Eukaryotic Gene Expression

Lectures 22-23

Several Features Distinguish Eukaryotic Processes From Mechanisms in Bacteria
Eukaryotic Gene Expression

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1. RNA Polymerases
   
   *E. coli* has one, eukaryotes have three nuclear enzymes
   
   - RNA pol I synthesizes rRNA
   - RNA pol II synthesizes mRNA
   - RNA pol III synthesizes tRNA and other small RNAs

2. Monocistronic Gene Structure
   
   - Most eukaryotic mRNAs encode single gene product. Many prokaryotic genes are polycistronic, multiple gene products per transcription unit (e.g. operons)
Eukaryotic Gene Expression, cont.

3. RNA processing
   - Eukaryotic messages are "capped" at 5' end with 7-methyl guanosine
   - Eukaryotic messages are polyadenylated at 3' end,
     i.e. AAAAAAAAₙ 3'
   - Internal portions of primary transcript are spliced of intervening sequences - this RNA splicing is due to their:

4. Split Gene Structure
   - Most prokaryotic DNA, RNA, and protein sequences of a gene are contiguous.
   - In eukaryotes, some (many) genes contain introns, whose RNA product is spliced out before mRNA transport to the cytoplasm, leaving only exon sequences in mature mRNA.
Split gene structure in eukaryotes

(a) Prokaryotes.

(b) Eukaryotes.
Why Genes in Pieces?

Current Thinking:

• All proteins (or most, anyway) have evolved from pre-existing sequences, shuffling of exons in the genome allows for the creation of new composites from pre-existing units.

• Domain structures of proteins often correlate with exon structure.

Advantage:

• Evolution is more rapid than "waiting" for new sequences to arise.

• It is the only way to create long open reading frames from pre-exiting sequences.
5. BUT The MOST CRUCIAL DIFFERENCE is the role of CHROMATIN in EUKARYOTES

To relieve repression by chromatin, much more must happen at eukaryotic promoters. The key to the process is controlling access to promoters.
Gene Regulation in Eukaryotes

• **Physiological aspects:**
  – Animals must generate many different cell types from a single egg (time & space).
  – Different cells are organized into different tissues/organs and express different proteins.

• **Structural aspects:**
  – Eukaryotic genes are often larger, containing more potential regulatory information.
  – Eukaryotic regulatory regions have more features of note.
Eukaryotic Promoters - in Contrast

RNA polymerase II promoters often tripartite

Mutations in these three elements may ↓↓ the level of transcription, while mutations outside them, or between them usually do not affect the basal level of transcription.

Temporal, tissue-specificity is generally not directed by promoter-binding factors but through more distant sites termed **ENHANCERS** or **UPSTREAM ACTIVATION SEQUENCES (UAS)**.
Properties of Enhancers

- can greatly increase transcription rates from promoters on same DNA molecule
- may act up to several thousand base pairs away
- function in either orientation (can flip 'em around) and can function upstream or downstream of the promoter they are enhancing

CONSIDER

1. They are sites for trans-acting factors
2. Action at a distance reflects conformation of the gene in chromatin - its protein-bound form, sites that are distant in linear DNA molecule may be adjacent in chromatin

- hence, distance & orientation are independent
Eukaryotic Transcriptional Regulatory Proteins

Often possess one or more functional domains:

• A domain that recognizes a DNA sequence
• A domain that interacts with one or more proteins of the transcriptional apparatus
• A domain that interacts with proteins bound to nearby regulatory sites (cooperativity)
• A domain that influences chromatin structure (directly or indirectly)
• A domain that acts as a sensor of conditions within the cell
KEY MODEL EUKARYOTE - YEAST

Unnumbered figure pg 390b
Introduction to Genetic Analysis, Ninth Edition
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MODEL SYSTEM -
THE GAL PATHWAY

Galactose (extracellular) → Gal2
Galactose (intracellular) → Gal1 → Gal7 → UDP-galactose → Gal10 → UDP-glucose → Gal7 → Glucose-1-phosphate → Glycolysis
Gal4 binds to UAS sequences upstream of genes in pathway and activates their transcription -

But only when galactose is present.
Gal4 is modular in structure with an ACTIVATION domain and a DNA-BINDING DOMAIN.
Gal4 is modular in structure with an ACTIVATION domain and a DNA-BINDING DOMAIN
Gal4 is modular in structure with an ACTIVATION domain and a DNA-BINDING DOMAIN

The activation domain works when grafted onto heterologous DNA-binding proteins
Gal4 activity is regulated physiologically through Gal80 and Gal3.

Gal80 binds Gal4 and blocks activation domain.

Galactose and Gal3 co-induce Gal4 activity by releasing Gal80.

Gal3, Gal4, Gal80 and the UAS form a “switch” that is flipped by galactose.

Figure 11-8
*Introduction to Genetic Analysis, Ninth Edition*
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How Do Activators Work?

• Some recruit the transcriptional machinery
• Some recruit proteins that modify chromatin structure and allow RNA polymerase II and other proteins access to DNA
• Some do both
Activators Recruit the Transcription Machinery

UAS

Gal4

(TFIID)

TBP

Mediator

RNA polymerase II

(TATA)

GAL genes

(“Co-activator”)
Some Activators Work Via “Chromatin Remodeling”

Eukaryotic DNA is “wrapped” around histones to form nucleosomes.
Some Activators Work Via “Chromatin Remodeling”

Chromatin remodeling exposes regulatory sequences

How do nucleosomes get shifted?
Chromatin Remodeling by Histone Modification

(There are many types of modifications in addition to these)
Some Activators Work Via “Chromatin Remodeling”

• Generally, increased acetylation is associated with increased gene expression; coactivators often possess histone acetylation (HAT) activity (Gal4 also recruits Swi-Snf complex, see reading)

• Decreased acetylation, or deacetylation is associated with decreased gene expression; co-repressors often possess histone deacetylation (HDAT) activity

• THE ACETYLATION /DEACETYLATION REACTIONS ARE REVERSIBLE
Repressors May Recruit Co-Repressors that Cause Chromatin Remodeling via Deacetylation

In presence of glucose, Mig1 binds downstream of UAS and recruits the Tup1 complex with histone deacetylase activity.
Eukaryotic Gene Regulation: Two Key Concepts

- **Cooperativity** - physical interactions between regulators increases occupancy of enhancers with synergistic effects on transcription
- **Combinatorial regulation** - the action of two or more regulators imparts greater specificity than single regulators
Cooperativity and the “Enhanceosome”

Binding of multiple interacting proteins leads to very high levels of gene expression (know concept, not details)
Differentiation and Development

• How are Different Cell Types Created?
  – in simple unicellular organisms
  – in complex animals

• Genetic Switches
  – in simple organisms
  – of single genes in complex organisms
  – of entire developmental pathways in complex organisms
Mating-type Regulation in Yeast

- **Primary Observations:** 3 cell types
  - a, α, and a/α
  - a cells and α cells (each haploid) can mate to form a/α diploid
  - mating type is under genetic control
  - some cells switch mating-type, sometimes as frequently as every division

- **Experimental Approach:**
  - Get mutants for the processes involved
  - Order pathways
  - Clone and identify molecular components
  - Determine molecular mechanism
Biology of the System

Switching
(mother cell switches to α)

Germination

Pheromone Response

Conjugation

a/α
(diploid)
Genetic Analysis

- **Find Mutants That:**
  - Can't switch
  - Can't mate

- **Switch Mutants**
  - include the *HO* gene, *SWI* genes, *HMRα, HMLα*
  - loss of these functions causes an inability to switch. *Why?*

- **Mating Mutants**
  - same strains won't mate, they are sterile (STE) - *Why?*
The Explanation

- The "cassette" model of mating-type switch.
- The $MAT$ locus determines the mating-type expressed.
- Alternative cassettes are inserted at the $MAT$ locus which determine the mating-type.
- $MAT\alpha$ encodes $\alpha_1, \alpha_2$ proteins which promote $\alpha$ phenotype, suppress a phenotype
- $MAT\alpha$ encodes $a_1$ protein which, when present with $\alpha_2$, functions to suppress haploid functions and to promote diploid functions.

i.e.

$MAT\alpha/ MAT\alpha$ heterozygotes are the diploid products of mating
THUS:

- A switch involves removal of the old cassette and insertion of the new.
- Switch is reversible because only copied information is transposed, silent information for a and α is always present at HM loci.
The process of mating type switching in yeast.
Role of \( HO \) Gene

- \( HO \) encodes an endonuclease which cuts DNA to initiate transposition event.
- \( HO \) is only activated in the mother cell and only at the correct time in the cell cycle (highly regulated).
- \( SWI \) genes regulate \( HO \) expression.

\textit{i.e.,}

\( SWI \) regulate the gene that regulates the switch, which contains a cassette encoding the proteins which regulate \( a, \alpha \), and \( a/\alpha \)-specific genes expression.
What do the MAT products do?

- $MAT_\alpha$ makes $\alpha_1$ - activates $\alpha$-specific genes.
- $MAT_\alpha$ makes $\alpha_2$ - represses a-specific genes
- MATa makes a1 -
- $MATa, MAT_\alpha$ makes - a1/$\alpha_2$ represses haploid specific genes
- $MAT$ encodes transcriptional regulators
- The MAT products recognize specific DNA sequences upstream of target genes which they regulate.
MAT proteins and MCM act combinatorially to regulate mating type.

WHO ARE THESE TARGET GENES?
TARGETS:

One set of targets is intimately related to mating process - these are the pheromone and pheromone receptor genes.

Recall:

After 2 divisions involving a switch there are $2\alpha$ cells and $2a$ cells in proximity. These cells secrete factors ($\alpha$ cell $\rightarrow \alpha$ factor; a cell $\rightarrow$ a factor) which are recognized by opposing cell ($\alpha$ cell $\rightarrow$ a factor receptor, a cell $\rightarrow$ $\alpha$ factor receptor).

- $MAT\alpha \rightarrow \alpha 1, MCM$, $\alpha 2, MCM \uparrow$ $\alpha$ factor, a factor receptor
  $\alpha$ factor, $\alpha$ factor receptor
- $MATa \rightarrow$ MCM $\rightarrow$ a factor, $\alpha$ factor receptor
- $MATa/\alpha \rightarrow a1/\alpha 2 \downarrow$ all haploid genes ($HO$, too)
  $\bullet$ does not mate
Overview of Mating-type

- Regulation of *HO* (*endonuclease*)
- Regulates *MAT* cassette transposition
- Which regulates which regulatory proteins are made (*α1, α2, a1*)
- Which regulate which cell-type specific gene are made (pheromones, etc.)
- Which regulate cell behavior (mating - more genes)
- Which regulate mating (process)
- Which regulates gene expression (new genotype)
- And sets the stage for switching and mating all over again (starvation → meiosis → haploid cells again)
Why is This Useful?

• **Exquisite** model of cell-type specific control of gene expression
• Mating type proteins work in concert with other regulatory factors to create sophisticated genetic switch
• Genetic analyses have allowed us to dissect both the regulatory pathways and the cell biology (transport of pheromone, signal reception, cell shape change etc., - too extensive to get into here)

Wait until you see how animal body formation is controlled - then you'll get it.