Транскрипция

Key Terms

The **coding strand (Sense strand)** of DNA has the same sequence as the mRNA and is related by the genetic code to the protein sequence that it represents.

The **antisense strand (Template strand)** of DNA is complementary to the sense strand, and is the one that acts as the template for synthesis of mRNA.

RNA polymerases are enzymes that synthesize RNA using a DNA template (formally described as DNA-dependent RNA polymerases).

A **promoter** is a region of DNA where RNA polymerase binds to initiate transcription.

Startpoint (startsite) (Startsite) refers to the position on DNA corresponding to the first base incorporated into RNA.

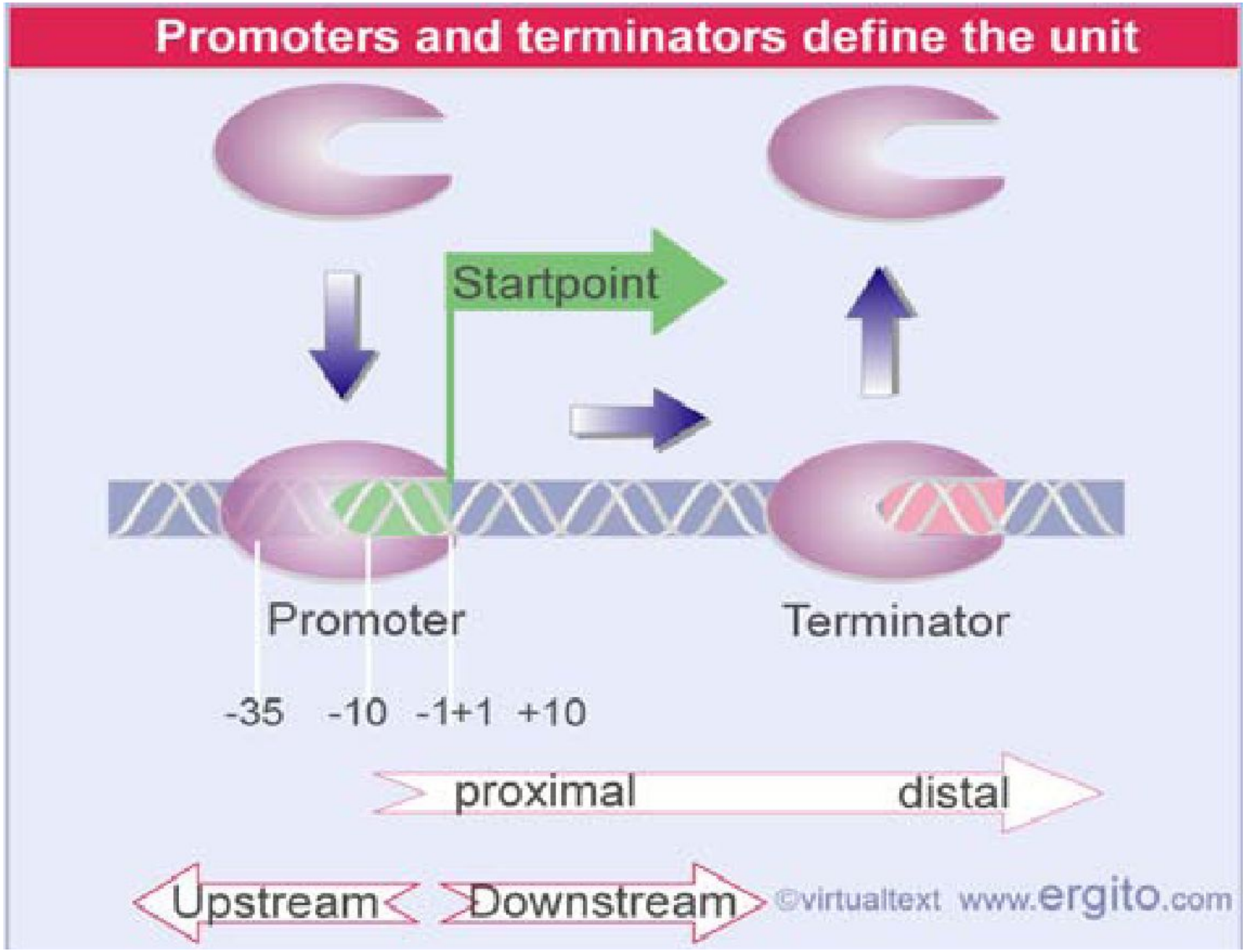
A **terminator** is a sequence of DNA that causes RNA polymerase to terminate transcription.

A **transcription unit** is the distance between sites of initiation and termination by RNA polymerase; may include more than one gene.

Upstream identifies sequences proceeding in the opposite direction from expression; for example, the bacterial promoter is upstream of the transcription unit, the initiation codon is upstream of the coding region.

Downstream identifies sequences proceeding farther in the direction of expression; for example, the coding region is downstream of the initiation codon.

A **primary transcript** is the original unmodified RNA product corresponding to a transcription unit.


Promoters and terminators define the unit
Startpoint
Promoter
Terminator
-35
-10
-1+1
+10
proximal
distal
Upstream
Downstream
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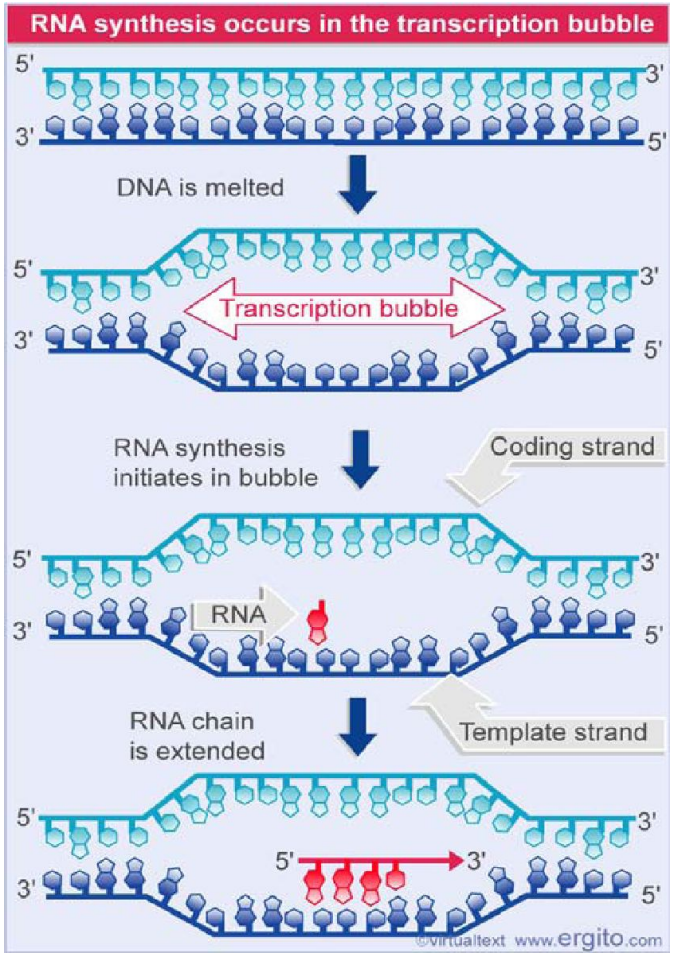
Transcription occurs by base pairing in a bubble of unpaired DNA

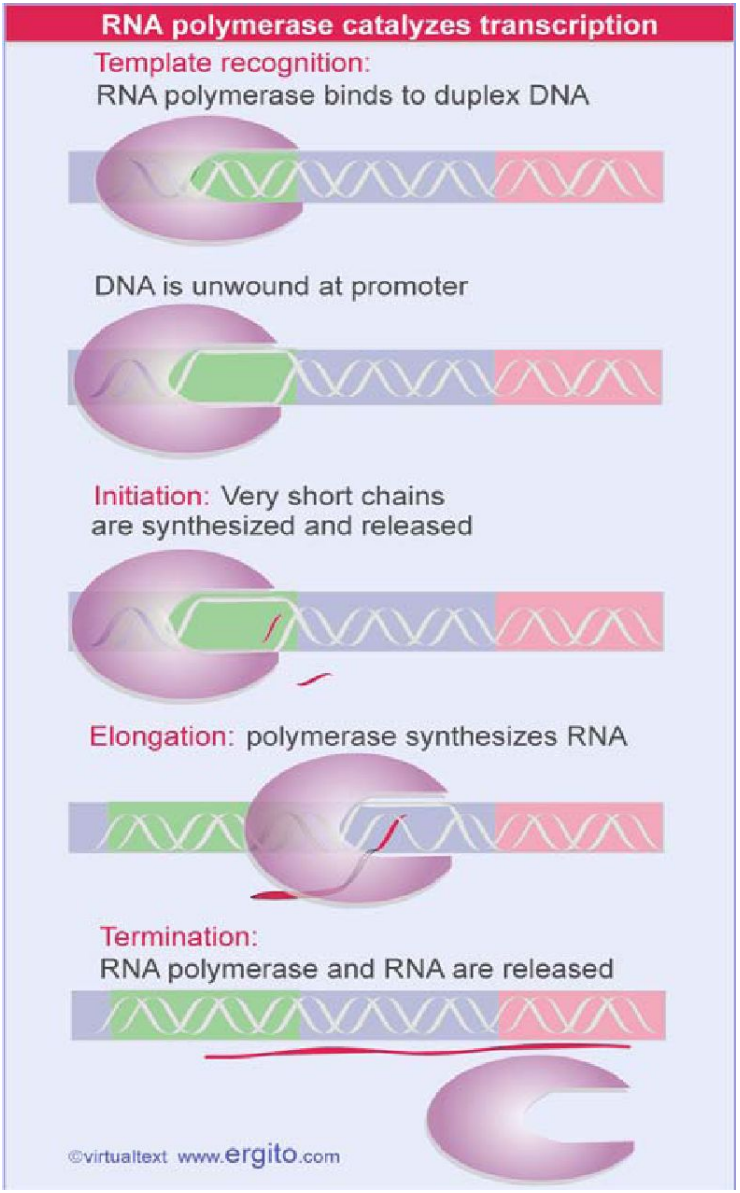
·RNA polymerase separates the two strands of DNA in a transient "bubble" and uses one strand as a template to direct synthesis of a complementary sequence of RNA.The length of the bubble is ~12-14 bp, and the length of RNA-DNA hybrid within it ~8-9 bp.



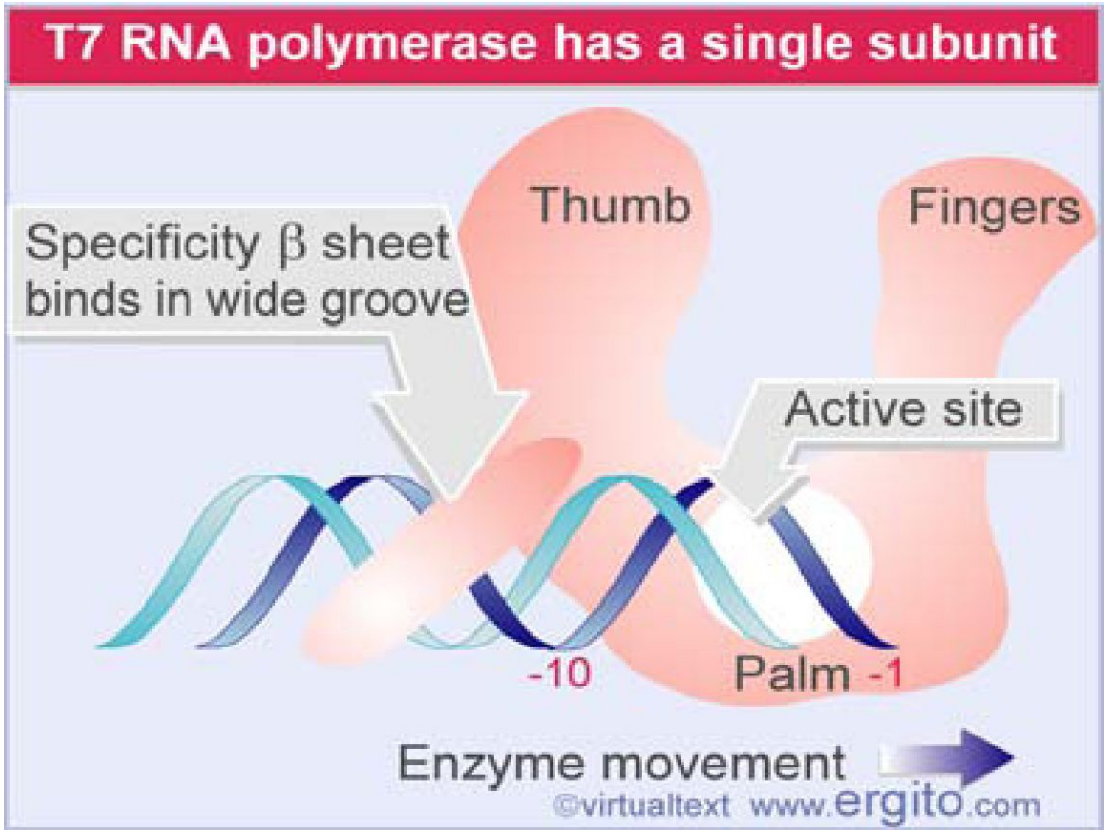
- **Initiation** describes the stages of transcription up to synthesis of the first bond in RNA. This includes binding of RNA polymerase to the promoter and melting a short region of DNA into single strands.

- **Elongation** is the stage in a macromolecular synthesis reaction (replication, transcription, or translation) when the nucleotide or polypeptide chain is being extended by the addition of individual subunits. During elongation the transcription bubble moves along DNA and the RNA chain is extended in the 5’ – 3’ direction.

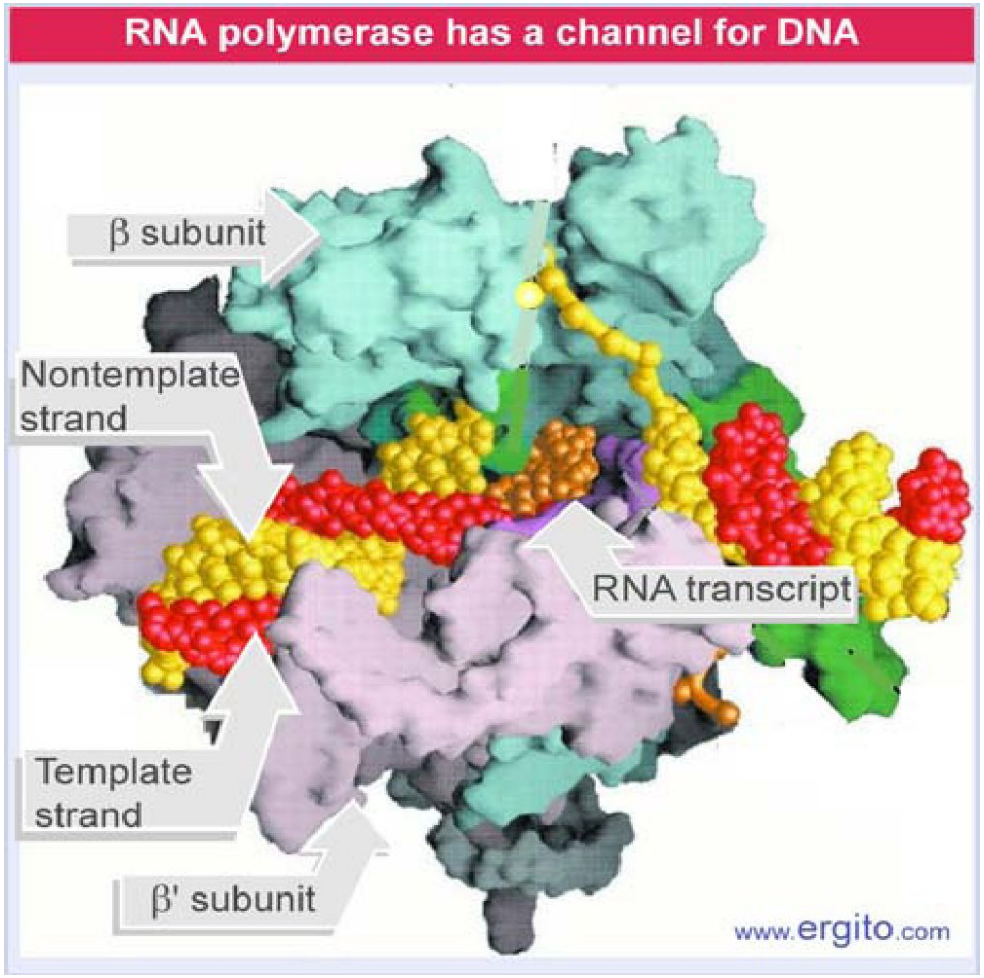
- **Termination** is a separate reaction that ends a macromolecular synthesis reaction (replication, transcription, or translation), by stopping the addition of subunits, and (typically) causing disassembly of the synthetic apparatus. Transcription stops, the DNA duplex reforms and RNA polymerase dissociates at a terminator site.



T7 RNA polymerase has a specificity loop that binds positions -7 to -11 of the promoter while positions -1 to -4 enter the active site.

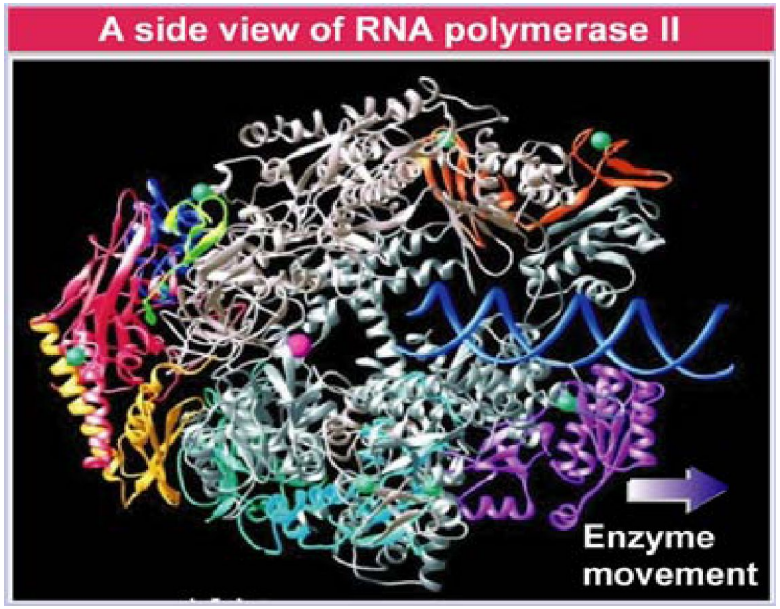


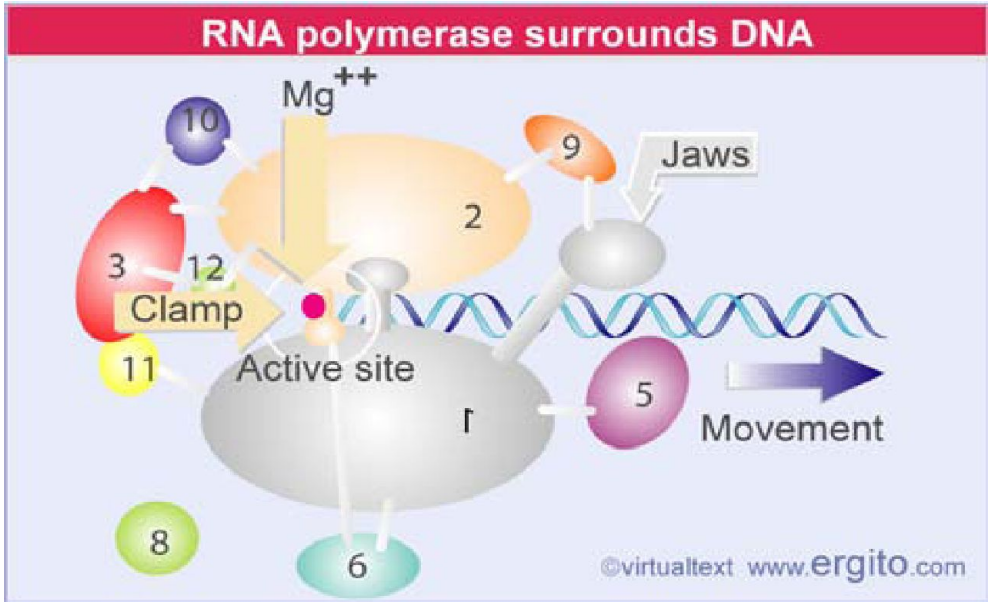
DNA moves through a groove in yeast RNA polymerase that makes a sharp turn at the active site. A protein bridge changes conformation to control the entry of nucleotides to the active site.



The b (cyan) and b' subunit (pink) of RNA polymerase have a channel for the DNA template. Synthesis of an RNA transcript (copper) has just begun; the DNA template (red) and coding (yellow) strands are separated in a transcription bubble.

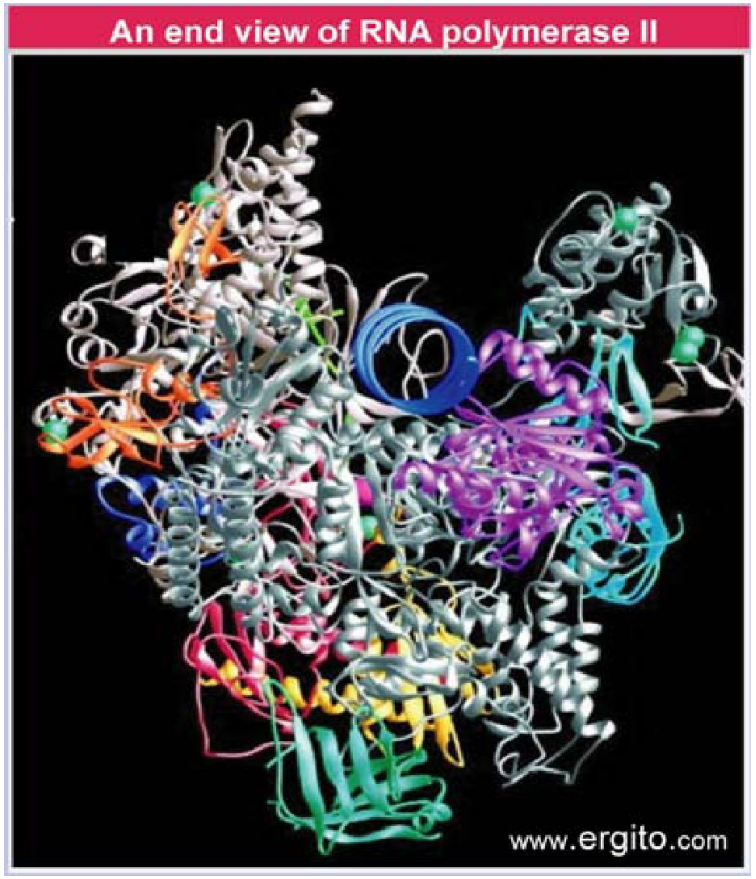
The side view of the crystal structure of RNA polymerase II from yeast shows that DNA is held downstream by a pair of jaws and is clamped in position in the active site, which contains an Mg++ ion.



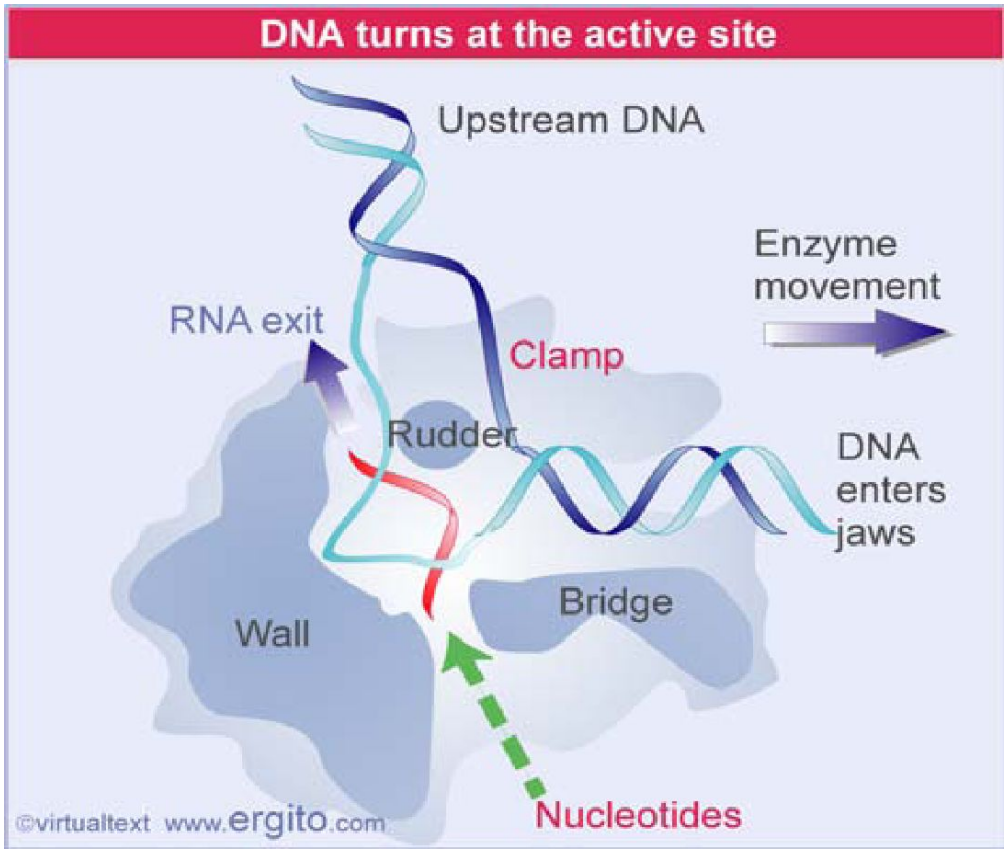


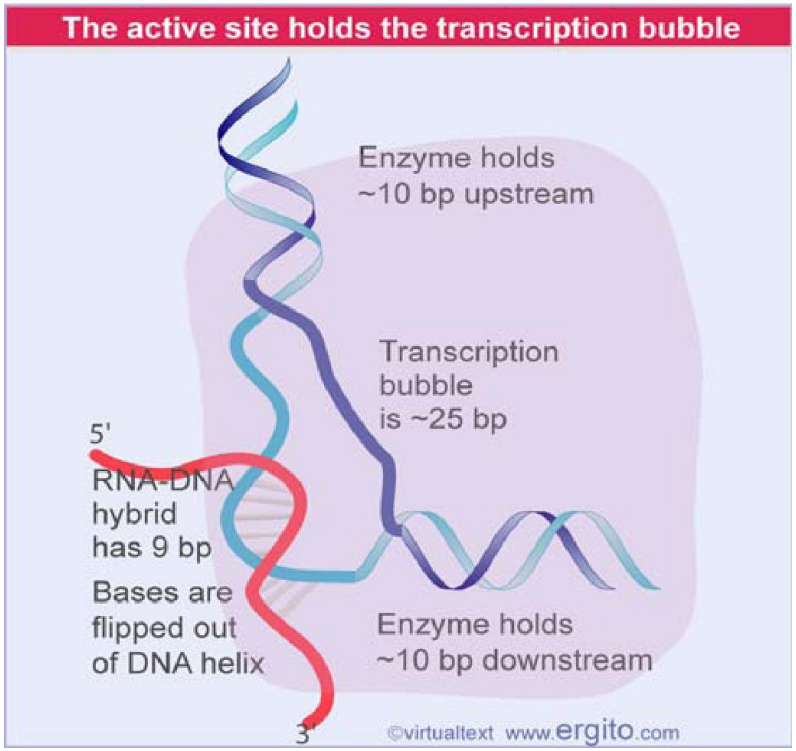
Ten subunits of RNA polymerase are placed in position from the crystal structure. The colors of the subunits are the same as in the crystal structures of the following figures.

The end view of the crystal structure of RNA polymerase II from yeast shows that DNA is surrounded by ~270° of protein.

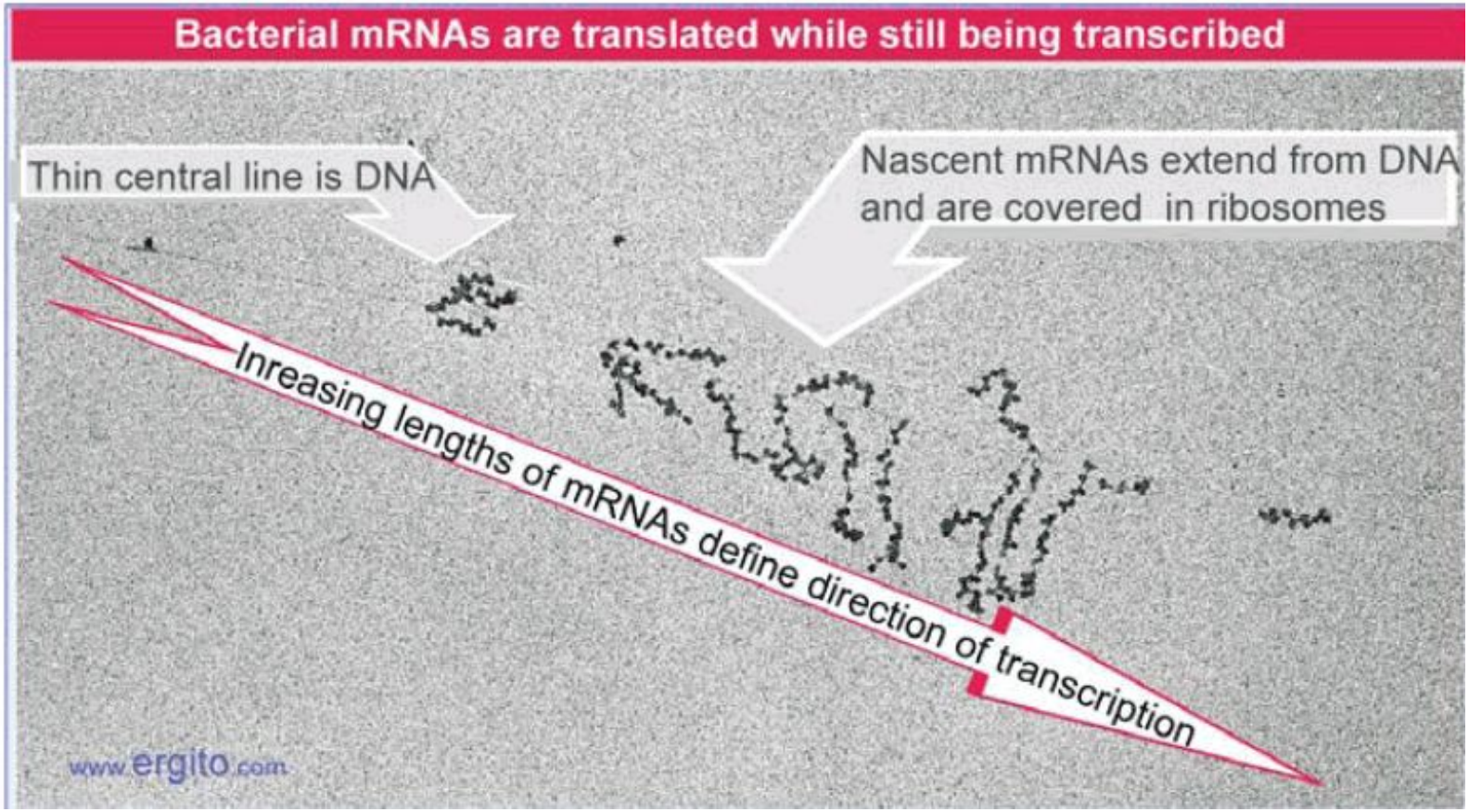


DNA is forced to make a turn at the active site by a wall of protein. Nucleotides may enter the active site through a pore in the protein





An expanded view of the active site shows the sharp turn in the path of DNA


Bacterial mRNAs are translated while still being transcribed
Thin central line is DNA
Nascent mRNAs extend from DNA and are covered in ribosomes
Inreasing lengths of mRNAs define direction of transcription
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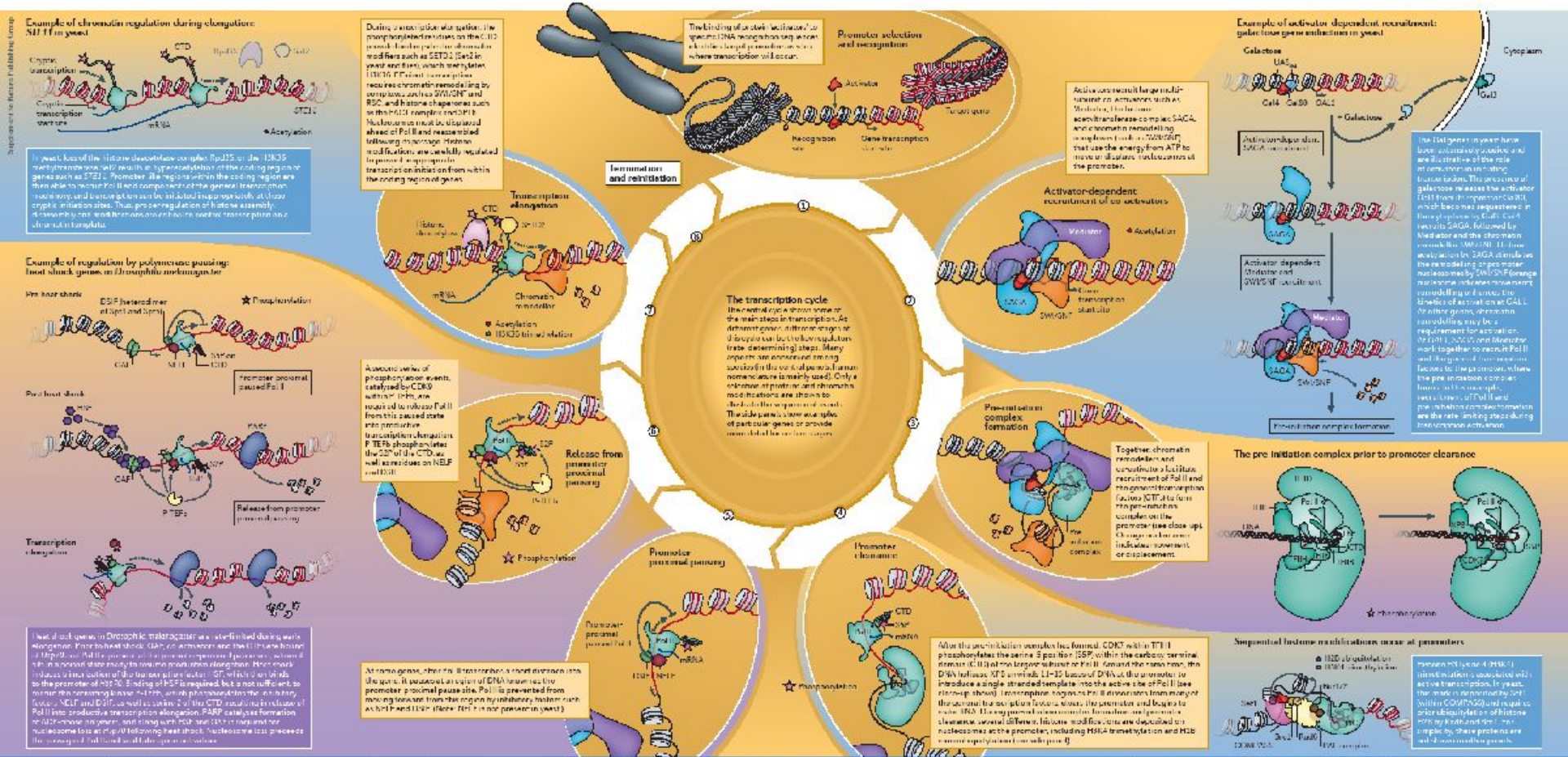
Chromatin remodelling and the transcription cycle

Vikki M. Weake and Jerry L. Workman

Transcription by RNA polymerase II (Pol II) occurs in the context of chromatin within a eukaryotic cell. Chromatin is generally inhibitory to transcription, so a variety of mechanisms are required to activate transcription from a nucleosomal template. One of the first steps is that large co-activator complexes interact with small activator proteins to identify gene promoters that are ready to be transcribed. Nucleosome remodelling complexes that use energy from ATP to move or displace nucleosomes from DNA facilitate the recruitment and assembly of these complexes on the promoter and enable rapid gene activation. Even during transcription elongation, nucleosomes must be removed for efficient passage of the polymerase. Furthermore, these same nucleosomes must be reassembled rapidly and modified appropriately following passage of the polymerase to prevent inappropriate initiation of transcription from promoter-like elements within the coding region.



References

Park, P. J., Arnsdorf, M. F. & Lis, J. T. Defining mechanisms that regulate RNA polymerase II transcription in vivo. Nature 361, 370–132 (1993). Weake, V. W. & Workman, J. L. Inducible gene expression: diverse regulatory mechanisms. Nature Rev. Genet. 33, 426–437 (2010). Kornberg, R. D. The molecular basis of eukaryotic transcription. Proc. Natl Acad. Sci. USA 104, 12955–12961 (2007). Orphanides, G. & Reinberg, D. A unified theory of gene expression. Cell 108, 439–451 (2002). Pavletich, N. P. & Pabo, C. O. Crystal structure of a five-finger GLI-DNA recognition complex: new perspectives on zinc fingers. Science 261, 1701–1707 (1993). Roeder, R. G. Transcriptional regulation and the role of diverse coactivators in animal cells. FEBS Lett. 579, 909–915 (2005).

Abbreviations

CTD, carboxy-terminal domain; COMPASS, complex proteins associated with Set1; DSIF, DRB sensitivity inducing factor; GAF, GAGA factor; HSF, heat shock factor; HSP, heat shock protein; IN, NELF, negative elongation factor; PARP, poly(ADP) ribose polymerase; P-TEFb, positive transcription elongation factor b; SWI/SNF, switch/sucrose non fermentable; TBP, TATA box binding protein; TFII, transcription factor II; TrxG, transcriptionally associated protein 1; UAS, upstream activating sequence

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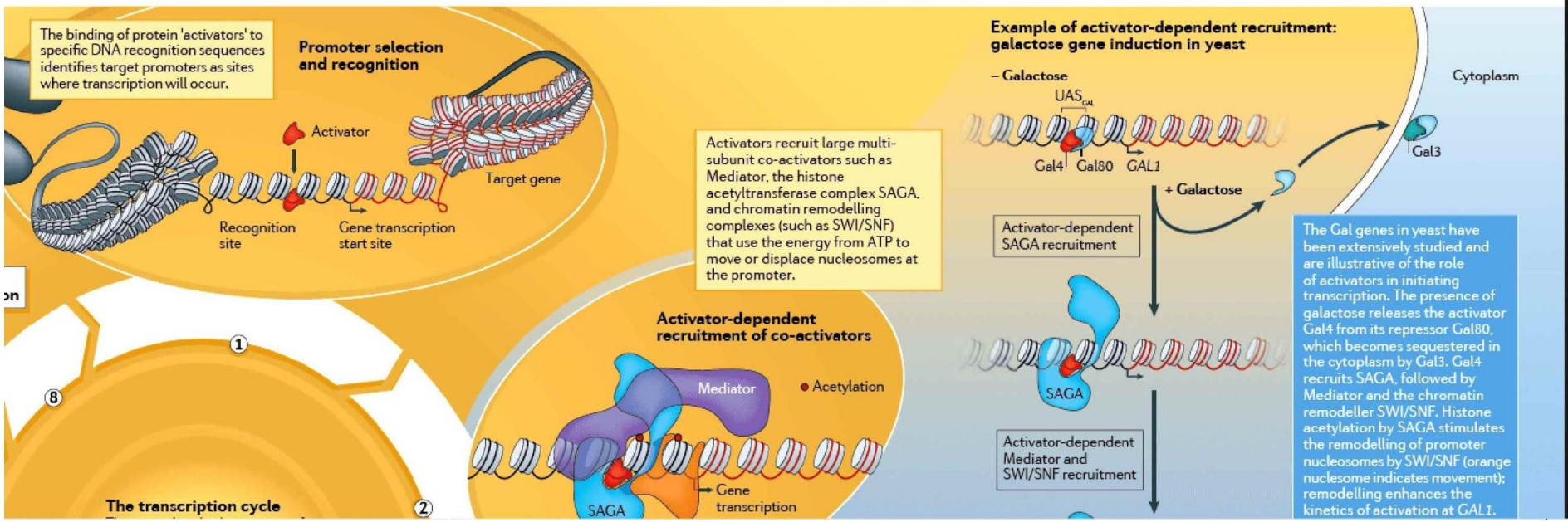
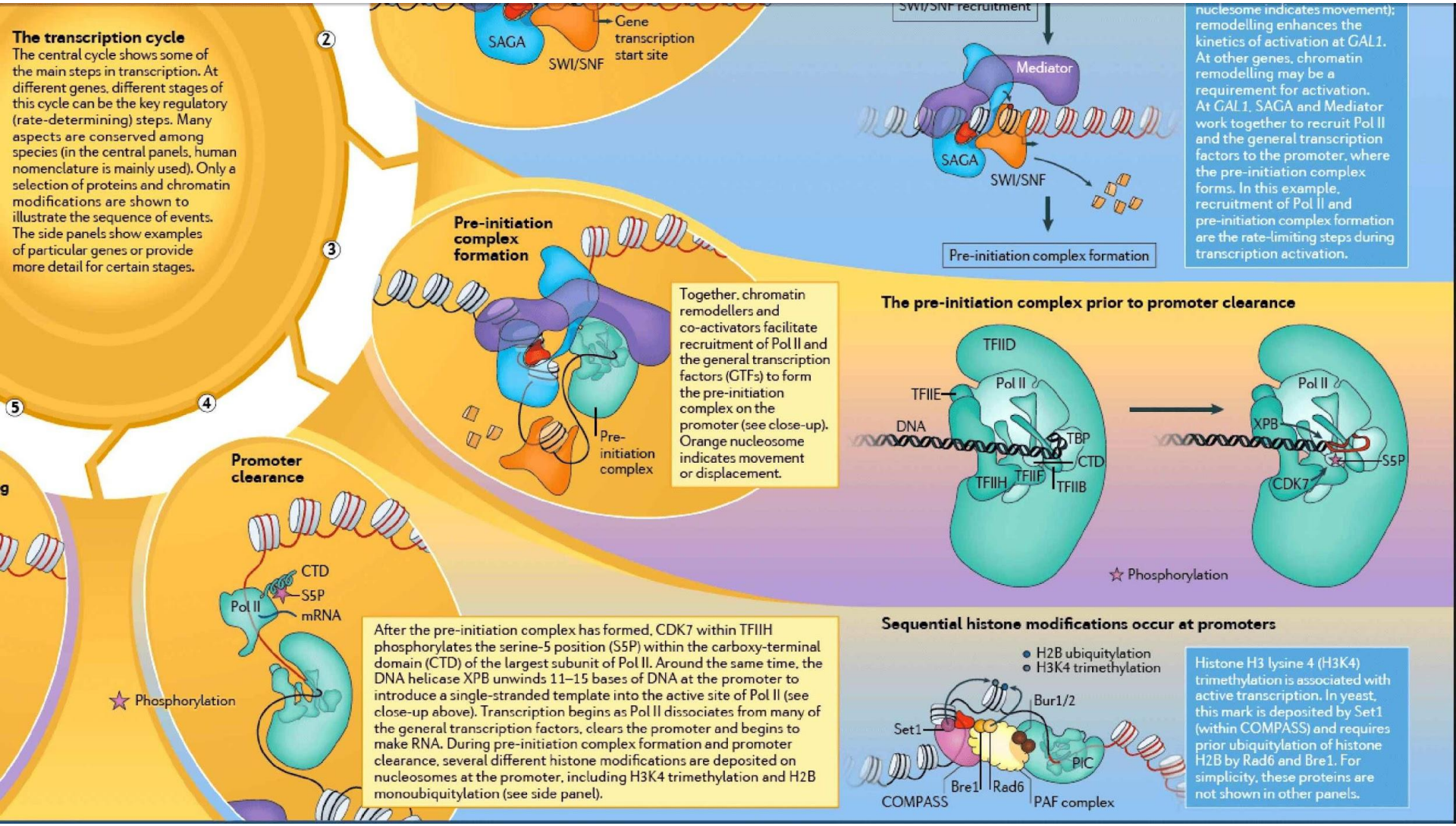
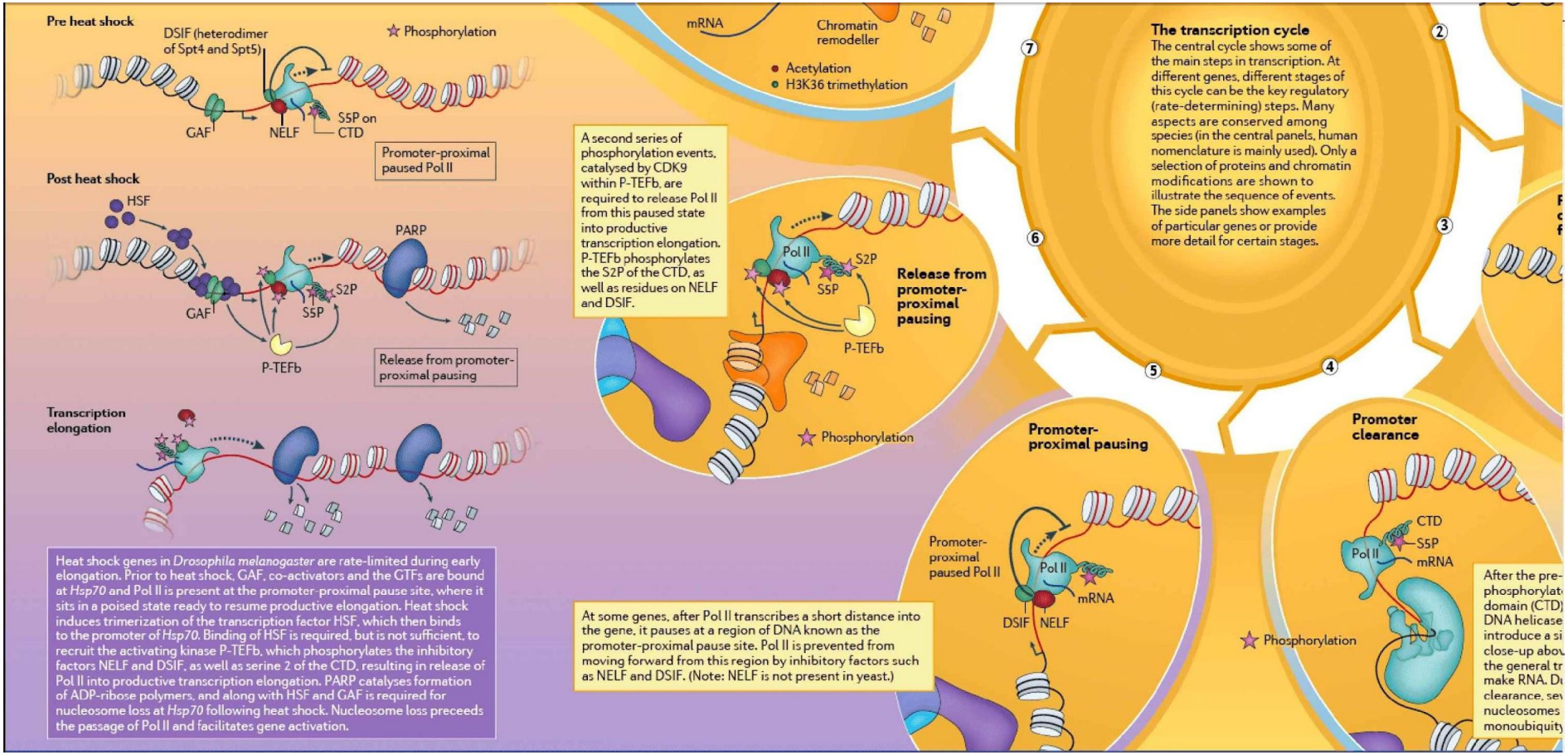



The transcription cycle
The central cycle shows some of the main steps in transcription. At different genes, different stages of this cycle can be the key regulatory (rate-determining) steps. Many aspects are conserved among species (in the central panels, human nomenclature is mainly used). Only a selection of proteins and chromatin modifications are shown to illustrate the sequence of events. The side panels show examples of particular genes or provide more detail for certain stages.
2
3
4
5
SAGA
SWI/SNF
Gene transcription start site
Pre-initiation complex formation
Pre-initiation complex
Together, chromatin remodellers and co-activators facilitate recruitment of Pol II and the general transcription factors (GTFs) to form the pre-initiation complex on the promoter (see close-up). Orange nucleosome indicates movement or displacement.
Promoter clearance
CTD
S5P
Pol II
mRNA
Phosphorylation
After the pre-initiation complex has formed, CDK7 within TFIIH phosphorylates the serine-5 position (S5P) within the carboxy-terminal domain (CTD) of the largest subunit of Pol II. Around the same time, the DNA helicase XPB unwinds 11–15 bases of DNA at the promoter to introduce a single-stranded template into the active site of Pol II (see close-up above). Transcription begins as Pol II dissociates from many of the general transcription factors, clears the promoter and begins to make RNA. During pre-initiation complex formation and promoter clearance, several different histone modifications are deposited on nucleosomes at the promoter, including H3K4 trimethylation and H2B monoubiquitylation (see side panel).
SWI/SNF recruitment
Mediator
SAGA
SWI/SNF
Pre-initiation complex formation
nucleosome indicates movement); remodelling enhances the kinetics of activation at GAL1. At other genes, chromatin remodelling may be a requirement for activation. At GAL1, SAGA and Mediator work together to recruit Pol II and the general transcription factors to the promoter, where the pre-initiation complex forms. In this example, recruitment of Pol II and pre-initiation complex formation are the rate-limiting steps during transcription activation.
The pre-initiation complex prior to promoter clearance
TFIID
Pol II
TFIIE
DNA
TBP
CTD
TFIIH
TFIIF
TFIIB
XPB
S5P
CDK7
Phosphorylation
Sequential histone modifications occur at promoters
H2B ubiquitylation
H3K4 trimethylation
Bur1/2
Set1
PIC
Bre1
Rad6
COMPASS
PAF complex
Histone H3 lysine 4 (H3K4) trimethylation is associated with active transcription. In yeast, this mark is deposited by Set1 (within COMPASS) and requires prior ubiquitylation of histone H2B by Rad6 and Bre1. For simplicity, these proteins are not shown in other panels.


Pre heat shock
DSIF (heterodimer of Spt4 and Spt5)
Phosphorylation
GAF
NELF
S5P on CTD
Promoter-proximal paused Pol II
Post heat shock
HSF
PARP
GAF
S2P
S5P
P-TEFb
Release from promoter-proximal pausing
Transcription elongation
Heat shock genes in Drosophila melanogaster are rate-limited during early elongation. Prior to heat shock, GAF, co-activators and the GTFs are bound at Hsp70 and Pol II is present at the promoter-proximal pause site, where it sits in a poised state ready to resume productive elongation. Heat shock induces trimerization of the transcription factor HSF, which then binds to the promoter of Hsp70. Binding of HSF is required, but is not sufficient, to recruit the activating kinase P-TEFb, which phosphorylates the inhibitory factors NELF and DSIF, as well as serine 2 of the CTD, resulting in release of Pol II into productive transcription elongation. PARP catalyses formation of ADP-ribose polymers, and along with HSF and GAF is required for nucleosome loss at Hsp70 following heat shock. Nucleosome loss preceeds the passage of Pol II and facilitates gene activation.
mRNA
Chromatin remodeller
Acetylation
H3K36 trimethylation
A second series of phosphorylation events, catalysed by CDK9 within P-TEFb, are required to release Pol II from this paused state into productive transcription elongation. P-TEFb phosphorylates the S2P of the CTD, as well as residues on NELF and DSIF.
Pol II
S2P
S5P
P-TEFb
Release from promoter-proximal pausing
Phosphorylation
The transcription cycle
The central cycle shows some of the main steps in transcription. At different genes, different stages of this cycle can be the key regulatory (rate-determining) steps. Many aspects are conserved among species (in the central panels, human nomenclature is mainly used). Only a selection of proteins and chromatin modifications are shown to illustrate the sequence of events. The side panels show examples of particular genes or provide more detail for certain stages.
1
2
3
4
5
6
7
Promoter-proximal pausing
Promoter-proximal paused Pol II
Pol II
mRNA
DSIF
NELF
At some genes, after Pol II transcribes a short distance into the gene, it pauses at a region of DNA known as the promoter-proximal pause site. Pol II is prevented from moving forward from this region by inhibitory factors such as NELF and DSIF. (Note: NELF is not present in yeast.)
Promoter clearance
CTD
Pol II
S5P
mRNA
Phosphorylation
After the pre-
phosphorylat
domain (CTD)
DNA helicase
introduce a si
close-up abov
the general tr
make RNA. Du
clearance, sev
nucleosomes
monoubiquity

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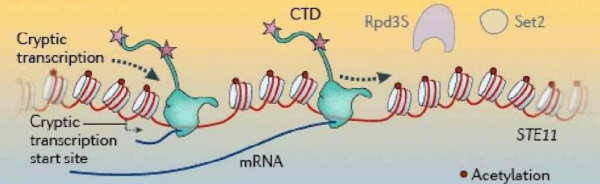
Example of chromatin regulation during elongation: *STE11* in yeast



In yeast, loss of the histone deacetylase complex Rpd3S, or the H3K36 methyltransferase Set2 results in hyperacetylation of the coding region of genes such as *STE11*. Promoter-like regions within the coding region are then able to recruit Pol II and components of the general transcription machinery, and transcription can be initiated inappropriately at these cryptic initiation sites. Thus, proper regulation of histone assembly, disassembly and modifications are critical to control transcription on a chromatin template.

Example of regulation by polymerase pausing: heat shock genes in *Drosophila melanogaster*

Pre heat shock

DSIF (heterodimer

Phosphorylation

During transcription elongation, the phosphorylated residues on the CTD provide binding sites for chromatin modifiers such as SETD2 (Set2 in yeast and flies), which methylates H3K36. Efficient transcription requires chromatin remodelling by complexes such as SWI/SNF and RSC, and histone chaperones such as the FACT complex and SPT6. Nucleosomes must be displaced ahead of Pol II and reassembled following its passage. Histone modifications are carefully regulated to prevent inappropriate transcription initiation from within the coding region of genes.