MCB        Chapter 11

Topic E

Splicing mechanism
Nuclear Transport
Alternative control modes

Reading : 419-449
Self-splicing group I introns were the first examples of catalytic RNA

Figure 11-51

A with 3 phosphoester bond
Small nuclear RNAs (snRNAs) assist in the splicing reaction

(a)

(b)

Mutation in pre-mRNA 5’ splice site blocks splicing

Compensatory mutation in U1 restores splicing
The spliceosomal splicing cycle
First transesterification
Second transesterification

Lariat intron + U2, U5, U6

Spliced exons
Spliced exons + Lariat intron + U2, U5, U6 → Debranching enzyme → Linear intron RNA

- Spliced exons
- Lariat intron
- U2, U5, U6
- Debranching enzyme
- Linear intron RNA
<table>
<thead>
<tr>
<th>Step (Name)</th>
<th>Event</th>
<th>Energy?</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (commitment complex)</td>
<td>U1 joins with the pre-mRNA (the splicing substrate), attaching at the 5' intron/exon boundary.</td>
<td>None required</td>
</tr>
<tr>
<td>A (A complex)</td>
<td>U2 binds to the complex at the branch point A</td>
<td>ATP</td>
</tr>
<tr>
<td>B1 (B1 complex)</td>
<td>The U4/U6 complex, along with U5 binds.</td>
<td>None required</td>
</tr>
<tr>
<td>B2 (B2 Complex)</td>
<td>U4 dissociates, allowing U6 to base pair with the snRNA in U2</td>
<td>None required</td>
</tr>
<tr>
<td>C1 (C1 complex)</td>
<td>Step one of the splicing mechanism (separation at the 5' exon/intron boundary and formation of the lariat).</td>
<td>ATP</td>
</tr>
<tr>
<td>C2 (C2 complex)</td>
<td>Step two of the splicing mechanism (joining of the exons and excision of the lariat structure intron).</td>
<td>ATP</td>
</tr>
<tr>
<td>I (I complex)</td>
<td>Joined exons dissociate from complex, leaving the lariat structure behind.</td>
<td>None required</td>
</tr>
<tr>
<td>(completion)</td>
<td>Spliceosome dissociates – snRNPs recycle – intron (lariat structure broken into monomers.</td>
<td>None required</td>
</tr>
</tbody>
</table>
Small nuclear RNAs (snRNAs) assist in the splicing reaction

Exchanging Base pairing of snRNA

All snRNPs - 5 RNA, 50 proteins = spliceosome

Assembly - require ATP (not the cleavage-ligation steps)
Cross exons - the basis for alternative splicing use of weak ‘leaky’ splicing sites...
Non standard GU-AG consensus

100 proteins are involved
Some like RNA helicases
The spliceosome is a ribonucleoprotein complex composed of multiple snRNPs
Trans-splicing
splicing and ligation of non-continuous RNAs

About 10% in C. elegans
Found in metazoa (trypanosomes)

140nt leader RNA found upstream to tandem repeat genes
39nt (mini exon) from it spliced to the 5’ of the gene
This leads to efficient translation
This leads to cleavage and poly A addition (at 3’ of exon)

Combination of polycistronic prokaryotic organization and eukaryotic mRNA
Self-splicing group II introns provide clues to the evolution of snRNPs

Figure 11-20
Self-splicing in group II introns

Highly structured (stem loops..)
Analogy to snRNA function in spliceosome
In vivo -self splicing is supported by maturases (stabilizing?)

Once CUT some domains and ADDED as ‘parts’
Self splicing resumes.

**RNA act on peptide bond formation on the ribosome**
Poly dT

DNA (blue)

Sc-35
Nuclear matrix
Main sub-topics
1. Termination control
2. hnRNA to mRNA
3. Capping,
4. hnRNPs
5. mechanism polyA/cleavage..
6. Splicing, snRNPs, mechanism
7. Group I, II, tRNA, nuclear mRNA
8. Self splicing
9. Nuclear matrix

Regulation of mRNA processing
Simple transcript

Complex transcript
Simple/complex transcriptional units

Complex transcriptional units are abundant (>25%, Multi-Cellular...)

Controlled at the level of poly A addition (few cases)

Example: U1A protein (in U1 snRNP) ‘clever’ design by which level of the pre-mRNA is regulated by the U1A protein binding (= a RNA binding protein) no poly A addition...
Tissue-specific RNA splicing controls expression of alternative fibronectins

And many more beautiful examples..

Figure 11-24
The sex determination pattern of Drosophila - regulation by RNA splicing

3 genes that are involved
Sxl (sex lethal)
tra (transformer)
dsx (double sex)

Each of these genes produces a pre-mRNA that has 2 possible splicing patterns, depending upon whether the fly is male (XY) or female (XX)

Timing for the expression of Sxl (Early), tra (late)
The sex determination pattern of Drosophila
Expression of Sex-lethal (Sxl) protein during *Drosophila* embryogenesis

**Figure 11-25**
(a) *sxl*

![Diagram of *sxl* pre-mRNA processing](image)

(b) *tra*

![Diagram of *tra* pre-mRNA processing](image)

(c) *dsx*

![Diagram of *dsx* pre-mRNA processing](image)

Rbp1 + Tra2

- [Female Sxl protein](image)
- [Female Tra protein](image)
- [Female Dsx protein](image)
- [Male Dsx protein](image)
The sex determination pattern of Drosophila

The inclusion of two exons (#3 in Sxl and #2 in tra) produces, in the case of the male mRNAs, messengers that have stop codons = inactive proteins.

The only active male product is the protein translated from dsx, which in turn inactivates all female -specific genes.
Functional variations by Alternative Splicing

Where??

Why??

How many??

In brain nerve cells developing brain, following learning, experience...

**Example:** The inner ear (hair cell)
Functional variations by Alternative Splicing

Ca level opens K channel

Slo gene
8 points
576 combinations
To and From the Nucleus

Signal-Mediated Transport through Nuclear Pore

Reading 426-436
To and From the Nucleus

Signal -Mediated Transport through Nuclear Pore

Reading 426-436
To and From the Nucleus

50-100 proteins
To and From the Nucleus

50-100 proteins

Ions and small molecules passively pass the ~9A pore

RNPs are large -~25 nm..

The key - Selectivity in transport

Experiment:
Gold coated with nuclear/cytosolic proteins
Model for passage of mRNPs through nuclear pore complexes
Human hnRNP A1 protein can cycle in and out of the cytoplasm but human hnRNP protein C cannot.
hnRNP with NES (nuclear export signal)

Several types:
- hnRNP A1 -38 nt signal
- hnRNP K
- PKI (inhibitor of PK)
- REV of HIV
A model for the export of nuclear cargo proteins bearing a leucine-rich nuclear-export signal (NES)

Figure 11-33

RCC (GEF)  
In nuclei

Ran GAP  
In cytosol

Figure 11-33
A model for hnRNP-mediated export of mRNAs from the nucleus
A model for the import of cytosolic cargo proteins bearing a basic NLS

Histons
Dna polymerases
RNA polymerase
TFs
Splicing proteins...

Must move to nuclei efficiently

mode **NLS**
Shuttle proteins -importins have NES,NLS
Proteins with a nuclear-localization signal (NLS) are recognized by receptors and transported into the nucleus.

**T-antigen**

**PKKKRKV**

Fused to Pyruvate Kinase

*Figure 11-35*
A model for the import of cytosolic cargo proteins bearing a basic NLS

Addition protein NTF2 (cytosol) ensures efficiency
HIV Rev protein regulates the transport of unspliced viral mRNAs

A mode that overcomes the block in exporting Un-spliced RNA (via NES related mechanism)
Other mechanisms for Post Transcriptional control

a. RNA editing
b. mRNA localization
c. Life time
d. Translation mRNA
e. Natural Anti-sense
f. RNA interference

yet unknown..

A. **RNA editing** - mostly in mitochondrial chloroplasts,
a. **RNA editing** alters the sequences of pre-mRNAs

A mammalian example

Information in the INTRON determine the RNA editing
a. RNA editing in protozoans

Editing in the kinetoplast of trypanosomes

Molecular fossil ??

Figure 11-40
b. Some mRNAs are associated with cytoplasmic structures or localized to specific regions.

The 3’ untranslated region of actin mRNA directs localization of actin transcripts.

Figure 11-41: Localization of actin transcripts.
Localization of Ash1 mRNA to the bud tip in yeast

Actin & Tubulin based mobility

Figure 11-42
c. The **stability** of cytoplasmic mRNAs varies

<table>
<thead>
<tr>
<th>Cell</th>
<th>Cell Generation Time</th>
<th>mRNA Half-Lives*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>20–60 min</td>
<td>3–5 min</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> (yeast)</td>
<td>3 h</td>
<td>22 min</td>
</tr>
<tr>
<td>Cultured human or rodent cells</td>
<td>16–24 h</td>
<td>10 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range Known for Individual Cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2–10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4–40 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min or less (histone and c-myc mRNAs)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3–24 h (specific mRNAs of cultured cells)</td>
</tr>
</tbody>
</table>


Specific sequences in 3’tail (AU rich)-shorten life time
The destabilizing effect of AUUUA sequences on mRNA half-life ($t_{1/2}$)

$\beta$-globin gene

$t_{1/2} \approx > 10 \text{ h}$

3' GMCSF sequence with AUUUA repeat

$t_{1/2} \approx 1-2 \text{ h}$

3' GMCSF sequence with G/C residues substituted for A/U residues

$t_{1/2} \approx 10 \text{ h}$
The degradation rate of some eukaryotic mRNAs is regulated

Iron-dependent regulation of the stability of transferrin-receptor mRNA
d. **Translation** of some mRNAs is regulated by specific RNA-binding proteins

Iron-dependent regulation of translation of ferritin mRNA

![Diagram of iron-dependent regulation of ferritin mRNA](image-url)
e. **Antisense** RNA regulates translation of transposase mRNA in bacteria

Figure 11-46
f.si-RNA small interference RNA
The end

Or just the beginning
Thank you