mRNA Processing and the coordination of nuclear events
The RNA processing “factory”

- the 5' capping enzymes
- TF=transcription factors (Pol II)
- SF=splicing factors
- pA=polyadenylation factors
- complexes “left” at the exon-exon junction after splicing

Bidirectional modulation of coordinated reactions

The 5' Cap (m\textsuperscript{7}Gppp): Structure and function

Several enzymatic reactions add the m\textsuperscript{7}Gppp cap co-transcriptionally. The capping enzymes bind to the phosphorylated CTD to carry out the following:

1. Step 1: $\square$-phosphate removal from 5'-end of RNA (triphosphatase: yeast Cet1)
2. Step 2: Transfer of GMP from GTP to the 5' diphosphate. (guanylyl transferase: yeast-Ceg1)
3. Step 3: Transfer of methyl groups from S-adenosylmethionine to the N7 position and the 2'-oxygen of ribose (guanine-7-methyl transferase and the 2'-o-methyl transferase)

Functions of the cap:

1. Splicing (CBC recruits U1 snRNP)
2. RNA stability
3. Export
4. Translation

* Distinguishing chemical features
The CTD of RNA polymerase affects recruitment of capping enzymes

The Cap Methyltransferase (Abd1) facilitates transcription

1. Abd1 recruitment to the promoter
2. Abd1 enhances pol II binding to the promoter
3. Abd1 enhances pol II transit from the promoter
4. Abd1 activity may involve CTD ser-5 phosphatase
   (Higher serine 2 phosphorylation associated with elongation)
ChIP (Chromatin immunoprecipitation assay)

Crosslink protein to DNA in living cells with formaldehyde

Break open cells/shear DNA

Add primary antibody of interest

Add Protein A agarose beads

Reverse crosslinks/remove proteins

IP to enrich for DNA bound by protein of interest

PCR amplify (Look for enrichment of precipitated DNA)

Adapted from Active Motif® scheme
ChIP analysis of Ceg1 association with the transcription complex
Chromatin IP of the CTD during active transcription

The making of a polyadenylated mRNA

1. Factors co-transcriptionally bind two sequences in the RNA: the upstream **AAUAAA** signal (CPSF) the downstream **GU- or U-rich** sequence (CStF) (CFI and CFII are part of this complex)

2. Poly(A) polymerase binds and facilitates cleavage at the poly(A) site.

3. Bound poly(A) polymerase, in a slow phase, adds about 12 A’s to the 3’ end of the RNA.

4. Poly(A)-binding protein accelerates poly(A) addition by poly(A) polymerase.

**Functions of polyadenylation:**

1. Protect 3’-ends of RNAs
2. Alternative processing generates different messages
3. RNA export
4. Translation
Polyadenylation and transcription are coordinated

Steps in co-transcriptional polyadenylation
1. After pol II passes polyA site, the polyA complex forms on pre-mRNA
2. Polymerase pauses (G-rich sequences)
3. Endonucleolytic cleavage of RNA (arrow) (Pin1 may be involved)
4. Cleavage and polyadenylation machinery, still tethered to the CTD carries out cleavage and polyadenylation

SOME OF THE EVIDENCE THAT POLYADENYLATION AND TRANSCRIPTION ARE COUPLED

1. RNAs transcribed by polymerase with a truncated CTD are not properly polyadenylated (McCracken et a. 1997)
2. CPSF binds directly to the CTD (GST-CTD pulldown).
3. PolyA machinery associates at the promoter & co-purifies with TFIID (Dantonel 97).
4. Purified Pol II A and II0 activate 3’ cleavage in a reconstituted system with other polyA factors (Hirose and Manley 1998).
5. Mutations in proteins involved in cleavage and the polyA site inhibit transcription termination (Birse et al. 1998)
Polyadenylation Complex

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 \( YS_2PTS_2PS \) (52 on the mammalian polymerase)
- 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

<table>
<thead>
<tr>
<th>Protein or complex</th>
<th>Specificity</th>
<th>Function</th>
<th>Comments</th>
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<tbody>
<tr>
<td>TBP</td>
<td>U</td>
<td>TATA-binding protein</td>
<td>Essential for transcription initiation</td>
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<tr>
<td>Mediator</td>
<td>U</td>
<td>Transcription coactivator</td>
<td>Holoenzyme subunits; CTD binding subunits unknown</td>
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<td>Rsp5</td>
<td>U</td>
<td>Ubiquitin ligase</td>
<td>requires WW domain to bind CTD</td>
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<tr>
<td>Spt4/Spt5</td>
<td>U, P</td>
<td>Elongation</td>
<td>DSIF; binds phospho and unphospho CTDs</td>
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<tr>
<td>Elongator</td>
<td>P</td>
<td>Transcription elongation</td>
<td>Six-subunit complex; CTD binding subunit unk</td>
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<tr>
<td>CA150</td>
<td>P</td>
<td>Transcription elongation</td>
<td>Requires FF domain to bind CTD</td>
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<tr>
<td>Ceg1</td>
<td>P</td>
<td>Capping guanylytransferase</td>
<td>Serine 5 specific; requires; Kin28/Cdk7</td>
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<tr>
<td>Abd1</td>
<td>P</td>
<td>Capping methyltransferase</td>
<td>Associated with pol II during transcription</td>
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<tr>
<td>Prp40</td>
<td>P</td>
<td>Splicing</td>
<td>Binds CTD with both FF and WW domains</td>
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<td>CstF50 subunit</td>
<td>P</td>
<td>Cleavage/poly(A) factor</td>
<td>Polyadenylation cleavage stimulatory factor</td>
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<td>Pcf11</td>
<td>P</td>
<td>Cleavage/poly(A) factor</td>
<td>Component of CFIA</td>
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<tr>
<td>Pta1 (CPSF)</td>
<td>P</td>
<td>Cleavage/poly(A) factor</td>
<td>Component of CFIIA</td>
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<tr>
<td>Ess1/Pin1</td>
<td>P</td>
<td>Peptidyl-prolyl isomerase</td>
<td>Req. WW domain to bind CTD; 3’ end proc.</td>
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<tr>
<td>Nrd1/SCAF8</td>
<td>P</td>
<td>3’ end formation</td>
<td>Directs poly(A)-independent 3’ end formation</td>
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<tr>
<td>Various SR proteins</td>
<td>P</td>
<td>Splicing</td>
<td></td>
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The speed of the polymerase affects splice site choice

Splicing can enhance transcription

“Heterologous introns can enhance expression of transgenes in mice” PNAS 1991.

TAT-SF1 and associated snRNP components stimulate transcription elongation


Outstanding questions about transcription-coupled splicing

- Can splicing factors modulate transcription by affecting chromatin structure?

- What is the phosphorylation state of the polymerase during transcription of intron-containing genes? Are changes in phosphorylation mediated by splicing factors?

- Do splicing factors alter polymerase speed to allow splicing to occur?

- Do spliceosomal RNAs play a role in coupling transcription and splicing? (U2 snRNA)

- What specific factors coordinate transcription and splicing? How? Ex. Cus2, the yeast homolog of TAT-SF1 and Bur2, the yeast homolog of P-TEFb). Ex. Mud2 (yeast) and the branchpoint binding protein interact with CTD modifying complexes, is branchpoint binding a transcription checkpoint?
mRNA splicing is coupled with RNA export

Premature stop codon upstream of exon-exon jxn. leads to further recruitment of the NMD machinery

Maniatis & Reed 2002
Factors deposited onto the mRNA after splicing affect message fate

Oskar mRNA in WT cell (at posterior pole of the oocyte) splicing of the first intron is essential for oskar or correct localization.

Proteins that mediate mRNP export

mRNA exporter has two subunits: N,M,C are part of large subunit (TAP/Mex67), which binds to the small (FG-associated) subunit (p15/Mtr2). The mRNA exporter transports RNPs through the pore.

Components of the exon-junction complex (e.g. Yra1/Aly) bind to the large subunit of the mRNA exporter. Yra1 is recruited during splicing.

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