GENE REGULATION IN PROKARYOTES

Course: Molecular Biology (02022312)

Instructor: Dr. M A Srour

Textbook:
Watson J, Baker TA, Bell SP, Gann A, Levine M, Losick R (2008). Molecular Biology of the Gene, 6th ed. Chap 12 pp. 377-387; Chap 16 pp. 547-87.

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Chap 12

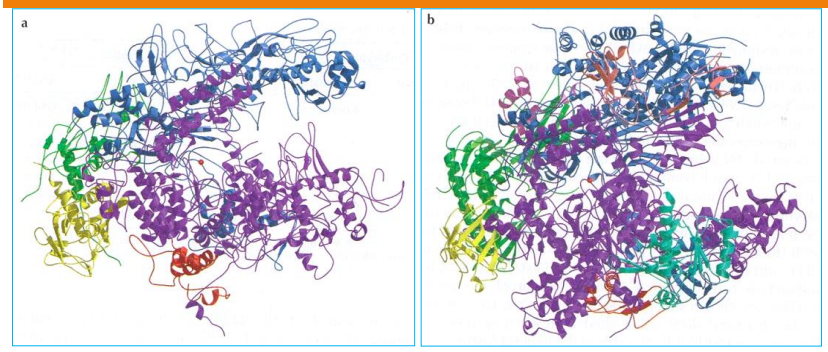
RNA Polymerases

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]

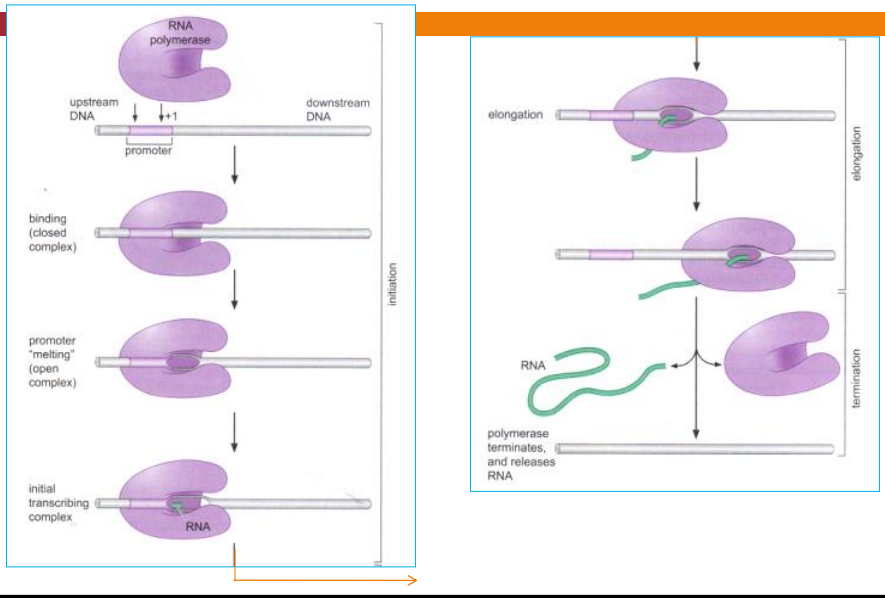
Adapted, with permission, from Ebright R.H. 2000. *J. Mol. Biol.* 304: 687–698, Fig. 1, p. 688. © Elsevier.
The subunits in each column are listed in order of decreasing molecular weight.

(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 by RNA Pol proceeds in a series of steps



The transcription cycle in bacteria

- Transcription initiation:
- Closed complex: RNA Pol binds to the promoter
- Open complex: DNA strands separate over a distance of ~14bp around TSS to form the transcription bubble
- Initial transcription & promoter escape: involves synthesis of first 10 or so nts, which is an inefficient step; once RNA Pol makes a transcript longer than 10 nts, it's said to have escaped the promoter

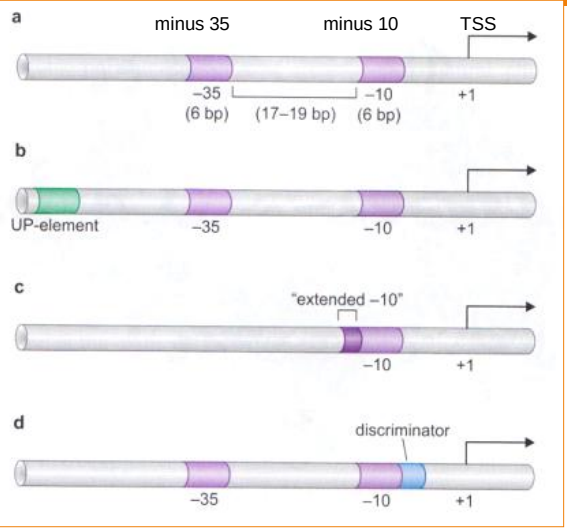
Bacterial promoters recognized by σ^{70} factor

Addition of σ^{70} factor to the core enzyme ($\alpha_2\beta\beta'\omega$) converts it into a holoenzyme that initiates only at promoter.

Promoter recognized by Pol containing $\sigma^{70,}$ have the following elements: Two conserved sequences, each 6 nts, separated by a nonspecific stretch of 17-19 nts.

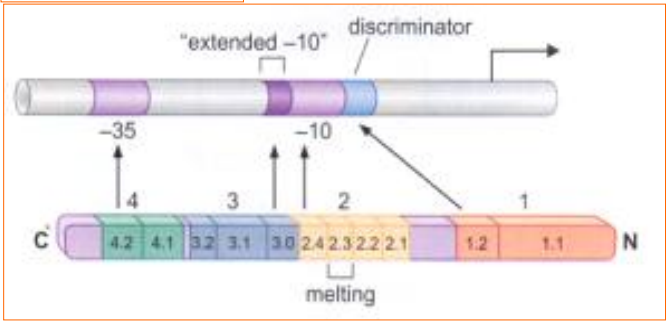
The strength of the promoter depends on how much its sequence is close to consensus sequence and the type of promoter elements present.

Different combinations of promoter elements



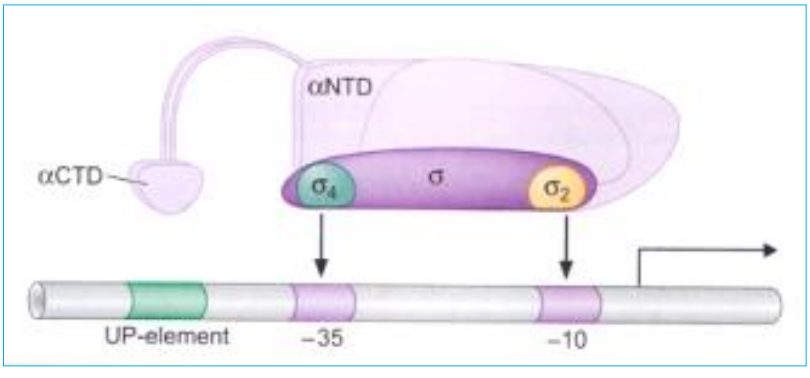
σ^{70} factor mediated binding of RNA Pol to the promoter

Regions of σ factor



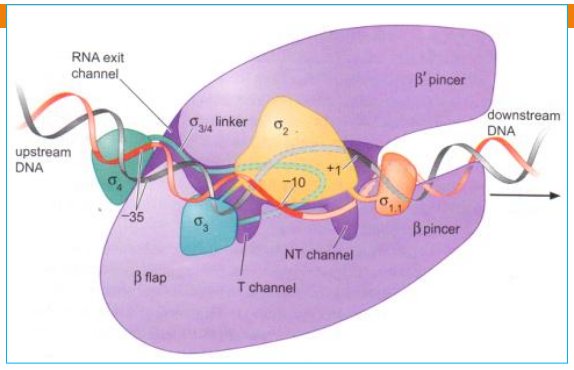
σ^{70} factor is divided into 4 regions 1-4, and each region binds a specific promoter element.
Region 4 forms a helix-turn-helix; one of these helices inserts into the major groove of DNA

The σ & α subunits recruit RNA Pol core enzyme (α2ββ'ω) to the promoter



αCTD: carboxyl terminal domain of α subunit,

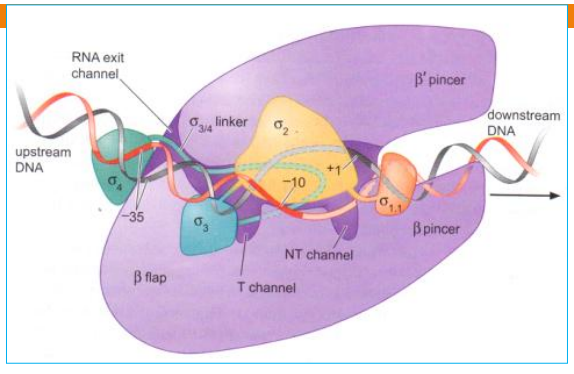
RNA Pol: channels into and out of the open complex



Transition from closed to open complex involves structural changes in enzyme & opening of duplex DNA to reveal template & non-template strands.
Melting occurs b/w positions -11 and +3 with respect to TSS.
In case of bacterial enzyme bearing σ^{70} factor this process is called isomerization, does not require energy.

RNA Pol: channels into and out of the open complex

Fig. 12-8



Active site is located at base of pincers, the so called active center cleft.
The enzyme has 5 channels: NTP-uptake channel (not shown in Fig), RNA exit channel, downstream DNA channel, non-template channel (NT) and template channel (T).

Chap 16

Principles of transcription regulation

- Regulation of gene expression can take place at different stages starting from transcription initiation to posttranslational control
- Regulation of gene transcription occurs at different stages
- The commonest stage is at the initiation of transcription

Regulatory proteins control gene expression

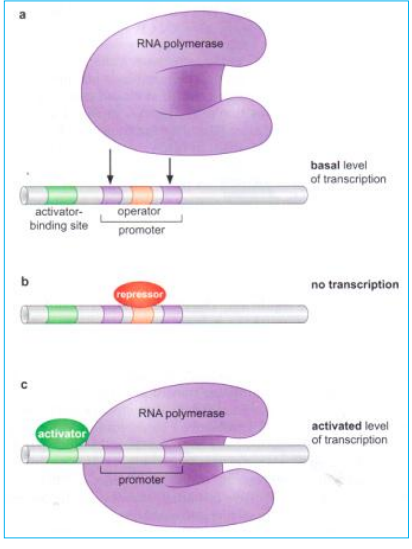
- Regulatory proteins: activators (positive regulators) & repressors (negative regulators) > mediate external signals to genes
- Most activators and repressors act at the level of transcription initiation, (Why?)
- Why regulation at transcription initiation is common?
  - This step is the most energetically efficient step to regulate
  - Regulation is easier to do well at this step

Regulatory proteins control gene expression

- Why not all regulation is focused on transcription initiation?
  - Allows for more input, regulation at more than one step allows more signals to modulate gene expression
  - Regulation at post-transcription steps can reduce response time
- Activators help RNA pol bind to promoter while repressors block binding to promoter
- In absence of regulatory proteins, RNA Pol binds weakly to promoter and can occasionally initiate transcription> gives a low level of constitutive expression called Basal level

Many promoters are regulated by activators that help RNA Pol bind DNA and by Repressors that block that binding:

Activation by recruitment of RNA Pol



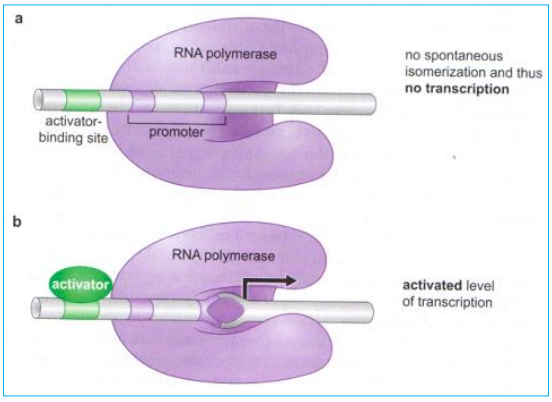
Allosteric activation of RNA Pol: regulation after RNA Pol binding

- In Allosteric activation RNA Pol binds a class of promoters efficiently unaided and forms a stable closed complex.
- But transition to open complex does not occur spontaneously, and an activator must stimulate this transition>> transition is the rate limiting step.
- The activator triggers transition to the open complex & high levels of transcription.

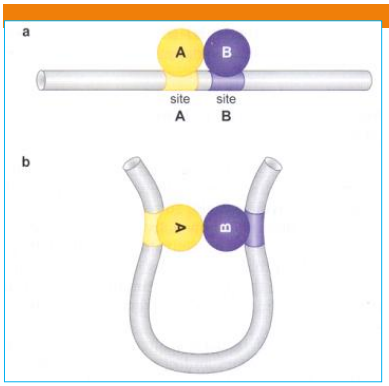
Allosteric activation of RNA Pol: regulation after RNA Pol binding

These activators interact with closed complex & induce conformational change in RNA Pol or DNA that triggers transition to open complex.

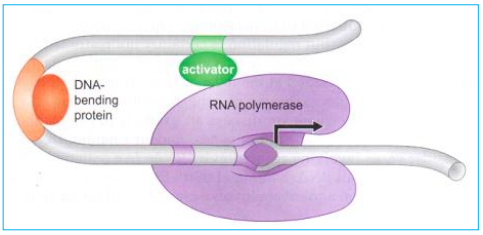
Some repressors interact with Pol & inhibit transition to open complex or promoter escape



Action at a distance & DNA looping



Cooperative binding of proteins to adjacent sites or separated sites.



A DNA-binding protein that bends the DNA binds between the activator-binding site and promoter

Cooperative binding & allosteric binding can act to integrate signals for different regulatory proteins.