CELL DIVISION: MITOSIS AND MEIOSIS

How do eukaryotic cells divide to produce genetically identical cells or to produce gametes with half the normal DNA?

BACKGROUND

One of the characteristics of living things is the ability to replicate and pass on genetic information to the next generation. Cell division in individual bacteria and archaea usually occurs by binary fission. Mitochondria and chloroplasts also replicate by binary fission, which is evidence of the evolutionary relationship between these organelles and prokaryotes.

Cell division in eukaryotes is more complex. It requires the cell to manage a complicated process of duplicating the nucleus, other organelles, and multiple chromosomes. This process, called the cell cycle, is divided into three parts: interphase, mitosis, and cytokinesis (Figure 1). Interphase is separated into three functionally distinct stages. In the first growth phase (G1), the cell grows and prepares to duplicate its DNA. In synthesis (S), the chromosomes are replicated; this stage is between G1 and the second growth phase (G2). In G2, the cell prepares to divide. In mitosis, the duplicated chromosomes are separated into two nuclei. In most cases, mitosis is followed by cytokinesis, when the cytoplasm divides and organelles separate into daughter cells. This type of cell division is asexual and important for growth, renewal, and repair of multicellular organisms.

Cell division is tightly controlled by complexes made of several specific proteins. These complexes contain enzymes called cyclin-dependent kinases (CDKs), which turn on or off the various processes that take place in cell division. CDK partners with a family of proteins called cyclins. One such complex is mitosis-promoting factor (MPF), sometimes called maturation-promoting factor, which contains cyclin A or B and cyclin-dependent kinase (CDK). (See Figure 2a.) CDK is activated when it is bound to cyclin, interacting with various other proteins that, in this case, allow the cell to proceed from G2 into mitosis. The levels of cyclin change during the cell cycle (Figure 2b). In most cases, cytokinesis follows mitosis.
Cyclins and CDKs do not allow the cell to progress through its cycle automatically. There are three checkpoints a cell must pass through: the G₁ checkpoint, G₂ checkpoint, and the M-spindle checkpoint (Figure 4). At each of the checkpoints, the cell checks that it has completed all of the tasks needed and is ready to proceed to the next step in its cycle. Cells pass the G₁ checkpoint when they are stimulated by appropriate external growth factors; for example, platelet-derived growth factor (PDGF) stimulates cells near a wound to divide so that they can repair the injury. The G₂ checkpoint checks for damage after DNA is replicated, and if there is damage, it prevents the cell from going into mitosis. The M-spindle (metaphase) checkpoint assures that the mitotic spindles or microtubules are properly attached to the kinetochores (anchor sites on the chromosomes). If the spindles are not anchored properly, the cell does not continue on through mitosis. The cell cycle is regulated very precisely. Mutations in cell cycle genes that interfere with proper cell cycle control are found very often in cancer cells.

As shown in Figure 3, different CDKs are produced during the phases. The cyclins determine which processes in cell division are turned on or off and in what order by CDK. As each cyclin is turned on or off, CDK causes the cell to move through the stages in the cell cycle.

Learning Objectives

- To describe the events in the cell cycle and how these events are controlled
THE INVESTIGATIONS

These questions are designed to see how well you understand and can explain the key concepts related to cell division before you begin your investigations.

1. How did you develop from a single-celled zygote to an organism with trillions of cells? How many mitotic cell divisions would it take for one zygote to grow into an organism with 100 trillion cells?

2. How is cell division important to a single-celled organism?

3. What must happen to ensure successful cell division?

4. How does the genetic information in one of your body cells compare to that found in other body cells?

5. What are some advantages of asexual reproduction in plants?

6. Why is it important for DNA to be replicated prior to cell division?

7. How do chromosomes move inside a cell during cell division?

8. How is the cell cycle controlled? What would happen if the control were defective?

Part 1: Counting Cells and Analyzing Data

1. Observe the cells at high magnification (400-500 X).
2. Look for well-stained, distinct cells.
3. Observe every cell in one high power field of view and determine which phase of the cell cycle it is in. This is best done in pairs. The partner observing the slide calls out the phase of each cell while the other partner
records. Then switch so the recorder becomes the observer and vice versa. Count at least three full fields of view. If you have not counted at least 200 cells, then count a fourth field of view.

4. Sketch and label a representative cell of interphase and each stage of mitosis in the space provided:

Table 1: Phases of the Cell Cycle

<table>
<thead>
<tr>
<th>Interphase</th>
<th>Prophase</th>
<th>Metaphase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anaphase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Telophase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

5. Collect the individual and class data for each group, and calculate the mean and standard deviation for each group.
6. Compare the number of cells from each group in interphase and in mitosis.
7. Use a chi-square distribution test to statistically analyze the data.
8. Calculate the percentage of cells in each phase.

Consider that it takes, on average, 24 hours (or 1,440 minutes) for onion root-tip cells to complete the cell cycle. You can calculate the amount of time spent in each phase of the cell cycle from the percent of cells in that stage. Percent of cells in stage \( \times \) 1,440 minutes = minutes of cell cycle spent in stage

Table 2 Individual Data:

<table>
<thead>
<tr>
<th>Field 1</th>
<th>Field 2</th>
<th>Field 3</th>
<th>Total</th>
<th>Percent of total cells</th>
<th>Time in each stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Prophase | Metaphase | Anaphase | Telophase  
|--------|----------|---------|-----------

Total cells:  

**Table 3 Class Data:**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Percent of total cells</th>
<th>Time in each stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prophase</td>
<td></td>
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<td>Anaphase</td>
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</tr>
<tr>
<td>Telophase</td>
<td></td>
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</tr>
</tbody>
</table>

Total cells:  

Use the data in the adjacent table as your expected values for comparison to perform a Chi-square analysis of the data collected from your mitosis observations.

1. Enter the number of treated cells in interphase and mitosis as observed (o).
2. Calculate the percentage of cells in interphase and mitosis in the control group from Table 2.
3. Multiply the percentages by the total number of cells in the treated group; this will give the expected numbers (e).
4. Calculate the chi-square ($\chi^2$) value for the test.
5. Compare this value to the critical value in Table 5.
1. The degrees of freedom (df) equals the number of groups minus one. In this case, there are two groups, interphase and mitosis; therefore, df = 2 - 1 or 1.

2. The p value is 0.05, and the critical value is 3.84. If the calculated chi-square value is greater than or equal to this critical value, then the null hypothesis is rejected. If the calculated chi-square value is less than this critical value, the null hypothesis is not rejected.

**DESIGNING AND CONDUCTING YOUR INVESTIGATION**

Now that you have worked with the root tip model system, design and conduct an investigation to determine what biotic or abiotic factors or substances in the environment might increase or decrease the rate of mitosis in roots. For instance, what factors in the soil might affect the rate of root growth and development? Consider, for example, abiotic soil factors such as salinity and pH or biotic factors, including roundworms, that might alter root growth.

**Part 2: Effects of Environment on Mitosis**

Scientists reported that a fungal pathogen, may negatively affect the growth of soybeans (*Glycine max*). Soybean growth decreased during three years of high rainfall, and the soybean roots were poorly developed. Close relatives of *R. anaerobis* are plant pathogens and grow in the soil. A lectin-like protein was found in the soil around the soybean roots. This protein may have been secreted by the fungus. Lectins induce mitosis in some root apical meristem tissues. In many instances, rapid cell divisions weaken plant tissues.

You have been asked to investigate whether the fungal pathogen lectin affects the number of cells undergoing mitosis in a different plant, using root tips.

9. What is your experimental hypothesis? Your null hypothesis? Are these the same?
10. How would you design an experiment with onion bulbs to test whether lectins increase the number of cells in mitosis?

Independent variable: _____________________________

Dependent variable: _____________________________

11. What would you measure, and how would you measure it?

12. What appropriate controls for your experiment?

Controlled experiment: ___________________________

Controlled variables: ___________________________

Review from Part 1 & 2

13. How is the cell cycle controlled in normal cells?

14. What are cyclins and cyclin-dependent kinases? What do these proteins do in a cell?

15. What was the importance of collecting the class data?

16. Does an increased number of cells in mitosis mean that these cells are dividing faster than the cells in the roots with a lower number of cells in mitosis?

17. What other way could you determine how fast the rate of mitosis is occurring in root tips?
18. If your observations had not been restricted to the area of the root tip that is actively dividing, how would your results have been different?

19. Why is it more accurate to call mitosis "nuclear replication" than cell division?

Part 3: Loss of Cell Cycle Control in Cancer
Many of us have family members who have or have had cancer. Cancer can occur when cells lose control of their cell cycle and divide abnormally. This happens when tumor suppressor genes, such as p53 or Rb (retinoblastoma), are mutated. There are many questions you should consider before beginning your investigation.

20. How are normal cells and cancer cells different from each other?

21. What are the main causes of cancer?

22. What goes wrong during the cell cycle in cancer cells?

23. What makes some genes responsible for an increased risk of certain cancers?

24. Do you think that the chromosomes might be different between normal and cancer cells?

The last question is the focus of this part of the lab. With your group, form a hypothesis as to how the chromosomes of a cancer cell might appear in comparison to a normal cell and how those differences are related to the behavior of the cancer cell.

25. Hypothesis: _____________________________________________________________________

For each of the following cases, look at pictures of the chromosomes (karyotype) from normal human cells. Compare them to pictures of the chromosomes from cancer cells. For each case, count the number of chromosomes in each type of cell, and discuss their appearance. Then answer the following questions.

26. Do your observations support your hypothesis?

27. If not, what type of information might you need to know in order to understand your observations?

28. If yes, what type of information can you find that would validate your conclusions?
**Case 1: HeLa cells**

HeLa cells are cervical cancer cells isolated from a woman named Henrietta Lacks. Her cells have been cultured since 1951 and used in numerous scientific experiments. Henrietta Lacks died from her cancer not long after her cells were isolated. Lacks's cancer cells contain remnants of human papillomavirus (HPV), which we now know increases the risk of cervical cancer. The following diagrams are karyotypes of HeLa cells.

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29. What happens in a normal cell if the DNA has mutations?

__________________________________________________________________________________

30. What would happen if cells with mutated DNA replicate?

__________________________________________________________________________________

31. How do cells monitor DNA integrity?

__________________________________________________________________________________

32. From your observations, what went wrong in Henrietta Lacks's cervical cells that made them cancerous?

__________________________________________________________________________________

__________________________________________________________________________________

33. How does infection with human papillomavirus virus (HPV) increase the risk of cervical cancer?

__________________________________________________________________________________

__________________________________________________________________________________
**Case 2: Philadelphia Chromosomes**

In normal cells, mitosis usually is blocked if there is DNA damage. Sometimes, though, DNA damage makes cells divide more often. Certain forms of leukemia have a unique feature called a Philadelphia chromosome. Look at the photos of the karyotype of leukemia cells in the following Figure:

34. How are the chromosomes different in the cancer cells compared to normal cells?

35. How could these differences lead to cancer?

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**Part 4: Modeling Meiosis**

Meiosis resembles mitosis but serves a very different purpose. Meiosis is a cell division resulting in the halving, or reduction, of chromosome number in each cell. A diploid organism has two sets of chromosomes (2n), while a haploid cell or organism has one set (1n). Meiosis produces gametes (ova and sperm) in animals and spores in fungi, plants, and protists. Three other important characteristics of meiosis are the exchange of genetic material ("crossing over") between homologous chromosomes, the independent assortment of the chromosomes, and the separation of alleles of the same gene (Figure 6). These characteristics, along with random fertilization, increase the genetic variability in the offspring. These mechanisms are essential to our understanding of genetics and evolution in sexually reproducing organisms.

The hallmark of sexual reproduction is the great diversity seen in the gametes and in the resulting offspring produced by fertilization. Meiosis is integral to this process because this type of cell division produces the sex cells, gametes. Before you begin the modeling exercise, your teacher will ask you to discuss these questions.
36. How do sexually reproducing organisms produce gametes from diploid progenitors?
__________________________________________________________________________________
__________________________________________________________________________________

37. How does the process increase gamete diversity? ____________________________
__________________________________________________________________________________
__________________________________________________________________________________

38. What are the outcomes from independent assortment and crossing over?
__________________________________________________________________________________
__________________________________________________________________________________

39. How does the distance between two genes or a gene and a centromere affect crossover frequencies
__________________________________________________________________________________
__________________________________________________________________________________

40. When is the DNA replicated during meiosis? ____________________________
__________________________________________________________________________________

41. Are homologous pairs of chromosomes exact copies of each other? ____________________________
__________________________________________________________________________________

42. What is crossing over? ____________________________
__________________________________________________________________________________

43. What physical constraints control crossover frequencies?
__________________________________________________________________________________
__________________________________________________________________________________

44. What is meant by independent assortment?
__________________________________________________________________________________
__________________________________________________________________________________

45. How can you calculate the possible number of different kinds of gametes?
__________________________________________________________________________________
__________________________________________________________________________________

46. What happens if a homologous pair of chromosomes fails to separate, and how might this contribute to genetic disorders such as Down syndrome and cri du chat syndrome?
__________________________________________________________________________________
__________________________________________________________________________________

47. How are mitosis and meiosis fundamentally different?
__________________________________________________________________________________
__________________________________________________________________________________
Part 5: Meiosis and Crossing Over in Sordaria

The fungus *Sordaria fimicola* exchanges genetic material when two mycelia meet and fuse. The resulting zygote undergoes meiosis to produce asci; each ascus contains eight haploid spores. A single gene determines the spore color.

*Sordaria fimicola* is an ascomycete fungus that can be used to demonstrate the results of crossing over during meiosis. *Sordaria* is a haploid organism for most of its life cycle. It becomes diploid only when the fusion of the mycelia of two different strains results in the fusion of the two different types of haploid nuclei to form a diploid nucleus. The diploid nucleus must then undergo meiosis to resume its haploid state.

Meiosis, followed by mitosis, in *Sordaria* results in the formation of eight haploid ascospores contained within a sac called an ascus (plural, asci). Many asci are contained within a fruiting body called a perithecium. When ascospores are mature the ascus ruptures, releasing the ascospores. Each ascospore can develop into a new haploid fungus. The life cycle of *Sordaria fimicola* is shown in Figure 7.

A cross was made between wild type (+; black) and tan (tn) strains. The resulting zygote produces either parental type asci, which have four black and four tan spores in a row (4:4 pattern), or recombinant asci, which do not have this pattern.

48. How do you explain the differences between the recombinant asci and the parental types?

__________________________________________________________________________________

_________________________________________________________

49. What meiotic event can account for this difference?

______________________________________

50. Using the model chromosomes from Part 4, predict the possible meiotic outcomes.

__________________________________________________________________________________

**Figure 8**

Formation of non-crossover asci

To observe crossing over in *Sordaria*, one must make hybrids between wild-type and mutant strains of *Sordaria*. Wild-type (+) *Sordaria* have black ascospores. One mutant strain has tan spores (tn). When mycelia of these two different strains come together and undergo meiosis, the asci that develop will contain four black ascospores and four tan ascospores. The arrangement of the spores directly reflects whether or not crossing over has occurred.

In Figure 8, no crossing over has occurred. Figure 9 shows the results of crossing over between the centromere of the chromosome and the gene for ascospore color.
Two homologous chromosomes line up at metaphase I of meiosis. The two chromatids of one chromosome each carry the gene for tan spore color (tn) and the two chromatids of the other chromosome carry the gene for wild-type spore color (+). The first meiotic division (MI) results in two cells each containing just one type of spore color gene (either tan or wild-type). Therefore, segregation of these genes has occurred at the first meiotic division (MI). The second meiotic division (MII) results in four cells, each with the haploid number of chromosomes (IN).

A mitotic division simply duplicates these cells, resulting in 8 spores. They are arranged in the 4:4 pattern.

**Figure 9**

In this example, crossing over has occurred in the region between the gene for spore color and the centromere. The homologous chromosomes separate during meiosis I. This time, the MI results in two cells, each containing both genes (1 tan, 1 wild-type); therefore, the genes for spore color have not yet segregated. Meiosis II (MII) results in segregation of the two types of genes for spore color. A mitotic division results in 8 spores arranged in the 2:2:2:2 or 2:4:2 pattern. Any one of these spore arrangements would indicate that crossing over has occurred between the gene for spore coat color and the centromere.

Two strains of *Sordaria* (wild-type and tan mutant) have been inoculated on a plate of agar. Where the mycelia of the two strains meet (Figure 10), fruiting bodies called perithecia develop. Meiosis occurs within the perithecia during the formation of asci. Prepare a wet mount of some perithecia (the black dots in figure 10).

**Figure 10**

1. **Use the following picture(s) directed by your teacher to determine the % crossing over.** Count at least 50 asci, and score them as either parental or recombinant (crossing over).

2. Enter the data in Table 6 and make the calculations. One map unit equals one recombinant per 100 total events. The percentage of asci showing crossover divided by 2 equals the map units in this activity. This is done because each spore produced by meiosis undergoes a mitotic division.
51. The published map distance between the gene and the centromere is 26 map units. How did the class data compare with this distance? ________________________________

52. What can account for disparities between the class data and the published data? ____________________________

The frequency of crossing over appears to be governed largely by the distance between genes, or in this case, between the gene for spore coat color and the centromere. The probability of a crossover occurring between two particular genes on the same chromosome (linked genes) increases as the distance between those genes becomes larger. The frequency of crossover, therefore, appears to be directly proportional to the distance between genes.
A map unit is an arbitrary unit of measure used to describe relative distances between linked genes. The number of map units between two genes or between a gene and the centromere is equal to the percentage of recombinants. Customary units cannot be used because we cannot directly visualize genes with the light microscope. However, due to the relationship between distance and crossover frequency, we may use the map unit.

Evaluating Results

53. Why did you divide the percentage of asci showing crossover (recombinant) by 2? _______________________

54. The published map distance between the spore color gene and the centromere is 26 map units. How did the class data compare with this distance? _______________________

55. How can you account for any disparities between the class data and the published data? ________________

56. Illustrate what happened during meiosis to produce the results you found.

57. Do you think the Philadelphia chromosome is a result of crossing over as seen in this part of the investigation or some other chromosomal abnormality? Explain your answer: _______________________

58. Do you think the cell cycle described for mitosis could be applied to meiosis as well? Explain your answer.

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