Control of Eukaryotic Gene Expression *(Learning Objectives)*

1. Compare and contrast chromatin and chromosome: composition, proteins involved and level of packing. Explain the structure and function of nucleosome, histones, scaffold proteins (metaphase chromosomes).

2. Explain the role of non-coding RNA and chemical modifications: methylation of DNA and acetylation of histones in control of gene expression. Define the term epigenetics.

3. Identify the main mechanism for turning on gene expression. Explain why control of gene expression in eukaryotic cells is like a “dimmer switch”, an “ON” switch that can be fine tuned.

4. Identify the major switch and all the fine-tuning steps that can modulate eukaryotic gene expression.

5. Identify and explain component of eukaryotic genes: coding and regulatory sequences (proximal and distal elements).

6. Compare and contrast pre and post transcriptional and translational controls of gene expression.


8. Define ubiquitin and proteosome and explain their roles in intracellular protein degradation.
Control of Eukaryotic Gene Expression
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- Cells express 3-5% of their genes
  - House-keeping genes - all the time
  - Genes turned on or off - internal and external signals (environmental - nurture)
  - Genes turned on only in some cell types not others while others are permanently shut down (Highly specialized nerves or muscle)
Structural organization of Eukaryotic DNA

- Nucleosom al beads of chromatin: DNA and histones “Beads on a string”
- Chromatin packing is the degree of nucleosome coiling
- Histones play major role in gene expression

Expose DNA when it is to be transcribed shield it when it is to be silenced

The human genome codes for ~21,000 genes
Interphase Chromatin states

Heterochromatin: compacted, transcriptionally inactive

Euchromatin: open, actively transcribed
Eukaryotic Chromatin Structure

Successive levels of chromatin packing
1. ds-DNA helix (naked DNA)
2. Nucleosomes (histones)
3. 30 nm chromatin
4. Looped domains (non-histone protein scaffold) 300 nm
5. Condensed scaffolded looped domains (700 nm)
6. Metaphase chromosome (1,400 nm)
Histones

- Positively charged proteins
- first level of DNA packing
- Leave DNA transiently during replication
- Remain associated with DNA during transcription (change position and shape to allow RNA polymerase move along the DNA)
Packing is highly specific and precise with particular genes located in the same places.
Eukaryotic gene expression

http://highered.mcgraw-hill.com/olc/dl/120080/bio31.swf
Control of Gene Expression

Nuclear level controls

1) **Chromatin remodeling** = “On/off” switch
2) Transcription Factors
3) Alternative splicing
Levels of control of gene expression include:

- Chromatin “remodeling” packing (*Epigenetic*)
- Transcription
- RNA processing: efficiency & alternative splicing
- RNA stability-microRNAs
- Translation
- Protein modifications
- Protein degradation
Maximizing Genetic Information

- Replication
- Transcription
- Translation

~21,000 genes
~100,000 mRNAs
~1 million proteins

- Chromatin remodeling
- Alternate splicing
- microRNAs block protein synthesis
- Protein folding
- Polypeptides shortened
- Sugars added
- Polypeptides aggregate

DNA → RNA → Protein

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• Proteins discrete structural and functional regions called **domains**.

• Different exons code for different domains of a protein.
Transcriptional Control

1. Chromatin-modifying enzymes
   - availability of DNA for transcription (*On Switch*)

2. Fine-tuning begins with the interaction of transcription factors with DNA sequences
Chromatin Remodeling

- Long non-coding RNAs coating the chromatin
- Specific Chemical modifications that bind to histones and DNA are:
  - Acetyl group - histones
  - Methyl groups - histones and DNA
  - Phosphate groups - histones

\[ + \text{acetyl} \left( \text{CH}_3\text{CO}_2 \right) \]
\[ + \text{phosphates} \left( \text{PO}_4 \right) \]

Transcription off - methyls \( (\text{CH}_3) \)  Transcription on
Histone tails protrude outward from a nucleosome.

Acetylation of histone tails promotes loose chromatin structure that permits transcription.

Unacetylated histones → Acetylated histones
Chromatin Chemical Modifications

a. DNA methylation
   - Active genes - unmethylated (Euchromatin)
   - Inactive genes - highly methylated (Heterochromatin)

b. Histone acetylation
   - Acetylated histones (Euchromatin)
   - De-Acetylated histones (Heterochromatin)

DNA methylation and histone de-acetylation cooperate to repress transcription
Transcriptional control

• Initiation of transcription- *universal* control of gene expression

• Controlled by interaction between proteins and DNA
Proximal and Distal Control elements of gene transcription

DNA

Enhancer (distal control elements)  Proximal control elements

Upstream  Promoter  Downstream

Exon  Intron  Exon  Intron  Exon

Transcription

Poly-A signal

Termination region

Primary RNA transcript (pre-mRNA)

Chromatin changes

Translation

RNA processing

mRNA degradation

mRNA

5' Cap  5' UTR (untranslated region)

Translation

Protein processing and degradation

Coding segment

mRNA  3' UTR (untranslated tail region)

Start codon

Stop codon

AAA...AAA

Poly-A tail

Cleaved 3' end of primary transcript

RNA processing: Cap and tail added; introns excised and exons spliced together

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Proximal control elements

- Control transcription initiation
- Binding sites for TATA binding protein RNA polymerase
- Other factors
- Coordinated interaction of a massive assembly
**Distal** control elements

*Action at a distance*

- Enhancers & Silencers (Tissue specific)
- Located far from the promoter upstream, downstream, or within introns
- Enhancers- bind activators
- Silencers- bind repressors

Transcription and complex enhancers

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter18/animations.html#

http://www.dnai.org/a/index.html
Binding of an **activator** to **enhancer** sequences bends DNA to make contact with the protein initiation complex at the promoter.
Role of Activators in Differential Gene Expression

(a) Liver cell
- Albumin gene expressed
- Crystallin gene not expressed

(b) Lens cell
- Albumin gene not expressed
- Crystallin gene expressed
Levels of control of gene expression include:

- Chromatin packing
- Transcription
- RNA processing
- Translation
- Various alterations to the protein product
- Protein degradation
Post-transcriptional control mechanisms

1. RNA processing
2. Alternate splicing
3. Half-life of RNA molecule
   - poly A tail
   - 5’ cap removal
   - Nucleotide sequences in the 3’ untranslated (3’-UTR) trailer region
4. RNA interference (non-coding RNA: miRNA, siRNAs and others)
Alternative RNA splicing
Regulates coding sequence of mRNA
Non-coding interference RNAs

Several groups:

- MicroRNAs (miRNA)- inhibit translation of same RNA
- Small interfering RNA (siRNA)- inhibit translation of other RNAs
- Long non-coding RNA (lncRNA)- variety of functions act in cytoplasm and nucleus

http://www.whatisepigenetics.com/non-coding-rna/

When a interfering RNA binds to a “target” mRNA, it prevents translation

- Specific degradation of an mRNA
- Specific blocking of translation

The human genome has about 1,000 distinct microRNAs that regulate at least 1/3rd of the protein-encoding genes
Post-transcriptional control
MicroRNAs

Transcriptional control
Chromatin remodeling

TF = Transcription factor

Figure 11.7
RNA interference (RNAi)

- Mechanism for silencing gene expression through a technology that uses RNA molecules called **RNA interference (RNAi)**

- Small **synthetic**, double-stranded RNA molecules are introduced into selected cells to block gene expression
Genome regulation by long noncoding RNAs

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3858397/
Translational control

1. Protein factors required to initiate
2. Specific sequences within the 5’-UTR (leader region) of mRNA bind to regulatory proteins preventing translation
3. Interfering RNAs
Post-translational modifications

Functional proteins

- enzymatic cleavage
- chemical modifications
- transport to the appropriate destination

Improperly modified proteins are promptly degraded
Normal proteins undergo selective **degradation** to limit half-life

- marked by **ubiquitin** proteins (76 amino acids)
- Giant proteosomes recognize the ubiquitin and degrade the tagged protein

http://bioisolutions.blogspot.com/2007/05/proteasomes.html
• Piwi-interacting RNAs (*piRNA*)
suppression of transposon activity in
germline and somatic cells

http://www.whatisepigenetics.com/non-coding-rna/