RNA Processing and Export

Pre-mRNA sequences involved in intron Recognition (mammals)
The spliceosome contains 5 small nuclear RNAs: U1, U2, U4, U5, and U6

The snRNAs:
1. Adopt defined secondary and tertiary structures
2. Are intimately involved in recognition of splice sites (through base-pairing interactions)
3. Appear to carry out the actual catalysis of splicing
4. Undergo dramatic, ATP-dependent structural rearrangements (mediated by specific proteins)
5. Are associated with proteins to form ribonucleoprotein complexes (snRNPs--small nuclear ribonucleoproteins)

The spliceosome contains over a hundred protein components:

Some properties of proteins associated with the spliceosome:
1. ATP-dependent RNA “unwindases” (DEAD-box like)
2. RNA binding proteins (eg. RRM, RGG, KH)
3. Proteins that bind to other proteins
Spliceosome Assembly

2. U1 and U4 are released (or become weakly associated with the spliceosome (ATP-dependent), leaving U2, U6, and U5. U2-U6-pre-mRNA form the catalytic center.
3. First transesterification step. A 2'-5' bond is generated to form a "lariat" intermediate.
4. After multiple ATP-dependent rearrangements of the snRNPs, the second transesterification step occurs. The resulting products are the spliced exons and the lariat intermediate. The snRNPs are then recycled for the next splicing events.
5. The lariat intron is "debranched" and the linear intron is degraded by exonucleases.

Exon recognition through cooperative binding of SR proteins and splicing factors to the pre-mRNA

Eukaryotic genes can have multiple introns
**The CTD of RNA polymerase coordinates transcription and splicing**

A. Splicing factors bind to the CTD

- The CTD is comprised of repeats of the sequence YS2PTS2PS (52 on the mammalian polymerase II).
- The CTD is phosphorylated on serines 2 and 5 during the transition from initiation to elongation.
- Splicing factors (and other processing factors) bind to the phosphorylated CTD.

B. The structure of RNA polymerase and its CTD

RNA polymerase II

![RNA polymerase structure](image)

**Regulated alternative splicing controls sex-determination in Drosophila**

(a) *sxl*  
(b) *tra*  
(c) *dsx*

![Regulated alternative splicing in sex-determination](image)

**Alternative Splicing Leads to Remarkable Genetic Diversity**

A striking example of alternative splicing: Drosophila DSCAM gene (an axon guidance receptor)

![Drosophila DSCAM gene](image)

38,000 possible gene products!!

**Regulated alternative splicing in sex-determination: A model of splicing activation by Tra and SR proteins**

![Regulated alternative splicing model](image)
Role of alternative splicing of slo mRNA in sound perception

The 5’ Cap (m7Gppp): Structure and function

Several enzymatic reactions add the m7Gppp cap co-transcriptionally. The capping enzymes bind to the phosphorylated CTD to carry out the following:

1. Step 1: γ-phosphate removal from 5’-end of RNA
2. Step 2: Transfer of GMP from GTP to the 5’ diphosphate.
3. Step 3: Transfer of methyl groups from S-adenosylmethionine to the N7 position and the 2’-oxygens of ribose

Functions of the cap:

1. Splicing
2. RNA stability
3. Export
4. Translation (see p. 131, fig. 4-31)

The making of a polyadenylated RNA

The RNA processing “factory”
mRNA’s are exported from the nucleus 5’-end first

Proteins that mediate mRNP export

mRNA exporter has two subunits: N, M, C are part of large subunit, which binds to the small (FG-associated) subunit. The mRNA exporter transports RNP through the pore.

Components of the exon-junction complex bind to the large subunit of the mRNA exporter. Components of the exon-junction complex are recruited during splicing!

The Cap-binding complex leads the way through the pore (providing directionality).