More Seminar Opportunities:
Dr. Carolyn Bertozzi from UC Berkeley
Friday 5/16 10-11 am Biophys class PH146
Chemical Approaches to Glycomics

Friday 5/16 5-6pm SL150  Plenary lecture
Sulfation Pathways in Inflammation and in Mycobacterial Infection

In the mouse embryo, four complexes of HOX genes (39 genes in all) occur on four different chromosomes. Not every gene is represented in every complex. The HOX genes are expressed in distinct domains along the AP axis.

As in Drosophila, the relative order of a gene within each vertebrate HOX complex is correlated with its spatial expression along the anteroposterior body axis.

Photomicrographs showing the mRNA expression patterns of three mouse Hox genes in the vertebral column of a sectioned 12.5 day old embryo.
Note that the anterior limit of each of the expression patterns is different.

How do we know that these variations in expression pattern have any meaning?
How can we directly address homeobox gene function in vertebrates?
Think about the following questions:

1. How do you clone a gene if all you know about it is the mutant phenotype?
2. If you have a clone of a gene, but don’t know the mutant phenotype, how do you generate a mutation in the gene?
Although the primary amino acid sequences of certain homeobox DNA-binding domains have been conserved in widely divergent organisms the regulatory roles that these transcription factors play have been altered over time to fit the specific needs of the host.

So far, we’ve only looked at *indirect evidence* suggesting the role of homeobox genes in vertebrate development.

*How can we address more directly the role of the HOX genes in vertebrate development?*

*Reverse genetics:*
1. Identify a molecular clone of a gene using standard molecular methodologies
2. Use targeted mutagenesis to produce an organism with a mutation in the gene
3. Determine the phenotype of the organism with the mutated gene
4. Deduce the role of the gene product in the functioning of the organism
Constructing a gene knockout

gene knockout = null mutation = complete loss-of-function
(a)

Zygote → Morula (2.5 d) → Blastocyst (3.5 d) → Culture ICM → Irradiated feeder layer → Introduce DNA and select clones containing genetic alterations → Make chimeras by blastocyst injection → Differentiate in vitro
Double selection:
for antibiotic resistance

against presence of Thymidine Kinase using
ganciclovir
Featured Papers:
Understanding BSE – 3
Serum-Free Culture of ES Cells – 8
New TACTECH™ PCR Cloning Enhancer – 15
New PROQUEST™ Two-Hybrid System – 19
Tips on Protein Molecular Weight Estimation – 24

(a)

Normal chromosome
m

Targeted mutation

ES cells
from brown
mouse

Black female

asMM

Blasckyst.
stage embryo

asMM plus AAMin

Altered embryo

Surrogate mother

Embryo

Newborn chimeric: male
(carrying cells from two mouse strains)

Brown mouse
What is the role of the *lim-1* homeobox gene in the development of vertebrates?

First look at a classic experiment in developmental biology:

**Vertebrate organizer:**
- originally described almost 75 years ago by Hans Spemann and Hilda Mangold
- region of a developing vertebrate embryo that can have long-range effects on the developmental fate of surrounding tissues

Transplantation of the dorsal blastopore lip of an early amphibian embryo can induce a secondary embryonic axis and embryo

*They named this transplanted region the “organizer”*

What is the molecular nature of the organizer?

- the molecular mechanisms involved in organizing the vertebrate axis appear to be evolutionarily conserved

- Several genes encoding presumptive DNA transcription factors are expressed in the Xenopus (huh?), chick and mouse organizers

The *lim-1* gene is one of the genes expressed
**LIM-homeodomain proteins**

- The LIM domain is a cysteine rich zinc-binding region responsible for protein-protein interactions
- LIM-homeoproteins possess two LIM domains together with the DNA-binding homeodomain
- Orphan homeobox gene (not part of a HOX cluster)
- Lim-1 gene was initially identified in a general screen for genes encoding homeoboxes

*What happens if this gene is knocked out in a mouse?*
targeted disruption of the lim-1 gene
a. Schematic representation of the genomic DNA, targeting construct and resulting knockout allele. Homologous recombination occurs in the genomic sequences flanking the coding region (bold line).
PGKneo = confers resistance to neomycin TK= thymidine kinase
The neo cassette introduces novel Eco RV (RV) and Eco RI {R} sites that allow genotyping by Southern blot. [PCR based genotyping is also possible.]
b. Southern blot analysis of wildtype (AB1) and two targeted ES clones after EcoRI digestion (5’ end) or Eco RV digestion (3’end).
c. Southern blots of EcoRI-digested DNA isolated from yolk sacs of embryos derived from crossing two mice heterozygous for the lim-1(knockout) allele.