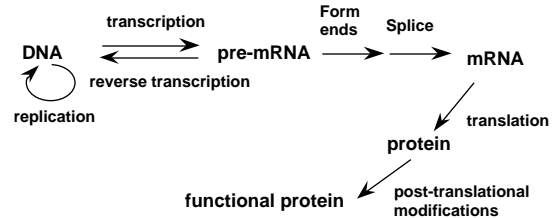


RNA polymerase #1

General properties
E. coli RNA polymerase
 Eukaryotic RNA polymerases

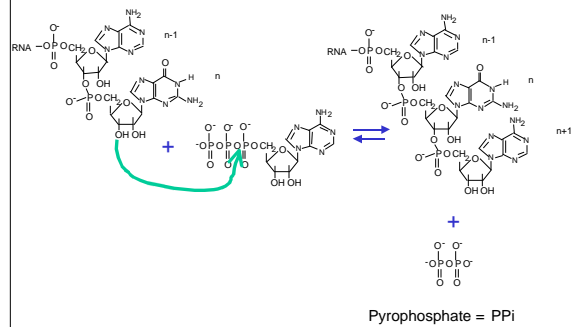
Pathway for Gene Expression



Reaction catalyzed by RNA polymerase

- Catalyzes the synthesis of RNA directed by DNA as a template = **transcription**
- Makes an RNA chain with a sequence complementary to the template strand of DNA
- Does NOT require a primer; can start RNA synthesis at a site on the DNA template

Sequential addition of ribonucleotides



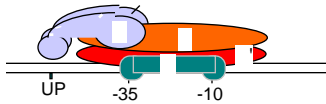
E. coli RNA polymerase

- Synthesizes all classes of RNA
 - mRNA
 - rRNA
 - tRNA
- Core = $\alpha_2\beta'$ catalyzes elongation of an RNA chain
- Holoenzyme = $\alpha_2\beta\beta'$ catalyzes initiation or RNA synthesis specifically at a promoter

Two Definitions of Promoter

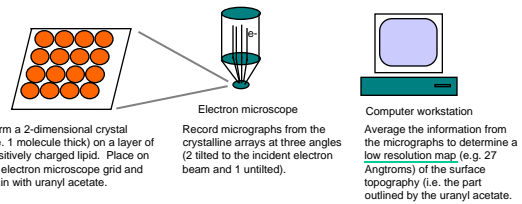
1. The sequence of DNA required for accurate, specific initiation of transcription
 2. The sequence of DNA to which RNA polymerase binds to accurately initiate transcription
- *In most cases*, RNA polymerase binds to a DNA sequence including the initiation site, but it can be directed there by sequences flanking the initiation site. Thus definition 2 can be a subset of definition 1.

RNA polymerase at a promoter

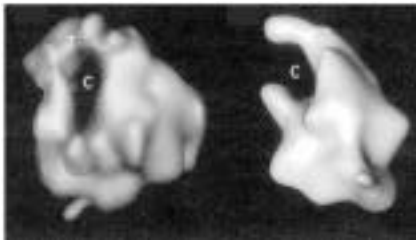


- : assembly and binds to UP
- + ' : form catalytic center
- : binds -10 and -35 of promoter to confer specificity during initiation

Determination of 3-D structure by electron crystallography



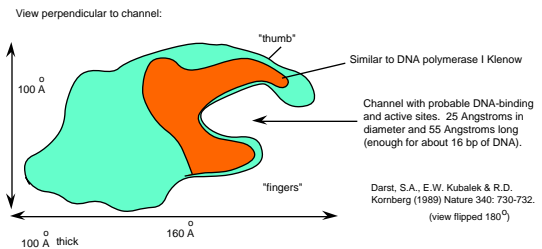
3-D images of core and holoenzyme



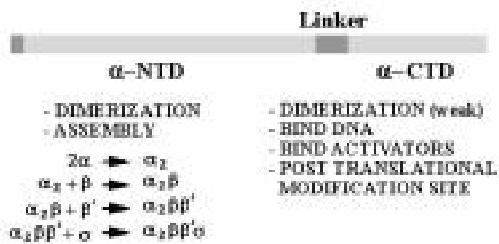
Core

Holoenzyme

Holoenzyme for RNA polymerase from *E. coli*

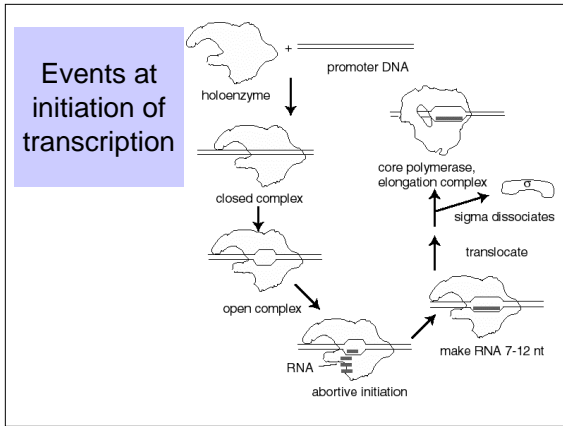


Role of subunit in assembly of RNA polymerase and other functions

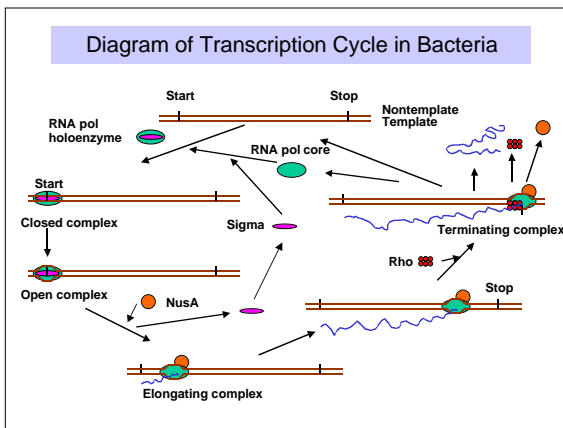


Mode of action of factors

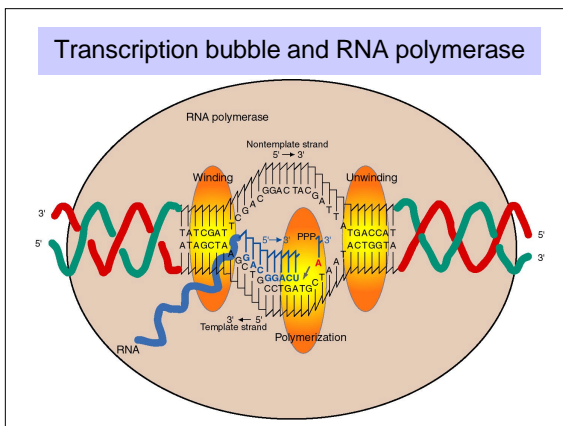
- The factor causes RNA polymerase to be selective in its choice of initiation sites by affecting the dissociation rate of polymerase from DNA.
 - Core dissociates from general DNA with a half-time of 60 min; use in elongation.
 - Holoenzyme dissociates from general DNA with a half-time of 1 sec!
 - Holoenzyme dissociates from promoter DNA with a half-time of hours (in absence of rNTPs).



- ### Transcription cycle
- **Initiation**
 - Holoenzyme binds to the promoter, unwinds DNA, and forms phosphodiester bonds between 7 to 12 nucleotides
 - Need
 - **Elongation**
 - dissociates
 - Core elongates RNA with high processivity
 - May use NusA
 - **Termination**
 - Polymerase dissociates from template DNA and releases new RNA
 - Often use



- ### Sites on RNA polymerase core
- Enzyme covers about 60 bp of DNA, with about 17 bp unwound = transcription bubble.
 - The bubble must contact the active site for polymerization.
 - At the beginning of the bubble, the DNA is unwound, implicating a helicase activity.
 - At the end of the bubble, the DNA is rewound.



- ### Effects of transcription on supercoiling of the template
- The unwinding and rewinding of the DNA template introduces positive supercoils ahead of the polymerase and negative supercoils behind it.
 - Unwinding will decrease T by 1 for every 10 bp unwound. Since $L=0$, $W=-T$, and $W=+1$ for every 10 bp unwound.
 - The opposite occurs upon rewinding. $T=+1$ for every 10 bp rewound, and thus $W=-1$ for every 10 bp rewound.

3 RNA polymerases in eukaryotes

Name	Makes	-amanitin
RNA Polymerase I	pre-rRNA	insensitive
RNA Polymerase II	pre-mRNA some snRNAs	very sensitive
RNA Polymerase III	pre-tRNA other small RNAs some snRNAs	less sensitive

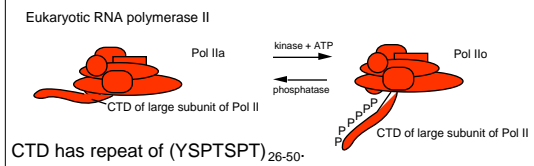
Subunit structure of eukaryotic RNA polymerases

- All 3 have multiple subunits (8 to 14).
- MW for each polymerase is about 500,000
- Some subunits are common to all 3 RNA polymerases
- All 3 RNA polymerases have subunits that are homologous to the bacterial α , β and β' subunits.

Subunits of yeast RNA Pol II

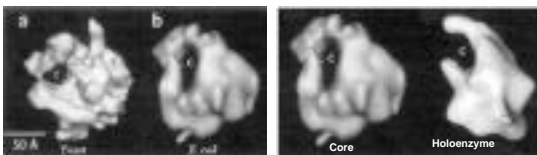
Approximate size (kDa)	subunits per polymerase	role / comment
220	1	related to β'
130	1	related to β
40	2	related to α
35	< 1	
30	2	common to all 3
27	1	common to all 3
24	< 1	
20	1	common to all 3
14	2	
10	1	

Phosphorylation of CTD of large subunit



Model: Phosphorylation of Pol IIa to make Pol IIo is needed to release the polymerase from the initiation complex and allow it to start elongation.

3-dimensional view of yeast RNA Pol II



Both yeast RNA Pol II and *E. coli* RNA polymerase core Have a similar shape and have the channel for DNA template.

Images from Dr. S. Darst

RNA Pol II bound to DNA and general transcription factors

