

Cell Cycle, I

I. Intro to cell cycle If you look through a microscope at a dish full of cultured cells, you can usually see two general cell types. One type, which constitutes about 80-90% of the population, has normal nuclei, and the other 10-20% have strange-looking nuclei with string-like structures lined up and dividing. The strange cells are those undergoing **mitosis**, or cell division, and the normal ones are those in **interphase**, the part of the life cycle that's not mitosis. Together these two phases are referred to as the **cell cycle**. The percentages of cells in each population represent the percent of the cell cycle a given cell spends in each phase, so it spends about 10-20% of its time in mitosis and 80-90% in interphase. In this lecture and the next, we'll see what happens during each phase and consider how the cell gets from one stage to the next. The mechanism of progression through the cell cycle involves an interplay between two systems we've been looking at, phosphorylation and proteolysis.

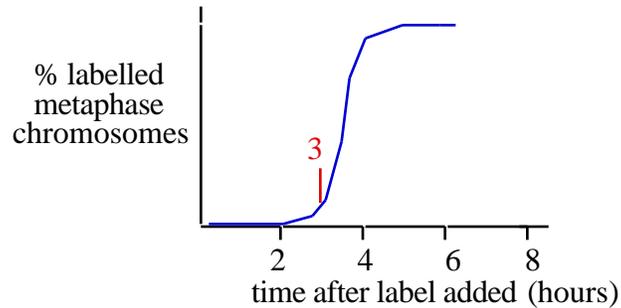
II. Interphase

A. DNA replication during S phase By the time mitosis starts the cell has doubled its normal DNA; when does the DNA replicate? Two ways to find out are 1) to feed the cells radioactive nucleotide and see when it becomes incorporated, and 2) to stain the cell's DNA with a dye and see when the amount of staining increases. Both methods reveal that DNA replication occurs during a distinct stage of interphase, called S phase for synthesis.

B. G1, S, and G2 phases DNA replication is separated from mitosis by a time gap called G1 that comes after mitosis and before S phase. After S phase another gap called G2 occurs before the next round of mitosis. The cell cycle thus consists of an ordered progression from mitosis to G1 to S phase to G2 to mitosis again, with G1, S, and G2 comprising interphase. How long does the cell stay in each phase, and what tells it to proceed in the right order?

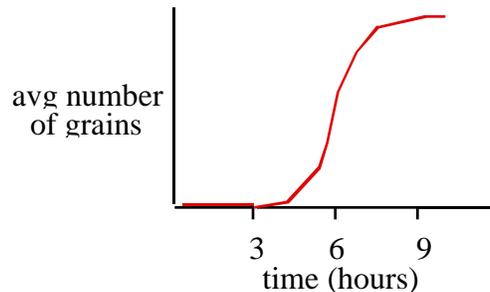
III. Length of phases in the cell cycle Looking at cells in a plate reveals that the average duplication time, or the time it takes to go through one cycle, is about 12 to 20 hours. Using 20 hours as the total time and 10% as the percent of time in mitosis (as discussed in the section I), the time the cell spends in **mitosis is 2 hours**.

While yeast have visible tags that make it possible to tell what stage they're in by looking through the microscope, animal cells require labeling experiments to determine how long they spend in each of the other stages. To figure out the length of G2, cells are treated with radioactive thymidine, a nucleotide which is incorporated into cells only during DNA synthesis. Cells that incorporate the label can be detected by autoradiography. (See **MBOC p. 866 Fig. 17-5.**) By seeing what percentage of the cells are labeled at mitosis reveals the time it takes the cell to get from the end of S phase to mitosis - that is, the length of G2.

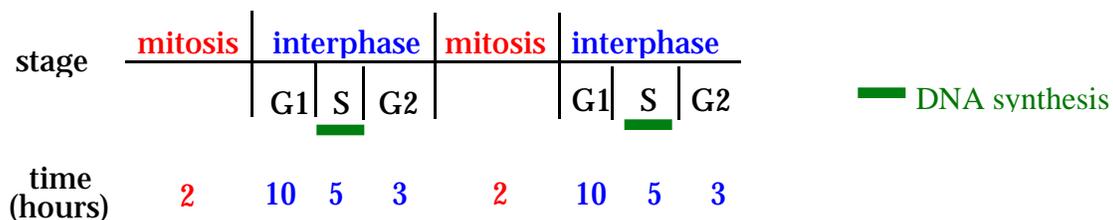


The results of the experiment showed that the label first starts appearing in mitotic cells after 3 hours. Since the cells that are labeled earliest must represent those that were at the end of S phase when the experiment started, the time between the end of S phase and metaphase is about 3 hours. Thus, it turns out that **G2 lasts about 3 - 4 hours.**

A similar labeling experiment was performed to determine the length of S phase. In this experiment the measured quantity was the average silver grain count per cell (corresponding to the exposure) over the population of metaphase cells. Saturation occurred when cells started incorporating radioactivity at the beginning of S phase, so the time it took to reach saturation reveals that the time it takes to go from the beginning of S phase to mitosis is about 8 hours. Since this time period represents G2 plus S, and we know G2 is 3 hours, **S must be 5 -6 hours long.**



Knowing that the total cell cycle time is 20 hours, that mitosis is 2 hours, S is 5 hours, and G2 is 3 hours, the remaining phase, **G1, can be calculated to last 10 hours.** The diagram below summarizes this information. (See [The Problems Book, #17-4.](#))



In yeast, the fact that yeast cells bud when they leave G1 for the S phase makes it possible to observe the transition directly under a microscope. An additional marker for the progression from G1 to S is the division of the spindle pole bodies, or the centrosome. At G2, the nucleus squeezes into the bud (since the nuclear membrane doesn't disintegrate as it does in animal cells).

IV. Mitosis Mitosis occurs through a series of stages illustrated in **MBOC pp. 916-917 Panel 18-1**.

A. Prophase Prior to cell division, the nucleus is surrounded by a membrane, and the DNA is diffuse. Also prior to mitosis, the **centrosome**, which contains the **centrioles** arranged perpendicular to each other, divides in two, resulting in two centrosomes each with one pair of centrioles. During prophase, protein motors move the centrosomes toward opposite ends of the cell. As they separate they serve as nucleation centers for **microtubules**, which radiate out from opposite ends of the cell once the centrosomes have reached their polar destinations. Another major event that occurs during prophase is **condensation of DNA (chromatin)** into defined, visible chromosomes, each consisting of two **sister chromatids** with a **centromere** at the center.

B. Prometaphase The next stage of mitosis starts when a **kinase** phosphorylates structural proteins called **lamins**, which make up the nuclear membrane. Lamin phosphorylation causes the nuclear membrane to break down, and the pieces form many small vesicles in the cytoplasm. Once the nuclear membrane is gone, the centrosomes situate themselves at opposite ends of the cell. Some of the microtubules growing out from the centrosomes reach the sister chromatids, and "capture" them.

C. Metaphase The microtubules push and pull on the sister chromatids from the ends of the cell until the chromatids are lined up halfway between the poles at metaphase. If the chromatids don't line up, cell division cannot progress to the next stage; thus this stage is a **checkpoint** in mitosis.

D. Anaphase Once the chromatids are lined up, a signal triggers the separation of the chromatid pairs, and they start moving toward the opposite poles as the microtubules shorten. Meanwhile the cell elongates as the poles of the cell move farther apart.

E. Telophase In telophase, the chromatids arrive at the poles. Pieces of the former nuclear membrane reassemble around the chromatids to form new nuclear envelopes, and the DNA starts to become diffuse again.

F. Cytokinesis After the nuclear material has divided, the cytoplasm divides by actin and myosin wrapping around the center of the cell and squeezing it until two cells are formed. After this stage, the cell reenters interphase.

V. The Mitotic Spindle and Kinetochore

The mitotic spindle is the structure consisting of the centrosomes and microtubules. During mitosis, the cell contains three types of microtubules radiating out from the centrosomes: **kinetochore microtubules** which "captures" the sister chromatids; **polar microtubules** which reach about halfway across the cell to interact with polar microtubules from the opposite centrosome, and are responsible for pushing the

centrosome away from each other; and the **astral microtubules** that radiate in all directions from the centrosomes, and which may be responsible for pulling the centrosomes away from each other and towards the cell membrane (see [MBOC p. 919 Fig. 18-10](#)).

Kinetochores are structures on the centromeres of the sister chromatids to which kinetochore microtubules attach. The actual composition of kinetochores is unclear, but it appears to have 3 layers. Spokes that emerge from the outer layer are probably involved in contact with microtubules.