7.016 Recitation 16 – Fall 2018

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

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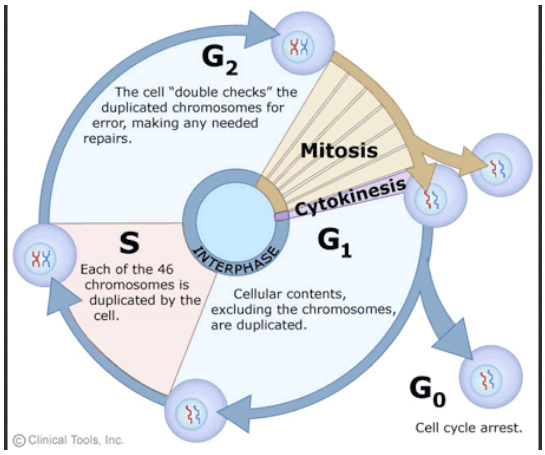
Summary of Lectures 23 (11/5) and 24 (11/7):

Cell cycle: Adult humans have approximately 10 trillion cells originating from a single diploid zygote that divides through the somatic cell division (mitosis). 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 genome) by DNA replication. The cell cycle consists of four stages: G1→S (DNA synthesis/ replication) → G2→ M (mitosis). G1 phase is when the cells are preparing to replicate their DNA and the cellular content other than DNA id duplicated. The genome replicates in S phase. G2 is when the cells are preparing to divide, which occurs in M phase. A schematic of the cell cycle is shown below.



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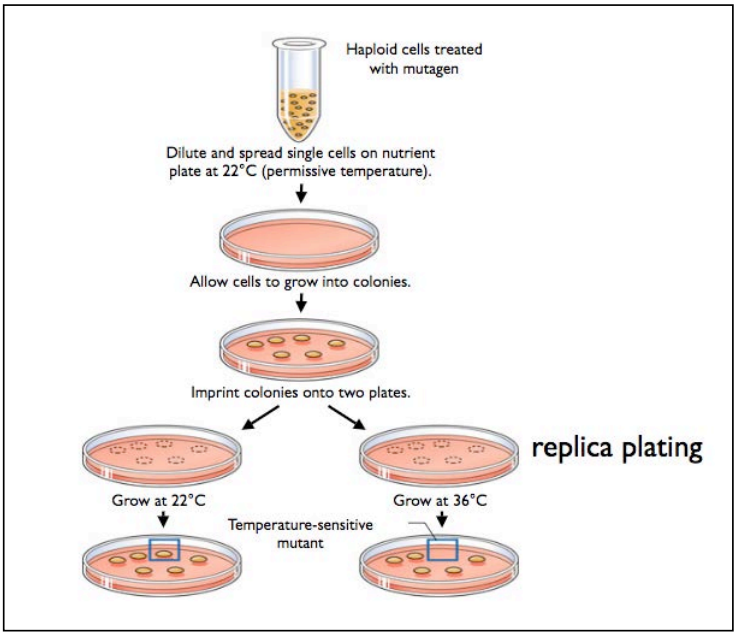
The transition from one phase to the next is regulated by a set of cell cycle checkpoints. The cell-cycle control system is based on two families of proteins: the cyclin-dependent protein kinases (CDK) and the cyclins. Lee Hartwell and Paul Nurse first discovered these in yeast. They were working with a yeast strain that had a nonfunctional CDK2 (encoded by cdk2 gene), which resulted in 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 progression through the cell cycle. This experiment demonstrated that 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.

Conditional mutants in cell cycle: "Conditional" mutations allow you to study regulators of cell cycle. Conditional mutations allow the encoded protein to function under one condition - e.g., lower temperature - while inhibiting its function under another condition, in this case, high temperature. Such mutations are special alleles, often caused by missense mutations that destabilize the protein or its interaction with other proteins. Because these alleles are far rare than general loss-of-function

alleles, they are most often isolated in organisms that enable rapid high-throughput screens, such as budding and fission yeast.



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Cell cycle stages can be recognized by the cell morphology (bud size) and nuclear division in budding yeast. Yeast grows as haploid or diploid organisms; you can identify recessive mutations in haploids and carry out complementation analysis in diploids.

The following schematic represents the identification of conditional mutants.

Here you mutagenize the haploid yeast cells and then plate them and allow them to grow at permissive temperature (22^{o}C). You replica-plate the colonies on two plates and let them grow at permissive (22^{o}C) or non-permissive (36^{o}C) temperature. You observe cells in all stages of cell cycle at 22^{o}C but you see an arrest of the same cells at a particular stage of the cell cycle when grown at 36^{o}C. However, if you move these cells from 36^{o}C->22^{o}C, they grow and divide normally indicating that you have identified a conditional mutant.

Stem cells: In an adult, the cells of most organs and tissues are differentiated and do not divide, but have a finite lifespan. Examples of such cells are the skin cells, blood cells and the cells of the intestinal lining. Cell replacement is accomplished by specialized stem cells, which are able to self-renew throughout the life of the organism. Stem cells are partially determined, while progenitors are more determined. Stem cells reside in a specific cellular environment, called a niche. Cell-cell signaling between the niche and stem cell regulates when the stem cell will divide and whether it will divide symmetrically, to create two stem cells, or asymmetrically, to create a new stem cell and a progenitor cell The progenitor goes on to divide further and create more progenitors which later differentiate into cells needed for replacement in a specific organ. Progenitors have limited division capability.

Each stem cell type has specific potency. The number of differentiated cell types originating from a stem cell defines its potency.

Cell	Definition
Totipotent	A cell that can form ALL cell types including the placenta > Zygote is the only totipotent cell
Pluripotent	A cell that can form all the cell types except placenta Ex: Embryonic stem cells, induced pluripotent stem cells etc
Multipotent	A cell that can form multiple cell types but NOT all cell types i.e. hematopoetic stem cells which form all the blood cell types and cells of immune system
Unipotent	A cell that can form only one differentiated cell type
Differentiated	A cell that has attained its final fate i.e. neuron

The number of differentiated cell types a stem cell produced defines its potency and the tree from a stem cell to the differentiated cell types that it produces is defined as a lineage. A set of cell types that arise from the same stem cell (or progenitor/ transit amplifying cells) is a lineage. Progenitors or transit amplifying cells may have less potency than the stem cell from which they arise. Stem cells may be able to repair or replace the damaged tissues in diseases (i.e. stroke, Parkinson's diabetes, muscular dystrophy and heart disorders). However, stem cells are rare and difficult to isolate. Stem cells can be generated from adult cells by expression of a small set of genes (induced pluripotent stem cells iPSCs).

Questions

1. One method for studying the essential components that regulate different phases of the cell cycle involves the use of conditional (i.e. temperature-sensitive) mutants.

You isolate a **temperature-sensitive cell division cycle X (Cdc X) yeast mutant.** Yeast cells that have this mutation grow normally at the permissive temperature (22 ºC) but they arrest at a specific phase in the cell cycle when they are shifted to a non-permissive/ restrictive temperature (36 ºC). You perform the following experiments in order to further characterize this mutant.

Experiment 1: You grow the temperature sensitive Cdc X cells at 22 ºC in the presence of **nocodazole**, **an inhibitor of microtubule assembly**, until they arrest. You then remove the nocodazole and shift the arrested cells to 36 ºC. You find that the cells divide once and then arrest.

Experiment 2: You grow the temperature sensitive Cdc X cells at 22 ºC in the presence of **p21cip1, which inhibits entry into mitosis**. You then remove **p21cip1** and shift the arrested cells to 36 ºC. You find that the cells divide once and then arrest.

Experiment 3: You grow the temperature sensitive Cdc X cells at 36ºC until they arrest. You then shift them to 22 ºC and add **hydroxyurea, an inhibitor of DNA replication**. You find that the cells divide once and then arrest.

Based on **Experiments 1-3**, where in cell cycle does the CdcX likely function: ***G1-S/ S-G2/ G2-M/ M checkpoints***? **Explain** why you selected this option.

2. Classify the following as totipotent, pluripotent, bipotent, unipotent or differentiated:

a) Cells that can differentiate into many cell types: _____

b) A zygote that is about to start cleavage: _____

c) Cardiac muscle cell: _____

3. A patient has a rare recessive genetic disorder due to the loss of a specific gene function. This disease causes a decrease in immune function. The patient receives a bone marrow transplant which is successful in alleviating the symptoms of the disease, such that patient can lead a normal life.

a) Why does the bone marrow transplant relieve the patient's symptoms?

b) The patient marries a man who happens to be carrier for the same genetic disorder. What is the probability that their offspring will inherit the disease if the couple had a child **a)** before and **b)** after the bone marrow transplant?

4. A major goal of stem cell research is to repair diseased brain tissue using neural stem cell lines.

a) Four human embryonic cell lines, originally prepared from the **SAME embryo**, were tested for their potency **in vitro**. Based on the data below, complete the table by ranking the potency of these cell lines.

Cell types	Cell types differentiated **in vitro**	Potency from 1-4 (1=most potent and 4= least potent).
A	motor	
B	motor, sensory, lateral, hippocampal	
C	sensory, lateral, hippocampal	
D	motor, sensory	

b) Draw a lineage tree for cell types A-D using the information in the table above.

Solution Key

involves the use of conditional (i.e. temperature-sensitive) mutants.

You isolate a **temperature-sensitive cell division cycle X (Cdc X) yeast mutant.** Yeast cells that have this mutation grow normally at the permissive temperature (22 ºC) but they arrest at a specific phase in the cell cycle when they are shifted to a non-permissive/ restrictive temperature (36 ºC). You perform the following experiments in order to further characterize this mutant.

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Experiment 2: You grow the temperature sensitive Cdc X cells at 22 ºC in the presence of **p21cip1, which inhibits entry into mitosis**. You then remove **p21cip1** and shift the arrested cells to 36 ºC. You find that the cells divide once and then arrest.

Experiment 3: You grow the temperature sensitive Cdc X cells at 36ºC until they arrest. You then shift them to 22 ºC and add **hydroxyurea, an inhibitor of DNA replication**. You find that the cells divide once and then arrest.

Based on **Experiments 1-3**, where in cell cycle does the CdcX likely function: ***G1-S/ S-G2/ G2-M/ M checkpoints***? **Explain** why you selected this option.

Based on experiment 1 the treatment with nocodazole blocks all the mutant cells in M phase, since microtubules are required for spindle formation and chromosome separation. Once nocodazole is removed and the cells are shifted to non-permissive temperature, the cells will progress through the cell cycle until the protein encoded by the CdcX gene is needed. Because the cells divide once before arresting, we know that the CdcX protein is not required in M phase. It likely has a function during the G1, S, or G2.

Following the same logic, based on experiment 2, we know that the CdcX protein is not required in the M phase (or G2/ M interphase) but instead it is needed in G1/ S/ G2 phase.

Based on experiment 3 the cells will block at the point in the cell cycle where the protein encoded by the CdcX gene is needed. You release the cells from this block and halt the progress through the cell cycle at S phase, where DNA synthesis occurs. Because the cells at the permissive temperature divide only once in the presence of hydroxyurea, you know the function of the CdcX protein is NOT required in G1 and S phase also. Most likely it is needed in the S/ G2 checkpoint.

2. Classify the following as totipotent, pluripotent, bipotent, unipotent or differentiated:

a) Cells that can differentiate into many cell types: *Pluripotent*

b) A zygote that is about to start cleavage: *Totipotent (it can form placenta also)*

c) Cardiac muscle cell: *Differentiated*

3. A patient has a rare recessive genetic disorder due to the loss of a specific gene function. This disease causes a decrease in immune function. The patient receives a bone marrow transplant which is successful in alleviating the symptoms of the disease, such that patient can lead a normal life.

a) Why does the bone marrow transplant relieve the patient's symptoms?
If the bone marrow transplant is successful, the bone marrow cells can proliferate to form the different blood cells that circulate in the patient's body and help them alleviate the signs and symptoms.

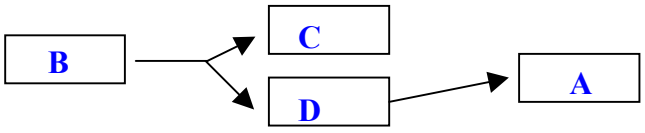
b) The patient marries a man who happens to be carrier for the same genetic disorder. What is the probability that their offspring will inherit the disease if the couple had a child **a)** before and **b)** after the bone marrow transplant?
The patient is (aa) before the transplant and she marries a carrier (Aa). So the chance that the child will have the disease is (aA, aa, aA, aa) = ½.The probability will remain the same even after bone marrow transplant since the gametes of this patient will still have the "aa" genotype.

4. A major goal of stem cell research is to repair diseased brain tissue using neural stem cell lines.

a) Four human embryonic cell lines, originally prepared from the **SAME embryo**, were tested for their potency **in vitro**. Based on the data below, complete the table by ranking the potency of these cell lines.

Cell types	Cell types differentiated **in vitro**	Potency from 1-4 (1=most potent and 4= least potent).
A	motor	*4*
B	motor, sensory, lateral, hippocampal	*1*
C	sensory, lateral, hippocampal	*2*
D	motor, sensory	*3*

b) Draw a lineage tree for cell types A-D using the information in the table above.



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