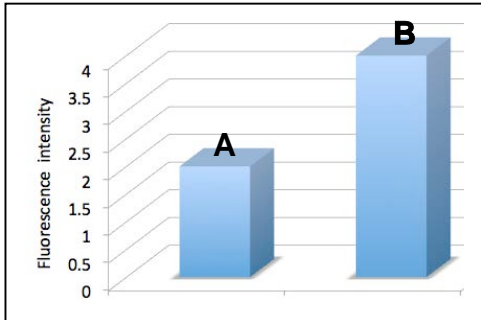


# 7.013 Problem Set 5- 2018

## Question 1

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.



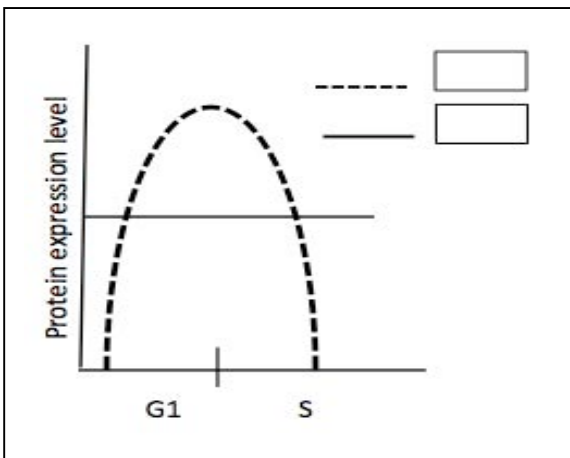
Which phase(s) of the cell cycle are the skin cells in?

**Cells in Plate A:** G1/ S/ G2/ M?

**Cells in Plate B:** G1/ S/ G2/ M?

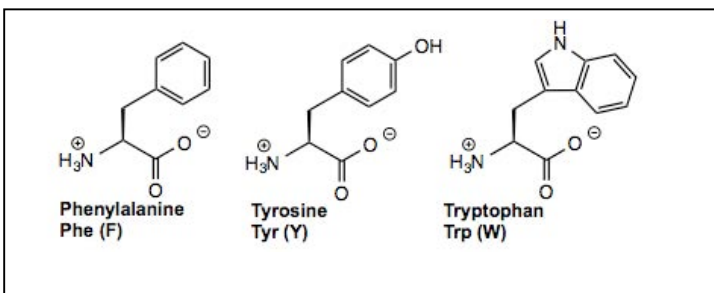
**Explain** why you selected the above options.

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

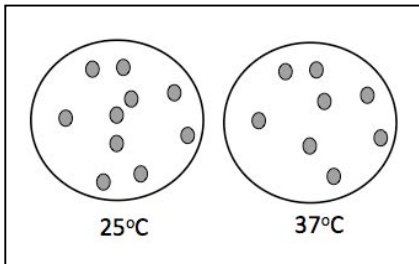
c) **Circle** the amino acid below that is likely phosphorylated by the Cyclin D-CDK4 complex.



## Question 2

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.

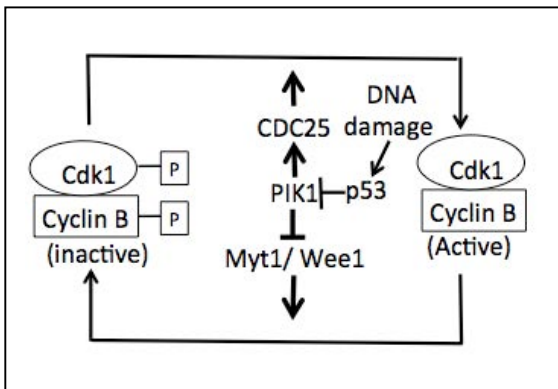
b) To create the temperature sensitive *cell division cycle* (*cdc*) mutants, you irradiate **budding yeast** with X rays, replica plate them at 25°C (permissive temperature) and 37°C (non-permissive or restrictive temperature), and then let them grow and form colonies as shown below.



- i. Circle the colony(s) that represents temperature sensitive mutants.
- ii. What feature of yeast allows you to check the phase of the cell cycle in which the *cdc* mutant is arrested?

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.

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/ 2x(2n)/ n**?

d) Would a **PIK1** temperature sensitive mutant, grown at the non-permissive temperature (37°C), progress from G2 -> M phase? **Why or why not?**

### Question 3

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.

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>Mutant Ras</i>	Encodes a G protein that that is constitutively (always) in its GTP bound active form and it stimulates growth signaling pathways		
<i>Bax</i>	Encodes a cytosolic protein that inhibits growth-signaling pathways		
<i>p16</i>	Encodes a protein that inhibits the CyclinD-Cdk4 complex which promotes the G1->S transition.		

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 that 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**?
- 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.

**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.

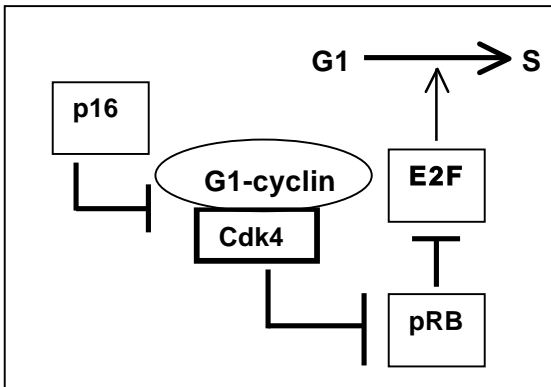
i. Why is RSV a rapidly mutating virus?

ii. Which of the following statements are true? Circle **ALL** the correct statements.

- 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**

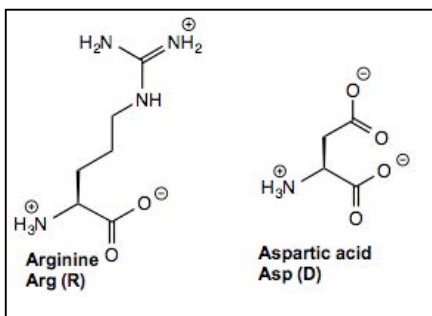
a) The RB gene encodes the retinoblastoma protein (pRB). **Note:** The dephosphorylated pRB protein is active in G1 phase. pRB binds to the transcription factor E2F and prevents E2F mediated G1 → S transition. The G1 cyclin-Cdk4 complex produced during G1 phosphorylates and thereby inactivates pRB. This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

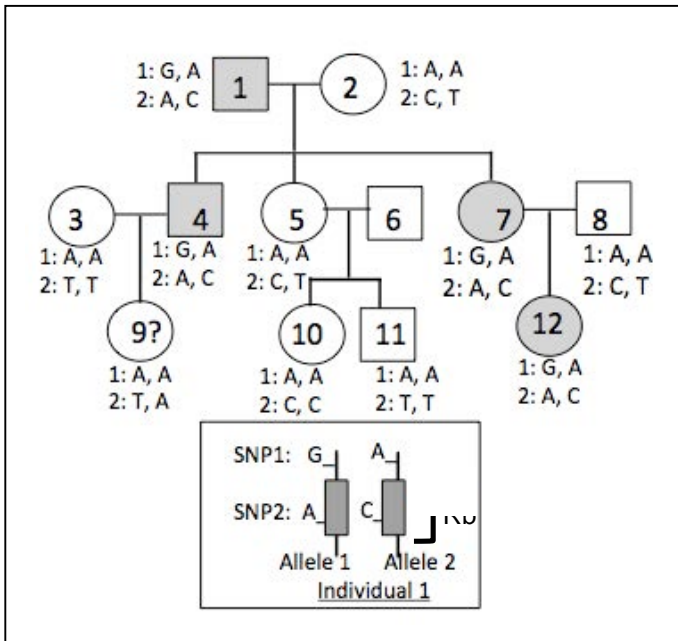
ii. Human papilloma virus (HPV) infection can result in cervical, head and neck cancer. The E7 protein of HPV binds to and inhibits pRB. Based on this observation, would you classify the gene encoding E7 as an oncogene or a tumor suppressor gene? **Explain** why you selected this option.

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?



**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: \_\_\_\_\_

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 the...

- i. Retinal cells at birth: \_\_\_\_\_
- ii. Retinoblastoma cancer cells: \_\_\_\_\_
- iii. His gametes: \_\_\_\_\_

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

- i. **SNP1:** \_\_\_\_\_
- ii. **SNP2:** \_\_\_\_\_

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

**Question 5**

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 growth (or proliferation) of a cancer cell prevented?
Adriamycin	Intercalates between DNA bases	
Vincristine	Microtubule inhibitor	
VEGF inhibitor	Inhibits blood vessel formation	

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.

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