20 pts: 1. With the aid of a diagram, indicate how initial dorsal-ventral polarity is created in fruit fly and frog embryos.

**Fly Embryo**

- **Dorsal Concentration:**
  - No Dorsal
  - Low [Dorsal]
  - High [Dorsal]

- **Graded Concentration:** Controlled by Mother

- **Activate D/V Genes**

**Frog Embryo**

- **Sperm Entry**
- **Cortical Rotation**

**In the fly embryo:** the maternal Dorsal gradient activates D/V genes in a threshold dependent fashion. High levels of Dorsal activate ventral mesoderm genes (e.g. snail, twist), lower levels activate neuroectodermal genes (e.g. AS-C, sog, rho), and the absence of dorsal permits expression of dorsal genes (e.g. dpp).

**In the frog embryo:** the point of sperm entry, which is located at the equator in the animal hemisphere (e.g. near the vegetal hemisphere), induces cortical rotation away from the point of fertilization. Latent dorsalizing factors, which are believed to lie at the vegetal pole, are thought to be swept to a location opposite to that of the sperm entry point where they are activated to define the dorsal pole of the embryo (ventral being the original point of sperm entry). The activated dorsalizing factors lead to high levels of β-catenin in the nucleus of dorsal cells which then functions as a morphogen to activate different genes along the D/V axis in a threshold dependent fashion.

In what respects are these pattern forming mechanisms similar and how are they different? (two sentences maximum)

**3 pts** Similarities: Both embryos rely on a transcription factor morphogen (Dorsal in flies and β-catenin in frogs) to activate tissue specific patterning genes in different D/V territories and maternal factors play a role in establishing D/V polarity.

**2 pts** Differences: An extrinsic factor (the point of sperm entry) plays a critical role in establishing the frog D/V axis but not that of the fly and the morphogens initiating D/V patterning are not related (e.g. Dorsal and β-catenin).
Explain briefly how VegT functions in vegetal cells to induce mesoderm in adjacent cells of the animal hemisphere.

5 pts  
*VegT activates expression of mesoderm inducing signals while also suppressing the response to these diffusible factors in the endoderm. The result of this “for-export-only” signaling is induction of mesoderm in adjacent animal cells that close enough to receive the signal.*

15 pts  
2. The gradient of maternal Dorsal protein in the *Drosophila* embryo leads to expression of the *snail* gene in the ventral mesoderm and the *rhomboid* (*rho*) gene in the lateral neuroectodermal region of the embryo. Draw a diagram of the regulatory region of the *snail* and *rho* genes indicating all relevant binding sites for activator and repressor transcription factors.

5 pts

- a. Why is it that *rho*, but not *snail*, can be expressed in lateral cells?
  5 pts  
  *The Dorsal binding sites in the rho enhancer are higher affinity sites than those in the snail enhancer.*

- b. Why is *rho* not expressed in ventral cells?
  5 pts  
  *Snail is a repressor expressed in ventral cell which binds to the rho enhancer. When Snail is bound to the rho enhancer, it overrides the positive activating effect of Dorsal.*

25 pts  
3. Draw a diagram indicating the relative expression patterns of the three neural identity genes in flies versus vertebrates. Indicate the relative locations of the Dpp and Dorsal gradients in flies and the BMP and Shh gradients in vertebrates.

5 pts
a. Explain how the graded activities of Dpp and Dorsal collaborate to create the pattern of neural identity genes.

5 pts  Dpp: Dpp represses neural identity genes in a dose-dependent fashion. The order of sensitivity of these genes to Dpp repression is:

- vnd (most sensitive) > ind > msh (least sensitive)

5 pts  DI: Dorsal activates vnd and ind in a dose-dependent fashion. vnd requires more Dorsal than ind to be activated. msh does not require Dorsal to be activated.

b. What is meant by ventral dominant cross-repression of neural identity genes?

4 pts  More ventral neural identity genes repress the expression of more dorsal ones. That is, Vnd represses both ind and msh, while Ind represses msh.

c. Using a diagram indicate for each row of neuroblasts how Dpp, Dorsal and ventral dominance act to produce the correct pattern of neural identity gene expression.

6 pts

<table>
<thead>
<tr>
<th>Row</th>
<th>Dpp activity</th>
<th>Ind activity</th>
<th>Vnd activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>r3</td>
<td>Dpp -I ind, vnd but not msh. msh is not repressed by Ind or Vnd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r2</td>
<td>Dpp -I vnd. ind is not repressed by Vnd. Low DI activates ind. Ind -I msh.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r1</td>
<td>Low Dpp cannot repress vnd. Moderate DI activates vnd. Vnd -I ind and msh.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

25 pts.  4. Draw a diagram of a developing vertebrate neural tube and surrounding tissues indicating the following: dorsal versus ventral, floorplate, serotonergic neurons, motor neurons, notochord, somites, neural crest.

7 pts

[Diagram of a developing vertebrate neural tube]
Draw a second diagram depicting the result of grafting a second notochord to an ectopic position relative to the neural tube.

3 pts a. Briefly describe the role of Hedgehog (Hh) in patterning the neural tube.  
Hh acts as a morphogen to determine ventral cell fates in the neural tube.  High levels specify floorplate, intermediate levels specify seratonergic neurons, and lower levels specify motor neurons.

2 pts b. Where is Hh first expressed during neural tube development?  
In the notochord

2 pts c. Where is Hh expressed next?  
In the floorplate

3 pts d. What is the relationship between Hh and the transcription factor HNF3β and how does this relationship help account for the second phase of Hh expression?  
Hh signaling activates expression of HNF3β which in turn activates (maintains) expression of Hh to define a positive feedback loop.  (2 of 3 pts) This positive feedback system can spread from the notochord to the floorplate by virtue of Hh in the notochord activating expression of HNF3β in the neighboring floorplate of the neural tube, which in turn activates expression of Hh to create a second stable source of Hh.  (1 of 3 pts)

3 pts e. What signal acts in opposition to Hh in patterning the neural tube?  Where are these factors produced?  
BMPs, which are produced by dorsal cells of the neural tube and overlying dorsal ectoderm.

2 pts f. What are two possible mechanisms by which BMPs could influence gene expression in the dorsal region of the neural tube?  
BMPs could activate or repress expression of target genes in a dose dependent fashion.
5a. Explain what is meant by neural competence. What genes are responsible for conferring neural competence in the fly neuroectoderm? What is the defect (or phenotype) in mutants lacking the function of these genes?

6 pts Neural competence means the potential of cells to become neural. Genes of the Achaete-scute Complex (AS-C) are responsible for conferring neural competence. In mutants lacking AS-C function cells in the neuroectodermal region of the embryo fail to form neuroblasts.

b. What is the process by which neuroblasts are selected from neural competent domains of cells? What signaling pathway mediates this process? What is the phenotype of fly mutants lacking this signaling pathway (indicate what happens in the neuroectoderm versus the dorsal epidermal region).

6 pts The process that selects neuroblasts from neural competent domains of cells is called lateral inhibition. Lateral inhibition is mediated by the Notch signaling pathway. In mutants of the Notch pathway, all cells in the neuroectoderm become neural (at the expense of epidermal cells). In the dorsal region, in which cells do not have neural competence, there is no effect in Notch mutants.

c. What is the phenotype of double mutants lacking the genes indicated in parts a and b.

3 pts AS-C; Notch- double mutants have the same phenotype as AS-C mutants (i.e., no neurons) since in absence of neural competence no cells can become neural.