

16.5 | Eukaryotic Post-transcriptional Gene Regulation

By the end of this section, you will be able to do the following:

- Understand RNA splicing and explain its role in regulating gene expression
- Describe the importance of RNA stability in gene regulation

RNA is transcribed, but must be processed into a mature form before translation can begin. This processing that takes place after an RNA molecule has been transcribed, but before it is translated into a protein, is called *post-transcriptional modification*. As with the epigenetic and transcriptional stages of processing, this post-transcriptional step can also be regulated to control gene expression in the cell. If the RNA is not processed, spliced, or translated, then no protein will be synthesized.

RNA Splicing, the First Stage of Post-transcriptional Control

In eukaryotic cells, the RNA transcript often contains regions, called introns, that are removed prior to translation. The regions of RNA that code for protein are called **exons**. (Figure 16.10). After an RNA molecule has been transcribed, but prior to its departure from the nucleus to be translated, the RNA is processed and the introns are removed by splicing. Splicing is done by spliceosomes, ribonucleoprotein complexes that can recognize the two ends of the intron, cut the transcript at those two points, and bring the exons together for ligation.

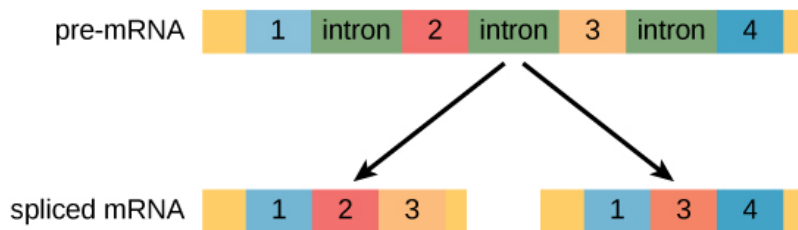


Figure 16.10 Pre-mRNA can be alternatively spliced to create different proteins.

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Alternative RNA Splicing

In the 1970s, genes were first observed that exhibited 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 (**Figure 16.11**). 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 now understood to be 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.

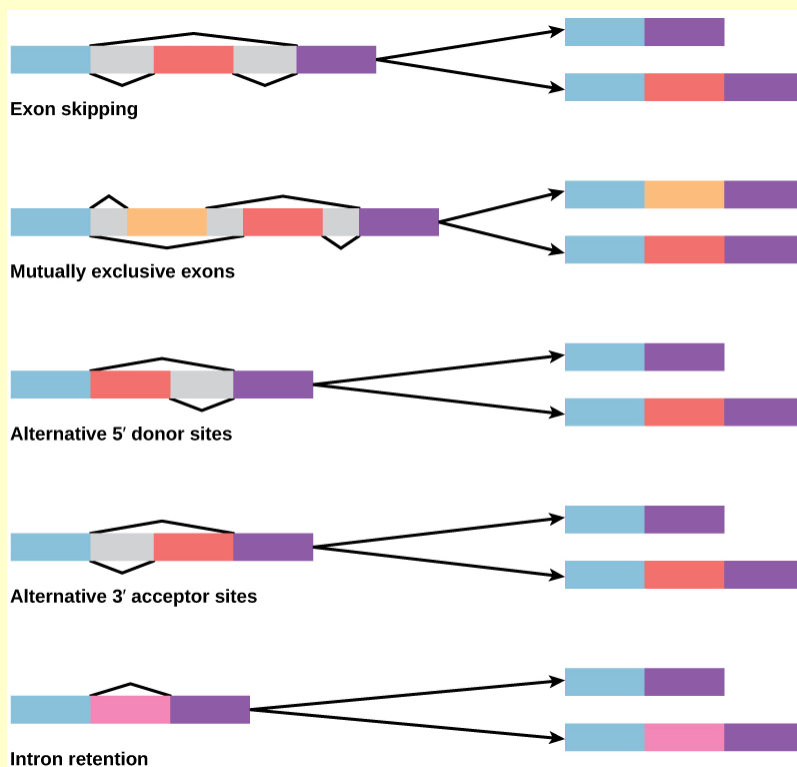


Figure 16.11 There are five basic modes of alternative splicing.

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How could alternative splicing evolve? Introns have a beginning- and ending-recognition sequence; it is easy to imagine the failure of the splicing mechanism to identify the end of an intron and instead find the end of the next intron, thus removing two introns and the intervening exon. In fact, there are mechanisms in place to prevent such intron skipping, but mutations are likely to lead to their failure. Such “mistakes” would more than likely produce a nonfunctional protein. Indeed, the cause of many genetic diseases is abnormal splicing rather than mutations in a coding sequence. However, alternative splicing could possibly create a protein variant without the loss of the original protein, opening up possibilities for adaptation of the new variant to new functions. Gene duplication has played an important role in the evolution of new functions in a similar way by providing genes that may evolve without eliminating the original, functional protein.

Question: In the corn snake *Pantherophis guttatus*, there are several different color variants, including amelanistic snakes whose skin patterns display only red and yellow pigments. The cause of amelanism in these snakes was recently identified as the insertion of a transposable element into an intron in the OCA2 (oculocutaneous albinism) gene. How might the insertion of extra genetic material into an intron lead to a nonfunctional protein?

LINK TO LEARNING

Visualize how mRNA splicing happens by watching the process in action in this video. (This multimedia resource will open in a browser.) (<http://cnx.org/content/m66507/1.5/#eip-id1171119155972>)

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. 5' and 3' exonucleases can degrade unprotected RNAs. 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. The **poly-A tail**, which is attached to the 3' end, is usually composed of a long chain of adenine nucleotides. These changes protect the two ends of the RNA from exonuclease attack.

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. This rate of decay is referred to as the RNA stability. If the RNA is stable, it will be detected for longer periods of time in the cytoplasm.

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. 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** (Figure 16.12). The binding of RBPs to these regions can increase or decrease the stability of an RNA molecule, depending on the specific RBP that binds.

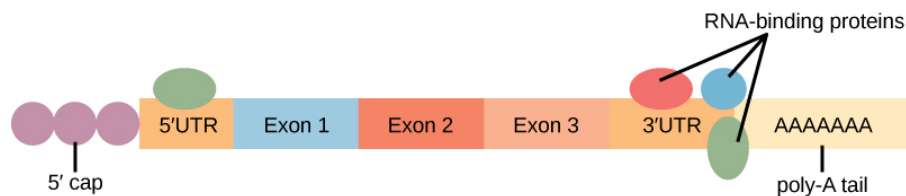


Figure 16.12 RNA-binding proteins. The protein-coding region of this processed mRNA is flanked by 5' and 3' untranslated regions (UTRs). The presence of RNA-binding proteins at the 5' or 3' UTR influences the stability of the RNA molecule.

RNA Stability and 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 21 to 24 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 base-pairs with complementary sequences on an mRNA and either impede translation of the message or lead to the degradation of the mRNA.

16.6 | Eukaryotic Translational and Post-translational Gene Regulation

By the end of this section, you will be able to do the following:

- Understand the process of translation and discuss its key factors
- Describe how the initiation complex controls translation
- Explain the different ways in which the post-translational control of gene expression takes place

After RNA has been transported to the cytoplasm, it is translated into protein. Control of this process is largely dependent on the RNA molecule. As previously discussed, the stability of the RNA will have a large impact on its translation into a protein. As the stability changes, the amount of time that it is available for translation also changes.

The Initiation Complex and Translation Rate

Like transcription, translation is controlled by proteins that bind and initiate the process. In translation, the complex that assembles to start the process is referred to as the translation **initiation complex**. In eukaryotes, translation is initiated by binding the initiating met-tRNA_i to the 40S ribosome. This tRNA 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)**. The tRNA-eIF2-GTP complex then binds to the 40S ribosome. A second complex forms on the mRNA. Several different initiation factors recognize the 5' cap of the mRNA and proteins bound to the poly-A tail of the same mRNA, forming the mRNA into a loop. 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. 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 16.13**). When eIF-2 remains unphosphorylated, the initiation complex can form normally and translation can proceed.