MCB  Chapter 11

Topic D

Post-Transcriptional Controls

Reading : 404-443
Post-Transcriptional Controls

All processes following transcription initiation

• Elongation - till the end
• Transport to cytoplasm
• Stability of mRNA
• Cellular localization

All added to the Regulation of gene expression
Transcription termination

• Several mechanisms in bacteria and eukaryotic cells

• **In bacteria** - two principle mechanisms involve RNA polymerase
  Requires the termination factor *Rho*
  *Rho* independent

• **In eukaryotes**, the mechanisms for terminating transcription differ for each of the 3 types of RNA polymerase
  
  Pol - I (pre-rRNA)
  Pol - II
  Pol - III (tRNA & 5S-rRNA)
Rho-independent termination occurs at characteristic sequences in *E. coli* DNA

1. Base-pairing A=U is low
2. Strong Stem-Loop
Premature termination by attenuation helps regulate expression of some bacterial operons

Attenuation - a mechanism that balances the elongation/termination of transcription

Figure 11-2
Mutations in the attenuator - leads to excess of tryptophan biosynthesis transcripts

How??

Figure 11-2
Mechanism of attenuation of \textit{trp}-operon transcription - a Rho independent

1. Alternative Base-pairing

2. Depend on the rate ribosomal translation of leader
3. This depend on tRNA-\textit{trp}

\(\text{trp}\)
A Rho independent mechanism of attenuation valid for Phe, His, Ile, Leu & Val

• The leader seq includes the relevant aa

• The leader seq is rapidly degraded after translation

• For attenuation in different cases - RNA binding proteins that stabilize base-pairing are essential.

E. coli bgl operon (for glucose containing polysacharadies)

RNA binding protein- stabilize a non-attenuated stem-loop.
The protein is activated by glucose phosphorylation
Thus, in the presence of glucose - bgl operon is functioning.
Rho-dependent termination sites are present in some $\lambda$-phage and *E. coli* genes

Rho was discovered after $\lambda$-phage infection, **How?**

- The Rho factor is a hexameric protein around which a 70- to 80-base segment of the growing RNA transcript wraps

- Rho then moves along the RNA in the 3′ direction until it eventually unwinds the RNA-DNA hybrid at the active site of RNA polymerase

- Whether transcription is terminated or not depends on whether Rho “catches up” to RNA polymerase

- Rho-dependent sites have no clear consensus sequence and Rho-dependent termination operates at relatively few operons
Anti-terminator by $\lambda$-phage N + E. coli proteins

If enough N- protein in the system..

If Nut seq (N-utilization)

If Nus proteins

Overrides other terminators
Three eukaryotic RNA polymerases employ different termination mechanisms

- **RNA polymerase I** is terminated by a mechanism that requires a polymerase-specific termination factor, which binds downstream of the transcription unit (A DNA-binding protein not a RNA binding as Rho)

- **RNA polymerase II** is terminated in a region 0.5-2 kb beyond the poly(A) addition site, and termination is coupled to the process that cleaves and polyadenylates the 3′ end of a transcript

- **RNA polymerase III** is terminated after polymerizing a series of U residues (no stem-loop is requested)
Transcription of HIV genome is regulated by an anti-termination mechanism.

 Unexpected similarity to λ-phage (Spt5 similar to NusG)

 Kinase - the substrate CTD of pol II

 TAR - RNA Copy sequence for Tat (HIV)
Eukaryotic RNA -pol II transcription termination

HIV example

**Drosophila HSP** (heat shock)
- polII pause but stay attached
- HS activates HSTF that relief pol II from pausing

Rapid response! No assembly time is wasted
Processing of eukaryotic mRNA
The 5′-cap is added to nascent RNAs after initiation by RNA polymerase II

~25 nt

7-methylguanosine

5′-5′ link

Dimeric capping enzyme - associated with CTD of pol II (only)

Methylation also on the ribose of the 1st (and) 2nd base
MOLECULAR PROCESS  POSSIBLE REGULATION

PRE-mRNA TRANSCRIPTION

Cotranscriptional RNA splicing and cleavage/polyadenylation

RNA editing (rare)

Nucleus

Regulation of alternative RNA splicing and cleavage/polyadenylation

Degradation of improperly processed RNA

Cell-type-specific RNA editing (rare)

Regulation of export (rare)

Nuclear export

Cytosol

Cytoplasmic localization (rare)

Translation initiation

Decapping and mRNA decay

Regulation of cytosolic localization

Regulation of translation initiation

Regulation of mRNA decay

mRNA decay

AMOUNT OF SPECIFIC PROTEIN PRODUCED
Pre-mRNA are associated with hnRNP proteins
Identifying hnRNP proteins

UV high dose - cross linking
Poly dT column from nuclear extract

Many proteins 30-120,000 daltons

Several proteins are alternatively spliced

Each binds to a ‘preferred’ site (I.e. 3’ of introns)

Mostly modular structure

RNP motif (=RBD) - most common
Many Arginine (as for N and Tat)

KH domain (45 aa), in Fragile X- gene (FMR-1)
hnRNP proteins may assist in processing and transport of mRNAs

A ‘unified’ mechanism for processing
IF hnRNAs are attached to hnRNP proteins
Pre-mRNAs are cleaved at specific 3′ sites and rapidly polyadenylated

All (but histones) have 3′-poly A

The ‘extra’ 3′ transcript very rapidly degraded

What are the signals for endonuclease?

5′- AAUAAA -3′ (10-35 nt upstream)
5′- AUUAAA -3′

If mutated -rapidly degraded
Pre-mRNAs are cleaved at specific 3′ sites and rapidly polyadenylated

**CPSF** - Cleavage and Polyadenylation Specificity factor

**CStF** - Cleavage Stimulatory factor

**CFI** - Cleavage factor I

**PAP** - poly (A) Polymerase

**Coupled - cleave + poly A**
Slow polyadenylation
Rapid polyadenylation

12 (A)n slow
200-250 (A)n fast
Pre-mRNAs are cleaved at specific 3′ sites and rapidly polyadenylated

PABII (in nuclei)

Signal for PAP to Add (A) and to stop
During the final step in formation of mature, functional mRNA, introns are removed and exons are spliced together.
Splicing occurs at short, conserved sequences

Consensus sequences around 5′ and 3′ splice sites in vertebrate pre-mRNA

How to determine the borders??
Genomic - cDNA   ESTs...

Fusion construct with half introns from genes - perfect product
Splicing mechanism
splicing type I, II, tRNA and mRNA

Primary transcript

Most dramatic processing -mRNA (euk) tRNA (euk+pro)

The introns -1977 Philip Sharp Richard Roberts

Exons  <1000 nt (ave. 100-200 nt)

Intron  up to 20,000 and more, some are 60 only
Splicing mechanism
splicing type I, II, tRNA and mRNA

**Type 1** - Pre-rRNA
Mitochondria, chloroplast tRNA, rRNA, mRNA

Use GMP for ‘creating OH end’
No energy (ie ATP)
Ligation

**Type 2** - mitochondria mRNA -
Fungi, algae, plant

Also rare in bacteria
Self-splicing group I introns were the first examples of catalytic RNA
All pre-tRNAs undergo cleavage and base modification

Figure 11-52
Splicing of pre-tRNAs differs from other splicing mechanisms

2-3 cyclic nucleotide phosphodiesterase
The splicing mechanisms type I, II

The introns are Self splicing!! T. Cech, 1982

No proteins are involved

Isolated DNA of protozoa - with intron + RNAp (bacteria) resulted in spliced RNA

In mRNA eukaryotes- with the aid of RNA-protein Complex - small nuclear ribonucleoproteins (snRNPs)
Splicing proceeds via two sequential transesterification reactions
Excised lariat intron

Second transesterification

Spliced exons

\[ O = 3' \text{oxygen of exon 1} \]
\[ O = 2' \text{oxygen of branch-point A} \]
\[ O = 3' \text{oxygen of intron} \]
Analysis of RNA products formed in an in vitro splicing reaction

Figure 11-15

Lariat Structure

Branch point
Small nuclear RNAs (snRNAs) assist in the splicing reaction

(a) [Diagram of splicing reaction with U1 and U2 snRNAs]

(b) [Comparison of wild-type and mutant pre-mRNA structures]

Mutation in pre-mRNA 5’ splice site blocks splicing

Compensatory mutation in U1 restores splicing
Diagram showing a gene structure with exons and introns. The diagram highlights conserved regions where splicing occurs. The 5' and 3' splice sites are indicated with arrows pointing to the intron.
The spliceosomal splicing cycle