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.

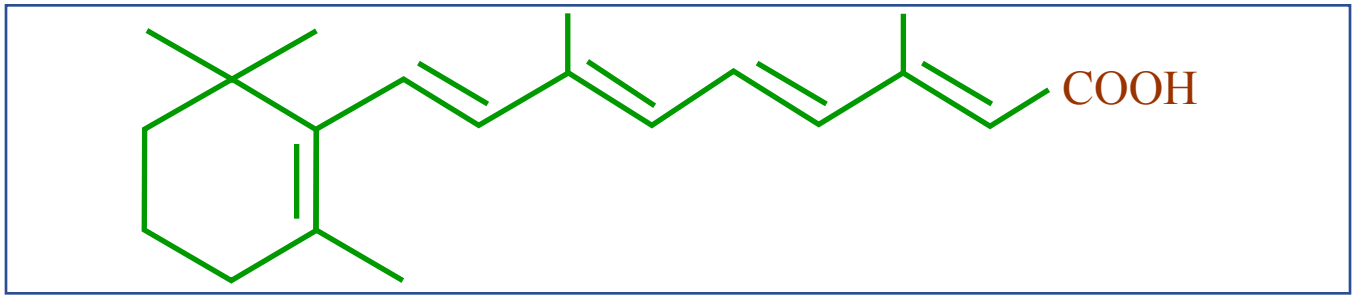
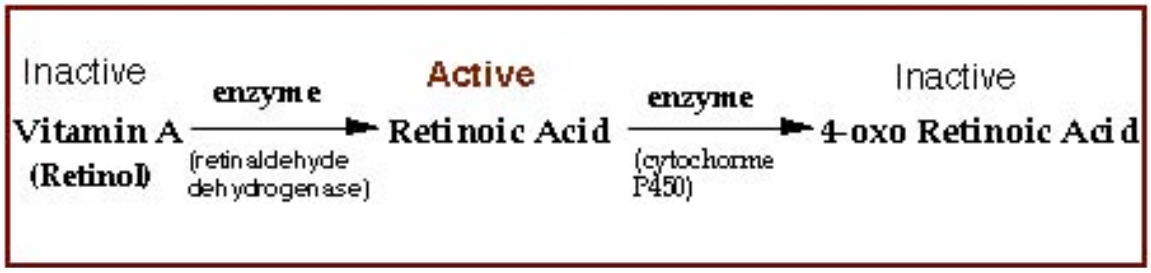


Figure by MIT OCW.

It is synthesized as indicated below.



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, the RARE, present in all target promoters.

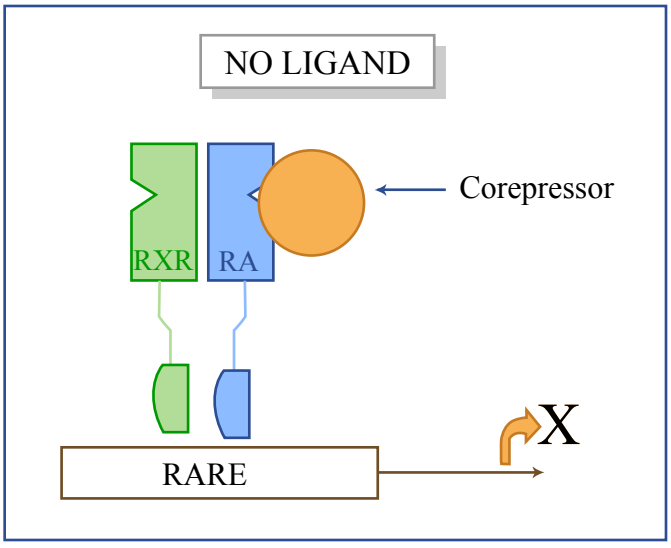


Figure by MIT OCW.

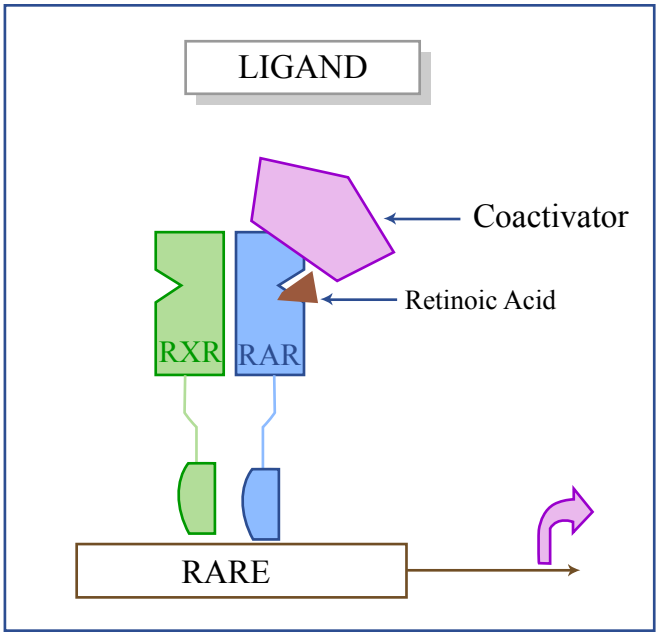


Figure by MIT OCW.

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.

Conclude that retinoic acid can change heart development, however this does not indicate it is normally involved in heart formation. One would need to show retinoic acid is present in the correct region at the correct time, and is required for heart development.

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.**

Can ablate function of the synthetic enzyme, retinaldehyde dehydrogenase and ask whether heart formation is affected. I would inject morpholino antisense oligonucleotides into the one cell embryo, directed against retinaldehyde dehydrogenase mRNA. These will prevent translation of the protein. Later, I will ask whether heart formation is affected. If retinoic acid is required for this process, its synthesis should have been ablated and levels therefore depleted.

Another good way would be to inject mRNA encoding a dominant negative RAR or RXR that would prevent retinoic acid from acting to change transcription.

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

My positive controls would check that retinoic acid was depleted by inhibition of the synthetic enzyme. Check for the presence of retinoic acid in the embryo after retinaldehyde dehydrogenase inhibition. Can do this by homogenizing embryos and isolating the retinoic acid and monitoring its concentration. This should be present at much lower levels after MO treatment than in control embryos.

Another way is to examine the expression of a gene you know to be normally activated by retinoic acid. Such a gene should not be expressed if endogenous retinoids have been depleted. This would show that the embryo can no longer respond to retinoic acid.

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

Inject a control MO oligo at the same concentrations used for the experiment. Monitor heart formation, this should be normal. This control will indicate that any effects seen are not a non-specific result of the MO.

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?

organogenesis

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

The process by which a cell assumes its final fate.

Determination

The process by which a cell decides its fate.

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?

One would want to remove retinoic acid at various times of development.

One way would be to add a soluble retinoic acid inhibitor at different times (blastula, gastrula, neurula and tailbud for example) and ask when later heart development is disrupted. For example, 4-oxo retinoic acid can inhibit retinoic acid function. One could use this as an inhibitor.

Another way is to have a dominant negative RAR or RXR under inducible control, and activate it at different times of development. For example, one could make a transgenic frog expressing dominant negative RAR under a heat shock inducible promoter. Embryos could be heat shocked at various times after fertilization to interrupt retinoic acid signaling, and heart formation later examined.

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?

   Neural tube

2 points Of what germ layer is it part?

Ectoderm

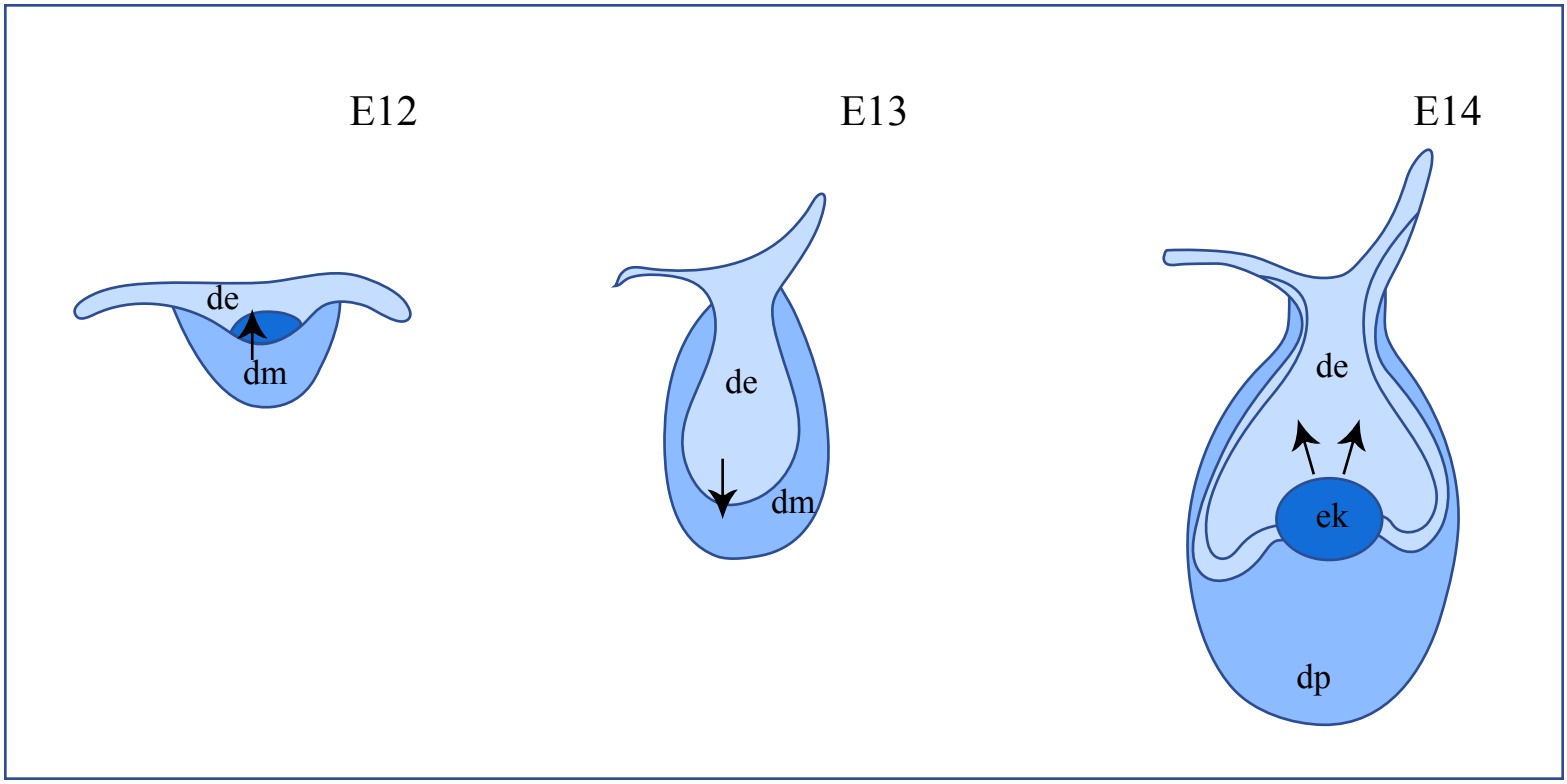


Figure by MIT OCW.

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**

Ask when each gene is expressed (most important) and where. Perform in situ hybridization for Lef1, Msx1 and Runx2 mRNA expression. Use antisense (RNA) probes labeled with a nucleotide that can later be detected. Could also use antibodies to each and perform immunocytochemistry to detect protein. Incubate embryos (or tooth primordia) of different stages with each probe. Ask whether the genes are expressed sequentially, which would indicate they are likely to act sequentially. Can also ask whether the proteins are expressed in different regions of the tooth primordium which would give more information regarding the tissues in which each acts.

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

Perform in situ hybridization or immunocytochemistry with a probe to a gene(s) known to be expressed at each time of development assayed. This will indicate the experimental protocol is working.

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

Use a sense probe (for each gene tested) or preimmune serum at each stage assayed. This will indicate that any signal seen is not a result of non-specific staining.

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**

Runx2 >>> Fgf3
Msx1>>> Shh + BMP2

Fgf3 +Shh + BMP2 >>> tooth formation

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

Ask in an Msx1 mutant whether Shh and/or BMP2 are expressed. Can use in situ hybridization or immunocytochemistry as described for b.

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

BMP2 and/or Shh are not expressed in the Msx1 mutant

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

BMP2 and Shh are expressed normally, at the protein level, in the Msx1 mutant. (Knowing just RNA expression is normal is less conclusive than knowing protein expression is normal).

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

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

A stem cell is self-renewing and also gives rise to progeny determined for a particular cell lineage.

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

Look for the presence of dividing cells in the developing tooth region. Use BrdU (a Thymidine analog) to label fish tooth primordial during tooth formation. As new teeth form, the putative stem cells will divide, to renew themselves and to give rise to the future tooth epithelium and/or mesenchyme. Other regions may also divide and this may complicate the analysis. One addition to this experiment that would help is to look where the BrdU labeled cells move to- the stem cells should stay in place, the cells that are renewing the tooth should move away. Thus a pulse/chase experiment with BrdU is best.

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**

Isolate the putative stem cells by dissection. Transplant these to the jaw region of a fish defective in tooth formation (a Runx2 mutant, for example). Could also do this in wild type fish. Use labeled putative stem cells (for example, labeled with GFP) and ask whether green teeth form after the transplant.

3.) Below is a long practice question that will reflect the type of exam question you will see tomorrow. I will send you answers later.

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.

No, you cannot say this. Since chemical mutagenesis affects multiple genes, many generations of outcrossing to wild type strains is necessary to isolate the mutant of interest from other mutations that co-segregate.

In order to have greater confidence in a role for CBFA1 in bone formation, I would perform an outcrossing experiment over many generations and screen for retention of the CBFA1 mutant allele. Any animals homozygous for mutant CBFA1 should have the bone phenotype.

One positive control would show that animals without the CBFA1 mutant gene have normal bone. This would further confirm that CBFA1 is required for bone formation, and that the mutagenesis did not cause weird effects that led to widespread bone disruption.

One negative control would be to show that unmutagenized animals do not display bone defects. This would indicate that there is a very low background of mutant genes prior to application of the chemical, and help confirm that mutant CBFA1 is causal of the phenotype.

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

Make a targeted deletion of the CBFA1 gene in a wild type mouse strain, using homologous recombination.

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.

Perform in situ hybridization for CBFA1 RNA. Use an antisense probe labeled with a modified nucleotide that can be detected later in a color reaction. This will hybridize with endogenous CBFA1 RNA. Ask whether color is observed in future bone cells.

Positive control. Use a probe for another gene that should be expressed at the same time of development, preferably in the same region of the embryo. This will show the in situ method is working.

Negative control. Use a sense probe from the CBFA1 cDNA. No signal should be observed. This will indicate the hybridization observed is specific for CBFA1.

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.

Express CBFA1 protein in non-bone cells. This can be done in mouse embryos by injecting an inducible promoter-driven CBFA1 DNA vector into the zygote and activating expression later. One can do this in a tissue-specific way. I would express CBFA1 in the somites from which most bone is derived and ask whether I later see extra bone made.

As a positive control, I would express a protein known to be bioactive via a similar vector. This would indicate that the embryo can respond to proteins expressed ectopically.

As a negative control, I would inject the vector alone, or one expressing what should be a non-bioactive protein, such as GFP.

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

BMP2 is a ligand of the TGFbeta superfamily. It binds to serine/threonine kinase receptors. When occupied, these phosphorylate Smads, transcription factors that can translocate to the nucleus when phosphorylated and alter transcription.

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.

One hypothesis is that if these proteins act in the same pathway, one might activate expression of the other. Examine BMP2 mutant embryos for CBFA1 RNA expression and vice versa. If CBFA1 expression is lost in BMP2 mutants (or vice versa), that is consistent with these proteins acting in the same pathway.

Positive control. Examine RNA expression of another gene known to be downstream of BMP2, and this should be lost in a BMP2 mutant. This would show that the mutant does have an effect on gene expression, and would also control for the in situ procedure working.

Negative control. Show that expression of genes known not be downstream of BMP2 is unaffected. Would indicate that BMP2 is not having a generally deleterious effect on the embryo.