

5' capping

Occurs very soon after RNA Pol II starts making the transcript

The 5' end is chemically modified by the addition of a **7-METHYLGUANOSINE RESIDUE**

This is called a **CAP** and occurs by the addition of a GMP nucleotide in the **REVERSE ORIENTATION** compared with normal 3' > 5' linkage

Cap forms a **BARRIER** to **5'-EXONUCLEASES** and thus **STABILISES** the TRANSCRIPT

Gives a 5'-5' **TRIPHOSPHATE BRIDGE**

Carried out by mRNA **GUANYLTRANSFERASE**

It is also important in other reactions of the pre-mRNA

Uncapped don't bind the ribosome so well

Probably also helps ribosome binding

Polyadenylation

In most mature mRNAs, the 3' end carries a "poly-A tail" - 200 nt long

Generated in two steps

This occurs at the **POLYADENYLATION SITE**

Consists of two signals

First, the 3' end is cleaved

Then, **POLY(A) POLYMERASE** adds up to 250 A residues to the 3' end of the cleaved mRNA

Thought to stabilise mRNA, since a poly(A)-binding protein binds to it which should act to resist 3'-endonuclease action

May also help with translation of mature mRNA in cytoplasm

Uses

RNA editing

Additional nucleotides are inserted into specific positions of the mRNA molecule

Requires a **GUIDE RNA** to specify what to include where

Neurofibromatosis is caused by a defect in this mechanism

The evolutionary rationale for RNA editing is not clear

Rather rare

RNA cleavage

The example, the rRNA genes transcribed by Pol I

Methylation

Certain bases are methylated - function unknown

Post-transcriptional modification of mRNA transcripts in eukaryotes

Introduction

In Euks, transcription and translation are spatially separated by the nuclear membrane

This means that the RNA can be extensively modified before translation

Pol II transcribes a huge variety of genes - the resulting RNA is referred to as **HETEROGENEOUS NUCLEAR RNA (hnRNA)**

Those that will eventually be processed to mRNA are called **pre-mRNAs**

This rapidly becomes coated with proteins to form **HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN (hnRNP)**

Various are known (A-U), and they are thought to help keep the RNA in single-stranded form and assist in the various RNA processing reactions

Pol II also transcribes most **snRNAs** (small nuclear RNA) which complex with specific proteins to form **snRNPs**

These are processed by a series of reactions in the nucleus and cytoplasm

They then return to the nucleus and are involved in **SPLICING** and **METHYLATING** tRNA

The phosphorylated carboxy-terminal domain (CTD) of RNA Pol II is involved in recruiting various factors that carry out **capping, polyadenylation and splicing**

It helps co-ordinate pre-mRNA processing events

Splicing

INTRONS are stretches of DNA that interrupt the coding sequence, but which are spliced out of hnRNA before translation

They can readily be observed through EM analysis of DNA/mRNA hybrids - the mRNA only binds to a small bit of the DNA

Almost always "5' - GU - 3'" at the 5' end

Often "5' - AG - 3'"

The AG at the 3' end is preceded by a pyrimidine-rich sequence called the **POLYPYRIMIDINE TRACT**

About 10-40 residues upstream of this is a conserved sequence called the **BRANCHPOINT SEQUENCE**, including a conserved "A"

This is what allows the cell to know where to splice

Allows snRNA to instruct the cell to splice

The 2' -OH of the "A" in the branch site attacks the 3' phosphate of the 5' exon

This creates a 3' -OH of the leaving group (the exon) and a tailed circular molecule called the **LARIAT**

The 3' -OH on exon 1 carries out a second nucleophilic attack on the 5' phosphate of exon 2 - the lariat leaves, and is eventually degraded

Splicing takes place in two steps

The RNA components of the snRNPs form base pairs with various conserved sequences at the 5' and 3' splice sites and the branching point

They then help keep the pre-mRNA in the correct orientation for splicing (eg: keeping the exons close to each other)

The whole complex is called a **SPLICEOSOME**

The process depends on snRNPs as well as other splicing factors

There is also a minor class of introns with different conserved sequences that are spliced by another type of spliceosome

What are introns? Why are they there?

To enlarge the **TRANSCRIPTOME**

A single gene can encode several different **ISOFORMS** of the same protein

Instead of having different genes in different tissue, every tissue has the same gene, but spliced differently to suit its needs

This is called **ALTERNATIVE SPLICING**

Each cell must have a certain tissue-specific protein for the correct splicing

They **promote evolutionary diversity**

Protein domains separated by introns and much higher chance of recombination

Some exons correspond to functional units of proteins and may allow a greater degree of "evolutionary mix-n-match"

SELFISH DNA

Introns are bits of DNA that have been picked up over time

Since they are essentially functionless, there is little selection pressure to lose them