Video Article

Blastomere Explants to Test for Cell Fate Commitment During Embryonic Development

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Abstract

Fate maps, constructed from lineage tracing all of the cells of an embryo, reveal which tissues descend from each cell of the embryo. Although fate maps are very useful for identifying the precursors of an organ and for elucidating the developmental path by which the descendant cells populate that organ in the normal embryo, they do not illustrate the full developmental potential of a precursor cell or identify the mechanisms by which its fate is determined. To test for cell fate commitment, one compares a cell's normal repertoire of descendants in the intact embryo (the fate map) with those expressed after an experimental manipulation. Is the cell's fate fixed (committed) regardless of the surrounding cellular environment, or is it influenced by external factors provided by its neighbors? Using the comprehensive fate maps of the *Xenopus* embryo, we describe how to identify, isolate and culture single cleavage stage precursors, called blastomeres. This approach allows one to assess whether these early cells are committed to the fate they acquire in their normal environment in the intact embryo, require interactions with their neighboring cells, or can be influenced to express alternate fates if exposed to other types of signals.

Video Link

The video component of this article can be found at <http://www.jove.com/video/4458/>

Introduction

Xenopus laevis embryos have been utilized extensively to identify the mechanisms by which embryonic cells acquire their specific fates because their eggs are large enough to permit microsurgical approaches. Additionally, they develop externally without the need for nutritional supplementation of the culture medium because each cell contains a rich intracellular supply of yolk platelets that provide an intrinsic energy store. An important asset for studying the mechanisms by which cell fate is determined is the comprehensive set of fate maps of the cleavage stage blastomeres (from 2- through 32-cell stages) ^{1, 2, 3, 4, 5, 6}. These maps were constructed by microinjecting a detectable molecule into a single, identifiable blastomere and monitoring later in development which tissues are populated by the labeled progeny. Consistent fate maps are possible because the cardinal axes of the embryos can be reliably identified in many embryos. First, in all wild type embryos the animal hemisphere is pigmented, whereas the vegetal hemisphere is not. Second, the entry of the sperm at fertilization causes a contraction of the animal hemisphere pigmentation towards the future ventral side; in many embryos a pigmentation difference therefore can be used to discriminate between dorsal and ventral sides. Third, the first cleavage furrow approximates the mid-sagittal plane in most embryos, and thus can be used to identify the right and left sides of the embryo. The fate maps also rely on the fact that naturally fertilized eggs frequently cleave in regular patterns that make each blastomere identifiable across a large population of embryos. While there is variability within and between clutches of eggs regarding pigmentation and cleavage patterns, using selection procedures described herein allows cells with prescribed fates to be identified with about 90% accuracy.

Cell fates can be determined during embryogenesis by several mechanisms. Intrinsic factors, such as differentially inherited cytoplasmic mRNAs or proteins, contribute to several aspects of early patterning. For example, specific maternal mRNAs determine which cells will contribute to the germ line, become the endoderm, or contribute to the dorsal body axis (reviewed in ⁷). Extrinsic factors provided locally by neighboring cells or more distantly from an embryonic signaling center, are responsible for inducing specific tissue types and patterning nearly every organ system. Examples of signaling centers include the organizer/node in the gastrula that induces the neural ectoderm and patterns the mesoderm, and the zone of polarizing activity that patterns the anterior-posterior axis of the limb bud. Although fate maps identify the precursors of the different organs and reveal the developmental path taken by their descendants in the normal embryo, they cannot distinguish between intrinsic and extrinsic influences on those cells. They also do not reveal the full developmental potential of a cell, whose descendants differentiate in the complex signaling environment of the embryo. Two experimental approaches can test whether a cell's fate is determined by intrinsic factors or is subsequently influenced by external factors: 1) transplantation of the cell to a novel location in the embryo; or 2) removal of the cell from the embryo followed by culture in the absence of exogenous signals.

Both experimental approaches have been feasible in *Xenopus* because the cells are large enough to be manually separated. For example, numerous studies have deleted single cells from embryos (to change cell-cell interactions) or transplanted cells to novel locations in host embryos to test for fate changes (reviewed in ^{7, 8, 9}). In addition, the second approach of explanting small numbers of cells from different regions of the embryo into culture to elucidate inductive tissue interactions in the absence of exogenous factors is possible because the cells of the *Xenopus* embryo are filled with an intracellular nutrient store, the yolk platelets. Therefore, they can be cultured for a few days in a defined salt medium without nutritional or growth factor supplementation of the culture medium. We have used this approach to show that dorsal animal blastomeres have an autonomous ability to produce neural and dorsal mesodermal tissues due to maternally inherited mRNAs ^{10, 11}, and others showed that mesoderm specification of 32-cell blastomeres relies on both intrinsic and extrinsic information ¹². An advantage of culturing cells as explants is that the medium can also be supplemented with defined signaling factors to determine which cell-to-cell communication pathway might influence the fate of the explanted cell ^{12, 13, 14}. In addition, one can inject the blastomere prior to culture with mRNA to over-express a gene, or with anti-sense oligonucleotides to prevent the translation of endogenous mRNAs. These, gain- and loss-of-function analyses can identify which molecules are required for an autonomously expressed fate. To analyze the fate of the explant, identification of specific cell types can be performed by standard gene expression (e.g., *in situ* hybridization, RT-PCR) and immunocytochemical assays. This protocol provides a simple, yet powerful, way to distinguish between intrinsic and extrinsic mechanisms that regulate how an embryonic cell develops into specific tissues.

Protocol

1. Preparation of Instruments, Culture Media and Dishes

1. Sharpen four forceps using Alumina abrasive film. Do this under a dissecting microscope to monitor the tip size. One pair of forceps serves as a back-up in case a tip is damaged during the procedure. Autoclave all four forceps and store in a sterile container.
2. Make 500 ml each of 0.1X and 1.0X culture medium (either Marc's Modified Ringers [MMR] or Modified Barth's Saline [MBS]; recipes in ¹⁵) and filter sterilize. We routinely use MBS (1X = 88 mM NaCl; 1 mM KCl, 0.7 mM CaCl₂, 1 mM MgSO₄, 5 mM HEPES [pH 7.8], 2.5 mM NaHCO₃). Store at 14–18 °C for months.
3. Make a 2% agarose solution in culture medium (2 g electrophoresis grade agarose in 100 ml 1X culture medium in a screw cap glass bottle). Autoclave to dissolve agarose. This can be stored at 4 °C for months, and microwaved to liquefy the agarose when it is next needed.
4. While the agarose is liquid, pour about 0.5 ml onto the bottom of each well of a sterile 24-well culture plate. This will prevent the explants from sticking to the plastic.
5. While the agarose is liquid, pour about 2–3 ml onto the bottom of two to three 60 mm Petri dishes, and swirl gently to make sure the bottom is covered. These will serve as dissection dishes and the agarose prevents embryos from sticking to the plastic once their membranes are removed.
6. When the agarose has cooled and hardened, flame the tip of a 6" Pasteur pipette until it melts into a ball, and lightly touch it on the surface of the agarose in each well of the culture plate. This will create a shallow depression into which the explant will be placed.
7. Fill each well of the culture plate with 1X sterile culture medium.
8. When the agarose has cooled and hardened, fill the dissection dish with diluted culture medium (0.1X MMR or 0.1X MBS) to facilitate blastomere separation.

2. Selection of Embryos

1. Obtain fertilized eggs and remove the jelly coats according to standard protocols ¹⁵. Transfer them to 0.5X culture medium in a 100 mm Petri dish.
2. When embryos reach the 2-cell stage, sort those in which the first cleavage furrow bisects the lightly pigmented area in the animal hemisphere (**Figure 1**) into a separate dish. These will be the donors for the explants. Keep the remaining 2-cell embryos in a separate dish next to the donor embryos throughout the procedure to serve as sibling controls to stage the explants.

3. Preparation of Explants

1. Place 5–10 embryos in a dissection dish, but only work on one embryo at a time.
2. Position the first embryo so the transparent vitelline membrane can be seen; it is separated by a clear space (perivitelline space) above the surface of the animal pole of the embryo. Using a sharpened forceps in one's subdominant hand (left hand if one is right handed), grasp the vitelline membrane above the perivitelline space. Using a sharpened forceps in the other hand, grasp the membrane close to the first forceps tip, and gently pull in opposite directions to peel the membrane away. Make the initial grasp at a distance from the cell that is to be dissected, so that the target cell is not damaged during removal of the membrane. One can tell that the membrane has been removed because the embryo will flatten.
3. Grab one neighboring cell with the forceps in the subdominant hand and use this cell as a "handle" so the cell to be dissected is not directly touched. With the forceps in the other hand, gently pull the remaining neighboring cells away from the desired blastomere. If midline cells, which share the same fate, are targeted, they can be removed together as a pair. Finally, dissect away the "handle" cell.
4. Pick up the blastomere (or blastomere pair), with a sterile, glass Pasteur pipette, avoiding air bubbles and excessive suction. The pipette can be fire-polished to remove sharp edges that can damage the cell, but we do not routinely do this. Place the tip of the Pasteur pipette under the surface of the culture medium in a well of the explant culture dish, and gently expel the blastomere. It should slide into the shallow depression by gravity. The same blastomere from more than one embryo can be combined into a single explant.
5. Repeat this procedure with the remaining embryos in the dissection dish, one-by-one. After about 10 embryos, the dissection dish will be filled with cellular debris. When this occurs, change to a fresh dissection dish.

6. After all dissections are done, check whether the explants are in the shallow depressions. If not, they can be gently pushed into the depression with a hair loop that has been sterilized in 70% ethanol and air dried.
7. About an hour after the last dissection, remove debris surrounding the healed explants with a sterile, glass Pasteur pipette.
8. Culture the plate of explants at 14-20 °C next to the Petri dish containing the sibling, control embryos. Sibling embryos will indicate the stage of development of the blastomere explants.

4. Harvesting Explants for Analysis

1. Harvest the explants when the siblings reach the desired developmental stage. These explants survive quite well even if some of the cells disintegrate. Therefore, if there is a cloudy mass in the culture well, explore it with a hair loop or forceps to determine if a healthy explant is buried within.
2. Pick up explants in a small volume of culture solution with a glass Pasteur pipette, and gently expel them into a fixative or lysis buffer appropriate for the assay to be conducted.

Representative Results

The ability of this assay to accurately assess the developmental potential of the cell relies upon dissecting out the correct blastomere based on the fate maps^{1, 2, 3, 4, 5, 6}. Therefore, it is critical to choose embryos with the correct pigmentation pattern at the 2-cell stage, as illustrated in **Figure 1**, that subsequently conform to regular cleavage patterns, as illustrated in **Figure 2**. If one observes the sorted embryos as they reach the required cleavage stage and separates out the embryos showing irregular cleavages, accuracy is greatly improved. As illustrated in **Figure 2**, regular cleavages often occur only on one side of the embryo. These embryos can be used as donors if the donor cell originates from the "regular" side. Because regular cleavage patterns are found in fewer embryos as cell division proceeds, sort about five times as many embryos as you intend to dissect. Naturally fertilized eggs tend to have more regular cleavages than *in vitro* fertilized eggs. Although cleavage patterns vary a great deal within a single clutch of eggs, one can expect at least 10-20% to be useful for this kind of a manipulation.

Blastomeres can be cultured alone or in small groups. When blastomeres are first dissected (**Figure 3A**), and transferred to the culture well (**Figure 3B, C**), they are soft and fragile. In our experience, animal and equatorial blastomeres are much easier to dissect and culture than vegetal blastomeres (**Figure 3C**). Perhaps because they are smaller, they are easier to transfer. They also seem to heal more quickly when nicked with the forceps. Vegetal cells often seem to complete cytokinesis more slowly and thus retain cytoplasmic bridges with sister cells, making them more "leaky" when dissected, and their cell membrane is more fragile and easier to damage. After about 1-2 hr in culture, the explanted blastomere(s) will have divided about 4 times and sloughed off most of the debris remaining from damaged neighboring cells (**Figure 3D**). After 20 hr, they form small, sturdy balls of cells (**Figure 3E**). While some may be buried in debris, often a healthy mass can be found (**Figure 3E, left**).

Although the explants are small, they survive processing for *in situ* hybridization as well as the more commonly used animal cap explants, which allows one to monitor later gene expression. In the experiment shown in **Figure 4A**, two dorsal blastomeres (D1.1) or two ventral blastomeres (V1.1) were dissected at the 16-cell stage and cultured in 1X MBS at 14 °C until sibling embryos reached early neural plate stages (st12-13; about 20-22 hr after dissecting). Each sample was assayed for the expression of a zygotic neural plate gene (*sox2*) by *in situ* hybridization (the blue reaction product). This assay demonstrates that the majority of dorsal animal blastomeres, which are precursors of the neural plate, can express *sox2* autonomously, that is, without additional signaling from other regions of the embryo. Those explants that do not express *sox2* may have been incorrectly identified at the time of dissection. In contrast, none of the ventral animal blastomeres, which are precursors of the ventral epidermis, express *sox2*. Thus, we conclude that dorsal animal blastomeres have an intrinsic ability to form neural tissue, whereas the ventral animal blastomeres do not. Note that some of the D1.1 explants have also elongated, a morphology indicative of dorsal mesoderm formation; these blastomeres also are fated to form the notochord in the intact embryo³, and in explant culture express a notochord marker protein¹⁰. Blastomere intrinsic fates can be altered by prior microinjection of mRNAs (gain-of-function) or anti-sense morpholino oligonucleotides (MOs; loss-of-function). In **Figure 4B**, the effect of altering the endogenous levels of a maternally expressed neural gene, *foxD5*^{16, 17}, is tested. When the endogenous level of *FoxD5* was increased by injecting *foxD5* mRNAs into the 8-cell precursor blastomeres of V1.1 (bottom row), the majority of the V1.1 explants (cultured to st 12-13) expressed *sox2*. Conversely, when the endogenous level of *FoxD5* was reduced by injection of MOs into the 8-cell precursor blastomeres of D1.1 (top row), only a few D1.1 explants (cultured to st 12-13) expressed *sox2* (compare to top row of **Figure 4A**). These experiments indicate that the maternal expression of *foxD5* plays a role in the intrinsic ability of dorsal blastomeres to acquire a neural fate.

Figure 1. Pigmentation patterns in 2-cell embryos from naturally fertilized eggs. The first cleavage furrow is indicated by an arrow. A) An example of an embryo that should not be used to identify blastomeres at later stages because the lightly pigmented region of the animal hemisphere of the embryo is contained mostly in one cell (on the left). Since the first cleavage furrow predicts the mid-sagittal plane of the embryo, the lightly pigmented region of this embryo will not identify dorsal. B) Two examples of embryos that should be sorted for explant cultures. In both, the lightly pigmented region of the animal hemisphere (*) is bisected by the first cleavage furrow. The embryo on the left is early in the first cell cycle, and the lightly pigmented region is only visible in about 25% of the cell surface. The embryo on the right is nearing the end of the first cell cycle, and the pigment has moved ventrally so the dorsal halves of the two cells are lighter. As a result, at later stages the lightly pigmented animal cells will predict dorsal (d) and darkly pigmented animal cells will predict ventral (v).

Figure 2. Examples of cleavage stage embryos from naturally fertilized eggs. All embryos are oriented with the animal pole facing the reader, dorsal to the top. A) 4-cell embryos. The embryo on the left has just entered the second cell cycle so the cleavage furrows are shallow. The embryo on the right has nearly completed the second cell cycle so the cleavage furrows are deeper and the cells appear more rounded. Black arrows point to the first cleavage furrow, and red arrows point to the second cleavage furrow. B) 8-cell embryos. The embryo on the left is early in the third cell cycle and the embryo on the right is near the end of the third cell cycle. C) 16-cell embryos. C' is an example of irregular cleavages to be avoided. C'' is an example of regular cleavages on the ventral side, but irregular on the dorsal side. C''' is an example of more regular cleavages on the dorsal side, but not on ventral side. C'''' is an example of regular cleavages at the midline, but not laterally. D) 32-cell embryos. D' is an example of regular cleavages on the ventral side, but irregular on the dorsal side. D'' is an example of regular cleavages on the dorsal side, but not on ventral side. D''' is an example of regular cleavages on the right side, but more irregular cleavages on the left side. E) 64-cell embryo. For a schematic representation of these stages, please refer to the Nieuwkoop and Faber staging series, which can be found on-line (<http://www.xenbase.org/anatomy/alldev.do>).

Figure 3. Explants from 16-cell embryos from naturally fertilized eggs. A) A ventral animal blastomere shortly after dissection. Arrow denotes remnants of the "handle" cell. B) A ventral animal blastomere transferred to a culture well that has divided once. C) A ventral vegetal blastomere shortly after dissection. Compared to animal blastomeres, these are larger, and more difficult to manipulate. D) Two ventral animal blastomere explants, 2 hr after dissection, have divided about four times. The left explant is viewed from the original, pigmented outer surface of the blastomere; the right explant is viewed from the original, unpigmented inner surface of the blastomere. E) Two ventral animal blastomere explants cultured for 24 hr. The left explant is sitting in a bed of cellular debris (arrows) whereas the right explant is free of any detritus. All images are at 50X magnification.

Figure 4. In situ hybridization analysis of gene expression in explants derived from 16-cell blastomeres dissected from naturally fertilized eggs. **A.** The 16-cell embryo on left illustrates the dorsal blastomere pair (D1.1) that was explanted for the results in the top row, and the ventral blastomere pair (V1.1) that was explanted for the results in the bottom row. Dissected blastomere pairs from uninjected embryos were placed in 0.1X MBS in culture wells and incubated at 14 °C for about 20 hr. They were collected in tubes of fixative and processed by *in situ* hybridization for expression of a zygotic neural gene, *sox2*. **B.** Blastomeres of 8-cell stage embryos were injected bilaterally either with *foxD5* MOs, to reduce endogenous expression in dorsal blastomeres, or with *foxD5* mRNA to increase endogenous expression in ventral blastomeres. When the injected embryos reached the 16-cell stage (20–30 min later), blastomere pairs were dissected, cultured and analyzed by *in situ* hybridization as in **A**. The number of explants that express *sox2* is significantly reduced in D1.1 explants that were injected with *foxD5* MOs (compare to top row in **A**). The number of explants that express *sox2* is significantly increased in V1.1 explants that were injected with *foxD5* mRNA (compare to bottom row in **A**). [Click here to view larger figure.](#)

Discussion

The most critical steps for successful culturing of individual blastomeres are: 1) correctly identifying the blastomere of interest; 2) maintaining a sterile, healthy culture; 3) dissecting cells at the correct part of the cell cycle; and 4) developing the necessary manual dexterity to prevent damage during dissection and transfer to the culture well.

To manipulate specific blastomeres, it is essential to be able to identify the cardinal axes. In *Xenopus* embryos, the dorsal side can be identified by three methods: (1) marking the sperm entry point with a vital dye^{18, 19}; (2) tipping *in vitro* fertilized eggs and marking one side^{18, 19}; or (3) selecting embryos during the 2-cell stage in which the first cleavage furrow bisects the lightly pigmented region in the animal hemisphere^{20, 21}. We use the third method, which accurately identifies blastomeres from naturally-fertilized 16-cell embryos in about 90% of cases^{20, 21}. At fertilization, the animal hemisphere pigmentation begins to contract towards the sperm entry point on the ventral side, causing the dorsal animal region to become less pigmented in a majority of embryos (**Figure 1**). There is considerable variation in the extent of this lightly pigmented region across clutches and even within clutches of *Xenopus* eggs. If the first cleavage furrow bisects this lighter area equally between the two daughter cells, then that lighter area can be used as the indicator of the dorsal side, and the first cleavage furrow will indicate the mid-sagittal plane. If the first cleavage furrow separates the daughters into one dark cell and one light cell, do not use it for the explant because the

pigmentation at later stages will not accurately identify the dorsal side. Previous studies showed that in these kinds of embryos the first cleavage furrow remained a marker for the mid-sagittal plane whereas the lightly pigmented region no longer indicated the dorsal side^{20, 21}. Embryos can also be selected at the early part of the 4-cell cleavage (left embryo in **Figure 2B**), when the first and the second furrows at the vegetal pole can still be distinguished; the first furrow should be complete and the second furrow not yet complete. If, however, one waits until the end of the 4-cell stage to select embryos (right embryo in **Figure 2B**), the first and second cleavage furrows can no longer be distinguished, and the lightly pigmented cells may be dorsal ones in only about 70% of embryos^{20, 21}. This will render targeting specific blastomeres much less accurate. It should be noted that the percentage of embryos in which the first cleavage furrow bisects the lightly pigmented region varies a great deal across clutches. In our experience they can account for <50% of the eggs in a clutch to >80% of the eggs in a clutch. Regardless, there almost always are enough eggs within a clutch of healthy eggs that meet this criterion to support an explant experiment.

The two recommended culture media (MMR, MBS) are virtually interchangeable; the preferences of different labs are mostly historical. MMR can be stored as a 10X solution for a year or more. MBS has a shorter shelf life because it is buffered with bicarbonate. Because embryos and explants can succumb to bacterial infection without the protective jelly and vitelline membranes, forceps and culture media should be sterile. If explants do not survive well, antibiotic can be added to the culture medium (e.g., 0.1% gentamicin). Solutions containing gentamicin have a short storage life, and thus should be made fresh on the day they are to be used. Cellular debris contains proteases that will damage the unprotected explants, and promotes bacterial growth. Therefore, it should be removed from the culture wells after the explants have had a chance to heal.

Blastomere dissections are performed in diluted culture medium (0.1X MBS or 0.1X MMR) in order to lessen the strength of cell-to-cell adhesions. During *Xenopus* cleavage stages, cell adhesion is mostly accomplished by calcium-/magnesium-dependent cadherins. Therefore, it is not necessary to use enzymatic dissociation; in fact this should be avoided because it removes surface proteins that may be important in fate determination events. Because embryonic cells from different batches of eggs adhere with different strengths, the dilution of the dissection medium may need to be adjusted on an ad hoc basis. If the cells are too adherent to dissect without tearing them, lower the concentration of the dissection medium stepwise from 0.1X. Conversely, if the cells fall apart when the vitelline membrane is removed and are very leaky, increase the concentration of the dissection medium stepwise. However, do not use calcium-/magnesium-free media because the cells become too fragile to transfer to the culture wells. Successful separation of blastomeres also depends on when during the cell cycle one performs the dissection. As shown in **Figure 2**, early in the cell cycle the cleavage furrows are shallow and the blastomeres are flattened. Later in the cycle the cleavage furrows are deeper and the cells are more rounded. Early in the cell cycle, there are cytoplasmic bridges that will cause cytoplasmic leakage if broken. Therefore, cells are more easily dissected late in the cell cycle. 4-cell embryos, 8-cell embryos, and vegetal cells at all stages are harder to dissect because the cytoplasmic bridges persist longer. One can anticipate a high death rate for these explants, meaning one needs to perform more dissections to recover a reasonable sample size. One can wait until the beginning of the next cleavage furrow to begin the dissection of these cells to ensure that the cytoplasmic bridges have closed. While single blastomeres taken from 8- and 16-cell embryos survive well in culture, in our experience older blastomeres fare less well as single cells. Although we do not know the cause of this, survival of older blastomere explants can be improved by dissecting several (2-6) of the same blastomere from different embryos and culturing them together in a single culture well^{10, 13, 14}.

This procedure requires practice and some manual dexterity. Because the time between cleavages is temperature dependent, it is convenient to store embryos at 14-16 °C to slow down cleavage and allow more time to perform the dissections. However, embryos will not tolerate temperatures lower than 14 °C. The first challenge in this procedure is removing the vitelline membrane. It is best not to move on to blastomere dissection until this first step is mastered. When dissecting out the blastomere, the "handle" cell is likely to leak and fall apart. This is not a concern because once all the other cells are peeled away from the desired cell, the "handle" cell can be removed with forceps. The handle cell remnants as well as other debris usually fall away when the blastomere is transferred to the culture well (**Figure 3B**). Blastomeres stick avidly to plastic, so use only glass pipettes to transfer them. Use minimal sucking pressure on the cell because it can disintegrate from the shear forces in the pipette. Also, avoid air bubbles in the pipette and make sure the tip of the pipette is below the surface of the culture medium when the explant is expelled because cells will explode if they touch an air-water interface.

The protocol described herein uses unmanipulated embryos as donors for the explants and simple salt media for culturing. These allow one to test the intrinsic ability of the explant to express particular fates. This method can be experimentally expanded in two ways. First, the culture media can be supplemented with signaling/growth factors to test whether these molecules can cause the explant to express alternative fates. Second, the gene expression of the donor embryos can be changed by injecting either mRNAs (gain-of-function) or anti-sense morpholino oligonucleotides (loss-of-function) into specific precursors prior to blastomere dissection. This can be done at the 1-cell stage, or in a more targeted manner at 1-2 cell divisions prior to dissection. With these approaches, one can test whether a particular exogenously supplied gene causes a blastomere to acquire an alternate fate, or whether a particular endogenous gene is necessary for the blastomere to express its autonomous fate (e.g., **Figure 4B**). These are powerful experimental approaches because they eliminate the confounding influence of the other cells and signaling centers present in the intact embryo.

Disclosures

No conflicts of interest declared.

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