Multiple choice (one point each; indicate the best answer)

1. Neural tube closure is accomplished by movement of the
   a. medial hinge point cells.
   b. medial and dorsolateral hinge point cells.
   c. medial and dorsolateral hinge point cells, and surface ectoderm.
   d. medial and dorsolateral hinge point cells, surface ectoderm, and somite mesoderm.

2. Spina bifida is a medical condition caused by failure of
   a. the anterior neuropore to close.
   b. the posterior neuropore to close.
   c. cerebrospinal fluid production by the central canal.
   d. none of the above.

3. In the chick, secondary neurulation occurs
   a. in the most anterior region.
   b. in the most posterior region.
   c. throughout the entire embryo
   d. none of the above.

4. In human skin, replacement cells are first formed in the
   a. cornified layer.
   b. granular layer.
   c. germinal layer.
   d. all of the above.

5. Neurons are produced in the _____________ of the neural tube.
   a. ventricular zone
   b. intermediate zone
   c. marginal zone
   d. sulcus limitans

6. In humans the rate of neuronal formation
   a. increases from birth until mid-childhood.
   b. decreases over the first several years of life.
   c. stops completely after the first year of life.
   d. remains at the same rate as seen in the late fetus until mid-childhood.

7. Cranial neural crest cells contribute to which structures?
   a. inner ear bones, cranial nerves
   b. thymus cells, jaw
   c. facial cartilage, facial bone
   d. all of the above
8. Trunk neural crest cells are specified
   a. prior to migration.
   b. during migration.
   c. by paracrine factors after arrival at their destination.
   d. none of the above.

9. Problems with cardiac neural crest cell migration might be expected to result in defects in the
   a. specification of atrial and ventricular regions.
   b. division of the aorta and pulmonary arteries.
   c. placement of the vitelline artery.
   d. none of the above.

10. Trunk neural crest cells that migrate into and remain in the adjacent somite become
    a. melanocytes.
    b. interneurons.
    c. dorsal root ganglia.
    d. parasympathetic ganglia.

11. Neural crest cells that form part of the adrenal gland
    a. originate in the vagal region.
    b. migrate late.
    c. migrate through the ventrolateral pathway.
    d. all of the above.

12. Osteocytes secrete a matrix consisting of
    a. calcium-phosphate
    b. collagen and proteoglycans.
    c. osteoid proteins.
    d. none of the above

13. Subdivisions of mesodermal lineages are specified by BMP gradients which are
    a. high anterior, low posterior.
    b. high dorsal, low ventral.
    c. high ventral, low dorsal.
    d. high distal (or lateral), low proximal.

14. Specification of somites
    a. occurs early, in the pre-somitic mesoderm.
    b. occurs early, as the individual somites are being formed.
    c. occurs midway through the formation of individual somites.
    d. occurs late, after formation of individual somites.

15. What tissue is induced by MyoD?
    a. Intramembranous bone
    b. Tendon
    c. Muscle
    d. Ligament
16. Which genes are probably responsible for the differential specification of somites producing cervical and thoracic vertebrae?
   a. BMPs
   b. Wnts
   c. Hox
   d. FGFs

17. Alignment of myoblasts into myotubes involves
   a. cell-to-cell communication.
   b. extracellular matrix proteins.
   c. cadherin.
   d. all of the above.

18. In male mammals, the mesonephric tubules and duct become
   a. ureters.
   b. metanephrogenic mesenchyme.
   c. efferent ducts, epididymus, and vas deferens.
   d. nothing; they disappear through apoptosis.

19. Cardiogenic mesoderm is induced by signals from the
   a. primitive streak.
   b. notochord.
   c. neurectoderm.
   d. endoderm.

20. Hemangiogenic mesoderm is induced by
   a. Wnt from the neural tube and ectoderm.
   b. Shh from the neural tube.
   c. Wnt from pharyngeal endoderm.
   d. none of the above.

21. Cushion cells, which form the heart valves, arise from
   a. ventricular myocytes.
   b. epidermal ectoderm.
   c. endoderm.
   d. endocardial endothelial cells.

22. Which gene activity is involved with cardiac looping?
   a. Notch.
   b. Nodal.
   c. Noggin.
   d. Neural.

23. Blood vessels consist of an inner layer of _________cells and an outer layer of _________ cells.
   a. hemangioblast, angioblast
   b. angiocyte, vasculocyte
   c. mesenchymal, epithelial
   d. endothelial, periendothelial
24. During early embryonic development, most of the blood supply comes from the
   a. blood islands.
   b. yolk sac.
   c. liver.
   d. bone marrow.

25. Hindlimbs are specified by the presence of
   a. Tbx4
   b. Tbx5
   c. Tbx6
   d. none of the above.

26. The AER function can be replaced by
   a. Shh
   b. BMP
   c. Wnt
   d. Fgf

27. Which proteins stabilize Fgf10 in the lateral plate mesoderm to initiate limb bud formation?
   a. Shh
   b. BMP
   c. Noggin
   d. Wnt

28. Gremlin acts in the early limb bud as
   a. a BMP antagonist.
   b. an Shh antagonist.
   c. a repressor of Gli3 transcription.
   d. none of the above.

29. In mice, digit 3 is specified by ______________ signaling.
   a. paracrine
   b. autocrine
   c. paracrine and autocrine
   d. juxtacrine

30. Limb dorsal-ventral patterning is under the control of
   a. Shh
   b. BMP
   c. Noggin
   d. Wnt
True / False (one point each)

31. The ventricular zone of the neural tube consists of multiple layers of germinal epithelium. True / False

32. Myelination of human brain neurons is completed during the third trimester of pregnancy. True / False

33. Trunk neural crest cells migrating via the dorsolateral pathway contact the ectoderm. True / False

34. The positional identity of an individual somite is established by the particular neural crest cells that migrate through it. True / False

35. Specification of somite subdivisions (e.g. sclerotome, dermamyotome, etc.) is established in the pre-somitic plate stage. True / False

36. The endothelial lining of the heart arises from mesoderm. True / False

37. Blood vessels are not specified as veins or arteries until after they have formed. True / False

38. The tonsils are derived from endoderm of the 2nd pharyngeal pouch. True / False

39. Wnt proteins stimulate the degradation of Fgf10 in the lateral plate mesoderm, thereby initiating limb bud formation. True / False

40. Overexpression of Shh in the limb bud would cause additional digits, all with digit 1 identity. True / False
**Fill in.** Indicate the location of each of the following structures, cells, or tissues. Label each with a line, arrow, or circle. (⅓ point each)

41 - 50.

Indicate where each of the following cells, structure or organs originate. Label each with a line, arrow, or circle. (⅓ point each)
Define. Write a brief definition or description of *any five* of the following terms or phrases (2 pts. each):

51. Dorsolateral hinge point
*Cells located in the dorsal and lateral areas of the folding neural plate that change from a columnar to a triangular shape, thereby pulling accomplishing the bending necessary to complete the dorsal portion of the neural tube.*

52. Myelination
*The process by which nerve axons are covered with multiple layers of lipid membranes donated by Schwann cells (in the peripheral nervous system) or oligodendrocytes (in the central nervous system). Myelination serves to insulate the nerves to conduct electrical signals.*

53. Intramembranous ossification
*Bone formation in which individual cranial neural crest cells differentiate into osteoblasts, which excrete proteoglycan matrices, which mineralizes into bone. This process forms most of the bones in the skull and face, as well as the scapula.*

54. Clock and wave mechanism
*Clock and wave describes a series of gene expressions that can produce a repeated function, such as the formation of somites. The clock function involves the expression of a gene that activates an effector gene and also activates its own transient inhibitor. Buildup of the inhibitor eventually shuts off the gene, which is reactivated after the inhibitor degrades. Activation of the effector takes place in a wave-like manner, building from the initial activation point and receding as the inhibitor shuts down activity.*

55. Hemangiblast
*Stem cells originating in the cardiogenic (lateral plate) mesoderm, induced by endodermal FGF. These cells are capable of producing both angioblasts and endothelial cells.*

56. Metanephrogenic mesenchyme
*Intermediate mesoderm and trunk neural crest tissue that produces the nephrons of the definitive kidney (metanephros) in vertebrates.*

57. Limb field
*Cells in the flank of a developing embryo that are capable of forming a limb. In addition to the limb proper, limb field cells also form the tissue surrounding the limb, such as the shoulder girdle and peribrachial flank in the forelimb.*

58. Zone of polarizing activity
*Cells in the posterior distal region of the developing limb bud that establish the posterior identity. Cells originating in the ZPA and producing sonic hedgehog become digits 5 and 4, while cells under the influence of Shh secreted from the ZPA become digits 3 and 2.*
**Short Answer.** Answer *any five* questions. Be certain to address all parts of the questions. (4 points each)

59. Describe the dorsolateral and ventrolateral migration of trunk neural crest cells. Provide details as to the timing of the migration, pathways taken, eventual fate, and location of the neural crest cells that follow each pathway or portion of the pathways.

*Early-migrating trunk neural crest cells travel through the ventrolateral pathway. The NC cells migrate ventrally between the neural tube the anterior portion of the somite. A portion of the NC cells move into the anterior somite and stay, forming dorsal root ganglia, and a portion move through the somite mesenchyme to form sympathetic ganglia, adrenal medulla, and aortic nerve clusters.*

*Later-migrating trunk neural crest cells move through the dorsolateral pathway between the somite and epidermal ectoderm. These NC cells become melanocytes.*

60. During the formation of the circulatory system, venous and arterial capillaries connect exclusively to each other. How is this accomplished?

*Angioblasts destined to become either arterial and venous endothelial cells are specified by VEGF (via Notch and Gridlock) to express the ligand ephrin-B2 or its receptor, EphrinB4 (receptor tyrosine kinase), respectively. Thus, the connection of veins to arteries is a function of ligand-receptor interactions, while connections between veins and veins or arteries and arteries are avoided.*

61. Describe the general molecular features of neural tube induction. What inducing molecules are involved and where do they originate?

*Sonic hedgehog from the notochord induces the ventral portion of the neural tube to become the floor plate, which then produces Shh on its own. Shh induces the ventral portion of the neural tube to fates including the motor nerves.*

*Ectodermal BMPs induce the dorsal region of the neural tube to become roof plate, which then produce additional BMPs and other members of the TGF-β superfamily. These induce the dorsal neural tube to fates including sensory nerves.*

62. Development of *Drosophila* segments and parasegments share some similarities with vertebrate re-segmentation of the sclerotome. Describe these similarities.

*Drosophila segments are made up of anterior and posterior compartments. Some gene expression patterns take place in sets of cells consisting of the posterior compartment of one segment and the anterior compartment of the adjacent segment.*

*Likewise, the sclerotome originating from a single somite is split into rostral (head) and caudal (tail) segments, which rejoin out of phase to form vertebrae, while the muscles from that somite and nerves inervating them are out of phase, which allows muscles to connect to and act against opposing bones.*
63. Amniote eggs demonstrate a variety of adaptations to development on land or inside the mother’s body. For any two of these adaptations, describe the structure, how it is produced, and what environmental challenge it addresses.

**Amnion** – is the membrane which encloses and protects the embryo in a fluid matrix; it is produced by the somatopleure (somatic mesoderm and ectoderm).

**Chorion** – forms the embryonic portion of the placenta, which provides nutrition, gas, and waste exchange for the embryo; it is produced by the somatopleure (somatic mesoderm and ectoderm) and trophoblast.

**Allantois** – is the nitrogenous waste repository or passageway for amniotes; it is produced by the splanchnopleure (splanchnic mesoderm and endoderm).

**Yolk sac** – provides nutrition in telolecithal organisms and is a source of blood cells for mammals; it is produced by the splanchnopleure (splanchnic mesoderm and endoderm).

65. Describe the circulatory system modifications found in human fetuses and the changes in the system that take place immediately after birth.

*Circulatory system modifications include:* umbilical veins and arteries, foramen ovale joining the left and right atria, and the truncus arteriosus joining the pulmonary artery and aorta.

*At birth, the foramen ovale closes via a septum due to a pressure change in the left ventricle.*

Prostaglandins constrict the truncus arteriosus closed, as well as the umbilical veins and arteries.

66. In an experiment individual cells in the progress zone of early limb buds were labeled with a permanent dye (which would be distributed among all of that cell’s progeny). In the fully grown limb the marked cells were each limited to a particular segment. For example, progeny from marked cells at the tip of the early limb bud were only found in the autopod. Do these results support the Progress Zone model or the Early Allocation and Progenitor Expansion model? Why?

*These results support the Early Allocation model. If the limb development was proceeding as suggested by the Progress Zone model, progeny from the marked cells would have been found in all segments of the fully grown limb, since cells from the tip of the limb bud would have contributed to all segments.*

*In the Early Allocation model, cells from the distal-most regions only contribute to the distal-most segment, the autopod, which was in fact the conclusion of the experiment.*

67. Explain why inserting a bead containing Shh into the anterior region of an early limb bud can result in a mirror image duplication of the limb.

*Shh specifies tissues in the posterior region of the autopod. Specifically, cells exposed to the highest concentration of Shh become the most posterior elements (digit 5), while cells exposed to decreasing amounts of Shh become, respectively, digits 4, 3, and 2 (digit 1 is not specified by Shh).*

*The Shh-containing bead establishes a morphogens gradient in the host tissue. Thus, the Shh highest concentration, located in the anterior region, becomes the most posteriorized*
Short Essay. Answer two of the following questions. (10 points each).

Be sure to address all parts of the questions and provide complete answers.

68. Listed below are a set of molecules (paracrine factors, growth factors, morphogens, receptors, and transcription factors) that we have repeatedly seen throughout our study of developing organisms. For any three molecules on the list:

A) Describe a developmental event or process associated with that particular molecule and describe its role in that event.

B) List another paracrine factor, etc. that is somehow associated with the molecule of choice (e.g. one stimulates production of the other; one antagonizes the other, etc.). You may use any associated paracrine factor, etc., not just those on the list. Also, you are not limited to examples from the most recent set of lectures; use anything we have covered throughout the semester.

<table>
<thead>
<tr>
<th>Developmentally active molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP (any)</td>
</tr>
<tr>
<td>Shh</td>
</tr>
<tr>
<td>Nodal</td>
</tr>
<tr>
<td>Fgf (any)</td>
</tr>
<tr>
<td>retinoic acid</td>
</tr>
</tbody>
</table>
69. Describe the formation of chick heart from gastrulation through formation of four distinct chambers.

*Lateral plate mesoderm in the distal anterior region is induced by BMPs and underlying endoderm to form cardiogenic mesoderm. Cardiac endothelial cells form into tubes, (dorsal aortae) on each side of the embryo. Folding of the gut brings the endothelial tubes and their associated cardiogenic mesenchyme together in the pharyngeal region, where they eventually fuse. At the posterior the sinoatrial region divides into the vitelline veins, while the anterior region forms the truncus arteriosus.*

*Cardiac looping, under the influence of Nodal and Lefty-2 genes, results in the sinoatrial region moving upwards and sideways, resulting in the reversal of the direction of blood flow through the body (originally flowing from the posterior to anterior), and the rearrangement of the two chambers to lie next to each other.*

*The two chambers join laterally, with cardiac endothelial cells forming septa that eventually separate the two chambers into four.*

70. Limb formation provides an ideal platform to study developmental processes using classic techniques, such as removal and transplantation. You have a hypothesis that a certain set of cells in the anterior limb bud progress zone specifies digit 1 of the forelimb. Describe the experiment(s) that you would conduct to try to prove this hypothesis. Also, describe your control experiment(s).

For example:
*Experiment 1 - Remove cells from the anterior region in question and observe the resulting digit development; does digit 1 develop?*  
*Control 1 - Remove cells from areas around the region in question; does digit 1 develop?*

*Experiment 2 – Transplant anterior cells to posterior regions; will the presence of anterior cells change the fate of posterior digits towards digit 1?*  
*Control 2 – Perform the same experiment with posterior cells or mesenchyme cells from an unrelated region; do the posterior digits change fates just because they are near transplanted cells?*