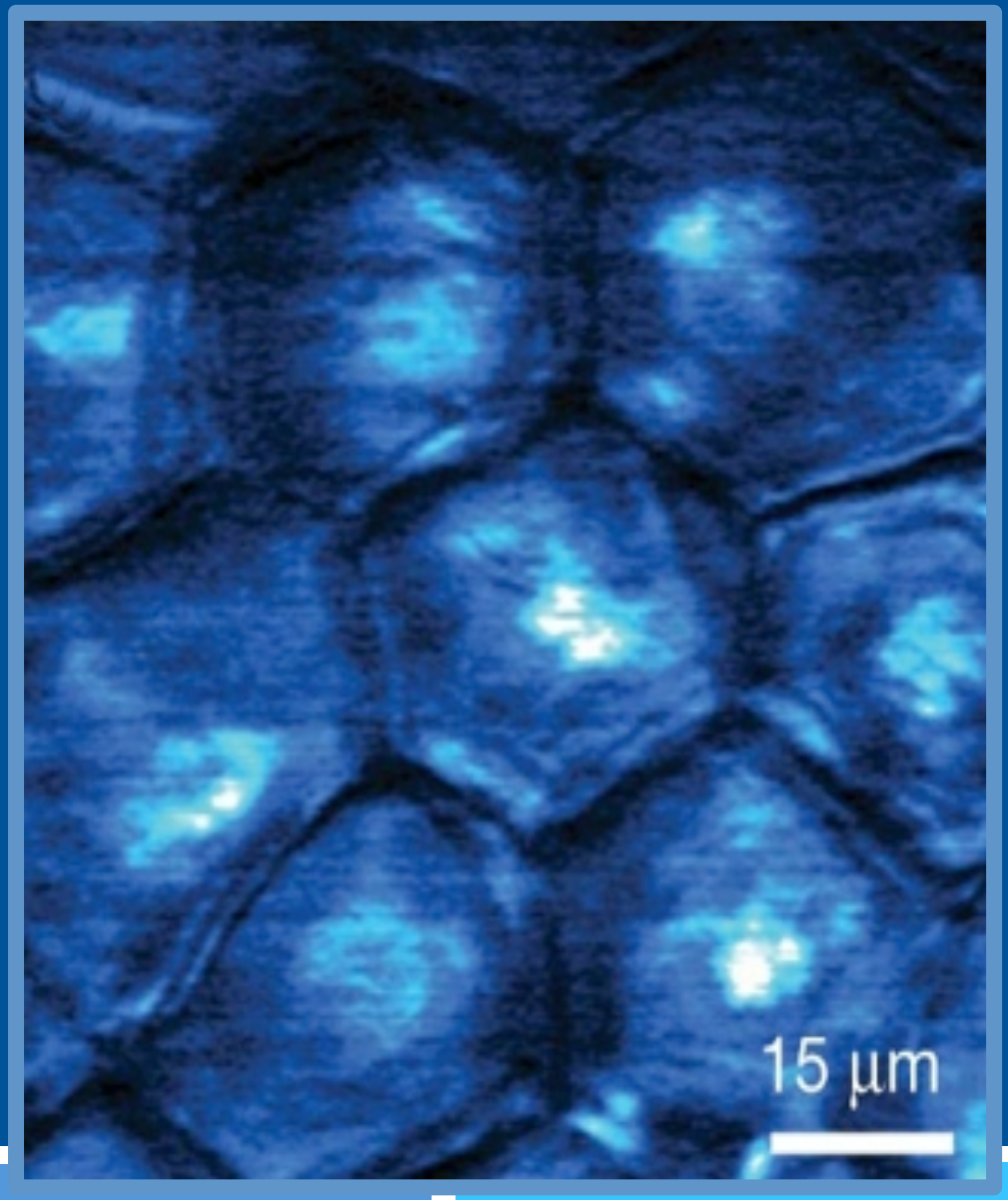




GENES AND CHROMOSOMES V

Lecture 7

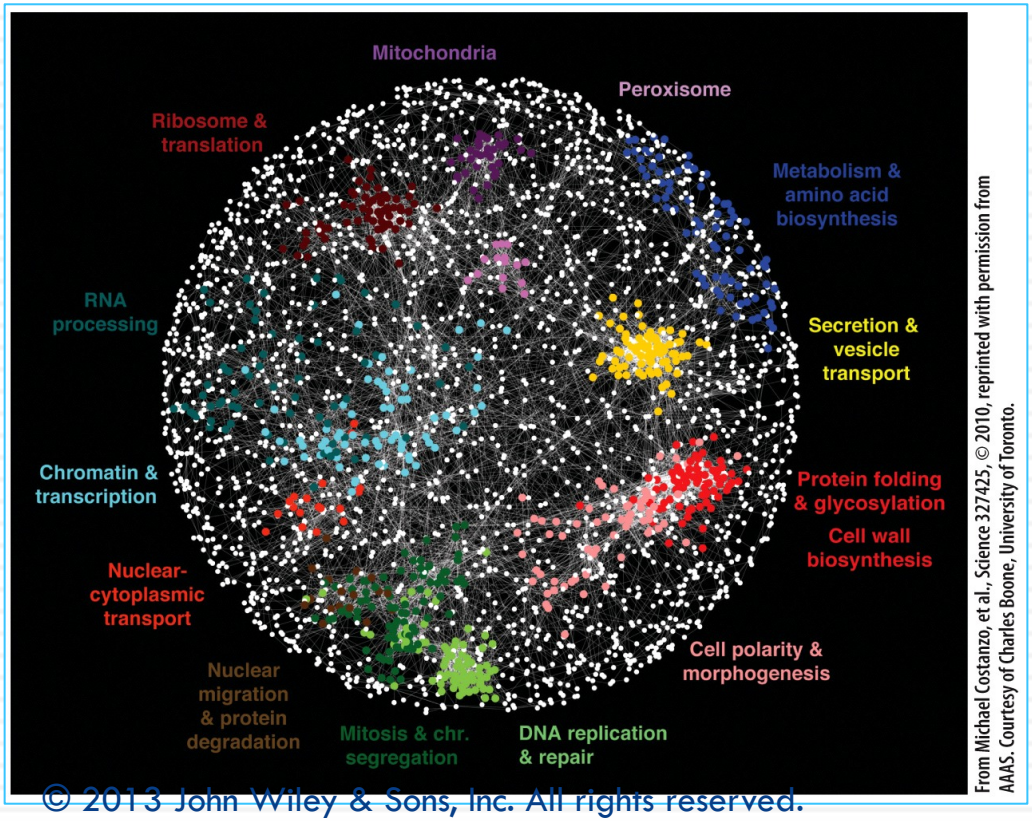
BIOL 266/4

2014-15

Dr. S. Azam

Biology Department
Concordia University

2 CELL NUCLEUS AND THE CONTROL OF GENE EXPRESSION



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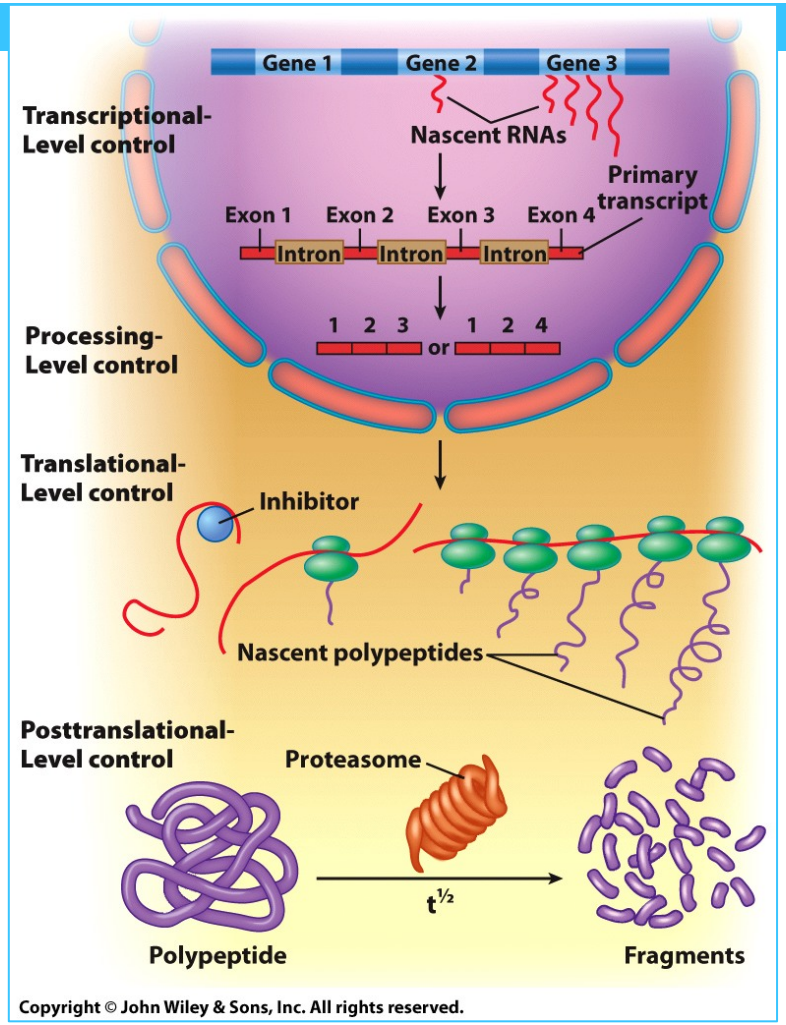
An Overview of Gene Regulation in Eukaryotes

- Genes are turned on and off as a result of interaction with regulatory proteins.
  - Each cell type contains a unique set of proteins.
  - Regulation of gene expression occurs on FOUR levels:
    1. **Transcriptional control**
    2. **RNA Process control**
    3. **Translational control**
    4. **Post-translational control (ubiquitination)**



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1. TRANSCRIPTIONAL CONTROL

Transcriptional control

- **Differential transcription** is the most important mechanism by which eukaryotic cells determine which proteins are synthesized.
- Differential gene expression is found in various conditions:
  a. Cells at different stages of embryonic development
  b. Cells in different tissues
  c. Cells that are exposed to different types of stimuli

Experimental demonstration of the tissue-specific expression of a gene involved in muscle cell differentiation. A transgenic mouse embryo that contains the regulatory region of the myogenin gene placed upstream from a bacterial β-galactosidase gene, which acts as a *reporter*.

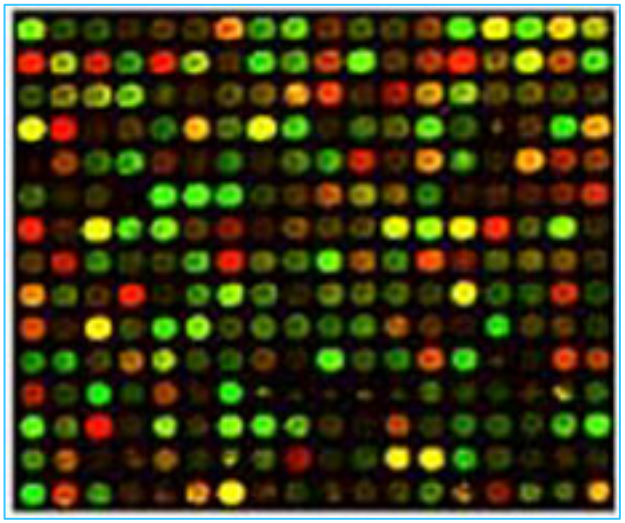
Transcriptional control

Technique: DNA microarrays

- **DNA microarrays** can monitor the expression of thousands of genes simultaneously.
  1. Immobilized fragments of DNA are hybridized with fluorescent cDNAs.
  2. Genes that are expressed show up as fluorescent spots on immobilized genes.
  3. Microarrays provide a visual picture of gene expression.

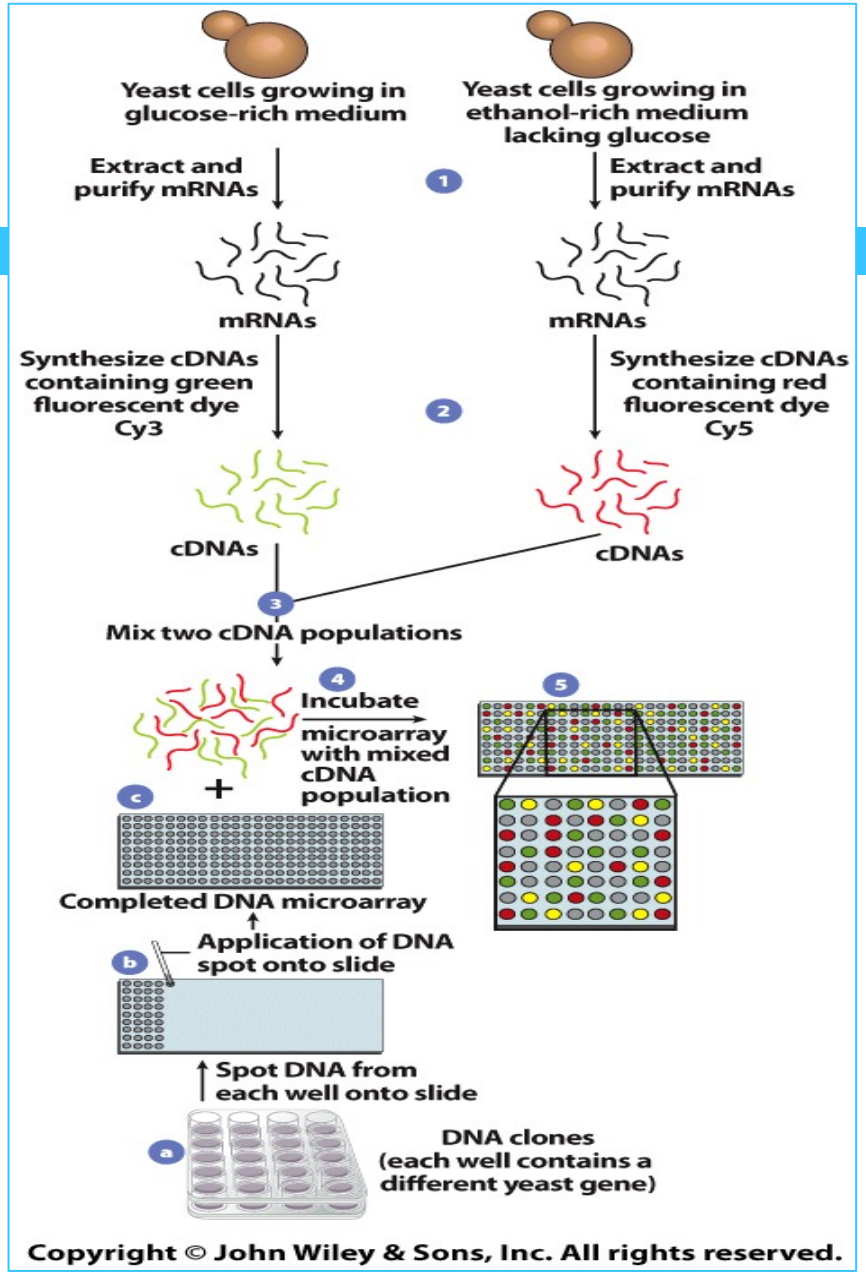
Transcriptional control

Technique: DNA microarrays



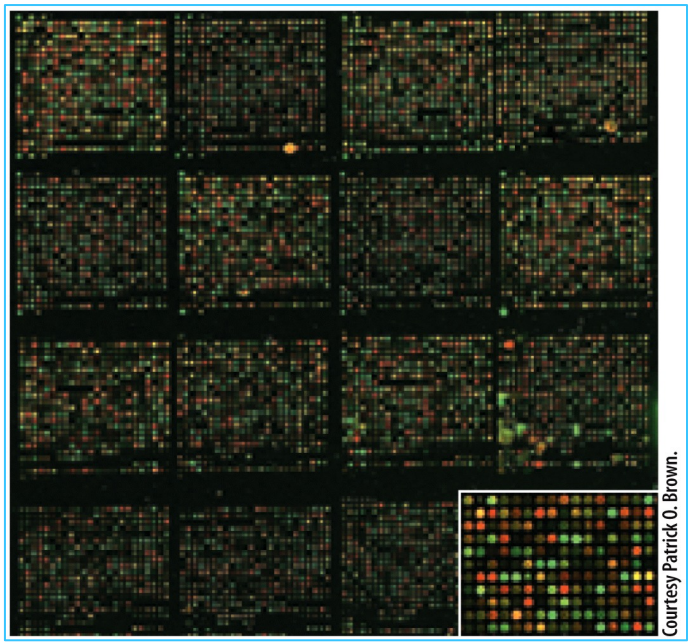
Intensity of the color of the spot is proportional to the cDNA bound there. What do the following colors represent?

- **Red + Green= Yellow**
- **Red**
- **Green**
- **Dark**

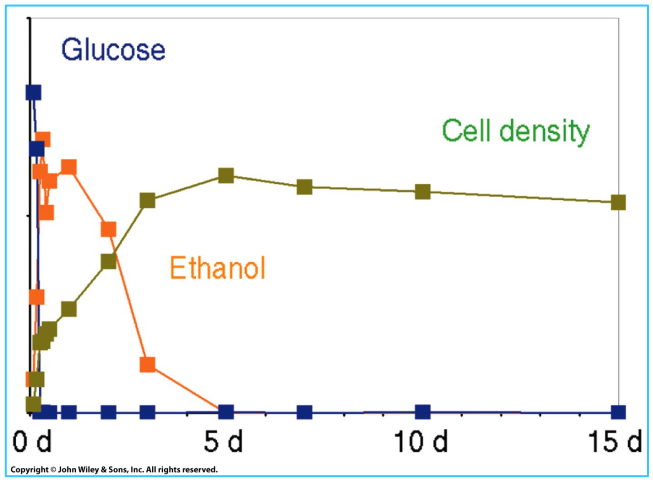


Transcriptional control

DNA microarrays



Experimental results comparing yeast in glucose or ethanol



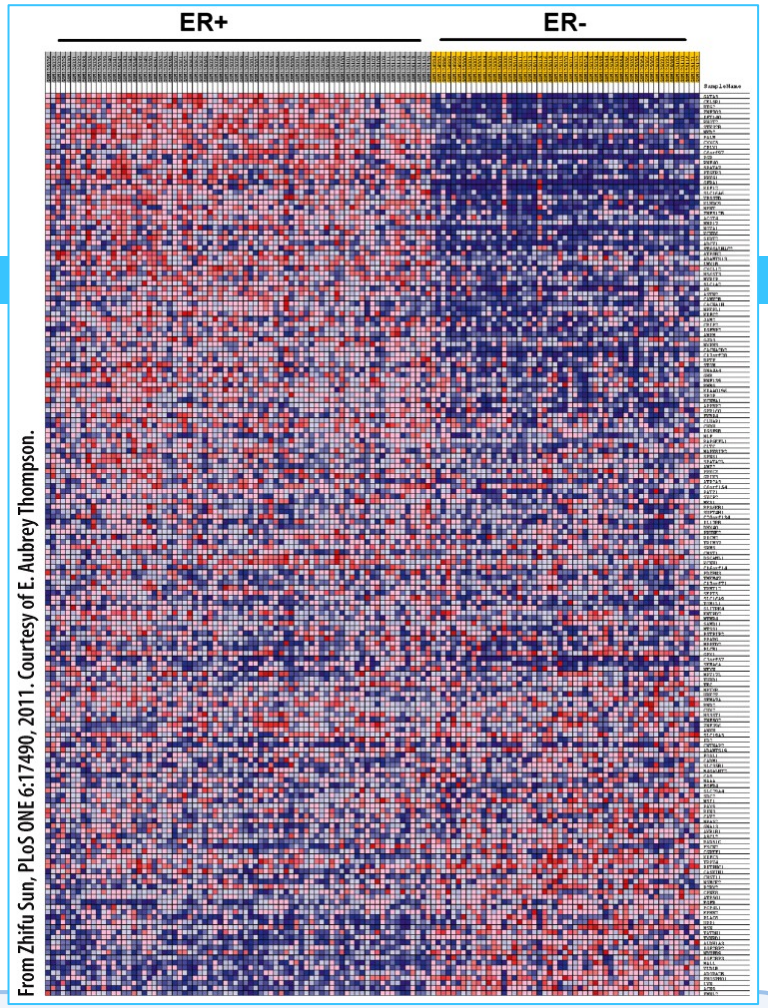
Plot showing changes in glucose and ethanol concentrations in the media and in cell density

Transcriptional control

Technique: DNA microarrays

DNA microarrays can facilitate the diagnosis and treatment of human diseases.

- Human breast tumors can be Estrogen receptor-positive (ER+) or Estrogen receptor-negative (ER-)
- These tumor subtypes show differences in a subset of genes.
- Personalized medicine in the future will be reliant upon transcription profiling, not only for the diagnosis and to establish a treatment plan, but also to monitor the effectiveness of the treatment.



Transcription profiling to personalize breast cancer therapy.

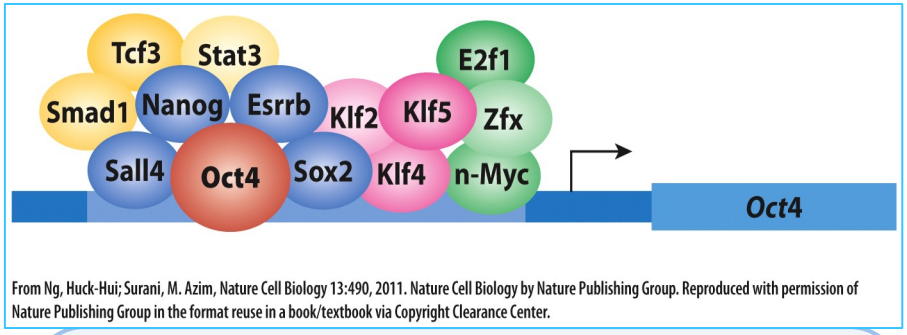
- ER+ and ER- tumors show 179 genes that are differentially regulated. Expression ranges from low (blue) to high (red).
- Each column of tiny square=single cancer patient

Transcriptional control

Transcription factors

Transcription factors are the proteins that either act as *transcription activators* or *transcription inhibitors*

- A single gene can be controlled by different regulatory proteins.
- A single DNA-binding protein may control the expression of many different genes.



From Ng, Huck-Hui; Surani, M. Azim, Nature Cell Biology 13:490, 2011. Nature Cell Biology by Nature Publishing Group. Reproduced with permission of Nature Publishing Group in the format reuse in a book/textbook via Copyright Clearance Center.

Combinatorial control of transcription. Transcription of the Oct4 gene requires the action of multiple transcription factors that bind upstream of the start site of transcription.

Transcriptional control

Transcription factors

From Tom K. Kerppola, Structure 6:550, 1998, with permission from Elsevier.

Interactions between transcription factors bound to different gene regions. Image of two separate transcription factors, NFAT-1 (green) and AP-1 (red and blue) bound to DNA.

Transcriptional control

Transcription factors

Gene expression gives rise to phenotypes within cells and tissues of an organism.

- Ectopic or forced gene expression can alter the phenotype.
  - Expression of MyoD causes fibroblasts to become muscle cells.
  - Expression of *eyeless gene* can trigger eye formation in the leg of flies.

Phenotypic conversion induced by abnormal expression of a single transcription factor. The leg of this fruit fly bears a fully formed eye that has developed due to the forced expression of the *eyeless* gene

Transcriptional control

Transcription factors

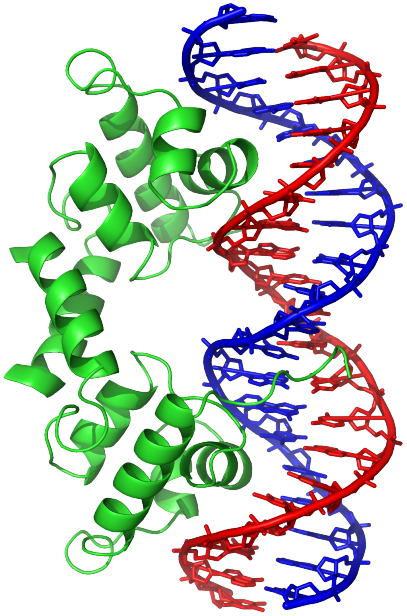
The Role of Transcription Factors in Determining a Cell's Phenotype

- Embryonic stem (ES) cells are:
  - Capable of indefinite self-renewal
  - Pluripotent: capable of differentiating into all of the different types of cells.
- Introducing a combination of genes encoding only four specific transcription factors (Oct4, Sox2, Myc, and Klf4) was sufficient to reprogram the fibroblasts and convert them into undifferentiated cells that behaved like ES cells.

Transcriptional control

Transcription factors

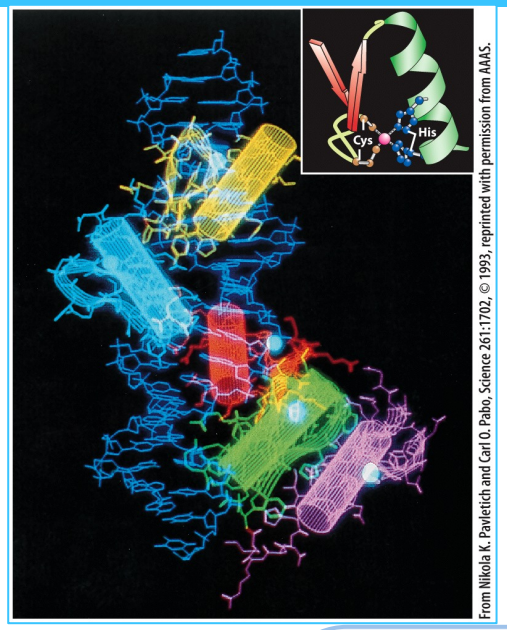
- The Structure of Transcription Factors
  - Transcription factors contain a *DNA-binding domain* and an *activation domain*.
  - Many transcription factors can bind a protein of identical or similar structure to form a dimer.
- Transcription Factor Motifs
  - The DNA-binding domains of most transcription factors have related structures (*motifs*) that interact with DNA sequences.
  - Most motifs contain a segment that binds to the major groove of the DNA.



Transcriptional control

Transcription factors

- Transcription factor motifs
  - **The zinc finger motif** – zinc ion of each finger is held in place by two cysteines and two histidines.
  - **The helix-loop-helix (HLH) motif** – has two α-helical segments separated by a loop.
  - **The leucine zipper motif** – has a leucine at every seventh amino acid of an α-helix.



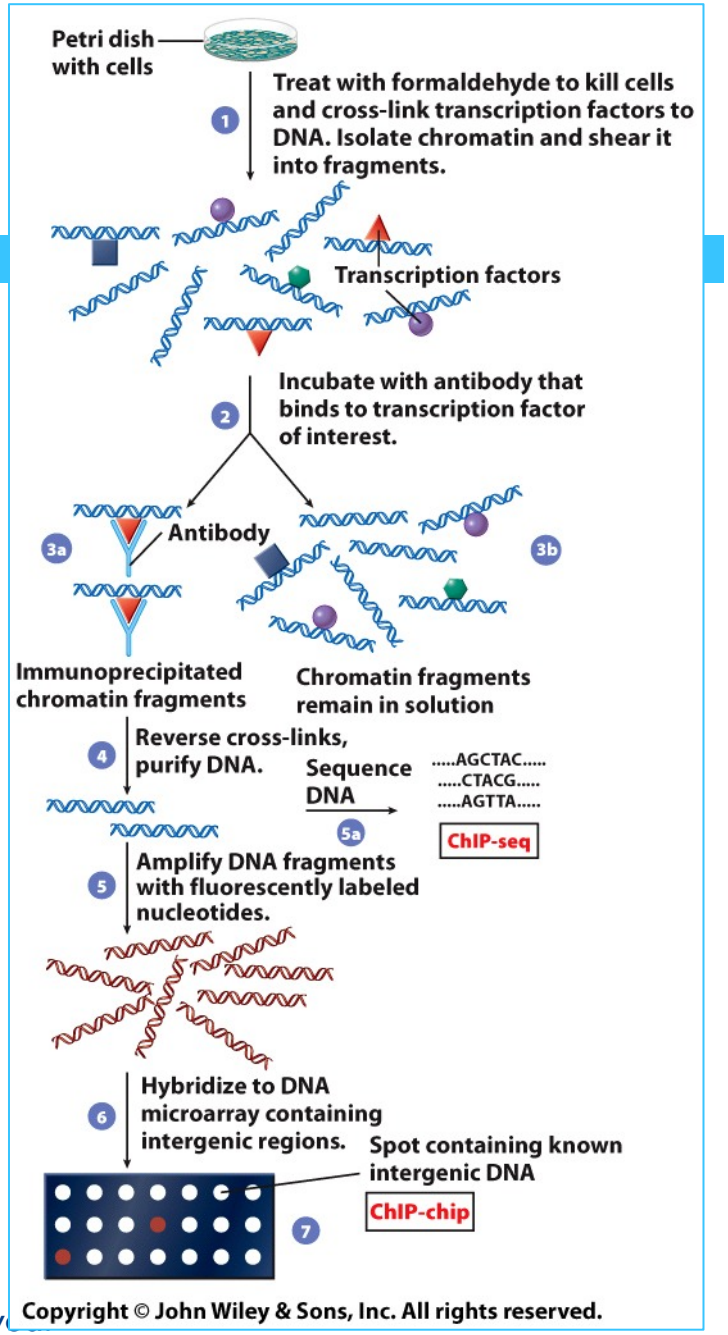
Complex between GLI (has five zinc fingers) and DNA. Each finger is colored differently. Inset: structure of a single zinc finger.

Transcriptional control
DNA binding sites

Researchers use several techniques to find DNA sequences involved in regulation:

- **Genome-wide location analysis**
  - Allows simultaneous monitoring of all the sites within the genome that are bound by a given transcription factor under a given set of physiologic conditions
  - Chromatin immuno-precipitation (ChIP): experimental technique used to investigate the interaction between proteins and DNA in the cell.

ChIP: global search for transcription-factor binding sites



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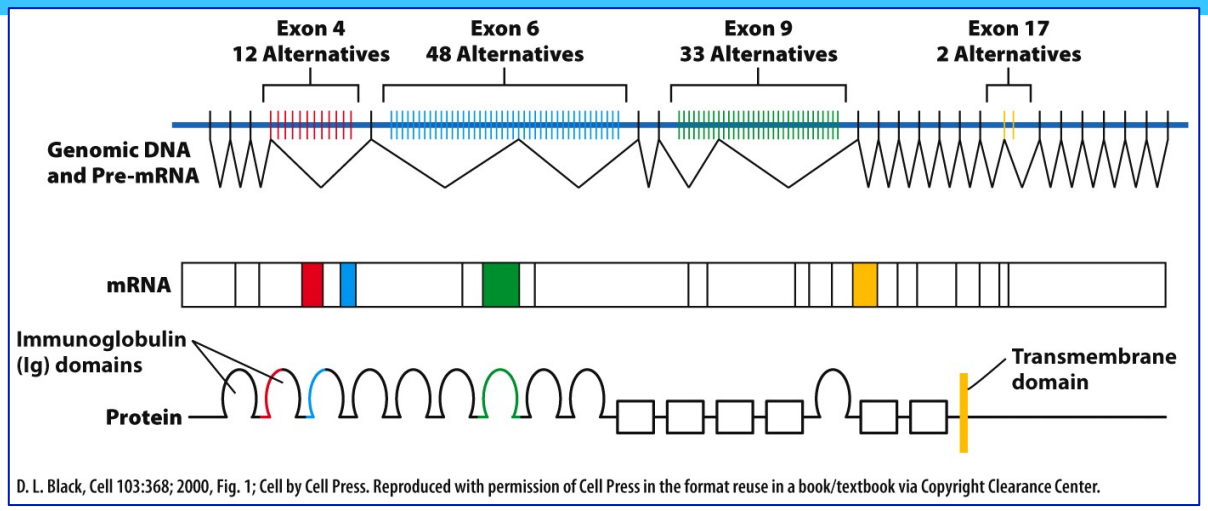
2. RNA PROCESSING CONTROL

RNA Processing Control



Alternative splicing. The *Drosophila Dscam* gene illustrates the diversity of transcripts (38,106 possible mRNAs)) from a single gene

- Introns must be removed by splicing and a poly-A tail is added before the mRNA goes out of the nucleus.
- Protein diversity can be generated by alternative splicing.
- Alternative splicing can become complex, allowing different combinations of exons in the final mRNA product.
- There are factors that can influence splice site selection.

Processing Control

RNA editing

- Specific nucleotides can be converted to other nucleotides through **mRNA editing**.
- RNA editing can create new splice sites, generate stop codons, or lead to amino acid substitutions.
- Example 1
  - It is important in the nervous system, where messages need to have A (adenine) converted to I (inosine) to generate a glutamate receptor.
  - Resultant glutamate receptor's internal channel is impermeable to calcium ions.
- Example 2
  - Conversion to cholesterol carrying protein (apolipoprotein B) mRNA to a truncated version in the intestines (apolipoprotein B-48) produces a smaller protein that works to help absorb fats.
  - Full length of mRNA is approximately 14,000 nucleotides long
  - Cytidine at 6666th position is converted to uridine: generates a STOP codon (UAA)

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3. TRANSLATIONAL CONTROL

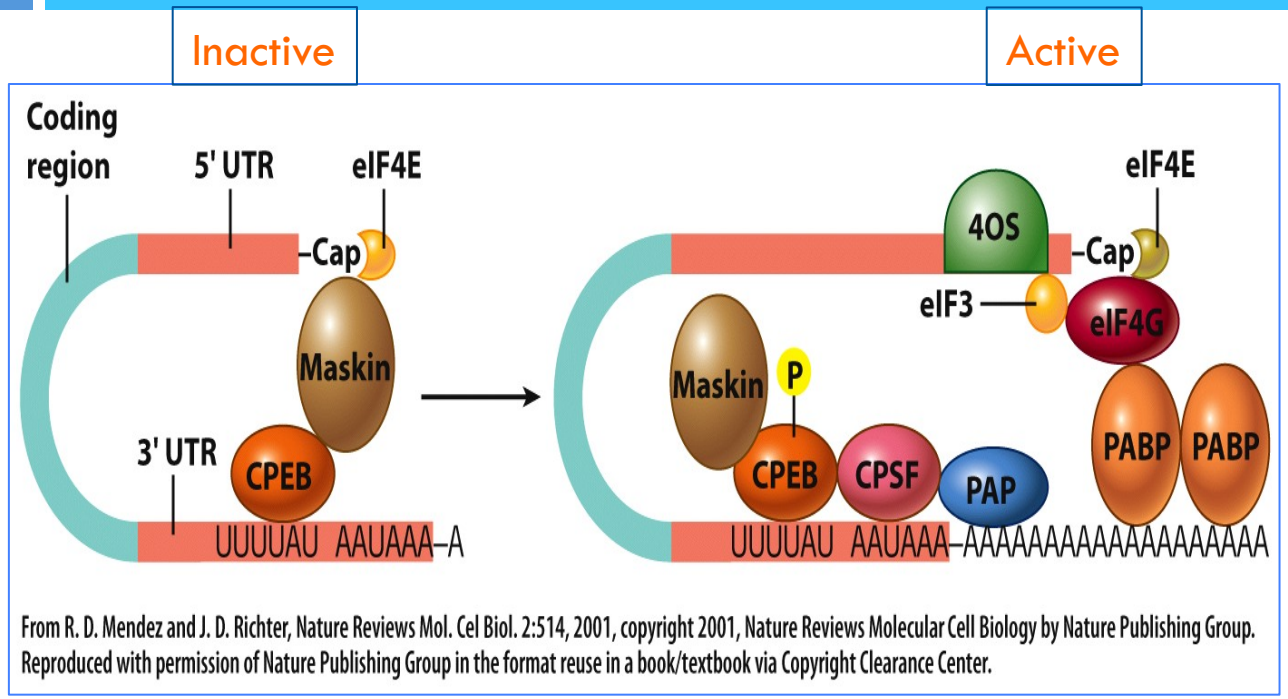
Translational Control

- Affects the translation of mRNAs previously transported from the nucleus to the cytoplasm.
- Types of control:
  1. Localization of mRNA to certain sites within a cell
  2. Whether or not a mRNA is translated?
  3. How often a mRNA is translated?
  4. Half life of mRNA: how long the message is translated for?

Translational Control

- mRNA contains non-coding regions called untranslated regions (UTRs) at both their 5' and 3' ends
- 5' UTR contains certain nucleotides to mediate translational control
- Many mRNA are stored in unfertilized eggs that remain inactive until fertilization and subsequent development
- Initiation of translation of mRNA during early development involves atleast 2 distinct events:
  1. Increase in the length of polyA tail
  2. Removal of bound inhibitory proteins

Translational control

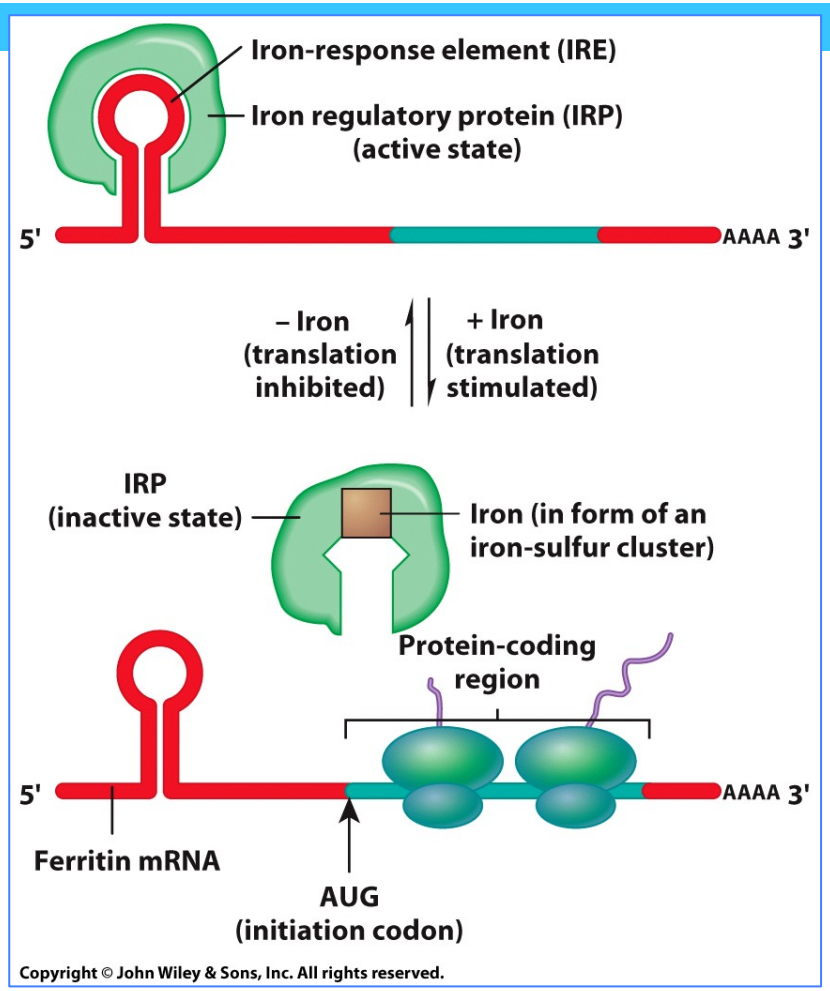


- Following fertilization, **CPEB** is phosphorylated, which displaces **Maskin**.
- **CPEB** recruits **CPSF** which recruits **poly (A) polymerase (PAP)** which adds adenosine residues to the poly(A) tail

A model for the mechanism of translational activation of mRNAs following fertilization of a *Xenopus* egg. mRNAs are maintained in the cytoplasm in an inactive state by their short poly(A) tails and a bound inhibitory protein Maskin.

Translational control

- The Control of mRNA Translation
  - When iron concentrations are low, iron regulatory protein (IRP) binds to the iron-response element (IRE) to prevent translation of ferritin *(intracellular iron storage protein)*
  - When iron becomes available, it binds to the IRP, changing its conformation and causing it to dissociate from the IRE, allowing the translation of the mRNA to form ferritin.



The control of ferritin mRNA translation

Some interesting links

https://www.youtube.com/watch?v=oBwtxdl1zvk
http://faculty.plattsburgh.edu/donald.slish/telomerase.html
https://www.youtube.com/watch?v=VNsThMNjKhM
http://www.bio.davidson.edu/genomics/chip/chip.html

Put your thinking cap on (Lectures 3-7)

1. What is the relationship between telomeres and aging? telomeres and cancer?
   - Find some articles on pubmed.ca that support your answer.
2. Compare and contrast the effect of a deletion in the operator of the lactose operon with one in the operator of the tryptophan operon.