Chapter 9

Transcriptional regulation and epigenetics

Lecture 11

Gene regulation in *E. coli*

Learning objectives

- Explain how lactose regulates transcription of the *lac* operon.
- Distinguish between positive and negative control
- Explain why repressors inhibit but activators stimulate transcription.

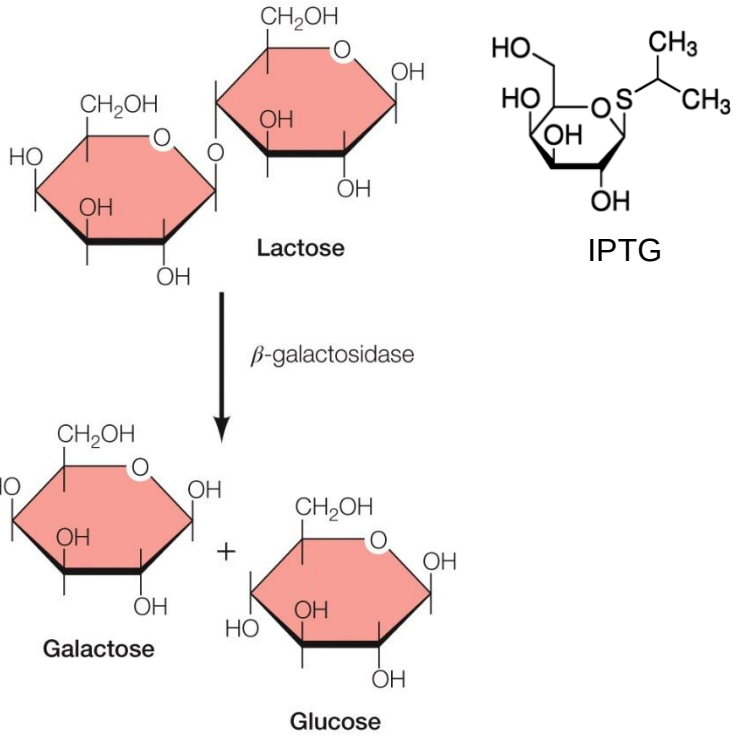
Operon

; a set of genes that is transcribed into a single mRNA in prokaryotes

Lac operon

Lactose induces the synthesis of enzymes involved in its own metabolism

- **β-galactosidase** catalyzes the hydrolysis of lactose to galactose and glucose
- **Lactose permease** transports lactose into the cell
- **Transacetylase** inactivates toxic thiogalactosides that are transported into the cell along with lactose by the permease

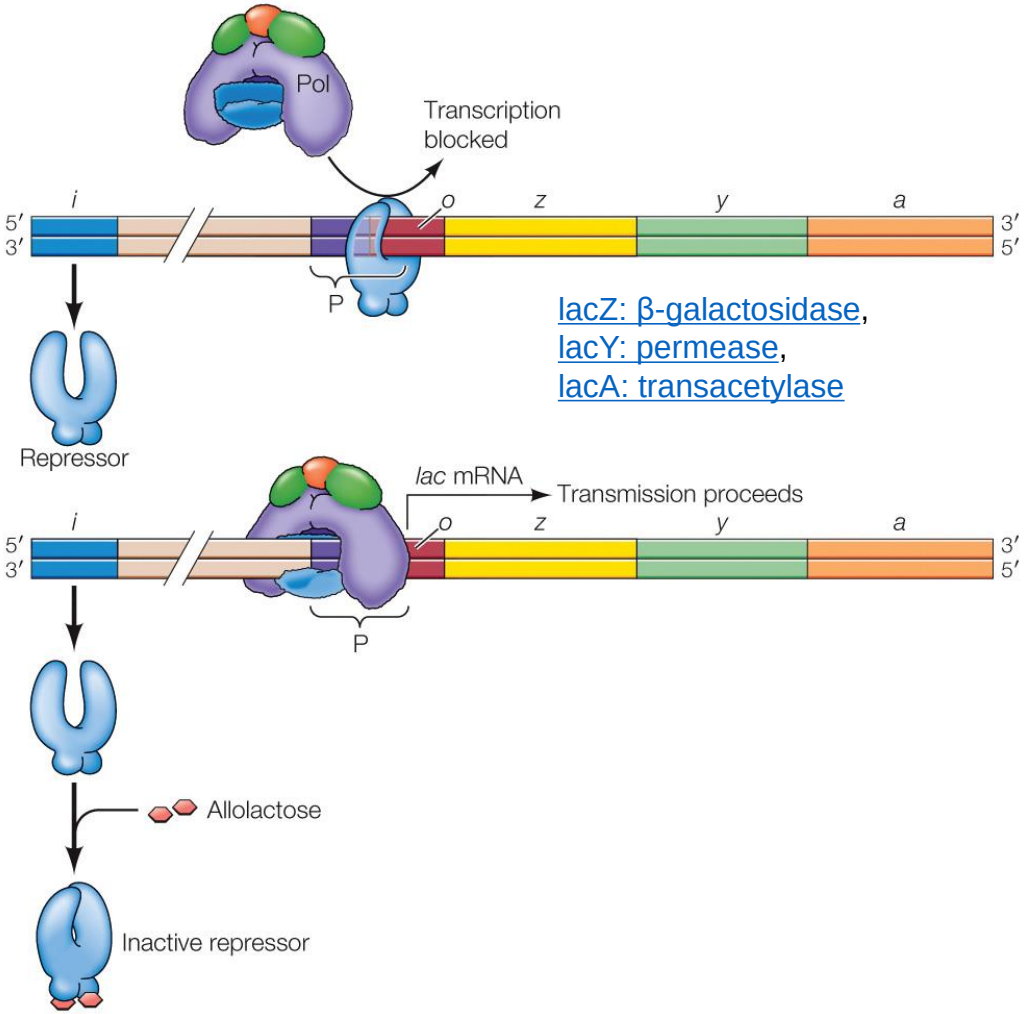


Negative control of the *lac* operon

i : operator에 결합하여 전사를 억제하는 repressor 유전자

o : repressor가 결합하는 전사개시부위 근처의 cis-acting control element

; Repressor의 결합을 통하여 유전자의 발현을 조절(억제): negative control



Positive control of the lac operon by glucose

<u>Glucose가 없는 조건에서만 lac operon이 발현됨</u>

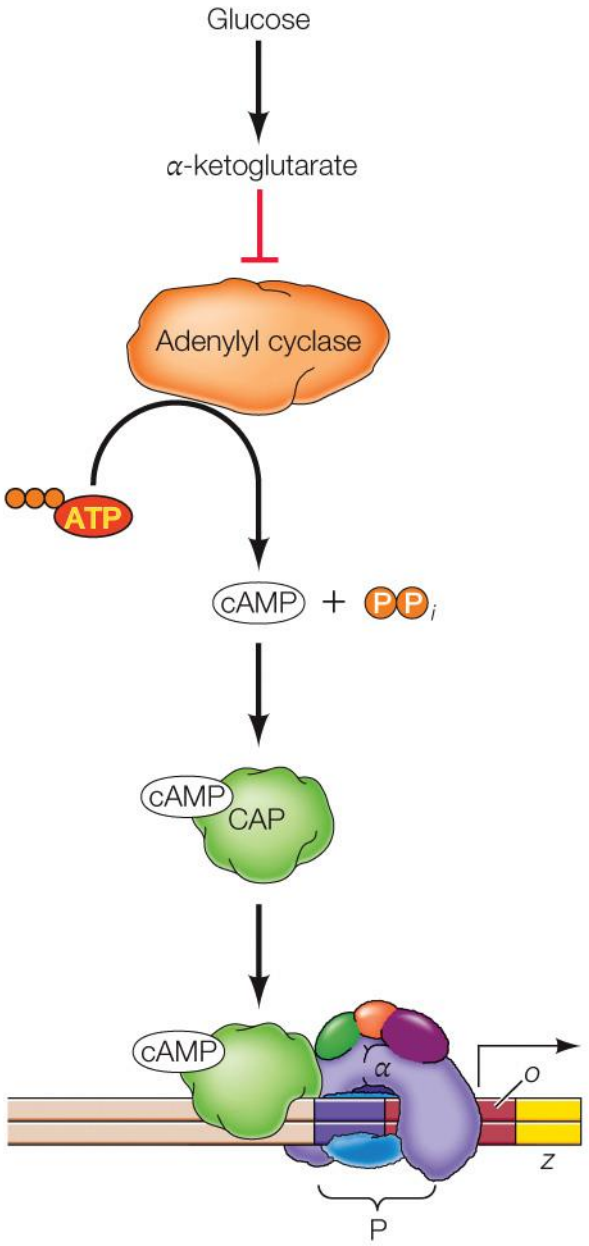
- Glucose 대사물질 α-ketoglutarate는 adenylyl cyclase를 저해함
- Glucose가 없는 조건에서 cAMP 양 증가

→ cAMP는 CAP(catabolite activator protein) 활성화

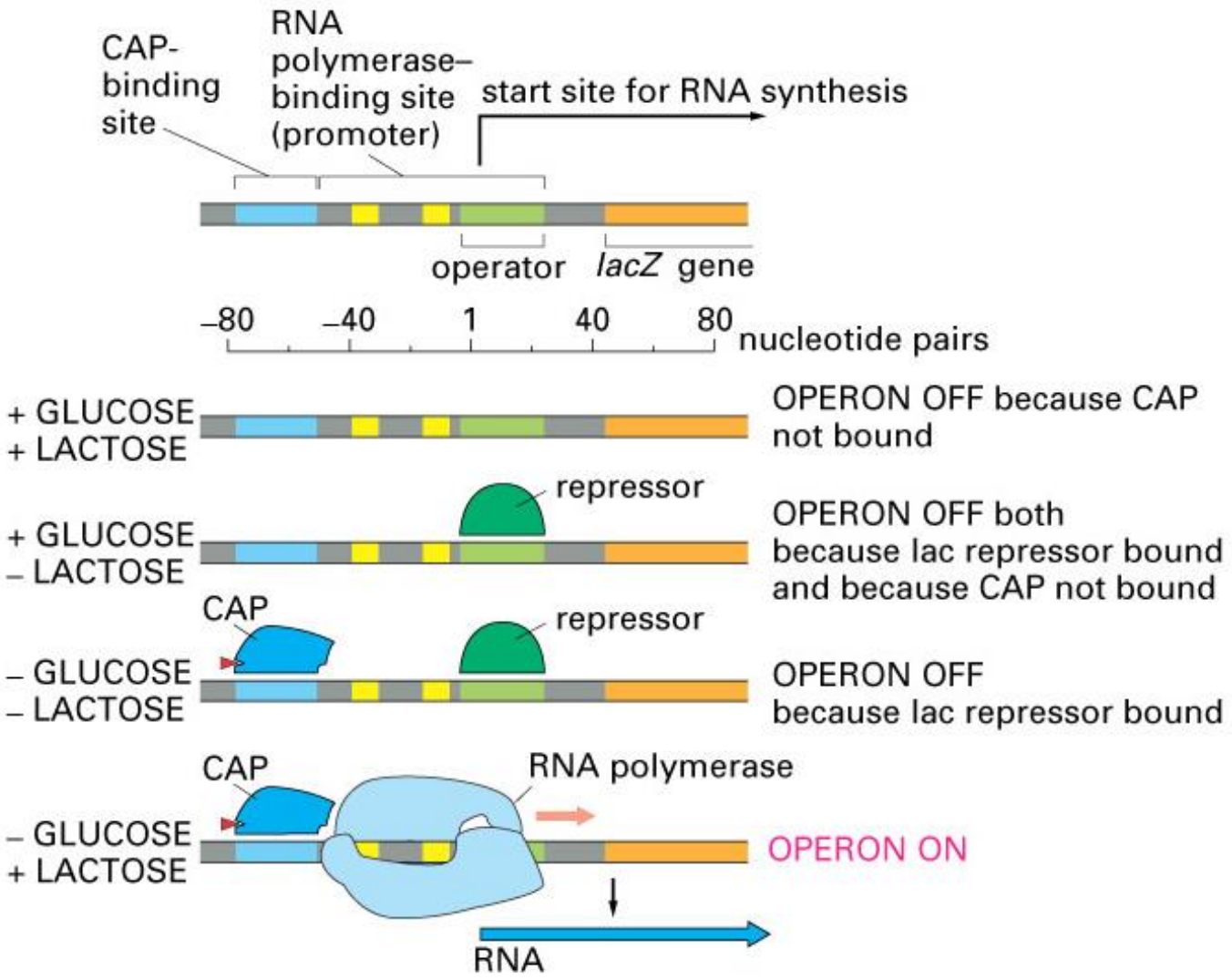
→ CAP이 DNA에 결합, RNA pol의 promoter 결합 촉진

→ Lac operon의 전사(단, lactose가 있어 repressor가 operator에 결합하지 않는 경우)

→Activator의 결합에 의한 유전자 발현의 positive regulation



Positive and negative regulation of lac operon



Transcription factors in eukaryotes

Learning objectives

- Compare and contrast promoters and enhancers
- Summarize the experimental approaches used to study the binding of transcription factors to DNA
- Describe how activators and repressors affect transcription
- Explain how transcriptional elongation is controlled

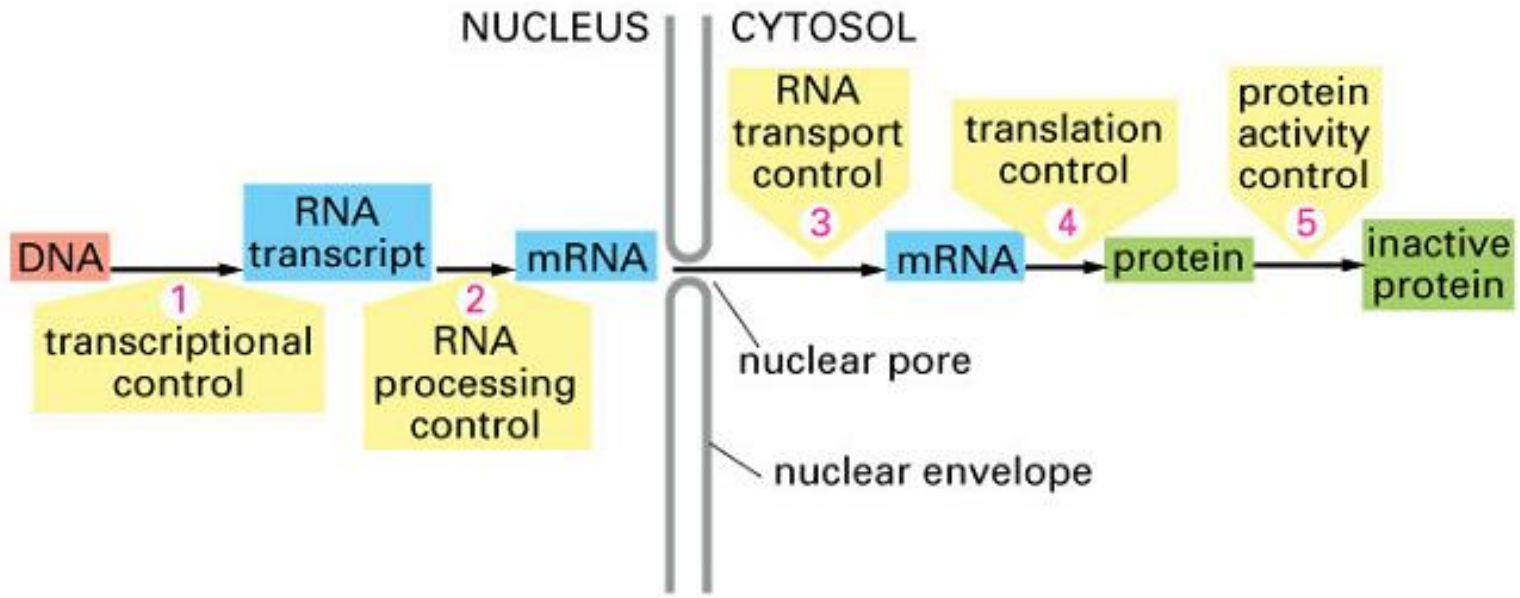
Regulation of transcription in eukaryotes

The expression of eukaryotic genes is controlled primarily at the level of initiation of transcription.

1. Transcription factors that bind to specific regulatory sequences and modulate the activity of RNA polymerase.

2. Packaging of eukaryotic DNA into chromatin limits its availability as a template for transcription

→ Modification of chromatin structure play key roles in the control of transcription in eukaryotic cells.



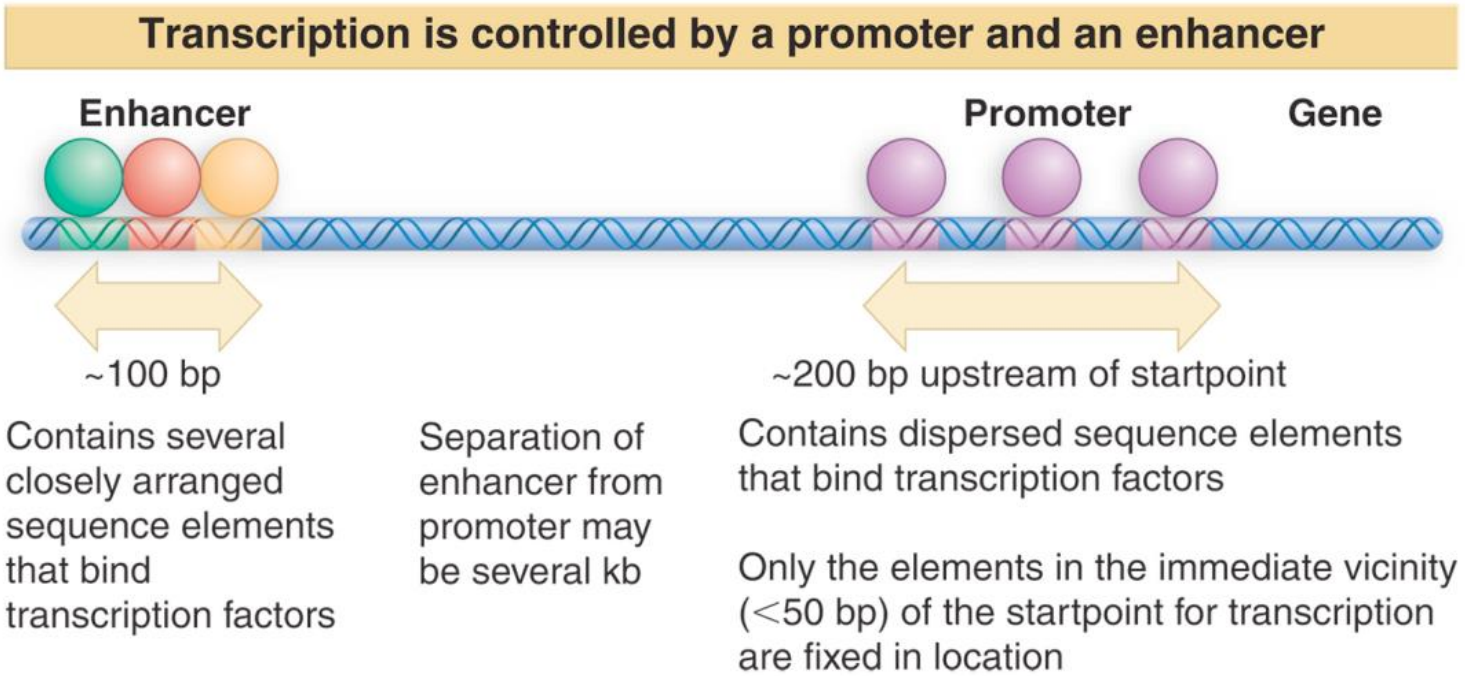
Cis-acting regulatory sequences: promoters and enhances

Promoters:

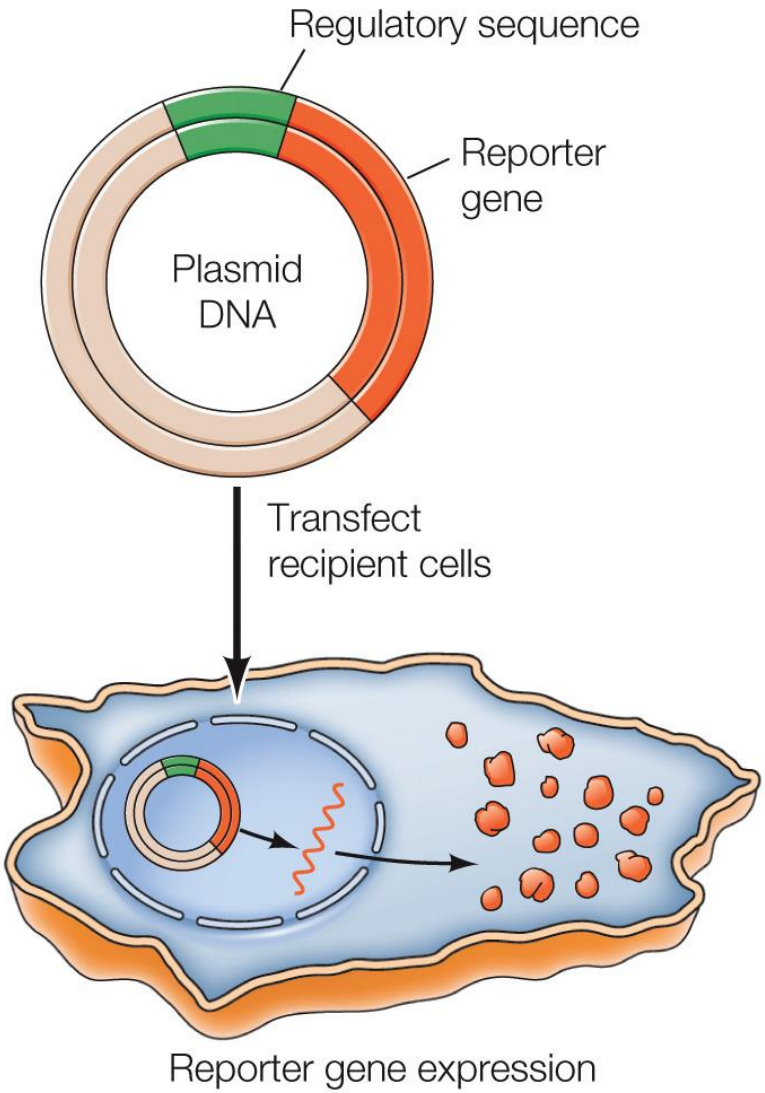
- a region of DNA that is required to initiate transcription of a particular gene.
- The promoter contains “several” short (<10 bp) sequence elements
- bind transcription factors
- dispersed over >200 bp

Enhancers

- An array short (<10 bp) sequence elements
- also bind transcription factors
- may be located several to many kb distant.



Identification of eukaryotic regulatory sequences : Reporter assay



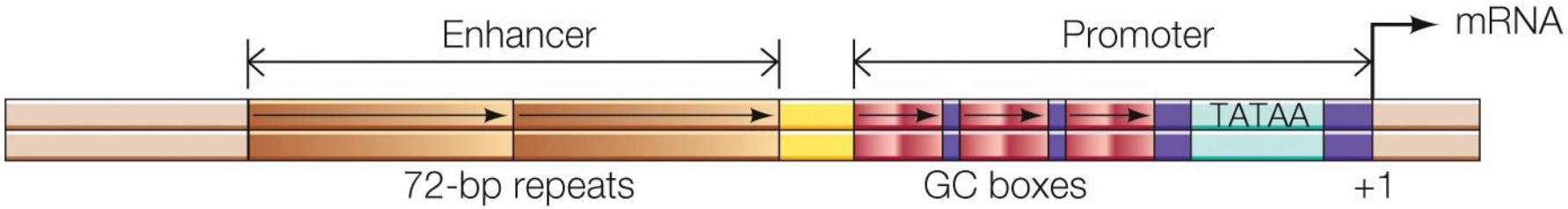
HSV TK promoter

Two *cis*-acting regulatory sequences were identified by studies of the promoter of the herpes simplex virus gene that encodes thymidine kinase.

- 1 CCAAT box: CCAAT-enhancer-binding protein (C/EBP)
- 2 GC boxes: Specificity protein 1 (Sp1)

The SV40 enhancer

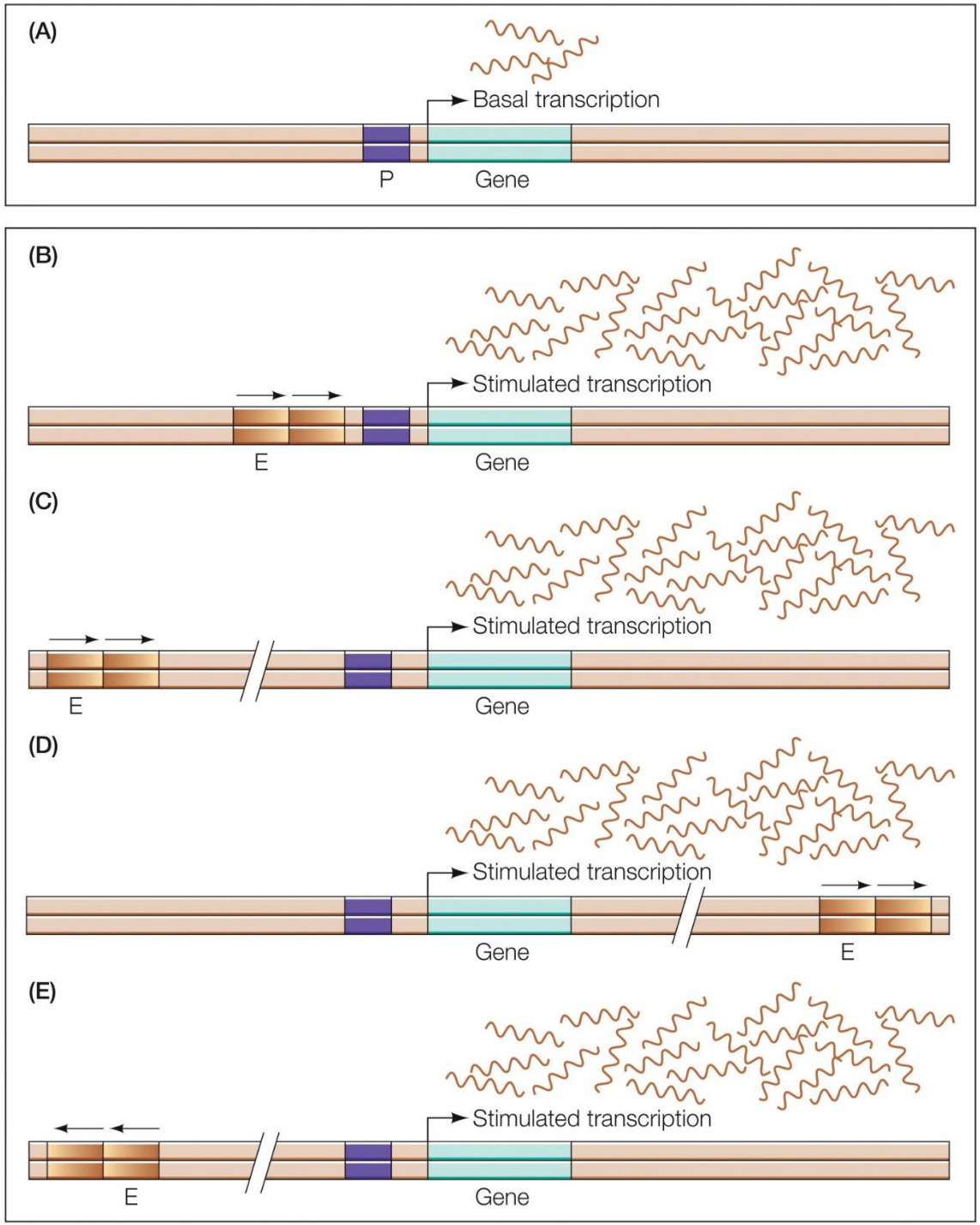
- SV40 early promoter: 1 TATA box and 6 GC boxes arranged in 3 sets of repeated sequences
- Enhancer consisting of two 72-bp repeats



Action of enhancers

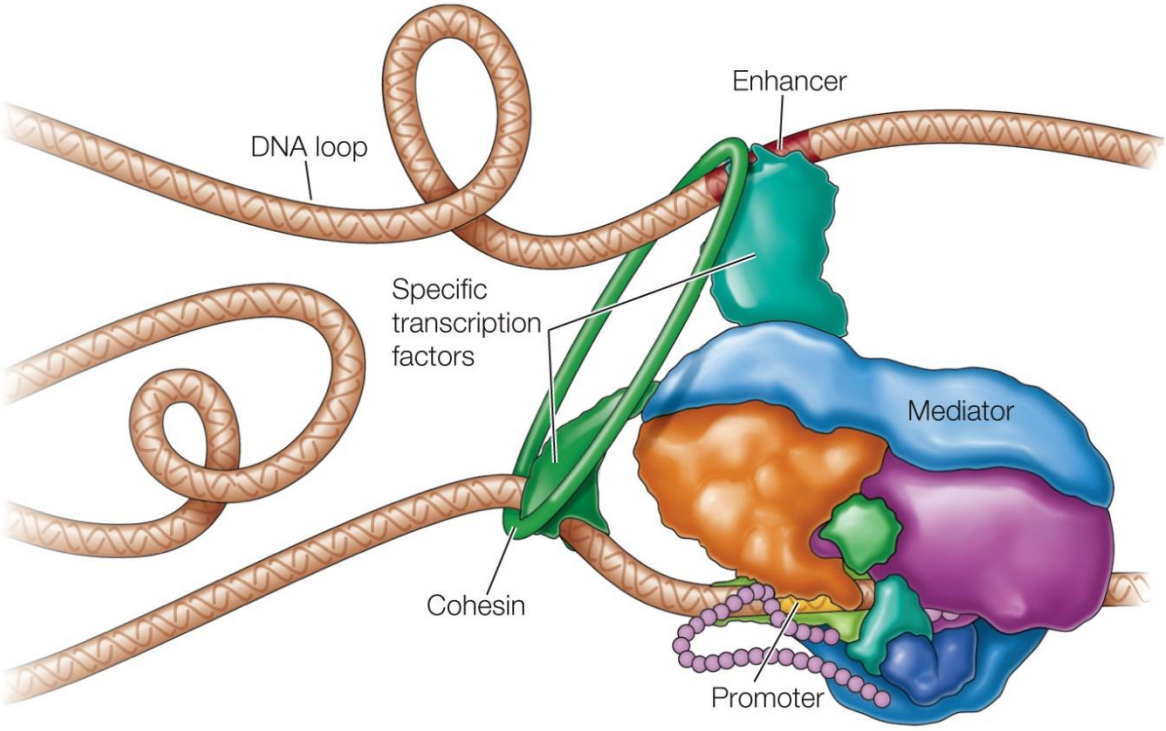
Distance, orientation, position-independent stimulation of transcription

***Silencer**



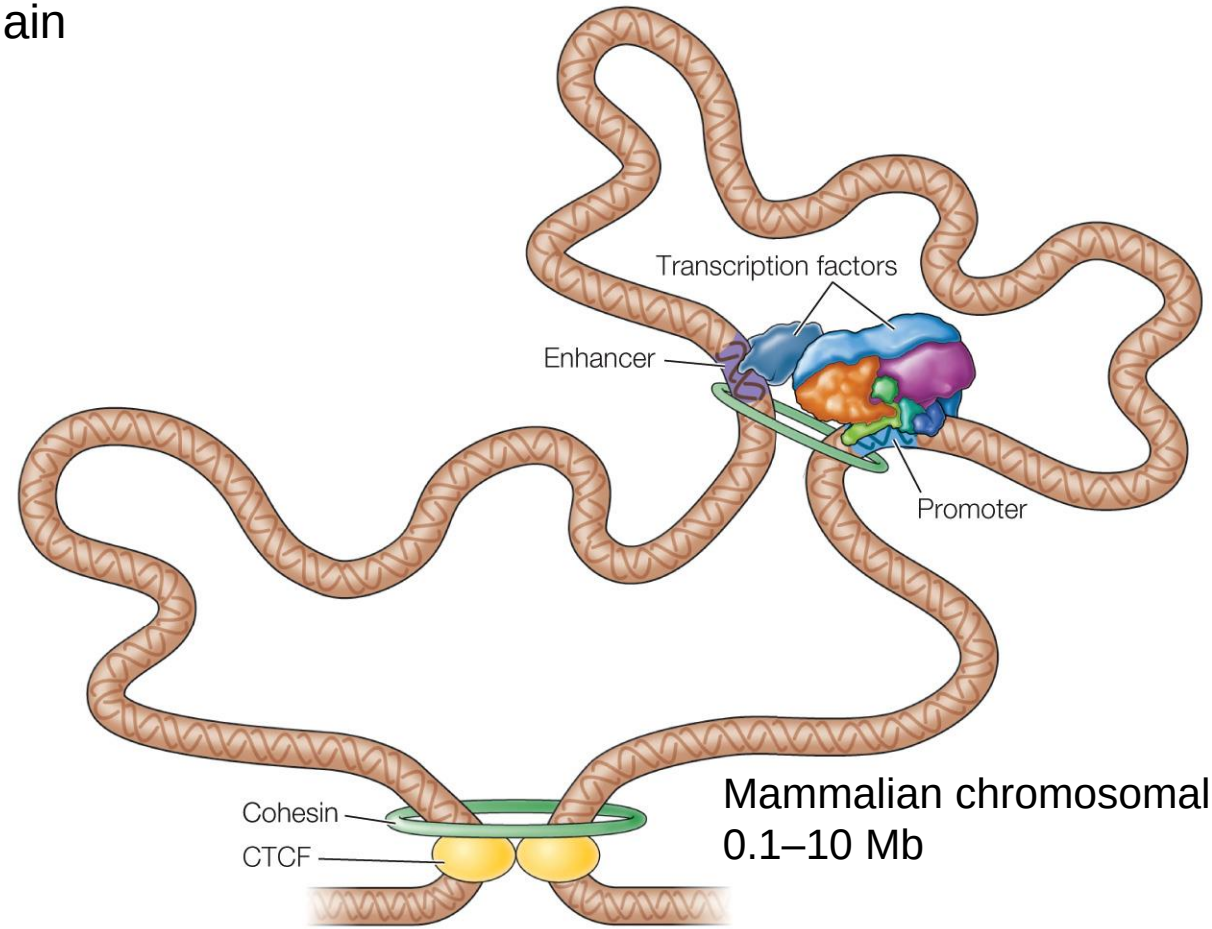
DNA looping

- Enhancers, like promoters, function by binding transcription factors that then regulate RNA polymerase.
- DNA looping allows a transcription factor bound to a distant enhancer to interact with proteins associated with the RNA polymerase/Mediator complex at the promoter.
- The loops are stabilized by cohesion, which forms a ring structure that encircles two strands of DNA



Chromosomal domains and CTCF

- Chromatin within the nucleus is organized into looped domains formed by the interaction of CTCF (CCCTC-binding factor) and cohesin.
- **Insulators**; enhancers are restricted to interacting with promoters in the same domain

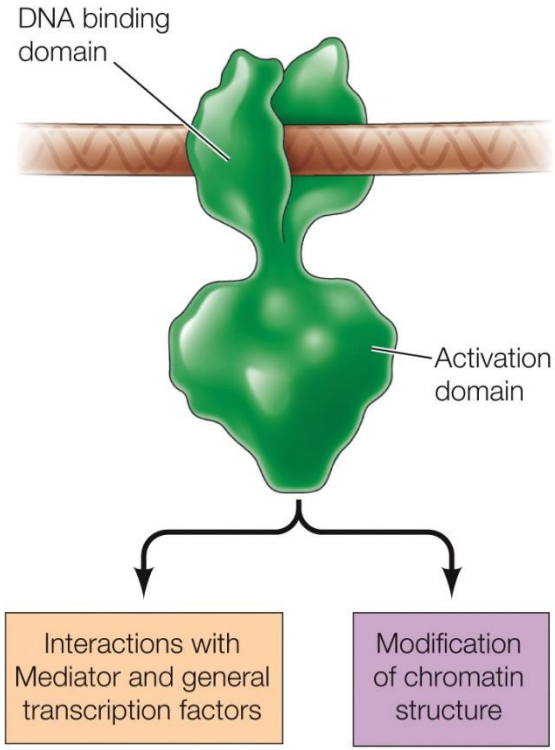


Mammalian chromosomal domain
0.1–10 Mb

Action of transcriptional activators

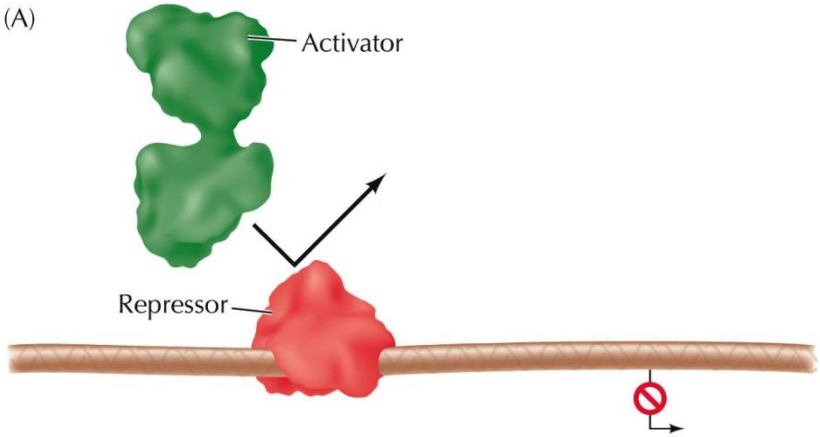
Activation domains stimulate transcription by two mechanisms:

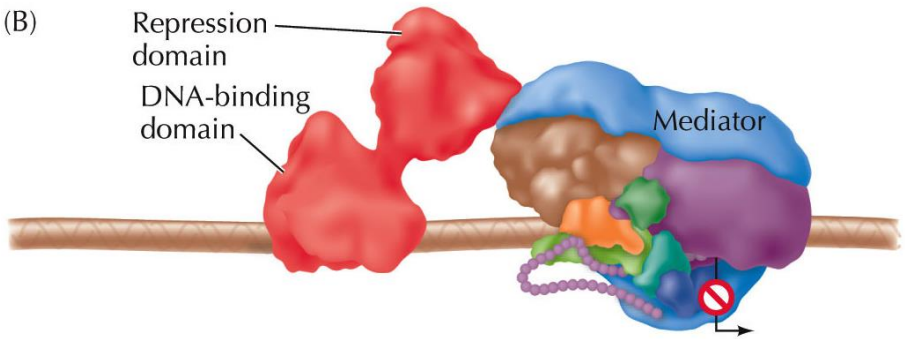
- Interact with Mediator proteins and general transcription factors
- Interact with **coactivators** to modify chromatin structure.



Action of eukaryotic repressors

- Some repressors block the binding of activators to regulatory sequences.
- Other repressors have active repression domains that inhibit transcription by interactions with Mediator proteins or general transcription factors, as well as with corepressors that act to modify chromatin structure.





Regulation of transcriptional elongation

1. Transcription is initiated following phosphorylation of the RNA pol II CTD at Ser 5 by TFIIH.
2. Factors involved in the initial stages of mRNA processing associate with the phosphorylated CTD.
3. NELF (negative elongation factor) and DSIF (DRB sensitivity inducing factor) associate with the polymerase and cause it to pause within about 50 nucleotides of the transcription start site.
4. Continuation of transcription results from the phosphorylation of NELF, DSIF, and serine 2 of the polymerase CTD by P-TEFb (positive transcription-elongation factor-b); Phosphorylated NELF dissociates from the complex, and additional factors required for elongation and processing associate with the polymerase.