

Lecture 7

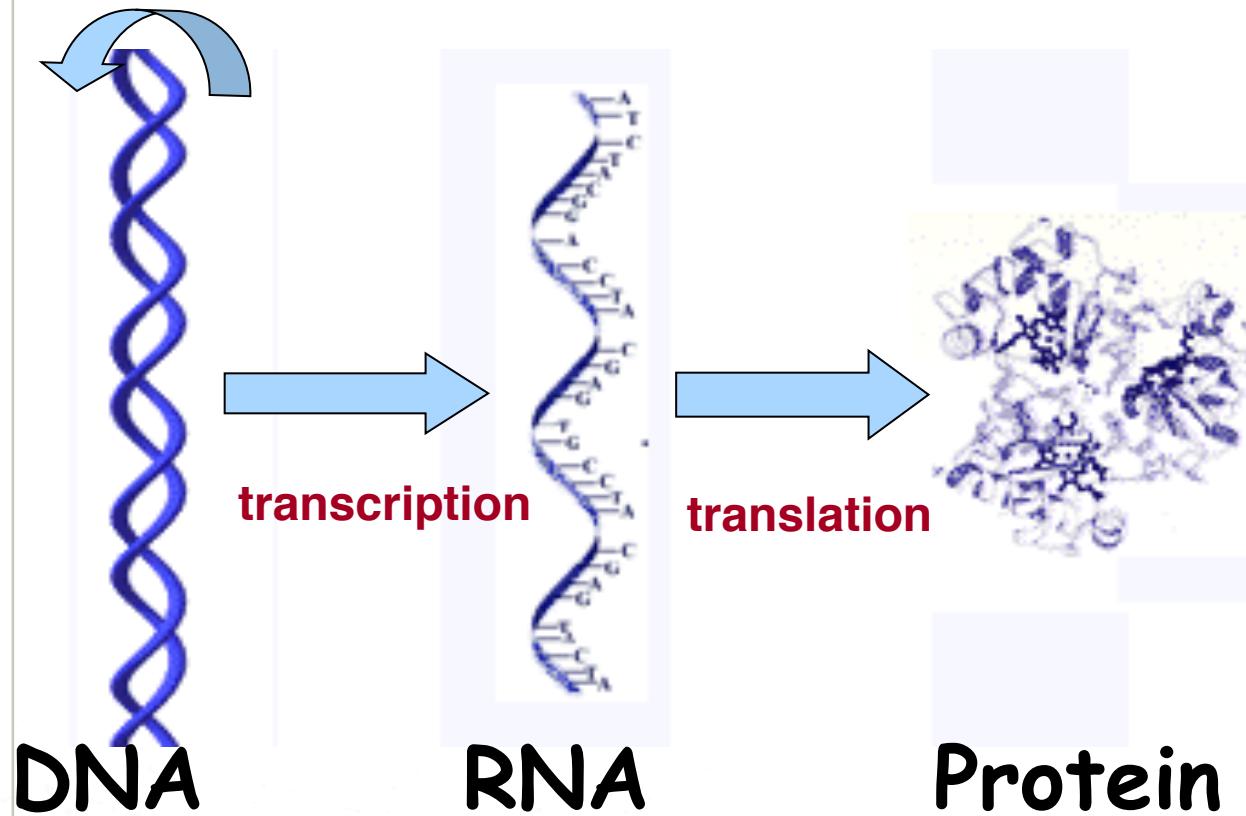
Transcription

April 19, 2016
Pyle

"Central Dogma" of the Flow of Genetic Information

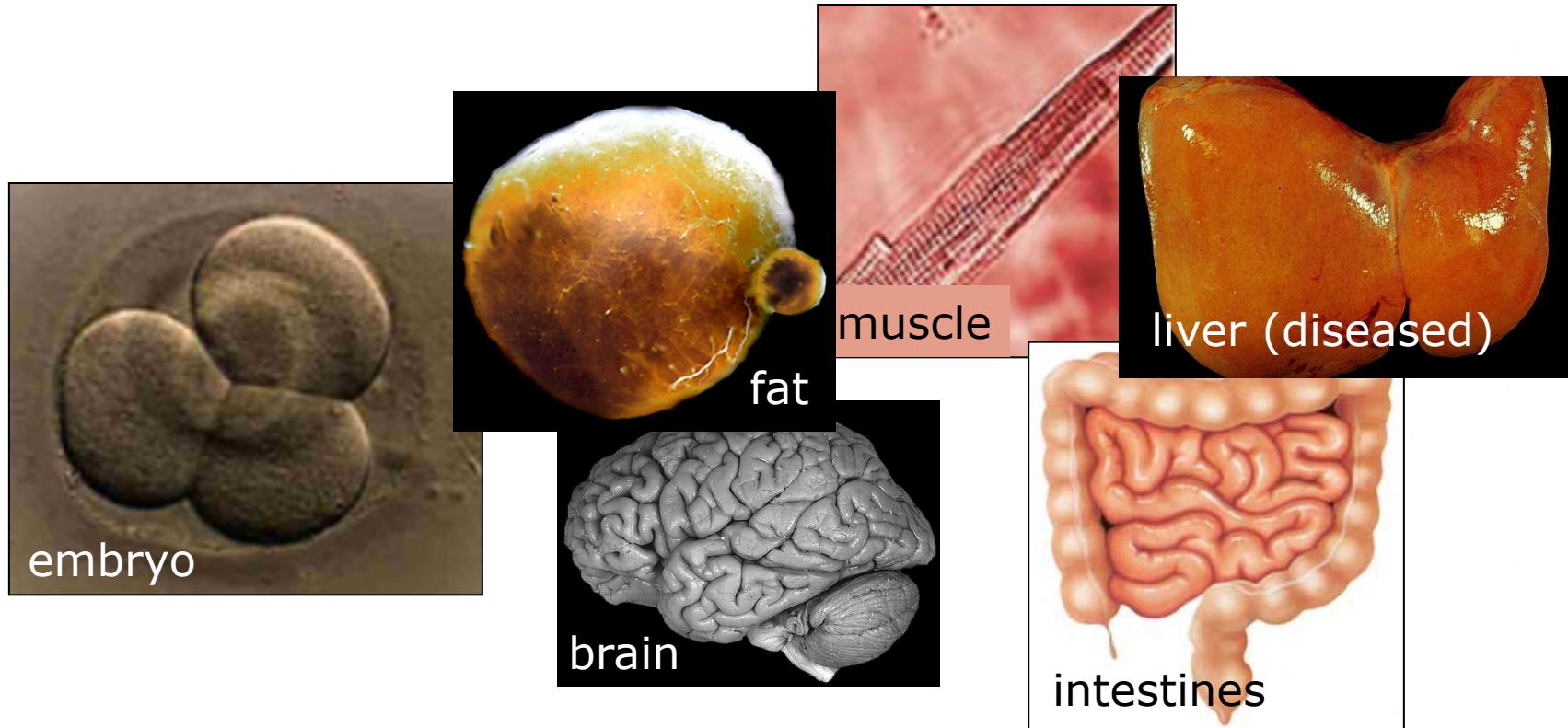
Central dogma, originally proposed by Francis Crick, states that the flow of the genetic information in a cell is always from DNA to RNA to protein.

replication



Although the principle of central dogma is generally correct, exceptions have been found. e.g. retroviruses like HIV make DNA from RNA.

Every cell has the same DNA and therefore the same genes. But different genes need to be "on" and "off" in different types of cells. Therefore, gene expression must be tightly regulated.



Gene expression is regulated in time, space and abundance. Gene regulation is important not only during development but also in mediating common variation between individuals, diseases, birth defects, and evolution.

Transcription

- Overview
 - Template - DNA
 - Enzyme - RNA polymerase
 - Steps of transcription
- Prokaryotic transcription
- Eukaryotic transcription

Transcription short movie

<https://www.hhmi.org/bioInteractive/dna-transcription-basic-detail>

Number of genes correlates generally with level of complexity of organism

<u>Organism</u>	<u>Genes</u>
Bacteria	2,000-6,000
Yeast	~4900
Fruit fly	~14,000
Mammals	~20,000

Start Signals

Stop Signals

Promoter

Gene Coding

**Transcription
terminator**



5'

3'

mRNA

Gene structure

- A gene is the entire DNA sequence required to encode a functional polypeptide or RNA (tRNA, rRNA, miRNA etc).
- A pseudogene is DNA sequence that looks like a gene but is not transcribed.
- Pseudogenes are DNA sequences similar to normal genes but non-functional; they are regarded as defunct relatives of functional genes.

Prokaryotic versus eukaryotic genes

1. Prokaryotic genes are **polycistronic**

- one promoter direct the synthesis of a mRNA that can encode more than one proteins

Proteins encoded by a prokaryotic polycistronic gene are usually all involved in the same biochemical pathway. This allows simple regulation of the whole pathway desired for fast growing bacterial cells.

2. Eukaryotic genes are **monocistronic**

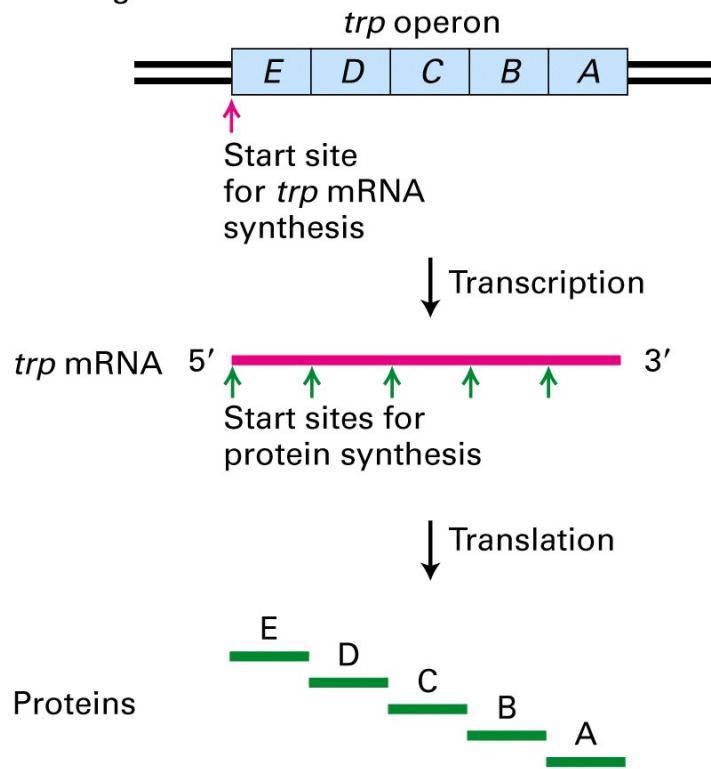
- one promoter direct the synthesis of a mRNA that encodes only one protein.

Eukaryotes prefer to do things in more sophisticated ways to achieve more control of the process, so every gene could have its own expression profile.

Tryptophan synthesis in both prokaryotes and eukaryotes

(a) Prokaryotes

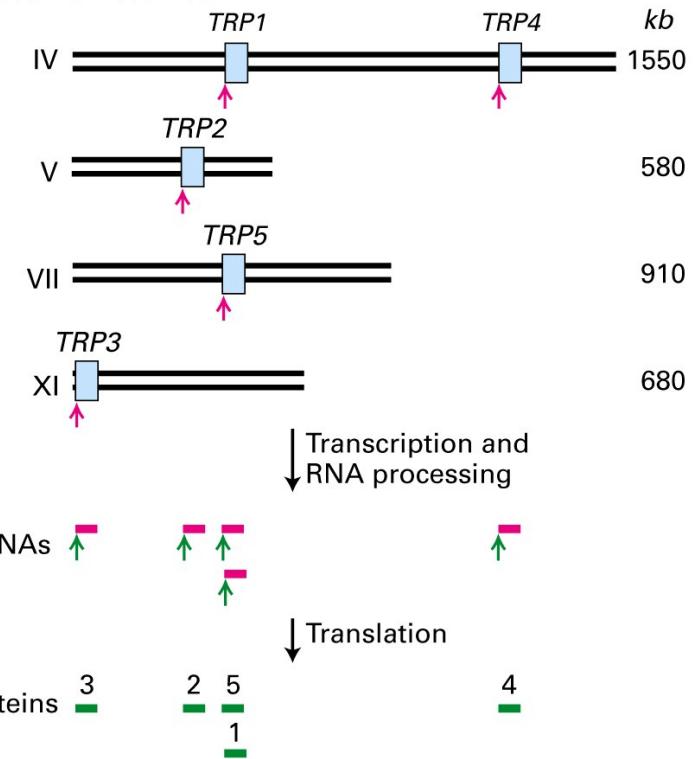
E. coli genome



One polycistronic gene in *E. coli*, transcribed into one mRNA, and translated into 5 proteins

(b) Eukaryotes

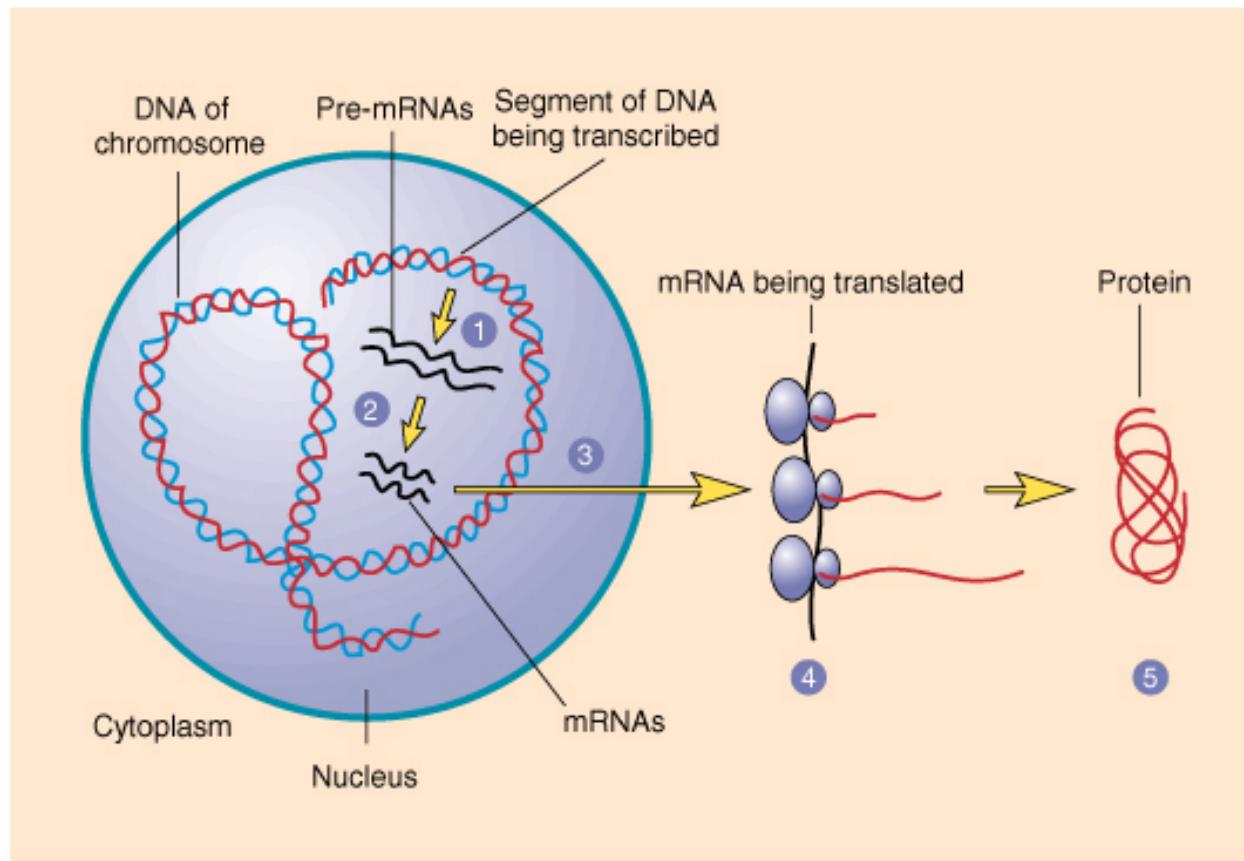
Yeast chromosomes



Five monocistronic genes in yeast, transcribed into 5 mRNA, and translated into 5 proteins

Transcription

A gene is expressed in two major steps: transcription for RNA synthesis, and translation for protein synthesis



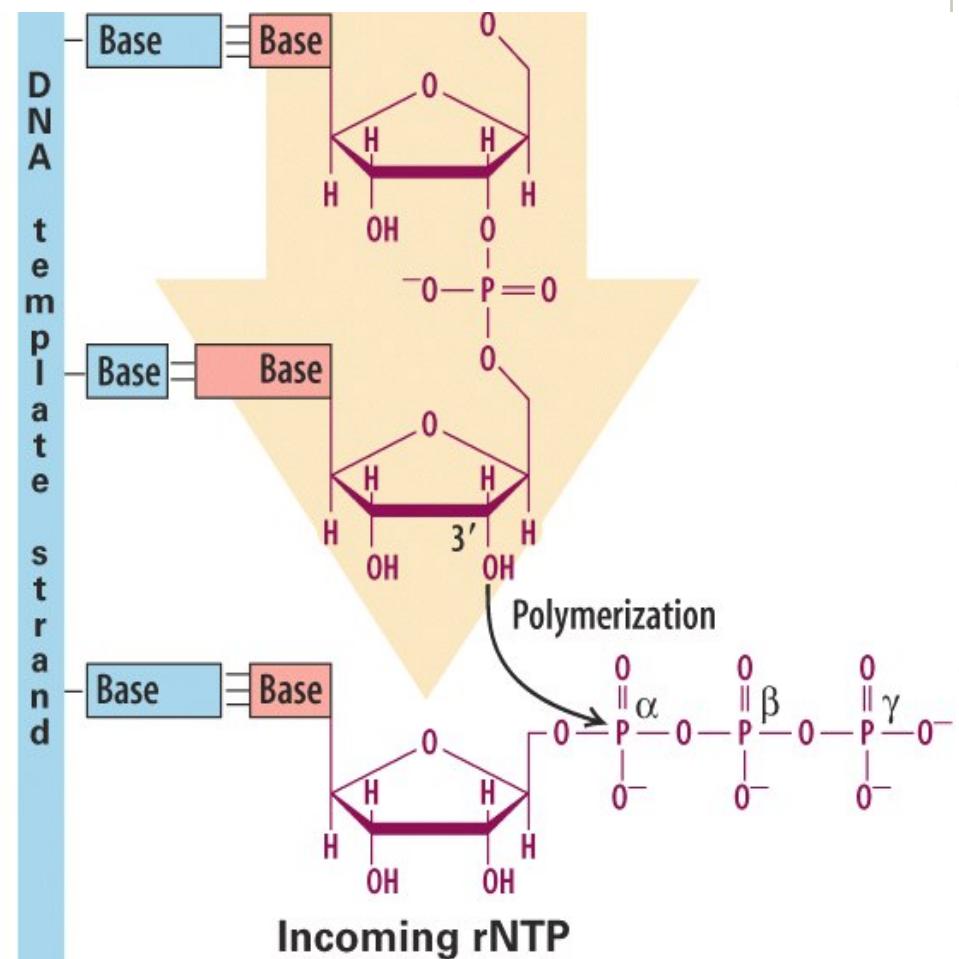
Transcription = RNA synthesis

- All cellular RNAs are synthesized by the transcription process.
- Transcription is a DNA-dependent RNA polymerization reaction catalyzed by the RNA polymerase.

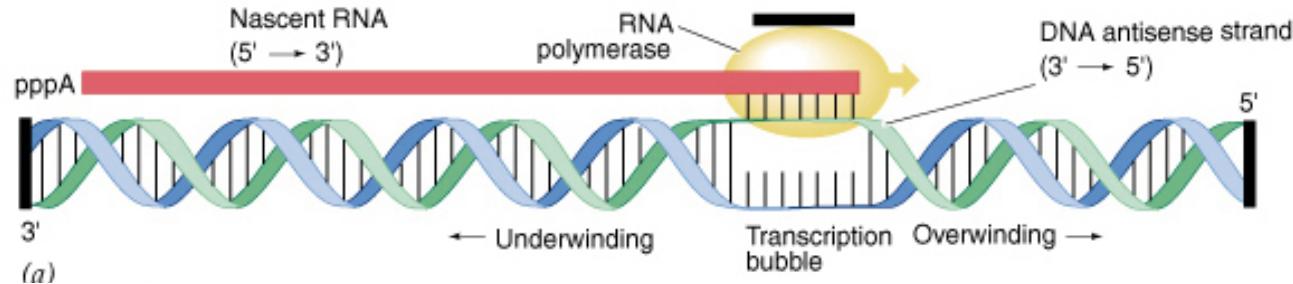
DNA 5' TACGTACGTACG 3'
3' ATGCATGCATGC 5'

RNA
polymerase

RNA 5' UACGUACGUACG3'



Direction of Transcription



1. RNA is always made in the $5' \rightarrow 3'$ direction.
2. Transcription produces single strand RNA that has the identical sequence with one of the two DNA strands of the gene.

DNA sense strand (non-template): with the same sequence as the RNA product. (coding)

DNA antisense strand (template strand) : with the sequence complementary to the RNA (non coding)

Upstream" : toward 5' of a given sequence

Downstream" : toward 3' of a given sequence

RNA is synthesized using DNA as a template - as with the double helix, the RNA-DNA hybrid is anti-parallel.

Only one of the two strands of DNA are copied into RNA in a given region.

DNA sense STRAND (NON-template)

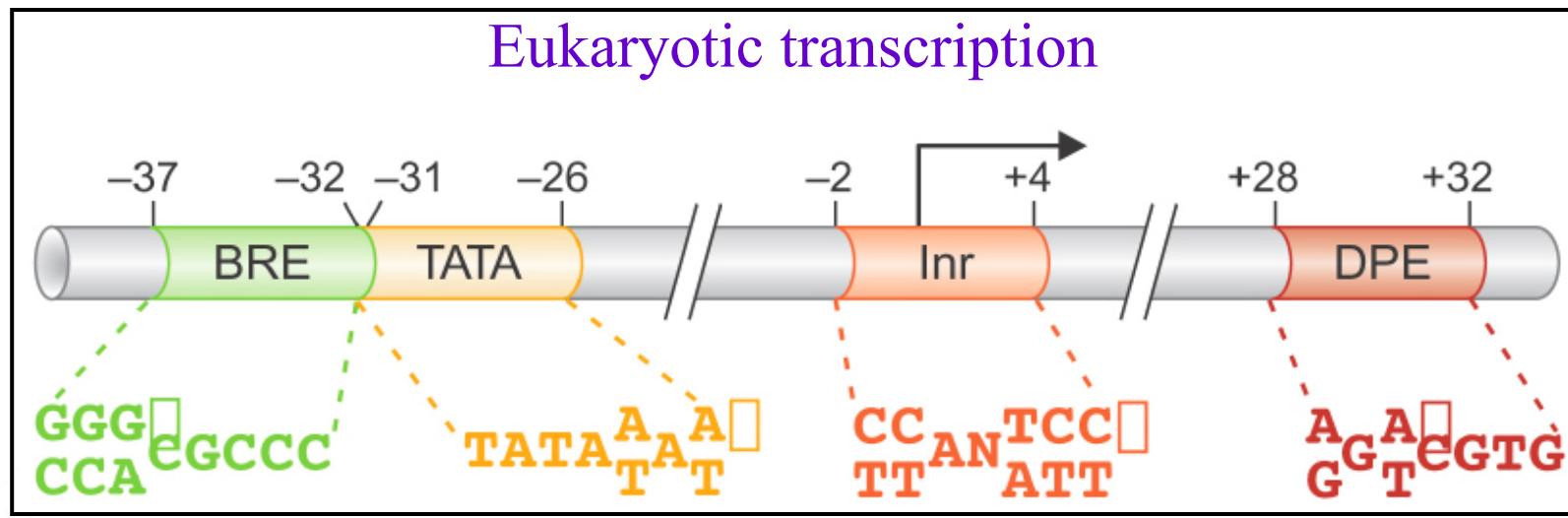
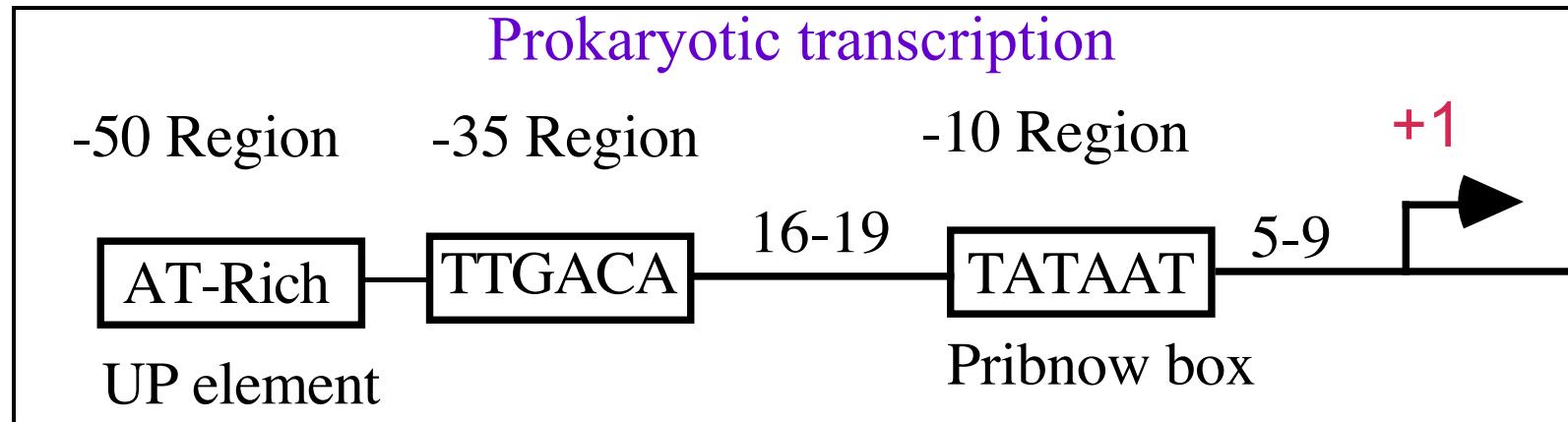
DNA 5' TATCGGCTCAAGATCGACTGA 3'

RNA 5' CUCAAGAUCGAC --> 3'

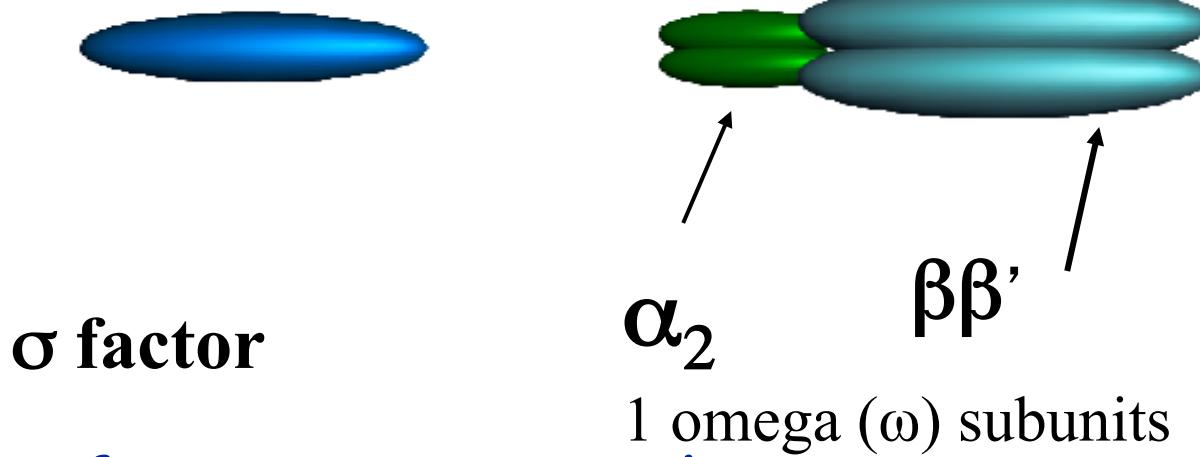
DNA 3' ATAGCCGÄGTTCTÄGCTGACT 5'

TEMPLATE STRAND

Sequence (cis) Elements in a Typical Minimal Promoter



E. coli RNA Polymerase Holoenzyme Subunits



σ factor

σ factor recognizes the promoter: There are multiple σ factors in the cell that recognize different promoters.
 σ^{70} is most common factor in *E. coli*

$\alpha_2\beta\beta'$ ω
constitutes
the core
polymerase
Addition of
sigma
creates the
holoenzyme

σ facilitates unwinding in -10 region

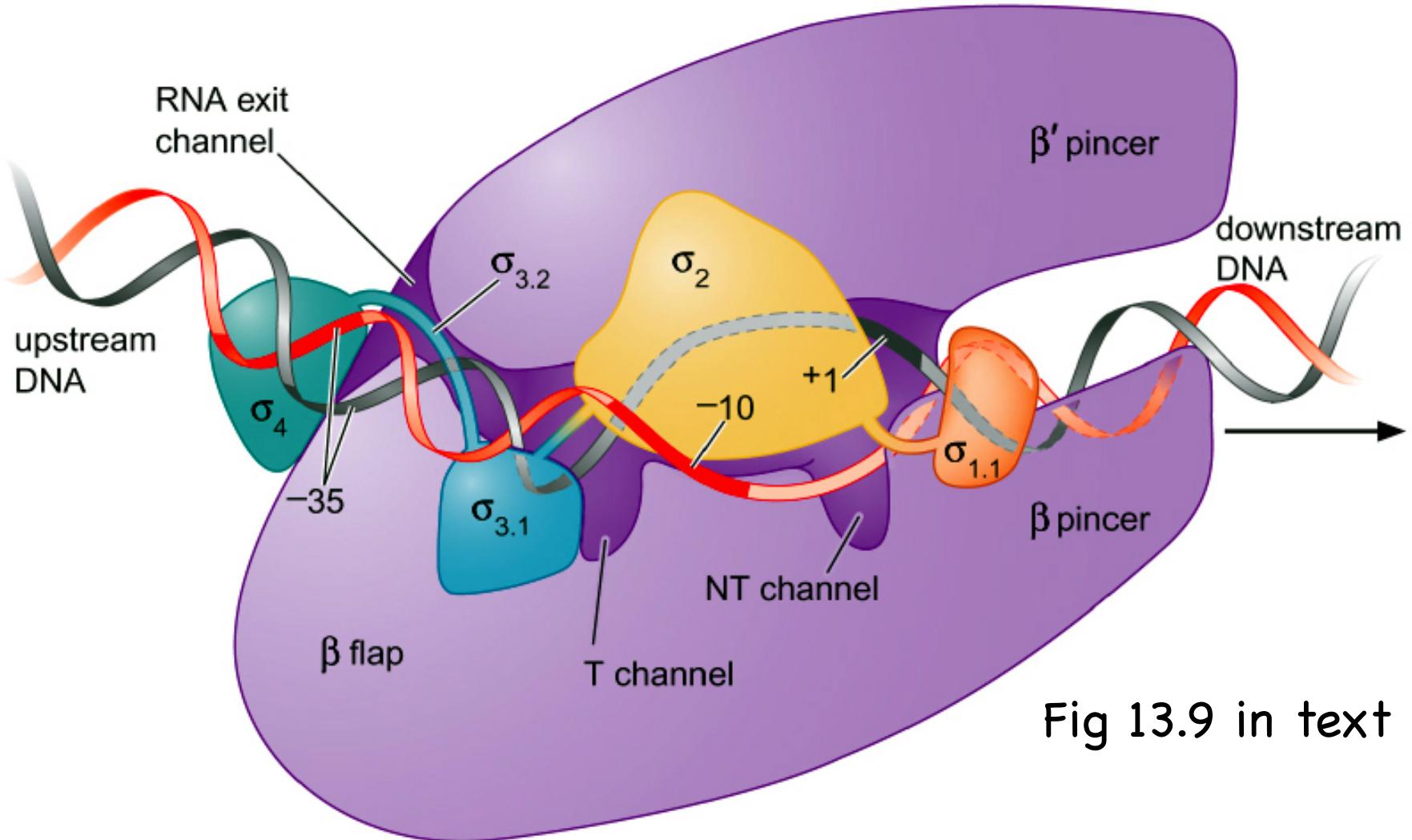


Fig 13.9 in text

Sigma reduces the affinity of RNAP for nonspecific DNA while increasing specificity for promoters, allowing transcription to initiate at correct sites

Binding of Polymerase subunits to the promoter sequences and CTD to UP-element

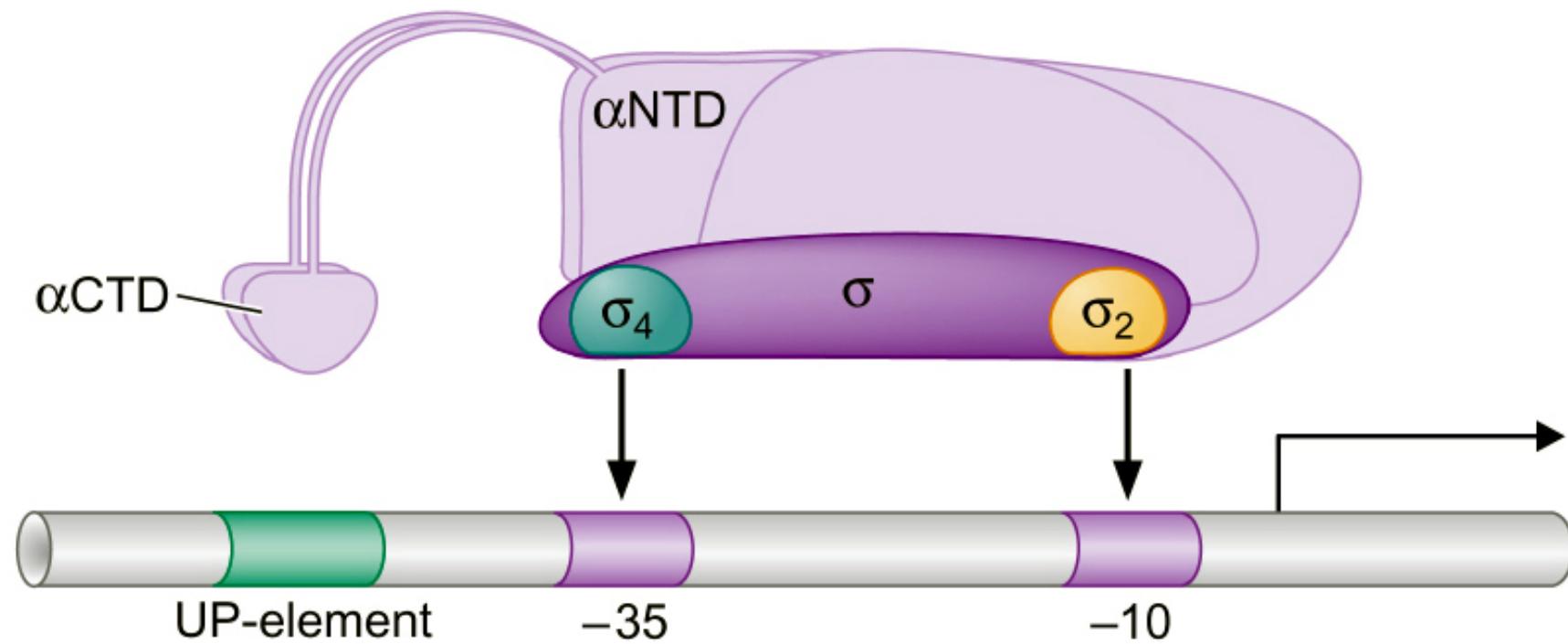


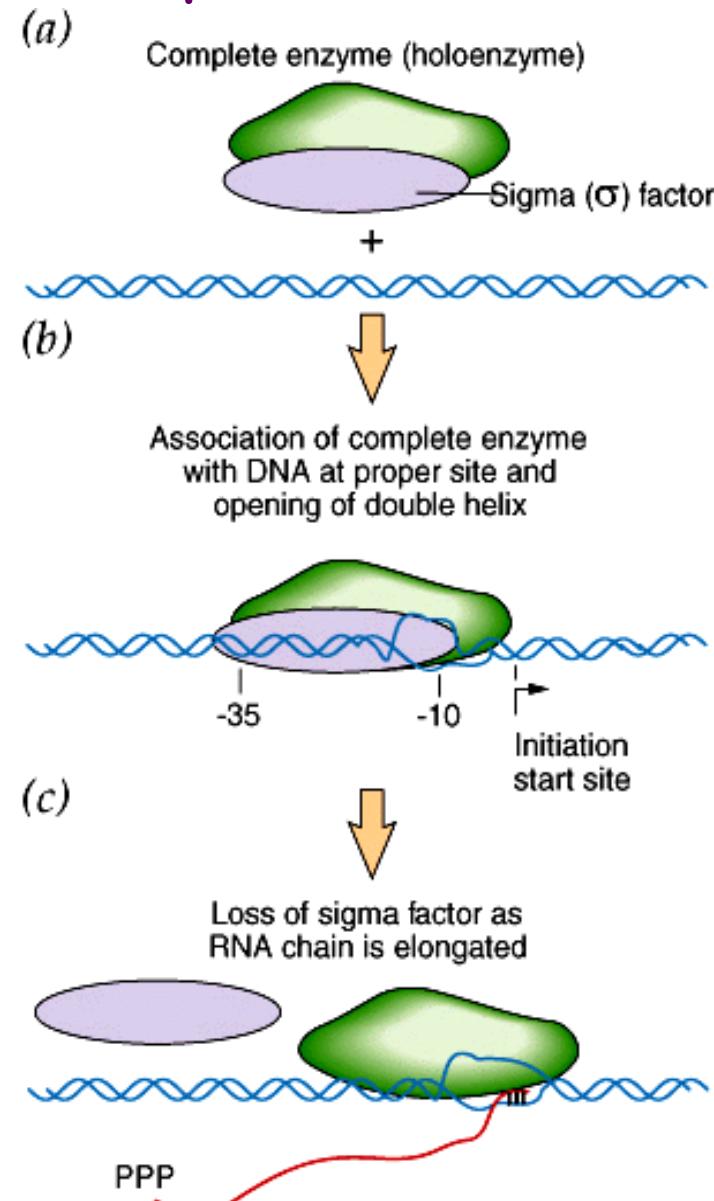
Figure 13.7 in text

Transcription proceeds by series of steps

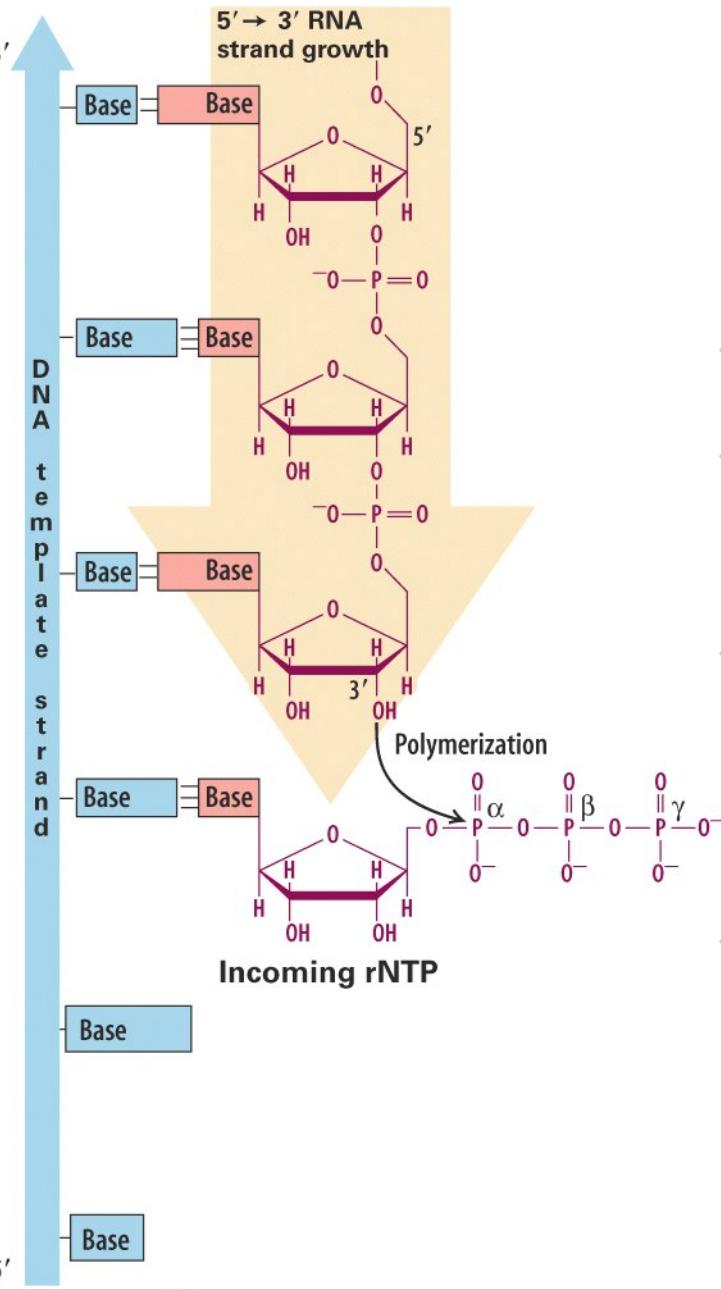
- Initiation
 - Binding (closed complex)
 - Unwinding DNA (open complex)
 - Initial transcript
- Elongation
- Termination

Steps in prokaryotic transcription initiation:

- Formation of the “closed complex”
- Unwinding of DNA to yield the “open complex”
- Conformational change initiates synthesis
- Synthesis of 5-10 phosphodiester bonds
- Release of sigma factor



Elongation

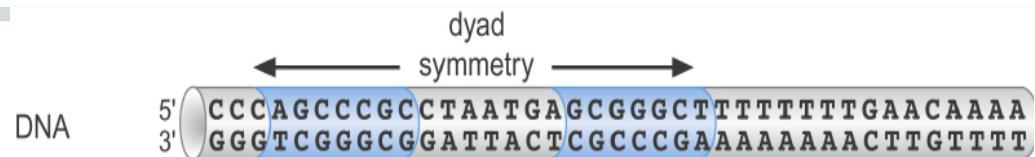


- ❖ Core RNA polymerase
- ❖ DNA template contains 17 bp transcription bubble
- ❖ Sequential addition of nucleotides to the growing RNA chain
- ❖ Transcription rate is approximately 50 nucleotides/second

Transcription Termination

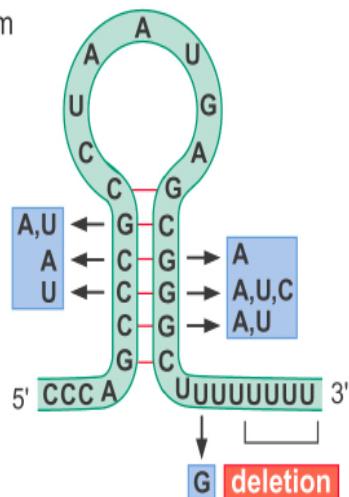
- Rho-dependent:
 - Pause sites become termination sites in presence of protein factor rho.
- Rho-independent:
 - GC rich stem-loop followed by run of U's

Rho-independent Transcription Termination



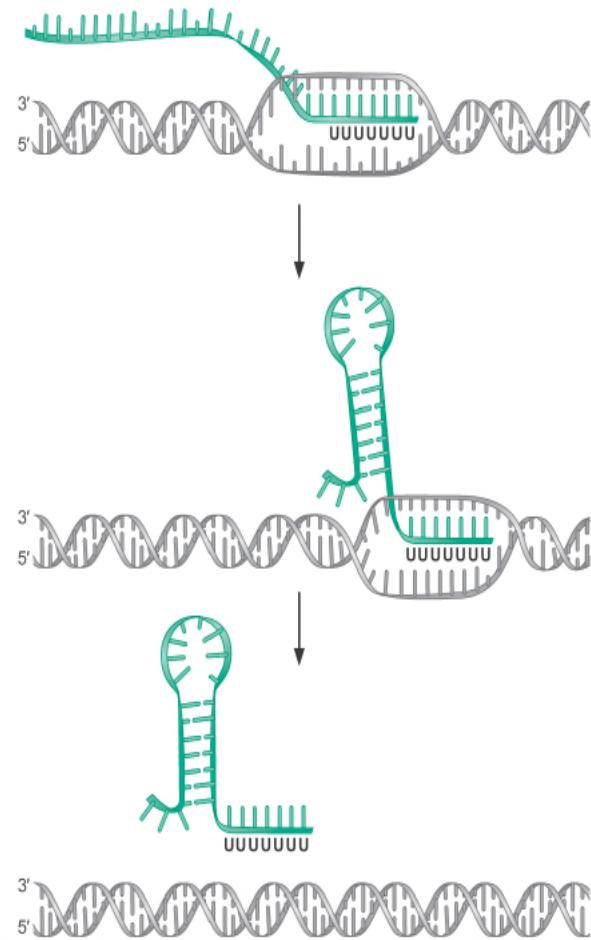
RNA 5' CCCAGCCCGCCUAUAGAGCGGGCUUUUUUUU 3'

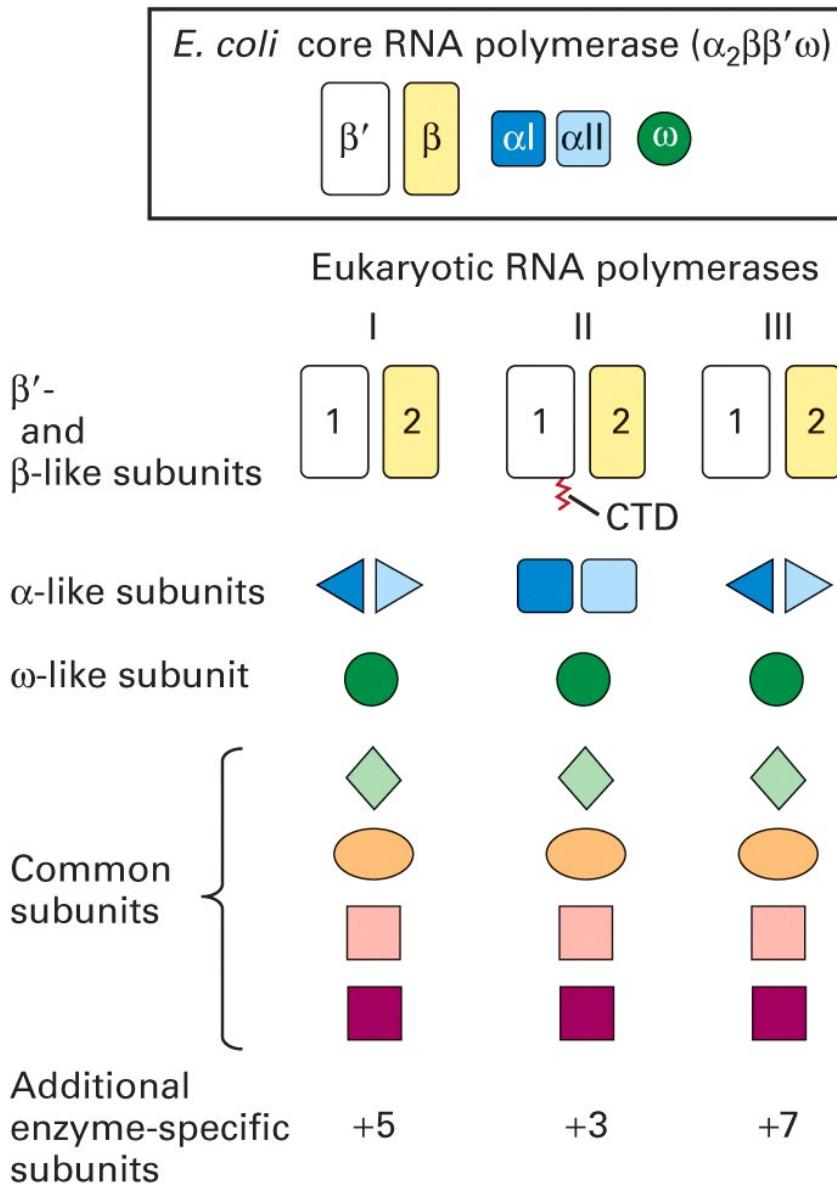
transcript folded to form termination hairpin



Hairpin formation forms since RNA:RNA hybrids are more stable than RNA:DNA

Release of RNA chain since A-U base pairs easily dissociate





RNA polymerases from prokaryotes and eukaryotes

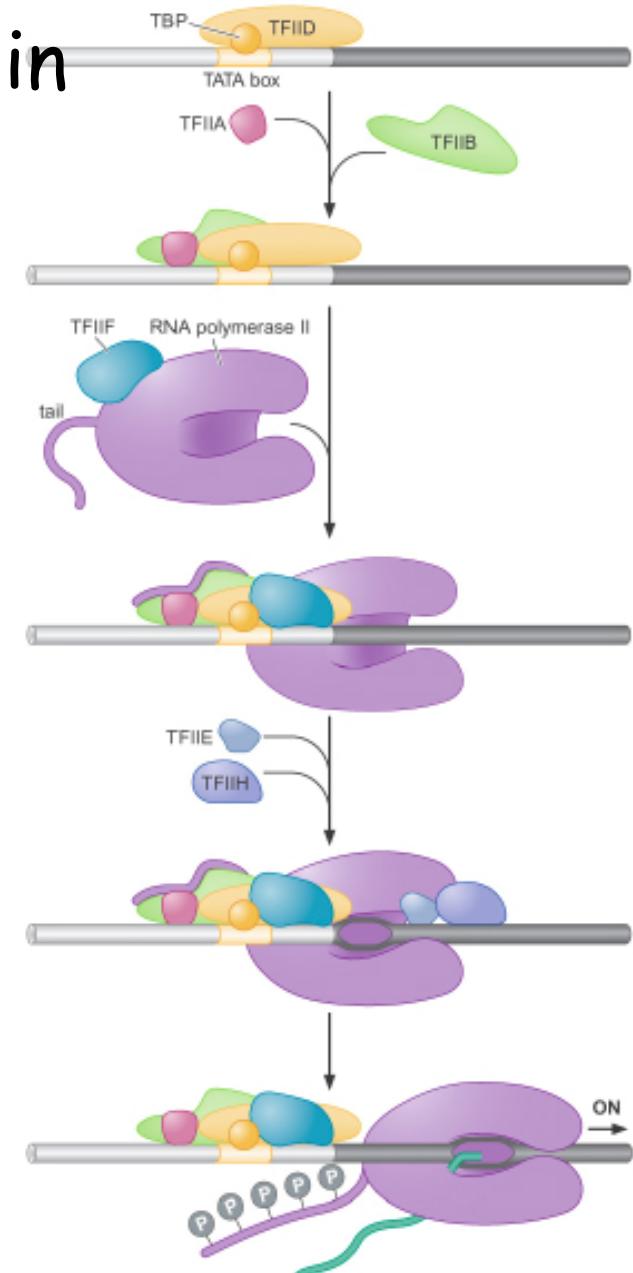
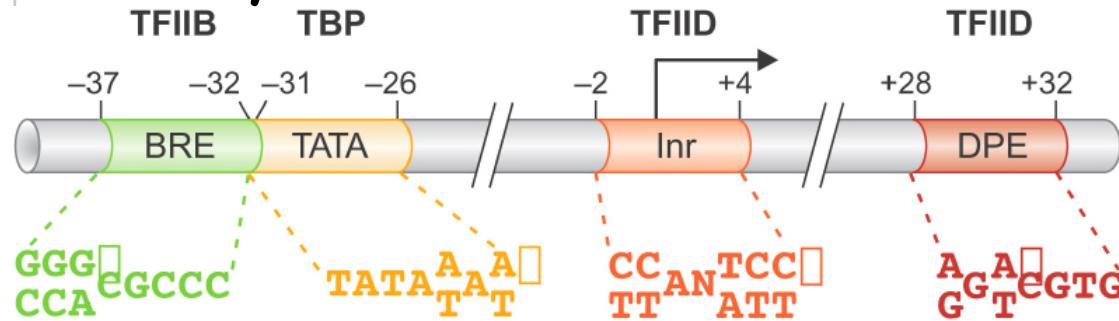
There are three types of RNA Polymerases in eukaryotic cells.

Pol I transcribes ribosomal RNA.

Pol II transcribes protein coding genes.

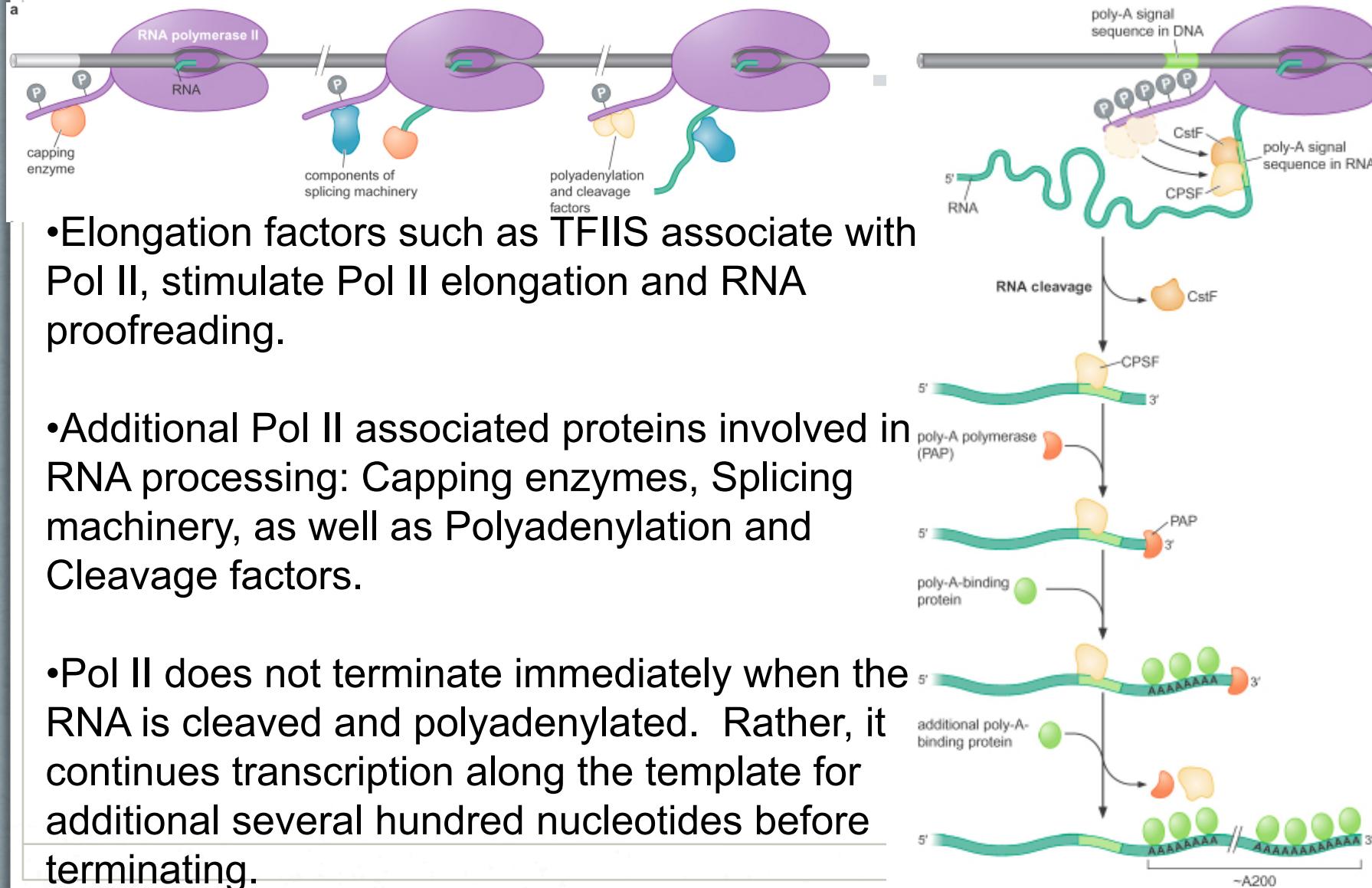
Pol III transcribes tRNA genes and other small RNAs

Pol II transcription initiation in Eukaryotes



- involves many more TFs binding Prior to bringing in RNAP II
- Ex. TFIIH unwinds dsDNA and phosphorylates the carboxyl terminal domain (CTD) of RNA polymerase II, resulting in the release of RNAPII from rest of the initiation complex and start of RNA synthesis.
- Promoter escape requires phosphorylation of the Polymerase "tail"

Pol II Transcription Elongation and Termination



Similarities between Prokaryotic and Eukaryotic Transcription

1. Both require promoter sequences for transcription initiation.
2. Both processes proceed in a 5' to 3' direction.
3. Both involve RNA polymerases that share similar structures.
4. Both involve other transcription activator and repressor proteins that bind specific DNA sequences and influence the rate of transcription initiation.

Differences between Prokaryotic and Eukaryotic Transcription

1. Eukaryotes contain 3 different RNA polymerases.
2. Eukaryotic RNA polymerases contain many more subunits.
3. Prokaryotic promoters are directly recognized by a subunit of the polymerase. Eukaryotic core promoters often contain a TATA box at -30, which is recognized by the TATA-binding protein (TBP). TBP then recruits the RNA polymerase.
4. In prokaryotes, “promoter” refers specifically to the RNA polymerase binding site. In eukaryotes “promoter” refers to all of the protein recognition sites between about -200 and +30, including the TATA box and binding sites for other activators and repressors.
5. Prokaryotic genes are regulated by the RNA polymerase plus one or two additional transcription factors (e.g. CAP and the lac repressor). A typical eukaryotic gene is regulated by dozens of transcription factors.

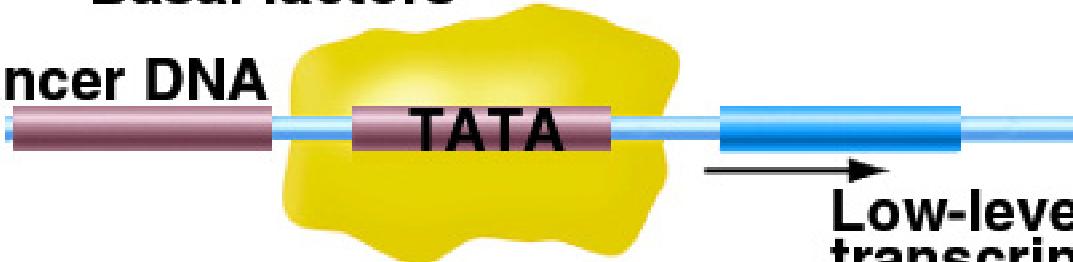
Enhancers and transcription activators

- 1. It is found that although initiation complex containing TFII factors and RNA Pol II can support transcription initiation in vitro, most eukaryotic genes need transcription activators for the efficient expression in vivo (in vivo means inside the cell).**
- 2. Transcription activators bind to specific DNA sequence and help either the formation of initiation or the efficient initiation after assembly of the initiation complex.**
- 3. Enhancers are DNA sequences that transcriptional activators bind to, which can be near the promoter region but more often are far away from the promoter region.**

Promoters and enhancers

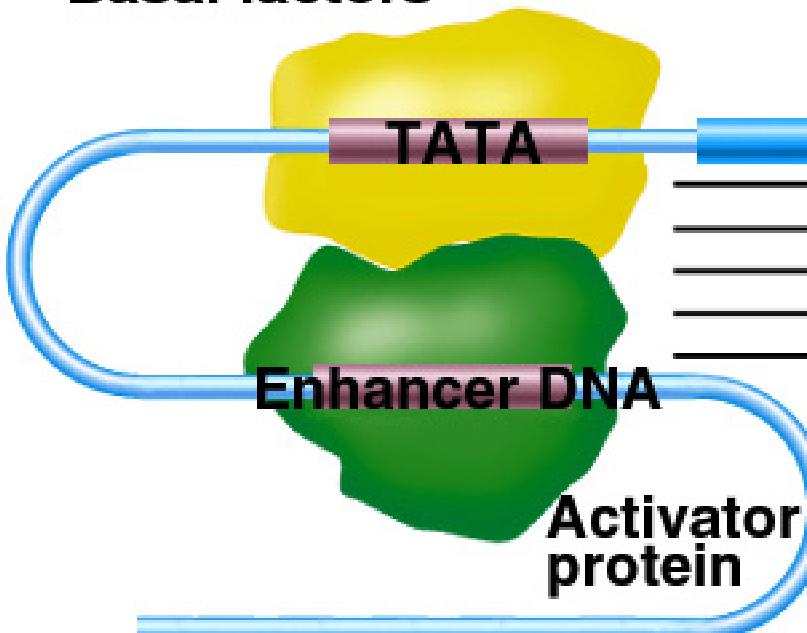
Basal factors

Enhancer DNA



Low-level transcription occurs with only basal factor bound to DNA.

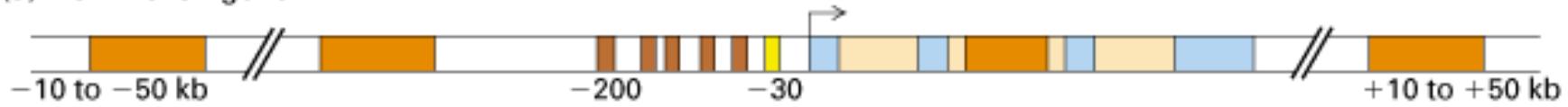
Basal factors



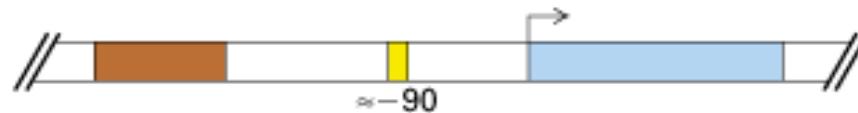
When basal factor and activator are bound to DNA, rate of transcription increases.

Cis-acting elements are DNA sequences that are linked to and involved in the transcription regulation of any given genes.

(a) Mammalian gene

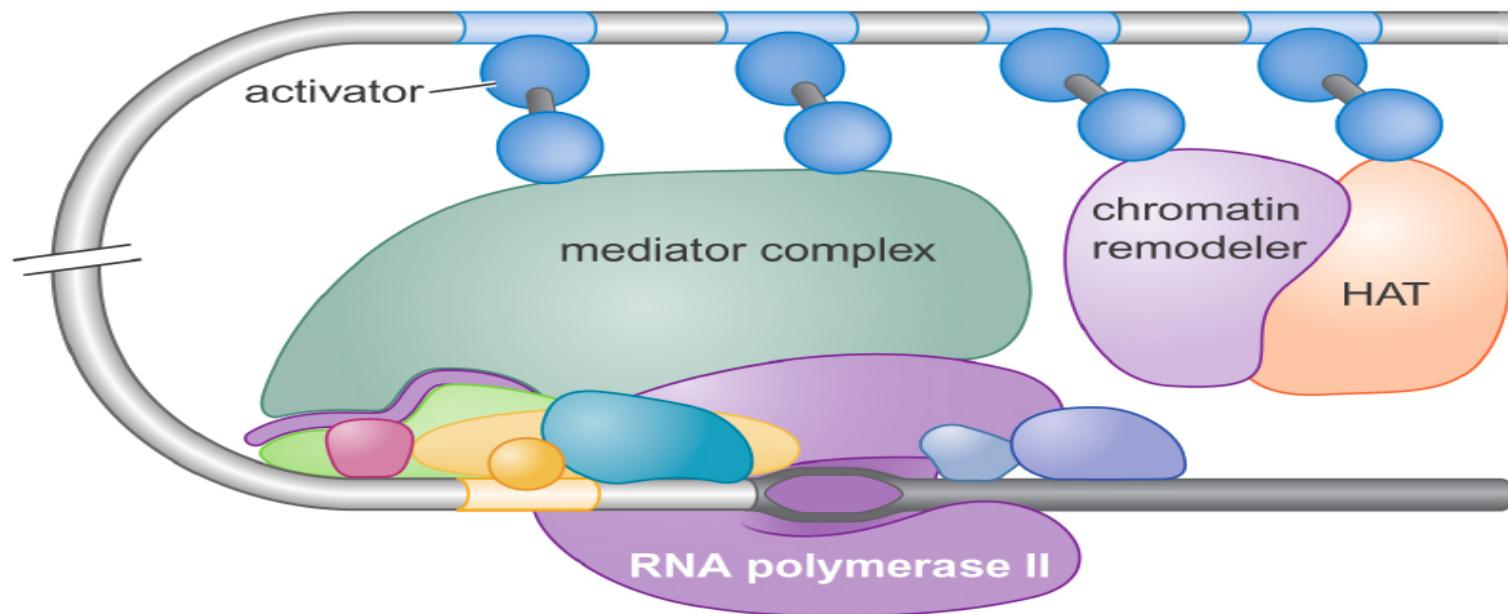


(b) *S. cerevisiae* gene



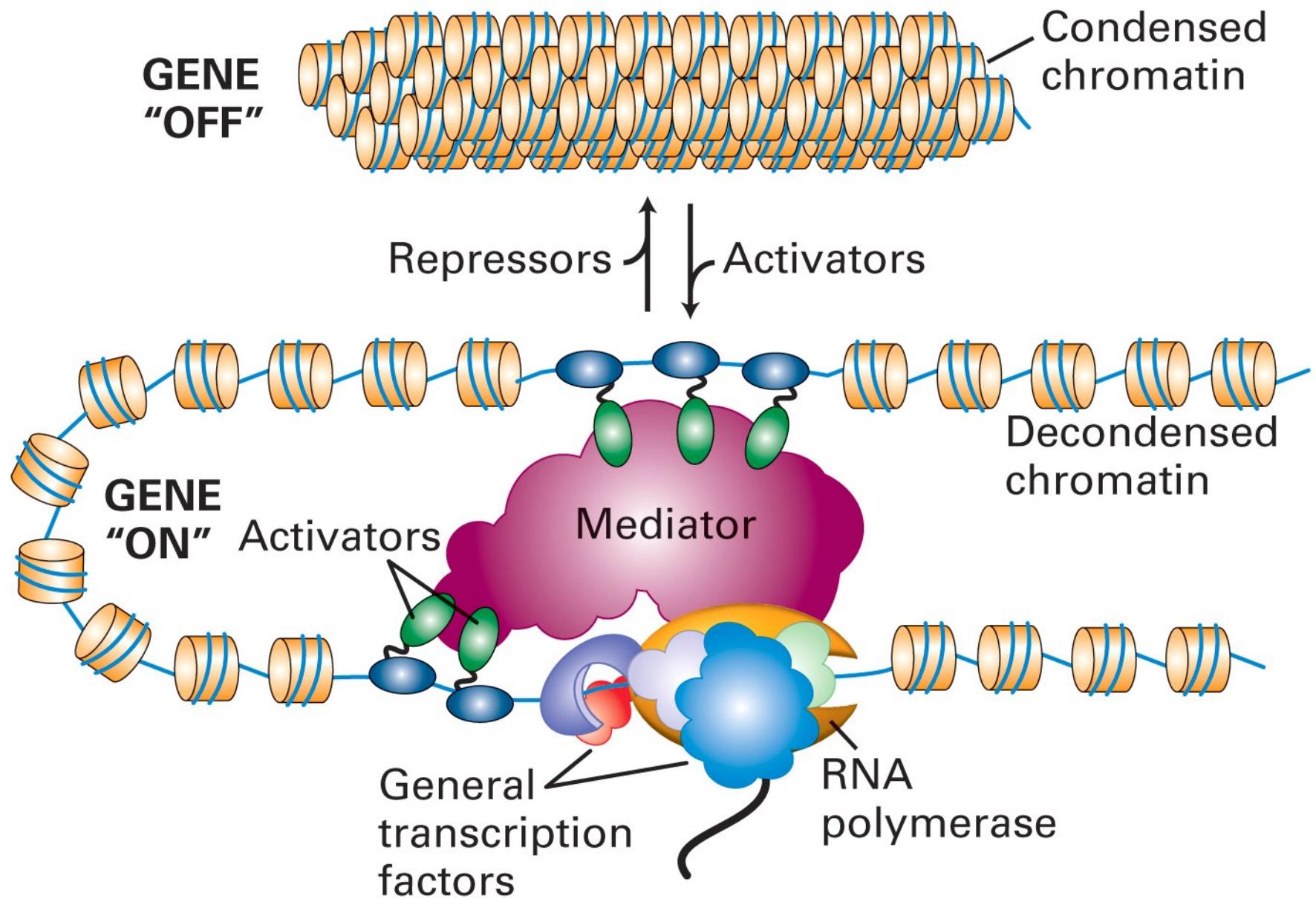
- Cis-acting elements of most eukaryotic genes include the TATA-box promoter, promoter proximal elements, and enhancers.
- Cis-acting DNA sequences only affect the gene adjacent to it (located in cis).

Trans-acting elements are proteins that bind to the *Cis* elements and regulate the transcription of any individual genes.



- Often called “transcription factors”.
- Diffusible within a cell.
- Same transcription factors can regulate the transcription of multiple gene through binding to their similar *cis* elements.

Multiple roles of transcription factors



Key Features of Transcription and RNA Polymerase

- RNA Polymerase does not need a primer. RNA polymerase can initiate transcription de novo.
- The RNA product gets displaced from the template DNA after only a few nucleotide addition. This ensures that multiple RNA polymerases can transcribe the same gene at the same time. This allows for synthesis of a large number of transcripts from a single gene/DNA sequence.
- Transcription is less accurate than replication (1 in 10 million) versus 1 in 10,000 for transcription. Proofreading is less efficient for RNA synthesis. This makes sense- any mutations occurring during DNA replication are potentially catastrophic!

A comparison between DNA replication and RNA transcription

	DNA replication	RNA transcription
template	DNA	DNA
direction	5' → 3'	5' → 3'
Bond formation	phosphodiester bond	phosphodiester bond
enzyme	DNA polymerase	RNA polymerase
Start from	replication origin	promoter
primer	needed	not needed
Proof reading	yes	No (less efficient)
Place of synthesis	nucleus	nucleus
Post-synthesis processing	no	Yes (eukaryote)*

Similarities between replication and transcription

- DNA replication and transcription are similar in a number of respects, starting with the central fact that both involve enzyme-mediated copying of a DNA template to create a new polynucleotide.
- Also, both reactions are carried out by complex molecular machines, containing multiple subunits, that carry out the diverse biochemical activities required for each process.

Differences between Replication and Transcription

- Transcription produces an RNA copy of the sequence, whereas replication produces a DNA copy.
- Replication generates a single copy of the entire genome, whereas transcription produces multiple copies of specific, limited sections of the genome. Another difference is that replication initiates at multiple locations that, in some cases at least (in particular multi-cellular eukaryotes) have flexible sequence requirements, whereas transcription begins (and stops) at very precise sequences.
- DNA replication requires a primer sequence, and the new DNA strand remains hybridized to the template, whereas transcription can begin de novo and the new RNA is displaced from the template. Finally, replication contains multiple proofreading mechanisms, giving rise to a very high level of accuracy, whereas transcription has fewer, less stringent methods of proofreading and is correspondingly less accurate.

Transcription= 1 in 10,000 nucleotides and 1 in 10 million for replication for errors

Implications and Critical Thinking-7

- DNA present *in vivo* differs from that used in *in vitro* systems because it is packaged into nucleosomes and higher order chromatin structures.
- These can interfere with the stable binding of polymerase and other transcription factors to the DNA, necessitating the presence of additional factors that can help promote polymerase binding.
- How does transcription proceed?