7.02 Fall 2001 **DEV Recitation #2**

Outline

1. Zebrafish observations and drawing
2. Retinoic acid treatment
3. Northern Blot
   -RNA isolation (completed)
   -Denaturing gel (today)

I. Zebrafish observations

A. Microscope

1) can read about the basics of the light microscope in your lab manual
2) we're using phase contrast- utilizes differences in the refractive index of the different objects in the specimen- different tissues, organ, organelles (see figure 1)

B. Drawings

1. use pencil
2. half-page per drawing
3. label:
   1. date/time of drawing
   2. date of fertilization
   3. stage of embryo (use appendix- drawings and text)
   4. magnification

- roll embryos around to see all around (3-D)
- try to label as many structures as you can
- try to notice positions of structures next to each other
- understand what structures you're looking at- use appendix drawings/descriptions

II. Retinoic Acid

A. Teratogen v. mutagen

- mutagens cause heritable change. changes to the genomic make-up of the individual.
- teratogens are exogenous agents disrupting development.

B. Retinoic Acid

- Related to vitamin A
- Acts like a steroid hormone $\Rightarrow$ RA binds to RA-receptor, heterodimer binds to RARE (retinoic acid responsive element) to drive transcription.
  - *Hox* genes are responsive to RA. *Hox* genes specify the anterior-posterior patterning of the embryo. (what do I mean by *specifying* the a-p patterning? Hint: determination vs. differentiation) Turn on *Hox* genes inappropriately thru exogenous RA, and embryo can lack posterior structures (see figure 2). This can mean that posterior vertebra are turned into anterior ones, or ribs grow more posteriorly than normal or whole posterior is missing! This depends on dosage and on day of RA administration. (Why would time matter? Again- det. vs. diff.)
  - Exogenous RA can mimic exogenous gene expression of the downstream target!
- RA is involved endogenously with limb development, brain and nervous system development, heart development.
  - humans: absent of defective ears, absent or small jaws, cleft palate, aortic arch abnormalities, thymic deficiencies, and CNS abnormalities
- other teratogens:
  - alcohol, lead, heroin, thalidomide
  - FAS (fetal alcohol syndrome): small head size, small brain, difficulty learning, mental retardation, cardiovascular defects, limb deformities

- Today:
  - We'll be adding RA to the media the zebrafish are in. Each group will be adding it at different times so that the class as a whole can observe the differences in effects due to when the RA is administered.

III. Northern blot (day 2)

A. Did the isolation work?

- Need to check:
  1. Quantity: Did we get enough RNA?
  2. Quality: Is the RNA undegraded?
- Best way to do determine is to run out on a gel. (what other ways could we use determine presence of RMA? hint: it's sitting on your benches and you used it turning PBC!)

B. Denaturing RNA gel:

- Agarose gel electrophoresis separates molecules on the basis of <u>size</u> and <u>shape</u>
  - RNA is linear with a fixed charge:mass ratio
    - RNA is single stranded – it H-bonds to itself and its neighbors forming secondary structure
  - **DENATURE!** Denaturing removes shape variation, so RNA will run true to size on the agarose gel (what did you do to deal with shape variation when you ran protein and DNA gels?)
    1. Formaldehyde (gel, denaturing mix). H bond acceptor
    2. Formamide (denaturing mix). H bond acceptor, H bond donor
    3. Heat (65°C)denaturation before loading.
  - Use HAE rather than TAE buffer in RNA gel because DEPC inactivates Tris.
  - Visualize nucleic acid using Ethidium bromide added to sample.
- Remember:
  1. keep gels in the hood until you're ready to load
  2. do not add Orange G (the loading dye) to the samples until *after* heating
  3. put the samples on ice <u>immediately</u> after heating

C. What do we expect to see on the gel?

- RNA components
  1. Ribosomal RNA 80-85%
  2. Transfer RNA 10-15%
  3. Messenger RNA 1-5%
     - rRNA
       - transcribed by RNA polymerase I as 45S particle (S denotes size)
       - 45S cleaved into 28S, 18S and 5.8S subunits
       - rRNA is so abundant because a cell needs a whole lot of protein synthesis for growing and rRNA makes up the ribosomes. more rRNA, more protein synthesis
     - So what do you think you'll see on the gel?
       - 28S migrates at 4.7 kb, 18S migrates at 1.9 kb. The prominence of these bands can be assumed to correlate with the amount and quality of the total RNA. If you have a lot of rRNA and it's not degraded, then you probably have enough undegraded mRNA as well.