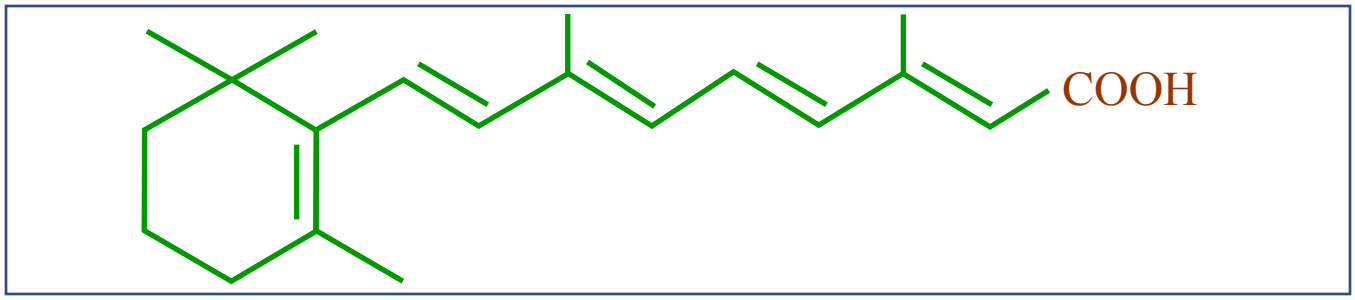
7.22 Fall 2005 exam 2 practice

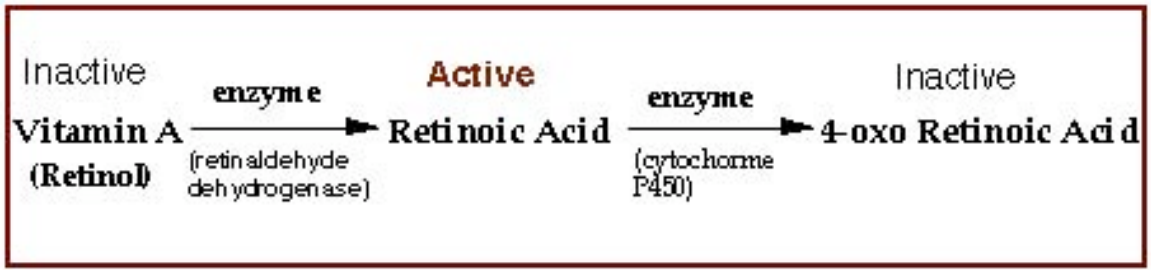
7.22 Exam II Practice Problems

100 points (105 possible)
Read the questions carefully! You will get more credit if you propose experiments appropriate for the organism involved and the stage of development assayed.

1. **40 points** Retinoic acid (structure below) is a small hydrophobic molecule that is derived from vitamin A.



It is synthesized as indicated below. Figure by MIT OCW.



As diagrammed below, retinoic acid binds to a nuclear receptor dimer (RAR+RXR) displacing a transcriptional corepressor. The retinoic acid/receptor complex binds a coactivator and then changes the transcription of target genes by binding to a promoter sequence, t

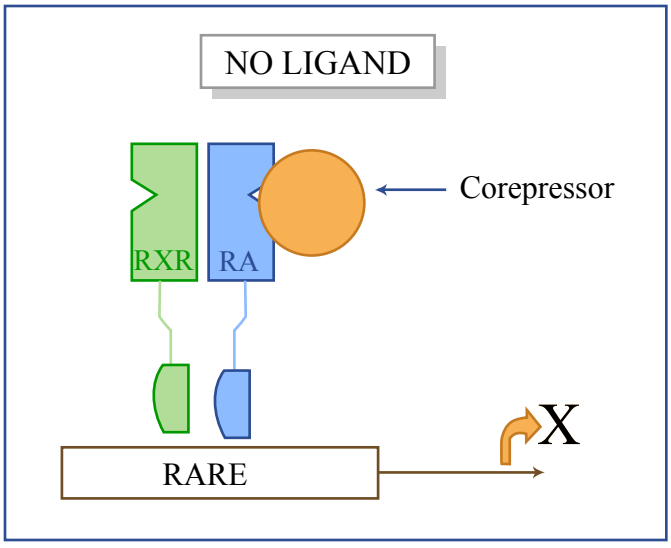


Figure by MIT OCW.

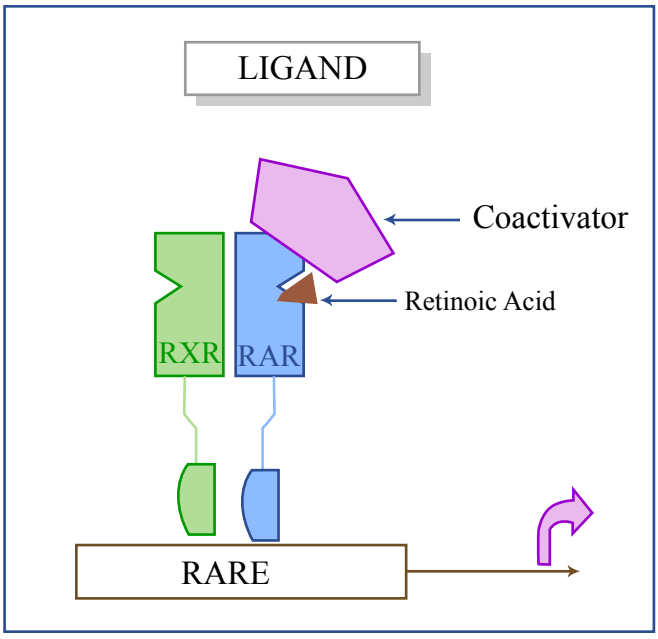


Figure by MIT OCW.

Putting gastrula stage (10 hours after fertilization) frog embryos into a solution of retinoic acid leads to huge expansion of the embryonic heart, as assayed at hatching stage (48 hours after fertilization).

a. **5 points** What can you conclude from this result, regarding the normal function of retinoic acid in heart formation? Explain your answer.

b. 20 points total How would you ask whether endogenous retinoic acid is required for heart formation? Suggest a plausible experiment based on above information, remembering that retinoic acid is not a protein.
10 points.

Describe a positive control for this experiment and explain the purpose of the control. **5 points.**

Describe a negative control for this experiment and explain the purpose of the control. **5 points.**

The heart forms by interaction of mesoderm and endoderm, beginning at early gastrula, and extending for 48 hours in frogs, until hatching stages when the heart begins to beat.

c. **1 point** What is the term for the process of organ formation?

d. **4 points** Distinguish between
Differentiation and

Determination

e. **10 points.** Assuming retinoic acid is important for normal heart formation, describe an experiment (or a series of related experiments) to ask <u>when</u> retinoic acid is important?

2. **60 points (65 possible)** Tooth loss is an important medical problem, since in humans, permanent teeth do not regenerate. There is therefore considerable interest in understanding tooth formation, with a view to getting teeth to regrow.
   a. Teeth develop from the neural crest.
      3 points. From what organ does the "neural crest" arise?

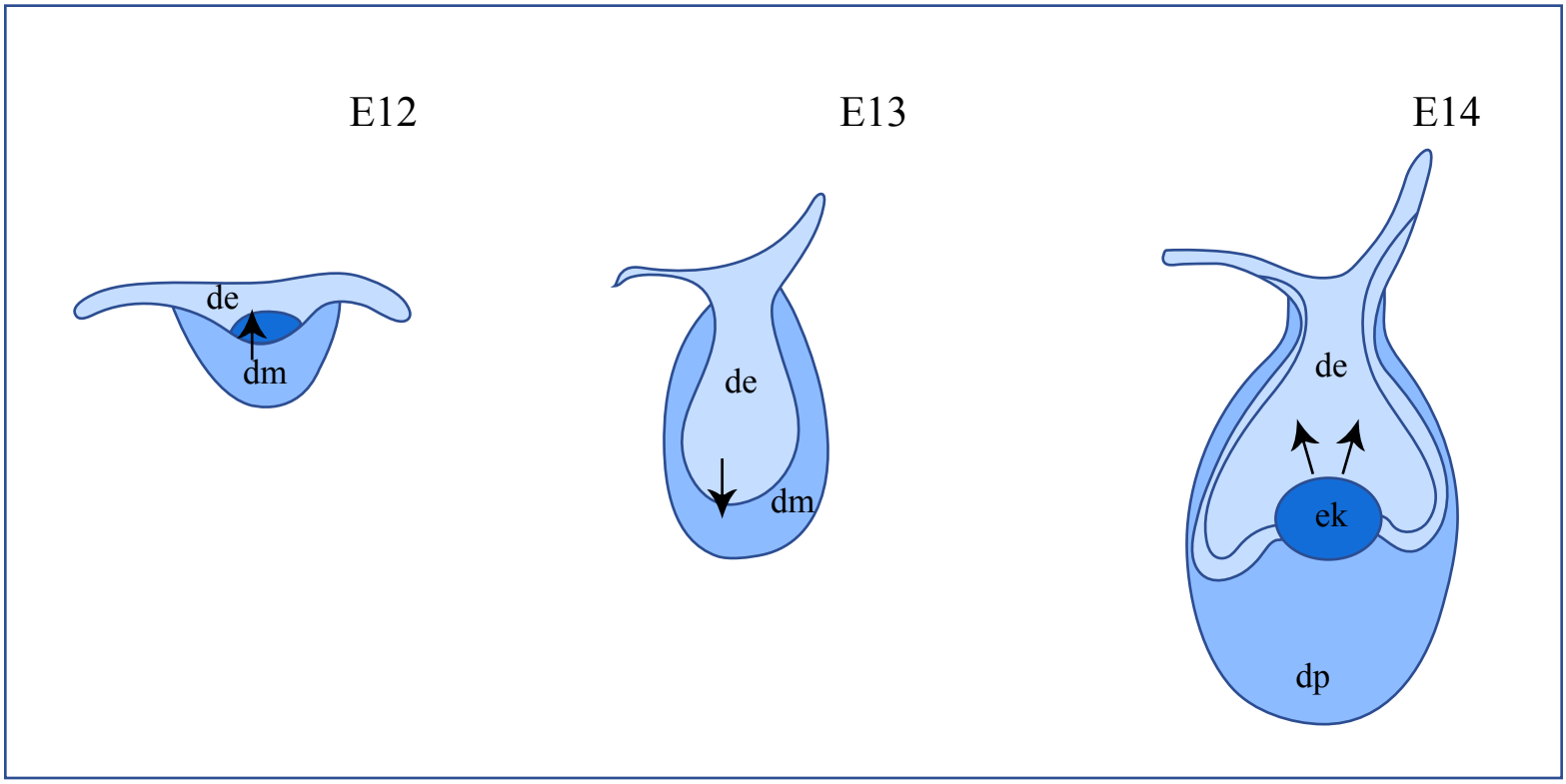


Figure by MIT OCW.

As diagrammed above, the tooth primordium forms from an interaction between mandibular (jaw) epithelium and mesenchyme. In mouse embryos, at E12 (12 days after fertilization), the dental mesenchyme (dm) induces (arrow) the dental epithelium (de) to form a bud. At E13, condensed mesenchymal cells (dm) surround the epithelial bud (de), and the dental epithelium now signals (arrow) the mesenchyme to form at E14, an enamel knot (ek). This is a non-dividing group of cells that induces (arrows) subsequent tooth formation. Ablation (removal) of the enamel knot prevents tooth formation.

Several genes have been identified that when deleted lead to absence of teeth in the newborn mouse (born at 21 days after fertilization). Three of these genes encode the transcription factors Lef1, Msx1 and Runx2.

b. 20 points total. Describe a simple experiment that would indicate whether Lef1, Msx1 and Runx2 are likely to act simultaneously or sequentially during tooth development. **10 points**

Describe a positive control and indicate the purpose of this. **5 points**

Describe a negative control and indicate the purpose of this. **5 points**

The enamel knot expresses Fgf3, Shh and BMP2. In the Runx2 mutant, teeth do not form. In this mutant, Fgf3 expression is absent, but BMP2 and Shh are expressed normally. In the Msx1 mutant, Fgf3 expression is normal, but teeth do not form.

c. **25 points** Based on the above information, formulate a hypothesis regarding genetic circuitry involving Runx2, Msx1, Fgf3, Shh and BMP2. Use a diagram to illustrate your hypothesis. **5 points**

Propose an experiment to test this hypothesis. **10 points**

Describe a result consistent with your hypothesis. **5 points**

Describe a result inconsistent with your hypothesis. **5 points**

In lower vertebrates, including the zebrafish, teeth regenerate continuously, suggesting involvement of stem cells.

d. 15 points What is a stem cell? **4 points**

What simple experiment would you perform to detect the position of potential stem cells in the zebrafish tooth region? **7 points**

Describe the idea behind an experiment to test whether the cells you have identified are tooth stem cells. You do not need to go into detail. **4 points**

3.) In mouse mutants resulting from a chemical mutagenesis screen involving the mutagen ethyl nitrosourea (ENU), mutation of the CBFA1 transcription factor is correlated with failure of the embryonic skeleton to make bone, when assayed in F2 embryos.

a. Can you say with certainty that CBFA1 is causal of bone formation? Explain your answer. Describe an experiment that would change your answer. Include a positive and negative control, and indicate what each is controlling for.

b. Describe a different experimental approach that would make you more certain that the CBFA1 gene is necessary for bone formation.

c. How would you determine whether CBFA1 activity is likely to play a direct role in bone formation (that is, in the future bone cells)? This is a correlation question. Describe a positive and negative control for the experiment you propose, and indicate what each is controlling for.

d. How would you determine whether CBFA1 is sufficient to cause bone formation? Describe a plausible experiment and the most important positive and negative controls (one of each) that you would perform. Indicate the purpose of each control.

e. Mutants in BMP2 also fail to form bone. What is BMP2? Briefly describe the pathway by which it acts.

f. How would you determine whether BMP2 and CBFA1 act in the same or different pathways? Describe an experimental approach, possible results and interpretations of each result. For the experimental approach, include one positive and one negative control, and indicate the purpose of each.