ONLINE INQUIRY & INVESTIGATION

A Picture Is Worth a Thousand Questions

STEPHEN RIBISI, JR. KRISTINA YU LORI LAMBERTSON

The old adage that a picture is worth a thousand words also holds true in cell biology. Much of the knowledge that we have of the structures and functions of cells has been acquired by biologists peering through the eyepieces of microscopes. In this lesson your students will have the chance to make observations and ask questions about a set of images of a developing sea urchin embryo.

The ability to ask questions that can be addressed by science is a critical science process skill that students must develop in order to progress in their mastery of science. When students design and pursue the answers to scientific questions, they are not only behaving as professional scientists do, but are more likely to be interested in the science content that is being taught. This lesson is a guided scientific inquiry in which students practice asking questions based on cell biological evidence, in this case photographs of a developing sea urchin embryo.

The concept of the cell is central to all of biology and is prominently featured in the *National Science Education Standards* (NRC, 1995) as well as in the curriculum standards of most states at the middle and high school levels. Generally, students are first introduced to the cell as part of a life science class at the middle school level. Many teachers, if they have access to a classroom set of microscopes, provide their students the opportunity to view living cells. But seeing real cells through the eyepiece of a microscope is not by itself sufficient to give students an accurate understanding of cell biology. Research has shown that students even at the high school level do not have an accurate understanding of the size and function of prokaryotic or eukaryotic cells (Arnold, 1983; Dreyfus & Jungwirth, 1988; Dreyfus & Jungwirth, 1989).

Developmental biology, the study of embryos, provides an excellent framework for teaching about growth, reproduction, and cell division (mitosis); concepts that are very important to understanding cell biology. The sea urchin, a marine invertebrate that can be found both in the deep ocean and intertidal zones worldwide, is an organism that has been critical to advancing our knowledge of cells and especially our knowledge of mitosis. During the development of the sea urchin, a single diploid cell that results from the combination of an egg and a sperm undergoes many rounds of mitosis to first produce a uniform ball of many cells and eventually a whole new spiny sea urchin! All of this occurs in isolation without any further contribution of food or resources from the parent sea urchins.

In this lesson your students will explore the secrets of the sea urchin embryo by observing and thinking about images of the first few hours of a sea urchin's new life and asking questions about the underlying biology. Students are led through a guided inquiry in which they will learn about the fundamental biological concept of nuclear/cytoplasmic (N/C) ratio. The N/C ratio is an important concept in understanding the early development of embryos when the number of cells increases without increasing the total volume of the embryo. This means that individual cells get progressively smaller while the nuclei remain roughly the same size. Through this lesson, students will come to understand the constraints under which developing sea urchins must operate while appreciating the beauty of biological images.

About the Images

The images used in this lesson were collected by the Microscope Imaging Station at the Exploratorium in San Francisco. The Microscope Imaging Station facility is equipped with research-grade compound microscopes and digital cameras that capture highly detailed images of cells such as the ones used in this lesson. The images and tables included with this article are also available for download from the Exploratorium server at the following URL:

http://www.exploratorium.edu/imaging_station/ti_cellactivity.

Once downloaded, prints suitable for use in this lesson can be made with any inkjet printer. Plain paper and low print qual-

STEPHEN RIBISI, JR. is Assistant Professor of Science Education, University of Massachusetts, Boston, MA 02125: e-mail: stephen.ribisi@umb.edu. KRISTINA YU is microscopist and co-director of the Microscope Imaging Station at the Exploratorium, San Francisco, CA 94123. LORI LAMBERTSON is a math educator at the Exploratorium Teacher Institute.

ity settings can be used to limit costs, but for optimum image quality we recommend photo paper and high quality print settings. The photomontage can then be placed into a plastic sheet protector. Students should be encouraged to mark on the plastic covering the images using overhead markers to make the task of measuring easier. For typical classroom use, one photomontage for every three students is optimal.

The sea urchin development photomontage used in this activity is composed of six images of a live sea urchin embryo taken with a digital camera attached to a compound microscope. Fertilized sea urchin eggs (*Lytechinus pictus*) were mounted in seawater and examined with an inverted compound microscope using a 40x objective and Normarski optics. The Nomarski technique, a type of differential interference contrast microscopy, enhances edges and produces images that have the illusion of depth. This is accomplished through the use of special light filters that enhance the contrast of the structures within the cells (NPACI, 2000). A series of still images were taken at 1-minute intervals for approximately two hours using a high-resolution digital camera attached to the microscope.

Materials

- sea urchin photomontage
- metric rulers
- transparency sheets
- transparency markers
- pencils
- graph paper
- calculators
- magnifying lenses
- plastic sheet protectors
- blank paper for drawing (optional)

Procedures

Student Instructions & Teaching Suggestions

Begin this lesson by briefly introducing your students to the sea urchin (see Figure 1 for interesting information about the sea urchin). A living specimen would be best, but if that is not practical in your classroom then photos of sea urchins will suffice. A good source of sea urchin images can be found online at: http://www.stanford.edu/group/Urchin/. This Web site, housed on Stanford University servers, is a collection of over 275 individual Web pages on sea urchin development with information that is appropriate for high school teachers. The entire collection is also available on CD-ROM as a compressed zip file. All of the material on the Web site is copyrighted by Stanford University and the URL is included here for your convenience. A photo of a sea urchin (Figure 2) is presented here for your use. The high resolution digital photo is also available for download from the Exploratorium server. After generating interest in the sea urchin, explain that the cells in the photos will eventually become a spiny sea urchin!

In the following section, student instructions are presented in *italics* to suggest a possible introduction for your students at the beginning of each of the four tasks. Suggestions on teaching (including suggested times for each section of the lesson, assuming a 50-minute period) follow in normal, non-italicized font.

Figure 1. Urchin Facts

- Sea urchins are invertebrate marine organisms that live only in salt water.
- There are about 700 species of sea urchin found worldwide.
- Sea urchins, along with starfish and sea cucumbers, belong to the phylum *Echinodermata*.
- Sea urchins mate by releasing gametes (eggs and sperm) into open water. Each male or female urchin will release millions of gametes at a time.
- Sea urchins are eukaryotic organisms (like us) that have a true nucleus.
- Sea urchins are a good experimental system: Their eggs are clear (you can see inside them as they develop), they produce lots of them, and the development of the early urchin is very similar to that of other animals, including humans.
- Sea urchins have been used to study the basic principles of development since the late 1800s.
- Sea urchins, eukaryotic organisms whose cells contain a "true" nucleus, are used as a model system in many areas of biology, including developmental, cell, and evolutionary biology.
- There are over 100 laboratories worldwide using urchins as a research organism.

Figure 2. The Star of the Show!

A sea urchin of the species *Lytechinus pictus* is shown with a penny for size comparison. This species of urchin is found in the shallow coastal waters of the Pacific Ocean ranging from Southern California into Mexico. All of the images of sea urchin embryos presented in this article are from this species of sea urchin. When spawning, adults shed millions of gametes into the sea producing many embryos. Only a small portion of these embryos goes on to become adult sea urchins.

Student Task #1. Observe, Describe & Ask Questions

Using your unaided eyes as well as the magnifying lens, take a close look at the images in each of the six small photos and write down as many observations as you can. There is a key in the lower right corner of the pho-

tomontage that points out some of the main features of the organism you are studying. What do you wonder about this developing organism? Work with your lab partners to generate questions about what you observe in the photos.

Teaching Suggestions
(10 minutes)

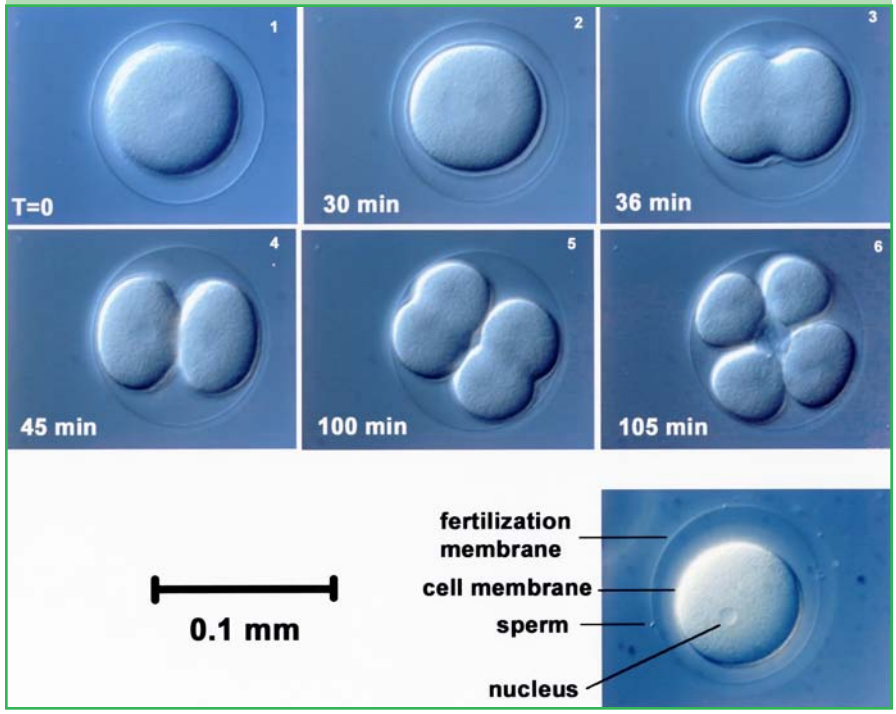
Allow your students time to make detailed observations of the photomontage. At this stage in the activity, have the magnifying glasses and the metric rulers available for the students to use if they wish. Have each student share one observation with the class.

Encourage your students to formulate their own questions based on their careful observation of the photomontage. Guide the students toward asking about the changes that are occurring to the cells of the embryo over time. Require that the students ask questions that are based on the evidence visible in the photos and are not inferences. Some examples of questions that students may ask are, "What kind of cells are these?" "How long does it take the cells to divide?" "Where do the cells get their food?" and "What's going to happen next?"

After you have provided students a few minutes to think of questions, ask them to think about which question they can answer from the photomontage and which questions would require more information to answer. For example, students may ask if there is a pattern to the intervals of time between each cell division or they may want to know how the dividing cells are getting their food. These are both examples of good biological questions that would require more information than is present in the photos alone to answer.

Figure 3. Photomontage of the Early Development of the Sea Urchin Embryo.

This figure is a photomontage consisting of a timed sequence of the early development of the sea urchin, *Lytechinus pictus*. Each panel labeled 1-6 shows the same embryo at different time points in minutes after fertilization (T=0). The major structures of the sea urchin embryo are labeled in the panel in the lower right corner of the photomontage.



Student Task #2. Determine the Magnification of the Images

There is a scale bar on the bottom left of the photomontage. The scale bar can be used to determine the magnification of the images. Use the ruler to measure the actual length of the scale bar in millimeters (thousandths of a meter). Compare this to the stated length (0.1 mm) that the scale bar represents. All of the small images that make up the photomontage are the same magnification. What is the magnification of the images? What is the actual diameter of the sea urchin embryo in millimeters? in micrometers (millionths of a meter)?

Teaching Suggestions (10 minutes)

Some students may quickly notice that the scale bar is about the same size as the sea urchin embryo in the photos and will say that the embryo is 0.1 millimeters in diameter. Ask the students to convert the embryo diameter into micrometers which is a much more common unit for measuring cells. Depending on the grade level and math skills of your students, you may need to help students understand how to use ratios to determine the magnification factor of the microscope images.

Student Task #3. Identify the Borders of the Cells & the Nuclei

You may have noticed that the embryo begins as a single cell that eventually begins to divide. After the first round of cell division there are two cells and after the second round of cell division there are four cells. Look closely at the cells and determine the location of the nucleus within each cell. The nucleus is visible as a round depression in the center of each cell. Everything outside of the nucleus but inside the plasma membrane is cytoplasm. At the edge of the cytoplasm is the cell membrane. The cell membrane is itself too thin to be seen with a light microscope but you can see where the cytoplasm ends. Using the transparency markers on the plastic cover sheet, carefully trace the boundary of each nucleus and each cell. What changes in the borders of the nuclei do you notice from panel to panel? Why do you think that the nuclei sometimes appear distinct and sometimes appear fuzzy?

Teaching Suggestions (10 minutes)

Students will need to make a decision as to which membrane they will use as the outer limit of the cells of the embryo. Students may want to determine the diameter of the one cell embryo by measuring from

the fertilization envelope (the outer membrane visible in the photos that functions to prevent multiple sperm from entering the egg and later protects the developing embryo) instead of the cell membrane. To head off this confusion you may wish to instruct students to measure from the cell membranes and NOT the fertilization envelope.

Student Task #4. Calculate the Ratio of The Cell Diameter to the Diameter of the Nucleus

As you were identifying the borders of the cells and their nuclei you may have noticed that the size of the cells changes dramatically as they divide, but what about the size of the nuclei? Do the nuclei get smaller as the cells of the embryo divide?

Now that you have identified the magnification and the location of the nucleus in each of the cells, use the following procedure to determine the ratio of the diameter of the nucleus to the diameter of its cell.

Provide the following directions to your students:

1. A worksheet has been provided to help you with the *calculations that you will do in the next steps. Fill in the worksheet as you complete Steps 2-5.*
2. *Use the ruler to determine the diameter of one cell and its nucleus in panels #1, #4, and #6. Pick a cell that has a nucleus that is clearly visible.*
3. *Convert the diameter you measured on the photo to the actual diameter of the cell in millimeters. To do this, divide the measured diameter by the magnification factor (÷ 500).*
4. *Millimeters are awfully big units to use to describe this embryo. A better unit would be the micrometer (also called the micron) which is one millionth (1/1,000,000) of a meter. Convert the actual diameters in millimeters to micrometers by multiplying the result you got from Step 2 above by 1000 (there are 1000 micrometers in each millimeter).*
5. *Divide the diameter of the cell by the diameter of the nucleus.*
6. *Graph the ratios to see if there is a trend in the data.*

Teaching Suggestions (20 minutes)

This task is the most challenging of this lesson. Start this part of the lesson by talking about the idea of the relative size of the nucleus of a cell to its total size. Details about the biology of the nuclear/cytoplasmic ratio are included later in this article. All of the previous tasks are designed to familiarize the students with how to work with the images. You may want to use a worksheet to help guide your students through the math they will need to do in order to determine the nuclear/cytoplasmic ratio. An example of a blank worksheet (Figure 4) as well as an example of a completed worksheet (Figure 5) are included with this lesson.

Figure 4. Cell Diameter/Nuclear Diameter Worksheet.

This is a worksheet designed to lead students through the calculations required to determine the ratio of the cell diameter to nucleus diameter of the cells of the developing sea urchin embryo. Note that due to break down of the nuclear envelope during the course of mitosis, these calculations are only possible using cells from panels 1, 4, and 6 from Figure 1. Abbreviations used: mm = millimeter (thousandth [10^{-3}] of a meter), µm = micrometer or micron (millionth [10^{-6}] of a meter), d_c = cell diameter, d_n = nucleus diameter.

	Measured Diameter (mm)	Calculate the actual diameter (divide diameter in mm by the magnification factor 500x)	Actual Diameter (mm)	Convert from mm to µm (multiply by 1000)	Actual Diameter (µm)	Ratio of cell diameter to nuclear diameter ($d_c \div d_n$)
Panel 1 Cell		÷ 500		x 1000 mm/µm	$\mathbf{d_c}$ =	
Panel 1 Nucleus		÷ 500		x 1000 mm/µm	$\mathbf{d_n}$ =	
Panel 4 Cell		÷ 500		x 1000 mm/µm	$\mathbf{d_c}$ =	
Panel 4 Nucleus		÷ 500		x 1000 mm/µm	$\mathbf{d_n}$ =	
Panel 6 Cell		÷ 500		x 1000 mm/µm	$\mathbf{d_c}$ =	
Panel 6 Nucleus		÷ 500		x 1000 mm/µm	$\mathbf{d_n}$ =	

Figure 5. Sample N/C Ratio Data Calculation Sheet.

This worksheet contains sample data and calculations of the ratios of cell diameter to nucleus diameter as determined by measurements of the cells of the sea urchin embryos from Figure 1.

	Measured Diameter (mm)	Calculate the actual diameter (divide diameter in mm by the magnification factor 500x)	Actual Diameter (mm)	Convert from mm to µm (multiply by 1000)	Actual Diameter (µm)	Ratio of cell diameter to nuclear diameter ($d_c \div d_n$)
Panel 1 Cell	38 mm	÷ 500	0.076 mm	x 1000 mm/µm	$d_c = 76$ µm	
Panel 1 Nucleus	6 mm	÷ 500	0.012 mm	x 1000 mm/µm	$d_n = 12$ µm	6.3
Panel 4 Cell	32 mm	÷ 500	0.064 mm	x 1000 mm/µm	$d_c = 64$ µm	
Panel 4 Nucleus	6 mm	÷ 500	0.012 mm	x 1000 mm/µm	$d_n = 12$ µm	5.3
Panel 6 Cell	24 mm	÷ 500	0.048 mm	x 1000 mm/µm	$d_c = 48$ µm	
Panel 6 Nucleus	6 mm	÷ 500	0.012 mm	x 1000 mm/µm	$d_n = 12$ µm	4.0

The Biological Context – Sea Urchin Development & Nuclear/ Cytoplasmic Ratio

Sea urchin embryos are small and are best observed using a microscope. The magnification of the images from this activity is 500x. That means that the actual embryos are 500 times smaller than the embryos in the pictures. The actual diameter of a sea urchin embryo is roughly 0.1 millimeter or 100 microns. This is roughly the diameter of a human hair. That may seem small but the red blood cells in our bodies are much smaller, measuring only 8 microns across.

The photos used in this activity show the first few hours in the life of a new sea urchin. The images were taken as the embryo completed two rounds of cell division over a 2½ hour period. There are six images in all, each taken at a specific time in the early development of the embryo. The embryo begins its development into a new sea urchin by dividing to make many small cells. The individual cells are clearly visible as are the nuclei within each of the new cells. There are times when the nuclei are harder to see against the background of the cytoplasm. This is due to the fact that as the cells divide, the nuclear envelope gradually breaks down so that the DNA can be divided into what will become the two new cells.

Cell division, or mitosis, occurs many, many times as the sea urchin embryo goes through its development until eventually the embryo is a ball of thousands of cells. At this point the embryo is called a blastula and each of the many diploid cells of the embryo is called a blastomere. In developmental biology the process by which a single-cell embryo divides into many blastomeres is known as cleavage. Cleavage allows the formation of many cells in the embryo without increasing the mass of the embryo. This is important because until the embryo has developed into a free-swimming larva and is able to feed itself, there is no input of nutrients or resources from the environment. Different organisms undergo cleavage in different ways. Sea urchin embryos divide by radial cleavage in which regular rows and columns of blastomeres are formed. Another common type of cleavage is spiral cleavage which occurs in organisms ranging from sponges to flatworms. Human embryos undergo a special kind of cleavage called rotational cleavage that is characteristic of mammalian embryos (Gilbert, 2000).

Even after the sea urchin embryo has completed cleavage and divided to form thousands of cells, it will still have far to go before becoming an adult sea urchin. As the cells of the sea urchin embryo divide, the amount of DNA in each nucleus remains the same while the volume of the resulting cells decreases. This results in an increasing ratio of nucleus volume to total cell volume. This ratio is the nuclear/cytoplasmic or N/C ratio and is a central concept in cell and developmental biology. It has important implications for the development of the sea urchin embryo. In most embryos, the first several rounds of cell division occur without any transcription (the process by which messenger RNA is made from the DNA) occurring in the nuclei of the embryonic cells. Instead the embryo uses its own supply of messenger RNA (mRNA) that was provided by the mother to make the proteins needed for development. At some point, however, this supply of stored mRNA derived from the egg cell begins to run low and the embryo must begin transcribing its own mRNA or face death.

The point at which embryonic transcription begins is called the mid-blastula transition (MBT) and is critical for normal devel-

opment (Gilbert, 2000). The onset of MBT is related to the ratio of the quantity of DNA to the quantity of cytoplasm in the cells of the developing embryo (Newport & Kirschner, 1982a; 1982b). The exact molecular mechanism for MBT is an active field of study in biology and is related to the phosphorylation of proteins (Cyclin E and Cyclin-dependent Kinase)) that control the onset of mitosis (Hartley et al., 1997). In medicine, the detection and staging of cancers sometimes require the calculation of the N/C ratio of the suspected cancer cells (Athanassiadou et al., 2000).

As your students calculate the ratio of cell diameter to nuclear diameter they may notice a downward trend. As the cells divide, they become significantly smaller while the nuclei stay about the same size. In the developing embryo the amount of cytoplasm per nucleus (known as the nuclear to cytoplasmic ratio) decreases as each round of cell division occurs. This ratio is normally calculated based on the total volume of a cell as compared to the volume of its nucleus. Here we simplified the calculations by comparing diameters instead of volumes. The N/C ratio starts at 6.3 in Panel #1 (one cell embryo), then drops to 5.3 in Panel #4 (two cell embryo), and finally to 4.0 in Panel #6 (four cell embryo).

Going Further

To make the measurements even more accurate, students can measure the horizontal and vertical diameters (the cells are not perfectly spherical) and determine the average cell diameter. Ask the students to use the average cell diameter to calculate the rough total volume of each cell. This will provide an opportunity to teach about sources of experimental error and the use of approximation in science. Students can compare each group's different values for total cell volume after using the approximation method. Ask your students to calculate by what percentage the ratios of cell to nuclear volume changed using average cell diameter versus simply using a single diameter measurement.

Biologists will use the ratio of cell to nuclear volumes rather than the ratio of diameters to help characterize cells. Your students can do the same calculations with the cells of the sea urchin embryo using the diameters that you have already determined. Ask your students to calculate the volumes of the cells and nuclei using the actual diameters in micrometers, keeping in mind that volume equals $4/3\ \pi\ r^3$ and π can be approximated by 3.14. Have the students compare the ratio of the total volume of the cell to the volume of the nucleus.

Conclusions

In this lesson students have an opportunity to work with biological data in order to practice asking questions about cell and developmental biology. While this lesson is geared for middle and high school students, it can be successfully used with older students as well. The point of this lesson is to provide an opportunity for students to observe cell biological data while practicing the critical skills of careful observation and scientific questioning. Early in the lesson students make detailed observations of a data set and develop their own questions. In the later stages of the lesson the inquiry is guided towards answering a question that requires the use of basic mathematical skills appropriate to the middle and high school life science classroom. Throughout the lesson there are ample opportunities for the teacher to introduce cell biological concepts including cell division and growth, development, the nuclear/cytoplasmic ratio, and more. This lesson provides a model for the integration of highly detailed images of cells into middle and high school classroom instruction in cell biology. We hope that you will find this lesson that integrates science, mathematics, and technology a welcome addition to your repertoire of life science lessons.

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