded depressions into an agarose surface.

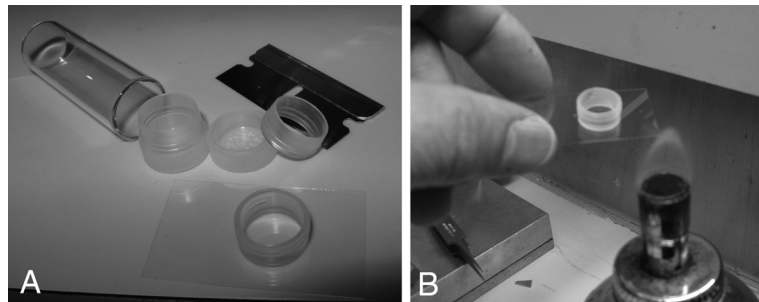


Fig. 2.3. Constructing an inverted-microscope observation chamber. (a) Nylon cap from shell vial is cut with razor. Cylinder is dipped briefly in molten wax and placed on clean coverslip. (b) Coverslip is briefly flamed to reheat wax which seals lip of cap to the glass. The wax seal is suitable for all aqueous media as well as Murray Clear.

specimens via inverted epifluorescence compound or confocal microscopy.

7. Observation chambers for upright compound microscopy. Cleared, whole-mount specimens, or cut fragments thereof, can be observed via a hanging drop method. A specimen, for example a cleared embryo half-stained via whole-mount immunocytochemistry (**Section 3.5**), is placed on a 22 mm × 22 mm coverslip, cut surface facing the glass, with a small droplet of Murray Clear surrounding the specimen. The coverslip is then inverted with the sample hanging by surface tension and placed over the well of a deep depression slide (**Fig. 2.4b**). This method works well for high-magnification, high-NA objectives because the surface tension of the drop (**Fig. 2.4c**) holds the specimen closely and stably near the optical surface of the coverslip.

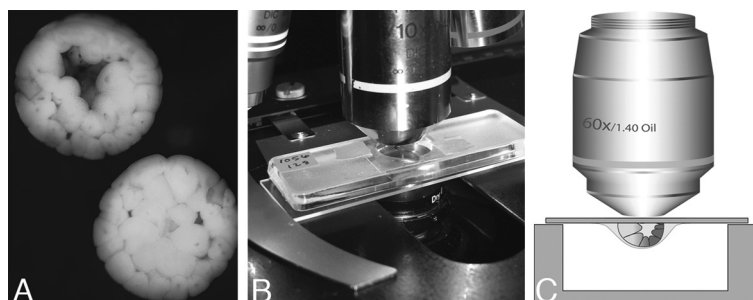


Fig. 2.4. Hanging drop method for upright confocal analysis of whole-mount specimens prepared for immunofluorescence detection. (a) Fixed embryo is cut in half with razor blade prior to staining, washing, and clearing. (b) Cleared embryo half is placed in a drop of Murray Clear on coverslip and flipped so that the cut surface is facing glass. The coverslip is inverted over a deep depression slide for confocal observation. (c) The surface tension of Murray Clear stably immobilizes specimen against the coverslip.

3.2. Fertilization and Embryo Culture

Collection of gametes, fertilization, and dejellying are described in extensive detail in **Chapter 3**, this volume. The abbreviated protocol below includes some variations on those techniques, reflecting the inherent tolerance of the *Xenopus* embryo to a wide variety of laboratory conditions.

1. Adult female *X. laevis* are induced to spawn by injecting 150 μL of 4,000 U/mL hCG solution (600 IU) into the dorsal lymph sac. Frogs are kept at 16–21°C overnight in their usual colony tank water in 1 gallon Tupperware® food containers with snap-secured lids which are perforated to allow adequate air exchange. At 16°C, spawning usually commences 12–14 h post-injection; at 21°C, spawning takes only about 8 h.
2. Spawning frogs are gently squeezed around the torso, mimicking the action of an amplexing male (*see Chapter 3*, this volume, for details on how to hold a frog). Eggs are extruded into a dry petri dish. Depending on the experiment, one may choose to collect as few as a dozen to as many as several thousand eggs for synchronous development from a single fertilization.
3. An ~ 1 mm thick fragment sliced off the end of the cultured testis (*see Chapter 3* for details of how to obtain testes) is macerated with a conical tissue grinder in a 1.5 mL Eppendorf tube containing ~ 1 mL MMR/3. This sperm suspension is immediately poured over the eggs, and a stopwatch is started to keep track of time elapsed since fertilization. The dish is gently tipped back and forth for about 30 s to ensure that all eggs are in contact with the sperm suspension. The dish is then filled with about 8 mL MMR/3 and allowed to stand for 20 min. During this time, eggs will exhibit signs of activation first by contracting the pigmented cap toward

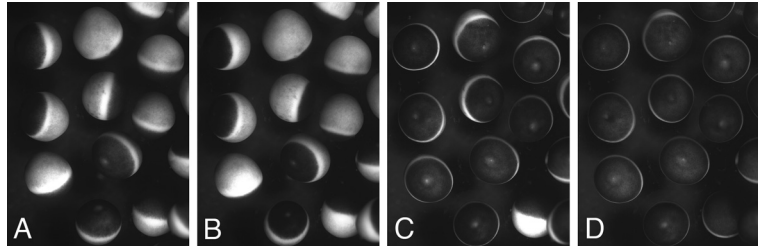


Fig. 2.5. Activation and righting of fertilized *Xenopus laevis* eggs. (a) Unfertilized eggs deposited in random orientations on substrate; (b) 8 min post-fertilization. Pigmented animal caps have contracted around their animal poles; (c) 16 min post-fertilization. Embryos have almost fully rotated within their fertilization (vitelline) envelopes; (d) 24 min post-fertilization. Animal caps have relaxed to 50% of the egg surface, and eggs have fully righted themselves.

the animal pole (Fig. 2.5a, b) and then by rotating within their vitelline envelope until the animal cap points upward (Fig. 2.5c, d).

4. Twenty minutes post-fertilization, dejelly the eggs by replacing the MMR/3 with fresh dejellying solution. Gently rock or agitate the dish for approximately 5 min until the jelly coat fully dissolves and embryos can settle closely together. Thoroughly rinse the dish of embryos with four or five exchanges of DeBoer's solution (1/20 \times) and then two exchanges of MMR/3. Embryos should be carefully inspected under the stereomicroscope at this point. Any broken, unfertilized, or abnormal eggs should be culled.
5. Embryos can be continuously cultured in MMR/3 to desired stages at temperatures ranging from 14 to 22°C (see Note 6). Because developmental rate is strictly dependent on temperature, one can use different incubation temperatures to manipulate the time at which embryos develop to given stages (see Table 2.1). For long-term culture, they should be kept at a density of fewer than 40 embryos per 10 mL dish and the MMR/3 should be replaced about twice a day. It is important to quickly remove any unfertilized, dead, or ruptured eggs to minimize bacterial infection.

3.3. Experimental Manipulations

In *Xenopus*, the point of sperm entry (SEP) normally defines the orientation of the embryo's dorsal–ventral axis (Fig. 2.6a). The fertilizing sperm contacts the egg at a random position around the animal pole, and the orientation of the dorsal–ventral axis is subsequently specified by development of an extensive array of microtubules that emanates unidirectionally across the vegetal cortex away from the eccentrically located sperm centrosome toward the prospective dorsal side (28). A 30° rotation of the

Table 2.1

Approximate time (hours) for *Xenopus laevis* embryos raised at different temperatures to reach developmental landmarks

	Stage 6	Stage 8	Stage 10	Stage 12	Stage 14
14°C	7	12	22	33	46
16°C	6	9	16	23	35
19°C	5	8	12	18	28
22°C	4	7	11	16	22
25°C ^a	3 ^a	5	8	12	17

^a25°C is too warm for the earliest cleavage stages: keep below 22°C until about stage 6.

inner cytoplasm relative to this microtubule array displaces vegetal pole determinants toward the equator on the side opposite to the SEP (22). This “vegetal cortical rotation” (29) results in the localized suppression of β -catenin degradation (30) and the initiation of a dorsoanterior-specific gene expression program (31). The pigment accumulation at the SEP normally persists through early cleavage stages, making possible the provisional identification of ventral and dorsal tiers of blastomeres for lineage-specific manipulations (**Fig. 2.6b, c**).

A classical demonstration of the dependence of dorsal–ventral axis formation on the vegetal cortical rotation is to irradiate the vegetal pole with UV light before the rotation has begun (32). This treatment results in the formation of ventralized embryos that display a characteristic spectrum of mild, moderate, or severe loss of dorsal anterior structures consistent with loss of dorsal mesodermal tissues. Ventralization produced by a treatment may

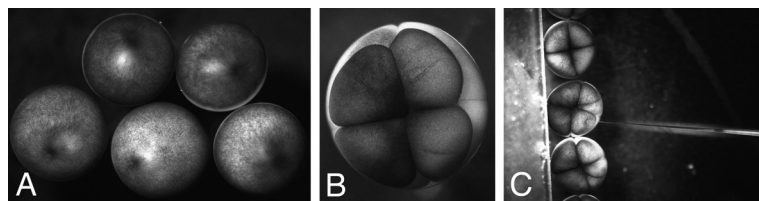


Fig. 2.6. Recognizing and using prospective dorsal–ventral pigmentation differences. (**a**) Fertilization occurs at a random location in the animal hemisphere. The sperm entry site (SEP) can be seen about 40 min after fertilization as a dark accumulation of pigment at a single site, marking ventral side of embryo. Pigmentation differences are retained during cleavage: in an eight-cell embryo (**b**), darker prospective ventral blastomeres (*left pair*) can be easily distinguished from lighter prospective dorsal (*right pair*) blastomeres. (**c**) Pigmentation differences permit identification of particular lineages during microinjection.

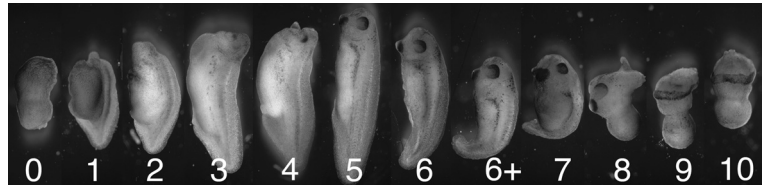


Fig. 2.7. Dorsoanterior index (DAI). Embryos 0–4 were ventralized via 55 s UV irradiation during the first cell cycle (**Section 3.3.1**). Control embryo 5 was not treated. Embryos 6–10 were dorsalized via 10 min exposure to LiCl during first cleavage (**Section 3.3.2**). At stage 36, they were arranged according to DAI criteria established by (3).

be scored using a nonparametric “dorsoanterior index” or DAI (3) (**Fig. 2.7**). Irradiated embryos can be rescued by various means, including tipping the egg 90° off-axis for the duration of the first cell cycle, using gravity to produce an internal cytoplasmic displacement similar to that produced by the normal rotation (33). The UV-irradiated embryo is highly responsive to localized injection of lithium chloride (34). Similarly, exogenous mRNAs coding for dorsalizing factors can be used to elicit nearly complete axis rescue (35); this sensitivity has served as a valuable assay for screening for new organizer genes (36).

3.3.1. Ventralizing Embryos with UV Irradiation

Ventralized embryos are easy to generate, requiring little more than a handheld UV light source (short wave, ~254 nm) and a stopwatch. Although one can go to the trouble of constructing quartz-bottomed cuvettes, we have found that it is just as effective to deposit embryos directly onto the UV lens after it has been waterproofed to prevent leakage of culture medium inside the lamp housing (*see Note 7* for waterproofing directions).

1. Fertilize and dejelly embryos as described in **Section 3.2**. In order to begin irradiation before the vegetal cortical rotation begins (approximately 30–35 min post-fertilization), the dejelling procedure should be initiated no later than 20 min post-fertilization.
2. With the UV lamp facing up and power *off*, pipet about 1 mL of MMR/3 directly onto the UV lens to form a pool about 2 mm high.
3. Pipet as many as 100 recently dejellied embryos directly into the MMR/3 pool on the UV lens. Gently nudge any embryos that are not facing animal pole into an upright orientation.
4. Remember to set aside an appropriate number of no-treatment control embryos.
5. *Caution:* don UV-protective goggles and warn nearby colleagues to avert eyes.
6. Turn the UV lamp *on* to irradiate embryos for desired number of seconds (*see Note 8* for calibration instructions), turn

it *off*, and immediately pipet the embryos to a new petri dish containing MMR/3 for culture to desired stages.

7. Severely irradiated embryos cannot hatch, since they lack dorsoanterior hatching glands (37). Their survival beyond hatching stage (stages 28–29) requires manual devitellination (**Section 3.3.3**) and frequent changes of fresh MMR/3.
8. Score embryos at stages 35–40 for dorsoanterior defects using the criteria of the DAI scale (3) (**Fig. 2.7**).

3.3.2. Dorsalizing Embryos by Exposure to LiCl

Embryos treated externally with lithium chloride (LiCl) during the first few cleavage cycles develop with a full range of phenotypes consistent with overproduction of dorsal anterior tissues. The resulting phenotypes at tadpole stage include macrocephaly, various degrees of twinning, and fully radialized dorsal structures (3) (**Fig. 2.7**). Because external treatment is only effective during the early cleavage stages, it is likely that external lithium gains access to the blastocoel along advancing cleavage furrows before tight junctions have fully sealed. Ventralized UV-irradiated embryos can be rescued for normal dorsal axial development by microinjecting single vegetal blastomeres at the 32-cell stage with LiCl (34). Similarly, secondary body axes can be generated in normal embryos via microinjecting lithium into single ventral blastomeres (38).

1. Fertilize and dejelly embryos as described in **Section 3.2**. Maintain in MMR/3 until after cleavage begins.
2. Do not forget to set aside an appropriate number of no-treatment controls in a separate dish of MMR/3.
3. Transfer cleaving embryos to a dish containing 0.3 M LiCl in MMR/3. Swirl the dish briefly to expose embryos thoroughly to the new medium.
4. Incubate in LiCl solution for 10 min. This interval may be varied to obtain different ranges of dorsalized phenotypes.
5. Transfer embryos to a fresh dish containing MMR/3. Thoroughly rinse out the LiCl via three 1 min exchanges of fresh MMR/3.
6. Culture embryos at room temperature to desired stages.
7. Perturbations will first become evident at gastrulation with the appearance of a uniformly circular blastopore lip. Depending on the severity of the phenotype, neurulation may or may not occur.
8. As with UV treatment, severely dorsalized embryos will not be able to hatch and require manual devitellination (**Section 3.3.3**) with frequent changes of fresh MMR/3.
9. Score embryos at stages 35–40 for dorsoanterior defects using the criteria of the DAI scale (3) (**Fig. 2.7**).

10. UV-ventralized embryos can also be rescued via microinjection of 0.2–1 nL of LiCl solution (**Section 3.3.4** for microinjection procedures).

3.3.3. Vitelline Envelope Removal

In addition to jelly coat layers, fertilized embryos are enclosed in a thin, transparent, extracellular coat, the vitelline envelope, which must be removed before isolating blastomeres or making explants. Also, as mentioned in **Section 3.3.1**, UV-irradiated embryos require manual devitellination since they cannot hatch. The vitelline envelope normally lifts away from the egg surface during egg activation at fertilization (*see Note 9*). Because the perivitelline fluid is hypertonic relative to pondwater (or MMR/3), the semipermeable vitelline envelope becomes turgid and adopts a nearly perfect spherical shape. Because there is very little loft between the vitelline envelope and the enclosed embryo, it is often a challenge to grasp the former without damaging the latter (*see Note 10* for two methods which can help with difficult batches or stages of embryos).

1. Fertilize and dejelly embryos as described in **Section 3.2**.
2. The plasma membranes of devitellinated embryos are very delicate and tend to stick to both glass and plastic. To avoid mechanical damage, devitellination should be done on an agarose surface, prepared by coating the bottoms of plastic petri dishes with 2 or 3 mL of molten agarose. For long-term culture, cleavage-stage embryos require mechanical support which can be provided by placing them in rounded pits of ~1 mm diameter melted into the agarose layer with the hot tip of a flamed capillary pipet (**Section 3.1**, Step 5 and **Fig. 2.2**).
3. While viewing under the stereomicroscope, grasp the vitelline envelope at the animal pole without pinching the embryo's plasma membrane, using a pair of fine, flat-tipped watchmaker's forceps (*see Note 4*). Do not let go.
4. With the other hand, use a pair of sharper, point-tipped watchmaker's forceps to grasp the pleat in the vitelline envelope formed by the first pair.
5. With a smooth, rapid movement, tear the vitelline envelope downward toward the vegetal pole. Because there is considerable hydrostatic pressure within the perivitelline space, it is important to rapidly tear a relatively large opening in the vitelline envelope: an embryo may undergo rupture if it is forced to extrude through a too small opening.

3.3.4. Microinjection

1. Fertilize and dejelly embryos as described in **Section 3.2**.
2. Prepare an injection chamber: cut a 3 or 4 mm wide strip of glass from a microscope slide. Immobilize the strip in the bottom of a clean polystyrene dish with a thin smear

Table 2.2
Calibration of microinjected volumes

Droplet diameter (μm)	volume (nL)
100	0.5
124	1.0
157	2.0
197	4.0
226	6.0
248	8.0
267	10.0

of vacuum grease. The lightly polished original edge of the slide provides a bumper against which embryos can be lined up (**Fig. 2.6c**).

3. Back-fill a microelectrode with injectate and attach it to the XYZ micromanipulator. Connect microelectrode to the pressure injection system via polyethylene tubing (Intramedic PE-100, 1.52 mm O.D., or equivalent).
4. Clip the tip of the microelectrode with watchmaker's forceps. Tip outer diameter should be about 10 μm .
5. Using a stage micrometer to measure drop size, calibrate the microinjection system to deliver pulses of desired volume of injectate (*see Table 2.2*). Useful volumes are typically in the range of 1–10 nL. With a pressure injection system, one can vary the pulse length and/or the pulse pressure to adjust to the variable tip diameter.
6. Transfer embryos of desired stage to the injection chamber.
7. It may be difficult to tip eggs off-axis to inject vegetal or marginal zone cells. If necessary, one can transfer 6% Ficoll to the injection dish. Ficoll will dehydrate the perivitelline fluid, causing the vitelline envelope to shrink a little, after which embryos can be rolled perpendicularly to expose vegetal tier cells for injecting.
8. Inject blastomeres as required.
9. Leave injected embryos in Ficoll for a few hours to minimize leakage through the small injection hole in the vitelline envelope. Transfer embryos out of Ficoll before gastrulation begins to avoid exogastrulation.

3.3.5. Dorsal Marginal Zone ("Keller") Explants

For several years Keller and colleagues have been studying explants of the dorsal marginal zone to investigate the cellular

basis of the dramatic morphogenetic movements of gastrulation (10, 26, 39, 40). Initially, these explants were viewed with low-magnification, long working distance optics, illuminated by obliquely directed white light, and filmed in black and white with 16 mm Bolex cameras. Now, a variety of refined optical and image capture techniques are available to analyze the role of protrusive activity in the control of cell polarity (14), the relationship of extracellular fibers to cell movements (10), and assessing the role of cytoskeletal components in morphogenesis (41). The procedure below describes construction of the basic Keller open-faced explant, which can be used as a starting point for various kinds of analysis, e.g., at low magnification for basic time-lapse analysis of cellular movements or with confocal microscopy and GFP-construct expression to study protrusive activities during particular morphogenetic movements under control or experimentally abrogated conditions.

1. Culture dejellied embryos in MMR/3 to stage 10. Embryos may be prepared beforehand, for example by injecting with mRNAs coding for various GFP-cytoskeletal protein or GFP-membrane tag constructs during early cleavage stages.
2. When the dorsal lip just begins to appear, transfer a few embryos, as needed, to an agarose-coated dish containing blastocoel buffer (**Fig. 2.8a**).
3. Devitellate embryos (**Fig. 2.8b**). Stage 10 embryos are difficult to devitellate without puncturing the underlying tissue. Since Keller explants are made with dorsal tissues, begin the vitelline envelope removal from the ventral side, where tissue damage will not matter.
4. Use a hair loop to flip the embryo upside down.
5. Orient the embryo so that the dorsal lip can be approached with the eyebrow knife.
6. Make two vertical cuts—one on each side of the dorsal lip—by forcing the tip of the eyebrow knife downward through the yolky vegetal cells toward the animal pole (**Fig. 2.8c, d**).
7. Use the hair loop to flip the embryo right side up (**Fig. 2.8e**).
8. Insert the eyebrow knife through the two slits up near the animal pole. Quickly flick outward so the eyebrow shaft cuts through the ectodermal layer, severing a wedge-shaped flap of dorsal equatorial tissue which is then free to fall away from the animal pole (**Fig. 2.8f**).
9. As it falls downward, this flap will peel away from the dorsal endodermal mass and expose the inner face of the dorsal

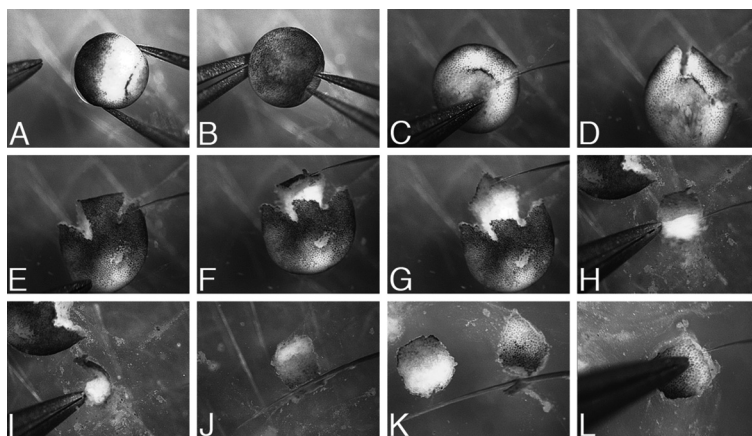


Fig. 2.8. Preparing a Keller sandwich. (a) Stage 10 embryo is placed on agarose surface; (b) embryo is devitellinated; (c) embryo is placed upside down and an eyebrow knife used to cut a slit from right edge of dorsal lip toward animal pole; (d) a similar cut is made on left edge of dorsal lip; (e) embryo is reoriented animal pole up; (f) eyebrow knife cuts dorsal flap away from animal pole; (g) dorsal flap falls away from remainder of embryo; (h) dorsal flap is cut away from remainder, using eyebrow knife; (i) dorsal tissue is turned on side to reveal large amount of yolkly vegetal tissue which must be removed by carefully carving with eyebrow knife; (j) edges of dorsal tissue fragment are trimmed to produce rectangular explant; (k) a second dorsal explant is prepared identically to the first; (l) for a double-faced sandwich, two dorsal explants are sandwiched together and held in place manually for about a minute until they begin to adhere. Entire operation requires only 4 or 5 min to complete.

marginal zone. The flap remains connected to the remainder of the embryo at its vegetal end by bottle cells of the blastopore lip (Fig. 2.8g).

10. Use the hair loop to gently push downward and gradually peel the preinvolution surface away from any already involuted head mesoderm.
11. At this point, the flap of tissue should lie relatively flat on the substrate, inner side up and still attached by bottle cells to the rest of the embryo. Use the eyebrow knife to sever this connection.
12. Trim the explant to desired shape and dimensions (Fig. 2.8i, j). See (42) for ideas and experimental rationales for subdividing the basic explant shown here.
13. Observe carefully whether any already involuted head mesodermal cells remain attached. These can be carefully flicked off one by one with the tip of the eyebrow knife. To make a double-faced explant, repeat Steps 1–13 and then gently push the two explants together (Fig. 2.8k, l).
14. To observe cellular activities in an open-faced explant, gently transfer the explant from the dissection dish, with a minimum of broken cell debris and yolk, into a depression slide

containing a drop of fresh blastocoel buffer. Orient the tissue with its inner, blastocoel surface cells facing upward.

15. Aspirate gently with a braking pipet to finish cleaning up debris along the explant's margins.
16. For time-lapse imaging of cell movements, the explant is immobilized and kept flat with a small rectangular glass bridge cut from a coverslip. A 1 mm bead of vacuum grease is placed at either end of the glass strip. Grasp the strip with watchmaker's forceps and place it atop the explant. Gradually tap the glass downward until it just makes contact with the explant.
17. Explants may be cultured in blastocoel buffer for several hours to overnight to score for extent of convergent extension or to image via low-magnification or compound microscopy.
18. Unlabeled specimens are imaged via time-lapse stereomicroscopy (*see* **Section 3.4**).
19. Specimens expressing GFP-construct mRNAs are imaged via time-lapse confocal microscopy (**Section 3.6.3**).

3.4. Time-Lapse Stereomicroscopy

Time lapse is an indispensable tool for recording and analyzing morphogenetic movements and quickly learning the results of experimental manipulations. Low-magnification time-lapse work with *Xenopus* embryos or various kinds of tissue explants is typically done at a stereomicroscope-based work station equipped with ready-at-hand gear for illumination, temperature control, micromanipulation, microinjection, and digital image capture. One example of many possible configurations is shown in **Fig. 2.9** (*see* **Notes 1–3**).

1. Place embryo or explant in appropriate observation chamber or petri dish.
2. Arrange lighting to accommodate constraints of the image capture system.
3. Calculate number of frames and frame capture rate to effectively record the event of interest (*see* **Note 11**).
4. Save captured frames to their own file folder. Use a file-name convention that includes an unambiguous numerical date-and-time stamp as part of the filename structure, e.g., "20080403_094837_a.tif".
5. Check on the progress of the movie while it is being generated: open the sequence via ImageJ ("File/Import/Image Sequence...") or Quicktime Pro ("File/Open/Image Sequence...").
6. If on-the-fly adjustments of framing, light intensity, or focus become necessary, they should be made gradually, i.e.,



Fig. 2.9. Live-embryo workstation. Stereomicroscope with digital camera mounted from side port. Microscope rests on a custom-built aluminum cooling stage which was channeled to circulate coolant from the temperature-controlled water bath located below the table. Note arrangement of fiber lights, micromanipulator, and embryo dishes on stage.

distributed over a series of five or more frames, to avoid visual discontinuities during playback.

7. For use in Powerpoint or Keynote presentations or web pages, open the movie sequence via Quicktime Pro ("File/Open/Image Sequence...") and export as a .mov movie via "File/Export/..."
8. For image analysis, open the image sequence as a .tiff stack via ImageJ ("File/Import/Image Sequence..."). Save the stack as ("File/SaveAs/Tiff...").
9. Archive the original, untouched file sequence in its own folder.

3.5. Whole-Mount Confocal Microscopy

Different cytoskeletal structures require different fixation protocols. Excellent protocols for fixing and visualizing microtubules in *Xenopus* oocytes and egg embryos have been optimized by David Gard and colleagues (*see* (27) and references within) and later adapted by others for embryo work (13, 20, 42, 43). In the sections below, two different fixation protocols are presented: one protocol for microtubules, in which specimens are optically cleared following immunostaining, and the other protocol for microfilaments, in which the specimens, while hydrated and non-transparent, still provide excellent imaging of microfilamentous structures.

3.5.1. Fixation and Staining for Microtubules

1. Dejellied embryos are cultured to desired stages and then fixed in FG fix buffer containing 3.7% formaldehyde (from commercial formalin), 0.25% glutaraldehyde, and 0.2% Triton X-100 for 2–4 h on a gently rocking nutator at room temperature (not overnight; bad for epitopes). Fixation is done in 4 mL glass shell vials with nylon snap caps, containing 1–2 mL fixative. Fix no more than 20 embryos/mL fixative. Post-fixation is overnight or longer in anhydrous MeOH at -20°C .
2. Pigment is bleached in bleaching solution. Bleaching is done under strong white-light illumination for 1 h or more and stopped when animal hemisphere pigment has faded to desired degree. Vials are laid on their side on a white fluorescent light table with a piece of aluminum foil placed over them. Use gloves to avoid contact with the peroxide solution.
3. Rehydrate embryos in PBS via three consecutive rinses for 10 min each in
50% MeOH/50% PBS.
25% MeOH/75% PBS.
100% PBS.
(possibly do devitellinating/bisecting at this point; *see Note 12*).
4. Reduce autofluorescence: Incubate embryos for 6–16 h (e.g., overnight) in freshly made borohydride solution (Caution: hydrogen gas! *see Notes 13 and 14*).
5. NTBS wash: Wash embryos via five 30 min exchanges of $1\times$ NTBS (possibly do devitellinating/bisecting at this point, *see Note 12*).
6. Primary antibody: Incubate embryos in primary antibody working solution or in FBS solution alone (no primary control). 250 μL is sufficient for 10 embryos or embryo

fragments. Use slow, gentle rocking overnight at 4°C. We sometimes leave embryos over weekend at this step.

7. Wash embryos with five 45 min exchanges with 1× NTBS. Extensive washing is necessary to reduce nonspecific staining.
8. Secondary antibody: Incubate embryos in secondary antibody working solution. Avoid unnecessary exposure to light from this point on, e.g., by wrapping vials in foil. Use slow, gentle rocking overnight at 4°C. We do *not* extend this incubation period past overnight, to avoid nonspecific background fluorescence.
9. Wash embryos with five 45 min exchanges with 1× NTBS. Again, extensive washing reduces nonspecific staining.
10. Dehydrate embryos via two consecutive rinses for 15–30 min each in anhydrous methanol.
11. Embryos are cleared via two 15–30 min changes of Murray's Clear. They are ready for confocal analysis when they have sunk to the bottom of vial and no opacity remains.

3.5.2. Fixation and Staining for Microfilaments

Xenopus oocytes (44), cleavage-stage embryos (13), blastulae (45), and gastrulae (10) are excellent targets for observing actin filament dynamics in both live cells expressing fluorescent-protein constructs (18) and fixed specimens stained with fluorochrome-coupled phalloidin. Since phalloidin does not remain bound to microfilaments during specimen dehydration, embryos must be examined while fully hydrated, and therefore opaque. Nevertheless, since most microfilaments, e.g., those of the contractile ring and filopodia, exist in the cell cortex, excellent visualization is possible via confocal microscopy.

1. Fix embryos in room temperature FG fix buffer containing 4% formaldehyde (from freshly made stock, not commercial formalin), 0.25% glutaraldehyde, and 0.2% Triton X-100. Store overnight at 4°C.
2. Rinse samples in NTBS.
3. Devitellinate manually.
4. Incubate overnight at 4°C in phalloidin working solution on a slow rocker or nutator.
5. Rinse thoroughly in NTBS.
6. For inverted microscopy, place NTBS-washed samples in coverslip-bottomed observation chambers (**Section 3.1**, Step 6). For upright microscopy, use the hanging drop method described in **Section 3.1**, Step 7.
7. Note that these hydrated specimens are opaque: staining will only be detected near the surface (*see* (13) for examples of phalloidin-stained contractile rings and apical filopodia).

3.6. Live-Cell Confocal Microscopy

Embryos are readily prepared for live-cell fluorescence microscopy by directly staining target membranes or vesicles with vital dyes, such as DiOC₆(3) (21, 45) or FM1-43 (13). Alternatively, one can express GFP or YFP constructs encoding proteins of interest by microinjecting mRNAs into specific blastomeres or the intact embryo. The following four experiments are given as examples of different live-cell approaches focused on different kinds of target regions, cells, or developmental phenomena.

3.6.1. Time Lapse of Aggregating Germ Plasm

Germ plasm is a complex aggregation of vesicles, mitochondria, intermediate filaments (46), mRNAs (47), and other exotic maternal transcripts (48). Germ plasm is found initially dispersed across the vegetal cortex in the form of hundreds of ~10 μ m islands. During the first two cell cycles, via a process related to surface contraction waves (49), these islands undergo a dramatic microtubule- and Xklp1-dependent relocalization (50) to produce a few large aggregates that will be inherited by a limited number of blastomeres committed to the germ lineage.

1. Dejellied embryos are transferred from MMR/3 to a dish containing 1 μ g/mL DiOC₆(3), made by 1:1,000 dilution of the stock DiOC₆(3) solution in MMR/3. To ensure exposure of the vegetal surface to the dye, the dish is rocked slowly (1–2 Hz) on a nutator or rocking platform.
2. After 3 min, transfer embryos to fresh MMR/3 and rinse thoroughly.
3. DiOC₆(3)-stained embryos are very light-sensitive and should be kept shaded from fluorescent room lights whenever possible.
4. To detect germ plasm on the vegetal pole, place stained embryo upright in glass-bottomed observation chamber.
5. Record motions of DiOC₆(3)-stained accumulations of germ plasm-associated mitochondria and endoplasmic reticulum via confocal microscope using FITC filters (488 nm excitation, >520 nm emission).

3.6.2. Time Lapse of Cortical Vesicle Translocations at the Embryo's Equator

Embryos quickly right themselves within their vitelline envelopes with respect to gravity shortly after fertilization (**Fig. 2.5**). They will not develop normally if this orientation is perturbed during the first few cleavage cycles. Because most microscopes are in either an upright or an inverted configuration, the equatorial region (aka the marginal zone) of live embryos is generally not available for viewing. However, use of a 45° mirrored prism (**Fig. 2.10a**) provides direct access to this region via long working distance compound microscope objectives (**Fig. 2.10b**) or stereomicroscopy (**Fig. 2.10c**).

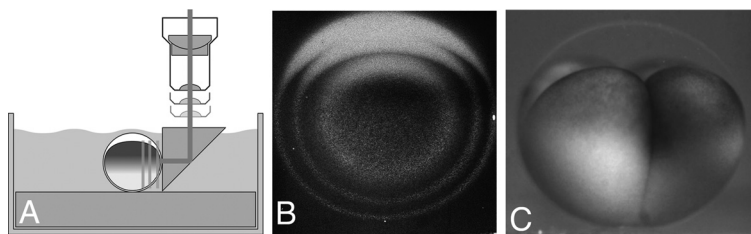


Fig. 2.10. Right-angle prism for imaging equatorial region of live embryos. Adaptable for low-magnification confocal (b) or stereomicroscopy (c). (a) Diagram shows arrangement of culture dish, embryo, prism, and long working distance objective (e.g., 4 \times , 0.3 NA). Image focus is subject to same working distance constraints as a conventional top-down arrangement, although the prism thickness may produce some spherical aberration. (b) Three superimposed confocal image planes trained on the equator of a *Xenopus* embryo stained with lipid dye DiOC₆(3). This image is a single frame from a 3-D time-lapse movie to detect motions of cortical vesicles and yolk platelets near the equator during the first cell-cycle rotation. (c) Side view of a normal living *Xenopus* embryo at second cleavage, observed through a stereomicroscope.

1. A side-viewing chamber is prepared by mounting a 5 mm right-angle aluminized hypotenuse prism on a lump of modeling clay in a polystyrene culture dish. The upper horizontal surface of the prism is not submerged in culture medium (Fig. 2.10a).
2. Stain embryos with DiOC₆(3) as in Section 3.6.1.
3. Using a hair loop, gently press embryo in upright orientation against the submerged vertical prism face.
4. Use low-magnification objective with at least 5 mm working distance on confocal microscope to observe or record through the horizontal prism surface (Fig. 2.10b).

3.6.3. Imaging Filopodia in the Blastocoel of Embryos Expressing GFP-Mem

Expression of fluorescent protein (FP) constructs has greatly advanced the analysis of cytoskeletal and membrane dynamics in early *Xenopus* embryos (10, 13, 14, 16, 18, 51, 52). One straightforward practice is to microinject synthetic mRNAs coding for FP-containing constructs into the fertilized egg. Expression of detectable levels of fluorescence generally develops within 2 h (four cleavage cycles). The example below uses an mRNA coding for a membrane-anchored GFP (GFP-mem) (14) to visualize filopodia and protrusive activity in the cleavage-stage blastocoel.

1. Microinject fertilized, dejellied eggs with 7 nL of mRNA (approximately 1 $\mu\text{g}/\mu\text{L}$) late in the first cell cycle.
2. Culture to desired stages in MMR/3.
3. Transfer embryo to a depression slide containing a drop of blastocoel buffer.
4. Devitellinate embryo, being careful to avoid puncturing any blastomeres.

5. Use a hair loop to gently immobilize the embryo. Grasp a single vegetal-tier blastomere with a pair of watchmaker's forceps and remove it.
6. Use braking pipet to very gently remove any broken cell debris, leaving a clean opening to the blastocoel (**Fig. 2.11a**). This opening will gradually sag open, affording a nearly unperturbed view of blastocoel contents.
7. Scrape each corner of a 22 mm \times 22 mm coverslip across the surface of a ball of modeling clay to build up \sim 1 mm clay feet.
8. Apply a small drop of blastocoel buffer to the coverslip, invert it, and carefully lower it directly onto the depression slide. With practice, this operation can be performed smoothly without introducing bubbles or disturbing the exposed blastomeres.
9. Press down each corner of the coverslip to bring it into close proximity to the opening between blastomeres.
10. Focus a high-magnification objective of the confocal microscope on the contacting surfaces of two blastomeres.

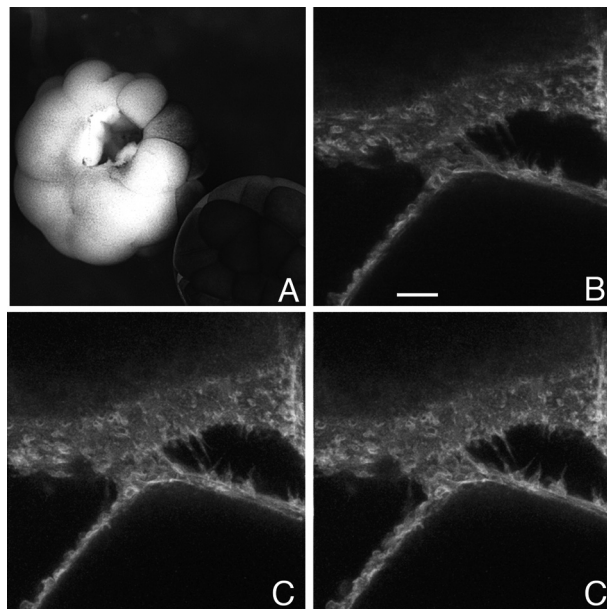


Fig. 2.11. (a) Live 32-cell embryo expressing eGFP-mem with single blastomere removed to permit contents of blastocoel to be imaged via 4-D confocal microscopy. Eleven confocal images were captured at 2- μ intervals in a region where filopodia extend between two blastomeres that are coming into contact. Image stack of a single time point was projected to produce a pair of images with $\pm 7^\circ$ of virtual rotation about the Y-axis. Confocal projections are presented as a red-blue anaglyph which may be viewed in color in the online version of this chapter (b) and as a stereo pair (the two panels may be viewed in stereo by fusing the two C's via either crossed or diverged eyes). Bar = 10 μ m.

11. Use manual focus and stage manipulation to locate a region of interest.
12. Record a preliminary “xyzt” sequence by specifying the capture of four or five optical slices 1.5 μm apart, repeating every 20 s, for 30 time points.
13. Modify the optical depth, step size, and time point interval as needed to capture events of interest.
14. Image stacks can be projected as stereo pairs using commercial software or ImageJ to produce red-blue anaglyphs (**Fig. 2.11b**) or side-by-side pairs (**Fig. 2.11c**).

*3.6.4. Time Lapse of
FM1-43-Stained
Filopodia of Dissociated
Blastomeres*

Early embryos display a lot of membrane protrusive activity along their basolateral (blastocoel-facing) surfaces that appears to be related to adhesion and shaping of the blastocoel. The water-soluble styryl dye FM1-43 becomes fluorescent when associated with plasma membranes and can be used to visualize membrane protrusive activity in living embryos. To record protrusive activity, blastomeres are first dissociated in low-calcium medium and then allowed to reassociate in the presence of FM1-43. The resulting fluorescence is sufficiently bright to enable 3-D time-lapse confocal imaging. The procedure below outlines a demonstration of membrane protrusive activity on the basolateral surfaces of 32-cell blastomeres that are reestablishing cell–cell contacts following low-calcium cell dissociation. Results and figures from a similar experiment can be found in (13).

1. Embryos undergoing first or second cleavage are placed in agarose-bottomed dish containing cell dissociation buffer and manually devitellinated, with care taken not to puncture any blastomeres.
2. At 10 min intervals over the next three cleavage cycles (about 90 min at room temperature), tip the dish back and forth gently a few times to gradually dissociate blastomeres from each other. Do not use a nutator, since regular agitation may cause incompletely-cleaved cells to drift apart too rapidly.
3. Use a braking pipet to gently clean up any broken-cell debris.
4. Prepare a depression slide by placing a 400 μL drop of blastocoel buffer containing 10 μM FM1-43.
5. Use a braking pipet to gently transfer (one at a time) two unbroken blastomeres to the culture dish.
6. Under the stereomicroscope, gently nudge the two cells together such that basolateral surfaces are in close contact.
7. Follow Steps 8–13 in **Section 3.6.3**.

4. Notes

1. High-quality stereomicroscopes suitable for work with *Xenopus* embryos are available from major optical suppliers (Leica, Nikon, Olympus, Zeiss, etc., see **Fig. 2.9** for an example setup.) A stereoscope should be capable of continuous zoom from about $5\times$ to at least $60\times$ and be equipped with a beamsplitter, a camera port (or a trinocular head), and C-mount adapter to accommodate image recording. Most modern systems are modular and can be customized to include not only a digital imaging system but also epi-fluorescence illumination and even motorized focus.
2. Because most tissues of *Xenopus* embryos are opaque, sub-stage illumination is useless and so may be omitted from the purchase of a new modular stereomicroscope system. Overhead illumination is generally provided by fiber-optic light systems. A variety of fiber-optic illuminators using halogen bulbs or LEDs are available from standard scientific equipment vendors. Flexible, dual-arm, focusable fiber-optic systems may be preferred over annular illuminators mounted on the objective (“ring lights”), because *Xenopus* tissues change quite drastically in their coloration (pigment distribution) during early development: different intensities or directions of illumination may be desired for adequate imaging. On the other hand, ring illuminators are sometimes useful to eliminate shadows that may be bothersome at particular stages.
3. For laboratory setups in rooms without adequate air-temperature regulation, seasonal fluctuations can be accommodated with a custom-built, water-cooled aluminum base (**Fig. 2.9**). Temperature of the base is maintained by a cooled, circulating water bath. For overnight culture at regulated temperatures, upright laboratory incubators are useful.
4. Although there is wide preference for forceps tips that are sharpened to fine points (e.g., Dumont’s “Biologic” tip), sharp tips are easily bent and can inadvertently puncture a *Xenopus* egg’s vitelline envelope before a firm grasp has been made. New users may find the more flattened (Dumont’s “Standard”) forceps tip to be just as effective as the pointed tip and better able to resist accidental bending. For some demanding operations, such as defolliculating early vitellogenic oocytes, forceps can be tuned by bending tips toward each other slightly for “tips first” grasping, but for most applications, the shanks and tips should

close flatly, nearly simultaneously, along their entire length. Sharpening of dulled or bent forceps is relatively easy to accomplish with a fine Arkansas stone or jeweler's-grade crocus (rouge) cloth. Progress should be followed under a stereomicroscope, with care taken that tips will meet precisely at their ends, simultaneous with contact along the shanks.

5. At a minimum, a digital camera system should be capable of capturing and displaying 8-bit, RGB, megapixel-range images both as still frames and as sequences of frames for time-lapse work. Although SLR and other handheld cameras can be used for capturing single frames at high resolution, time-lapse work typically requires a computer to store and play back movie files and requires software control to specify frame capture rate. Choose a digital capture system that saves frames as individual files. One then has random access to the image sequence while it is being generated, making possible minor on-the-fly adjustments or the decision to scrub a session altogether if something does not look right.
6. Later stage *X. laevis* embryos develop comfortably within a broad temperature range (~ 14 – 25°C). However, the earliest cleavage stages should be kept cooler than 22°C .
7. Handheld UV lights should be waterproofed by building up an ~ 2 mm wall of dental wax or silicone rubber cement along the joint between the UV filter and instrument housing.
8. The appropriate dose of UV is determined empirically by irradiating groups of ~ 25 embryos as in **Section 3.3.1** for durations varying by 5 or 10 s increments from 30 to 90 s and scoring for DAI (3) when the embryos have reached stages 35–40. A useful target dose will yield highly ventralized embryos (DAIs of 0–2; **Fig. 2.7**) that are not moribund and capable of responding to rescue, e.g., via LiCl or *Siamois* injected into vegetal blastomeres (34, 35).
9. The vitelline envelope undergoes progressive crosslinking and gradually hardens over an ~ 30 min period following fertilization (53). Thus, when embryos are dejellied after 30 min post-fertilization, the fully hardened vitelline envelope retains its original diameter, just slightly greater than that of the embryo itself. However, vitelline envelope hardening can be blocked by earlier dejelling treatment: when embryos are dejellied within ~ 5 min post-fertilization, the vitelline envelope remains distensible and swells greatly, resulting in a flattened embryo (*see* Supplemental Figure S1 in (13)). This flattening forces normally horizontal cleavage

planes (e.g., 3rd and 5th) to a more vertical orientation, but usually has no impact on later development.

10. It is relatively easy to remove the vitelline envelope before first cleavage. However, the amount of loft between it and the embryo steadily diminishes with each cleavage cycle, making removal progressively more difficult. Two methods make the vitelline envelope easier to remove, particularly at later stages. One method is to dejelly eggs within about 10 min after fertilization: cysteine blocks the hardening of the vitelline envelope which normally takes about 30 min to complete. The softened vitelline envelopes of early dejellied embryos expand greatly and are easier to remove mechanically than fully hardened ones. A second method is to place embryos in 6% Ficoll solution which, by dehydrating the perivitelline fluid, causes the vitelline envelope to deflate slightly, providing a deformable, wrinkly surface, easier to grasp with forceps.
11. To effectively compress a developmental event, one should know in advance something about its duration. Some events (e.g., fertilization waves), occur in only minutes, while others (e.g., neurulation) take several hours. Whatever an event's realtime duration, its playback for presentation purposes (e.g., Powerpoint, Keynote) should last only 15 s or so. Assuming one uses a standard 24 frame-per-second (fps) playback, then the number of frames generally needed to record is $24 \times 15 = 360$ frames. As an example, to record a single 30 min cleavage cycle in 360 frames, one would set the realtime capture rate to 12 frames per minute ($360 \text{ frames}/30 \text{ min} = 12 \text{ fpm}$). In contrast, the 6 h of neurulation would be recorded at a capture rate of $360 \text{ frames}/360 \text{ min} = 1 \text{ fpm}$. For motion analysis work, e.g., of morphogenetic movements, lamellipodial motion, or exocytotic events, the frame capture rate should be increased as needed.
12. At some point vitelline envelopes need to be removed, and desired surfaces exposed for enhancing antibody penetration. In principle, this could occur at any step following fixation, but the earlier it is done, the more beat up the cut surfaces become. The borohydride treatment is particularly damaging to cut surfaces. On the other hand, devitellination and cutting happen to be particularly easy while embryos are still in 50% methanol; they are somewhat more brittle.

Devitellination can be done manually at some stages, embryo by embryo, using watchmaker's forceps, as described in **Section 3.3.3**. However, if the vitelline envelopes are very closely applied to the embryo surface,

as happens from about stage 8 onward, this may be time consuming, tedious, and potentially damaging to the specimen. An alternative approach is to cut a Pasteur pipet to a diameter just able to accommodate the fixed embryo itself. Do not fire-polish the pipet; the sharp edge will actually be helpful here. Simply pipet up and down (somewhat violently) a few times, and the embryos will be freed of their vitelline envelopes.

Bisection: Transfer devitellinated embryos into a plastic Falcon dish in current medium (you are either at Step 3 or Step 5 of the immunostaining procedure of **Section 3.5.1**). Grasp a small, triangular-shaped razor fragment (broken from a NEW razor blade with pliers; wear goggles while breaking razor), and *slice*—do not push—in desired plane. Straight cuts will generally result if one begins the cut on the vegetal surface. Tissue should pop cleanly into two fragments. Sometimes it is helpful to crack along older cleavage planes. Some cleavage stages, such as eight-cell embryos, are particularly difficult to cut except along established cleavage planes.

13. Since borohydride emits hydrogen gas, the solution will bubble vigorously at first (avoid nearby flame or spark!). Vial caps should be punctured with a needle to prevent them from popping off. Embryos will tumble and float to surface at first. Vials may be shaken occasionally to ensure that embryos stay submerged.
14. Expanding H₂ bubbles in the blastocoel sometimes blow large holes in stages 8–11 embryos. Two possible solutions: (a) use the borohydride solution after its effervescence has subsided somewhat; (b) bisect embryos to open up the blastocoel prior to this step.

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References

1. Wallingford, J. B., Liu, K. J., and Zheng, Y. (2010) *Xenopus*. *Curr. Biol.* **20**, R263–R264.
2. Nieuwkoop, P. D. and Faber, J. (1994) *Normal Table of *Xenopus laevis* (Daudin): A Systematical and Chronological Survey of the Development from the Fertilized Egg Till the End of Metamorphosis*. Garland Pub, New York, NY.
3. Kao, K. R. and Elinson, R. P. (1988) The Entire Mesodermal Mantle Behaves as Spemann's Organizer in Dorsoanterior

- Enhanced *Xenopus laevis* Embryos. *Dev. Biol.* **127**, 64–77.
4. Moody, S. A. (1987) Fates of the Blastomeres of the 16-Cell Stage *Xenopus* Embryo. *Dev. Biol.* **119**, 560–578.
5. Moody, S. A. (1987) Fates of the Blastomeres of the 32-Cell Stage *Xenopus* Embryo. *Dev. Biol.* **122**, 300–319.
6. Dale, L. and Slack, J. M. W. (1987) Fate Map for the 32-Cell Stage of *Xenopus laevis*. *Development* **99**, 527–551.
7. Keller, R. E. (1975) Vital Dye Mapping of the Gastrula and Neurula of *Xenopus laevis*. I. Prospective Areas and Morphogenetic Movements of the Superficial Layer. *Dev. Biol.* **42**, 222–241.
8. Keller, R. E. (1976) Vital Dye Mapping of the Gastrula and Neurula of *Xenopus laevis*. II. Prospective Areas and Morphogenetic Movements of the Deep Layer. *Dev. Biol.* **51**, 118–137.
9. Keller, R. and Danilchik, M. V. (1988) Regional Expression, Pattern and Timing of Convergence and Extension During Gastrulation of *Xenopus laevis*. *Development* **103**, 193–209.
10. Davidson, L. A., Dzamba, B. D., Keller, R., and Desimone, D. W. (2008) Live Imaging of Cell Protrusive Activity, and Extracellular Matrix Assembly and Remodeling During Morphogenesis in the Frog, *Xenopus laevis*. *Dev. Dyn.* **237**, 2684–2692.
11. Wallingford, J. B., Sater, A. K., Uzman, J. A., and Danilchik, M. V. (1997) Inhibition of Morphogenetic Movement During *Xenopus* Gastrulation by Injected Sulfatase: Implications for Anteroposterior and Dorsoventral Axis Formation. *Dev. Biol.* **187**, 224–235.
12. Fredieu, J. R., Cui, Y., Maier, D., Danilchik, M. V., and Christian, J. L. (1997) Xwnt-8 and Lithium Can Act Upon Either Dorsal Mesodermal or Neurectodermal Cells to Cause a Loss of Forebrain in *Xenopus* Embryos. *Dev. Biol.* **186**, 100–114.
13. Danilchik, M. V. and Brown, E. E. (2008) Membrane Dynamics of Cleavage Furrow Closure in *Xenopus laevis*. *Dev. Dyn.* **237**, 565–579.
14. Wallingford, J. B., Rowning, B. A., Vogeli, K. M., Rothbacher, U., Fraser, S. E., and Harland, R. M. (2000) Dishevelled Controls Cell Polarity During *Xenopus* Gastrulation. *Nature* **405**, 81–85.
15. Park, T. J., Gray, R. S., Sato, A., Habas, R., and Wallingford, J. B. (2005) Subcellular Localization and Signaling Properties of Dishevelled in Developing Vertebrate Embryos. *Curr. Biol.* **15**, 1039–1044.
16. Bement, W. M., Sokac, A. M., and Mandato, C. A. (2003) Four-Dimensional Imaging of Cytoskeletal Dynamics in *Xenopus* Oocytes and Eggs. *Differentiation* **71**, 518–527.
17. Houlston, E. and Elinson, R. P. (1991) Patterns of Microtubule Polymerization Relating to Cortical Rotation in *Xenopus laevis* Eggs. *Development* **112**, 107–117.
18. Burkel, B. M., Dassow, G. V., and Bement, W. M. (2007) Versatile Fluorescent Probes for Actin Filaments Based on the Actin-Binding Domain of Utrophin. *Cell Motil. Cytoskeleton* **64**, 822–832.
19. Noguchi, T. and Mabuchi, I. (2001) Reorganization of Actin Cytoskeleton at the Growing End of the Cleavage Furrow of *Xenopus* Egg During Cytokinesis. *J. Cell. Sci.* **114**, 401–412.
20. Danilchik, M. V., Funk, W. C., Brown, E. E., and Larkin, K. (1998) Requirement for Microtubules in New Membrane Formation During Cytokinesis of *Xenopus* Embryos. *Dev. Biol.* **194**, 47–60.
21. Savage, R. M. and Danilchik, M. V. (1993) Dynamics of Germ Plasm Localization and Its Inhibition by Ultraviolet Irradiation in Early Cleavage *Xenopus* Embryos. *Dev. Biol.* **157**, 371–382.
22. Weaver, C. and Kimelman, D. (2004) Move It or Lose It: Axis Specification in *Xenopus*. *Development* **131**, 3491–3499.
23. Ubbels, G. A., Hara, K., Koster, C. H., and Kirschner, M. W. (1983) Evidence for a Functional Role of the Cytoskeleton in Determination of the Dorsoventral Axis in *Xenopus laevis* Eggs. *J. Embryol. Exp. Morphol.* **77**, 15–37.
24. Keller, R. E., Danilchik, M. V., Gimlich, R., and Shih, J. (1985) The Function and Mechanism of Convergent Extension During Gastrulation of *Xenopus laevis*. *J. Embryol. Exp. Morphol.* **89**, 185–209.
25. Sargent, T. D., Jamrich, M., and Dawid, I. B. (1986) Cell Interactions and the Control of Gene Activity During Early Development of *Xenopus laevis*. *Dev. Biol.* **114**, 238–246.
26. Doniach, T., Phillips, C. R., and Gerhart, J. C. (1992) Planar Induction of Anteroposterior Pattern in the Developing Central Nervous System of *Xenopus laevis*. *Science* **257**, 542–545.
27. Becker, B. E. and Gard, D. L. (2006) Visualization of the Cytoskeleton in *Xenopus* Oocytes and Eggs by Confocal Immunofluorescence Microscopy. *Methods Mol. Biol.* **322**, 69–86.
28. Elinson, R. P. and Rowning, B. (1988) A Transient Array of Parallel Microtubules in Frog Eggs: Potential Tracks for a

- Cytoplasmic Rotation That Specifies the Dorso-Ventral Axis. *Dev. Biol.* **128**, 185–197.
29. Gerhart, J. C., Vincent, J. P., Scharf, S. R., Black, S. D., Gimlich, R. L., and Danilchik, M. V. (1984) Localization and Induction in Early Development of *Xenopus*. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **307**, 319–330.
 30. Miller, J. R., Rowning, B. A., Larabell, C. A., Yang-Snyder, J. A., Bates, R. L., and Moon, R. T. (1999) Establishment of the Dorsal-Ventral Axis in *Xenopus* Embryos Coincides with the Dorsal Enrichment of Dishevelled That Is Dependent on Cortical Rotation. *J. Cell Biol.* **146**, 427–437.
 31. Rowning, B. A., Wells, J., Wu, M., Gerhart, J. C., Moon, R. T., and Larabell, C. A. (1997) Microtubule-Mediated Transport of Organelles and Localization of β -Catenin to the Future Dorsal Side of *Xenopus* Eggs. *Proc. Natl. Acad. Sci. USA* **94**, 1224–1229.
 32. Scharf, S. R. and Gerhart, J. C. (1983) Axis Determination in Eggs of *Xenopus laevis*: A Critical Period Before First Cleavage, Identified by the Common Effects of Cold, Pressure and Ultraviolet Irradiation. *Dev. Biol.* **99**, 75–87.
 33. Black, S. D. and Gerhart, J. C. (1986) High-Frequency Twinning of *Xenopus laevis* Embryos From Eggs Centrifuged Before First Cleavage. *Dev. Biol.* **116**, 228–240.
 34. Kao, K. R., Masui, Y., and Elinson, R. P. (1986) Lithium-Induced Respecification of Pattern in *Xenopus laevis* Embryos. *Nature* **322**, 371–373.
 35. Sokol, S., Christian, J. L., Moon, R. T., and Melton, D. A. (1991) Injected Wnt RNA Induces a Complete Body Axis in *Xenopus* Embryos. *Cell* **67**, 741–752.
 36. Smith, W. C. and Harland, R. M. (1992) Expression Cloning of Noggin, a New Dorsalizing Factor Localized to the Spemann Organizer in *Xenopus* Embryos. *Cell* **70**, 829–840.
 37. Drysdale, T. A. and Elinson, R. P. (1991) Development of the *Xenopus laevis* Hatching Gland and Its Relationship to Surface Ectoderm Patterning. *Development* **111**, 469–478.
 38. Busa, W. B. and Gimlich, R. L. (1989) Lithium-Induced Teratogenesis in Frog Embryos Prevented by a Polyphosphoinositide Cycle Intermediate or a Diacylglycerol Analog. *Dev. Biol.* **132**, 315–324.
 39. Keller, R. and Danilchik, M. V. (1988) Regional Expression, Pattern and Timing of Convergence and Extension During Gastrulation of *Xenopus laevis*. *Development* **103**, 193–209.
 40. Keller, R. and Shook, D. (2008) Dynamic Determinations: Patterning the Cell Behaviours That Close the Amphibian Blastopore. *Philos. Trans. R. Soc., B, Biol. Sci.* **363**, 1317–1332.
 41. Rolo, A., Skoglund, P., and Keller, R. (2009) Morphogenetic Movements Driving Neural Tube Closure in *Xenopus* Require Myosin IIB. *Dev. Biol.* **327**, 327–338.
 42. Davidson, L. A., Hoffstrom, B. G., Keller, R., and Desimone, D. W. (2002) Mesendoderm Extension and Mantle Closure in *Xenopus laevis* Gastrulation: Combined Roles for Integrin, Fibronectin, and Tissue Geometry. *Dev. Biol.* **242**, 109–129.
 43. Lee, C., Scherr, H. M., and Wallingford, J. B. (2007) Shroom Family Proteins Regulate Gamma-Tubulin Distribution and Microtubule Architecture During Epithelial Cell Shape Change. *Development* **134**, 1431–1441.
 44. Mandato, C. A. and Bement, W. M. (2001) Contraction and Polymerization Cooperate to Assemble and Close Actomyosin Rings Around *Xenopus* Oocyte Wounds. *J. Cell Biol.* **154**, 785–797.
 45. Nandadasa, S., Tao, Q., Menon, N. R., Heasman, J., and Wylie, C. C. (2009) N- and E-Cadherins in *Xenopus* Are Specifically Required in the Neural and Non-Neural Ectoderm, Respectively, for F-Actin Assembly and Morphogenetic Movements. *Development* **136**, 1327–1338.
 46. Wylie, C. C., Heasman, J., Parke, J. M., Anderton, B., and Tang, P. (1986) Cytoskeletal Changes During Oogenesis and Early Development of *Xenopus laevis*. *J. Cell Sci. Suppl.* **5**, 329–341.
 47. Houston, D. W. and King, M. L. (2000) A Critical Role for Xdazl, a Germ Plasm-Localized RNA, in the Differentiation of Primordial Germ Cells in *Xenopus*. *Development* **127**, 447–456.
 48. Kloc, M., Wilk, K., Vargas, D., Shirato, Y., Bilinski, S., and Etkin, L. D. (2005) Potential Structural Role of Non-Coding and Coding RNAs in the Organization of the Cytoskeleton at the Vegetal Cortex of *Xenopus* Oocytes. *Development* **132**, 3445–3457.
 49. Quaas, J. and Wylie, C. (2002) Surface Contraction Waves (Scws) in the *Xenopus* Egg Are Required for the Localization of the Germ Plasm and Are Dependent Upon Maternal Stores of the Kinesin-Like Protein xklp1. *Dev. Biol.* **243**, 272–280.
 50. Robb, D. L., Heasman, J., Raats, J., and Wylie, C. (1996) A Kinesin-Like Protein Is Required for Germ Plasm Aggregation in *Xenopus*. *Cell* **87**, 823–831.

51. Wallingford, J. B., Ewald, A. J., Harland, R. M., and Fraser, S. E. (2001) Calcium Signaling During Convergent Extension in *Xenopus*. *Curr. Biol.* **11**, 652–661.
52. Weaver, C., Farr, G. H., Pan, W., Rowning, B. A., Wang, J., Mao, J., Wu, D., Li, L., Larabell, C. A., and Kimelman, D. (2003) GBP Binds Kinesin Light Chain and Translocates During Cortical Rotation in *Xenopus* Eggs. *Development* **130**, 5425–5436.
53. Larabell, C. A. and Chandler, D. E. (1990) Stepwise Transformation of the Vitelline Envelope of *Xenopus* Eggs at Activation: a Quick-Freeze, Deep-Etch Analysis. *Dev. Biol.* **139**, 263–268.

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