

iBiology.org Teaching Tools

Anthony Hyman's Lecture Part 1

Teaching Tools were prepared by Lisa Dennison and Anthony Hyman.

Contents

1. Keywords and Terms
 2. Lecture Notes
 3. Recommended Reading
 4. Review Questions
 5. Answers to Review Questions
 6. Discussion Questions
 7. Answers to Discussion Questions
 8. Explain or Teach These Concepts to a Friend
 9. Research the Literature on Your Own
-

1. Keywords and Terms

Cytoplasmic organization, cell division, asymmetric cell division, RNAi, *C. elegans*, genome-wide screening, protein complexes, cell cortex, emergent properties

2. Lecture Notes

How does complexity arise from molecular interactions?

Cells are often 5 or 6 orders of magnitude bigger than the molecules of which they are composed. What are the rules by which individual molecules can interact and create much larger complex structures? This lecture will address this question using the model organism *C. elegans*, a nematode with an embryo that is 50 μ m in diameter.

Advantages of *C. elegans*

The cell lineage of the worm *C. elegans* is invariant. This means that every worm develops the same way, with the exact same cell divisions leading from embryo to adult. The process of cell division itself is also invariant. This reproducibility allows us to study how the cell is organized after fertilization.

Early cell divisions of the *C. elegans* embryo

Two pronuclei, each containing the haploid genome of one parent, are visible in the cytoplasm of the new embryo shortly after fertilization. Following pronuclear migration, the two pronuclei fuse near the middle of the cell and form a mitotic spindle. Mitosis proceeds and the cell divides asymmetrically, such that one cell is smaller than the other, and the fate of each cell is different. One daughter cell will give rise to the germline of the worm, while the other will give rise to somatic cells. The next round of cell division is asynchronous as well as asymmetric, producing a 4-cell embryo approximately 30 minutes after fertilization.

Thus, within this 30-minute window, the *C. elegans* embryo can be used to study:

- a) the basic cell division processes, which are likely to be common to all cells
- b) the process of asymmetric cell division
- c) the timing of cell divisions, and cell cycle duration

Cells reorganize themselves after fertilization

The majority of the cytoplasm of a fertilized egg comes from oocyte, while the sperm functions to trigger the fertilization process and contribute 50% of the DNA. The oocyte in *C. elegans* is a relatively undifferentiated soup of cytoplasm components. Before the first cell division, the cytoplasm must be organized into a complex, choreographed set of events necessary for this process. What are the genes required for this organization and the cell division that follows?

The importance of sequencing and RNAi

C. elegans was the first multi-cellular eukaryotic organism to have its complete genome sequenced. This complete DNA sequence determines the potential catalog of genes, ~20,000 in total, required for the life of the worm. But simply knowing the sequence of the worm's genome is not enough. How can we assign a function to each gene? A key step forward in this cataloging process in worms was the discovery of RNA interference (RNAi), an advancement for which the Nobel Prize was awarded in 2006. The function of an individual gene can be silenced, or essentially turned off using RNAi. Observing the consequences of the removal of a given gene then provides clues as to what the normal function of that gene might be.

How can a gene be silenced using RNAi?

To silence the function of a specific gene using RNAi, double-stranded RNA (dsRNA) matching the sequence of the gene is first made in vitro, in a test tube in the lab. This dsRNA is then introduced into the mother, either by injection or feeding. The enzyme Dicer next chops up the dsRNA into smaller pieces known as small interfering RNAs (siRNAs) that are 21-25 base pairs in length. These siRNAs are then bound by the RISC protein complex, which unwinds them into single-stranded components. These small single-stranded pieces of RNA then bind the complementary portion of the mRNA of the target gene that has been expressed in the worm. Dicer now targets this mRNA for cleavage, effectively removing the mRNA of the gene of interest from the worm. Since mRNA is translated into protein, no more protein is made once the RNAi is introduced. While RNAi destroys mRNA very quickly, it does not destroy the protein that was already made. The levels of protein decline more slowly as the protein is degraded over time, which can complicate subsequent analysis of the missing gene's function. In *C. elegans*, however, it is possible to create embryos that lack a protein of interest entirely because of the way in which oocytes develop.

Embryogenesis and RNAi in *C. elegans*

C. elegans rapidly creates 200 embryos early in its life cycle. Oocytes are made within the gonad of the worm, a syncytial organ where the cytoplasm is not enclosed by cell membranes. New oocytes are created by enclosing some of the cytoplasm within a membrane as it rounds the bend of the gonad. Thus, the maternal cytoplasm can be accessed and altered before an oocyte is created. dsRNA is injected or fed to the mother, degrading the corresponding mRNA.

After time, any remaining protein is flushed out of the gonad, and no new protein is made because the mRNA is missing. At that point, all new oocytes are made in the complete absence of the protein of interest, and the effects of this protein's absence on development can be assessed.

A genome-wide screen to find genes important for cell division

How do we search the 20,000 genes of the worm for those that are important for cell division? RNAi can be used to screen the entire genome for genes whose removal leads to cell division defects. The sequencing of the worm genome was thus a key event in trying to understand cell division, because it made a systematic screen of every gene possible. To carry out this screen, dsRNAs matching every gene were designed, synthesized, and injected into worms. The embryos of those worms were then filmed while they were developing and examined for cell division defects. To ensure that the analysis of each movie was quantitative, not qualitative, cell division defects were separated into 47 different phenotypic categories. For every category, each movie was manually scored with a '1' if there was a defect, and a '0' if there was no defect.

This genome-wide RNAi screen uncovered 800 genes that are required for the first cell division in a *C. elegans* embryo. Organizing the results by phenotypic category revealed that clusters of genes are required for certain phenotypes, pointing to genes that may work together to coordinate different aspects of cell division. However, knowing that a particular set of genes is required for a particular aspect of cell division still does not tell us how the functions of these genes are coordinated to accomplish their respective tasks.

Biology as a hierarchy of organization

Biological systems have a hierarchy of organization. Genes are translated into proteins, but each protein rarely acts alone. One of the first levels of organization in a cell is that of proteins into protein complexes. Protein complexes have a wide variety of functions and possible formations in the cell. Some are polymers, such as microtubules and centrioles. Others are extremely complex supramolecular structures composed of dozens or even hundreds of different proteins, such as ribosomes and nuclear pore complexes.

Proteomics is a term used to describe the large-scale analysis of proteins, protein complexes, and their functions. How do we determine which proteins are in a particular

protein complex? An antibody that specifically binds one protein in the complex can be used to perform an immunoprecipitation, which will pull down the protein and anything else in the same complex. Next, the enzyme trypsin is added to cleave the proteins into smaller peptides. These peptides can then be precisely measured and identified using mass spectrometry.

Proceeding up the organizational hierarchy, protein complexes are organized into even bigger complexes, which are then organized into cellular compartments and organelles. Much less is known about how protein complexes are organized into compartments and organelles. Many cellular compartments are not membranebound and have no obvious structure by electron microscopy; these include centrosomes, kinetochores, nuclear bodies, ribonuclearproteins, and even the cell cortex.

While components in cellular compartments turn over quickly, on the order of minutes, the protein complexes that make up the compartments turn over slowly, on the order of hours. For example, gamma-tubulin is a component of the centrosome, as a part of the gamma tubulin complex. In an experiment wherein gamma-tubulin is labeled with GFP and the entire centrosome is photobleached, fluorescence recovers very quickly, indicating that new gamma-tubulin has arrived at the centrosome. This is likely because new gamma-tubulin complexes have arrived at the centrosome, because the individual subunits within a single gamma-tubulin complex turn over very slowly. Scientists want to understand the rules that keep protein complexes in their respective compartments, despite the fact that they are turning over very quickly.

Emergent properties of collections of individuals at every scale

When individual components are organized into larger groups, interesting new properties and capabilities emerge. Scientists are investigating these emergent properties at every scale, asking:

- What properties of protein complexes emerge from the combination of their proteins?
- What properties of compartments emerge from the combination of their protein complexes?
- What properties of cells emerge from the combination of their compartments?
- What properties of tissues emerge from the combination of their cells?

These questions must be addressed differently at every scale, using different techniques, theories, and microscopy. The next 3 talks in this lecture series will address these various levels of organization in more detail, highlighting the different kinds of microscopy and the unique problems that occur at each level.

3. Recommended Reading

1. The following reading recommendations are from the 5th edition of *Molecular Biology of the Cell*, Alberts et al.
2. For more on protein complexes, see Chapter 3, pages 148-149, 184.
3. For more on mass spectrometry, see Chapter 8, pages 519-521.
4. For more on RNAi, see Chapter 7, pages 495-497, and Chapter 8, pages 571-572.
5. For more on reverse genetics and genetic screens, see Chapter 8, pages 563-576.
6. For more on *C. elegans* development, see Chapter 22, pages 1321-1328.
7. For more on using *C. elegans* to study cell division read sections 1 and 2 of the
8. Cell Division chapter of the Worm Book, here:
http://www.wormbook.org/chapters/www_celldivision/celldivision.html

4. Review Questions

1. What best describes the development of the worm *C. elegans*?
 - a. Each worm develops in a unique way. No two worms have exactly the same cell lineage or total number of cells.
 - b. Each worm has the same, invariant cell lineage, and the process of cell division is also invariant from worm to worm.
 - c. Each worm has the same total number of cell divisions and total cells as an adult, but the process of cell division can vary from one worm to the next.
2. What important biological processes can be studied within the first 30 minutes after fertilization of a *C. elegans* embryo?
 - a. Basic cell division processes

- b. Asymmetric cell division
 - c. Timing and duration of cell divisions
 - d. All of the above
3. Where does the cytoplasm of a fertilized *C. elegans* embryo come from?
- a. The majority comes from the oocyte (the mother)
 - b. The majority comes from the sperm (the father)
 - c. The mother and father each contribute ~50% of the cytoplasm to the embryo
 - d. Newly fertilized embryos have no cytoplasm
4. Approximately how many genes does the *C. elegans* genome contain?
- a. 200
 - b. 2,000
 - c. 20,000
 - d. 200,000
5. RNAi can be used to silence the function of a specific gene, but how? Put the following steps in order.
- a. siRNAs are unwound into single-stranded RNAs
 - b. inject or feed dsRNA to the worm
 - c. siRNAs are bound by the RISC complex
 - d. Dicer cleaves mRNA bound by small RNAs
 - e. synthesize dsRNA in vitro
 - f. single-stranded RNAs bind complementary mRNAs
 - g. Dicer cleaves dsRNA into siRNAs
6. True or False? Once the mRNA transcripts of a gene are destroyed using RNAi, the function of that gene is immediately silenced.
7. Approximately how many genes are required for the first cell division in *C. elegans*, as determined by genome-wide RNAi screening?
- a. 8
 - b. 80
 - c. 800
 - d. 8,000
8. What is an example of a protein complex?
- a. Microtubule

- b. Centriole
 - c. Ribosome
 - d. Nuclear Pore Complex
 - e. All of the above
9. Name 2 features of a non-membrane bound cellular compartment.
10. This lecture discussed the hierarchy of organization in biological systems. Start with “proteins” and work your way up this hierarchy, naming at least 3 other levels of organization in order.

5. Answers to Review Questions

1. b. Each worm has the same, invariant cell lineage, and the process of cell division is also invariant from worm to worm.
2. d. All of the above
3. a. The majority comes from the oocyte (the mother)
4. c. 20,000
5. Synthesize dsRNA in vitro, inject or feed dsRNA to the worm, Dicer cleaves dsRNA into siRNAs, siRNAs are bound by the RISC complex, siRNAs are unwound into single-stranded RNAs, single-stranded RNAs bind complementary mRNAs, Dicer cleaves mRNA bound by small RNAs
6. False. Before the gene can be effectively silenced, any protein that was made prior to the destruction of the mRNA must be degraded.
7. c. 800
8. e. All of the above
9. Answers include: no obvious structure; made up of many protein complexes working together; protein complexes that make up compartments turn over slowly; subunits of protein complexes within the compartment turn over more quickly
10. Proteins → Protein Complexes → Compartments/Organelles → Cells → Tissues

6. Discussion Questions

1. What does it mean to be a “germline” progenitor cell or “somatic” progenitor cell? What parts of the worm will each of these cells produce?
2. What are the big advantages to using RNAi to study biological processes in *C. elegans*? Describe the advantages of RNAi, as well as why *C. elegans* is particularly well suited to this technique.
3. What does it mean to perform a “genetic screen”? Why would a scientist use a screen to begin studying a biological process?
4. In the RNAi screen described in this lecture, observable cell division defects were separated into 47 distinct phenotypic categories. What is the advantage of categorizing the phenotypes this way, as opposed to just noting that a cell division defect exists?

7. Answers to Discussion Questions

1. The germline progenitor cell will produce the gonad of the worm. This includes all of the sperm and egg cells. Somatic cells, derived from the somatic progenitor cell, include all parts of the worm that are not the gonad.
2. RNAi is a very useful tool for studying *C. elegans* because:
 - a. It is easy to administer dsRNA to worms, via injection or feeding.
 - b. RNAi allows scientists to effectively turn off the expression of a specific gene without altering the genome of the worm.
 - c. RNAi can be used to study genes essential to development and cell division by injecting dsRNA into the gonad as oocytes are forming. The resulting embryos will lack the targeted RNA and protein all along, which avoids the complication of protein run-down.
 - d. Large-scale screening is relatively easy with RNAi.
3. A genetic screen aims to identify genes responsible for a particular biological process. In a mutagenesis screen, not described in this lecture, scientists randomly mutagenize the genome of an organism and then examine the resulting genetic mutants for a certain phenotype. The mutated genes that produce the desired phenotype are then identified in a process known as forward genetics. An RNAi screen is an example of reverse genetics, where the identities of the genes being disrupted are known from the outset. A screen is a useful way to begin studying a

biological process because it provides an unbiased, often largescale approach to find any genes that might be involved.

4. Categorizing phenotypic defects when conducting a screen provides more information about the possible function of each gene. For instance, using the categories in the screen described in this lecture, we could learn that a particular gene is important for cytokinesis but not for chromosome segregation. To follow up on the function of this gene, we would then specifically focus on its role in cytokinesis. Furthermore, categorizing phenotypes gives clues as to what genes might work together for specific parts of the biological process in question.

8. Explain or Teach These Concepts to a Friend

1. You have discovered a protein that is localized to the centrosome at specific times within the cell cycle. You know the gene's sequence, and you also have in your possession an antibody that binds specifically to this protein. Explain to your friend how you would study the function of this protein and how you would determine with what other proteins (if any) it forms a complex.
2. You have invented a special microscope that allows you to discover a new phenomenon about *C. elegans* development: the embryos turn a series of 6 different colors after fertilization, always in the same order. Describe to your friend how you would design and carry out a screen to determine which genes are necessary for each step of this remarkable color-changing process.

9. Research the Literature on Your Own

1. This lecture describes an RNAi screen to study cell division in *C. elegans*. RNAi screens have been used to study many other diverse biological processes in *C. elegans*, including aging, stress resistance, neuronal development, and much more. Research the literature to learn more about one such RNAi screen. Describe what phenotypes the researchers were looking for and at least one conclusion from the screen.
2. Protein complexes are one of the first levels of organization within a cell. Two different protein complexes mentioned briefly in this lecture were the gamma-tubulin complex and the Par-polarity complex. Pick one of these complexes and research

the literature to learn more about it. What proteins make up the complex? What is a function of the complex?