GENE REGULATION IN EUKARYOTES

Course: Molecular Biology (02022312)
Instructor: Dr. M. A. Srour

Reference:
Watson et al. Molecular biology of the gene, 6th ed. 2008,
Chap 17 pp. 589-632; Chap 12 pp. 396-414

Lec # 11 Wed 28.03.2012

Chap 12

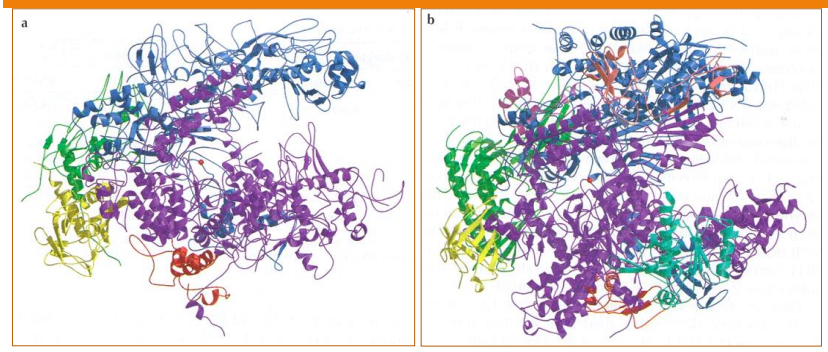
RNA Polymerases

TABLE 12-1 The Subunits of RNA Polymerases

Prokaryotic		Eukaryotic		
Bacterial	Archaeal	RNAP I	RNAP II	RNAP III
Core	Core	(Pol I)	(Pol II)	(Pol III)
β′	A′/A″	RPA1	RPB1	RPC1
β	B	RPA2	RPB2	RPC2
α^{I}	D	RPC5	RPB3	RPC5
α^{II}	L	RPC9	RPB11	RPC9
ω	K	RPB6	RPB6	RPB6
	[+6 others]	[+9 others]	[+7 others]	[+11 others]

Adapted, with permission, from Ebright R.H. 2000. *J. Mol. Biol.* 304: 687–698, Fig. 1, p. 688. © Elsevier.
The subunits in each column are listed in order of decreasing molecular weight.

(a) Prokaryotic & (b) eukaryotic RNA Pol



The shape of each enzyme resembles a crab claw, a reminiscent of the "Hand" structure of DNA Pol.
In prokaryotic RNA Pol, the two pincers of the crab claw are made up predominantly of the two largest subunits β and β'.
The active site or active center cleft is located at the base of the pincers.

Transcription in Eukaryotes

- **Transcription in eukaryotes vs. prokaryotes:**
- RNA polymerases are similar in both eukaryotes & prokaryotes
- Eukaryotes have 3 RNA Pol, prokaryotes have one RNA Pol
- Eukaryotes require several factors (General transcription factors or GTFs) for efficient & promoter-specific initiation; while prokaryotes require only one initiation factor (σ)
- In eukaryotes the DNA template is incorporated into nucleosomes ***in vivo*** & thus GTFs alone are not sufficient, & require additional factors including DNA-binding regulatory proteins called Mediator complex & chromatin-modifying enzymes

RNA Polymerase II core promoters

- Core promoter: minimal set of sequence elements required for accurate transcription initiation by the Pol II machinery
- Class II promoters are made up of a combination of 4 different sequence elements
- Core promoter elements:
  - TFIIB recognition element (BRE),
  - TATA element,
  - Initiator (Inr),
  - Downstream promoter elements (DPE, DCE or MTE)
- Core promoter is typically 40-60 bp, extending either upstream or downstream of TSS.

RNA Polymerase II core promoters

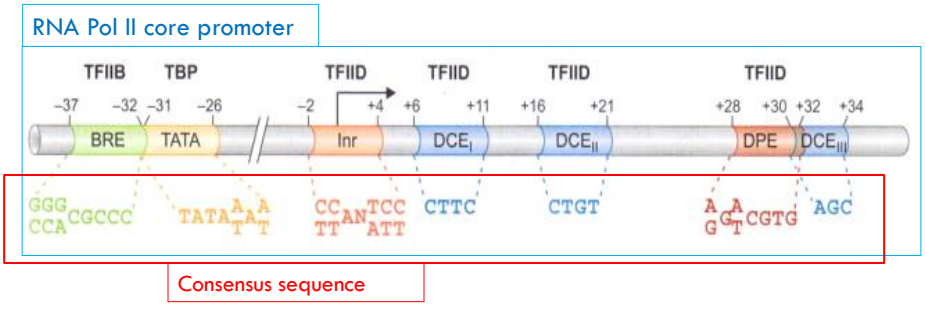


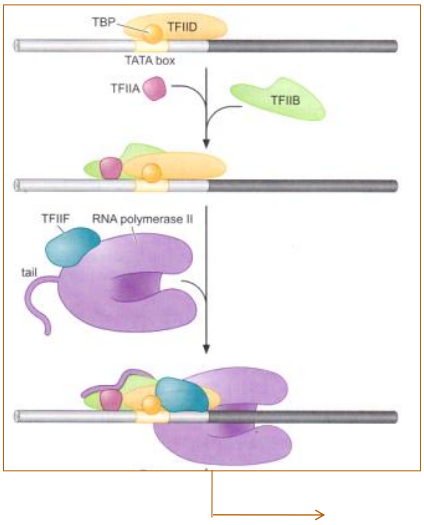
Fig 12-14/ p. 397

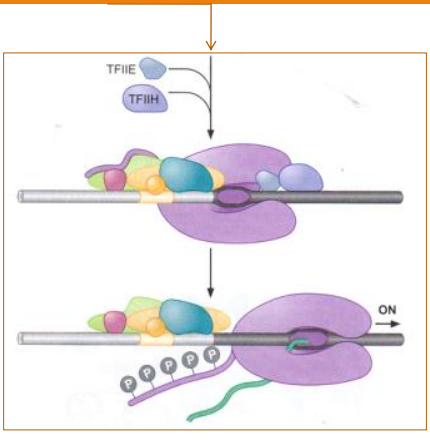
Additional Eukaryotic regulatory sequence

- Promoter proximal elements
- Upstream activator sequences (UASs)
- Enhancers
- Silencers
- Insulators or Boundary elements

- All these regulatory sequences bind regulatory proteins called **ACTIVATORS & REPRESSORS** which help coordinate transcription from core promoter
- Some of these regulatory sequences can be located tens or even hundreds of kbs away from core promoters!

RNA Pol II forms a pre-initiation complex with GTFs at the promoter





GTFs bind in the order: TFIID, A,B, & TFIIF together with Pol. Then TFIIE & H (helicase & ATPase)

Fig 12-15/ p.399

- TFIID binds the TATA element and TFIID is critical for promoter recognition and pre-initiation complex formation
- TFIID is a multisubunit structure and includes TBP (TATA box Binding protein) and other subunits called TAF (TBP-Associated Factors)
- Some TAFs recognize other core promoter elements such as the Inr, DPE and DCE
- Why its important that TAFs bind elements other than TATA element??

TBP-DNA complex

- TBP extensively distorts TATA sequence & the resulting TBP-DNA complex provide a platform to recruit other GTFs & RNA Pol

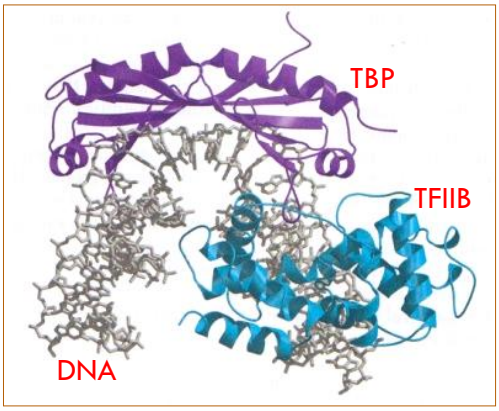


Fig 12-17/ p.402

Promoter escape requires phosphorylation of the RNA Polymerase tail

- Following preinitiation complex formation, follows a period of abortive initiation
- Promoter escape in eukaryotes require:
  - ATP hydrolysis (in addition to ATP hydrolysis required for melting DNA)
  - Phosphorylation of Pol CTD-Tail
- The CTD-tail of Pol has a heptapeptide sequence: Tyr-Ser-Pro-Thr-Ser-Pro-Ser which is repeated 27X in yeast to 52X in humans
- Each heptapeptide repeat has sites for phosphorylation by specific kinases including one that is a subunit for TFIIH

GTFs and their roles

- TBP: binds TATA element, distorts DNA using a B-sheet inserted in the minor groove
- TAFs: TBP is associated with ~ 10 TAFs
- TFIIB: enters the preinitiation complex after TBP. It appears that it bridges the TATA bound- TBP and RNA Pol
- TFIIF: binds Pol II and recruited to promoter with it. Binding of TFIIF & RNA Pol II stabilizes the DNA-TBP-TFIIB complex

- TFIIE: plays a role in recruitment and regulation of TFIIH
- TFIIH: has ~ 10 subunits, the largest and most complex of GTFs. it has 2 subunits that function as ATPase & kinase

In vivo, Transcription Initiation requires the "Mediator complex" & "Nucleosome-modifying enzymes"

- Mediator binds Pol via its CTD tail and present other surfaces for interaction with other DNA-binding activators
- Mediators aids initiation by regulating the CTD kinase in TFIIH
- Mediators in human consist of > 20 subunits
- Nucleosome-modifiers are recruited by activators

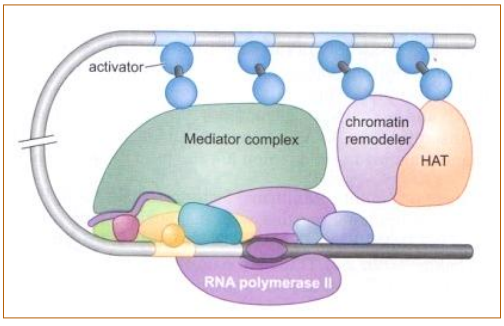
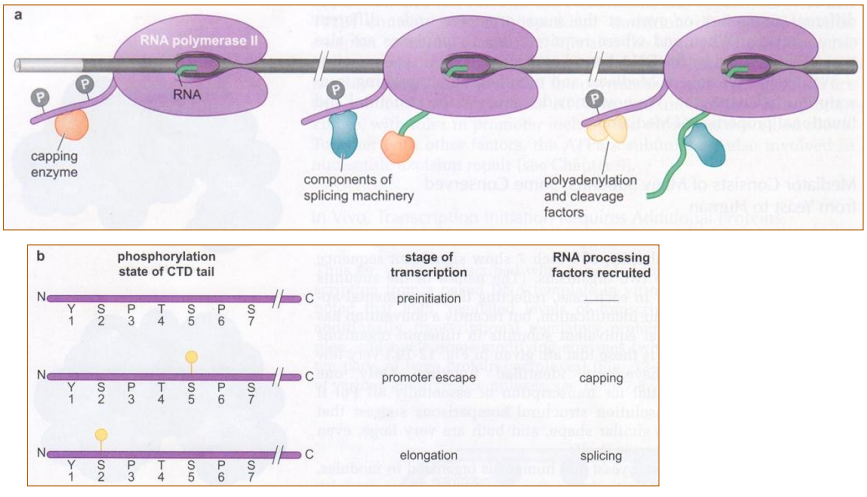


Fig 12-18/ p. 402

A new set of factors stimulate Pol II elongation and RNA proofreading

- Following promoter escape, Pol II sheds most of initiation factors- GTFs & Mediator
- New factors are recruited like Elongation factors are recruited (TFIIS & hSPT5) and factors for processing > favors the phosphorylated CTD

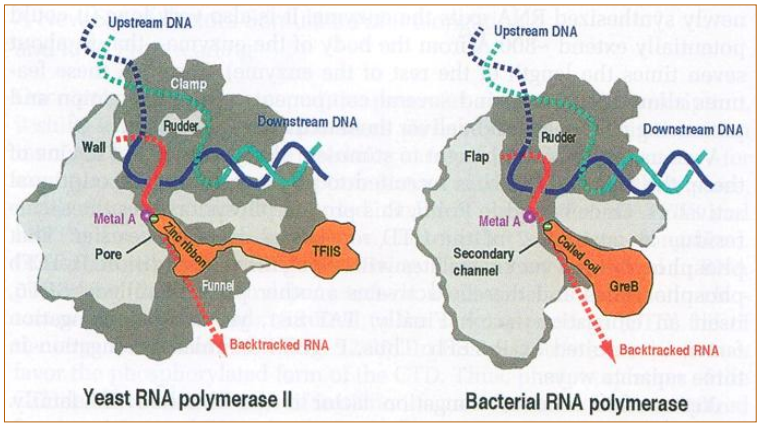
RNA processing enzymes are recruited by the CTD tail of Pol



A new set of factors stimulate Pol II elongation and RNA proofreading

- Elongation:
- Kinase P-TEFb is recruited to Pol II, phosphorylates CTD and activates hSPT5, also recruits TAT-SF1
- ELL family bind elongating Pol II and suppress transient pausing by enzyme
- TFIIS: limits the length of time that Pol II pauses when it encounters seq that slow the enzyme's progress
- TFIIS contributes to proofreading by RNA Pol II. TFIIS stimulates an inherent RNase activity in Pol allowing it to remove misincorporated bases

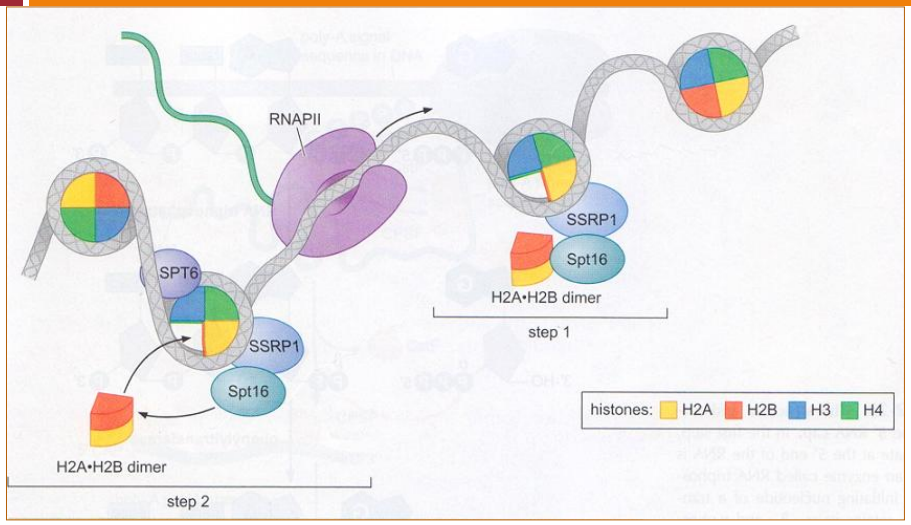
TFIIS and GreB act in analogous ways



Elongating RNA Pol must deal with Histones in its path

- How does RNA Pol transcribe through histones?
- FACT (facilitates chromatin transcription) is a heterodimer of 2 proteins: Spt16 & SSRP1> It makes transcription on chromatin templates more efficient
- How does FACT work?
- Spt16 binds to H2A/H2B & SSRP1 binds to H3/H4
- FACT can dismantle and reassemble histones
- FACT has a histone chaperon activity> allows it to restore H2A/H2B dimer to histone octamer

A model for FACT-aided elongation through nucleosomes



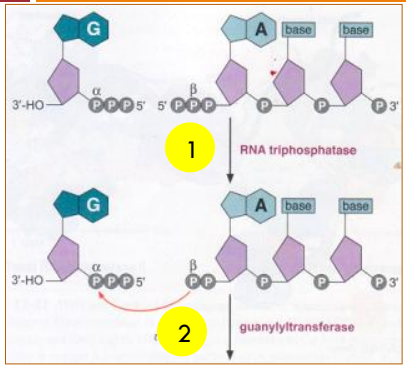
Elongating Pol is associated with a new set of protein factors required for various types of RNA processing

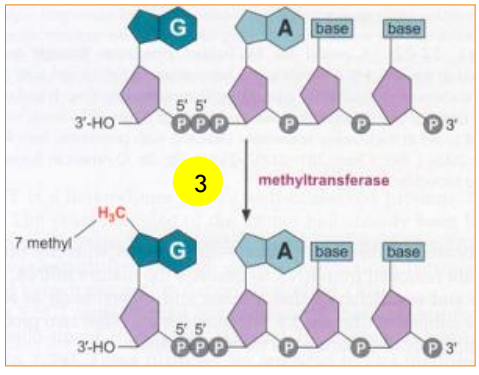
- Transcribed RNA is processed in different ways:
  - Capping of the 5' end of RNA
  - Splicing
  - Polyadenylation of the 3' end of the RNA
- The elongat factor hSPT5 helps recruit the 5'-capping enzyme elongation factor TAT-SF1 recruits splicing machinary

- After capping, dephosphorylation of CTD causes dissociation of the capping machinery
- Once RNA Pol reaches the end, it encounters specific DNA seq that after being transcribed into RNA, trigger transfer of polyadenylation enzymes to that RNA and leading to 4 events: cleavage of message, addition of many A residues to its 3' end & degradation of RNA remaining associated with Pol by a 5' to 3' exonuclease & termination of transcription

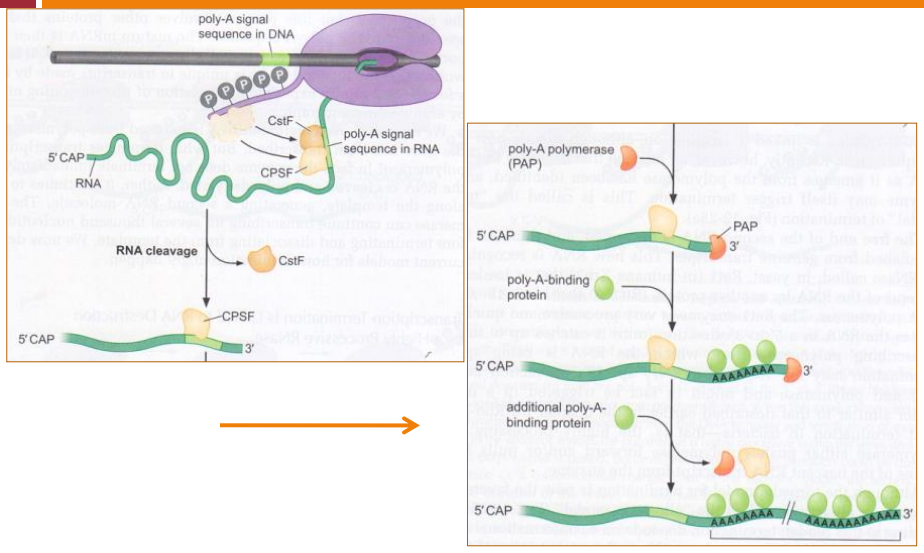
- Two proteins are carried by CTD of Pol as it approaches the end of the gene:
- CPSF (cleavage & polyadenylation specificity factor) & CstF (Cleavage stimulation factor)

Structure and formation of the 5' RNA cap





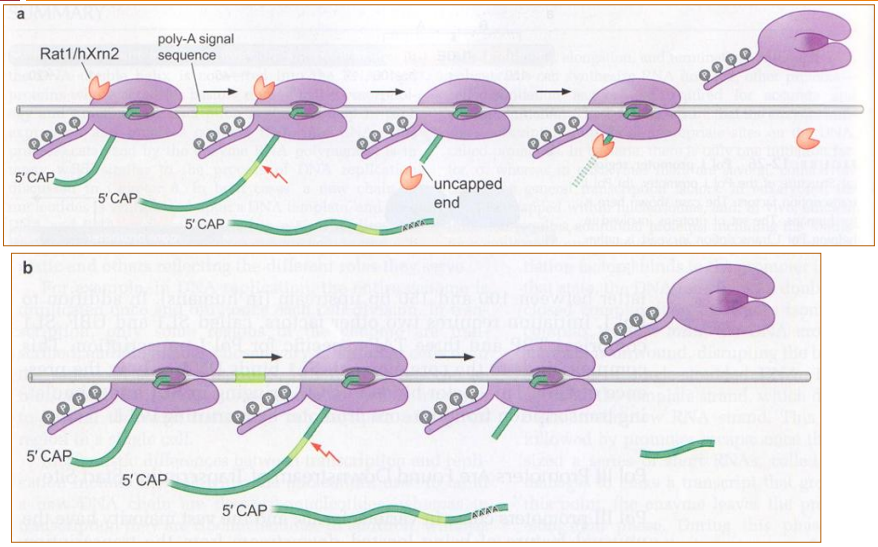
Polyadenylation and termination



Transcription termination is linked to RNA destruction by a highly processive RNase

- Polyadenylation is linked to termination. How??
- The free end of RNA emerging from Pol is uncapped and is recognized by an Rnase called Rat1 in yeast and Xrn2 in human that is loaded on CTD by Rtt103

Model of termination: (a) Torpedo & (B) allosteric



End