Simulating Cortical Rotation, Axis Induction, and Experimental Embryology in Amphibian Embryos Using Clay Models

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INTRODUCTION

Animals and plants have complex bodies composed of numerous cell types that are organized with respect to multiple body axes. Yet, most of these organisms begin life as a single cell—the zygote—with little initial patterning, if any. How complexity arises during embryogenesis has captured the interest of developmental biologists for centuries (1). The study of amphibian embryonic development yielded foundational insights into this question and is taught as a case study in most developmental biology courses (2–4). For example, in what is widely regarded as the most famous experiment in developmental biology, Hilde Mangold and Hans Spemann demonstrated that certain cells in newt embryos can induce neighboring cells to adopt a particular fate (5). These “organizer” cells are formed in a region of the embryo that is made distinct by a symmetry-breaking process called cortical rotation. Though developmental mechanisms vary from species to species, symmetry breaking and induction are recurring themes in the emergence of complex body plans during development and are core concepts in the field of developmental biology (6).

This lesson article describes an activity used to reinforce the material taught in a lecture about early amphibian development, with an emphasis on symmetry breaking, axis formation, and induction. The general premise of the lecture is as follows: the early amphibian embryo is radially symmetric about a pre-established animal-vegetal (A-V) axis. Fertilization initiates cortical rotation, in which certain maternally-deposited, polarized factors such as wnt mRNAs rotate ~30°...
away from the sperm entry point in a microtubule-dependent manner (7). This rotation breaks radial symmetry and creates a unique zone of overlap between two important signaling pathways: Nodal, which is produced in the vegetal pole in a cortical rotation-independent manner and induces mesoderm along the entire equatorial midline, and Wnt, which is polarized opposite the sperm entry point by cortical rotation and modifies mesoderm to adopt an “organizer” identity (8). The organizer cells secrete additional signals that cause nearby cells to adopt dorsal fates by inhibiting ventralizing cues, thus establishing a dorsal-ventral axis (9). The necessity of the organizer region for establishing the body axis has been demonstrated through classical embryology experiments such as halving whole embryos at an early stage in development. Halves that inherit some of the future organizer region develop into fully-patterned animals with a defined body axis, but halves that inherit none of the future organizer region fail to establish a body axis and develop instead as a ventralized “belly piece” (10).

An understanding of the three-dimensional anatomy of embryos is fundamental to the lesson but can be difficult for students to conceptualize from two-dimensional renderings. Since multiple other developmental biology teaching contexts have used modeling of embryos to help students visualize the anatomical features of embryos (11–16), I hypothesized that clay modeling would be a well-suited tool for this particular learning context. Additionally, students would be able to easily manipulate clay models to simulate “cut-and-paste” embryology experiments outside of an authentic lab setting.

In the activity described here, students assemble into small groups and construct simple clay models of *Xenopus* frog oocytes. They simulate the processes of fertilization, cortical rotation, and the first two rounds of cell divisions, then replicate various embryological manipulations such as bisecting an embryo in half along the first or second division plane and comparing the outcome. Throughout the activity, the instructor prompts students to discuss and answer reflection questions, which guide them to recognize key results and takeaways themselves. This lesson lets students get a sense of embryo anatomy and how embryological manipulations are conducted in 3D space.

As an alternative active-learning approach to clay modeling and simulated experimentation, students can observe real embryos and recreate classical experiments themselves in the lab, as described by Olive et al. (17). Lab practical exercises like this are useful for teaching laboratory skills and methods and may increase student interest and motivation (18–22), though the pedagogical utility of conducting lab exercises that simply recreate an experiment with a known outcome as a way to teach content is contested (18–25). Additionally, recreating classic experiments using real amphibian embryos is not possible in many developmental biology classrooms for a variety of reasons. Some classes do not have an associated lab, classes with labs may not have adequate time or resources (e.g., animal facilities, lab reagents) for students to conduct the experiments, the manipulations are technically difficult and therefore may be unsuitable as a teaching exercise for novices, and animal use restrictions may prohibit experimentation on vertebrate embryos in undergraduate teaching laboratories. By contrast, the modeling and simulation activity described here is low-cost, does not require a lab, and involves no live animals, making it accessible to students in a wide variety of education settings. It also provides additional instructive value over experimenting on actual embryos, since some structures that can’t be seen in live embryos (e.g., localized mRNAs) can be represented visually in the model.

**Intended Audience**

I designed this activity for an upper-division (juniors and seniors), majors undergraduate developmental biology lecture course at a large, public, 4-year, research-intensive university on the US West Coast. The course enrolls about 100 students a semester, and the activity was done in the smaller, graduate teaching assistant-led discussion sections (~25 students per section). The course has no associated lab section.

**Required Learning Time**

This activity took twenty minutes for students to complete, not including the time spent introducing the concepts in lecture.

**Prerequisite Student Knowledge**

To complete this activity exactly as described here, students must already be aware of the organization of the pre-fertilization *Xenopus* oocyte, the roles of sperm and microtubules in cortical rotation, the signals required to induce the dorsal organizer, and the organizer's role in inducing the dorsal-ventral body axis. However, this activity can be easily adapted for students with less prior knowledge or for classes that do not go into as much molecular detail (see Discussion, Suggestions for Modification).

In the field test described here, students had been introduced to all associated content knowledge in advance of the activity as part of the main course lecture, including a description of the methods and results of the experiments that this activity simulates. I conducted this activity in discussion sections, which were led by graduate student teaching assistants and were exclusively used to review and reinforce the previous week’s lessons rather than to present new information.

**Prerequisite Teacher Knowledge**

The instructor should have sufficient knowledge of *Xenopus* embryonic development to anticipate and answer relevant student questions. This activity could be led by an undergraduate or graduate teaching assistant.

**SCIENTIFIC TEACHING THEMES**

**Active Learning**

Overwhelming evidence suggests that active learning—engaging students in learning through processes other than merely listening to an instructor, which can take many forms but typically requires higher-order thinking and reflection—benefits students as compared to learning through lecture exclusively (26–28). Furthermore, the use of physical models in appropriate contexts can support students’ understanding of three-dimensional structures and phenomena (29). Hands-on modeling and structured reflection are the core components of this activity; both actively engage students and position them as knowledge creators rather than passive recipients of information. Instead of simply looking at diagrams of classical embryological experiments, students construct model embryos which they subsequently manipulate to simulate...
those experiments. Rather than having a lecturer state key takeaways from each step of the activity, the accompanying worksheet prompts students to consider the consequences of different changes made to the models, such that students reach these understandings themselves through individual reflection and small group discussion.

Assessment

To determine students’ knowledge of the lesson topics prior to this activity (but after the initial lecture covering the relevant content), I administered an ungraded pre-assessment (Supporting File S1) during discussion section, immediately before the activity. The pre-assessment consisted of a single, multi-part question that tested the following concepts: (a) the relationship between the sperm entry point and the direction of cortical rotation, (b) the relationship between \textit{wnt11} RNA localization and the site of the future organizer, and (c) the necessity of microtubules during cortical rotation. Students had five minutes to complete the pre-assessment.

During class when the activity was administered, students filled out an activity worksheet (Supporting File S2). This worksheet, composed of four short-response questions related to the lesson objectives, doubled as an opportunity for student self-assessment and a way for the instructors to measure students understanding of the topics. The in-class worksheet also contained a Likert-style question asking students to report their perceptions of the effectiveness of the activity.

I collected data from the pre-assessment and activity worksheet from students who consented to have their de-identified responses used for research purposes. IRB approval for this research was granted by the University of California, Berkeley Committee for Protection of Human Subjects (CPHS #2021-11-14787).

Inclusive Teaching

I created this lesson as a way to incorporate active learning into a course which is otherwise taught in a traditional lecture format. Incorporation of active learning activities into college science classes increases student achievement overall (26) with disproportionately positive impacts on minoritized groups in STEM (27, 30). Additionally, students benefit from engaging with information in multiple modalities that are appropriate for the kind of information being taught (31, 32); in this case, a hands-on, tactile modality is appropriate for teaching about spatially-complex embryological manipulations and complements the slide deck and verbal explanation of classical experiments the professor gave in lecture.

When I conducted this activity, some sections of students were initially reluctant to engage in group work. Group work can support learning and feelings of classroom community, but it can also induce anxiety in some students (33). To help students to engage with their groups in a low-stress way, I added an impromptu icebreaker prompt: give their model embryos names and introduce them to the group. As an example, I told the students my clay embryos were named Hilde and Ethel, in honor of Hilde Mangold and Ethel Browne Harvey, the scientists who first discovered organizer activity in amphibians and hydra, respectively (34). This icebreaker succeeded in stimulating enthusiastic group participation from all students, with the additional benefit of highlighting two women in developmental biology whose crucial contributions to the field are often overlooked.

LESSON PLAN

An overview and timeline of the entire lesson is included in Table 1.

Preparation

The course professor introduced the topics listed under the Prerequisite Student Knowledge section during whole-course lecture periods the week before this activity was conducted in discussion sections (Supporting File S3).

Before the activity was conducted, I prepared kits for students to use to construct their clay embryo models. Each kit included the following:

- Four colors of modeling clay (approximate amounts are listed in parenthesis based on common objects of similar volume). I used Play-Doh (Hasbro) or Art-Time Dough (Sargent), but any brand of similar modeling clay should suffice.
  - Blue (approx. volume: 1 golf ball)
  - White (approx. volume: 1 golf ball)
  - Orange (approx. volume: 1 green olive)
  - Yellow (approx. volume: 1 chickpea)
- Dental floss or string (at least 10” length)

Progression Through Activity in Class

I conducted the activity in discussion sections, which are smaller subsections of the entire class that are led by Graduate Student Instructors (GSIs) and are intended to reinforce material taught in lecture. GSIs began by presenting a brief slide deck “mini-lecture” reviewing some of what the professor taught in lecture the week prior. ~5 minutes were spent covering material directly related to this activity, namely the organization of the frog oocyte before and after fertilization and cortical rotation, with specific mention of the role of microtubules in these processes (Supporting File S4). After the mini-lecture, I gave students five minutes to complete an ungraded pre-assessment. The pre-assessment asked students to indicate the direction of cortical rotation, the location of the future organizer(s), and the final location of \textit{wnt11} mRNAs in \textit{Xenopus} oocytes with and without a microtubule inhibitor (Supporting File S1). Importantly, the pre-assessment had the sperm entry point on the right side of the animal pole, unlike all other diagrams that students had previously seen in their course materials which showed sperm entry on the left side of the animal pole. This let me discriminate between students who had simply memorized the layout of similar diagrams from those who understood the relationship of sperm entry to the direction of cortical rotation, the movement of localized determinants, and the ultimate location of the future organizer.

The modeling activity began immediately following the pre-assessment. Step-by-step pictures of the activity are shown in Figure 1 and Supporting File S5. All students received an activity worksheet (Supporting File S2) and a kit containing...
modeling clay and dental floss. Each student had their own kit and worksheet, but I directed them to form small groups with their neighbors (2–4 students per group) so they could discuss the reflection questions before writing down their answers on their own worksheet. The worksheet includes instructions for all the steps of the activity. In the first of four discussion sections that were included in this field test, students read the instructions on the worksheet and completed the activity at their own pace. However, only 43% (6/14) of students in the first section were able to complete the whole worksheet in the allotted time. To ensure students progressed through the activity with adequate pacing in the subsequent three sections, I guided students through the activity by giving verbal instructions while completing the steps of the activity with the students in real time at the front of the classroom. These verbal instructions were identical to the instructions written on the worksheet, which students still had access to. After modifying the activity to include the instructor guiding students through the activity, the worksheet completion rate increased to 100% (44/44).

TEACHING DISCUSSION

Learning Outcomes

Students were exposed in lecture to all the course content covered by this lesson, including descriptions of the simulated experiments and their outcomes. To assess the effectiveness of the modeling and simulation activity, I compared levels of mastery that students demonstrated through their answers on the pre-assessment (Supporting File S1) and the activity worksheet (Supporting File S2). Data from the pre-assessment and activity worksheet were collected from 58 students who gave informed consent, with IRB approval granted by the University of California, Berkeley Committee for Protection of Human Subjects (CPHS #2021-11-14787). If a student did not complete a question on the pre-assessment because they arrived to class late, or if they did not complete a question on the activity worksheet because they ran out of time, I excluded them from analyses involving those questions.

Relationship of Sperm Entry Point to Future Body Axes

I tested students’ initial understanding of the relationship between sperm entry and the location of future dorsal structures using a pre-assessment, in which students marked the location of the future dorsal organizer on an image of an oocyte with a sperm fertilizing the right side of the animal pole. I marked student responses as correct if they drew an X on the left side of the diagram. The deliberate placement of the sperm on the right let me distinguish students who understood the relationship of sperm entry to the site of the future organizer from students who uncritically reproduced diagrams previously seen in class, which all showed sperm entry on the left side and dorsal structures on the right. 43.9% (25/57) of students demonstrated an understanding of the relationship between sperm entry and the future body axis on the pre-assessment. To retest this same concept, the activity worksheet Reflection Question #1 asked students to “defend the following statement: ‘Before fertilization, all sides of the embryo are capable of becoming future dorsal structures.’” I marked students as correct if their answer indicated that the location of dorsal structures depends on the location of sperm entry, which is not predefined. On the activity worksheet, the percentage of students who correctly described this relationship increased to 73.7% (42/57).

Relationship of Wnt and Nodal Signaling to Organizer Formation

The formation of the dorsal organizer requires both Wnt and Nodal signaling. On the pre-assessment, only 34.0% (18/53) of students indicated that the organizer would form on the midline of the embryo, where they predicted wnt11 mRNA to overlap with adjacent Nodal signaling. On the activity worksheet, in response to Reflection Question #3 (“Without cortical rotation, no organizer forms. Why?”), 73.6% (39/53) of students correctly indicated that wnt11 mRNA must rotate for the organizer to form. 52.8% (28/53) of students correctly indicated that wnt11 mRNA must rotate for the organizer to form and that it must overlap with Nodal/the location where Nodal signaling occurs, e.g., the midline, the equator, or the animal hemisphere. Four students in the self-paced section were excluded from this analysis because they did not reach Reflection Question 3 on the worksheet.

Necessity of Organizer for Axis Induction

The dorsal organizer produces necessary and sufficient signals to dorsalize other embryonic structures. During this activity, students replicated a classical experiment that provided evidence for the necessity of the dorsal organizer for axis induction: bisecting the embryo along the A-V axis through the organizer, or along a perpendicular plane that is also along the A-V axis. Reflection Question #2 asked students to explain “why the plane of bisection changes the outcome of the experiment.” 82.8% (48/58) of students correctly reported that both halves may or may not inherit regions fated to become the organizer depending on the plane of bisection. Additionally, Reflection Question #4 asked students to identify which regions of a bisected wild-type embryo “could ‘rescue’ the UV-irradiated phenotype [no cortical rotation or subsequent organizer formation] when grafted onto one half of the second embryo.” 92.2% (47/51) of students who answered...
Reflection Question #4 indicated that a region containing the future organizer would be capable of rescue. Although there is no question on the pre-assessment that directly tested students’ baseline understanding of this concept, the overall high success rate on these questions indicates that most students understood this critical concept by the end of the activity. Additionally, they could use their knowledge to explain the results of classical experiments.

**Student Perceptions**

In addition to reflection questions, the activity worksheet contained a Likert-style survey question to assess students’ self-reported perceptions of the effectiveness of the activity. Regarding the statement, “This activity increased my understanding of the material taught in class,” 87% (46/53) of students either agreed or strongly agreed, 13% (7/53) neither agreed nor disagreed, and none disagreed. Many noted in the optional feedback section of the activity worksheet that they enjoyed the activity and found it useful, and quite a few students also told me this at the end of class when I conducted the activity.

**Suggestions for Modification**

In the context that I field-tested this activity, students had already been taught all essential content covered by this activity during in-class lectures. It would be possible, with slight modifications to the lesson protocol and materials, to introduce many of these concepts concurrently with the activity, rather than in advance. Additional scaffolding could be added to the activity, such as having students draw/make/describe the region of the embryo experiencing Nodal signaling, rather than just labeling the region of Nodal/ Wnt overlap. Alternatively, references to specific molecular determinants and mechanisms (e.g., wnt11 mRNA, Nodal protein, the microtubule basis for cortical rotation) could be removed, which would make the activity better suited to classes which do not discuss the formation of the grey crescent/dorsal organizer in specific molecular detail.

While I had students work together in groups, this activity could be done individually by students (although this would remove the pedagogical benefits of group work). Because the lesson worksheet lists all activity instructions, students could also complete the activity independently at home if they were provided with kits containing the modeling materials.

Many aspects of the clay models—e.g., color of clay, size of models—are amenable to modification. Any color clay can be substituted, though I recommend ensuring that color blind-accessible color combinations are used. Many students aptly pointed out that real *Xenopus* embryos have pigmented animal poles and unpigmented vegetal poles; switching the blue clay and white clay so that they represent the animal and vegetal poles respectively would more accurately reflect the appearance of real *Xenopus* embryos. To make this activity accessible to blind or visually disabled students, the clay could be textured by mixing in small objects like sand or beads that distinguish each color tactiley.

I suggest modifying reflection question #1 (“Based on what you have done so far, defend the following statement: ‘Before fertilization, all sides of the embryo are capable of becoming future dorsal structures.’”) to improve clarity. I intended the word “side” to be interpreted in contrast to the top and bottom (in this case, the animal and vegetal poles), but a valid alternative interpretation could be “any point on the spherical embryo.” A clearer question would read, “Based on what you have done so far, defend the following statement: ‘Before fertilization, all lateral sides of the embryo are capable of becoming future dorsal structures.’”

This activity has students simulate a small number of embryological manipulations (sections of embryos, with and without cortical rotation). The activity could be extended to involve additional experimental simulations, such as replicating the Spemann-Mangold organizer transplant (5). The materials can be reused for other learning activities such as constructing gastrulation-stage model embryos to understand their complex anatomy, as described elsewhere (11).

**Conclusion**

This activity effectively reinforced and strengthened students’ understanding of early amphibian development, from fertilization to axis induction. This activity engaged students in active learning and improved their understanding of material taught in lecture.

**SUPPORTING MATERIALS**

- S1. Clay Embryology – Pre-assessment
- S2. Clay Embryology – Activity worksheet
- S3. Clay Embryology – Class lecture slides
- S4. Clay Embryology – Discussion section slides
- S5. Clay Embryology – Steps of activity

**ACKNOWLEDGMENTS**

Special thanks to Richard Harland for encouraging me to develop this activity for CourseSource and for jumping through numerous bureaucratic hoops to sponsor the associated IRB application, and for sharing his slide deck from the lecture associated with this activity. Additional thanks to Anne Chen and Anna Rogers who also shared their slide decks and graciously allowed me to run this activity in their discussion sections. I would not have prepared this lesson for CourseSource if I hadn’t attended the CourseSource Writing Studio, sponsored by the Society for the Advancement of Biology Education Research (SABER) and led by Erin Vinson. I am thankful to the many co-participants at the Writing Studio who provided valuable feedback during the earliest stages of this article’s planning and preparation. Sophia Friesen provided helpful feedback and edits which greatly improved the final manuscript.

**REFERENCES**

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Table 1. Lesson timeline. The activity takes about 20 minutes to complete.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Estimated Time</th>
<th>Notes (language in quotes indicate the lesson script)</th>
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<tbody>
<tr>
<td>Instructor Preparation for Activity</td>
<td></td>
<td></td>
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<tr>
<td>Prepare materials</td>
<td>Assemble kits containing clay and dental floss. Print out activity worksheets.</td>
<td>Variable [depends on number of students]</td>
<td>Need at least 1 kit per group; one kit per student ideal. Need one activity worksheet per student.</td>
</tr>
<tr>
<td>In-Class Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assemble into groups</td>
<td>Students assemble into groups, 2–4 students in size.</td>
<td>1 minute</td>
<td></td>
</tr>
<tr>
<td>Distribute kits</td>
<td>Hand out a supply kits to students.</td>
<td>2 minutes</td>
<td></td>
</tr>
<tr>
<td>Assemble model oocytes</td>
<td>Students assemble clay oocyte.</td>
<td>2 minutes</td>
<td></td>
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</table>
| Simulate fertilization + cortical rotation | Students poke the model and rotate the clay disc.                       | 30 seconds     | See Figure 1C–D  
“3. Poke the animal pole to simulate sperm entry. Based on where the sperm entered, move the orange disc ~30° away from the sperm entry point to simulate cortical rotation.”  
Note: only use half of the clay; save enough to make an entire second embryo later.  
“2. Make a disc of orange clay representing maternally deposited, cortically localized cytoplasmic determinants such as \textit{wnt11} and \textit{vg1} mRNAs. Place the disc at the vegetal pole. The size of the disc should be such that it covers a significant portion of the vegetal pole, but not all of it (40–60%).”  
| Mark the organizer            | Students mark the site of the future organizer.                             | 30 seconds     | See Figure 1E  
“4. Use a small piece of yellow clay to label the site of the future organizer. It should be located approximately along the midline where you would expect an overlap between Wnt and Nodal signaling.”  
| Group reflection Q1           | Group discussion; students write answer on worksheet.                      | 3 minutes      | Discussion prompt: Based on what you have done so far, defend the following statement: “Before fertilization, all sides of the embryo are capable of becoming future dorsal structures.” |
| Bisect the embryo along two planes | Students cut model in half along two axes and examine the resulting halves. | 1 minute       | See Figure 1F–I  
“5. Use the dental floss to cut the embryo in half twice, along the A-V axis:  
• One cut that goes through both the sperm entry point and the middle of the orange clay disc  
• Another cut perpendicular to the first  
“6. Separate the two halves of the embryo based on the first cut. Compare the two halves.  
“7. Separate the two halves of the embryo based on the second cut. Compare the two halves.” |
<p>| Group reflection Q2           | Group discussion; students write answer on worksheet.                      | 3 minutes      | Discussion prompt: If you separate the embryo into halves along one of the cut planes and allow development to proceed, you get two complete embryos. If you separate the embryo into halves along the perpendicular cut plane and allow development to proceed, you get one complete embryo (with a big head), and one “belly piece.” Explain why the plane of bisection changes the outcome of the experiment. |</p>
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</table>
| Assemble a second embryo       | Students assemble another clay oocyte.                                     | 2 minutes      | See Figure 1J–K  
“8. With the remaining clay, repeat steps 1 and 2 to assemble a second embryo.” |
| Simulate fertilization without cortical rotation | Students poke the second model.                                             | 30 seconds     | See Figure 1L–M  
“9. Poke the animal pole to simulate sperm entry. This time, pretend it is a UV-irradiated embryo—do not rotate the orange disc.” |
| Group reflection Q3            | Group discussion; students write answer on worksheet.                      | 3 minutes      | Discussion prompt: Without cortical rotation, no organizer forms. Why? |
| Bisect the embryo              | Students cut second model in half along two axes.                          | 30 seconds     | See Figure 1N  
“Use the dental floss to cut the second embryo in half, along the A-V axis.” |
| Group reflection Q4            | Group discussion; students write answer on worksheet.                      | 3 minutes      | Discussion prompt: Which sections of embryo 1, when grafted onto a half of the second embryo could “rescue” the UV-irradiation phenotype and result in a complete embryo? |