CHAPTER 9 THE CELL CYCLE

Scientific Skills Exercise

Teaching objective: Students will practice reading a histogram in this exercise. The students then relate the data to phases of the cell cycle based on relative amounts of DNA.

Teaching tips: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

The method used to measure DNA content in this experiment is flow cytometry, also sometimes called fluorescence-activated cell sorting (FACS). The fluorescent dye (propidium iodide in this example) binds to the cell's DNA, so the strength of the fluorescence signal corresponds to the amount of DNA the cell contains. The flow cytometer instrument passes individual cells through a small tube that counts a cell while recording the cell's size and fluorescence level. The analysis software calculates how many cells in each sample had a given range of fluorescence and graphs the relationship as cell frequency (or cell count) versus fluorescence interval. In the experiment producing the results shown here, the researchers ran 10,000 cells through the flow cytometer for each treatment.

The concept of dependent and independent variables is not obvious in this exercise. The *x*-axis, fluorescence level, is the independent variable: The researcher specifically set the instrument to count cells within each small interval of fluorescence along the *x*-axis. The number of cells thus becomes the dependent variable, as the value on the *y*-axis depends on the fluorescence interval within which cells are being counted. The treatment versus control is a separate level of analysis and is presented in two histograms for that reason.

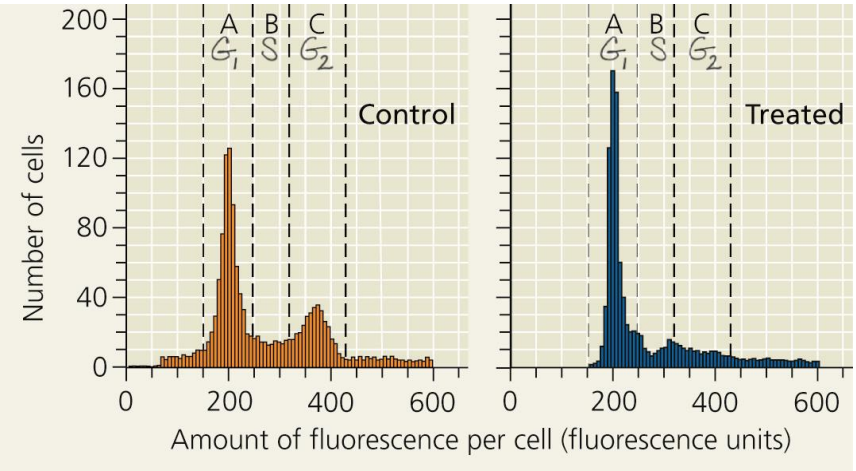
Students may need some help with the idea of frequency data in the form of cell counts. Students may be used to seeing DNA content of cells over time, and may tend to fall into thinking of the *x*-axis as representing time and the height of the peak as representing DNA content. A clearer way for students to think of it is that in the case of the cell cycle data seen here, there are basically two states that most cells will be in: G_1 (less DNA) and G_2 (more DNA). The spread of cells in between these two states represents cells in S phase, transitioning from the low to high amount of DNA. After a cell enters mitosis, it will have the doubled DNA content until it goes through cytokinesis, at which point it will be counted as two cells, and those cells would immediately show up in the G_1 peak to the left along the *x*-axis. An analogy to use: If you were to count the number of students in two sequential classrooms along a hallway at any one time, you get relatively high head counts for each classroom location. But there will be some students walking from classroom G_1 to classroom G_2; they are in transition between locations and will represent a low head count spread out along the hallway between the two rooms (S phase). The M to G_1 transition would be like teleporting back to the first classroom and never showing up in the hallway at all!

Students may note that while there are distinct peaks for G_1 and G_2, there is still a range of DNA content for each state. This most likely represents differences in dye uptake and binding efficiency of individual cells in the cell population, or damaged cells.

Answers:

1. (a) The relative amount of DNA is shown on the *x*-axis. Because the fluorescent dye is binding to DNA in each cell, the amount of fluorescence corresponds to the amount of DNA (the more DNA, the more fluorescence). (b) The second peak (region C) represents the group of cells with the higher amount of DNA per cell because the cells at the second peak have a higher level of fluorescence. (Note that the peak in C is shorter, because it has fewer cells, but each cell has a higher level of fluorescence, thus more DNA.)

2. (a) The first peak (region A) represents cells in G_1, when the cells have only one copy of DNA; the second peak (region C) represents cells in G_2—as the cell prepares to divide, there are two copies of the DNA; in S (region B), the cells are synthesizing DNA, and so have a quantity of DNA between the G_1 and G_2 phase.



(b) The S-phase group of cells does not have a distinct peak because as the cells transition from G_1 to G_2, they are all "caught" at different stages of DNA synthesis and thus have different amounts of fluorescing dye/DNA present.

3. (a) The G_1 phase of the cell cycle has the greatest number of cells because the highest peak in the treated sample corresponds to the lower fluorescence level, comparable to the G_1 cells in the control sample. (b) Most of the treated cells are in G_1 instead of in S or G_2, which strongly suggests that treatment causes the cell cycle to arrest at G_1. (c) The G_1 checkpoint might be inhibited; molecules could be affected at any stage of the relevant signal transduction pathway (signaling molecules, receptors, second messengers, or proteins involved in the cellular response). For example, the inhibitor could block action of a G_1 checkpoint protein or a cyclin. The inhibitor could block DNA replication at an early step while allowing other later events to occur, so the cells "look" like they're still in G_1 on the graph when they are really past the G_1 checkpoint. Or the inhibitor could be acting as a signal that triggers the cell to enter G_0.

Suggested Answers for End-of-Chapter Essay Questions

See the general information on grading short-answer essays and the suggested rubric at the beginning of this document.

8. Scientific Inquiry
(a) The motor proteins at the kinetochore ends of kinetochore microtubules walk the chromosomes toward the poles, so these motor proteins must be minus end-directed. In cells in which the "reeling-in" mechanism is observed, plus end-directed motor proteins must be present at the poles. (b) The motor proteins between associated nonkinetochore microtubules must move toward the middle of the spindle to push the microtubules away from each other, so they are plus end–directed.

9. Focus on Evolution
Natural selection favors organisms with attributes that lead to most offspring, and cell division is a crucial function for both unicellular and multicellular eukaryotes. Although the *number* of chromosomes would be correct in each daughter cell, the two cells would *not* contain identical genetic information. Imagine that a cell had ten chromosomes and that they were divided equally in number, five each into two daughter cells, followed by chromosome duplication. Each daughter cell would end up with ten chromosomes but would have double copies of whatever genetic information was on the five chromosomes it happened to receive from the parent cell. We have noted in this chapter, though, that the chromosomes are made up of two sets, one from each

parent. (You'll learn more about this in Chapter 10.) Therefore, if the random division of chromosomes resulted in one parental set going into each daughter cell, the daughter cells might each end up with two complete sets of genetic information. However, the probability of this happening is very low—much like putting five differently colored pairs of socks into a bag, blindly reaching in to pull out five socks, and having them be five different colors. As a consequence, the vast majority of the daughter cells would not have a complete set of genetic information. They would therefore most likely not be viable, and would not be able to survive and reproduce.

10. Focus on Information

Sample key points:

- The continuity of life depends on cells dividing and faithfully passing heritable information to daughter cells.
- The chromosomes of a eukaryotic cell duplicate in the S phase.
- The stages of mitosis include prophase, prometaphase, metaphase, anaphase, and telophase.
- In this sequence of stages, the chromosomes condense, a spindle forms, chromosomes line up
- on the metaphase plate, sister chromatids separate and move to opposite ends, and nuclei re-form.
- Cytokinesis divides the cytoplasm, forming two genetically identical daughter cells.

Sample top-scoring answer:

The continuity of life—reproduction of unicellular organisms and the development, growth, and repair of multicellular organisms—depends on the transmission of identical copies of heritable information as cells divide. The DNA of a eukaryotic cell is divided among several chromosomes. In the S stage of the cell cycle, DNA is replicated, and each duplicated chromosome now consists of identical sister chromatids. Following a G_2 phase of growth, the cell divides. In the highly orchestrated stages of mitosis (prophase, prometaphase, metaphase, anaphase, and telophase), the chromatin of the duplicated chromosomes condenses, a mitotic spindle forms, and spindle microtubules attach to the kinetochore of each sister chromatid, moving the duplicated chromosomes to the metaphase plate. The sister chromatids separate and move to opposite ends of the cell. Nuclear envelopes re-form, completing mitosis, and cytokinesis divides the cytoplasm. A cell has divided to form two genetically identical cells.

11. Synthesize Your Knowledge

Cancer cells divide without being subject to the usual cell cycle controls. They can divide in the absence of growth factors or other molecules that are necessary for normal cells to pass particular checkpoints. Furthermore, cancer cells are not inhibited by density and do not require anchorage to a surface in order to divide. They may not respond appropriately to signals that normally trigger apoptosis, such as those resulting from mistakes in DNA replication. The underlying basis for this altered behavior is a series of genetic and cellular changes, including mutations in genes whose protein products normally regulate the cell cycle. These gene products are often proteins that function in cell signaling pathways.