

# **iBiology.org Teaching Tools**

## **David Morgan's Lecture Part 1:**

### **An Introduction to Cell Cycle Regulation**

Teaching Tools were prepared by David Morgan.

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#### **1. Keywords and Terms**

Cell cycle, cell division, chromosome, mitosis, sister chromatid, metaphase, anaphase, cytokinesis, protein kinase, cyclin-dependent kinase, ubiquitin ligase, anaphase-promoting complex

#### **2. Lecture Notes**

Why do we care about cell division?

All living things are composed of cells. The growth, development, and survival of all organisms depends on their ability to produce new cells. The production of new cells is

also important in human health: one of our major diseases, cancer, is essentially a disease of excess cell number.

How are new cells made? When it was first realized in the early 19<sup>th</sup> century that cells make up all living things, prominent scientists argued that new cells were assembled in the intercellular fluid or inside other cells. By the 1850s, however, it became clear that all new cells arise by the duplication and division of pre-existing cells. Thus, all cells in existence today are derived by division from some ancestral cell that divided billions of years ago.

## Cells reproduce by a series of events called the cell cycle

Cell reproduction depends on two processes: (1) duplication of the cell's components, and (2) distribution of those components into two daughter cells. It is particularly important that the chromosomes containing the cell's genetic information are duplicated and distributed precisely, to ensure that each daughter is genetically identical.

The processes of duplication and division are divided into a series of events called the cell cycle. Chromosomes are duplicated in S phase, while the duplication of most other components (organelles, proteins, etc.) occurs continuously throughout the cycle. The duplicated chromosomes and other components are distributed equally into two daughter cells during M phase. M phase includes two major events. During mitosis, or nuclear division, the duplicated chromosomes are separated and packaged into individual daughter nuclei. During cytokinesis, the entire cell divides to distribute the daughter nuclei and other components into a pair of daughter cells. In most cells, gap phases exist between S and M phases: G1 before S phase, and G2 before M phase.

Mitosis is a dramatic and beautiful process that depends on a protein machine called the mitotic spindle, a bipolar array of protein polymers called microtubules, which radiate outward from two organizing centers, or spindle poles. In early mitosis, the duplicated chromosomes (called sister-chromatid pairs), are attached to the mitotic spindle with one sister attached to each pole of the spindle. In metaphase, the entire set of sister-chromatid pairs is aligned at the middle of the spindle. At anaphase, the sisters detach from one another and are pulled by the microtubules of the spindle to opposite ends of the cell – where they are then packaged in new nuclei and distributed into the daughter cells by cytokinesis.

## Cell cycle events are controlled by a complex regulatory system

A key problem in biology is how the events of the cell cycle are controlled, to ensure that they occur at the correct time and in the correct order, and are coordinated with each other to ensure that later events do not occur until preceding events are completed.

This problem has been studied in many model experimental organisms, including the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. Yeasts are particularly powerful experimental systems because of the ease with which it is possible to carry out genetic screens or manipulate gene expression and other processes in the cell. Studies in budding yeast led to the identification of the first 'cell-division cycle' or *cdc* mutants: mutants that fail to progress past a specific cell cycle stage. Important work has also been done with the eggs and early embryonic cells of the frog *Xenopus laevis*; these cells are so large that it is possible to inject them with test substances or isolate their cytoplasm and recreate many features of cell cycle control in a test tube.

Studies in frog embryos first revealed that cell-cycle control depends on a regulatory system that is essentially independent of the events it controls. Further work revealed that this cell-cycle control system is based on a linked series of biochemical switches that trigger progress through several major regulatory transitions: the Start transition, where commitment to a new cell cycle is controlled in late G1; the G2/M transition, where entry into mitosis is controlled; and the metaphase-anaphase transition, where the initiation of anaphase is controlled.

Progress through the major cell-cycle transitions is regulated by numerous intra- and extra-cellular signals, which are often called checkpoint mechanisms. Entry into a new cell cycle at Start occurs only when environmental conditions are appropriate for cell reproduction. Entry into mitosis at the G2/M transition is allowed only when S phase has been completed successfully, ensuring that mitosis does not occur until the chromosomes are duplicated. Similarly, the metaphase-anaphase switch is triggered only when all sister-chromatid pairs are correctly aligned on the mitotic spindle. By

these and other mechanisms, cell-cycle events are coordinated with each other and with environmental conditions.

The central components of the control system are cyclin-dependent kinases and the anaphase-promoting complex

The key components of the cell-cycle control system were first revealed in studies of yeasts and frogs. These studies told us that the master regulators of the cell cycle are protein kinases called the cyclin-dependent kinases or Cdks. Protein kinases are common signaling enzymes that catalyze the attachment of phosphate to amino acid residues on their substrates, thereby altering the function of the substrate. Cdks, as their name implies, are activated upon binding to regulatory subunits called cyclins. Several different cyclins are present in the cell, and their levels rise and fall at different cell cycle stages, resulting in the formation of an ordered series of Cdk-cyclin complexes that initiate the events of the cell cycle. When activated, Cdk-cyclin complexes phosphorylate large numbers of proteins in the cell to bring about the events of chromosome duplication in S phase and chromosome segregation in M phase.

Cdks are responsible for driving the cell to metaphase, when the sister-chromatid pairs are aligned on the mitotic spindle. Progress beyond this point requires a different regulator called the anaphase-promoting complex or cyclosome (APC or APC/C). The APC is an enzyme called a ubiquitin ligase which promotes the attachment of multiple copies of a small protein called ubiquitin to other proteins. These ubiquitins are recognized by a large protease in the cell called the proteasome, which destroys the ubiquitin-tagged protein. By this mechanism, the APC triggers the destruction of several regulatory proteins that control the onset of anaphase.

A key target of the APC is a protein called securin. Securin destruction leads to activation of a protease called separase, which cleaves a protein complex that holds the sister chromatids together. The APC also promotes the destruction of cyclins, resulting in the loss of Cdk activity in anaphase and late mitosis. Cdk inactivation is important at this point because it allows the many substrates of Cdks to become dephosphorylated, which is essential for the cell to complete mitosis and enter the following G1.

### 3. Recommended Reading for the Classroom

These three sources provide overviews of cell cycle events and their control, ranked in order of increasing depth:

1. Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. *Essential Cell Biology*, 3<sup>rd</sup> edition. New York: Garland Science, 2010. Chapter 18.
2. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. *Molecular Biology of the Cell*, 5<sup>th</sup> edition. New York: Garland Science, 2008. Chapter 17.
3. Morgan, D.O. *The Cell Cycle: Principles of Control*. London: New Science Press, 2007.

### 4. Review Questions

1. What are the two major phases of the cell division cycle, and what are the events that occur in those stages?
2. Why are yeasts so useful for the analysis of fundamental cellular processes like cell division?
3. Where does the cell cycle arrest if chromosomes are not aligned properly on the mitotic spindle?
4. Why does the activity of cyclin-dependent kinases oscillate in the cell cycle?
5. How do cyclin-dependent kinases trigger cell cycle events?
6. How does the ubiquitination of a protein lead to its destruction?
7. How does the anaphase-promoting complex initiate anaphase?

## 5. Answers to Review Questions

1. S phase (chromosome duplication) and M phase (mitosis and cytokinesis).
2. There are many reasons. Yeasts can grow in a haploid state in which it is easy to analyze the effects of gene mutations without interference from a second gene copy. Yeasts have a short generation time (~90 min) that makes it easy to study their cell cycle. They have high rates of gene recombination, which makes it easy to knock out genes or manipulate gene expression in various ways. They can be grown in large amounts, allowing the preparation of large quantities of proteins for detailed biochemical analysis.
3. Metaphase (i.e. before the metaphase-anaphase transition). This arrest depends on a regulatory system called the spindle assembly checkpoint, which monitors the attachment of sister chromatids to the spindle. If correct attachment is not achieved, the unattached chromosome produces a negative signal that inhibits the APC, thereby preventing the initiation of anaphase.
4. Cdk oscillations depend primarily on changes in the cellular concentration of cyclins. Cdk activity is also regulated by phosphorylation at inhibitory and stimulatory sites on the Cdk subunit, and also by inhibitory proteins that bind and inhibit cyclin-Cdk complexes at certain cell-cycle stages.
5. Cdks are protein kinases, which act by phosphorylating large numbers of specific substrates in the cell. Phosphorylation changes some feature of the activity of the substrate, resulting in the initiation of cell cycle events.
6. The attachment of multiple ubiquitins to a protein, often in the form of ubiquitin chains, results in the protein being recognized by a large, multi-subunit protease called the proteasome. The proteasome binds the ubiquitinated protein, unfolds it, and chops it into short pieces. The ubiquitin is not destroyed and is recycled for further use.
7. The APC is a ubiquitin ligase that attaches ubiquitin to numerous proteins, triggering their destruction in the proteasome. The key targets of the APC are securin, the destruction of which initiates sister-chromatid separation, and cyclin, the destruction of which causes Cdk inactivation and the exit from mitosis.

## 6. Discussion Questions

1. Why is cell reproduction important? In other words, what is the evolutionary advantage for a cell (or organism) that is able to reproduce?
2. Imagine you know nothing about where new cells come from. What are some of the possible mechanisms by which a new cell could be built? How would you begin to address this problem?
3. In the early days of cell biology, confusion resulted from the fact that cells in some tissues seemed to divide into four daughters, not two. What sort of tissues would this be?
4. Chromosomes are duplicated precisely once in S phase. What other major cellular component is also duplicated precisely once per cell cycle, and why?
5. When does the cell duplicate its major membrane-bounded organelles, such as the mitochondria, endoplasmic reticulum, and Golgi apparatus, and how does the cell ensure that daughter cells receive an equal share of these organelles?
6. When chromosomes are duplicated in S phase, they are held together by a protein complex called cohesin. As a result, the sister-chromatid pairs are tightly linked when the cell enters mitosis. What is the advantage to the cell of keeping the sisters linked when they reach mitosis?
7. Unicellular organisms like yeast divide as rapidly as possible when abundant nutrients are available, but they stop dividing when conditions are not ideal. At what point in the cell cycle do these cells arrest? Why is this an ideal point at which to stop progress? How might environmental nutrients influence progression through this point?
8. In multicellular organisms, cells divide only when a tissue needs new cells; in many tissues, the rate of cell division is quite low. Cancer is a disease in which cells divide inappropriately, and is often promoted by mutations that influence the cell cycle control system. Which regulatory transition in the cell cycle is most commonly influenced by cancer mutations? What effects do you expect in cells carrying mutations that stimulate inappropriate progression through other cell cycle transitions?

9. When a cell commits itself to progression through a cell-cycle transition like the metaphase-anaphase transition, it does so in an all-or-none, irreversible manner. Why is it important for the cell to make these total commitments?
10. Cells cannot proliferate if they carry mutations that block cell division. How was it possible for yeast geneticists to produce and study mutations in genes that are essential for cell division?

## 7. Answers to Discussion Questions

1. The ability to reproduce is a defining feature of all living things. No matter how complex or beautiful, a cell (or any organized 'living' entity) cannot exist for long if it cannot reproduce. A cell that is able to reproduce can increase its population size and thus compete for limited resources more effectively than a cell that does not divide or divides less frequently. The evolution of large, complex organisms would clearly have been difficult or impossible without cell reproduction to provide the cells that form specialized tissues. Because chromosome duplication and segregation inevitably cause a low frequency of genetic changes in the daughter cells, the process of cell reproduction also helps provide the random variation that fuels the natural selection of more successful descendants. The evolution of all living things is therefore greatly facilitated by the ability of cells to reproduce.
2. When cells were first analyzed in detail in the early 19<sup>th</sup> century, most scientists believed that new cells were simply assembled from their component parts in the intercellular space – in the same way that we humans make our complex machines. As is often the case in biology, the key to understanding this problem came from simply looking carefully at cells with microscopes, which eventually revealed that new cells always arise by the division of an existing cell into two daughters. Thus, cells are not like typical man-made machines: they have evolved from the beginning to be able to duplicate and divide. Imagine building a Boeing 747 that could do this!
3. In sexually reproducing organisms, most cells are diploid: that is, they contain two slightly different copies (homologs) of each chromosome – one from each parent. The production of haploid gametes in the reproductive tissues of these organisms involves a specialized form of nuclear division called meiosis, in which a cell goes through a single round of chromosome duplication and then two rounds of chromosome segregation to produce four haploid gametes, each containing one homolog of each chromosome.



4. Most animal cells contain a large protein structure called a centrosome, which serves as the central organizing center for microtubules: most microtubules in the cell are anchored in the centrosome. Early in the cell cycle, the centrosome is duplicated precisely once, so that the cell enters mitosis with two centrosomes. The centrosomes then provide the two radiating sources of microtubules at the two poles of the mitotic spindle. It is important to enter mitosis with only two centrosomes, to ensure that the mitotic spindle is bipolar. Cells with too many centrosomes can sometimes form multipolar spindles, and this can result in errors in the attachment and segregation of sister chromatids.
5. These organelles, as well as most of the other components of the cell (except chromosomes and the centrosome) grow continuously throughout the cell cycle, so that they double in size by the time the cell reaches M phase. Membranes of the endoplasmic reticulum and Golgi apparatus break up into smaller pieces in mitosis, and these pieces are distributed in the cytoplasm around the spindle poles; as a result, they are distributed roughly equally when the cell is cleaved in two by cytokinesis. It is not essential that the daughter cells receive exactly the same amounts of these organelles and other components. In fact, there are many examples in animal development of asymmetric cell divisions, in which the two daughters receive different amounts of cytoplasmic components (but always the correct number of chromosomes and a centrosome).
6. The easiest way to think about this problem is to imagine what would happen if duplicated chromosomes separated after they were duplicated, so that they were completely unattached when the cell entered mitosis. How would it then be possible to attach one sister chromatid to one spindle pole and the other sister chromatid to the other spindle pole? By having the two sister chromatids attached, it is relatively straightforward for the cell to evolve mechanisms for achieving the correct bipolar attachment.
7. When extracellular nutrients or other growth factors become limiting, most cells arrest in G1 of the cell cycle, just before the Start transition. Cells with insufficient resources are thereby prevented from making a commitment to a complex, risky, and energetically costly process (cell division). A G1 arrest is preferable to an arrest elsewhere in the cell cycle because the cell then contains a single copy of its genome. Cells arresting in G2, for example, contain two copies of the genome, and prolonged arrest at this point might lead to genetic damage due to inappropriate recombination of chromosomes or other mechanisms. It would be even riskier to arrest the cell cycle in the middle of S phase or mitosis.

External nutrients and other factors typically regulate progression through Start by using various signaling mechanisms to influence activation of the Cdk-cyclin complexes that drive progression through Start. For example, a decrease in external nutrients turns off expression of genes encoding the cyclins that are required for activation of Cdks at Start.

8. Cancer-causing mutations typically act by stimulating the signals that drive entry into the cell cycle at the Start transition. For example, these mutations can cause inappropriate activity in a signaling pathway that responds to external factors that promote cell division. Other cancer mutations cause defects in signals that normally restrain cell division. By such mechanisms, these mutations increase the rate of cell reproduction.

Mutations that cause inappropriate progression through the G2/M transition or the metaphase-anaphase transition are likely to be lethal in many cases and would therefore be less commonly seen in cancer. Successful cell division requires that progress through these transitions occurs only when the cell is ready, and premature progression into mitosis or anaphase might lead to lethal errors in chromosome segregation. On the other hand, minor defects in the control of mitosis can lead to chromosome segregation errors that are not lethal but can cause changes in gene expression that promote excessive cell reproduction.

9. The simplest way to think about this question is to imagine what would happen if the cell initiated a cell cycle event only partly and then gave up and turned back. What would happen, for example, if a cell began to separate its sister-chromatid pairs but then returned to metaphase: the result would likely be lethal errors in chromosome segregation due to partial separation of some but not all chromosomes. Similarly, it is very dangerous for a cell to initiate chromosome duplication and then turn back, because the cell would then contain some chromosome regions that are duplicated and some that are not, and re-entry into S phase under these conditions might eventually lead to errors when these unevenly duplicated chromosomes were pulled apart by the mitotic spindle.
10. It is possible to identify mutations in essential genes by searching for mutants that are conditional: that is, they are functional under one condition and nonfunctional under another. In yeast genetics, the most common approach is to find temperature-sensitive mutations, so that the mutant protein functions normally at one temperature (room temperature) but fails to function at a higher temperature (typically 37 degrees).

## **8. Explain or Teach These Concepts to a Friend**

1. Explain the major events of the cell division cycle.
2. Explain why alignment of sister chromatid pairs on a bipolar mitotic spindle is important to allow equal segregation to the daughter nuclei.
3. Explain the key components of the cell-cycle control system and how they govern cell-cycle events.

## **9. Research the Literature on Your Own**

1. Learn how progression through the Start checkpoint is controlled by the mating pheromone alpha factor in budding yeast.
2. Learn about the mechanisms that ensure that the chromosomes are duplicated once and only once per cell cycle. What mechanisms prevent the cell from accidentally duplicating chromosomes more than once, which might result in daughter cells with incorrect chromosome numbers?
3. Learn how inhibitory phosphorylation of Cdks governs Cdk activation at the G2/M transition. Learn how these mechanisms generate switch-like, irreversible activation of Cdk-cyclin complexes at the beginning of mitosis.
4. Learn how a mechanism called the spindle assembly checkpoint restrains the onset of anaphase when sister chromatids are not properly attached to the spindle.
5. Learn how the cell responds when its DNA is severely damaged, and how DNA damage affects progression through the cell cycle.
6. Learn about the complex series of enzymes involved in protein ubiquitination.
7. Research the latest studies of sister-chromatid cohesion, and assess current models for how the cohesin complex holds sister chromatids together.
8. A major question in cell cycle biology is how cells coordinate cell division with cell growth (i.e. increase in cell mass) to ensure that cell size remains constant. In most

dividing populations of cells, cells double their mass in each cell cycle. What are some potential mechanisms for linking cell size with progress through the cell cycle?