12.1: Eukaryotic gene regulation

Control of gene expression can be exerted at many levels and can be broadly divided into: changes in DNA content or position and changes in gene activity (e.g., expression patterns).

Changes to DNA content and rearrangement are addressed elsewhere. Briefly, DNA of different cell types does not vary in either amount or type. However, highly specialized cases are known to exist where DNA loss, rearrangement, and amplification profoundly influence gene expression in isolated situations.

This section will focus on changes in gene expression.

Regulation is known to occur at several different points of a multistep gene expression pathway. Four main levels of control include:

1. Transcriptional control: Determines if, how much, and when an mRNA is made.

2. Processing or post-transcriptional control: Determines if, how much, and when an mRNA is available for translation into a protein.

3. Translational control: Determines if, how much, and when a protein is made.

4. Post-translational control: Determines if, how much, and when a protein is functional.

Transcriptional control

Control of transcriptional initiation is a primary means used to regulate gene expression in eukaryotic organisms. Most eukaryotic genes are controlled at the level of transcription by proteins (trans-acting factors) that interact with specific
Transcription factors: Enhancers

Along with general transcription factors, there are additional regions that help increase or enhance transcription. These regions, called enhancers, are not necessarily close to the genes they enhance. They can be located upstream of a gene, within the coding region of the gene, downstream of a gene, or thousands of nucleotides away.

Enhancer regions are binding sequences, or sites, for specific transcription factors. When a protein transcription factor binds to its enhancer sequence, the shape of the protein changes, allowing it to interact with proteins at the promoter site. However, since the enhancer region may be distant from the promoter, the DNA must bend to allow the proteins at the two sites to come into contact. DNA-bending proteins help bend the DNA and bring the enhancer and promoter regions together (figure 12.1). This shape change allows for the interaction of the specific activator proteins bound to the enhancers with the general transcription factors bound to the promoter region and the RNA polymerase. Two different genes may have the same promoter but different distal control elements, enabling differential gene expression.

Transcription factors: Repressors

Like prokaryotic cells, eukaryotic cells also have mechanisms to prevent transcription. Transcriptional repressors can bind to promoter or enhancer regions and block transcription. Like the transcriptional activators, repressors respond to external stimuli preventing the binding of activating transcription factors. This is often done by histone deacetylation,
which increases the interaction of DNA and histones (figure 12.2).

Figure 12.2: Modification of DNA and histones can alter DNA accessibility and therefore transcription.

Transcription factors: Structure and function

Structurally, transcription factors share similar characteristics but can take on very different secondary structures. Common examples of transcription factors include: Zn fingers, helix-loop-helixs, and leucine zippers. Regardless of structure, common characteristics include:

1. A positively charged DNA-binding domain,
2. Activation domain, and
3. Dimerization domain.

As noted above, one of the major roles of transcription factors is to bend or remodel the DNA in a way to allow for interactions of transcription factors and their binding sites. Chromatin remodeling by modifications of the histones (through acetylation or shifting) is common (figure 12.2).

Processing or post/cotranscription

Alternative RNA splicing

Alternative RNA splicing is a mechanism that allows different protein products to be produced from one gene when different combinations of exons are combined to form the mRNA. This alternative splicing can be haphazard, but more often it is controlled and acts as a mechanism of gene regulation, with the frequency of different splicing alternatives controlled by the cell as a way to control the production of different protein products in different cells or at different
stages of development. Alternative splicing is a common mechanism of gene regulation in eukaryotes; according to one estimate, 70 percent of genes in humans are expressed as multiple proteins through alternative splicing. Although there are multiple ways to alternatively splice RNA transcripts, the original 5'-3' order of the exons is always conserved. That is, a transcript with exons 1 2 3 4 5 6 7 might be spliced 1 2 4 5 6 7 or 1 2 3 6 7, but never 1 2 5 4 3 6 7 (figure 12.3).

Figure 12.3: Five common modes of alternative splicing.

Translational control

Like transcription, translation is controlled by proteins that bind and initiate the process, restrict access to the mRNA, or control the localization of the transcript itself.

Localization

One fundamental way in which translation is controlled is physically by where the mRNA is located within the cell or organism. This is extremely important in development where restriction of a transcript to one side of a cell can influence the phenotype of a localized cellular region. This is largely mediated by interactions with the 5’ untranslated region (UTR).

Translational initiation

In translation, the complex that assembles to start the process is referred to as the translation initiation complex, and similar to transcription, this complex can be activated or inhibited. In eukaryotes, translation is initiated by binding the initiating met-tRNAi to the 40S ribosome.

Initially the met-tRNAi is brought to the 40S ribosome by a protein initiation factor, eukaryotic initiation factor-2 (eIF-2). The eIF-2 protein binds to the high-energy molecule guanosine triphosphate (GTP), and the tRNA-eIF2-GTP complex
then binds to the 40S ribosome.

The cap-binding protein eIF4F brings the mRNA complex together with the 40S ribosome complex. The ribosome then scans along the mRNA until it finds a start codon AUG. When the anticodon of the initiator tRNA and the start codon are aligned, the GTP is hydrolyzed, the initiation factors are released, and the large 60S ribosomal subunit binds to form the translation complex. Insulin increases the efficiency of formation of the cap-binding complex, therefore increasing the rate of protein synthesis.

The binding of eIF-2 to the RNA is controlled by phosphorylation. If eIF-2 is phosphorylated, it undergoes a conformational change and cannot bind to GTP. Therefore, the initiation complex cannot form properly, and translation is impeded (figure 12.4).

[Diagram showing the regulation of translational initiation with phosphorylation of eIF-2]

When eIF-2 is not phosphorylated, translation occurs.

When eIF-2 is phosphorylated, translation is blocked.

Figure 12.4: Regulation of translational initiation.

When eIF-2 remains unphosphorylated, the initiation complex can form normally, and translation can continue.

Control of RNA stability

Before the mRNA leaves the nucleus, it is given two protective “caps” that prevent the ends of the strand from degrading during its journey. These changes protect the two ends of the RNA from exonuclease attack.

1. The 5′ cap, which is placed on the 5′ end of the mRNA, is usually composed of a methylated guanosine triphosphate molecule (GTP). The GTP is placed “backward” on the 5′ end of the mRNA, so that the 5′ carbons of the GTP and the terminal nucleotide are linked through three phosphates.

2. The poly(A) tail, which is attached to the 3′ end, is usually composed of a long chain of adenine nucleotides.

Once the RNA is transported to the cytoplasm, the length of time that the RNA resides there can be controlled. Each RNA molecule has a defined lifespan and decays at a specific rate. This rate of decay can influence how much protein is in the cell.

• If the decay rate is increased, the RNA will not exist in the cytoplasm as long, shortening the time available for translation of the mRNA to occur.

• Conversely, if the rate of decay is decreased, the mRNA molecule will reside in the cytoplasm longer and more protein can be translated.
**RNA-binding proteins**

Binding of proteins to the RNA can also influence its stability. Proteins called RNA-binding proteins, or RBPs, can bind to the regions of the mRNA just upstream or downstream of the protein-coding region. These regions in the RNA that are not translated into protein are called the untranslated regions, or UTRs (figure 12.5). They are not introns (those have been removed in the nucleus). Rather, these are regions that regulate mRNA localization, stability, and protein translation. The region just before the protein-coding region is called the 5’ UTR, whereas the region after the coding region is called the 3’ UTR. The binding of RBPs to these regions can increase or decrease the stability of an RNA molecule, depending on the specific RBP that binds.

One classic example of this is the regulation of transferrin receptor (TR) and ferritin levels in response to iron.

- Iron is transported in the blood bound to the iron transport protein, transferrin. Transferrin receptors (TRs) are required to bring extracellular iron from the bloodstream into cells (i.e., iron uptake).
- Ferritin, a protein involved in iron storage, is also regulated post-transcriptionally. The half-life of these mRNAs is regulated by iron concentrations.
- The translation of ferritin mRNAs is regulated by the concentration of iron.
- The 5’ UTR of transferrin receptor mRNA contains a single iron response element (IRE) that mediates the effect.
  - When iron is low, the iron-responsive protein (IRP) binds the IRE to block ferritin mRNA translation.
  - When iron is high, IRP cannot bind IRE, and ferritin mRNA is translated.

**microRNAs**

In addition to RBPs that bind to and control (increase or decrease) RNA stability, other elements called microRNAs can bind to the RNA molecule. These microRNAs, or miRNAs, are short RNA molecules that are only twenty-one to twenty-four nucleotides in length. The miRNAs are made in the nucleus as longer pre-miRNAs.

These pre-miRNAs are chopped into mature miRNAs by a protein called dicer. Like transcription factors and RBPs, mature miRNAs recognize a specific sequence and bind to the RNA; however, miRNAs also associate with a ribonucleoprotein complex called the RNA-induced silencing complex (RISC). The RNA component of the RISC basepairs with complementary sequences on an mRNA and either impede translation of the message or lead to the degradation of the mRNA.

**Post-translation regulation**
Chemical modifications

Proteins can be chemically modified with the addition of groups including methyl, phosphate, acetyl, and ubiquitin groups.

The addition or removal of these groups from proteins can have many effects and can be in response to many cellular changes. For example:

- Covalent modifications can regulate protein activity.
- Sometimes these modifications can regulate where a protein is found in the cell — for example, in the nucleus, in the cytoplasm, or attached to the plasma membrane.
- Chemical modifications occur in response to external stimuli such as stress, the lack of nutrients, heat, or ultraviolet light exposure.
- These changes can alter epigenetic accessibility, transcription, mRNA stability, or translation — all resulting in changes in expression of various genes.

This is an efficient way for the cell to rapidly change the levels of specific proteins in response to the environment. Because proteins are involved in every stage of gene regulation, the phosphorylation of a protein (depending on the protein that is modified) can alter accessibility to the chromosome, can alter translation (by altering transcription factor binding or function), can change nuclear shuttling (by influencing modifications to the nuclear pore complex), can alter RNA stability (by binding or not binding to the RNA to regulate its stability), can modify translation (increase or decrease), or can change post-translational modifications (add or remove phosphates or other chemical modifications).

Protein degradation

The addition of an ubiquitin group to a protein marks that protein for degradation. Ubiquitin acts like a flag indicating that the protein lifespan is complete. These proteins are moved to the proteasome, an organelle that functions to remove proteins, to be degraded. One way to control gene expression, therefore, is to alter the longevity of the protein (figure 12.6).

Figure 12.6: Proteasome-mediated degradation.

References and resources

Text

Clark, M. A. *Biology*, 2nd ed. Houston, TX: OpenStax College, Rice University, 2018, Chapter 10: Cell Reproduction, Chapter 11: Meiosis and Sexual Reproduction, Chapter 16: Gene Expression.


**Figures**

Grey, Kindred, Figure 12.1 Example of transcriptional complex involving two separate genes. 2021. [CC BY 4.0](https://creativecommons.org/licenses/by/4.0). Adapted from Biology 2e Figure 16.10 Interaction between proteins at the promoter and enhancer sites. [CC BY 4.0](https://creativecommons.org/licenses/by/4.0). From OpenStax.

Grey, Kindred, Figure 12.2 Modification of DNA and histones can alter DNA accessibility and therefore transcription. 2021. [CC BY 4.0](https://creativecommons.org/licenses/by/4.0). Adapted from Biology 2e. Figure 16.8 Nucleosomes can slide along DNA. [CC BY 4.0](https://creativecommons.org/licenses/by/4.0). From OpenStax.

Grey, Kindred, Figure 12.3 Five common modes of alternative splicing. 2021. [https://archive.org/details/12.3_20210926.](https://archive.org/details/12.3_20210926. CC BY 4.0).

Grey, Kindred, Figure 12.4 Regulation of translational initiation. 2021. [https://archive.org/details/12.4_20210926. CC BY 4.0](https://archive.org/details/12.4_20210926. CC BY 4.0).

Lieberman M, Peet A. Figure 12.5 RNA Binding proteins can increase stability of the transcript. Adapted under Fair Use from Marks’ Basic Medical Biochemistry. 5th Ed. pp 312. Figure 16.21 Translational regulation of ferritin synthesis. 2017.

Lieberman M, Peet A. Figure 12.6 Proteasome mediated degradation. Adapted under Fair Use from Marks’ Basic Medical Biochemistry. 5th Ed. pp 312. Figure 35.6 The proteasome and regulatory proteins. 2017.