Key points:

- Even after a gene has been transcribed, gene expression can still be regulated at various stages.
- Some transcripts can undergo **alternative splicing**, making different mRNAs and proteins from the same RNA transcript.
- Some mRNAs are targeted by **microRNAs**, small regulator RNAs that can cause an mRNA to be chopped up or block translation.
- A protein's activity may be regulated after translation, for example, through removal of amino acids or addition of chemical groups.

Introduction

The genes that a eukaryotic cell turns "on" largely determine its identity and properties. For instance, a photoreceptor cell in your eye can detect light because it expresses genes for light-sensitive proteins, as well as as genes for neurotransmitters that allow signals to be relayed to the brain.

In eukaryotic cells like photoreceptors, gene expression is often controlled primarily at the level of transcription. However, that doesn't mean transcription is the last chance for regulation. Later stages of gene expression can also be regulated, including:

- RNA processing, such as splicing, capping, and poly-A tail addition
- Messenger RNA (mRNA) translation and lifetime in the cytosol
- Protein modifications, such as addition of chemical groups

In the sections below, we'll discuss some common types of gene regulation that occur after an RNA transcript has been made.

Regulation of RNA processing

When a eukaryotic gene is transcribed in the nucleus, the primary transcript (freshly made RNA molecule) isn't yet considered a messenger RNA. Instead, it's an "immature" molecule called a pre-mRNA.

The pre-mRNA has to go through some modifications to become a mature mRNA molecule that can leave the nucleus and be translated. These include splicing, capping, and addition of a poly-A tail, all of which can potentially be regulated – sped up, slowed down, or altered to result in a different product.

Alternative splicing

Most pre-mRNA molecules have sections that are removed from the molecule, called **introns**, and sections that are linked or together to make the final mRNA, called **exons**. This process is called **splicing**.

In the process of **alternative splicing**, different portions of an mRNA can be selected for use as exons. This allows either of two (or more) mRNA molecules to be made from one pre-mRNA.

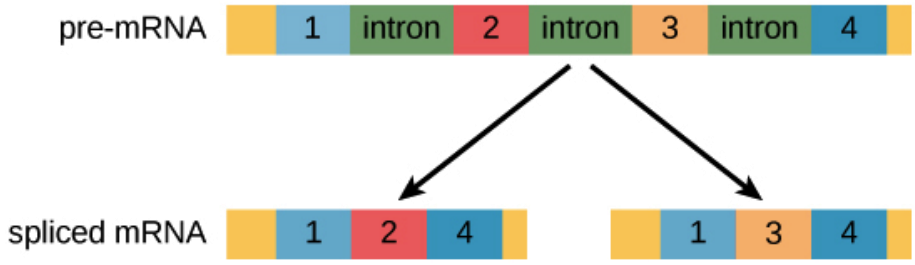

Diagram of a pre-mRNA being spliced into two different variants. There are four possible exons in the pre-mRNA: 1, 2, 3, and 4

Variant 1 contains exons 1, 2, and 4, but not exon 3.

Variant 2 contains exons 1, 3, and 4, but not exon 2.

Alternative splicing is not a random process. Instead, it's typically controlled by regulatory proteins. The proteins bind to specific sites on the pre-mRNA and "tell" the splicing factors which exons should be used. Different cell types may express different regulatory proteins, so different exon combinations can be used in each cell type, leading to the production of different proteins.

Small regulatory RNAs

Once an mRNA has left the nucleus, it may or may not be translated many times to make proteins. Two key determinants of how much protein is made from an mRNA are its "lifespan" (how long it floats around in the cytosol) and how readily the translation machinery, such as the ribosome, can attach to it.

A recently discovered class of regulators, called **small regulatory RNAs**, can control mRNA lifespan and translation. Let's see how this works.

microRNAs

microRNAs (**miRNAs**) were among the first small regulatory RNAs to be discovered. A miRNA is first transcribed as a long RNA molecule, which forms base pairs with itself and folds over to make a hairpin.

Next, the hairpin is chopped up by enzymes, releasing a small double-stranded fragment of about 22 nucleotides. One of the strands in this fragment is the mature miRNA, which binds to a specific protein to make an RNA-protein complex.

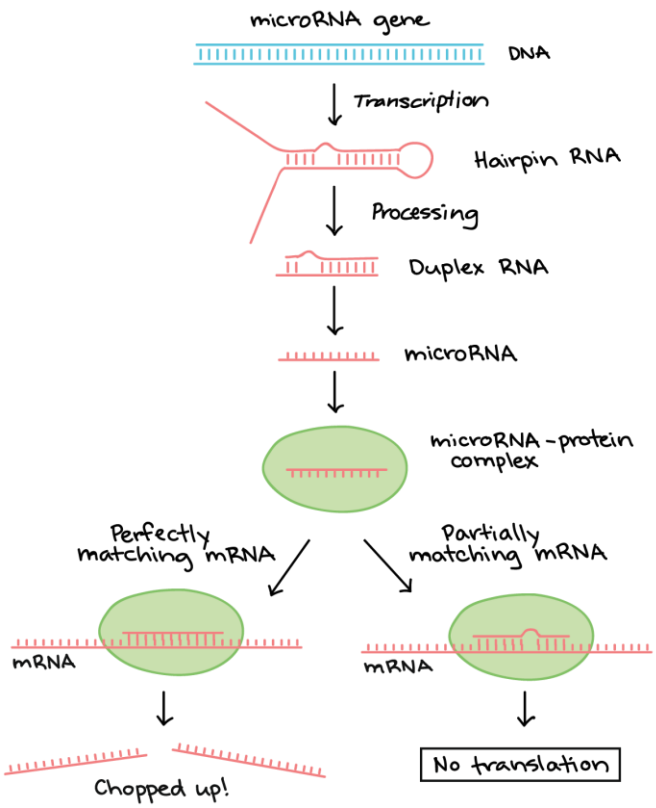

Diagram of where miRNAs come from and how they regulate targets.

(Diagram explanation: First, a microRNA precursor is transcribed from a microRNA gene. The precursor folds into a hairpin, which is then processed by enzymes so it is as short duplex (double-stranded) RNA that's imperfectly complementary. One strand of this duplex is the miRNA, which associates with a protein to form an miRNA-protein complex.

The miRNA directs the protein complex to mRNAs that are partially or fully complementary to the miRNA. When the miRNA is perfectly complementary to the mRNA, the mRNA is often cut in two by an enzyme in the protein complex. When the miRNA is not perfectly complementary to the mRNA, the miRNA-protein complex may remain bound to the mRNA and block translation.**)**

Image modifed from "miRNA biogenesis," by Narayanese, CC BY-SA 3.0. The modified image is licensed under a CC BY-SA 3.0 license

The miRNA directs the protein complex to "matching" mRNA molecules (ones that form base pairs with the miRNA). When the RNA-protein complex binds:

- If the miRNA and its target match perfectly, an enzyme in the RNA-protein complex will typically chop the mRNA in half, leading to its breakdown.
- If the miRNA and its target have some mismatches, the RNA-protein complex may instead bind to the mRNA and keep it from being translated.

These are not the only ways that miRNAs inhibit expression of their targets, and scientists are still investigating their many modes of action.

What do miRNAs actually do in organisms? Their direct role is to reduce the expression of their target genes, but they may play this role to produce many different outcomes.

For instance, in mice, a specific miRNA plays a key role in the development and function of the vascular (circulatory) system. Mice without function of this miRNA had defects in heart development and were unable to survive. Changes in expression levels of miRNAs are also associated with human diseases, including various types of cancer and cardiac hypertrophy.

Regulation of translation

We already saw how miRNAs can inhibit translation, but there are a number of other ways that translation of an mRNA can also be regulated in a cell. One key step for regulation is translation initiation.

In order for translation to begin, the ribosome, an RNA-and-protein complex that houses translation, must assemble on the mRNA. This process involves many “helper” proteins, which make sure the ribosome is correctly positioned. Translation can be regulated globally (for every mRNA in the cell) through changes in the availability or activity of the “helper” proteins.

For example, in order for translation to begin, a protein called eukaryotic initiation factor-2 (eIF-2) must bind to a part of the ribosome called the small subunit. Binding of eIF-2 is controlled by phosphorylation, or addition of a phosphate group to the protein.

When eIF-2 is phosphorylated, it's turned "off"—it undergoes a shape change and can no longer play its role in initiation, so translation cannot begin. When eIF-2 is not phosphorylated, in contrast, it's "on" and can carry out its role in initiation, allowing translation to proceed.

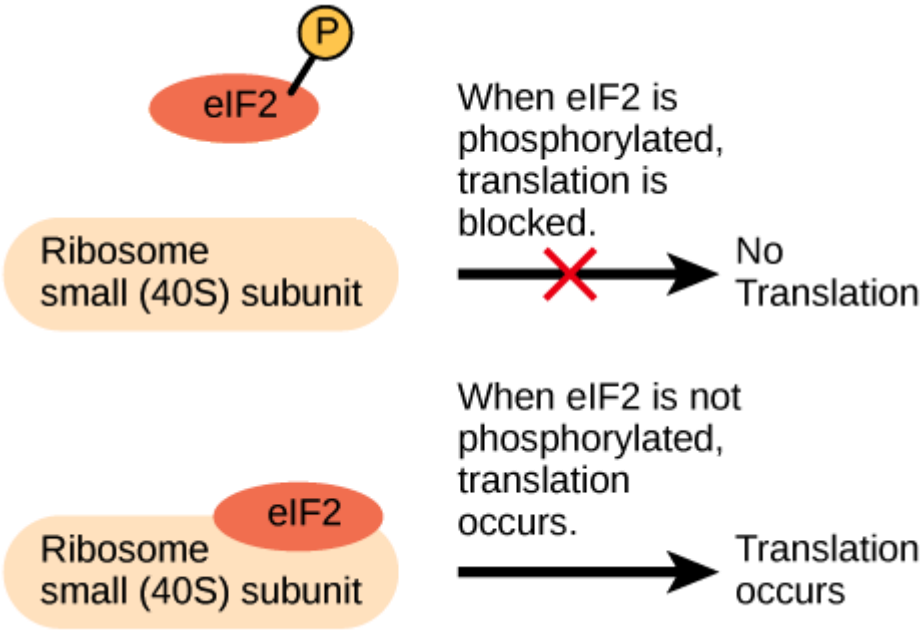

Image credit: "Eukaryotic translational and post-translational gene regulation," by OpenStax College, Biology, CC BY 4.0

In this way, phosphorylation of eIF-2 acts as a switch, turning translation on or off. Inactivation of translation can be a good strategy in periods when the cell can't "afford" to make new proteins (e.g., when the cell is starved for nutrients).

Proteins can be regulated after translation

There are also regulatory mechanisms that act on proteins that have already been made. In these cases, an "edit" to the protein – such as removal of amino acids, or addition of a chemical modification – can lead to a change in its activity or behavior. These processing and modification steps can be targets for regulation.

For example, some proteins must be proteolytically cleaved (chopped up) in order to become active. The insulin used by diabetics is one example. Other proteins may have chemical groups added to them, including methyl, phosphate, acetyl, and ubiquitin groups. Often, these groups can be added and removed dynamically to control activity.

Addition or removal of chemical groups may regulate protein activity or the length of time a protein remains in the cell before it undergoes "recycling." Sometimes, chemical modifications can also determine where a protein is found in the cell—for example, in the nucleus or cytoplasm, or attached to the plasma membrane.

Phosphorylation

One of the most common post-translational modifications is **phosphorylation**, in which a phosphate group is attached to a protein. The effect of phosphorylation varies from protein to protein: some are activated by phosphorylation, while others are deactivated, and others yet simply change their behavior (interacting with a different partner, or going to a different part of the cell).

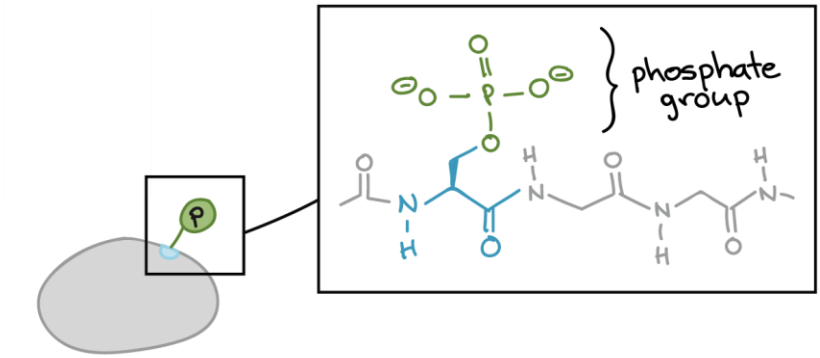

Image of a protein with a phosphate group attached, showing the chemical structure of the phosphate group, which bears a negative charge.

We saw one example of this above, when we examined how eIF-2 is inactivated by addition of a phosphate group (blocking translation). However, many different proteins can be selectively phosphorylated, producing various effects depending on the protein's role in the cell.

Ubiquitination

Proteins can be tagged for degradation by the addition of a chemical marker called **ubiquitin**. Ubiquitin-tagged proteins are taken to the proteasome, or "recycling center" of the cell, and broken down into their component parts. Ubiquitination is an important way of controlling the persistence of a protein in the cell.