7.02 Fall 2001 **DEV Recitation #1**

Outline

1. intro to development
   - themes
   - classical & molecular approaches
2. organism (zebra fish)
3. module intro
4. today
   - RNA
   - embryo lysaes
   - RNA isolation

Development- a process of progressive change.

-Can be the process by wh/ our red blood cells or skin cells are replaced every day
-by which a single totipotent fertilized egg grows into a adult multicellular organism, with all its different cell types and tissues.

major *themes* of developmental biology: **totipotency, pleuripotency, differentiation, cell fates**

1. **POTENCY**
   - *totipotent* cell has the potential to differentiate into any other cell type
   - *pleuripotent* cell has the potential to differentiate into many, but not all cell types
2. **DIFFERENTIATION**
   - *differentiation*- development of specialized cell types from the single fertilized egg. examples are: skin cells, muscle cells, macrophages, nerve cells. a cell that is terminally differentiated is set as that cell type and cannot change
   - *determination*- even before a cell has the appearance or phenotype of the differentiated cell type it will become, it will commit to that endpoint. (*Determined/ specified*- see your lecture notes from 11/29)

How does one *study development*:

1. **Classical approach** is to *observe* and record normal and abnormal development. abnormal development can be caused by <u>mutations</u> to the genome and thus the internal programming of development or by disruption of the process thru use of <u>teratogens</u>. We will be using the light microscope to accomplish our observations.
   a) <u>good observation skills</u> are important to acquire in any career path. (doctors need observation skills for diagnosis)
   b) observations, like any form of experimentation, much be <u>repeated</u> several times. living organisms vary. make sure different observations are due to the experiment, not idiosyncratic to the individual.
   c) zebrafish websites on the 7.02 web page
   d) zebrafish observations begin after thanksgiving

2. **Molecular approach**. study how molecules affect development- changes in gene expression leading to changes in protein expression (leading to changes in cell phenotype/ physical changes in the cell).
    - What do I mean by gene expression? (RNA levels, protein levels. we express our selves in words, the genome uses RNA)
        - We will be observing the changes in expression of an epidermal gene, *z-cyt* . this encodes a cytokeratin protein.
            - Cytokeratins are structural protein abundant in the epidermis, a protective outer layer of skin.
            - Because we know that *z-cyt* expression is linked to epidermal development, we are using it as a marker to examine epidermal development on a molecular level.
            - Rather than looking at the whole, which might involve a hundred or a thousand genes turning on or off, we are looking one player as a stand-in for the whole.
                - like saying if I'm here in the auditorium talking, then recitation must be going on.

Model organism choice:

organism	generation time	fit in $1ft^3$	use
Bacteria	20 min	10^16	Intracellular (but no nucleus!), cell-cell talk
Fish	3 months	~30	Early development- i.e. heart, neural
Mice	3 months	1	Disease modeling, development
Humans	30 years	2 x 10^-4	retrospective studies

Zebra fish pros:
- small and easy to maintain
- short generation time (3 months) and rapid development (larva by end of day 3), multigenerational work
- external fertilization, easy to obtain and manipulate embryos
- transparent embryos, possible to observe development of internal structures in live organism
- can produce mutant embryos
- zebrafish is a vertebrate

four stages of embryonic development: (look in your lab manual for pictures! p.32-5)

-**blastula** (~3 hrs) –ball of cells
-**gastrula** (~6 hrs) –cells begin to move and shape themselves into structures

-straightening-
-hatching-

So obviously expression levels will change or we wouldn't be doing the experiment. Any ideas on how the expression will change? When will it begin? determined vs. differentiated!

In order to examine the expression level of *z-cyt* we will be using a technique called **Northern Blotting**. This allows *detection* and *quantitation* of a specific RNA amidst the mess of RNAs extracted from the cells. We can use the northern blot to compare expression between cell/tissue types or between different stages. Remember: we're looking at temporal changes and ignoring spatial ones.

the northern blot experiment is outlined on p.27 of the manual.

TODAY

1. we'll begin working with RNA. RNA is not as easy to work with as DNA.
   -less stable!
   -single stranded
   -has 2'OH group
   -permits attack by that OH group on the phosphodiester bond between bases
   -RNAses are EVERYWHERE! on your hands, for instance**. BE CAREFUL WHAT YOU TOUCH!**
   -you must go to extraordinary lengths to inactivate RNAses.
   -rough prep for isolating RNAse is to boil a protein prep and anything left active is an RNAse.
   -remember the RNAse you used for your miniprep? you don't want any DNAse in there, but the less expensive RNAses you can buy have some DNAse contamination. so what you can do is buy the cheaper stuff, boil it for 5 minutes and then voila, you've got DNAse-free RNAse.
   -**WEAR GLOVES**
   -**USE RNASE FREE TIPS AND TUBES**
   -**DEPC WATER** for all solutions. diethyl pyrocarbonate esterifies proteins at certain residues, such as histidines. RNAse has 2 histidines in its active site, which are covalently (irreversibly) modified by DEPC.
2. Preparation of the embryo lysates
   -mated fish, collected embryos in the morning, let them develop to the desired stages.
   -homogenized (crushed) 1600 embryos in 50 mL of lysis buffer, aliquoted 0.5 mL and froze at –80C
   -each group gets 0.5mL aliquots of each stage
   (figure out how many embryos per stage you have!)

   lysis buffer

-guanidine isothiocyanate- denatures proteins, both hydrophobic and hydrophilic regions
-sarkosyl- detergent, solubilizes membranes, keeps things from sticking together
-BME- strong reducing agent, prevents disulfide bond formation. mimics the cellular environment
-sodium citrate (pH 7.0) buffer. what are buffers for?

Isolation of RNA!

Roles of Reagents:
-**phenol** and **chloroform** make up the *organic, non-polar phase*
-**Isoamyl alcohol** reduces surface tension, prevents beading and foaming
-**NaOAc, pH 4** added to the *aqueous phase*
(?which phase is on top?)
-contains Na+ which will be necessary when you want to precipitate the RNA in the latter steps (how? remember DNA preps from RDM!)
-the low pH is necessary now when you're trying to remove the DNA and leave RNA during the phenol/chloroform preps
-DNA backbone is protonated (at pH 4.0), making it non-polar, goes into the organic phase
-RNA is more soluble, because it's single stranded and has a 2'OH, remains in the aq. layer

-why would you find some proteins at the interphase between aqueous and organic- polar and nonpolar- phases?
-proteins have both hydrophobic and hydrophilic regions, most are at the **interphase**, some in organic layer
-**therefore**, leave the white stuff at the intephase in the tube with the organic layer