Fill in the blanks (3 pts each).
Pick the term from the list provided that best completes each sentence. A term is provided for each question, but you may substitute your own term if it satisfactorily completes the sentence.

1. The term “differentiation” refers to the progressive acquisition of cellular characteristics that allow a cell to perform its ultimate function.

2. A(n) _______________ is a sheet of cells, usually connected by tight junctions, that typically forms linings of organs and body cavities.

3. Gain-of-function mutations in (proto)-oncogenes can lead to cancer.

4. In autonomous specification, if we remove a blastomere (cell) from an early embryo and culture it, it would produce the same set of cell fates as if it were left in an intact embryo.

5. Differential affinity among cells is mediated primarily by _______________ molecules.

6. The concept that all cells of an organism have the same genetic composition and that cellular differences arise through differential gene expression is known as _______________.

7. Blastomere B.4.1 of the tunicate embryo normally produces muscle but a.4.2 does not. MACHO mRNA is normally present only in B.4.1, but if MACHO mRNA is injected into a.4.2, the a.4.2 cell now gives rise to muscle cells. MACHO mRNA is a(n) _______________ of muscle cell fate.

8. Second messengers include non-protein molecules, such as diacylglyceride (DAG), Ca++ and inositol tris-phosphate (IP3), that are involved in intracellular signaling.

9. Induction _______________ is when one cell or tissue alters the activity, behavior or fate of another cell or tissue.
10. Steroids have nuclear receptors that directly function as transcription factors upon activation.

Term list for questions 1-10

- Apoptosis
- Autonomous specification
- Caspases
- Cell adhesion
- Cell fate
- Cell migration
- Cytoskeleton
- Competence
- Conditional specification
- Differential splicing
- Differentiation
- Determinant
- Epithelium
- Extracellular matrix
- G proteins
- Gap junctions
- Genomic equivalence
- Golgi
- Induction
- Integrins
- MAP kinases
- Mesenchyme
- Morphogen
- Oncogene
- Peptide hormones
- Potency
- Receptor kinase
- Second messengers
- Signal transduction
- Steroids
- Tumor suppressor
- Ubiquitin

Multiple choice (3 pts each)
Pick the choice that best answers each question. You may qualify your answers with an explanation if you want.

11) The following is a class of proteins that is part of the complex that regulates the central events of the cell cycle:

a. cyclins
b. caspases
c. tubulins
d. integrins
e. none of the above

12) We assay the activity of a particular MAP kinase in two different cell types and find that activity is present in one cell type but not the other. Possible reasons for this differential activity include:

a. differential MAP kinase gene transcription
b. differential mRNA processing of the MAP kinase transcript
c. differential translation of the MAP kinase transcript
d. differential post-translational modification of the MAP kinase protein
e. all of the above
13) Tumor suppressors include genes whose normal functions are:

   a. activators of cell cycle progression
   b. cell adhesion
   c. activators of apoptosis
   d. inhibitors of apoptosis
   e. inhibitors of differentiation

14) If we transplant an amphibian optic cup subjacent to head ectoderm, a lens forms from the ectoderm. However if we transplant the optic cup subjacent to trunk ectoderm, no lens forms. The differential response by the head and trunk ectoderm could be due to differential expression of

   a. extracellular matrix
   b. initiator caspases
   c. receptors
   d. signal ligands
   e. actin

15. “Death receptors” are likely to activate

   a. paracrine signals
   b. cyclin dependent kinases
   c. ubiquitin
   d. cyclin dependent kinase inhibitors (CKIs)
   e. initiator caspases

**Short answer.**
Write in complete sentences and be sure to explain your answers where indicated.

16) (5 points) Brassinolide (BR) is a plant hormone that regulates cell elongation via the to the following signal transduction pathway:

   \[ \text{BR} \rightarrow \text{BRI1} \rightarrow \text{BIN2} \rightarrow \text{BES1} \rightarrow \text{cell elongation} \]

BRI1 is a receptor kinase, BIN2 is a cytoplasmic protein kinase, BES1 is a transcription factor.

Does brassinolide promote or inhibit cell elongation? Explain.

**BR promotes cell elongation.**

\[ \text{BR activates BRI1. Once activated BRI1 inhibits BIN2. This relieves the inhibition of BIN2 on BES1 and allows BES1 to promote cell elongation} \]**
17) (10 pts) Two different loss-of-function mutants, *sevenless* and *boss*, have the exact same phenotype of not differentiating an R7 cell in the *Drosophila* eye. In genetic mosaics, the following is observed:
- For *sevenless*, in eyes with a mutant pre-R7 cell and a wild type R8 cell, the R7 cell does not differentiate.
- In eyes with a R8 cell that is mutant for *sevenless*, but with a pre-R7 cell that is wild type, a normal R7 cell differentiates.
- For *boss*, eyes with a pre-R7 cell that is mutant but with a R8 cell that is wild type, the R7 cell differentiates normally.
- Eyes with a pre-R7 cell that is wild type, but with a R8 cell that is mutant for *boss*, the R7 cell does not differentiate.

a) (2 pts) Which gene acts cell-autonomously and which acts non-cell-autonomously?
- *Sevenless* is cell autonomous
- *Boss* is non-cell-autonomous

b) (4 pts) Which gene is likely to be involved in producing an inductive signal? Explain.
- *Boss* because it is required in the R8 cell for the induction of R7 cell differentiation.

c) (4 pts) Which gene is likely to be involved in establishing competence to respond to an inductive signal? Explain.
- *Sevenless* because it is required in the pre-R7 cell for that cell to respond to the induction by R8.

18) (10 pts) A marker dye is injected into a particular region of a frog embryo, and it is determined that that region gives rise to neural tissue. Describe how one would determine experimentally at what point in embryogenesis neural cell fate was specified. Describe potential outcomes of this experiment and how they would be interpreted with relation to the question of specification.

Mark the cells of interest in several embryos. At different times in development, explant the cells to a neutral environment (i.e. culture). If the cells develop as neural cells, they were specified. If they do not form neural cells, they were not yet specified.
19) (16 points) Choose either the Retinoblastoma (Rb) or p53 protein (circle one) and answer the following questions in relation to the protein you chose.

a) (10 points) Describe the molecular mechanism by which this protein regulates the cell cycle. How is this protein regulated? What proteins does it regulate and how? What is the function of the proteins it regulates? What is the net effect on the cell cycle?

Rb is a key checkpoint regulator. It functions by binding to and inhibiting the activity of a transcription factor called E2F. The function of E2F is to promote the expression of genes required for S-phase, including the S-phase cyclin. It also promotes expression of a CKI that blocks the G1-phase cyclin/cdk. Rb is regulated through phosphorylation by the G1-cyclin/cdk which causes it to dissociate from E2F. Thus, Rb inhibits the cell cycle until phosphorylated.

b) (2 points) What influence does this protein have on apoptosis?

Rb inhibits apoptosis. Phosphorylation of Rb de-represses both cell cycle and apoptosis.

c) (4 points) Of what particular importance is this protein to cancer?

Rb is a tumor suppressor gene. Loss-of-function mutations in Rb de-repress the cell cycle allowing cell proliferation. Mutations in other genes that cause cancer must also somehow result in Rb mis-regulation or the cell cycle will not progress.

20) (4 pts) Upon activation, many signal transduction pathways form a negative feedback loop to inactivate the receptor. Why is this important to a cell?

If there was not a mechanism to turn signal transduction pathways back off, cells would not be able to respond to changes in their signaling environment. It is required to re-sensitize the cell.
19) (16 points) Choose either the Retinoblastoma (Rb) or p53 protein (circle one) and answer the following questions in relation to the protein you chose.

a) (10 points) Describe the molecular mechanism by which this protein regulates the cell cycle. How is this protein regulated? What proteins does it regulate and how? What is the function of the proteins it regulates? What is the net effect on the cell cycle?

p53 is a transcription factor. Its expression is activated by cell/DNA damage. It activates the transcription of p21, a CKI that binds and inhibits the cyclin/CDK complex at all stages of the cell cycle. Thus p53 blocks cell cycle progression in response to cell damage.

b) (2 points) What influence does this protein have on apoptosis?

p53 promotes apoptosis.

c) (4 points) Of what particular importance is this protein to cancer?

p53 is a tumor suppressor that prevents cancer formation by blocking proliferation and promoting elimination of damaged cells that would be at risk of becoming malignant. p53 is also important for many cancer therapies that work by inducing apoptosis in tumorous cells.

20) (4 pts) Upon activation, many signal transduction pathways form a negative feedback loop to inactivate the receptor. Why is this important to a cell?
21) (10 pts) Describe the key components of the mitochondrial pathway, how they are regulated and how they regulate apoptosis.

Cytochrome C → Apaf → Initiator Caspase → Effector Caspase → Apoptosis

Bax → Bcl2

Effector caspases are proteases that break down cellular components and proteolytically regulate other proteins. They are activated through proteolytic cleavage of pro-effector caspases by initiator caspases. Apaf activates initiator caspases by proteolytic cleavage.

Apaf is activated by cytochrome C, thus cytochrome C promotes apoptosis. Cytochrome C is released from mitochondria when stress or damage causes membrane leakage.

Apaf is also regulated by inhibitor from Bcl2, a protein located on the cytosolic face of mitochondrial membranes. Bcl2 can be inhibited by Bax, which releases Apaf and thus induces apoptosis.