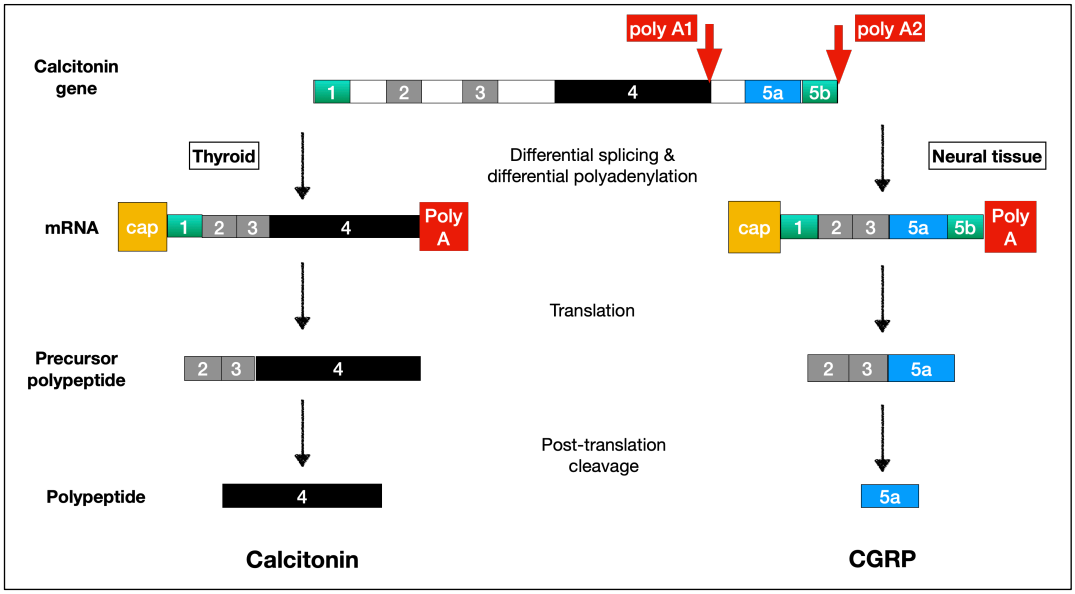
SECTION 4 POST-TRANSCRIPTIONAL CONTROL

ALTERNATIVE SPLICING AND POLYADENYLATION

Regulation of most genes occur at the transcription level. However, after transcription there are still various ways that gene expression is regulated. After DNA is transcribed, the primary transcript undergoes various processing reactions, including 5' capping, 3' cleavage/polyadenylation and RNA splicing to yield mature mRNA. Splicing involves the ligation of exons with the concomitant excision of introns. This mRNA is then transported from the nucleus to the cytoplasm. These steps constitute additional levels of gene control and together are called post-transcriptional control.

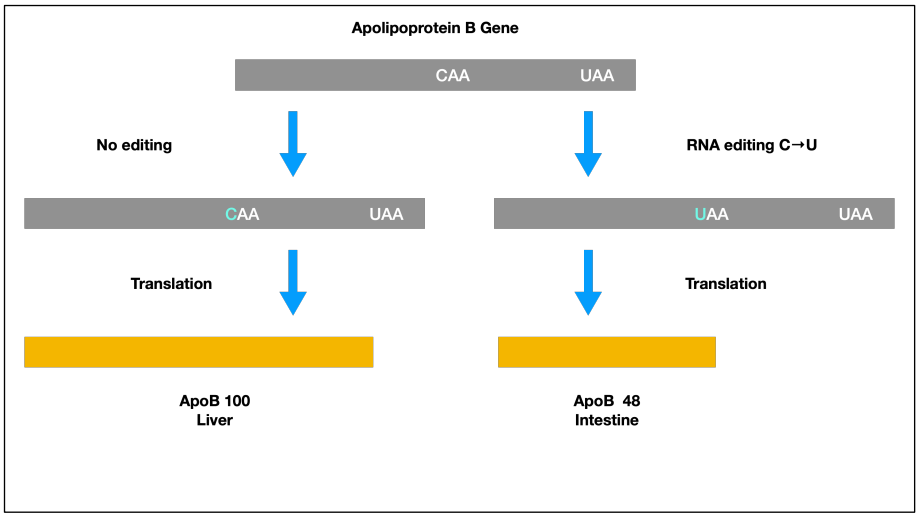
In higher eukaryotes, transcriptions may be simple or complex. In the simple type, the primary transcripts contain one poly(A) site and have only one pattern of RNA splicing. This means that they encode only a single mRNA. In the complex transcription unit, the primary transcripts produced may be processed in alternative ways, yielding different mRNA, which ultimately will produce different proteins. Which pathway the complex transcription units will take is cell-type specific. That means that one pattern will occur in one type of cell while another pattern occurs in a different cell.

An example of both alternate splicing and polyadenylation is the calcitonin gene. In the thyroid the calcitonin gene yields the hormone calcitonin. In neural tissue, the product is calcitonin gene-related peptide (CGRP).



RNA EDITING

RNA editing is a process where nucleotides are inserted, deleted or substituted. It is one more way that RNA is modified other than splicing. An example of editing is that which occurs in Apolipoprotein B. This protein exists in two isoforms; one in the liver (ApoB100) and one in the intestines (ApoB48). Both isoforms are coded by *APOB* gene and by a single mRNA transcript. By virtue of C→U editing, a premature stop codon is generated resulting in two proteins with the same N-terminal but with different lengths (ApoB100 is 4563 amino acids & ApoB48 is 2152) and with different C-terminals.



Another example of RNA editing involves a change from A→I in glutamate receptors. Glutamate is a neurotransmitter and its receptors are important for memory and learning. When glutamate binds to these receptors, ion channels open, causing initiation of nerve impulses. Some glutamate receptor channels conduct both Na^+ and Ca^{++} ions, whereas others only conduct Na^+. This difference is due to the A→I editing in one of the subunits, changing a CAG codon (glutamine) to a CIG codon (arginine). This change in the amino acid sequence of the receptor is enough to change the function of the ion channel.

RNA TRANSPORT

The movement of mRNA from the nucleus to the cytoplasm is not a random event. In fact, it is a tightly regulated process that requires active transport. This provides another avenue where regulation is demonstrated. The average primary transcript RNA is around ten times longer than the mature mRNA and only around 5% of RNA made in the nucleus leaves it to enter the cytoplasm. A big portion of RNA synthesized in the nucleus is either degraded there or is blocked from exit.

Newly formed RNA transcripts bind to a variety of proteins and the complexes are called heterogenous ribonucleoproteins (hnRNPs). Those that carry mature mRNA are called messenger RNPs (mRNPs). It is critical that immature RNA that have not undergone splicing not be transported from the nucleus, lest introns be included in translation. Pre-mRNA bound to small nuclear RNAs (snRNA) and assembled within spliceosomes (a complex molecule in the nucleus that removes introns from pre-mRNA) are prevented from being transported out of the nucleus. The mechanism for this is still not entirely understood.

Clinical Correlation. Thalassemia is an inherited disease where there is decreased hemoglobin production. This is due to mutations in the globin-gene splice sites. The pre-mRNAs do not undergo the proper splicing within the spliceosome and thus are not processed into mature mRNAs. This results in the unspliced globin pre-mRNAs being retained in the nucleus and subsequently being degraded. As a consequence, the affected globin is either not produced or produced at low levels.

RNA LOCALIZATION

The products of mRNA translation are diverse and are needed in various parts of the cell or even outside the cell as in the case of proteins that are exported from the cell. The translation of these mRNA does not take place just anywhere in the cytoplasm. They are tightly regulated as to where they are transported and where they undergo translation. As the mRNP leaves the nucleus, the proteins bound to them are exchanged for another set from the cytoplasm. The polyribosomes that these mRNA are associated with localize to different areas. Polyribosomes (with their mRNA) intimately associated with the rough endoplasmic reticulum synthesize proteins that are meant to be bound within vacuoles to be transported to the cell membrane or out of the cell. Actin microfilament mRNA and their polyribosomes associate with the cytoskeleton to be transported to where they are eventually going to be used to synthesize structural proteins. Translation does not proceed until mRNA reaches the location where it is meant to undergo this process.

mRNA STABILITY

The amount of mRNA for a particular gene product is determined not only by the amount transcribed but also how quickly or slowly they are degraded. While the activity of a particular transcription factor has a profound effect on how much a gene is transcribed, the stability of the mRNA of that gene also affects its ultimate expression. Unstable mRNAs have very short half-lives and are degraded within minutes while other mRNAs stay viable within the cytoplasm for hours or even up to a day. The implication of this is that proteins whose production should be shut down quickly have mRNAs with short half-lives and those that perform functions over a matter of hours or longer are more stable. One feature common in many short-lived mRNAs is

the presence of the sequence AUUUA in the 3’ untranslated region. How this results in destabilization is yet to be fully understood.

An example of this regulation is the translation of the protein transferrin, whose mRNA is subject to degradation in response to iron levels. In humans, ingested iron is carried in the blood by transferrin. The transferrin-iron complex enters the cell with the aid of the transferrin receptor (TfR) through receptor-mediated endocytosis. When iron in the cells is sufficient, there is an increase in the degradation rate of the TfR mRNA, which leads to a decrease in the level of this receptor. This ultimately leads to a decrease in the entry of the transferrin-iron complex into the cell. The reverse is true when iron levels are low; TfR mRNA is stabilized, increasing its half-life, leading to increased synthesis of the receptor protein.