# **Pol II - mRNA synthesis**

Pol II, S. cerevisiae (12 subunits)

- core by specific Rpb1-3, 9 and 11

specific subcomplex Rpb4/7 not essential



CTD: Tyr<sub>1</sub>Ser<sub>2</sub>Pro<sub>3</sub>Thr<sub>4</sub>Ser<sub>5</sub>Pro<sub>6</sub>Ser<sub>7</sub>

26 (yeast) - 52 (human) repeats

- Rpb5-6, 8, 10 and 12 - shared by Pol I-III

## **Co-transcriptional mRNA processing**

CTD posphorylation status



#### Phospho-CTD Associated Proteins

- transcription
- chromatin structure
- RNA processing
- (splicing, 3' end formation)
- RNA export
- RNA degradation
- snRNA modification
- snoRNP biogenesis
- DNA metabolism
- protein synthesis and degradation



#### CAPPING





Co-transcriptional capping - occurs after the synthesis of 10-15 nt of RNA

- CE recruitment to CTD requires high Ser5-P

GT/Ceg1-guanylyltransferase MT/Abd1-methyltransferase (promote early elongation) Cet1-RNA triphopshatase (inhibits re-initiation) CBC-cap binding complex

## Co-transcriptional mRNA processing: SPLICING



Chrissie Barrass, 2011, cover of Mol. Cell

# **Pre-mRNA SPLICING**







de Almeida and Carmo-Fonseca, FEBS Lett, 2008

#### Pre-mRNA SPLICING: CIS ELEMENTS



#### The consensus splicing sequences are not so conserved after all



Warf and Berglund, 2010, TiBS; Reddy, Ann.Rev.PlantBiol., 2007

#### Pre-mRNA SPLICING: TRANS ELEMENTS



Warf and Berglund, 2010, TiBS; Reddy, Ann.Rev.PlantBiol., 2007

# RNA catalyses nuclear pre-mRNA splicing

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#### Chemistry of pre-mRNA splicing and U2/U6 model

### **TRANSCRIPTION AND SPLICING**



## **ALERNATIVE SPLICING (AS)**



### **ALERNATIVE SPLICING (AS)**



Exons and introns often contain sequences that facilitate or inhibit splice site usage.

These elements bind splicing activators or repressors.

ESR – exonic splicing regulatory elements ISR – intronic splicing regulatory elements ESS/ISS – exonic/intronic splicing silencers ESE/ISE - exonic/intronic splicing enhancers

SR – Ser/Arg rich proteins
PTB – polypyrimidine tract-binding proteins
hnRNP – heterogenous nuclear RNP

AS occurs at the level of recognition of splice sites and other regulatory elements by RNA-binding proteins

# AS – splicing CODE: chromatin, ncRNAs, SF



### **CLEAVAGE AND POLYADENYLATION**



Millevoi and Vagner, NAR, 2008

### TRANSCRIPTION TERMINATION: hybrid allosteric- torpedo model





Luo and Bentley, Gene Dev, 2006

3'-end processing factors are recruited to Ser2-P CTD at 3' end of genes via CID (CTD-interacting domain) of Pcf11 for CP and Rtt103 for Rat1 5'-3' exonuclease and its activator Rai1.

<u>Pcf11</u> and <u>Rat1</u> coordinately contribute to the recruitment of 3'- end processing factors

#### Nrd1/Nab3/Sen1-dependent TERMINATION





 Histone pre-mRNA contains conserved stem-loop (SL) structure, recognized by the SLBP (SL-binding protein)

• SLBP, ZFP100 and HDE (histone downstream element) stabilize the binding of U7

 U7 snRNP, specificaly Lsm11, recruits cleavage factors and the cleavage by endonuclease CPSF-73 generates mature 3' end of histone mRNA

Dominski and Marzluff, Gene, 2007

## **POLYMERASE BACKTRACKING**



Regulatory pauses and arrests

Termination mechanisms

Transcriptional fidelity

Elongation rate control

Coupling transcription to translation in bacteria

Cotranscriptional RNA folding and processing

Genome instability



**Polymerase backtracking in genome stability** Double-strand break (DSB) formation as a result of codirectional collisions between the replisome and backtracked RNA polymerase in bacteria. Transcript cleavage factor (Gre) prevents polymerase backtracking and R loop formation, preserving genome integrity.

Nudlerr, Cell, 2013





## **RNA EXPORT**



## mRNA EXPORT – ALL FACTORS





**SAGA** histone acetyltransferase complex (including **Spt, Ada, Gcn5**); trx activation **THO** mRNP biogenesis and export: **Hpr1, Mft1, Tho2** and **Thp2** (human **THOC1-7**)

**TREX** transcription-export complex: **THO/Sub2/Yra1**, interacts with NPC via Mex67-Mtr2 **TREX-2** transcription-export complex: **Cdc31/Thp1/Sac3** and **Sus1** from **SAGA** 

<u>TREX-2</u> and <u>TREX</u> complexes link transcription (Pol II via THO, initiation complex SAGA via Sus1) to export receptors (Mex67, Yra1) and Nuclear Pore Complex

### **EXPORT of other RNAs**



#### **Mature mRNA: TRANSLATION**



UTR- <u>UNT</u>RANSLATED <u>R</u>EGION EJC- <u>E</u>XON <u>J</u>UNCTION <u>C</u>OMPLEX



- eIF4E interacts with m7G cap to form translationally active mRNA:
   cap dependent translation
  - circular mRNA protects agains degradation and stimulates translation
  - eIF4E/eIF4G/PAB recruits small ribosomal subunit
- tRNA-bound 40S scans mRNA to locate START

# THE RIBOSOME



