

RNA Processing in Eukaryotes

mRNA Processing

Eukaryotic pre-mRNA receives a 5' cap and a 3' poly (A) tail before introns are removed and the mRNA is considered ready for translation.

LEARNING OBJECTIVES

Outline the steps of pre-mRNA processing

KEY TAKEAWAYS

Key Points

- A 7-methylguanosine cap is added to the 5' end of the pre-mRNA while elongation is still in progress. The 5' cap protects the nascent mRNA from degradation and assists in ribosome binding during translation.
- A poly (A) tail is added to the 3' end of the pre-mRNA once elongation is complete. The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation.
- Introns are removed from the pre-mRNA before the mRNA is exported to the cytoplasm.

Key Terms

- **intron:** a portion of a split gene that is included in pre-RNA transcripts but is removed during RNA processing and rapidly degraded
- **moiety:** a specific segment of a molecule
- **spliceosome:** a dynamic complex of RNA and protein subunits that removes introns from precursor mRNA

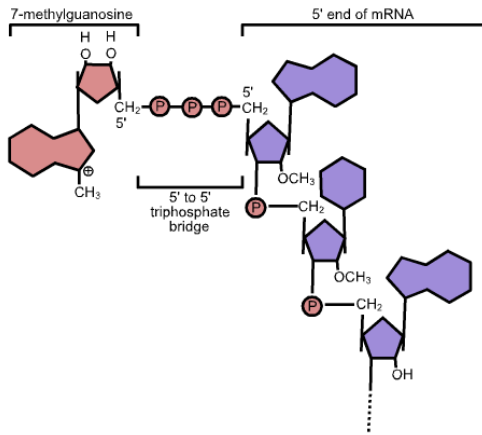
Pre-mRNA Processing

The eukaryotic pre-mRNA undergoes extensive processing before it is ready to be translated. The additional steps involved in eukaryotic mRNA maturation create a molecule with a much longer half-life than a prokaryotic mRNA. Eukaryotic mRNAs last for several hours, whereas the typical *E. coli* mRNA lasts no more than five seconds.

Pre-mRNAs are first coated in RNA-stabilizing proteins; these protect the pre-mRNA from degradation while it is processed and exported out of the nucleus. The three most important steps of pre-mRNA processing are the addition of stabilizing and signaling factors at the 5' and 3' ends of the molecule, and the removal of intervening sequences that do not specify the appropriate amino acids. In rare cases, the mRNA transcript can be "edited" after it is transcribed.

5' Capping

While the pre-mRNA is still being synthesized, a 7-methylguanosine cap is added to the 5' end of the growing transcript by a 5'-to-5' phosphate linkage. This moiety protects the nascent mRNA from degradation. In addition, initiation factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.



5' cap structure: Capping of the pre-mRNA involves the addition of 7-methylguanosine (m^7G) to the 5' end. The cap protects the 5' end of the primary RNA transcript from attack by ribonucleases and is recognized by eukaryotic initiation factors involved in assembling the ribosome on the mature mRNA prior to initiating translation.

3' Poly-A Tail

While RNA Polymerase II is still transcribing downstream of the proper end of a gene, the pre-mRNA is cleaved by an endonuclease-containing protein complex between an AAUAAA consensus sequence and a GU-rich sequence. This releases the functional pre-mRNA from the rest of the transcript, which is still attached to the RNA Polymerase. An enzyme called poly (A) polymerase (PAP) is part of the same protein complex that cleaves the pre-mRNA and it immediately adds a string of approximately 200 A nucleotides, called the poly (A) tail, to the 3' end of the just-cleaved pre-mRNA. The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation.



image

Poly (A) Polymerase adds a 3' poly (A) tail to the pre-mRNA.: The pre-mRNA is cleaved off the rest of the growing transcript before RNA Polymerase II has stopped transcribing. This cleavage is done by an endonuclease-containing protein complex that binds to an AAUAAA sequence upstream of the cleavage site and to a GU-rich sequence downstream of the cut site. Immediately after the cleavage, Poly (A) Polymerase (PAP), which is also part of the protein complex, catalyzes the addition of up to 200 A nucleotides to the 3' end of the just-cleaved pre-mRNA.

Pre-mRNA Splicing

Eukaryotic genes are composed of exons, which correspond to protein-

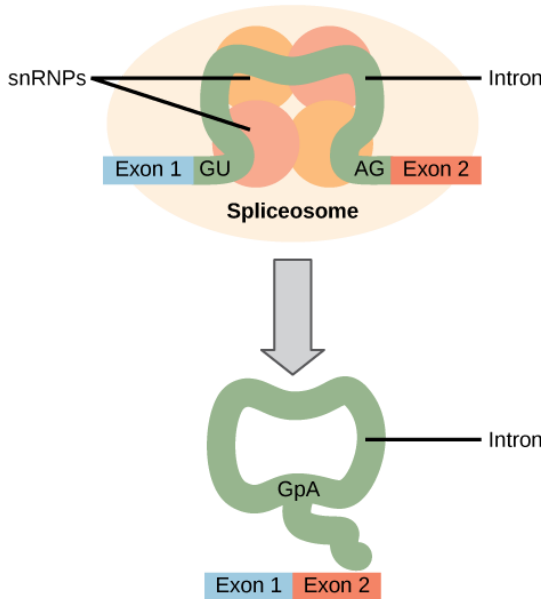
coding sequences (*ex-on* signifies that they are expressed), and intervening sequences called introns (*int-ron* denotes their *intervening* role), which may be involved in gene regulation, but are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins.

Discovery of Introns

The discovery of introns came as a surprise to researchers in the 1970s who expected that pre-mRNAs would specify protein sequences without further processing, as they had observed in prokaryotes. The genes of higher eukaryotes very often contain one or more introns. While these regions may correspond to regulatory sequences, the biological significance of having many introns or having very long introns in a gene is unclear. It is possible that introns slow down gene expression because it takes longer to transcribe pre-mRNAs with lots of introns. Alternatively, introns may be nonfunctional sequence remnants left over from the fusion of ancient genes throughout evolution. This is supported by the fact that separate exons often encode separate protein subunits or domains. For the most part, the sequences of introns can be mutated without ultimately affecting the protein product.

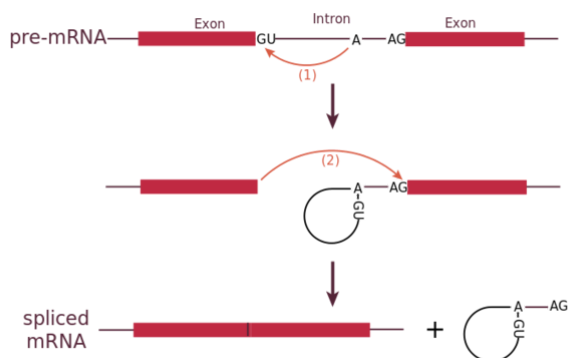
Intron Processing

All introns in a pre-mRNA must be completely and precisely removed before protein synthesis. If the process errs by even a single nucleotide, the reading frame of the rejoined exons would shift, and the resulting protein would be dysfunctional. The process of removing introns and re-connecting exons is called splicing. Introns are removed and degraded while the pre-mRNA is still in the nucleus. Splicing occurs by a sequence-specific mechanism that ensures introns will be removed and exons rejoined with the accuracy and precision of a single nucleotide. The splicing of pre-mRNAs is conducted by complexes of proteins and RNA molecules called spliceosomes.



Pre-mRNA splicing: Pre-mRNA splicing involves the precise removal of introns from the primary RNA transcript. The splicing process is catalyzed by large complexes called spliceosomes. Each spliceosome is composed of five subunits called snRNPs. The spliceosome's actions result in the splicing together of the two exons and the release of the intron in a lariat form.

Each spliceosome is composed of five subunits called snRNPs (for small nuclear ribonucleoproteins, and pronounced “snurps”.) Each snRNP is itself a complex of proteins and a special type of RNA found only in the nucleus called snRNAs (small nuclear RNAs). Spliceosomes recognize sequences at the 5' end of the intron because introns always start with the nucleotides GU and they recognize sequences at the 3' end of the intron because they always end with the nucleotides AG. The spliceosome cleaves the pre-mRNA's sugar phosphate backbone at the G that starts the intron and then covalently attaches that G to an internal A nucleotide within the intron. Then the spliceosome connects the 3' end of the first exon to the 5' end of the following exon, cleaving the 3' end of the intron in the process. This results in the splicing together of the two exons and the release of the intron in a lariat form.



Mechanism of pre-mRNA splicing: The snRNPs of the spliceosome were left out of this figure, but it shows the sites within the intron whose interactions are catalyzed by the spliceosome. Initially, the conserved G which starts an intron is cleaved from the 3' end of the exon upstream to it and the G is covalently attached to an internal A within the intron. Then the 3' end of the just-released exon is joined to the 5' end of the next exon, cleaving the bond that attaches the 3' end of the intron to its adjacent exon. This both joins the two exons and removes the intron in lariat form.

Processing of tRNAs and rRNAs

rRNA and tRNA are structural molecules that aid in protein synthesis but are not themselves translated into protein.

LEARNING OBJECTIVES

Describe how pre-rRNAs and pre-tRNAs are processed into mature rRNAs and tRNAs.

KEY TAKEAWAYS

Key Points

- Ribosomal RNA (rRNA) is a structural molecule that makes up over half of the mass of a ribosome and aids in protein synthesis.
- Transfer RNA (tRNA) recognizes a codon on mRNA and brings the appropriate amino acid to that site.
- rRNAs are processed from larger pre-rRNAs by trimming the larger rRNAs down and methylating some of the nucleotides.
- tRNAs are processed from pre-tRNAs by trimming both ends of the pre-tRNA, adding a CCA trinucleotide to the 3' end, if needed, removing any introns present, and chemically modified 12 nucleotides on average per tRNA.

Key Terms

- **anticodon:** a sequence of three nucleotides in transfer RNA that binds to the complementary triplet (codon) in messenger RNA, specifying an amino acid during protein synthesis

Processing of tRNAs and rRNAs

The tRNAs and rRNAs are structural molecules that have roles in protein synthesis; however, these RNAs are not themselves translated. In eukaryotes, pre-rRNAs are transcribed, processed, and assembled into ribosomes in the nucleolus, while pre-tRNAs are transcribed and processed in the nucleus and then released into the cytoplasm where they are linked to free amino acids for protein synthesis.

Ribosomal RNA (rRNA)

The four rRNAs in eukaryotes are first transcribed as two long precursor molecules. One contains just the pre-rRNA that will be processed into the 5S rRNA; the other spans the 28S, 5.8S, and 18S rRNAs. Enzymes

then cleave the precursors into subunits corresponding to each rRNA. In bacteria, there are only three rRNAs and all are transcribed in one long precursor molecule that is cleaved into the individual rRNAs. Some of the bases of pre-rRNAs are methylated for added stability. Mature rRNAs make up 50-60% of each ribosome. Some of a ribosome's RNA molecules are purely structural, whereas others have catalytic or binding activities.

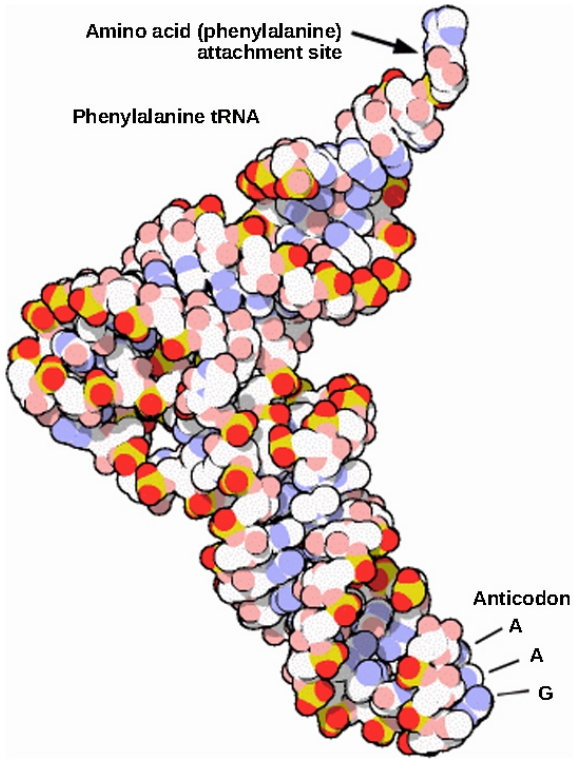
The eukaryotic ribosome is composed of two subunits: a large subunit (60S) and a small subunit (40S). The 60S subunit is composed of the 28S rRNA, 5.8S rRNA, 5S rRNA, and 50 proteins. The 40S subunit is composed of the 18S rRNA and 33 proteins. The bacterial ribosome is composed of two similar subunits, with slightly different components. The bacterial large subunit is called the 50S subunit and is composed of the 23S rRNA, 5S rRNA, and 31 proteins, while the bacterial small subunit is called the 30S subunit and is composed of the 16S rRNA and 21 proteins.

The two subunits join to constitute a functioning ribosome that is capable of creating proteins.

Transfer RNA (tRNA)

Each different tRNA binds to a specific amino acid and transfers it to the ribosome. Mature tRNAs take on a three-dimensional structure through intramolecular basepairing to position the amino acid binding site at one end and the anticodon in an unbasepaired loop of nucleotides at the other end. The anticodon is a three-nucleotide sequence, unique to each different tRNA, that interacts with a messenger RNA (mRNA) codon through complementary base pairing.

There are different tRNAs for the 21 different amino acids. Most amino acids can be carried by more than one tRNA.



Structure of tRNA: This is a space-filling model of a tRNA molecule that adds the amino acid phenylalanine to a growing polypeptide chain. The anticodon AAG binds the codon UUC on the mRNA. The amino acid phenylalanine is attached to the other end of the tRNA.

In all organisms, tRNAs are transcribed in a pre-tRNA form that requires multiple processing steps before the mature tRNA is ready for use in translation. In bacteria, multiple tRNAs are often transcribed as a single RNA. The first step in their processing is the digestion of the RNA to release individual pre-tRNAs. In archaea and eukaryotes, each pre-tRNA is transcribed as a separate transcript.

The processing to convert the pre-tRNA to a mature tRNA involves five steps.

1. The 5' end of the pre-tRNA, called the 5' leader sequence, is cleaved off.

2. The 3' end of the pre-tRNA is cleaved off.

3. In all eukaryote pre-tRNAs, but in only some bacterial and archaeal pre-tRNAs, a CCA sequence of nucleotides is added to the 3' end of the pre-tRNA after the original 3' end is trimmed off. Some bacteria and archaea pre-tRNAs already have the CCA encoded in their transcript immediately upstream of the 3' cleavage site, so they don't need to add one. The CCA at the 3' end of the mature tRNA will be the site at which the tRNA's amino acid will be added.

4. Multiple nucleotides in the pre-tRNA are chemically modified, altering their nitrogen bases. On average about 12 nucleotides are modified per tRNA. The most common modifications are the conversion of adenine (A) to pseudouridine (ψ), the conversion of adenine to inosine (I), and the conversion of uridine to dihydrouridine (D). But over 100 other modifications can occur.

5. A significant number of eukaryotic and archaeal pre-tRNAs have introns that have to be spliced out. Introns are rarer in bacterial pre-tRNAs, but do occur occasionally and are spliced out.

After processing, the mature pre-tRNA is ready to have its cognate amino acid attached. The cognate amino acid for a tRNA is the one specified by its anticodon. Attaching this amino acid is called charging the tRNA. In eukaryotes, the mature tRNA is generated in the nucleus, and then exported to the cytoplasm for charging.



image

Processing of a pre-tRNA.: A typical pre-tRNA undergoing processing steps to generate a mature tRNA ready to have its cognate amino acid attached. Nucleotides that are cleaved away are shown in green. Chemically-modified nucleotides are in yellow, as is the CAA trinucleotide that is added to the 3' end of the pre-tRNA during processing. The anticodon nucleotides are shown in a lighter shade of red.

[Previous](#)

[Next](#)



Privacy Policy