Morgan Lecture 1: Introduction

Key words and terms

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

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 19th 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.

Basic recommended reading for the classroom

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

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. *Essential Cell Biology*, 3rd edition. New York: Garland Science, 2010. Chapter 18.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. *Molecular Biology of the Cell*, 5th edition. New York: Garland Science, 2008. Chapter 17.

Morgan, D.O. *The Cell Cycle: Principles of Control*. London: New Science Press, 2007.

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?

Facilitator 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?

Explain/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.

Research the literature on your own (or suggested assignment)

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?