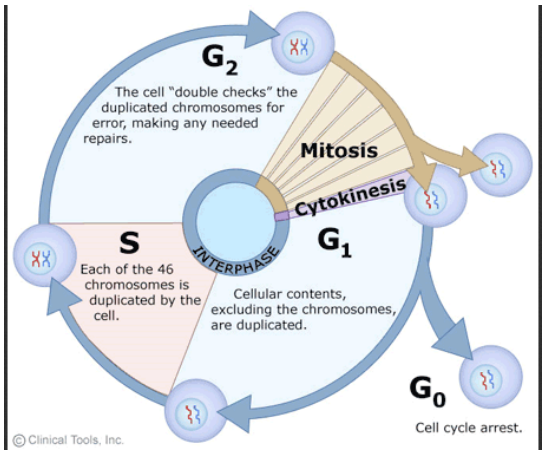
7.013 Recitation 12 – Spring 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

Summary of Lecture 19 (3/23) and Lecture 20 (4/2)

Cyclins, CDKs and Cell cycle: The cell cycle is the process by which one cell becomes two identical cells. The cell cycle is the chain of events that occur in only those cells that are actively growing and dividing. A dividing cell can be visualized by using microscopy techniques of appropriate resolving power i.e. ability to see two close objects as two separate objects. Using fluorescence or chemical dyes such as ethidium bromide, annexin or propidium iodide one can also quantitate the replicating DNA within a dividing cell or the cells at different phases of the cell cycle.

Cells preparing to undergo cell division must first copy each of their double-stranded DNA molecules (or chromosomes) by DNA replication. The cell cycle consists of four stages – G1, S (DNA synthesis/ replication), G2, and M (mitosis). The phase between two cell cycles in often referred to as the G0 phase (***note:*** *interphase is the time between two mitosis*). G1 phase is when the cells are preparing to replicate their DNA, which occurs in S phase. G2 is when the cells are preparing to divide, which occurs in M phase. Of these, the S and M phases have fixed lengths but the G1 (and G0) phase shows variable length in different cell types. A schematic of the cell cycle is shown below.



The transition from one phase to the next is regulated by a set of cell cycle checkpoints. The cell-cycle control system is based on two families of proteins: the cyclin-dependent protein kinases (CDK) and the cyclins. Lee Hartwell and Paul Nurse first discovered these in yeast. They were working with a yeast strain that had a nonfunctional CDK2 (encoded by cdk2 gene), which resulted in their cell cycle arrest. They found that, if these yeast strains were transformed with the human version of cdk2 gene that encoded a functional CDK2 protein, they exhibited normal cell cycle. So the cyclins and CDKs are present ubiquitously in different species and their sequence and function is highly conserved.

Hartwell & Nurse received the Nobel Prize in 2001 for their contribution (http://www.nobelprize.org/nobel_prizes/medicine/laureates/2001/illpres/index.html)

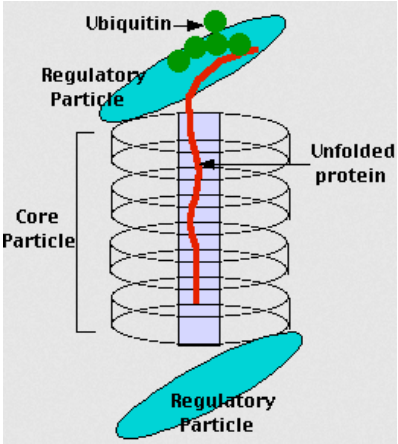
Specific cyclins bind and activate specific CDKs, which allosterically change the conformation of the corresponding CDKs so that they are phosphorylated and activated to promote cell division. Expression of cyclins is transient; they undergo a cycle of synthesis and degradation with each division cycle. The expression of cyclins is regulated by transcriptional control (expressed only in the right stage of the cell cycle) and proteasomal mediated irreversible degradation and phosphorylation of specific serine/ threonine and tyrosine amino acids residues in the proteins. This in turn regulates the activation of corresponding CDKs. The cyclic assembly, activation, and disassembly of cyclin-CDK complexes are the pivotal events that drive the cell cycle.

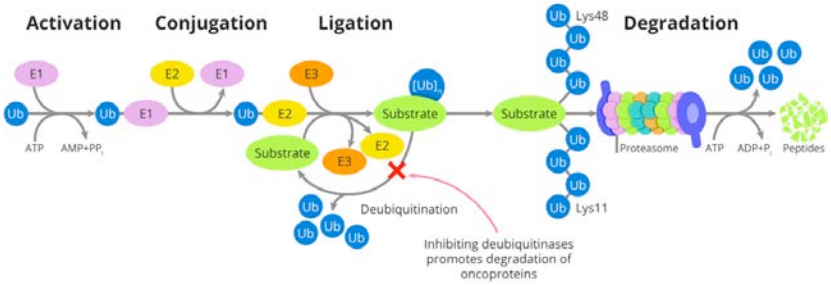
Any alteration of the cyclin-CDK function either results in excessive cell proliferation as observed in diseases like cancer or increase cell death as is observed in neurodegenerative disease like Alzheimer's.

Proteasomes: Protein can either be degraded by the lysosomal enzymes or through the proteasome complex. **Lysosomes** deal primarily with **extracellular** proteins, e.g., plasma proteins, that are taken into the cell (endocytosis), cell-surface membrane proteins that are used in receptor-mediated endocytosis, autophagy that engulfs the proteins (and other macromolecules).

In comparison, **Proteasomes** deal primarily with endogenous proteins that are synthesized within the cell such as: transcription factors, cyclins, proteins encoded by viruses or other intracellular pathogens, proteins that have been damaged by other molecules within the cell and proteins that are folded incorrectly due to errors in translation or mutations within the gene.

The proteasome is made of core particle (CP) and regulatory particle (RP) and is a multimeric protein complex. The proteasome complex within the cell may chew of Proteins that have the incorrect amino acid sequence.





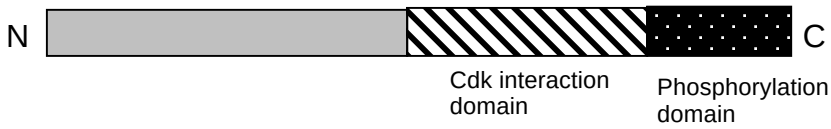
Proteins destined for destruction are conjugated to a molecule of ubiquitin. The ubiquitin-protein complex binds to the ubiquitin-recognition site(s) on RP. The protein is unfolded by ATPases using the energy from ATP and then translated into the central cavity of CP. As it passes through the CP it is degraded by hydrolysis of the peptide bonds. This produces a set of peptides that leave the CP and are broken down into individual amino acids by peptidases in the cytosol. The regulatory particle releases the ubiquitins for reuse.

A loss of these processes can result in neurodegenerative diseases and prions related diseases.

Proteasome mediated degradation is energy requiring, it is irreversible, requires ubiquitinylation of target proteins and this is regulated by E2 and E3 enzymes.

Questions

1. The cyclin D protein has the following structure.



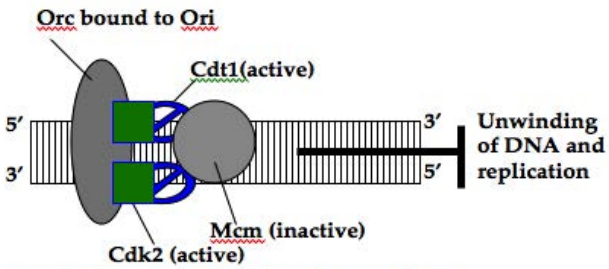
a) What effect would the deletion of the Cdk-binding domain of Cyclin D have on cell proliferation? Explain.

b) Using a temperature sensitive variant of Cyclin D, you find that the cells fail to divide at the non-permissive temperature. The arrested cells have a diploid DNA content (2n) in a single nucleus. At what stage of cell cycle is cyclin D likely to act? Explain.

c) How would the Cdk protein levels vary during the normal cell cycle?

2. The origin recognition complex (ORC) is a multi- subunit protein complex that binds to the **ori site(s)** and serves as a platform for the assembly of kinases like Cdk6 and Cdt1.

During the G1 phase of the cell cycle in yeast, ORC forms a pre- replication complex by recruiting Cdk6 and Cdt1 that bind to both strands of DNA. These factors bind and inhibit the Mcm protein that functions as a helicase as is shown in the schematic below. ***Note:*** *The activation is shown by an → and inhibition by ⊥ sign.*



Pre-replication complex in G1 phase

a) Activation of the pre-replication complex occurs during the S phase and this requires its interaction with Cdk2 and Cyclin E proteins that degrade Cdk6 and Cdt1. This results in the replication of DNA. Draw a schematic, similar to the one above, to show the regulatory interactions between ORC, Cdk6, Cdt1, Mcm, Cdk2 and Cyclin E proteins. ***Note:*** *Please indicate the activation by an → and inhibition by ⊥ sign.*

b) In a cell showing a Cdk2 **loss-of–function** mutation, in which phase (*choose from G1, S, G2, M, all or none*) would the cell arrest? **Explain** why you selected this option.

c) If the cdk-2 gene encodes Cdk-2 protein, in which phase (*choose from G1, S, G2, M, all or none*) will the cdk-2 gene be expressed? **Explain** why you selected this option.

d) If the cyclin E gene encodes Cyclin E protein, in which phase (*choose from G1, S, G2, M or all*) will the cyclin E gene be **optimally expressed**? **Explain** why you selected this option.

e) You further create two mutant cells each having a mutation in Cdk-2 genes as described below.

- **Mutant cell- type 1:** The Cdk-2 protein lacks its kinase domain.
- **Mutant cell- type 2:** The Cdk-2 lacks its Cyclin E binding domain.

Predict what would happen to the **cell cycle** in…

i. **Mutant 1:**

ii. **Mutant 2:**

3. The proteasomal complex can degrade any misfolded protein.

a) What is the highest order of protein structure for the proteasomal complex?

Primary **Secondary** **Tertiary** **Quaternary**

b) Circle the correct option. The degradation of protein by proteasome is example of …

Exergonic reaction **Endergonic reaction**

c) If, within a cell, the proteasome complex has a mutation as a result of which it cannot bind to the ubiquitin binding site, would you expect the cell to survive and have normal functions? Why or why not?

7.013 Introductory Biology

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