Name: _____
Student #: _____

University of Toronto
Faculty of Applied Science and Engineering
Division of Engineering Science
Final Examination

BME205H1 – Cells and Biomolecules
Thursday April 24, 2014, 2:00 – 4:30 pm
Duration: 150 minutes
Examiners: J. Rocheleau and P. Gilbert

ANSWER ALL QUESTIONS ON THESE SHEETS

1. No cell phones are allowed.
2. Type A: Closed book examination, no aids permitted.
3. Calculator: Type 2 – All non-programmable calculators
4. Part 1 Multiple Choice Questions are provided in this booklet. **Your answers are to be placed on the Scantron Sheet**.
5. Part 2 Questions have the mark available in the square brackets [ ]. Each question has a strict sentence limit restriction, **each sentence written above the limit will be deducted half a mark**.
6. Please note that a help sheet containing the Genetic Code Table has been provided on the final page of the test booklet.

Last Name: _____

First Name: _____

Student Number: _____

Tutorial section:

[ ] TUT 01 1 Tue 13:00 14:00 BA2195 (Jennifer)

[ ] TUT 02 1 Tue 13:00 14:00 BA3116 (Michelle)

[ ] TUT 03 1 Thu 16:00 17:00 BA3008 (Aric)

[ ] TUT 04 1 Thu 16:00 17:00 BA2135 (Ben)

[ ] TUT 05 1 Fri 12:00 13:00 BA2135 (Richard)

[ ] TUT 06 1 Fri 12:00 13:00 BA3008 (Alex)

[ ] TUT 07 1 Fri 10:00 11:00 BA2159 (Gabrielle)

[ ] TUT 08 1 Fri 10:00 11:00 BA2135 (Yonatan)

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PART I _____ out of 60

PART 2: Short Answer Questions

Question	1	2	3	4	5	6	7	8	9	10	11	12
Marks Available	3	4	3	3	3	4	3	3	3	4	4	3
Marks Achieved												

PART 2 _____ out of 36

TOTAL MARKS _____ out of 94

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PART I: Multiple Choice Questions (1 mark each)
You answer **must** be recorded on the Scantron. Anything you circle on these sheets will **not** be counted towards your grade.

(1) Which of the following is not a general description of the gene expression regulation mechanisms that operate in eukaryotic organisms?
a. transcriptional-level controls
b. processing-level controls
c. translational-level controls
d. post-translational-level controls
e. denaturation-level controls

(2) In glycolysis, _____ is converted to pyruvate and ATP is generated by _____ in the _____ of oxygen.
a. Glucose, substrate level phosphorylation, absence
b. Glucose, proton motive force, absence
c. Lipids, proton motive force, absence
d. Glucose, substrate level phosphorylation, presence
e. Glucose, proton motive force, presence

(3) Which part of the cell cycle is the most variable?
a. G_1 phase
b. S phase
c. G_2 phase
d. M phase
e. G_0 phase

(4) Which statement below is a correct statement about the abilities of normal cells and cancer cells to grow and divide when cultured under conditions favorable for cell proliferation?
a. Malignant cells grow and divide at a somewhat faster rate than normal cells.
b. Normal cells grow and divide at a faster rate than malignant cells.
c. Malignant and normal cells grow and divide at similar rates.
d. Neither type of cell grows well in culture.
e. Normal cells do not grow at all, while malignant cells grow very rapidly.

(5) Which technique below can be used to measure the mechanical properties of purified cytoskeletal elements?
a. PCR
b. Atomic force microscopy
c. Transmission electron microscopy
d. Scanning electron microscopy
e. SDS-PAGE

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(6) RNA polymerase activity is regulated by transcription factor bound enhancer sequences kilobases away from the start codon via:
a. myosin II
b. centrioles
c. The activity of chromatin remodeling factors
d. TFIIE
e. σ factor

(7) When bound to ATP, the myosin head:
a. Moves the actin filament to the left
b. Produces a power stroke
c. Rotates into the 'cocked' state
d. Binds to actin
e. Is released from actin

(8) What enables the interaction between the mitotic spindle and the chromosomes to occur?
a. nuclear envelope breakdown
b. nucleosome breakdown
c. nucleolus breakdown
d. chromatin dissolution
e. chromosome condensation

(9) Covalent linkage to what small, highly-conserved protein marks proteins for destruction?
a. phosphate
b. actin
c. ubiquitin
d. cyclin
e. acetyl

(10) The citric acid cycle (aka TCA cycle) primarily takes place in the:
a. Matrix domain of the mitochondria
b. Intermembrane domain of mitochondria
c. Cytoplasm
d. Lysosome

(11) The contractile ring theory suggests that a thin band of _____ and _____ generates the force to cleave the cell during _____.
a. tropomyosin; myosin II; muscle regeneration
b. actin; dynein; mitosis
c. microtubules; kinesin; mitosis
d. microtubules; myosin filaments; cytokinesis
e. actin; myosin filaments; cytokinesis

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(12) While RNA polymerase is a processive enzyme that remains attached to the DNA over long stretches of template, it must be associated _____ enough so that it can move from nucleotide to nucleotide along the template.

a. tightly
b. loosely
c. rapidly
d. angularly

(13) During the second step of the PCR cycle:

a. DNA binds and extends the sequence
b. Both the DNA and DNA polymerase are denatured by NaOH
c. The PCR primers anneal to the template strand
d. DNA is denatured by high temperature

(14) The nucleotide at which transcription is initiated is called _____.

a. 0
b. +1
c. –1
d. +2
e. –2

(15) What is the effect of a competitive inhibitor on an enzyme-mediated reaction?

a. V_{max} stays the same, K_M decreases
b. V_{max} decreases, K_M is unchanged
c. V_{max} stays the same, K_M increases
d. V_{max} stays the same, K_M is unchanged
e. V_{max} increases, K_M is unchanged

(16) Where is the RNA polymerase II phosphorylated during its activation?

a. on the N-terminal end of the largest RNA polymerase II subunit
b. on the central 20 amino acids of the largest RNA polymerase II subunit
c. on the 3' end of the largest RNA polymerase II subunit
d. on the 5' end of the largest RNA polymerase II subunit
e. in the carboxyl-terminal domain (CTD) of the largest RNA polymerase II subunit

(17) The electron transport chain:

a. Is coupled to proton pumping
b. Produces conformational changes in transport chain complexes
c. Generates a series of reduction/oxidation (redox) reactions
d. Is mediated by complexes containing prosthetic groups
e. All of these choices

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(18) What is the name of the protein that blocks myosin II movement along actin fibres in muscle cells in the presence of calcium?

a. Titin
b. GTP
c. ATP
d. Tropomyosin
e. Myosin II movement in muscle cells is not impeded when calcium is present.

(19) Why can cancer cells proliferate in the absence of serum?

a. Their nuclei depend on these serum growth factors to maintain their structure.
b. The serum inhibits their growth, while it is necessary for normal cells.
c. The cell cycle of cancer cells does not depend on signals transmitted from serum growth-factor receptors located at their surface.
d. The cell cycle of cancer cells depends on signals transmitted from serum growth-factor receptors located in their cytoplasm.
e. Their mitochondria depend on serum growth factors for their activity.

(20) _____ is/are required for cell cycle entry such that if the gene(s) is/are deleted in mice, embryos will fail to develop from the single cell (oocyte) stage.

a. Cdk 4 and Cdk6
b. Cdk1
c. Cdk1 and Cdk4
d. Cdk4
e. Cdk6

(21) TFIIH is the only known general transcription factor that functions as:

a. A kinase
b. A GTPase
c. A helicase
d. Both a kinase and a helicase
e. Both a kinase and a GTPase

(22) The _____ is a network of filamentous structures comprised of _____:

a. Muscle myofibre; microtubules
b. ATP synthase; actin and microtubules
c. Cytoskeleton; microtubules, actin and intracellular vesicles
d. Cytoskeleton; microtubules, intermediate filaments, and microfilaments
e. Histone; microfilaments and myosin

(23) Which amino acids can be phosphorylated by protein kinases?

a. Serine, threonine, tyrosine
b. Tyrosine, threonine, glycine
c. Threonine, serine, tryptophan
d. Serine, leucine, tyrosine
e. Phenylalanine, serine, tyrosine

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(24) The 3' end of most eukaryotic mRNAs contains a _____, while the 5' end has a _____.

a. poly(A) tail, methylated guanosine cap
b. poly(U) tail, methylated guanosine cap
c. methylated guanosine cap, poly(A) tail
d. poly(A) tail, sulfonated guanosine cap
e. methylated guanosine cap, poly(U) tail

(25) Neural tube closure during embryonic development occurs via:

a. changes in cell shape produced by contraction of microfilaments and cell migration
b. cell division such that the new cells bridge the gap
c. a combination of cell division and cell migration
d. layering a second piece of tissue atop the open neural tube

(26) What part of the cell cycle does the pRB protein help to regulate?

a. the S – G_2 transition
b. the G_1 - S transition
c. the G_2 - M transition
d. the G_0 – G_1 transition
e. the M – G_2 transition

(27) The regulatory subunit of maturation-promoting factor _____.

a. transfers a phosphate group to certain serine and threonine residues of specific protein substrates
b. is called cyclin because its concentration rises and falls predictably as the cell cycle progresses
c. converts ATP to ADP
d. converts ADP to ATP
e. transfers a phosphate group to certain tyrosine residues of specific protein substrates

(28) Most of the cellular RNA is in what form?

a. hnRNAs
b. snoRNAs
c. mRNAs
d. rRNAs
e. hmRNAs

(29) If the FRAP technique is used to bleach a small zone of fluorescent microtubules in a cell, which of the following is a possible explanation for the recovery of fluorescence in the region of the cell previously bleached?

a. the dynamics of the microtubules turning over in that bleached zone of the cell
b. the growth of new microtubules into the bleached zone
c. movement of microtubules through the bleached zone
d. the dynamics of the microtubules turning over in that bleached zone of the cell and the growth of new microtubules into the bleached zone
e. all of these choices

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(30) Which of the following is not a macromolecule formed by polymerization?
a. proteins
b. polysaccharides
c. DNA
d. lipids

(31) In bacteria, the _____ is located about 10 bases upstream from the initiation site. It has the consensus sequence _____ and is responsible for identifying the precise nucleotide at which _____ begins.
a. Pribnow box, TATAAT, transcription
b. Pribnow box, TTGACA, transcription
c. Pribnow box, TATAAT, translation
d. Portnoy box, TATAAT, transcription
e. Portnoy box, TATAAT, translation

(32) Which stage of mitosis starts as sister chromatids split apart and begin to move toward opposite poles?
a. prophase
b. metaphase
c. prometaphase
d. anaphase
e. telophase

(33) It is suggested that solid tumors _____.
a. might be destroyed by enhancing their ability to form new blood vessels
b. might be dissipated by enhancing the ability of cancer cells to separate from each other
c. might be destroyed by inhibiting their ability to form new blood vessels
d. might be enlarged by breaking down neighboring tissues
e. might be desiccated by osmotic action

(34) As a fibroblast moves, its leading edge extends from the cell as a broad, flattened, veil-like protrusion called a _____.
a. pseudopod
b. filopodia
c. lamellipodium
d. podium
e. extensor

(35) How is the RISC directed to the target mRNA that it is destined to destroy?
a. The ribonuclease slicer directs the RISC to the cleavage site.
b. The single-stranded siRNA is complementary to the target mRNA and directs the RISC to it.
c. The passenger strand acts like a guide to direct the RISC to the target.
d. The siRNA is complementary to the slicer ribonuclease.
e. The siRNA is magnetically attracted to the target mRNA.

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(36) Which of the following statements about the proton-motive force is FALSE?
a. It is a combination of a proton concentration gradient and the membrane potential
b. It is an anaerobic process.
c. It is necessary for ATP generation by the ATP synthase
d. It lends to the pumping of protons into the intermembrane space
e. It is achieved by the electron transport chain

(37) The two opposing events driving chromosome segregation during anaphase are:
a. Astral microtubule depolymerization and chromosomal microtubule depolymerization
b. Astral microtubule polymerization and chromosomal microtubule polymerization
c. Polar microtubule polymerization and chromosomal microtubule depolymerization
d. Polar microtubule depolymerization and chromosomal microtubule polymerization
e. Polar microtubule polymerization and astral microtubule depolymerization

(38) What is always the first amino acid incorporated at the N-terminus of a nascent polypeptide chain?
a. cysteine
b. cystine
c. methionine
d. asparagine
e. glycine

(39) You isolate DNA from a particular organism and analyze it. The amount of cytosine was 1.5 μmoles and the A+T/C+G ratio is 4. How much thymine should be in the sample?
a. 6 μmoles
b. 3 μmoles
c. 1.5 μmoles
d. 4 μmoles
e. 12 μmoles

(40) The genetic code has 64 codons, while there are only 20 amino acids. Thus, some amino acids are coded for by more than one codon. As a result, the genetic code is said to be _____.
a. regenerate
b. degenerate
c. overlapping
d. nonoverlapping
e. nonspecific

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(41) If an exon is not supposed to be included in the mature mRNA, it must be excised. With what other RNA sequences is it removed together with?

a. the flanking exons
b. the flanking introns
c. one flanking exon and one flanking intron
d. the promoter
e. the poly(A) sequence

(42) You are studying two rodent cell lines, one malignant and one normal. You carry out an experiment in which you fuse malignant and normal cells. What happens?

a. All of the hybrid (fused) cells behave like malignant cancer cells.
b. Some of the hybrid cells lose malignant traits.
c. Some of the hybrid cells gained more extreme malignant traits.
d. Most of the hybrids died shortly after fusion.
e. The hybrids began to fuse together spontaneously making giant multinucleate cells.

(43) What chemical below is known to bind free actin monomers and block their incorporation into the actin polymer?

a. cytochalasins
b. nocodazole
c. phalloidin
d. latrunculin

(44) Which of the phenomena below is responsible for the ability of one gene to code for more than one polypeptide?

a. transcription
b. alternative splicing
c. transposition
d. hybridization
e. exon shuffling

(45) Beadle and Tatum's research suggested that _____ ?

a. A gene carries the information to build lipids
b. A gene is made of RNA
c. A gene is made of DNA
d. A gene carries the information for the construction of a particular enzyme

(46) Which of the following is <u>not</u> a normal property of eukaryotic mRNAs?

a. They contain a continuous nucleotide sequence encoding a specific polypeptide.
b. They are found in the cytoplasm and inside the Golgi complex.
c. They are attached to ribosomes when they are translated.
d. Most have a significant noncoding segment that does not direct assembly of amino acids.
e. Eukaryotic mRNAs have special modifications at their 5' and 3' termini.

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(47) What is thought to drive the initial separation of centrosomes?
a. motor proteins associated with microfilaments
b. motor proteins associated with adjacent microtubules
c. contraction of individual microfilaments
d. sliding of adjacent microfilaments over one another
e. contraction of adjacent microtubules

(48) You are interpreting data on a DNA microarray. You expose the chip to a mixture of two cDNA populations: one from cells that were not treated with a glucocorticoid hormone (untreated controls; labeled with a red fluorescent dye) and a population from cells that were treated with glucocorticoid hormones (glucocorticoid-treated; labeled with green fluorescent dye). You look at a spot on the chip representing the gene for phosphoenolase, a gene that is turned off by glucocorticoid treatment, but is expressed in control, untreated cells. What color should the spot representing the phosphoenolase gene be?
a. red
b. green
c. yellow
d. no color, the spot is not labeled.

(49) Hair cells are named from the bundle of stiff, hair-like cilia that project from the apical surface of the cell into the fluid filled cavity of the inner ear. Cilia shape is supported by:
a. bundles of intermediate filaments
b. bundles of parallel actin filaments
c. bundles of microtubules
d. the surrounding extracellular matrix
e. fluid shear flow

(50) Once bound to the promoter, RNA polymerase _____.
a. breaks the DNA chain in the start site region
b. melts the two DNA strands in the start site region
c. degrades the two DNA strands in the start site region
d. melts the two DNA strands in the termination site region
e. chemically alters the DNA template

(51) How short must the poly(A) tail get to cause the mRNA to be degraded rapidly?
a. about 10 adenosine residues
b. about 100 adenosine residues
c. about 30 adenosine residues
d. about 75 adenosine residues
e. one adenosine residue

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(52) At the outer surface of the centromere of each chromatid is a proteinaceous, buttonlike structure called the _____.

a. primary constriction
b. centrosome
c. kinetochore
d. proteasome
e. centriole

(53) Which of the following is/are a method(s) used to label microtubules with a fluorescent dye to permit the investigator to follow the microtubules in a living cell?

a. Coupling tubulin to a fluorescent dye
b. Coupling actin to a fluorescent dye
c. Inducing a cell to express the gene for tubulin that has been fused to the gene for GFP
d. Coupling tubulin to a fluorescent dye or inducing a cell to express the gene for tubulin that has been fused to the gene for GFP
e. Inject cells with a fluorescent dye

(54) The building blocks of a nucleotide are _____.

a. a pentose sugar, a phosphate group and a nitrogenous base
b. a pentose sugar, a phosphate group and an amino acid
c. a pentose sugar and a phosphate group
d. a pentose sugar and a nitrogenous base
e. a phosphate group and a nitrogenous base

(55) You are carrying out experiments in cell fusion by fusing together cells at different stages of the cell cycle. You then observe the behaviour of each nucleus residing in the combined cytoplasm of the two cells. Which of the following responses would occur if you fused an S-phase cell to a cell in the M-phase?

a. Fluorescence activated cell sorting (FACS)
b. The S-phase nucleus undergoes premature chromosomal condensation.
c. The M phase nucleus is affected in such a way that its compacted chromosomes decondense.
d. The S-phase nuclei undergoes premature cytokinesis.

(56) Why does an actin thin filament manage to move continuously during a contraction cycle?

a. All of the myosin heads beat synchronously.
b. All of the myosin heads beat out of synchrony with one another.
c. They use an enormous amount of ATP.
d. They use an enormous amount of GTP.
e. None of the above

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(57) Mutant forms of tumor-suppressor genes act _____; both copies of the gene must be _____ before their protective function is lost.

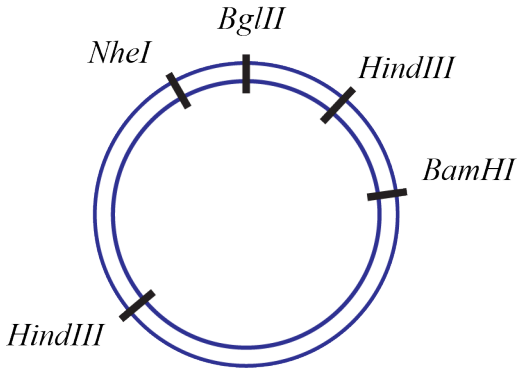
a. as dominant Mendelian traits, mutated or deleted
b. as dominant Mendelian traits, preserved
c. recessively, mutated or deleted
d. recessively, preserved
e. like sex-linked recessive genes, mutated or deleted

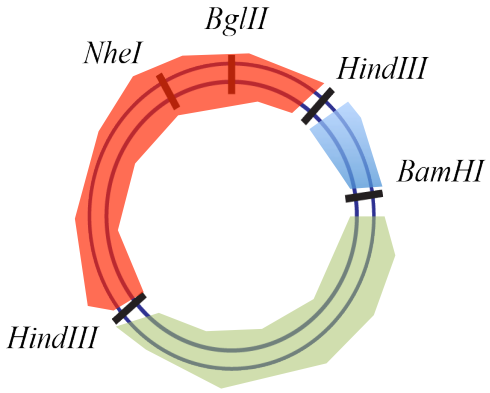
(58) You are working with adult mouse fibroblast cells. The genes for four transcription factors from pluripotent stem cells are transduced into the adult fibroblast cells as part of viral vectors, and once inside the cells they were expressed. What results were obtained?

a. The fibroblasts die.
b. The combination of transduced genes for only four specific transcription factors was sufficient to reprogram the fibroblasts into undifferentiated fibroblast cells.
c. The combination of transduced genes for only four specific transcription factors was sufficient to induce the fibroblasts to behave like pluripotent ES cells.
d. The combination of transduced genes for only four specific transcription factors was sufficient to cause a terminal differentiation of the fibroblast cells.

(59) The plasmid below was digested with the restriction enzymes EcoRI, HindIII, and BamHI. How many bands will appear on an agarose gel electrophoresis?

a. 1
b. 2
c. 3
d. 5
e. 7





Digested plasmid indicating fragments

(60) Integral membrane proteins have been engineered to lack the portion that normally projects into the extracellular space. What happens to the mobility of this engineered protein in the plasma membrane of cells?:

a. They do not move at all
b. They move at a much greater rate than the wild-type protein
c. They are not inserted into the membrane so nothing can be learned about their mobility
d. They move at a much slower rate than the intact protein

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PART 2: Short Answer Questions

Q1. [3 marks] You treat breast cancer cells with vorinostat, a drug that inhibits histone deacetylases, and are now curious to know what genes are now expressed compared to the untreated control.

a. [1 marks] To answer this question you perform Chromatin ImmunoPrecipitation (ChIP) to see which DNA sequences RNA Pol II is bound to after the treatment. Indicate what order each step of ChIP occurs by labeling within the chart (e.g. Step 1, Step 2, Step 3 or Step 4).

Order	Steps in ChIP Protocol
3	Immunoprecipitate to isolate Pol II cross-linked to DNA
4	Reverse cross-links, isolate DNA and perform PCR or deep sequencing
1	Treat living cells or tissues with a cross-linker such as formaldehyde
2	Sonicate to shear chromatin into small fragments and add antibody to Pol II

b. [2 marks] Being an enthusiastic scientist, as soon as you get your ChIP data you immediately search the gene list for known tumor suppressors and oncogenes. Think about what you know about cancer, tumor suppressor and oncogene expression in cancer cells, and the effect of histone acetylation on gene expression. How do you anticipate the histone deacetylate inhibitor will impact tumor suppressor and/or oncogene expression and why? Limit your answer to 3 sentences or less.

ANSWER: One of the known mechanisms for inhibiting tumor suppressor expression in the setting of cancer is the overexpression of histone deacetylases. Histone acetylation opens up chromatin for transcriptional activity, and histone deacetylases remove acetyl groups and tighten up the chromatin where tumor suppressor genes reside in the genome. Therefore, you are hoping to see RNA Pol II bound to the promoter of tumor suppressor genes in the cancer cells treated with vorinostat.

Marking scheme:

+1 mark for correctly describing the impact of histone acetylation on gene expression

+1 mark for indicating that you expect to see RNA Pol II bound to the promoter of tumor suppressor genes in vorinostat treated cells

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Q2. [4 marks] Below is a segment of the sequence of epithelial cadherin containing the transmembrane segment. Circle the region that is the most likely the transmembrane segment. In three sentences or less, state the reason for your selection.

QVTTLDVHVCDCE**QLLILILLLLLLVGILGGGILAFLPAILR**GTVNNCMKAGIVAAGLRRTVVK EPLLPPDDDT

ANSWER:

Looked for a sequence with consecutive amino acids with **hydrophobic** R-groups around 27 aa long (in this case 26 aa) that interact with **the fatty acyl tails of the phospholipids**. The transmembrane space also includes **hydrophilic amino acids** flanking the hydrophobic portion (at the ends) that interact with the **hydrophilic head group of the phospholipids**. The selected sequence is therefore 27 amino acids long, with one hydrophilic amino acid on each side (flanking) the 25 hydrophobic amino acids.

Marking scheme:

+ 2 marks for selecting the exact region, +1 mark for mostly correct
+ 1 mark for mentioning fatty acyl tails interact with hydrophobic R-groups (or amino acids)
+ 1 mark for mentioning the hydrophilic head group of the phospholipid interact with hydrophilic aa

Q3. **[3 marks]** You isolate cells from a person who has a mutation in a single allele of p53, a person with a mutation in both alleles of p53, and an individual with normal p53 expression. In three sentences or less, what do you expect if you expose the normal and p53 mutant cells from each of the three patients to a chemotherapeutic agent that induces DNA damage (e.g. etoposide) in culture?

ANSWER: In the cells with one or both alleles of p53 mutated, the p53-p21 G1-S checkpoint would compromised and cell cycle could progress despite the presence of DNA damage caused by the chemotherapeutic agent. Hence, while the normal cells would undergo apoptosis, the cells with both alleles of p53 mutated would continue to divide similar to untreated control, while those with a single mutated allele of p53 will proliferate better than the normal patient cells, but less well than the patient with two mutated alleles of p53.

Marking scheme:
+1 marks for mentioning the role of p53 in halting cell cycle in response to DNA damage
+1 marks for noting that normal cells will respond to the agent by dying
+1 marks for noting that cells with two mutated alleles of p53 will continue to grow unaffected by the agent, while those with a single mutated allele will have a phenotype that appears in-between that observed for normal cells and those with two mutated alleles.

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Q4. [3 marks] If a reaction vessel is cold to the touch and the reaction results in an increase in order in the reaction vessel, is the reaction spontaneous or nonspontaneous? Explain your answer in four or fewer sentences. (Hint: $\Delta G = \Delta H - T\Delta S$)

ANSWER: The reaction is nonspontaneous. The ΔH is positive and the ΔS negative. When these values are plugged into the equation, $\Delta G = \Delta H - T\Delta S$, the result is a positive ΔG and, therefore, a nonspontaneous reaction.

Marking scheme:
+1 mark for stating that the reaction would be nonspontaneous
+2 marks for indicating that this is due to a positive ΔH and a negative ΔS

Q5. [3 marks]. You have been asked to study the biological effects of a new anti-cancer drug, *Nucleisin* (fictional). Preliminary tests indicate that Nucleisin deregulates the nuclear pore complexes of highly mitotic cells, allowing large molecules to freely pass through the nuclear envelope and thereby affecting the size and shape of the nucleus. Based on your experiences in the teaching lab, propose an experiment to study the effect of nucleisin on nucleus size and shape.

ANSWER:
1) Treat a cancer cell line (e.g. HeLa) with and without Nucleisin
2) Stain these cells with DNA-binding/nucleus staining dye (e.g. Fast Blast DNA stain)
3) Take images of these cells at 40X (you should be able to see at 10X, but it's harder to distinguish subcellular features)
4) Measure and compare the size and shape of the nuclei between the two treatments

Marking Scheme: +1 for noting 1 step, +2 for noting 2-3 steps, +3 for completely correct

Q6. [4 marks] You are a macrophage migrating toward a foreign bacterium that you fully intend to engulf and destroy within your lysosome.

a. [1 mark] The bacterium left a trail of cytokines that receptors embedded within your plasma membrane detect and indicate that the bacterium has taken a sharp turn towards the left. Below are the steps involved in migration. Indicate what order each step of migration occurs by labeling within the chart (e.g. Step 1, Step 2, Step 3 or Step 4).

Order	Steps in cell movement
Step 2	A new focal adhesion at the front of the cell is formed
Step 4	The rear of the cell detaches from the substrate and adhesion receptors are recycled to the front of the cell
Step 3	The nucleus, organelles and cytoplasm are pushed toward the front of the cell in a myosin-dependent manner
Step 1	Lamellipodia at the front of the cell extends forward (extension)

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a. [1 mark] The extension step of migration is induced by a rapid burst of actin polymerization at the leading edge of the cell that pushes the cell membrane forward. Critical to this polymerization burst is the generation of branched actin structures. Name a protein required to nucleate actin and generate branched actin polymers. **ANSWER**: Arp2/3

c. [2 marks] Directed cell movement requires actin reorganization. The small GTPases Rac, Rho and Cdc42 are known to be involved in this process by organizing actin into three distinct types of actin-containg structures. Indicate in the table below, the type of actin-containing structure each GTPase generates.

Small GTPase	Actin-containing structure
Rac	Lamellipodia
Rho	Actin stress fibres
Cdc42	Filopodia

Marking scheme:
+1 mark for 1 correct answer
+1 mark for 2 or more correct answers

Q7. [**3 marks**] DNA mutations are often classified by their potential to disrupt protein function. Consider the following codon sequence: AUGCCUCCUCAG (original sequence)

a. Translate the amino acid polypeptide encoded by the sequence above.
ANSWER: Methionine-proline-proline-glutamine [1 mark]

b. What type of mutation results from the following single base change?
ATGCCTCCTTAG (mutated sequence)
ANSWER: nonsense mutation [1 mark]

c. Rewrite the original sequence to indicate a frameshift mutation.
ANSWER: Insertion or deletion of one or two bases [1 mark]

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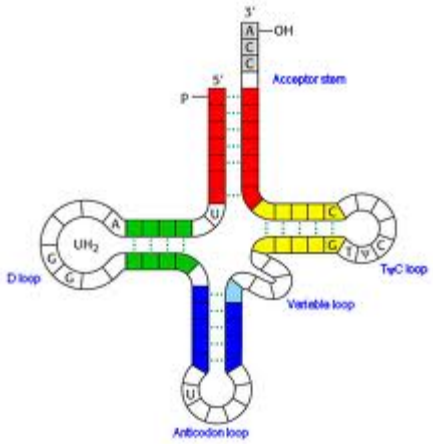
Q8 [3 marks]. The anticodon sequence of a tRNA is indicated just below the tRNA image. Write the codon(s) that this tRNA can recognize as well as the amino acid (s) that can associate with the tRNA.

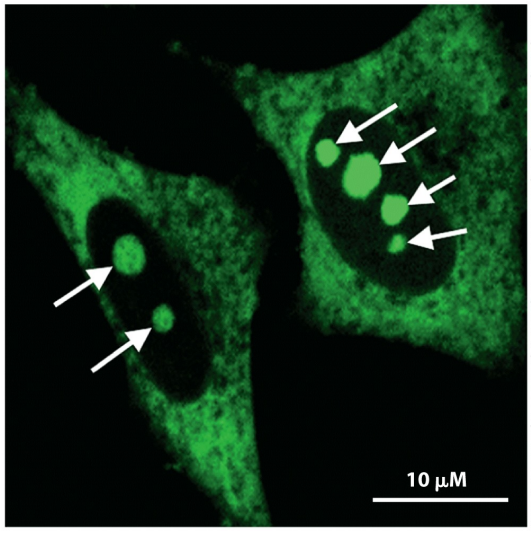


Anticodon = CAA

ANSWER: Amino acid = valine [1 mark]; Codons = GUU, GUC, GUA, and GUG [1 mark for partially correct, 2 marks for listing all four sequences]

Q9 [3 marks]. As a side project in the teaching lab, you decide to generate a plasmid containing the green fluorescent protein (GFP) gene fused to the c-terminal end of the cyclin D1 gene. You introduce the plasmid into HELA cells and track the dynamic localization of the cyclin D1-GFP fusion protein and you immediately realize something is wrong. The image below is what you saw when you looked at your cells with the fluorescence microscope. You sequence your plasmid to find out what gene sequences are contained in your plasmid, though you already have a pretty good idea what gene it contains. The GFP gene is indeed in the plasmid, but as you expected, the cyclin D1 gene is not! You are starting to understand why they call it 're-search' ☺

Based on what you know about where cyclins should be localized in the cell and your knowledge of various subcellular regions of the cell, what tipped you off that something wasn't right? Rather than cyclin-D1, what gene do you expect you fused to GFP? Explain your answer in <u>4 sentences or less</u>.



From C. E. Lyon and A. I. Lamond, Curr. Biol. 10, p. 323, 2000, Fig. b, with permission from Elsevier.

ANSWER: You see GFP localized to the nucleolus, a subcellular region of the nucleus where ribosomal RNA is transcribed and where rRNA is assembled into the ribosomal proteins to create the small (40S) and large (60S) ribosome. Cyclins are found in the nucleus and cytoplasm, but not the nucleolus. As you expected, when you sequence the plasmid you find that the sequence codes for a ribosomal protein, which is why you see it in the nucleolus, where the ribosome is assembled, and in the cytoplasm, where translation occurs.

Marking Scheme: +1 for noting GFP in the nucleolus, +1 for recognizing that the sequence encoded for a ribosomal protein, and +1 for indicating that cyclins are not found in the nucleolus.

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Q10 [4 marks]. Consider what you learned about cancer cells.

a. [3 marks] What metabolic pathway do cancer cells utilize to generate ATP and why is this advantageous to them? Answer in three or fewer sentences. Glycolysis [1 mark]. Because it generates ATP quickly [1 mark] and does not require oxygen [1 mark], which is important since tumours grow rapidly and tend to have lower access to oxygenated blood than normal tissues (low oxygen, hypoxia).

b. [1 mark] What is the name of the process that tumours initiate to increase blood and oxygen flow into the center of the rapidly growing tumour? Angiogenesis [1 mark]

Q11[4 marks]. Watching brightfield videos of cell migration fascinates you. You decide it would be even more fun to watch cells move if you could also visualize changes in the actin cytoskeleton. Since your most recent adventure in cloning was a bit frustrating, you are not particularly excited by the idea of creating an actin-GFP fusion protein. Fortunately, talking with a colleague you find out that you can purchase the pharmacologic agent, Phalloidin, that is tagged with GFP and which readily enters living cells. You follow the manufacturers instructions and in no time you've captured a fluorescent image of the cellular cytoskeleton. You run a fluorescence time-lapse experiment overnight and to your disappointment, when you arrive the next morning all of your cells are dead! You wonder if you added too much Phalloidin or if you exposed them to too much light. Then all of a sudden you recall what you learned in BME205 about how Phalloidin works and you know why your cells died. In four sentences or fewer, describe why your cells died.

ANSWER: Phalloidin binds to intact actin filaments and prevents their turnover (no removal or addition of actin subunits). As a result, the cytoskeleton is paralyzed. Since the cytoskeleton is essential to more than just cell movement, Phalloidin exposure ultimately results in cell death.

Marking scheme: +2 for correctly indicating the mechanism of Phalloidin action, +1 for the effect it has on the cytoskeleton (paralysis), and +1 for noting that the cytoskeleton is important for more than just migration.

Q12 [3 marks]. Enzymes are biological catalysts. Below is a chart showing the kinetics of a reaction catalyzed by an enzyme. The three curves correspond to an uninhibited (control) reaction, or reactions treated with a competitive or non-competitive enzyme inhibitor.

a. Indicate which line corresponds to which condition [1 mark].
A. Uninhibited B. +Competitive inhibitor C. +Non-competitive inhibitor

b. Two or fewer sentences, explain how competitive and non-competitive enzyme inhibitors function to disrupt enzyme action [2 marks]. **ANSWER**: Competitive enzyme inhibitors compete with the enzyme for active sites on the substrate [1 mark], while non-competitive enzyme inhibitors bind to sites other than the active site and inactivate the enzyme [1 mark].

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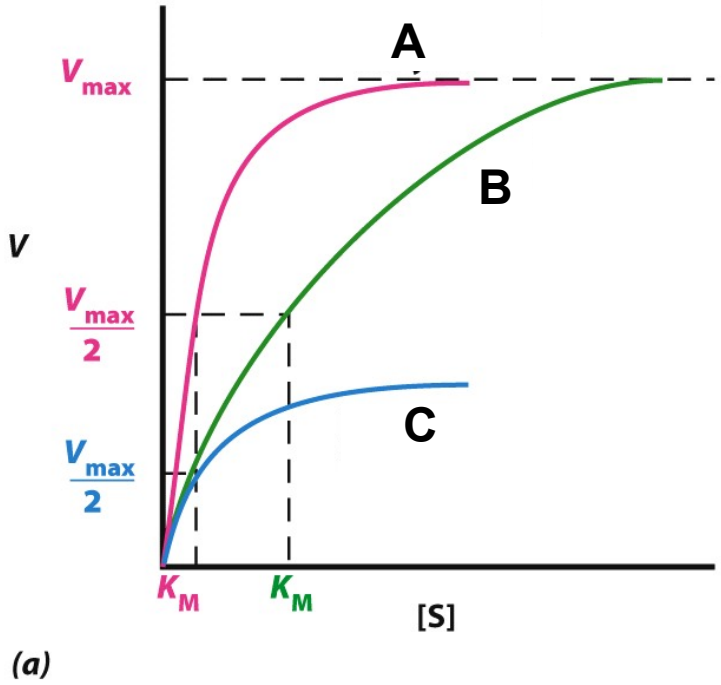
Student #: _____

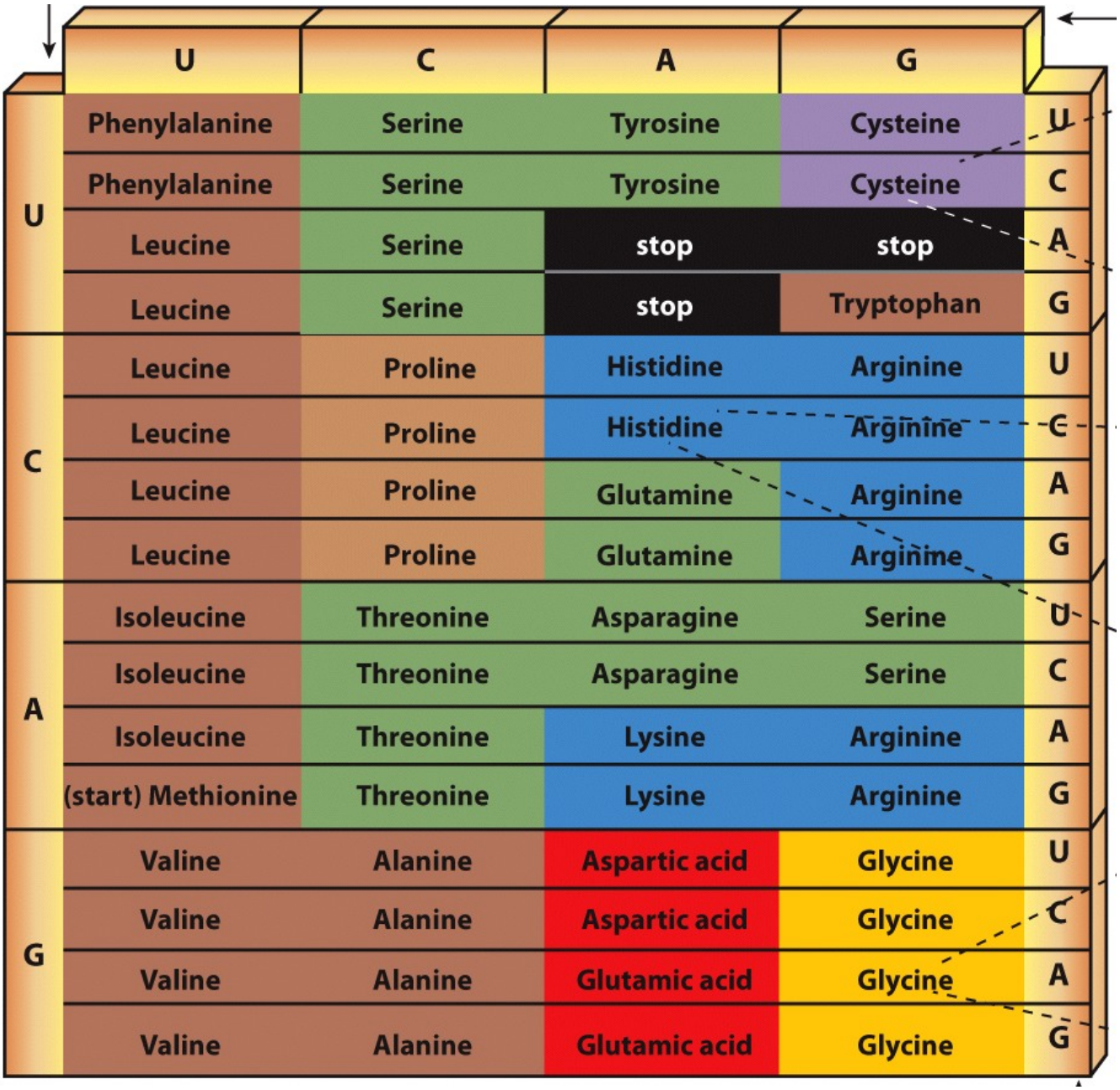


(a)

Name: _____
Student #: _____

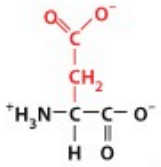
RESOURCE

The Genetic Code Table

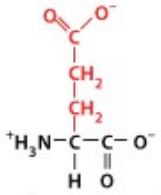


	U	C	A	G	
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
	Phenylalanine	Serine	Tyrosine	Cysteine	C
	Leucine	Serine	stop	stop	A
	Leucine	Serine	stop	Tryptophan	G
C	Leucine	Proline	Histidine	Arginine	U
	Leucine	Proline	Histidine	Arginine	C
	Leucine	Proline	Glutamine	Arginine	A
	Leucine	Proline	Glutamine	Arginine	G
A	Isoleucine	Threonine	Asparagine	Serine	U
	Isoleucine	Threonine	Asparagine	Serine	C
	Isoleucine	Threonine	Lysine	Arginine	A
	(start) Methionine	Threonine	Lysine	Arginine	G
G	Valine	Alanine	Aspartic acid	Glycine	U
	Valine	Alanine	Aspartic acid	Glycine	C
	Valine	Alanine	Glutamic acid	Glycine	A
	Valine	Alanine	Glutamic acid	Glycine	G

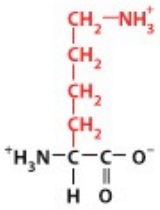
Polar charged



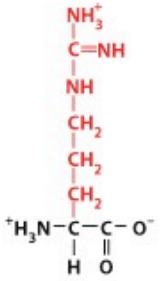
Aspartic acid
(Asp or D)



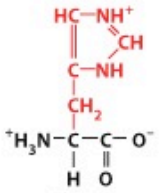
Glutamic acid
(Glu or E)



Lysine
(Lys or K)

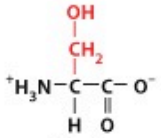


Arginine
(Arg or R)

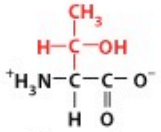


Histidine
(His or H)

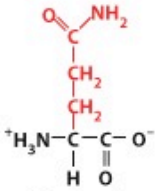
Polar uncharged



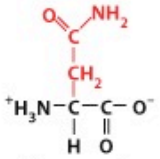
Serine
(Ser or S)



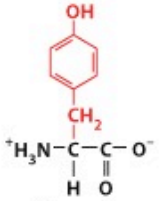
Threonine
(Thr or T)



Glutamine
(Gln or Q)

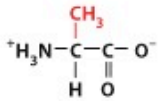


Asparagine
(Asn or N)

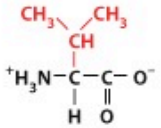


Tyrosine
(Tyr or Y)

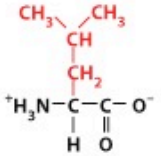
Nonpolar



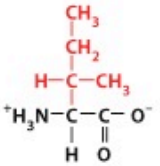
Alanine
(Ala or A)



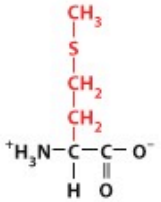
Valine
(Val or V)



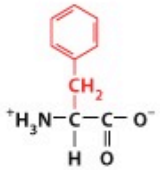
Leucine
(Leu or L)



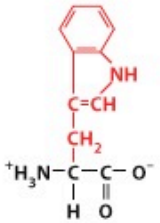
Isoleucine
(Ile or I)



Methionine
(Met or M)

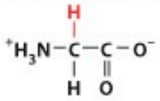


Phenylalanine
(Phe or F)

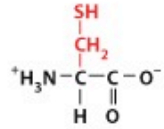


Tryptophan
(Trp or W)

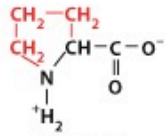
Side chains with unique properties



Glycine
(Gly or G)



Cysteine
(Cys or C)



Proline
(Pro or P)