A transcriptional network for specification of a light-sensing organ

A plausible chronology for animal locomotory abilities, visual function and the occurrence of master control genes.

The “need” (evolutionary pressure) for fast locomotion and image forming vision are likely to have originated during the Cambrian explosion, whereas master control genes (HOX complexes) in general would have been needed much earlier.

Bilaterally symmetric animals with prominent eyes appeared “suddenly” in the fossil record from the Cambrian explosion.

Eyes were image-forming and vision was binocular
PAX-6: homeobox and paired box DNA binding motifs

heterozygotes for loss-of-function mutation:

LOOK up DNA binding motifs here:
http://www.biochem.ucl.ac.uk/bsm/prot_dna/prot_dna_cover.html
Hierarchical Gene Regulatory Circuits

Progressive restriction of cell fate

molecular specification of *embryonic axes*  
\[ \downarrow \]
molecular specification of *head* structures  
(*lim-1* homeobox gene and others)  
\[ \downarrow \]
molecular specification of *eye*  
(*pax-6* gene and others)  
\[ \downarrow \]
\[ \downarrow \]
\[ \downarrow \]
*lens* specific genes (*δ* crysallin)  
(*crx* gene)  
molecular specification of *photoreceptor*  
\[ \downarrow \]
*rhodopsin* and other proteins specific to photoreceptor cells

Pax-6 protein binds the enhancer poorly in the absence of SOX2/3 transcription factor
**lim-1, pax-6 and crx** all code for transcription factors with homeodomains

**One important goal of molecular developmental biologists is to elucidate the transcriptional networks that underly specification of cell fate**

Who’s controlling expression (transcription) of whom and when and where?

*In this context:*

- which genes are controlled by the Lim-1 transcription factor?
- which genes are controlled by the Pax-6 transcription factor?
- which genes are controlled by the Crx transcription factor?

**Strategies to address these questions?**

*How do we figure out which genes are regulated by any given regulator?*
• Survey cis-acting sites for sequences that will bind to the transcription factor in vitro (also some in vivo methods have been worked out)

• Then find out where the cis-acting sites are located in the genome-- which gene they are next to

• OR -- look at various combinations of regulator/regulatee double mutants

• OR -- Measure transcription of candidate genes (regulatee genes) in regulator mutant strains
What strategies does the genome era have to offer us?

**Genome scale experiments: DNA chips and microarrays**

**Required reading**

“Microarray analysis of the transcriptional network controlled by the photoreceptor homeobox gene Crx”

http://fire.biol.wwu.edu/trent/trent/cepko.pdf

**Supporting material in Watson:**

Chapter 18  pgs. 575 & 577 on microarrays
Chapter 19  pgs. 613-622
Chapter 20 pgs. 651-653 on hybridization; pgs. 656-657 on libraries of cloned molecules
Chapter 21 pgs. 705-709 targeted gene knockout in mice
Jargon Review:

**Genome:** the entire genetic complement of an organism

**Genomics:** the cloning and molecular characterization of whole genomes

**Proteome:** the complete set of protein-coding genes in a genome or the complete set of proteins that a living cell is capable of synthesizing

**FUNCTIONAL GENOMICS:** characterization of the proteome and overall patterns of gene expression.
A SPECIFIC GOAL OF FUNCTIONAL GENOMICS:

Delineate the transcriptional networks controlled by transcription factors and determine how they contribute to cellular and organismal phenotypes

This goal may now be within our grasp:

Genomics, Gene Expression and DNA Arrays  
(from Nature 405: 827  June 15, 2000)

Biological research is in the midst of a transition that is being driven by two primary factors:

1. the massive increase in the amount of DNA sequence information available
2. the development of technologies to exploit this information and to allow us to pose different types of questions

New types of experiments are possible and observations, analyses and discoveries are being made on an unprecedented SCALE
**Required reading**

“Microarray analysis of the transcriptional network controlled by the photoreceptor homeobox gene Crx”

Don’t stress over all the different gene names in the paper


Each cone cell senses either red or green or blue light (depending on whether the red or green or blue opsin genes are expressed). Rod cells are sensitive to low light but don’t distinguish color.
Crx gene: **cone, rod homeobox**

- codes for a transcription factor that has a pivotal role in the morphological differentiation of both rod and cone photoreceptors
- mutations in human Crx result in either congenital blindness or photoreceptor degeneration

> Details of Retinal Circuitry

[Crx also has a paired domain. Want to find out about the paired domain?]
Strategy: Knock-out Crx gene in mice and compare gene expression in Crx+/Crx+ and Crx-/Crx- neonates

Logic: Transcription patterns of genes controlled (directly or indirectly) by CRX+ will be altered in the mutants

Pre-genomic era scale of experiment: analyze effect of mutation on transcription -- one gene at a time

Genomics era scale of experiment
In the CRX paper: 960 cDNA clones examined at once using DNA microarrays
Differential gene expression: Scaling up
DNA microarrays
http://www.gene-chips.com/

Jargon: cDNA microarrays

DNA microarrays are commonly used to:
• monitor expression of arrayed genes in different mRNA populations

• DNA microarrays are solid (often glass) surfaces bearing arrays of DNA fragments at discrete locations. This tethered DNA is called the PROBE

• These tethered DNA fragments are available for hybridization to a complex sample of fluorescently labelled RNA or DNA in solution

• The hybridization of a sample of nucleic acid in solution (called the TARGET) to the nucleic acid on the chip (that is, the probe) is, in effect a parallel search by each molecule for its complementary partner on an affinity matrix

• The pairing of the molecules is determined by the rules of base pairing and the physical conditions of the hybridization
GeneChip arrays or simply “chips”

- various types of DNA can be fixed to chip
- oligonucleotides and be synthesized on the chip in situ

**cDNA arrays are made by robotic deposition of DNA spots that are 50-150 µm in diameter onto a coated glass surface**

*One of the most important applications for arrays so far is the monitoring of gene expression (mRNA abundance)*

**DNA arrays are often used to measure levels of gene expression (mRNA abundance) for thousands of genes simultaneously**
Review of cDNA synthesis

1. LYSE CELLS AND PURIFY mRNA
2. HYBRIDIZE WITH POLY(T) PRIMER
3. MAKE DNA COPY WITH REVERSE TRANSCRIPTASE
4. DEGRADE RNA WITH RNase H
5. SYNTHESIZE A COMPLEMENTARY DNA STRAND USING DNA POLYMERASE; RNA FRAGMENT ACTS AS PRIMER

double-stranded cDNA copy of original mRNA

Figure 8–34. Molecular Biology of the Cell, 4th Edition.
Just one mRNA is illustrated in this figure, but the mRNA pool would typically be a complex mixture of sequences at varying concentrations.

For Crx paper: a cDNA library made from retinal cell DNA is generated:

cDNAs are generated from the mixed pool of retinal mRNAs and then cloned via traditional recombinant DNA methods.

PCR amplification with T3/T7 (vector specific primers)

960 cDNA clones picked at random from the retinal cDNA library and arrayed onto a glass chip.
Making DNA microarray for PCRex paper

1. Obtain retinal cells from adult mouse
2. Harvest mRNA
3. Make cDNA copies of mRNA (this gives you a heterogeneous population of DNA representing the mRNA population)
4. Clone cDNA molecules into a bacteriophage vector
5. Each virus particle carries a different cDNA insert
6. Plate clones on E. coli
7. Each clear spot on lawn is a pool of identical viruses
8. Pick 960 clones at random
   a. Hybridize DNA on microarray with 45/C3- specificity labeled target DNA
   b. Amplify each cDNA insert with PCR

Note: Colors indicate different genes - not Cys/G3 fluorescence

**Note: rhodopsin came up 13 times in the random pool**
Gene A is equally expressed in samples 1 and 2.

Gene B is highly underexpressed in sample 2.

Gene C is highly overexpressed in sample 2.

In sample 2, relative to sample 1, Gene D is moderately overexpressed, Gene E is equally expressed, and Gene F is moderately underexpressed.
collection of gene-specific DNA molecules

PCR amplification

robotic 'printing' onto glass slide

mRNA from sample 1 labeled with red fluorochrome

mRNA from sample 2 labeled with green fluorochrome

HYBRIDIZE

WASH

SCAN RED AND GREEN SIGNALS AND COMBINE IMAGES

small region of microarray representing expression of 110 genes from yeast

Figure 8–62. Molecular Biology of the Cell, 4th Edition.
Compare the scale of these experiments with the old-style Northern blot (one gene at a time.....)

NOTE: for Northern and Southern blots the PROBE is cloned DNA that is radiolabelled (and is in solution). The DNA or RNA to be examined is fixed onto a nylon membrane (equivalent to the TARGET)
Figure 8–27 part 2 of 2. Molecular Biology of the Cell, 4th Edition.
Figure 1  CRX paper
Figure 2   CRX paper
Figure 4  CRX paper
Figure 5  CRX paper