

**Directions:** This exam will be divided into separate pages to facilitate grading. **Write your name on every page!**

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page 5 (4) \_\_\_\_\_

Answer in the spaces provided, they should suffice.  
If you absolutely need more space, put an arrow on the front and a number on the back of the same page as the question is on.

TOTAL (75) \_\_\_\_\_

**Be concise with the short answer questions, a few words will usually suffice!**

1) (6 points) Predict (and explain why) what would happen to skeletal muscle development in a mouse with normal Myf5 activity, but without any functional MyoD, MRF4, or myogenin genes.  
*Without myogenin, skeletal muscle development would halt at the myoblast stage. Technically you were also to know that since MyoD activity is absent, you also might predict that the formation of hypaxial myoblasts could be delayed.*

2) You have discovered a colony of mice with defects in skeletal muscle development. For each example, note which gene(s) were mutated to cause the observed defect and explain why.

a) (2 points) A mouse embryo has normal looking somites, including a normal myotome, but no myoblasts.  
*The embryo likely has mutations in both the MyoD and Myf5 genes since the two genes have overlapping functions in producing myoblasts. Both must be defective to prevent myoblast formation*

b) (2 points) An embryo has myoblasts, but no myotubes.  
*A mutation in myogenin would allow myoblast production, but prevent the transition to myotubes.*

3) (4 points) Name the two different processes that form bone, tell how they differ from each other, and provide one example of a bone that is produced by each type of osteogenesis.  
*intramembraneous ossification involves the direct conversion of mesenchyme into bone (example= skull bones), while endochondrial ossification involves the initial production of cartilage and its subsequent replacement by bone (examples= vertebrae, pelvis, limb bones) (2 pts each)*

4. (6 pts) It has been postulated that Joan of Arc may not have been a normal XX female since she apparently never menstruated. Propose two possible genotypes she might have had and explain why these genotypes would have resulted in a female somatic phenotype but lack of a functional female reproductive system.

*Two of the following answers (there are other possibilities too—like XY with only one copy of Sox-9, but these are the most likely). 3 pts each*

*1. She could have been either XY with androgen insensitivity, XO, or XY with an SRY mutation. With androgen insensitivity, testes are formed because the individual has a Y chromosome. Mullerian duct degenerates due to AMH production. However, the Wolffian duct cannot be maintained because the testosterone receptors can't bind testosterone. Later, enough estrogen is produced in these individuals to give them external female characteristics, but on the inside they have testes with no other reproductive structures or tracts. 2. XO individuals look more or less female, but are infertile and have menstruation problems, due to the fact that they are missing an X chromosome and there are regions required for fertility that are normally not inactivated. 3. The third possibility is an XY individual with an SRY mutation or absence. Without SRY, they would form ovaries and generate the female ductal structures. However, because they only have one X, they would not be totally normal females, and would probably be infertile.*

5. In mammals, primary and secondary sex determination are distinct.

- a. (2 pts) Briefly describe the mechanics of primary sex determination, and the principle molecule involved.

*Primary sex determination is determined by a single chromosome, the Y chromosome. The presence of the SRY gene on the Y chromosome directs the formation of testes rather than ovaries.*

- b. (3 pts) Briefly describe the main factors involved in secondary sex determination in both males and females.

*In males, testosterone and AMH (or MIS) are secreted by cells in the testes. AMH makes the mullerian duct degenerate while testosterone allows the maintenance of the Wolffian duct.*

*In females, the Mullerian duct is maintained by estrogen while the Wolffian duct degenerates due to lack of estrogen.*

*These ductal systems make up all the rest of the female and male reproductive tracts, and the hormones that will be released from the gonad are required for later obvious male and female external differences.*

6. (2 pts) Briefly describe the mechanism by which **ONE** of the following organisms accomplishes sex determination : *Drosophila*, *C. elegans* , or reptiles.

*Drosophila and C. elegans: X to A ratio. High X/A is female (or hermaphrodite), low X/A is male.*

*Reptiles: lower temperature seems to promote male development while higher temperatures seem to promote female development. This may be due to the activity of aromatase, which makes testosterone into estrogen, and is inactive at low temps, active at higher temps.*

7. (4 pts) During the formation of tissues, cells undergo transitions from mesenchyme to epithelium or from epithelium to mesenchyme. Describe **one example of each** of these transitions.

*Mesenchyme to epithelial:*

*--In heart development, lateral plate mesoderm (mesenchyme) coalesces on either side of the neural tube to form endocardial tubes, which are epithelial.*

*--In somites, they start off mesenchymal in the PSM, then become epithelial after they segment.*

*--mesenchyme of the kidney ultimately aggregates and then epithelializes into a nephron*

*Epithelial to mesenchyme:*

*--Endocardial cells of the heart leave their epithelial layer to migrate out into the cardiac jelly to make the endocardial cushion which will form the septae and valves of the heart*

*--Somitic cells become mesenchymal again, first the sclerotome, and later the myotome.*

*--neural crest cells start out part as part of the ectoderm, then become mesenchymal as they migrate*

8. (4 pts) Two axes of the limb are affected in a *Wnt7a* knock-out mouse. Which two axes, and why? Briefly explain.

*The D/V axis and the A/P axis. Wnt 7a is required for dorsal structures. Without it, the limb has ventral structures on both surfaces. Wnt 7a also induces Shh expression in the ZPA. Thus in the absence of Wnt 7a, Shh are also absent (or lower), and posterior digits are missing.*

9. (6 pts) In the experiment diagrammed on the board, interdigital space 3 is removed from a developing limb. What is the consequence of this removal on digits 3 and 4? What molecule is present in the interdigital space that is most likely responsible for this result? What is an additional role that this molecule plays in the interdigital spaces?

*Digit 3 is transformed into digit 2 because it is now affected by the interdigital space anterior to it. Digit 4 is unaffected. BMP-7 is present in the interdigital spaces. BMP-7 also induced apoptosis in these interdigital regions so that the digits are eventually not connected.*

10. (2pts) What would be the outcome on cartilage and bone production in the limb if steroid hormones (estrogen or testosterone) were blocked?

*Chondrocytes would continue to proliferate, would not become hypertrophic. Thus cartilage would not be converted to bone. Limbs would be entirely cartilaginous, and probably long.*

11. (4 points) Apoptosis and necrosis have different features.

Describe 2 features of **apoptosis** that are distinct from those of necrosis.

*e.g. cells shrink, DNA is cleaved into fragments, no inflammation, can be internally programmed (natural)*

Describe 2 aspects of **necrosis** that are different from apoptosis.

*e.g. cells explode, always caused by damage of some sort, inflammation of tissue often results*

12. (4 points) Caspases function in cell death pathways.

What type of enzyme is a caspase? *A protease*

We discussed two general classes of caspases functioning in death pathways. What were they, and for each class, what molecules are responsible for their activation?

<u>Class</u>	<u>Molecules involved in activation</u>
1- <i>initiator caspases (ie, caspase 9 and CED3)</i>	<i>Apaf-1/bcl-2 family proteins/cytochrome c CED-4</i>
2- <i>effector or execution caspases (ie, caspases 3,4,6)</i>	<i>other caspases</i>

13. (6 points) In the mouse, mutations affecting the \_\_\_*Notch*\_\_\_ signaling pathway affect somite size, by influencing the process of somite formation from the \_\_\_*pre-somitic/paraxial*\_\_\_ mesoderm. After somites form, they differentiate into different components. The \_\_\_*sclerotome*\_\_\_ portion gives rise to vertebrae, after its specification through the action of the signal \_\_\_*sonic hedgehog*\_\_\_ from the notochord. The remaining portion of the somite gives rise to the \_\_\_*dermis or dermatome*\_\_\_ of the skin and \_\_\_*muscle/myotome*\_\_\_.

14. (6 points) After graduating from MCDB, you've been hired by a biotech company. The powers that be in the company have decided that there's a market for 6-legged cats. Given that you've just learned about limb development in 4650, you're assigned to make these cats. How would you go about making them?

*Implant FGF8 beads in the flanks, or make a transgene causing expression of FGF8 there*

You realize that the cats may be more mobile if they have 4 forelimbs and 2 hindlimbs. What type of gene might be useful to manipulate to cause the extra limbs to be forelimbs?

*You'd need to manipulate Tbx gene expression, Tbx-4 and Tbx-5 specify forelimb vs hindlimb identity*

Some customers seem to want the cats to have feet with opposite A/P ordering of toes. How might you manipulate toe order? *Cause Shh or RA to be expressed on the anterior side of the limb bud (or use a bead)*

15. (2 points) You have identified a mutation that interferes with the differentiation of chondrocytes before they hypertrophy. What would you predict about formation of the bones in this animal.

*If chondrocytes can't differentiate, the most likely outcome is that neither cartilage or bone will form.*

*However, if this question was taken to mean that chondrocytes don't become hypertrophic, then the chondrocytes aren't differentiating to the point that they'll make the matrix needed for blood vessel invasion, so the bones won't form, remaining cartilaginous. Either answer will be given full credit.*

16. (2 points) You are analyzing a mouse mutation in which the animals die a few days after birth due to kidney failure. You find that they have enormous amounts of branching in their ureteric ducts, much more than normal. Suggest 2 different possible mechanisms to explain this phenotype.

*Overexpression of GDNF*

*Hyperactivity of the GDNF receptor, or of signal transduction components downstream*

17. (2 points) You have identified a mouse mutation in which the hearts are enormous, beginning very early in development. Suggest a mechanism to explain this relating to establishment of cardiogenic mesenchyme.

*Excess specification of cardiogenic mesenchyme would be due to either excessive positive signals from the endoderm or an absence of negative signals from the notochord/neural tube*

*1 of these answers suffices for credit*

18. (2 points) True/false, circle the statements that are **true**.

- The heart begins development as a single tube *technically, it's 2 tubes that fuse later—but because of the way this is worded, I think we should give credit for people who answered that this was true—ie, by the time it's a heart, it is a single tube. So, don't take off if this was selected as true.*
- The atria always found anterior of the ventricles *no, only after looping are they anterior*
- Nkx2.5 is a transcription factor important early in heart development. **True -- important in early differentiation**
- MyoD is necessary for synthesis of cardiac actin *no, there's a different pathway from skeletal muscle*
- Purkinje fibers are derived from neural crest cells *no, they come from ventricular myocytes*
- The dHand and eHand are important in distinguishing ventricles from atria *no, they specify differences between the left and right sides (l/r atria and l/r ventricles)*

19. (4 points) A question relating to the papers we discussed. Following the removal of the AER in a chick limb bud at different stages, how does resulting limb phenotype if development is allowed to proceed, and the zone of cell death in the underlying mesenchyme shortly after AER removal lend support to the prepattern model?

*There was consistently cell death ~200um below the surface of the mesenchyme  
Depending upon the stage of development when the AER was removed, different parts of the limb were lost, suggesting that the cells giving rise to that specific part of the limb were eliminated by death. Earlier removal caused loss of more of the limb because the bud was smaller, and thus the zone of apoptosis would encompass more of the prepattern.*