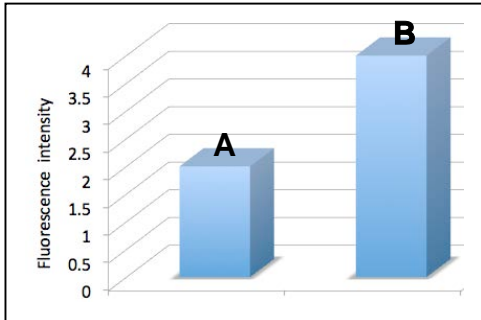


# Solution key-7.013 Problem Set 5- 2018

## Question 1 (3pts)

a) Fluorescent probes such as DAPI (4, 6-Diamino-2-phenylindole) are often used to study cells that are in the different phases of cell cycle. The following schematic represents the variation in fluorescence intensity of human skin cells, growing in Plate A and Plate B that have been stained with DAPI and are in different phases (A & B) of the cell cycle.



Which phase(s) of the cell cycle are the skin cells in?

**Cells in Plate A:** G1/ S/ G2/ M? **G1 phase (0.25pts)**

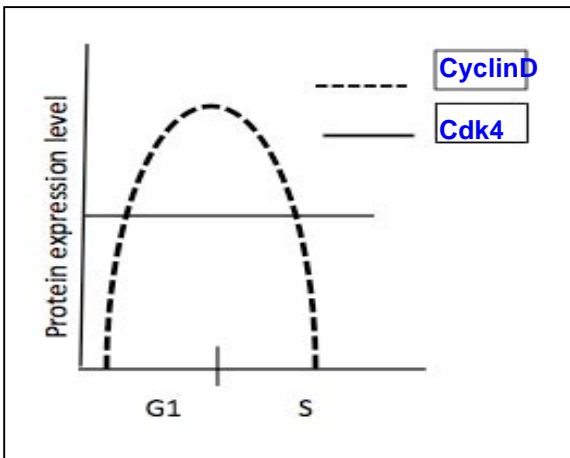
**Cells in Plate B:** G1/ S/ G2/ M? **S/G2/ M phase (0.25pts)**

**Explain** why you selected the above options.

*The intensity of DAPI fluorescence in Condition A is  $t \frac{1}{2}$  of that in Condition B. This means that the cells in condition A have still not replicated their genomic DNA (ploidy  $2n$ ) and are in G1, unlike that in condition B. In condition B, the cells have*

*replicated their DNA in S phase (ploidy  $4n$ ), so they are either in S/ G2/ M phase. (0.5pts or 0.25 for each)*

b) The Cyclins and the Cyclin dependent kinases (Cdks) are two different classes of proteins, which play a critical role in regulating the cell cycle. For example, Cyclin D is a protein that promotes the **G1 - > S phase transition** by activating Cyclin dependent kinase 4 (Cdk4).

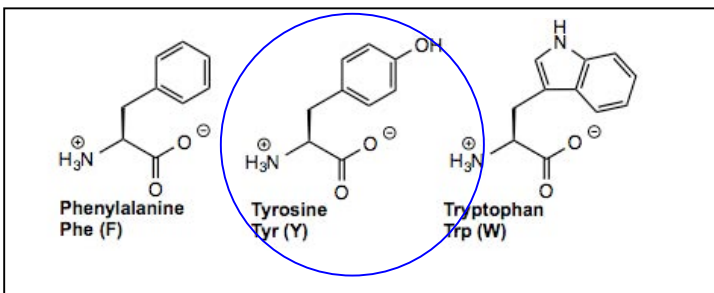


I. On the schematic below, identify the profile that represents the levels of Cyclin D and Cdk4 by filling in the boxes. **(0.5pts)**

II. Briefly explain why it is critical for most cyclin proteins to be expressed only at a specific phase of the cell cycle. **(1pt)**

*Each Cyclin binds to a unique CDK to activate it. The active cyclin-CDK complex allows the cell to cross a specific cell cycle check point (G1->S, S->G2, G2->M etc). If the cyclins were ALWAYS expressed, their corresponding Cdk would always be active, and the cell cycle would proceed uncontrollably.*

c) **Circle** the amino acid below that is likely phosphorylated by the Cyclin D-CDK4 complex. **(0.5pts)**

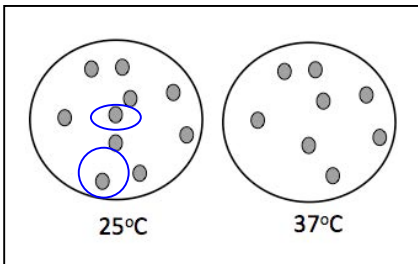


**Question 2 (4pts)**

a) One method for studying the essential components that regulate different phases of the cell cycle involves the use of conditional temperature-sensitive mutants. Briefly **explain** the mechanism that allows a temperature sensitive mutant to function at the permissive temperature but become non-functional at the non-permissive temperature.

*The temperature sensitive mutant has a protein that can reversibly shuttle between the active 3D-conformation (when it is at permissive temperature) and denatured, inactive 3D-conformation (when placed at non-permissive temperature). This allows us to use them to study different phases of the cell cycle. (1pt)*

b) To create the temperature sensitive *cell division cycle (cdc)* mutants, you irradiate **budding yeast** with X rays, replica plate them at 25°C (permissive temperature) and 37°C (non-permissive or restrictive temperature), and then let them grow and form colonies as shown below.



i. Circle the colony(s) that represents temperature sensitive mutants. **(0.5pts)**

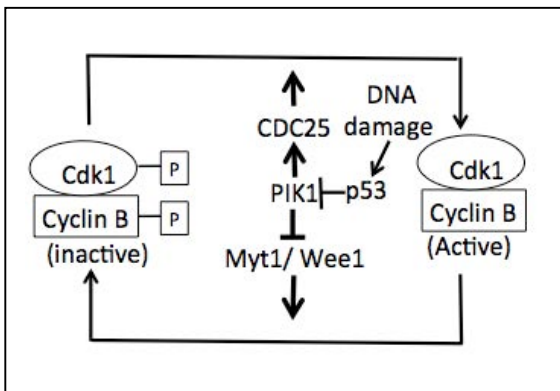
ii. What feature of yeast allows you to check the phase of the cell cycle in which the *cdc* mutant is arrested?

*Yeast cells have a specific phenotype at each phase of the cell cycle. One can look observe the phenotype to determine the phase of the cell cycle that a yeast cell is in. (0.5pts)*

iii. Briefly **outline an experiment** that can help you check if the human variant of *cdc* can rescue the phenotype of the *cdc* yeast mutants. **(0.5pts)**

*You transform the yeast *cdc* mutant with the wild-type variant of human *cdc* gene and then plate them on growth medium and see if they are able divide at restrictive temperature (37°C). If they divide and form colonies at 37°C then they have transformed into the prototrophs (i.e. wild-type phenotype).*

The following is the regulatory network that controls the activity of the mitotic cyclin dependent kinase (Cdk1-Cyclin B) that is critical for the G2->M phase transition of a cell undergoing the cell cycle.



- DNA damage or failure to complete DNA replication results in activation of p53.
- The activated p53 regulates Cdk1/ Cyclin B function by inhibiting PIK1.
- PIK1 inhibits Myt1/ Wee1 kinase and activates CDC25.
- The Myt1/Wee1 kinase inhibits Cdk1 whereas CDC25 activates Cdk1.
- The Cdk1-CyclinB is active when dephosphorylated and inactive when phosphorylated.

c) In a **diploid yeast** going through the cell cycle, you identify a Cyclin B (or Mitotic-Cyclin) temperature sensitive mutant which arrests in the cell cycle at 37°C. Give the DNA content of the arrested cell: **2n/ 2(2n)/ n? 2(2n) (0.5pts)**

d) Would a **PIK1** temperature sensitive mutant, grown at the non-permissive temperature (37°C), progress from G2 -> M phase? **Why or why not?** *It will progress through G2 phase less rapidly since P1K1 at non-permissive temperature will not be able to inhibit Myt1/Wee1, which is critical for converting Cdk1-Cyclin B from active to inactive state. This will prevent G2->M transition. (1pt)*

**Question 3 (6pts)**

Mutations in genes that promote uncontrolled cell division and inhibit programmed cell death (apoptosis) can result in cancer.

a) Complete the table below for each of the following genes. (4pts, 1 for each row, 0.5 for each cell)

Gene	Function of encoded protein	Is this a proto-oncogene/ oncogene/ tumor suppressor gene?	Would cancer cells show dominant/ recessive/ gain-of- function/ loss-of-function mutation for this gene? Include <b>ALL</b> that apply.
<i>c-Myc</i>	Encodes a transcription factor that increases expression of growth promoting genes	<i>Proto-oncogene</i>	<i>Dominant, gain-of-function mutation</i>
<i>Mutant Ras</i>	Encodes a G protein that is constitutively (always) in its GTP bound active form and it stimulates growth signaling pathways	<i>Oncogene</i>	<i>Dominant, gain-of-function mutation</i>
<i>Bax</i>	Encodes a cytosolic protein that inhibits growth-signaling pathways	<i>Tumor suppressor gene</i>	<i>Recessive, loss-of-function mutation</i>
<i>p16</i>	Encodes a protein that inhibits the CyclinD-Cdk4 complex which promotes the G1->S transition.	<i>Tumor suppressor gene</i>	<i>Recessive, loss-of-function mutation</i>

b) The Ames test is a standard method used to evaluate the mutagenic potential of a chemical agent. You want to test the mutagenic potential of a carcinogen by doing the following experiments 1-3.

**Experiment 1:** You subcutaneously inject the carcinogen into a mouse once every three days for 6 weeks. You observe the mouse develops a solid tumor. These tumor cells grow uncontrollably and form foci (pile of clonal cell population) when grown on cell culture plates.

**Experiment 2:** You perform a **standard Ames test** by incubating His<sup>-</sup> bacterial cells with the carcinogen and plate them on cell culture plates containing growth medium that lacks histidine. You observe 500 bacterial colonies.

**Experiment 3:** You perform a **modified Ames test** by first incubating the carcinogen with liver extract. You then incubate the His<sup>-</sup> bacteria with the liver extract treated carcinogen. You plate them on cell culture plates containing growth medium that lacks histidine. You observe 10,000 bacterial colonies.

- i. Why does the incubation of the carcinogen with the liver extract increase the number of bacterial colonies in **Experiment 3** as opposed to **Experiment 2**? (0.5pts)  
*This compound is likely a pro- mutagen. In its native form, it is a weak mutagen producing only 500 His<sup>+</sup> colonies in Experiment 2. However, in experiments 1 and 3, it is converted to a metabolic form that is potentially mutagenic by the enzymes of the liver extract. The metabolites generated by Compound 3 can now convert His<sup>-</sup> cells to His<sup>+</sup> cells resulting in 10,000 His<sup>+</sup> colonies.*
- ii. Normal, healthy cells when cultured in cell culture plates divide and form a monolayer. In comparison, the tumor cells, as shown in Experiment 1 can grow and form foci. **Explain** what feature/ property of the tumor cells allows them to form foci. (0.5pts)  
*The tumor cells have lost the property of contact inhibition and hence can grow and form clumps to form foci.*

**Question 3 continued**

c) Rous sarcoma virus (RSV) is a cancer causing **retrovirus** (it has an RNA genome, which is reverse transcribed by its reverse transcriptase enzyme to make the cDNA copy of the viral genome that integrates into the host cell genome). It is a modified form of Avian leucosis virus (ALV). However, in addition to the genes found in the ALV genome, the genome of RSV also has v-src oncogene, which causes cancer.

i. Why is RSV a rapidly mutating virus?

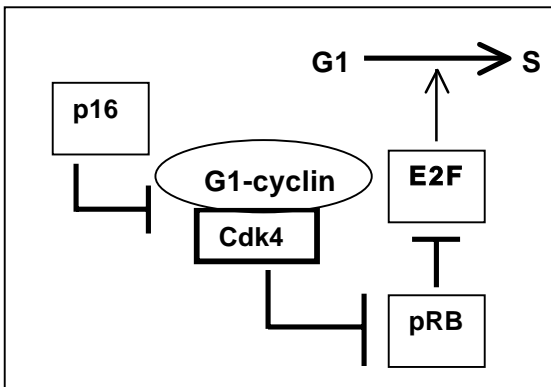
*RSV being a retrovirus has an RNA genome. Once it infects a target cell it reverse transcribes its genome using Reverse transcriptase enzyme. This enzyme lacks proofreading ability, unlike the host cell DNA polymerase enzyme. As a result, the viral genome can acquire mutations at a much faster rate unlike the genome of the target host cell. (0.25pts)*

ii. Which of the following statements are true? Circle **ALL** the correct statements. (0.75pts or 0.25 each)

- [RSV causes sarcoma by encoding a Src kinase that has lost its regulatory domain](#)
- [The v-Src of RSV hybridizes with the exons of c-Src in humans](#)
- The v-Src of RSV hybridizes with the introns of c-Src in humans
- The v-Src of RSV hybridizes with the promoter region of c-Src in humans
- [The Src gene is present across different species.](#)

**Question 4 (5pts)**

a) The RB gene encodes the retinoblastoma protein (pRB). **Note:** The dephosphorylated pRB protein is active in G1 phase. pRB binds to the transcription factor E2F and prevents E2F mediated G1 → S transition. The G1 cyclin-Cdk4 complex produced during G1 phosphorylates and thereby inactivates pRB. This allows the cell to enter S phase. The p16 protein inhibits the G1cyclin-Cdk4 complex.



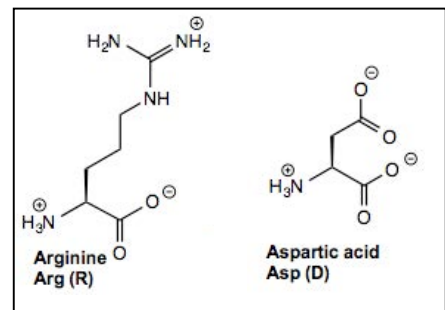
i. Identify pRb as a product of a **tumor suppressor gene or an oncogene**. (0.5pts)

ii. Human papilloma virus (HPV) infection can result in cervical, head and neck cancer. The E7 protein of HPV binds to and inhibits pRB. Based on this observation, would you classify the gene encoding E7 as an oncogene or a tumor suppressor gene? **Explain** why you selected this option.

*E7 is an oncogenic protein since it promotes cell proliferation by inhibiting pRB, a tumor suppressor protein. (0.5pts)*

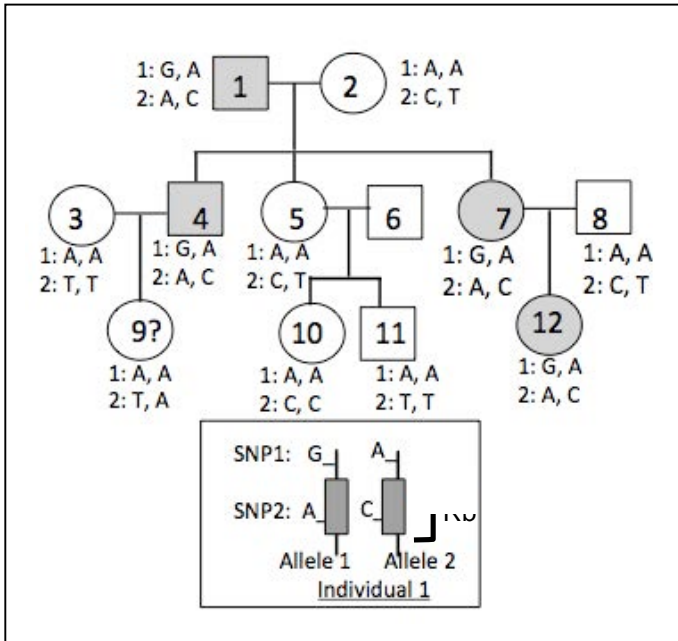
iii. You note a version of E2F that has an **Arg<sup>28</sup>->Asp<sup>28</sup> mutation** within its DNA-binding domain. How might this mutation **chemically** affect the DNA binding ability of E2F?

*Arginine has a positively charged side-chain whereas Aspartic acid as a negatively charged side-chain. Mutating Arg-> Asp will promote repulsion between E2F and negatively charged sugar-phosphate backbone of DNA. Therefore, E2F will not be able to bind to DNA. (1pt)*



**Question 4 continued**

The following human pedigree shows **familial predisposition to retinoblastoma** due to a mutation in the *Rb* gene. **Note:** All shaded individuals (except for #9 that shown as a “?”) develop retinoblastoma. Individuals 3, 6 and 8 do not have the disease-associated alleles of *Rb* gene. SNP1 is 2cM away from the *Rb* gene whereas SNP2 is located within the *Rb* gene. The location of SNPs with respect to *Rb* gene is shown as a small insert in the drawing below.



b) Give the **mode of inheritance** of predisposition to retinoblastoma: Autosomal dominant (0.25pts)

c) Using  $Rb^{WT}$  for the allele associated with the normal phenotype and  $Rb^{MUTANT}$  for the allele associated with the mutant phenotype, for Individual 1, give the genotype of ...

- i. Retinal cells at birth:  $Rb^{WT} Rb^{MUTANT}$  (0.25pts)
- ii. Retinoblastoma cancer cells:  $Rb^{MUTANT} Rb^{MUTANT}$  (0.25pts)
- iii. His gametes:  $Rb^{WT}$  OR  $Rb^{MUTANT}$  at a frequency of 1: 1 (0.25pts)

d) Assuming no recombination, give the genotype of **Individual 6** for...

- i. **SNP1:** A, A (0.5pts)
- ii. **SNP2:** T, C (0.5pts)

e) Propose how Individual 9 may have acquired her alleles for SNP1 and SNP2?

*The gametes produced by Individual 3 would all have SNP1: A and SNP2: T since she is homozygous for these SNPs. In comparison, Individual 4 is heterozygous for SNP1 and SNP2. Since the distance between SNP1 and *Rb* gene is 2cM there is crossing over which may result is gametes that has following combinations of SNP1 and SNP2: Gametes1: G, A (parental), Gamete 2: G, C (recombinant), **Gamete 3: A, A (recombinant)** and Gamete 4: A, C (parental). The fusion of gamete 3 from Individual 4 with a gamete from Individual 3 results in the Individual 9 who has a different SNP profile compared to his parents. (1pt)*

**Question 5 (2pts)**

a) The following are examples of anti-tumor agents that are often used as a part of chemotherapeutic regimens for cancer patients. **Explain** how the use of the following drugs may prevent cancer cell growth and / or proliferation.

Drug	Target of drug	How is cancer cell growth and / or proliferation prevented?
Adriamycin	Intercalates between DNA bases	<i>The DNA strands will not be able to unwind. This is critical for the replication of genome and transcription of the gene. Hence the cells will not divide (since there is no S phase) or undergo apoptosis (if critical genes are not transcribed) (0.5pts)</i>
Vincristine	Microtubule inhibitor	<i>Prevents the formation of mitotic spindle and hence inhibits cell proliferation (0.5pts)</i>
VEGF inhibitor	Inhibits blood vessel formation	<i>Prevents supply of nutrients and removal of waste thus contributive to cell death (0.5pts)</i>

b) Two retinoblastoma patients (A and B) have the same mutations. However, Patient A acquires an additional gain-of-function mutation in “Gene A” that encodes transcription factor A (TFA)”. The TFA normally functions by suppressing the expression of cell-cell adhesion proteins.

**Explain** why Patient A now has a worse prognosis (i.e. poorer treatment outcome) compared to Patient B for retinoblastoma.

*The patient’s cells have acquired mutations that allow them to detach from each other and infiltrate other tissues, metastasize and form secondary tumors at additional sites. Therefore they are malignant and this leads to a poor prognosis. This is an oncogenic, gain-of-function mutation. (0.5pts)*

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Spring 2018

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