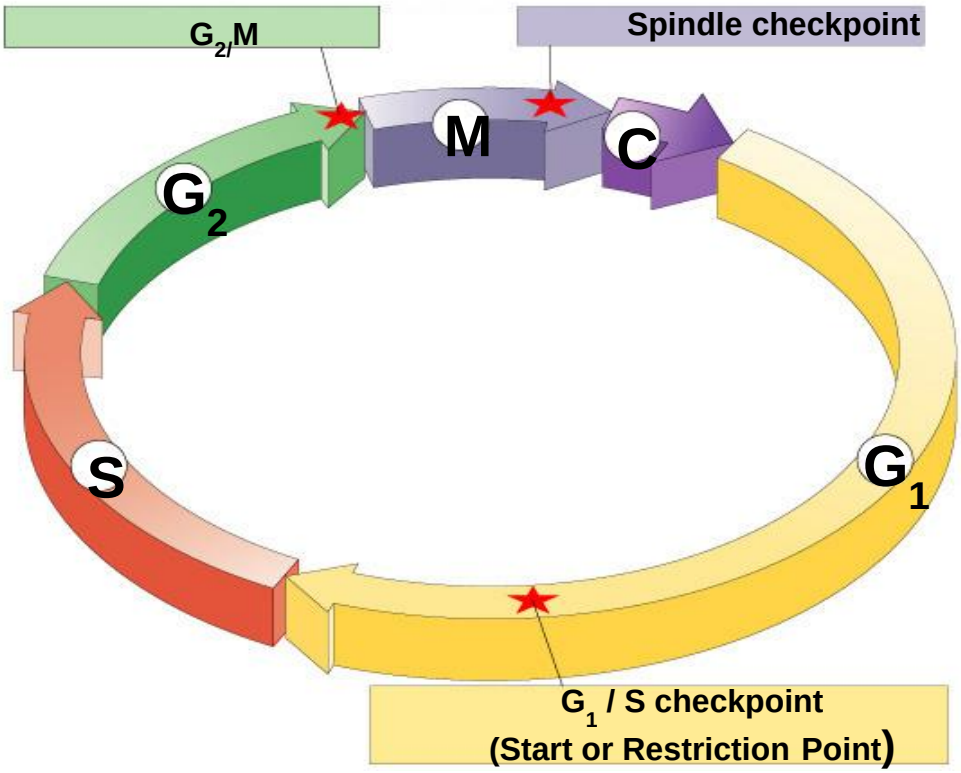
The eukaryotic cell cycle

The growth and development of any organism is dependent on the ability to duplicate the genetic information and then divide this material equally into the two resulting daughter cells. The term **cell cycle** encompasses the entire series of events that lead to the duplication of the eukaryotic cell. Earlier in the course you learned about mitosis, or the orderly segregation of chromosomes; mitosis is just one stage of the cell cycle. The use of the term *cycle* refers to the alternating pattern of cell growth and DNA synthesis followed by segregation of chromosomes. This doubling of cell number can rapidly produce an enormous population of cells. For example, a fertilized zebra fish egg goes from the single cell stage to an embryo with 512 cells in a period just over two hours, which means the cell doubling time in the early embryo is roughly 15 minutes.

Stages of the cell cycle

Biologists divide the cell cycle into four phases called **G1, S, G2, and M**.

G1: During the *first gap phase* (**G1)**, the cell grows, monitors DNA damage, and prepares for the process of replicating the genome. The cell is small in size and needs to replace organelles and membranes that were divided between the two daughter cells in the previous round of cell division. In addition to cell growth, many proteins need to be newly synthesized to carry out the replication of the genome and segregation of newly synthesized DNA.



Many regulatory controls are in place to ensure that the cell is ready for chromosome duplication and cell division. The time a cell spends in G1 varies more than any other stage of the cell cycle. In the rapidly dividing human embryo, G1 may last only a few hours. At the other end of the

spectrum, many mature cells enter a non-dividing phase referred to as **G0**. Some cells, such as brain cells, will permanently leave the cell cycle. Other cells can be induced to reenter the G1 phase, and prepare for cell division if growth factors are present.

S phase: The chromosomes are duplicated in the **S (synthesis) phase**. The replication of DNA in S phase in a mammalian cell can requires a few hours. The resulting pair of connected chromosomes is referred to as **sister chromatids** (remember they are held together by proteins called **cohesins** that encircle the two chromatids).

G2: During the *second gap phase* (**G2**), the cell prepares for the separation of the sister chromatids into the two daughter cells. This is a very important time in the cell cycle for determining if the cell is ready to enter the next phase of the cell cycle. If breaks in the DNA occur, or unfinished regions of DNA replication exist, then signals are relayed to arrest the cell cycle. In order to prevent lethal consequences to the resulting daughter cells, the cell must repair the errors before it can proceed to the next stage of the cell cycle. Although the duration of this phase varies depending on the cell type, a typical mammalian cell may spend up to four hours in G_2 preparing for mitosis.

Mitosis: The physical separation of the sister chromatids occurs during the **M** (**mitosis) phase** of the cell cycle. The precise division of chromosomes during mitosis is what ensures the faithful transmission of genetic information from parent to daughter cells. The entire process of mitosis actually occurs quite rapidly, often on the order of 30-60 minutes, and makes up a small fraction of the entire cell cycle. During much of the cell cycle the genetic material cannot be detected as individual chromosomes under the light microscope. Instead, the chromosomes appear as very fine, tangled threads inside the nucleus, resembling the line or string used on a fishing pole. This non-compacted state of the chromosome exists during G1, S, and G2 phases of the cell cycle, a duration also referred to as **interphase**. Only when the cell prepares for chromosome segregation during the M phase are the chromosomes compact enough to see as individual entities under the microscope. Remember this compaction helped prevent tangling during mitosis (if you have ever gone fishing, you know how easy it is to tangle your fishing line!).

Mitosis is typically followed by **cytokinesis**, which involves the division of the cytoplasm to produce two distinct daughter cells. However there are cells (some fungi for example) that undergo mitosis without cytokinesis – the result is a cell with multiple nuclei.

Cell cycle regulation

The cell cycle is a highly regulated process, as all organisms must carefully coordinate the timing of DNA replication and chromosome segregation in order to survive. Multicellular organisms face the further challenge of developing differentiated organs. This process critically depends upon the correct cells dividing at the right time. But just as importantly, some cells must stop dividing. It is essential that multicellular organisms be able to prevent cells from dividing too often. For example, regulated cell division during embryonic development produces organs with the correct shape and structure for their proper function. Each organ is composed of the correct number of cells that are organized in a particular arrangement. Imagine your heart: cells must divide to form the four chambers of the heart, but must then stop dividing to leave an opening in

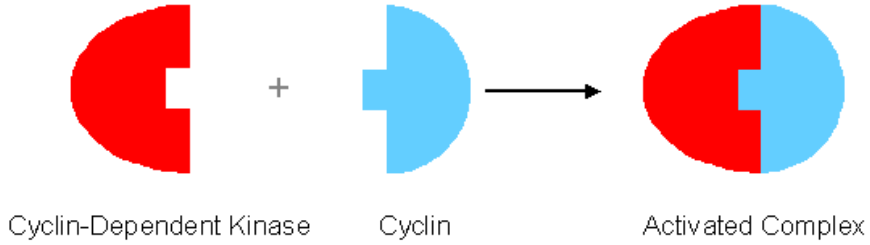
each chamber to pump blood. The continued mitotic division of heart cells throughout an individual's lifetime would disrupt the organ's ability to effectively pump blood.

An organism must also prevent cell division when conditions are unfavorable, such as when there is DNA damage. If mutations in the DNA are allowed to be copied and transmitted to daughter cells, the likelihood of an organism developing unregulated cell division increases. There is a high risk to excessive cell division, as cells that have lost the ability to control cell division can become cancerous. Even single celled organisms must carefully balance cell growth with cell division. If the cell doesn't have a chance to grow to a certain size between cell divisions, it will eventually become too small to continue functioning.

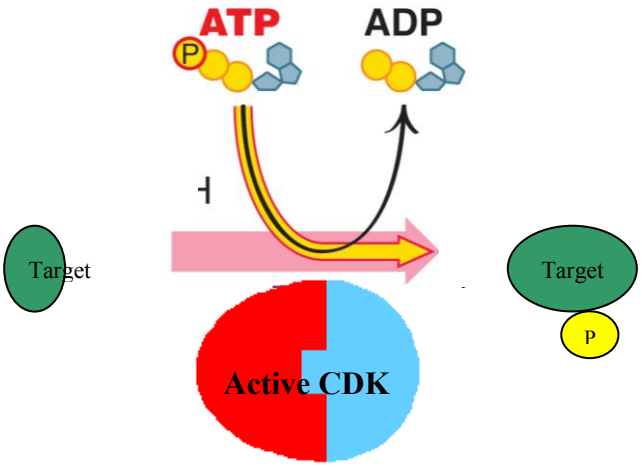
To regulate division, the cell uses information from a variety of extracellular signals, including the presence of growth factors and the availability of nutrients. The cell balances these extracellular signals with information from internal checkpoint proteins before committing to DNA replication and mitosis.

Role of cyclins and cyclin dependent kinases in cell cycle regulation

The regulation of the cell's progression through the various phases of the cell cycle is due to the activity of two families of proteins known as **cyclins** and **cyclin dependent kinases (CDK).** CDK is active only when bound to a cyclin, hence its name, ***c***yclin ***d***ependent ***k***inase.



The active cyclin-CDK complex functions to phosphorylate target proteins, thus rapidly altering the target's function (remember that phosphorylation causes a change in the conformation of a protein and therefore a change in function).



Timothy Hunt discovered cyclins in 1982 while researching the control of protein synthesis in sea urchin eggs. To track the synthesis and degradation of proteins in the cells, Hunt used radioactively labeled amino acids. Cells actively synthesizing protein took up the labeled amino acid and incorporated it into protein. The proteins were then separated according to size on an SDS-polyacrylamide gel and the radioactive bands of protein visualized using autoradiographic film. Hunt found that following fertilization of the eggs, the majority of the protein bands continued to increase in strength as time went on, indicating an increase in their production. Unexpectedly, one particular protein band that had been among the strongest in the unfertilized egg, disappeared following fertilization. The disappearance of the band suggested that the protein was undergoing degradation. Further studies showed that the concentration of this protein oscillates, or "cycles," throughout the cell cycle, so the protein was named **cyclin**. We now know that the oscillations in cyclin concentration are due to an increase in expression of the cyclin gene followed by cyclin protein degradation at a later stage in the cell cycle. Budding yeast cells and multicellular organisms have multiple, distinct cyclin proteins, with each cyclin functioning during a different phase of the cell cycle.

Throughout each turn of the cell cycle, the expression (transcription and translation) of the cyclin gene must increase in response to nutrient or growth factor cues from the environment. Although yeast cells produce cyclins in a nutrient-dependent manner, animal cells divide in response to specific growth factors, which can be produced either by neighboring cells or by cells located quite far away in the body. In the latter case, the growth factor travels throughout the body via the bloodstream to the target cell.

Due to its dependence on cyclin concentration, CDK activity also oscillates throughout the cell cycle. Let's take a closer look at how CDK activity is involved in the cell's transition from G1 to S phase in multicellular organisms. As the cell responds to nutrients and growth factors in G1 phase, the concentration of the G1-cyclin protein increases resulting in CDK activation. Before DNA replication can occur in S-phase, the genes for certain enzymes required for DNA replication must be transcribed and translated, a process that requires the binding of transcription factors to regulatory regions. Active CDK transfers a phosphate group from ATP to an inhibitor protein known as **Retinoblastoma** (Rb). Phosphorylated Retinoblastoma protein releases a transcription factor called E2F. Released E2F is free to bind DNA and increase the expression of genes required for DNA synthesis during S phase, including the genes for DNA polymerase and thymidine kinase. Thymidine kinase is an enzyme required by the cell for the synthesis of deoxythymidine (one of the building blocks of the DNA molecule). Although the details differ for single celled organisms, such as yeast, active CDK still leads to increased gene expression and DNA synthesis. ***Diagram the process this paragraph just explained in words, beginning with a cell expressing a growth factor receptor and ending with expression of the DNA polymerase gene.***

It is essential that the cell regulate the destruction as well as the synthesis of cyclins in order to prevent cells from proliferating unchecked. Once growth factor signals have initiated sufficient cyclin expression to activate CDKs, the CDKs will not only stimulate a pathway leading to initiation of DNA synthesis, but they will also negatively regulate their own activity by stimulating the destruction of cyclin. This process begins with CDKs activating enzymes that add ubiquitin tags to the cyclin protein. Proteins tagged with ubiquitin molecules are marked for

destruction and are sent to the proteasome – the garbage disposal center of the cell - for digestion. This built in self-regulatory mechanism functions to ensure that the cyclins, and, therefore, CDK activity, peak during G1, but then decrease during the remainder of the cell cycle. This decrease in CDK activity is essential for the cycle to be able to repeat itself. Cyclin degradation helps ensure that the cell cycle moves in one direction only: *forward*. If cyclin levels were maintained at high levels throughout all phases of the cell cycle, CDKs would also remain active, and the unregulated cell division could result in excessive growth or cancer. In fact, cells from many breast cancers have been shown to have higher than normal levels of cyclin proteins. The discovery of how the cell cycle is regulated by cyclins and CDKs had a tremendous impact on our understanding of cell biology, cell growth, and development. Knowledge of cell cycle regulation is key to understanding cancer development, and has opened new avenues for possible cancer therapies.

And finally, the G1 and G2 phases of the cell cycle require different preparatory steps for the S and M phases, respectively, and distinct cyclin-CDK complexes are active in the G1/S and G2/M transitions. Cells express different cyclin proteins in the G1 and G2 phases, and, in addition, multicellular organisms express multiple, cell cycle-phase specific CDKs. The phosphorylated targets of the G2/M specific cyclin-CDK complex prepare the cell for mitosis by contributing to cytoskeleton restructuring, nuclear envelope breakdown, and chromosome condensation—all necessary events for mitosis.

Answer the following questions before class:

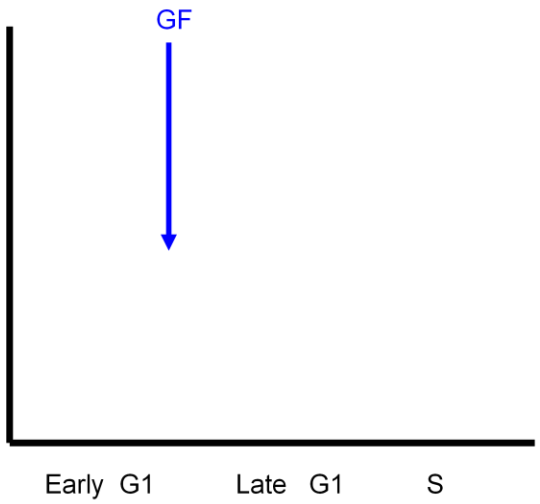
1. You are growing a population of cells all at the same stage of the cell cycle.

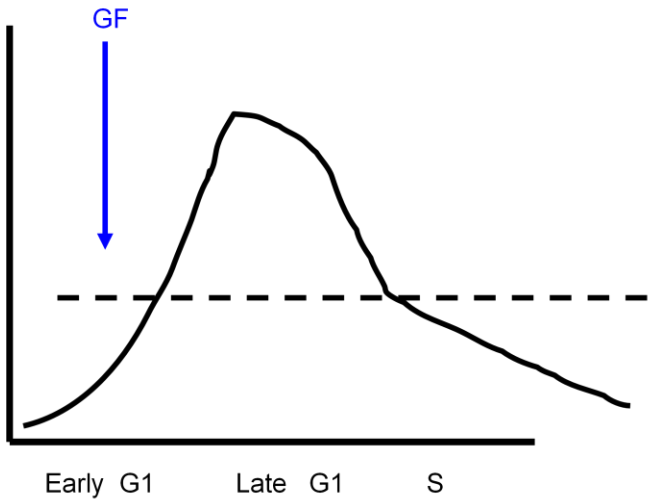
Using a solid line, indicate the cyclin protein concentration on the graph below:

a) At the beginning of G1
b) Following growth factor (GF) binding and signaling

Using a dotted line, indicate the cyclin-dependent kinase (CDK) protein concentration:

a) At the beginning of G1
b) Following growth factor (GF) binding and signaling





2. You observe that the concentration of cyclin protein increases in a clone of yeast cells growing in media containing adequate nutrients. Unexpectedly, the cells all stop - or arrest - in the G1 phase of the cell cycle and no DNA is synthesized. Predict which proteins in the cell may not be functioning. Experimentally how would you determine if your prediction is accurate?

Cells require active cyclin-CDK complexes to move from the G1 to S phase of the cell cycle. Because cyclin proteins are being synthesized, it suggests that the problem may be with CDK. There may be a mutation in the CDK gene or in its regulatory region, resulting in the lack of functional CDK protein in the cell. Without CDK, the cell cannot move from the G1 phase to S phase and no DNA will be synthesized. The essential role of CDKs in the cell cycle was demonstrated by Leland Hartwell. He was able to determine that mutant yeast cells lacking the gene for CDK stopped, or arrested, in the G1 phase of the cell cycle, just as observed in this problem. This work, along with many subsequent experiments, demonstrated that without a functional CDK gene, cells will not progress through the cell cycle and divide. Leland Hartwell shared the 2001 Nobel Prize with Paul Nurse and Timothy Hunt for their contributions to our understanding of the regulation of the eukaryotic cell cycle.

3. Explain why the Retinoblastoma protein serves as the brakes of the cell cycle in animals.

Although it may not be intuitive, the default setting for the cell cycle in multicellular organisms is actually the "off" position. This prevention of cell division is due to the action of a regulatory protein known as **Retinoblastoma**, or Rb. Rb functions as a *brake* for the cell cycle by binding to, and blocking, the activity of a transcription factor that is required for the expression of the genes needed for DNA synthesis. The Rb protein received its name due to the tumor of the retina that occurs when both copies of the gene are mutated and the cell is missing its "brakes". A tumor is a growth caused by cells that are constantly dividing. The Rb protein is a member of a class of proteins called **tumor suppressors** due to their ability to prevent unregulated cell division, and the retinoblastoma gene was one of the first tumor suppressor genes to be studied. Mutations in tumor suppressor genes result in increased cell division, and the loss of functional Rb protein in a cell leads to a very high risk of cancer.

So how does a normal cell duplicate itself if the default condition is that Rb prevents cell division? Before a cell can divide, the Rb brake must be released in the G1 phase of the cell cycle by the action of CDK. A growth factor signal binds to a receptor and following receptor binding, the signal is relayed through the cytoplasm to the nucleus resulting in cyclin gene expression and CDK activation. Rb is one substrate of

the activated CDK-cyclin complex, and the transfer of a phosphate group from ATP to Rb leads to a conformational change in the Rb protein. Phosphorylated Rb releases the bound transcription factor known as E2F. The released E2F transcription factor is now free to activate expression of the DNA polymerase gene as well as other genes requires for entry into S phase. In the absence of DNA damage, the cell will continue through the remaining phases of the cell cycle, dividing to produce two daughter cells.

After division, the brakes must be reset so that the cell does not continue through another cycle in the absence of the appropriate growth factor signal. Resetting the brakes is dependent upon the oscillation of cyclins. Their degradation by the proteosomes reduces CDK activity and allows Rb to return to an unphosphorylated state able to associate with E2F, thus preventing progression to S phase. Growth factor must continue to be present to induce the cell to again synthesize cyclin protein in G1 and initiate another round of CDK activation and cell division. In the absence of growth factor, the cell will not divide: no expression of the cyclin gene means that CDK will not be active, and thus the Rb brake remains firmly in place.

Clicker Questions:

1. Cyclin
   A) is a protein able to bind and activate CDK
   B) is a protein that is directly able to transfer phosphate from ATP to target proteins
   C) is a protein eaten by cyclists
   D) concentration stays steady throughout the cell cycle
   E) is a protein unlikely to be involved in any cancer

2. During the G2 phase of the cell cycle
   A) the cell is synthesizing new DNA
   B) the cell is segregating sister chromatids
   C) the cell is not metabolically active (generating and using ATP)
   D) the cell is checking DNA for errors and preparing for mitosis
   E) none of the answers are correct
   F) all of the answers are correct

3. Growth factor
   A) signal relay pathways often involve kinases
   B) signaling lead to an increase in CDK activity in the cell
   C) signaling can move the cell from the G1 phase to S phase
   D) Signaling can lead to the synthesis of DNA by the cell
   E) all of the answers are correct
   F) none of the answers are correct