

Cellular and Molecular Biology I

BIO-205-7

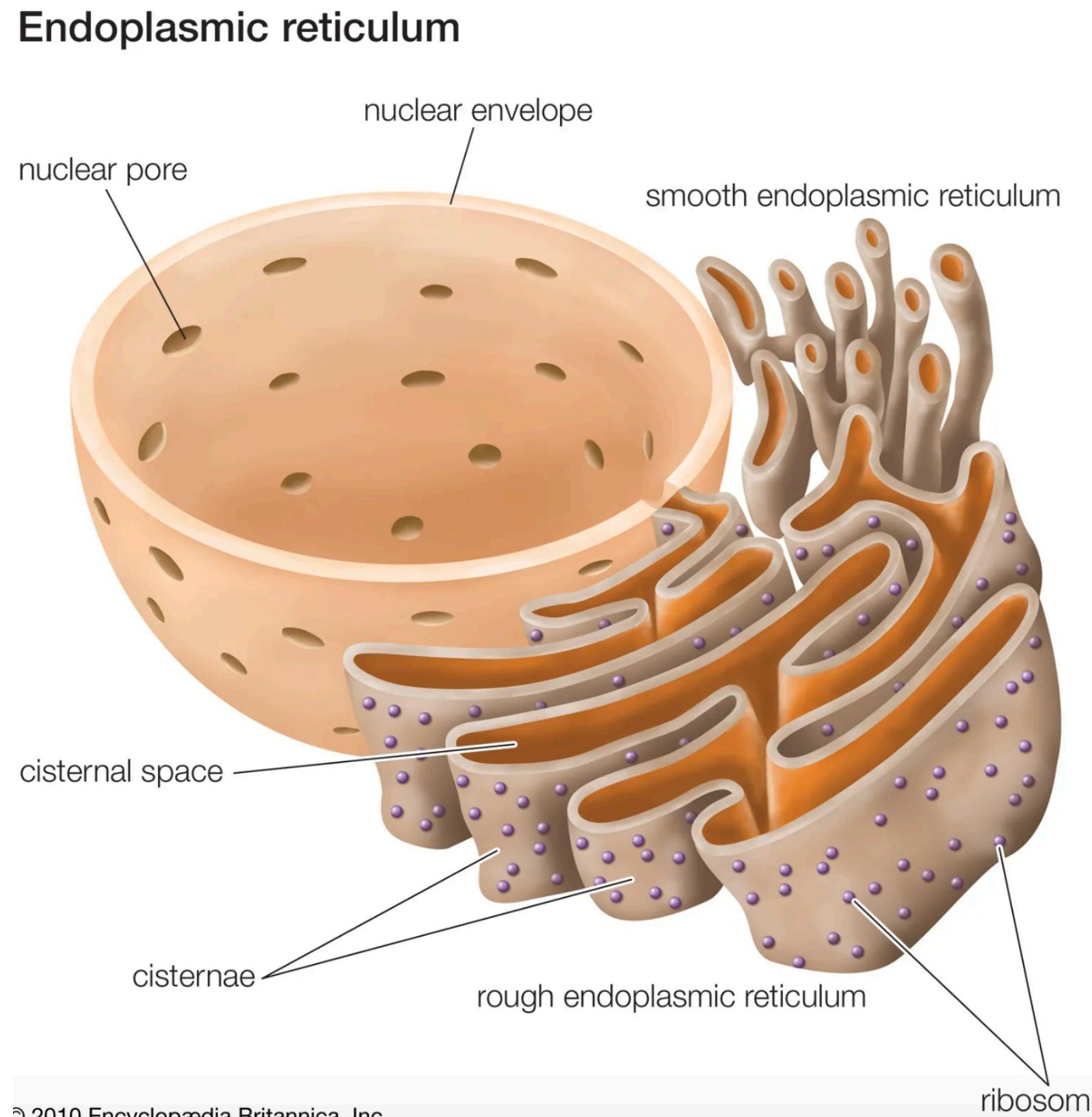
Camille Goemans - 2024

Welcome back!



A few things to discuss

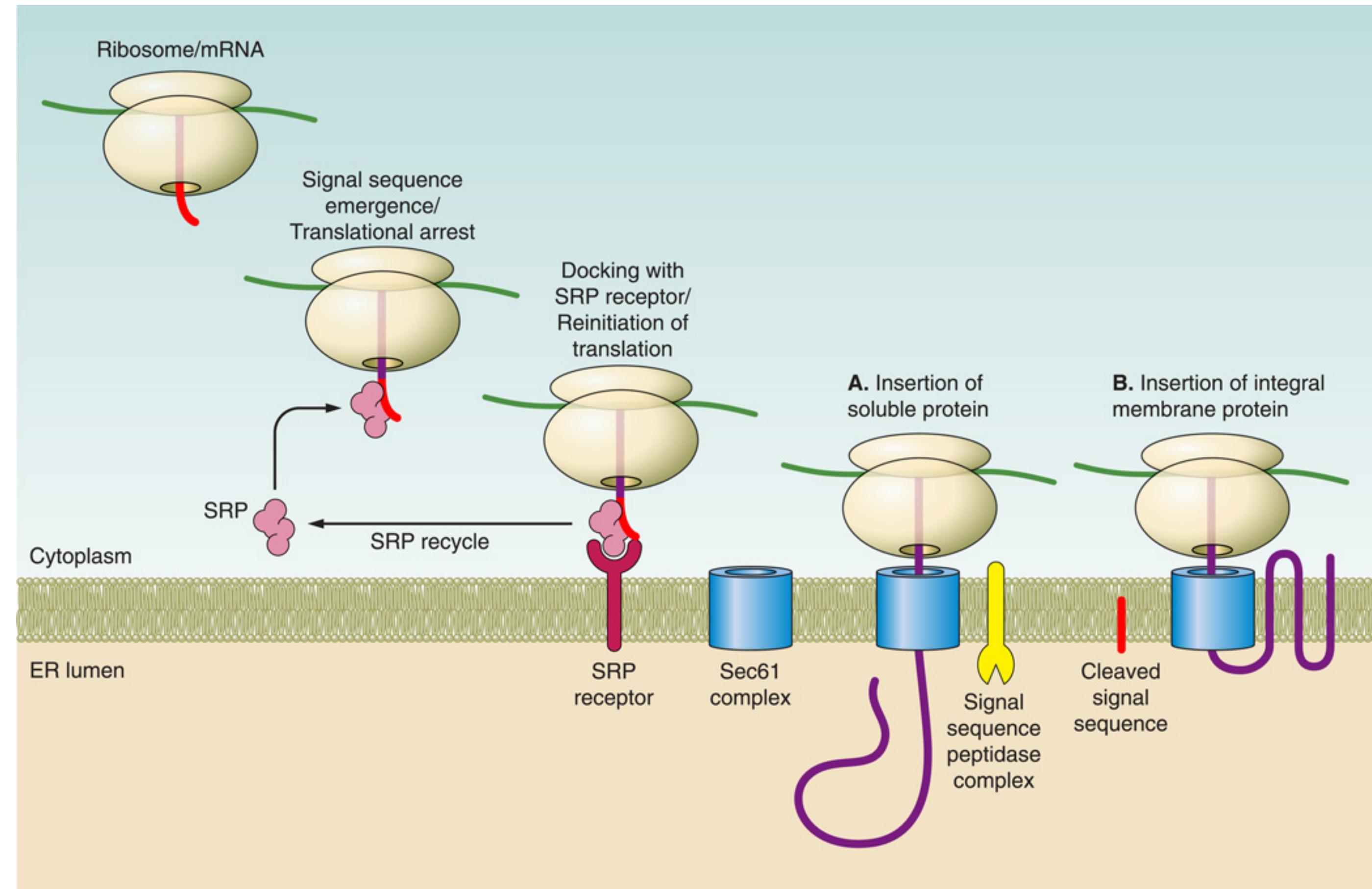
- How does translation/folding work for membrane proteins which have hydrophobic parts?
- How are ribosomes located on the surface of the endoplasmic reticulum?



- ER: proteins (RER) and lipids (SER) synthesis, folds and modifies proteins, detoxifies substances, and stores calcium.
- Golgi: Further modifies, sorts, and packages proteins and lipids from the ER for transport to their final destinations.

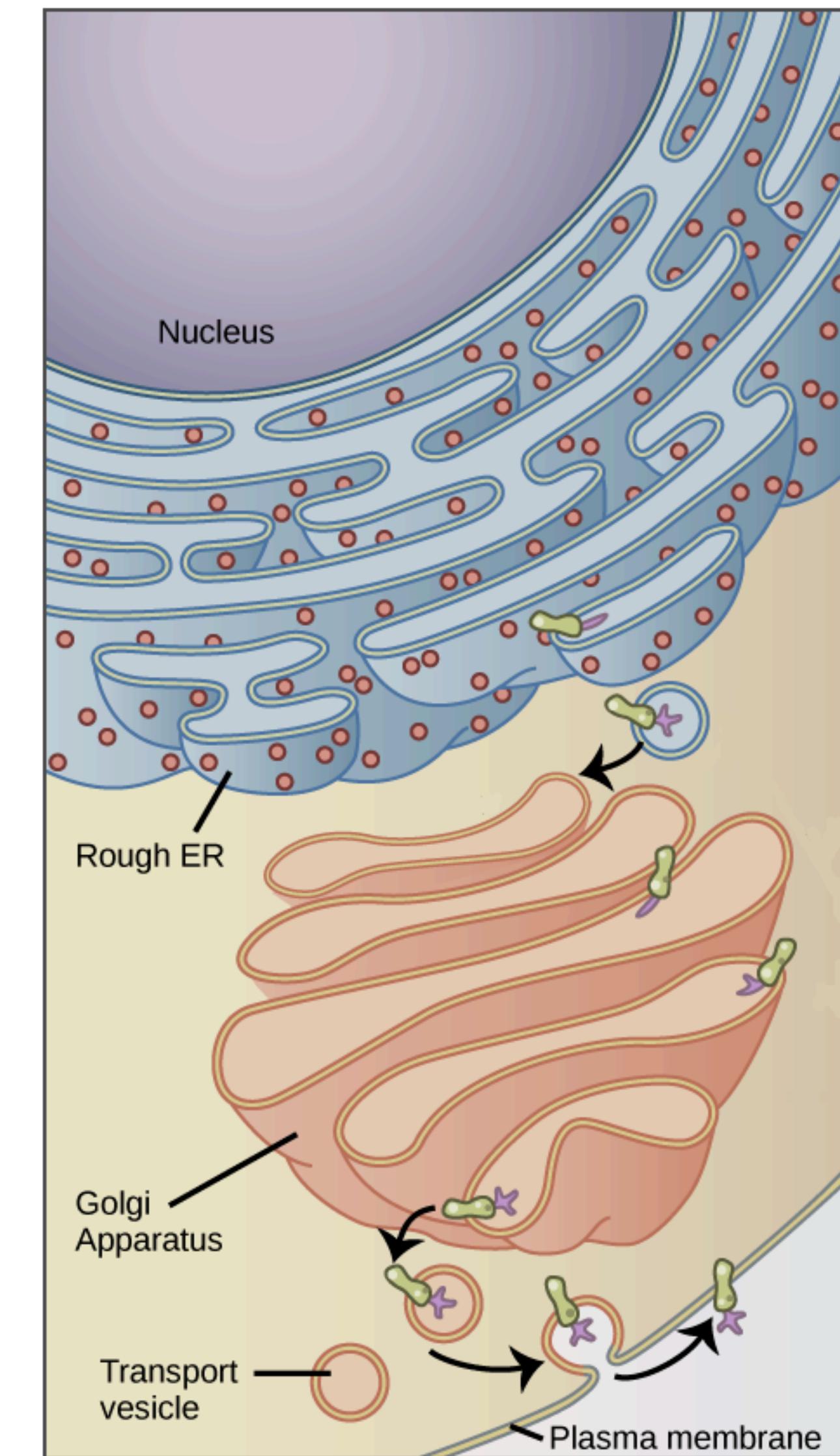
A few things to discuss

- How does translation/folding work for membrane proteins which have hydrophobic parts?
- How are ribosomes located on the surface of the endoplasmic reticulum?



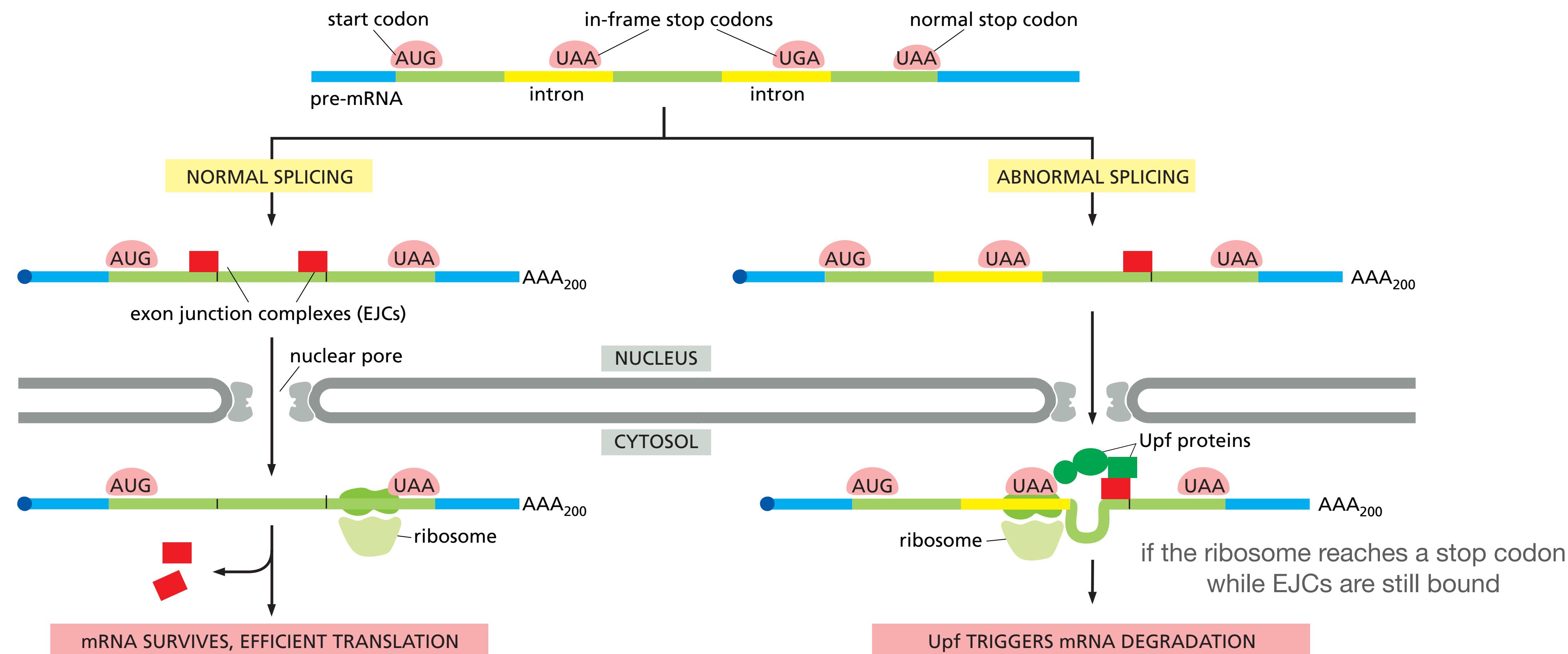
A few things to discuss

- How does translation/folding work for membrane proteins which have hydrophobic parts?
- How are ribosomes located on the surface of the endoplasmic reticulum?



Quality control mechanism: erratum

- Nonsense-mediated mRNA decay eliminates defective mRNA as they move away from the nucleus
- active when an mRNA has a **stop codon** at the wrong location



Quality control mechanism: erratum

- The nonsense-mediated mRNA decay mechanism begins as an mRNA molecule is being transported from the nucleus to the cytosol.
- As its 5' end emerges from a nuclear pore, the mRNA is met by a ribosome, which begins to translate it. As translation proceeds, the exon junction complexes (EJCs) that are bound to the mRNA at each splice site are displaced by the moving ribosome.
- The normal stop codon will lie within the last exon, so by the time the ribosome reaches it and stalls, no more EJCs will be bound to the mRNA. In this case, the mRNA “passes inspection” and is released to the cytosol where it can be translated in high amounts.
- However, if the ribosome reaches a stop codon earlier, when EJCs remain bound, the mRNA molecule is rapidly degraded. In this way, the first round of translation allows the cell to test the fitness of each mRNA molecule as it exits the nucleus.

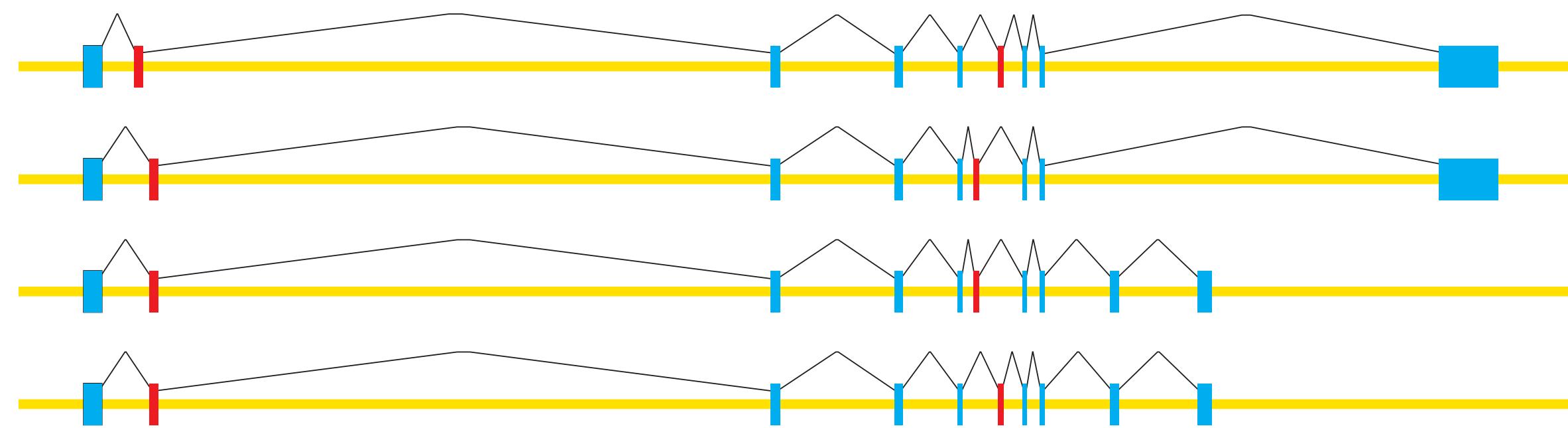
Exercise

The human α -tropomyosin gene is alternatively spliced to produce different forms of α -tropomyosin mRNA in different cell types. For all forms of the mRNA, the protein sequences encoded by exon 1 are the same, as are the protein sequences encoded by exon 10. Exons 2 and 3 are alternative exons used in different mRNAs, as are exons 7 and 8.

(A) HUMAN α -TROPOMYOSIN GENE



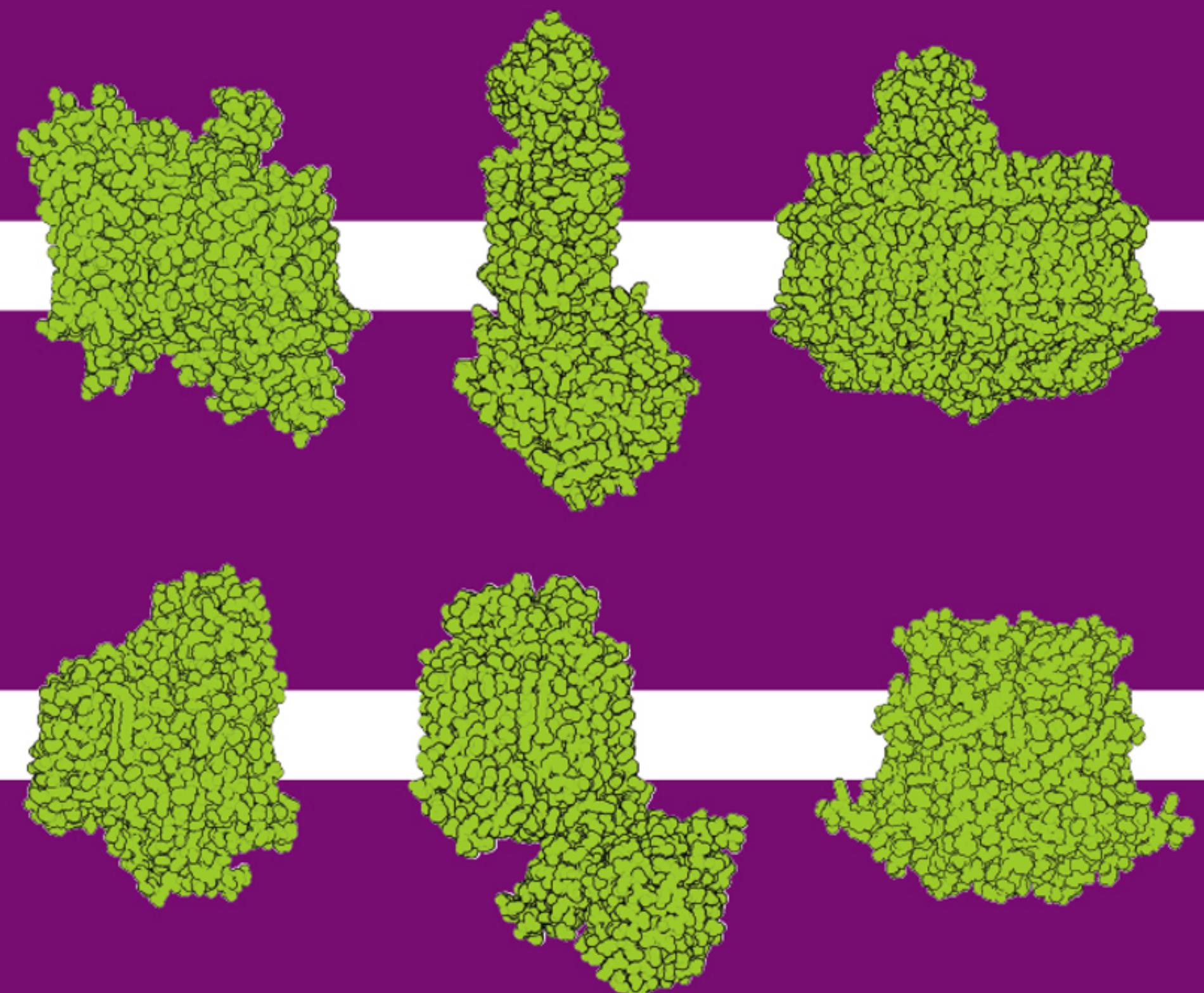
(B) FOUR DIFFERENT SPLICING VARIANTS



Which of the following statements about exons 2 and 3 is the most accurate? Is that statement also the most accurate one for exons 7 and 8? Explain your answers.

- Exons 2 and 3 must have the same number of nucleotides.
- Exons 2 and 3 must each contain an integral number of codons (that is, the number of nucleotides divided by 3 must be an integer).
- Exons 2 and 3 must each contain a number of nucleotides that when divided by 3 leaves the same remainder (that is, 0, 1, or 2).

MOLECULAR BIOLOGY OF
THE CELL
SEVENTH EDITION



ALBERTS HEALD JOHNSON MORGAN RAFF ROBERTS WALTER

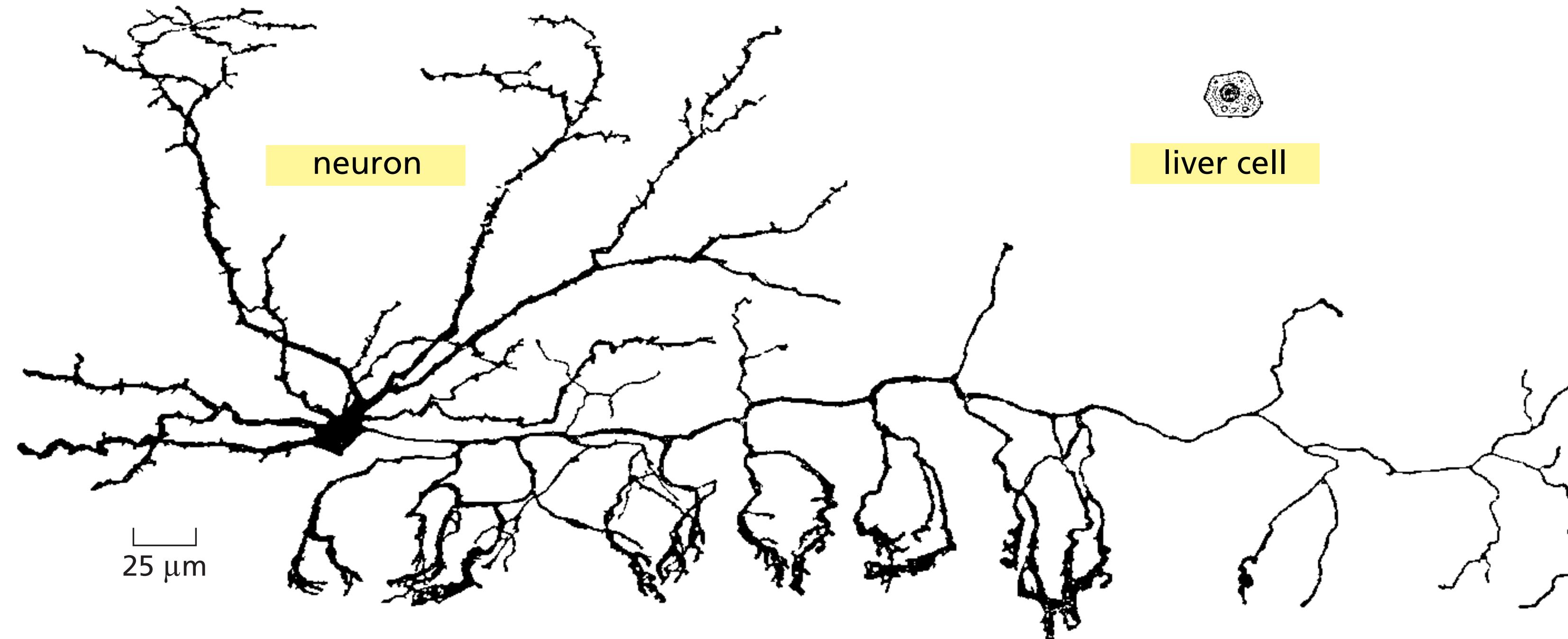
Chapter 7

Control of Gene Expression

Plan

- **Different cell types have the same DNA**
- Transcriptional control
 - Transcriptional regulators
 - Activators
 - Repressors
 - Understanding other regulatory systems
 - Combinatorial gene control and cell types

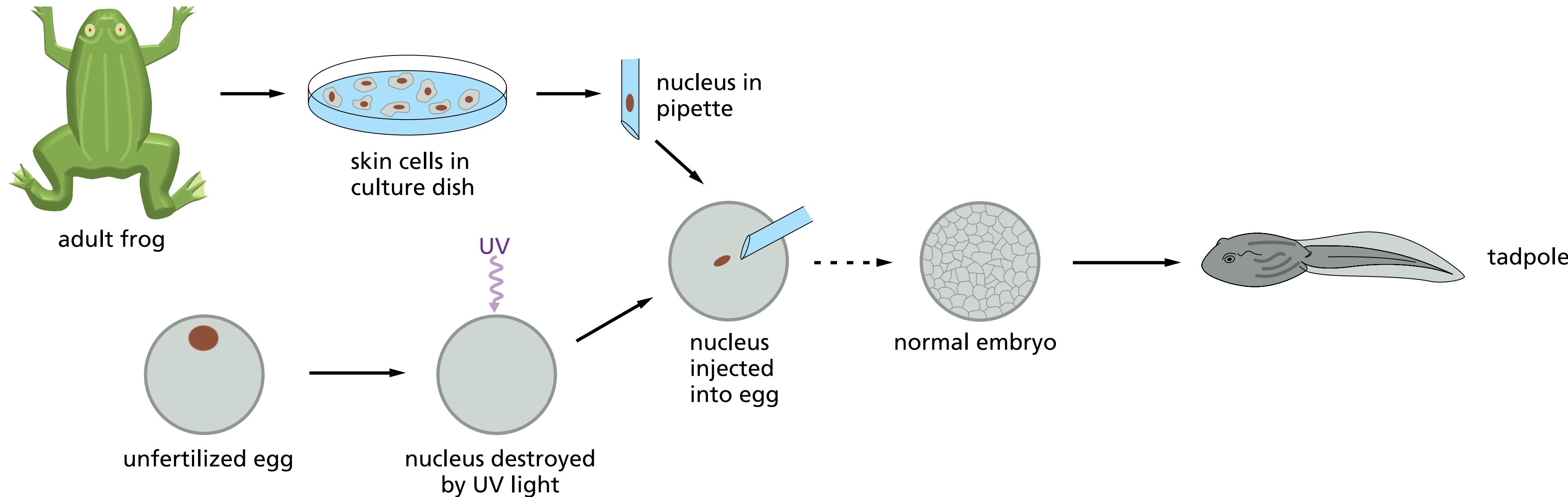
Different cell types contain the same DNA



They have **different sets** of mRNA and proteins

Different cell types contain the same DNA

How do we know this?



Different cell types contain the same DNA

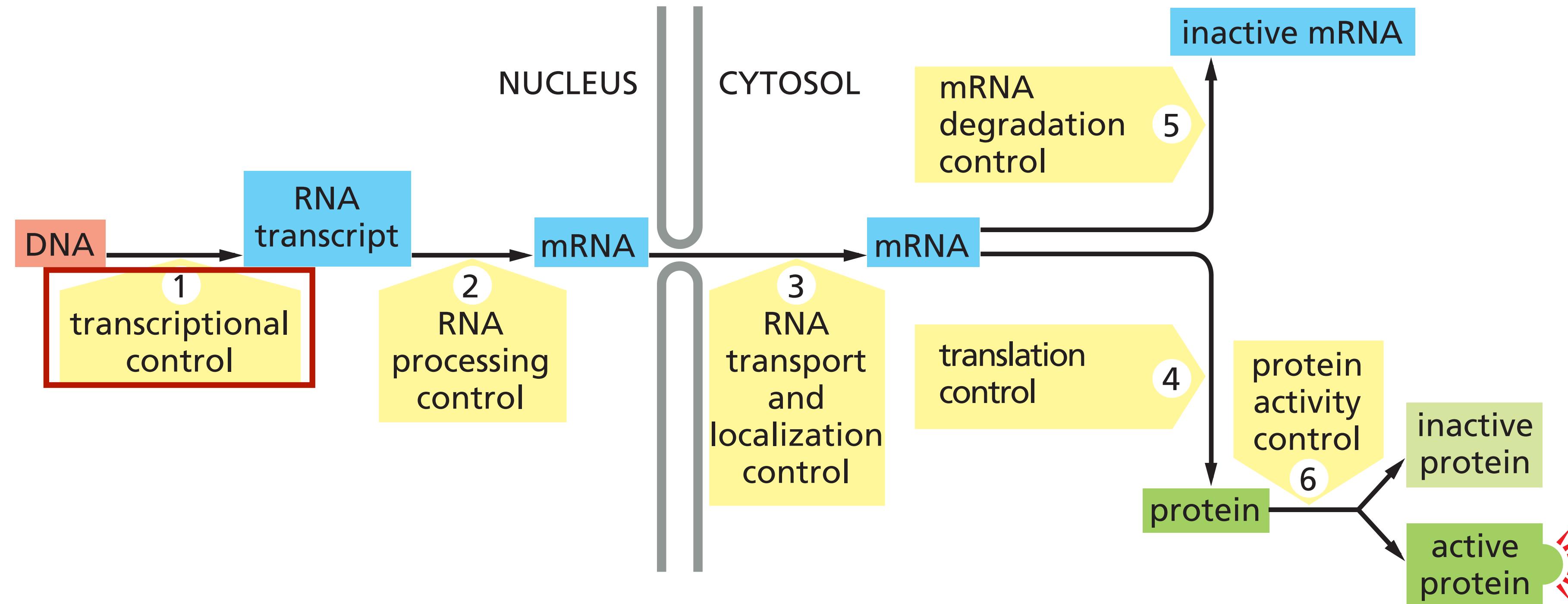
What are the **differences** between a cell type and another?

- Many processes are **common** (e.g. chromosomes, RNA and DNA polymerases, ribosomes, etc.)
- Some RNAs and proteins are abundant in **specialized cells**
- At any time, cells express **~30-60% of their ~ 30 000 genes**

Plan

- Different cell types have the same DNA
- **Transcriptional control**
 - Transcriptional regulators
 - Activators
 - Repressors
 - Understanding other regulatory systems
 - Combinatorial gene control and cell types

Levels of gene expression regulation



Plan

- Different cell types have the same DNA
- Transcriptional control
 - **Transcriptional regulators**
 - Activators
 - Repressors
 - Understanding other regulatory systems
 - Combinatorial gene control and cell types

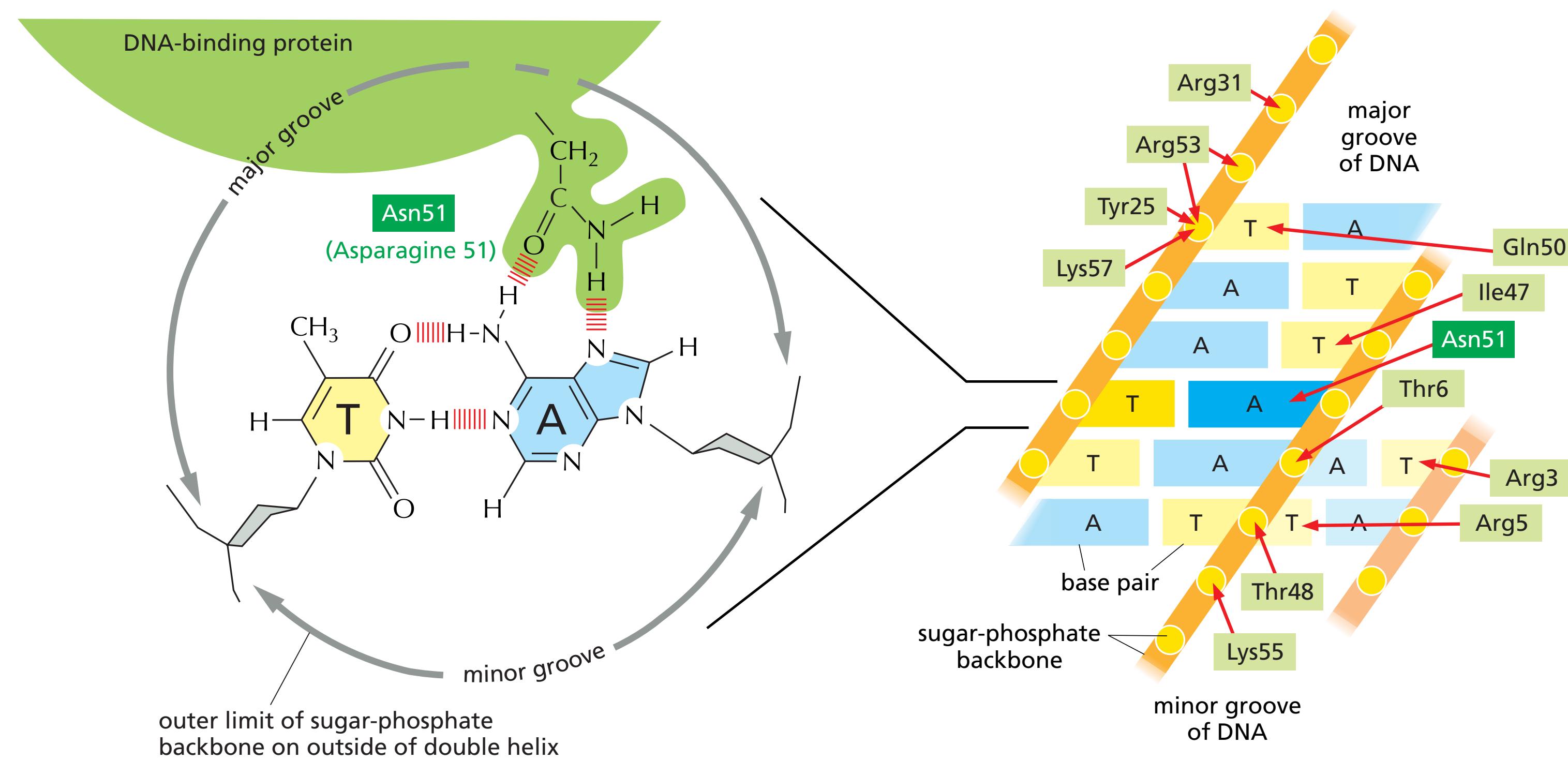
Control of transcription by DNA-binding proteins

- Proteins known as **transcription regulators**
- **DNA sequences** (5-10 nucleotides) known as **cis-regulatory sequences** (on the chromosome of the gene to regulate)
- **10%** of the genes in most organisms encode for **transcription regulators**
- **Each gene** is controlled by its own cis-regulatory sequences (often in the intergenic region upstream of the transcription start)
- **Complex** arrangement of sequences and transcription regulators
- They determine the **time and place** each gene is transcribed

Control of transcription by DNA-binding proteins

How does the **recognition** work?

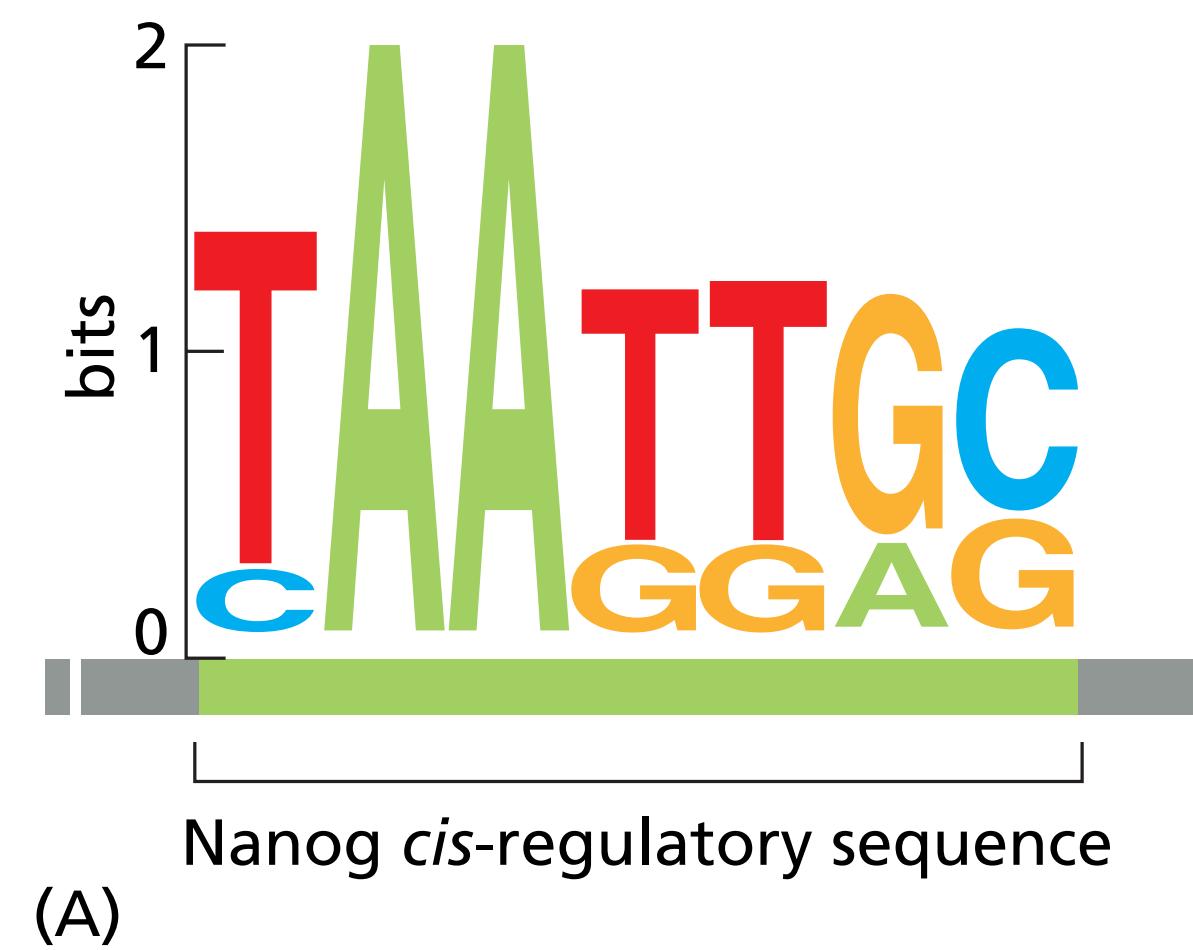
- the surface of the protein is **complementary** to the special surface features of a portion of DNA
- contacts involve **hydrogen bonds, ionic bonds and hydrophobic interactions**



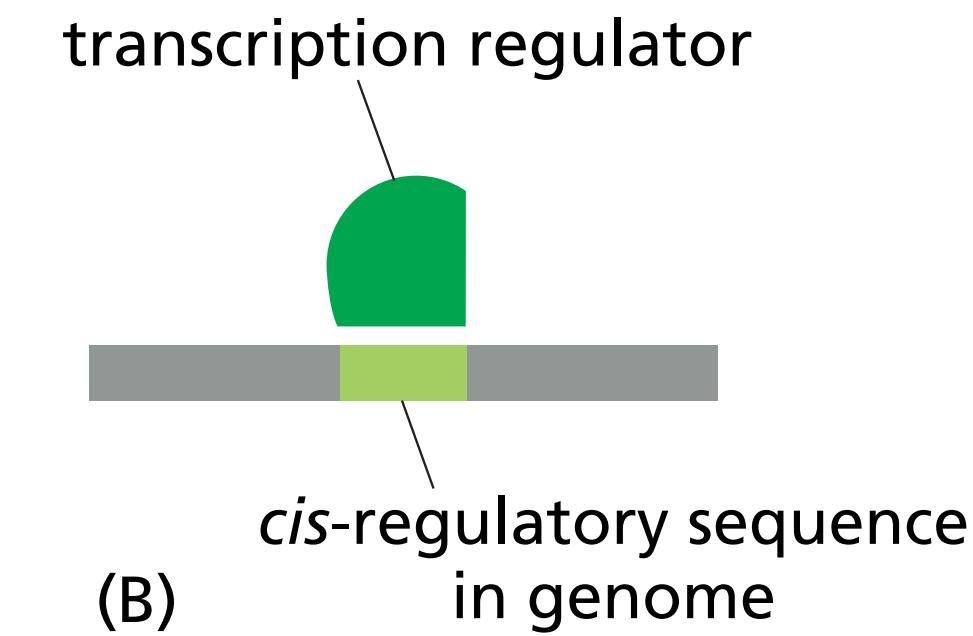
Control of transcription by DNA-binding proteins

How does the **recognition** work?

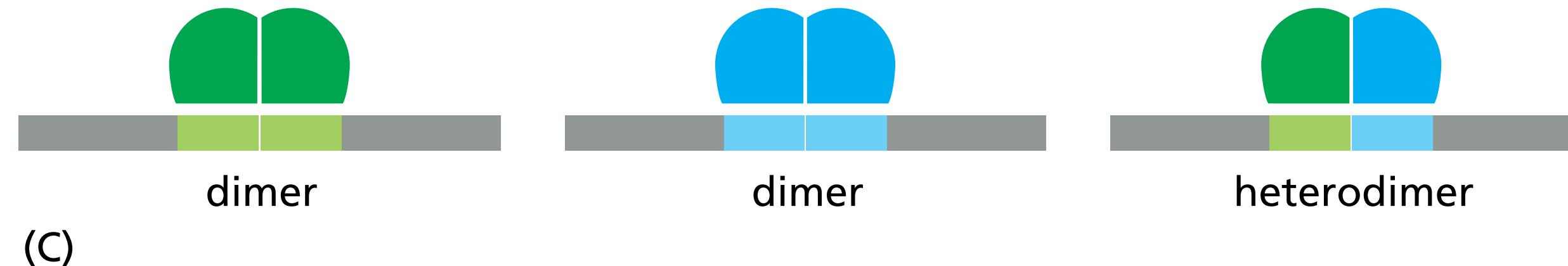
- DNA binding proteins bind to different **closely-related sequences** (depicted as a logo)
- Specificity is increased by **dimer formation** which doubles the length of the cis-sequence



(A)



(B)



(C)

Examples

Homeodomain



Homeodomain

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Examples

Zinc-finger domain

Zinc Finger Domain

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Examples

Leucine Zipper



Leucine Zipper

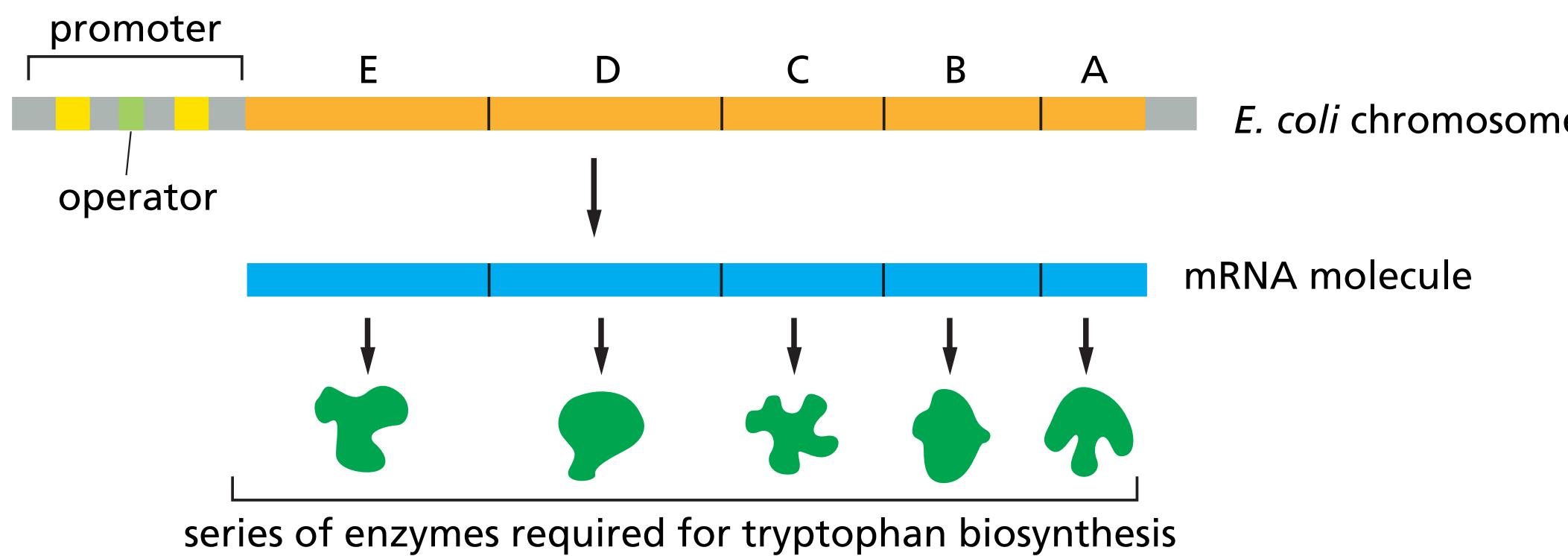
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Control of transcription by DNA-binding proteins

Transcription regulators **switch genes on and off**

- The tryptophan repressor (bacteria)

The tryptophan operon

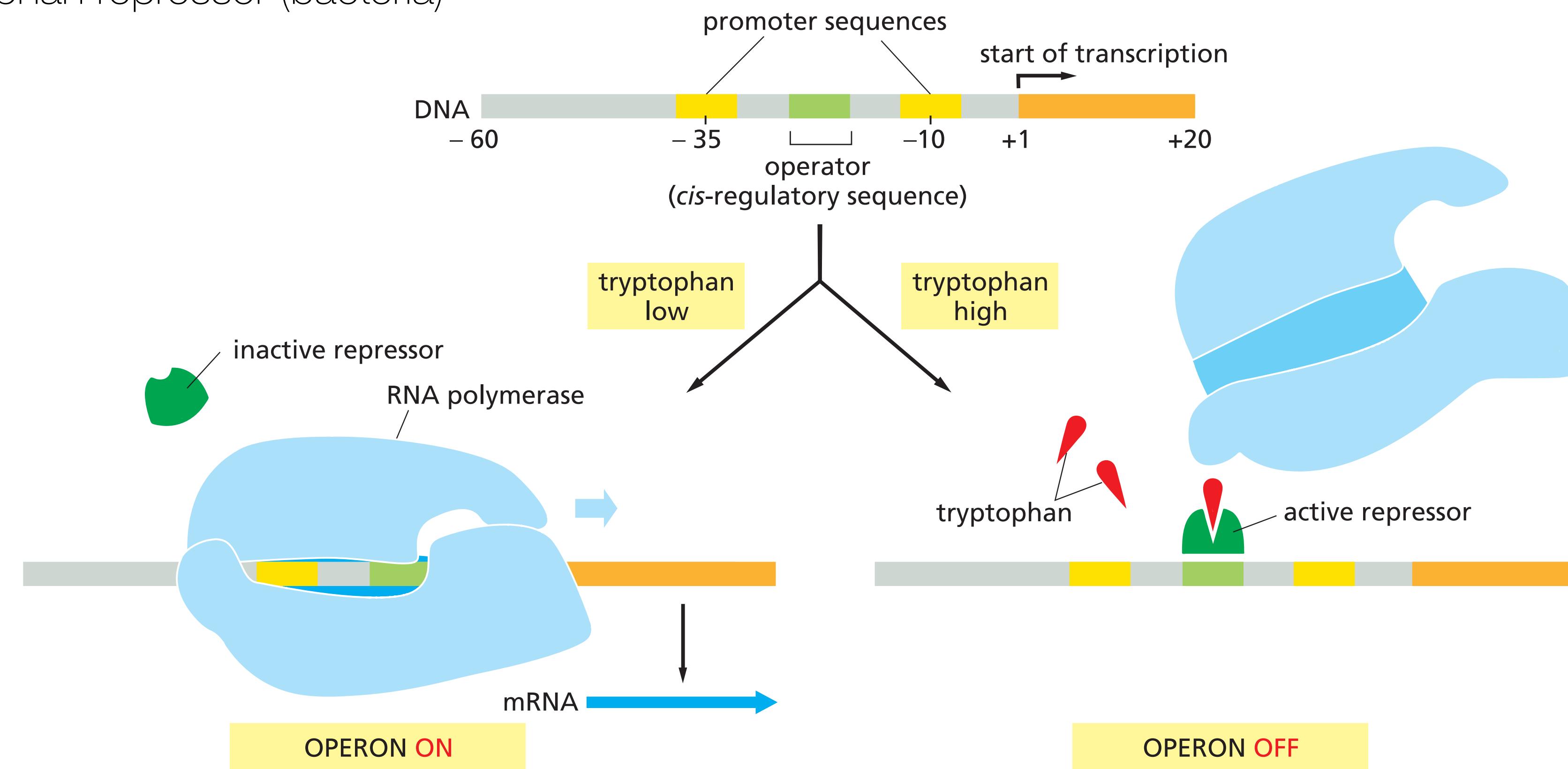


- When tryptophan levels are low, the operon is transcribed
- When tryptophan levels are high, the operon is not transcribed

Control of transcription by DNA-binding proteins

Transcription regulators **switch genes on and off**

- The tryptophan repressor (bacteria)

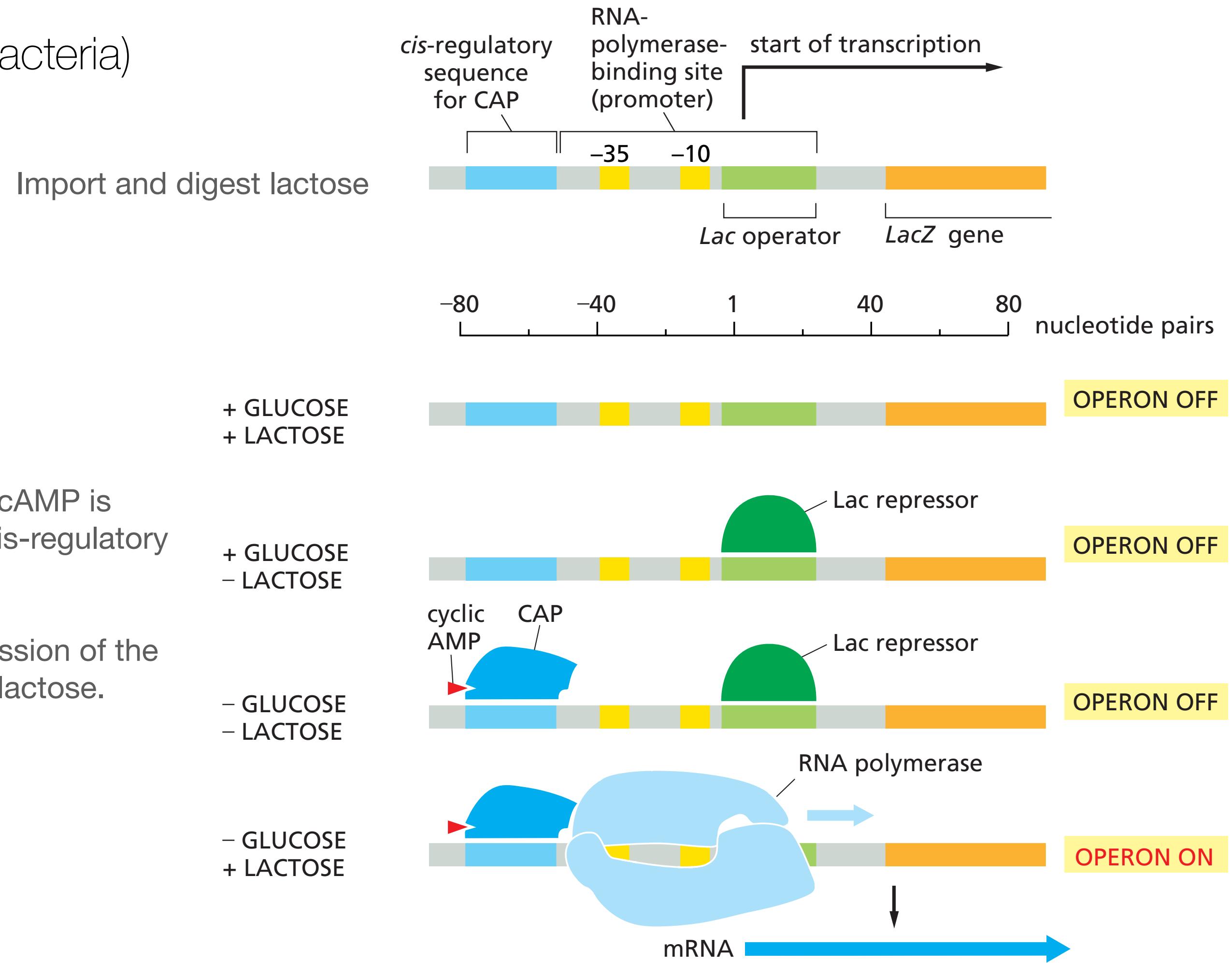


Transcriptional **repressors** turn genes off, transcriptional **activators** turn genes on

Control of transcription by DNA-binding proteins

Transcription regulators **switch genes on and off**

- The lac operon (bacteria)



When glucose levels are low, cAMP is produced and CAP binds to the *cis*-regulatory sequence.

Lac repressor prevents the expression of the lac operon in the absence of lactose.

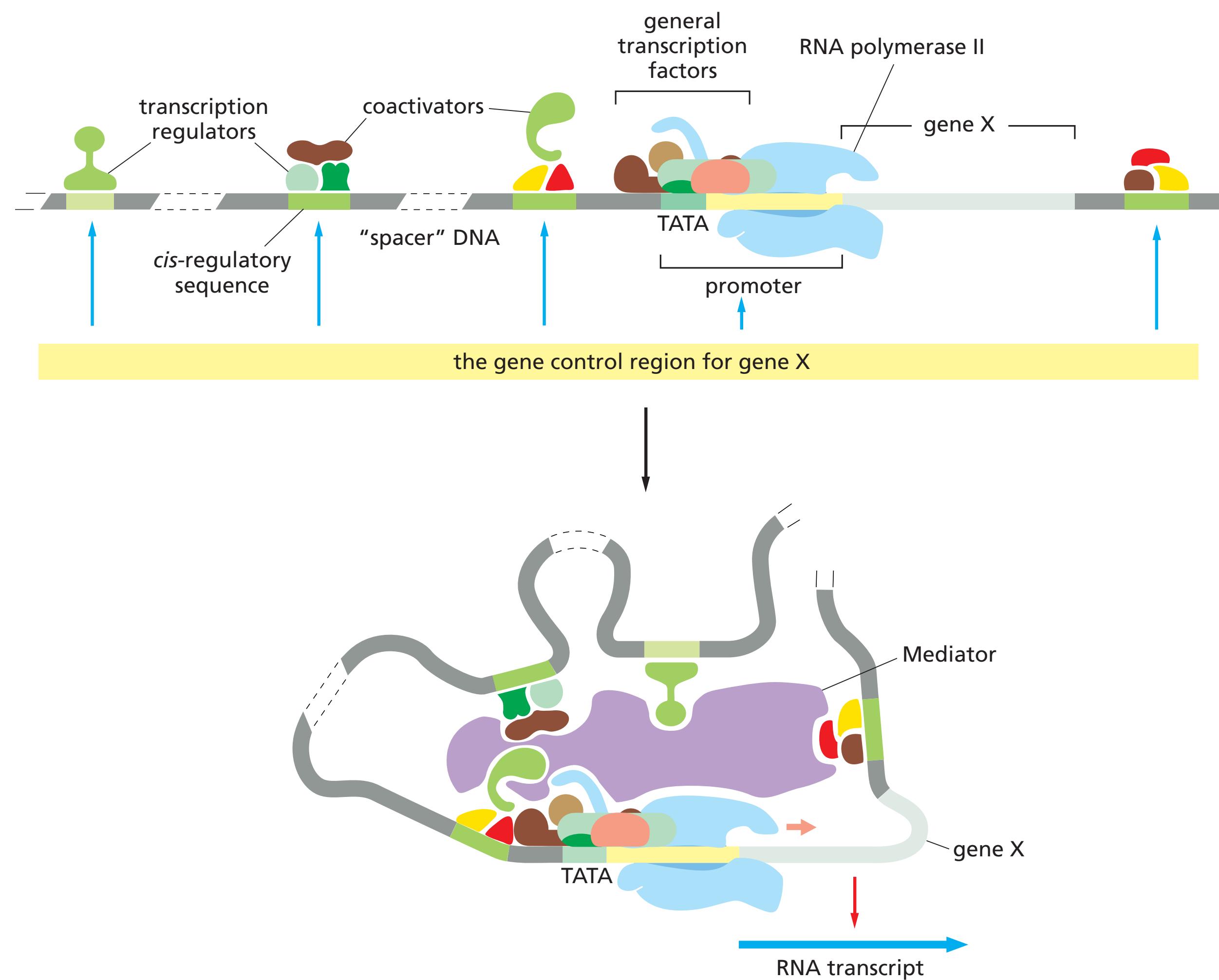
The lac operon

The *lac* Operon

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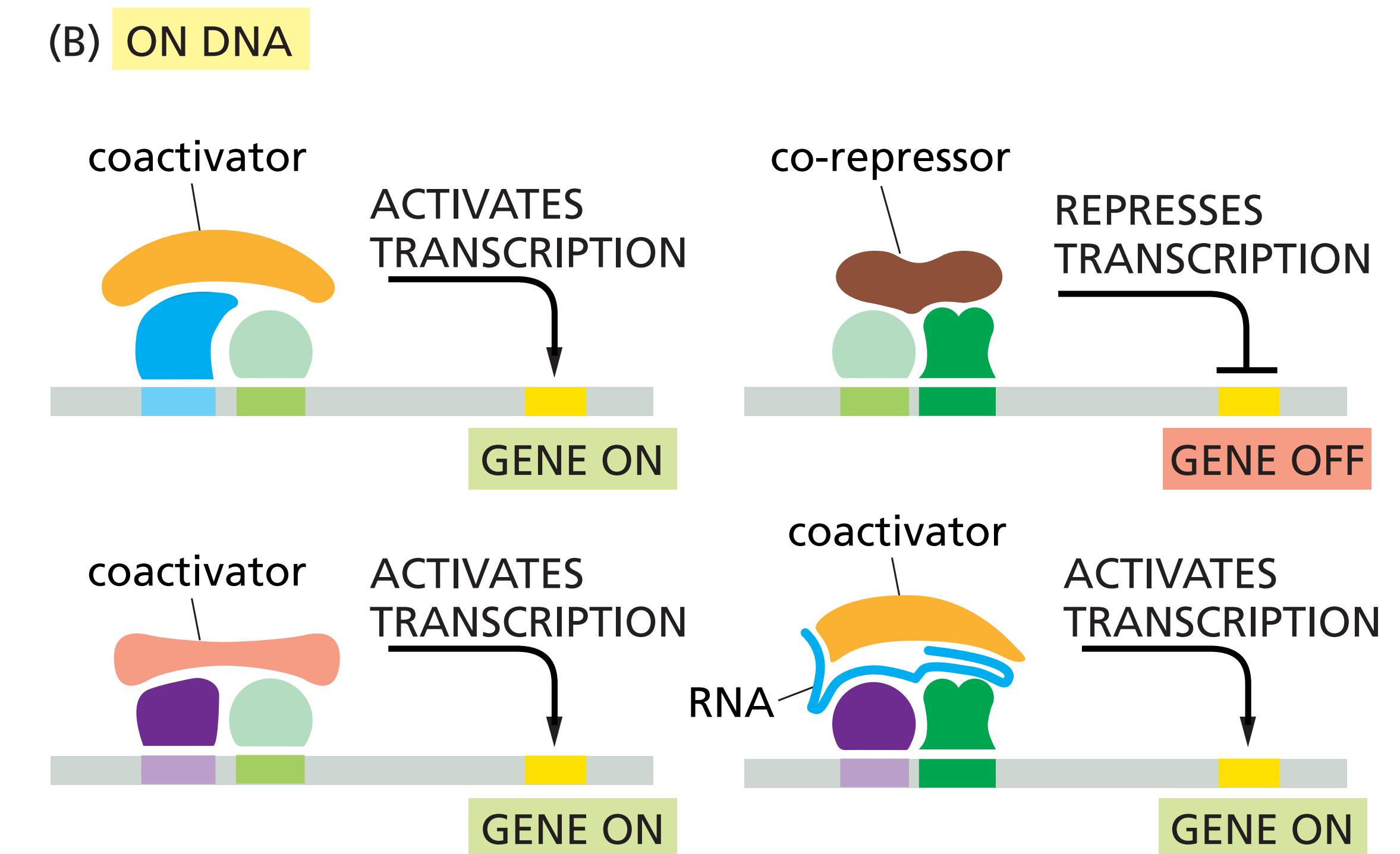
Control of transcription by DNA-binding proteins

- More complex in **Eukaryotes**
- RNA polymerase II requires **5 general transcription factors** - their stepwise assembly already controls the rate of transcription initiation
- Cis-regulatory sequences spread over a long stretch of DNA = **gene control region**
- The gene control region includes the **promoter + all cis-regulatory sequences**
- There are **thousands of different transcription regulators**



Control of transcription by DNA-binding proteins

- More complex in **Eukaryotes**
- Transcription regulators tend to work **in groups**

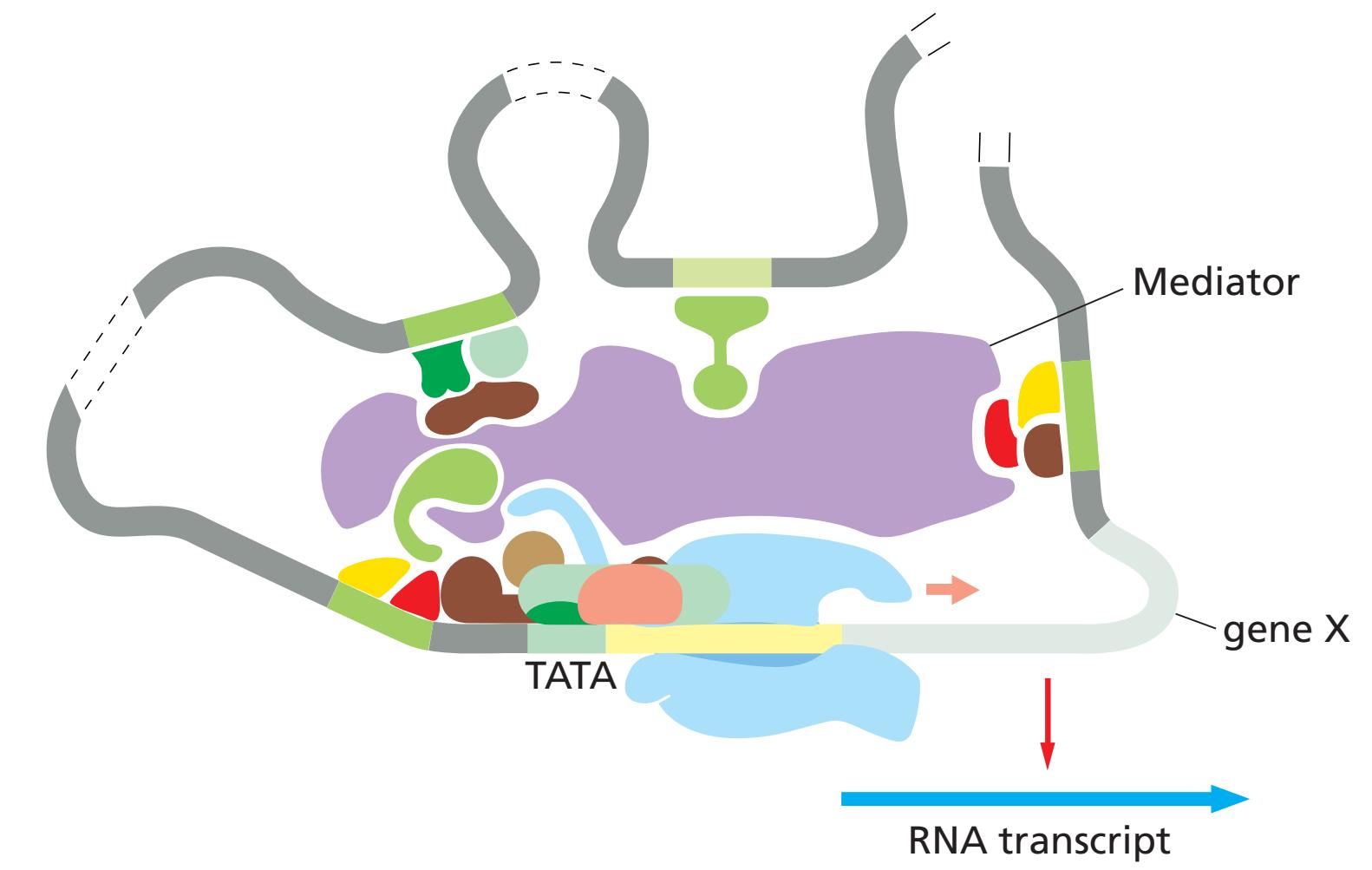


Plan

- Different cell types have the same DNA
- Transcriptional control
 - Transcriptional regulators
 - **Activators**
 - Repressors
 - Understanding other regulatory systems
 - Combinatorial gene control and cell types

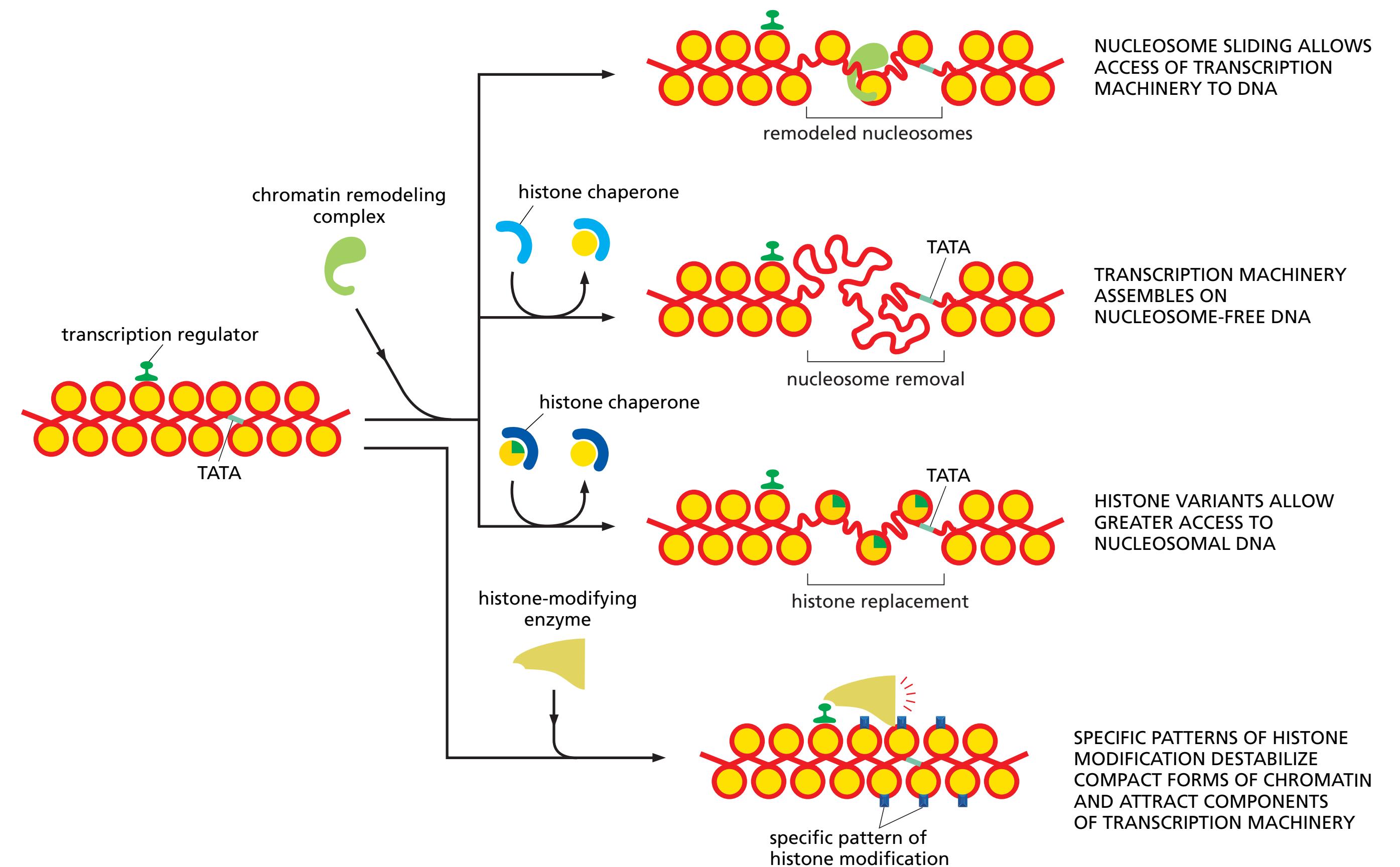
Activator proteins

- cis-regulatory sequences to which activator bind were called **enhancers**
- they can be **far** from the promoter
- activators **attract and position** the RNA polymerase II and **release** it so transcription can begin
- Example: Mediator is an activator composed of 30 subunits that forms a bridge between transcription activators, RNA polymerase and general transcription factors



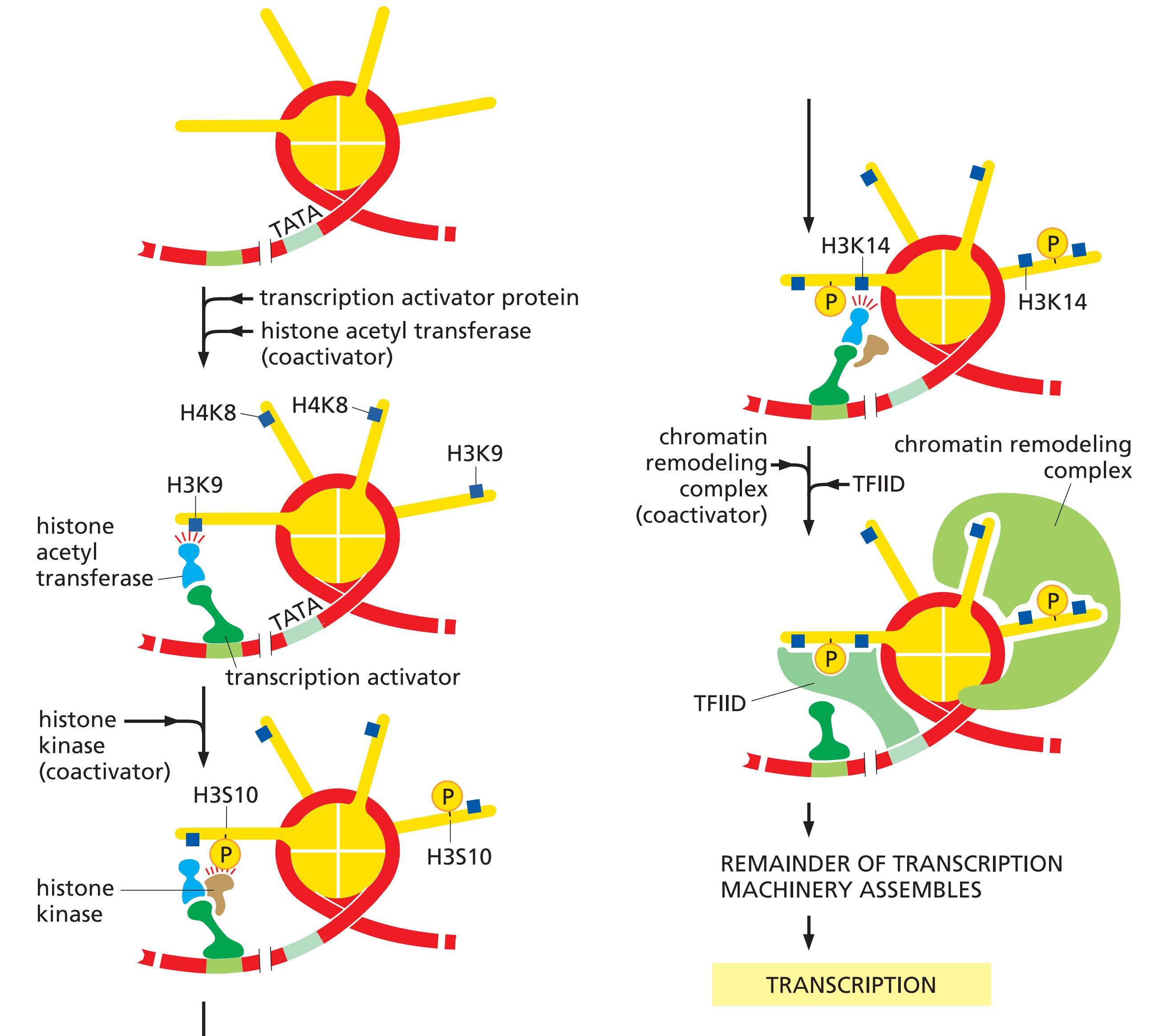
Activator proteins

- RNA polymerase and general transcription factors are not able to assemble on a promoter that is **packaged into chromatin**
- Activators trigger changes in **chromatin structure** making DNA more accessible
- To do this they use **histone modifications, nucleosome remodelling, nucleosome removal and histone replacement** (co-activators)

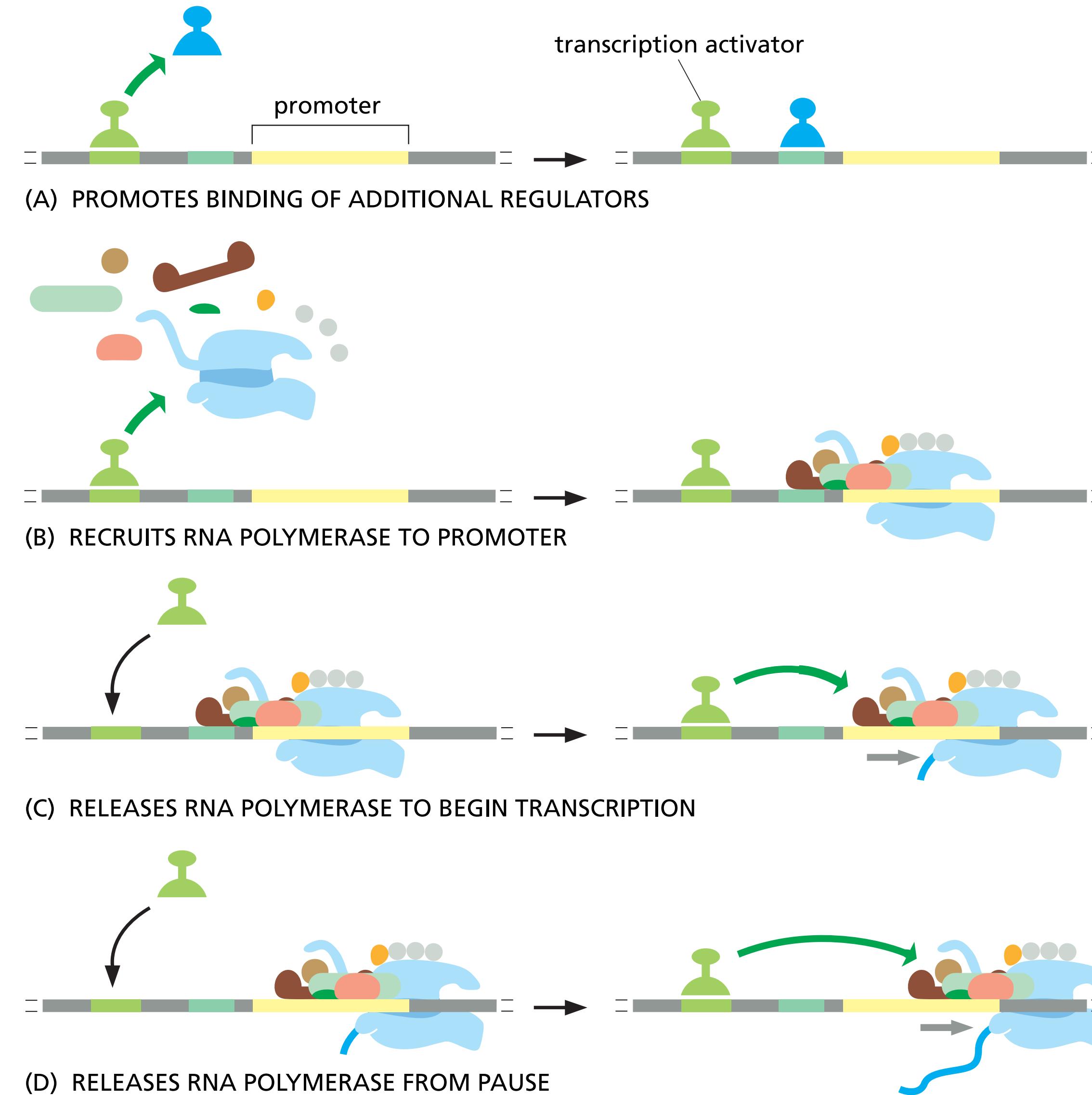


Activator proteins

- These mechanisms work together during **transcription initiation**
- The altered chromatin can be **rapidly reversed** (quick on-off switches) or **not**



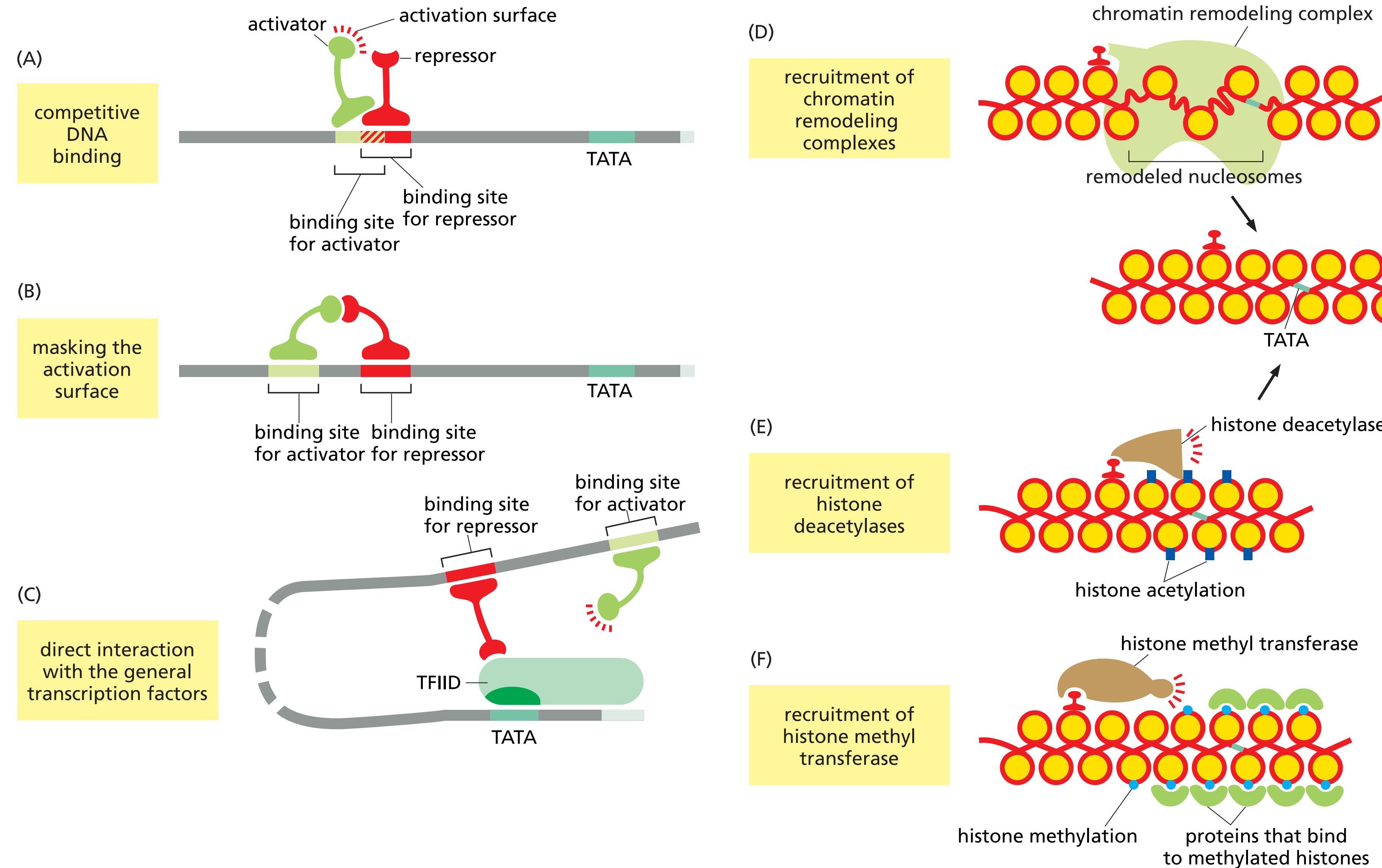
Activator proteins



Plan

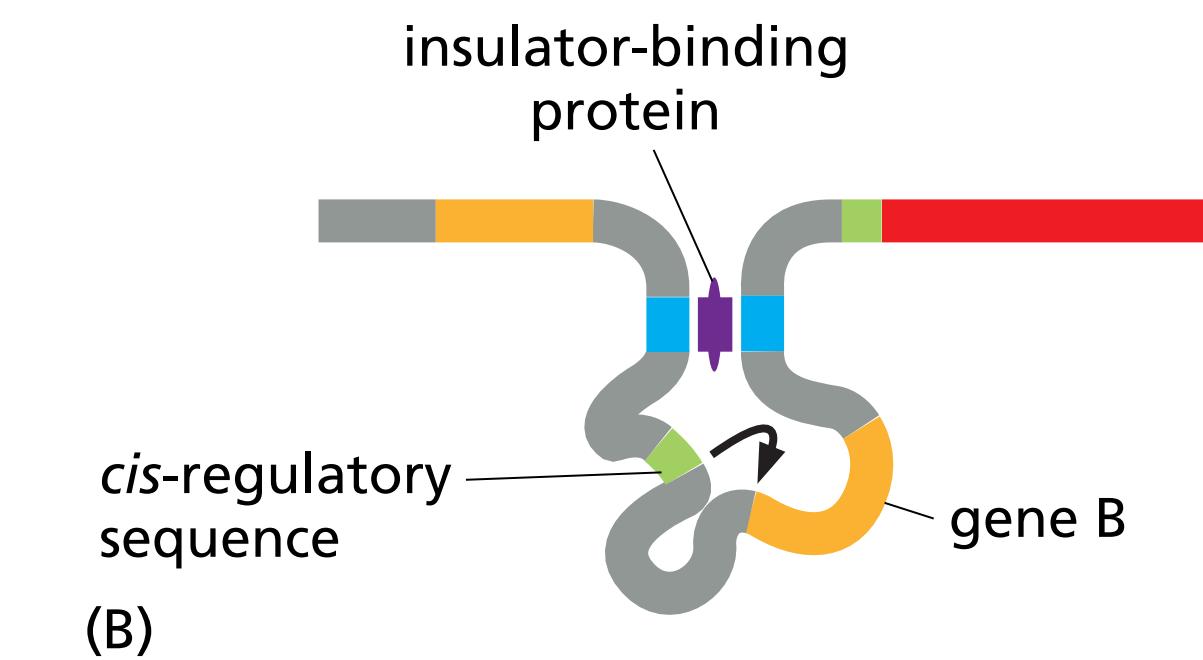
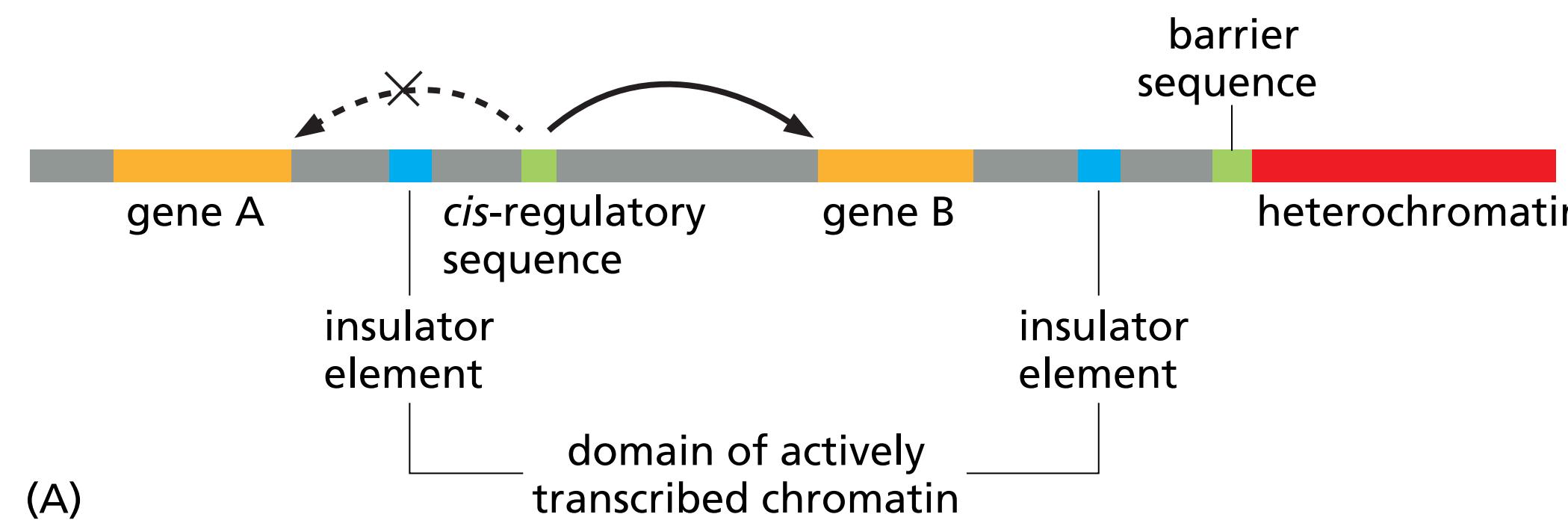
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Repressor proteins



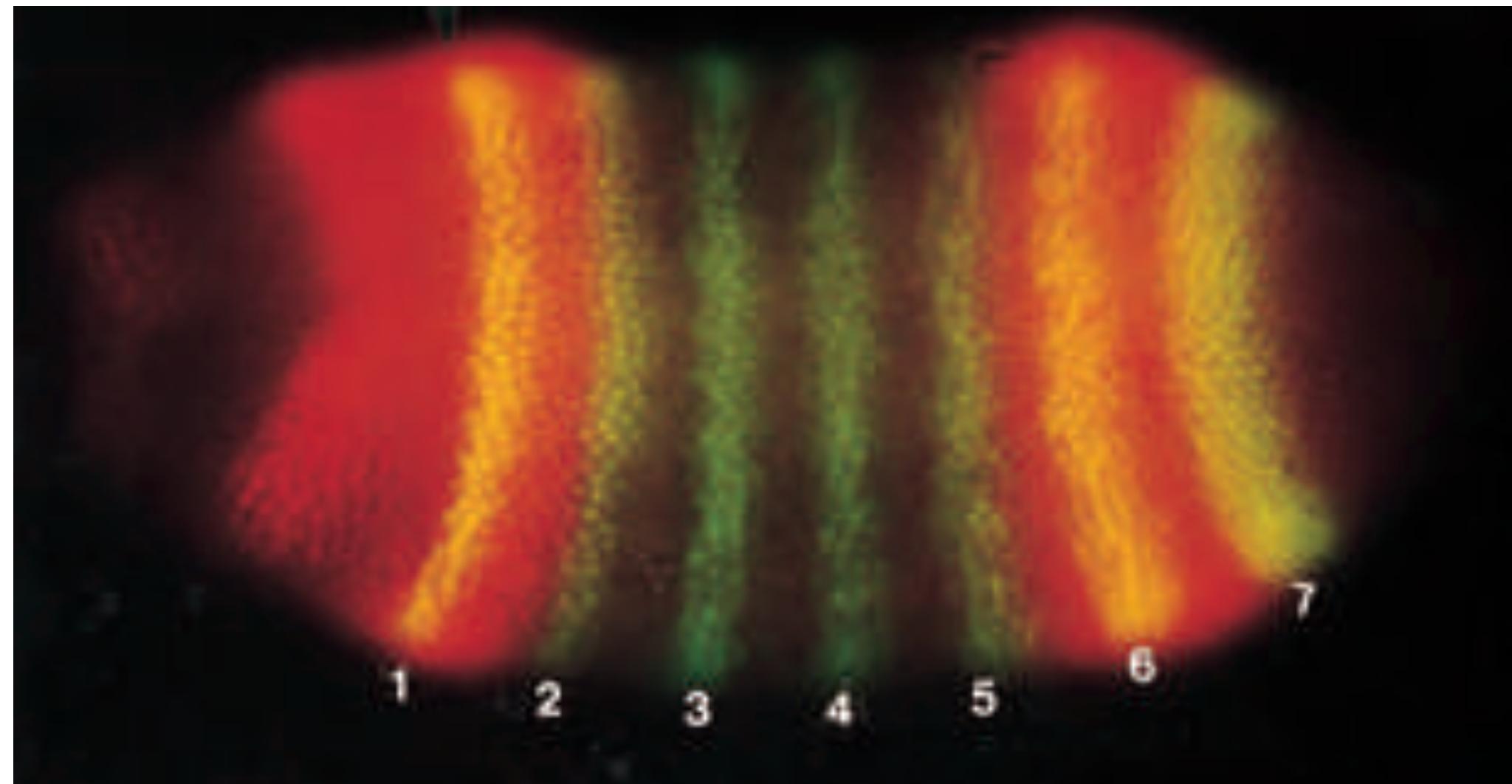
Insulator DNA sequences

- Prevent cis-regulatory elements to **activate inappropriate** genes



Drosophila Even-skipped gene

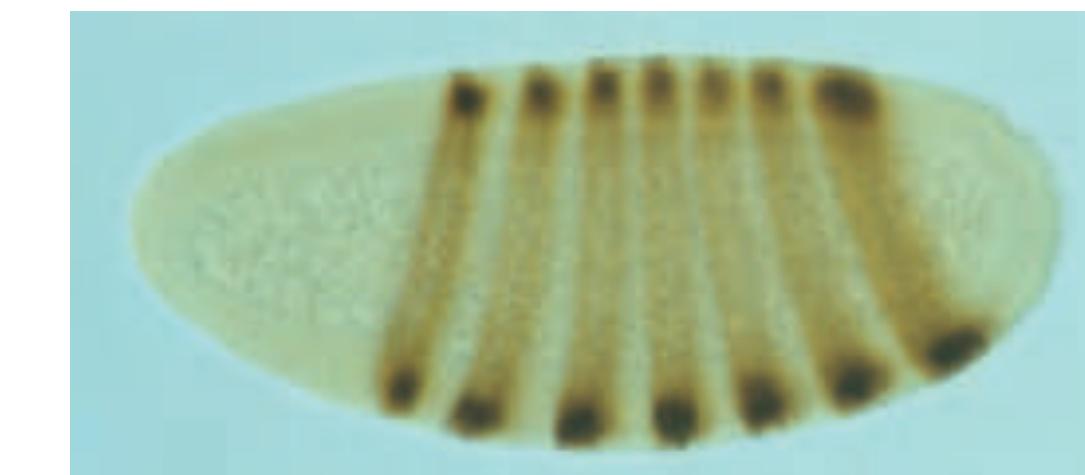
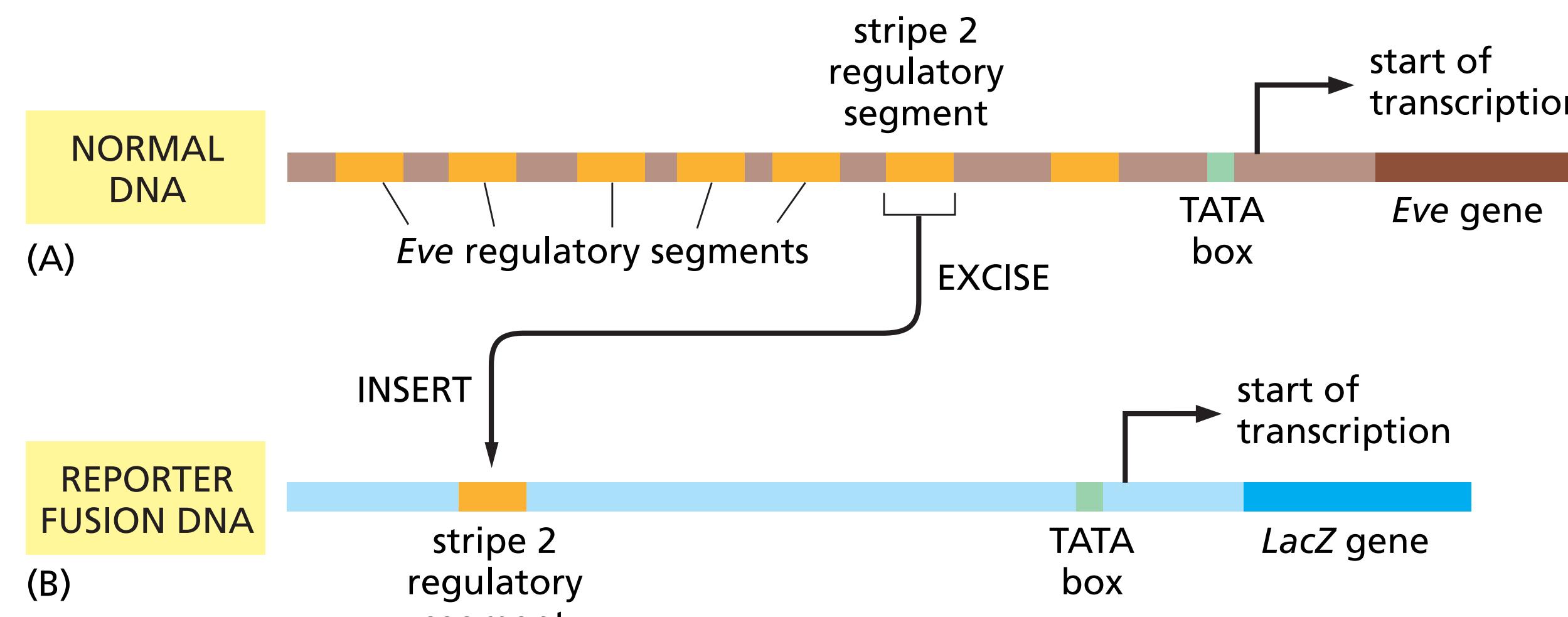
- Plays an important role in Drosophila embryo
- The embryo is a single giant cell with multiple nuclei in a giant cytoplasm with a mixture of transcription factors
- Those are distributed unevenly
- The nuclei rapidly start to express different genes because they are exposed to different transcription regulators



Eve is expressed in 7 precisely positioned stripes

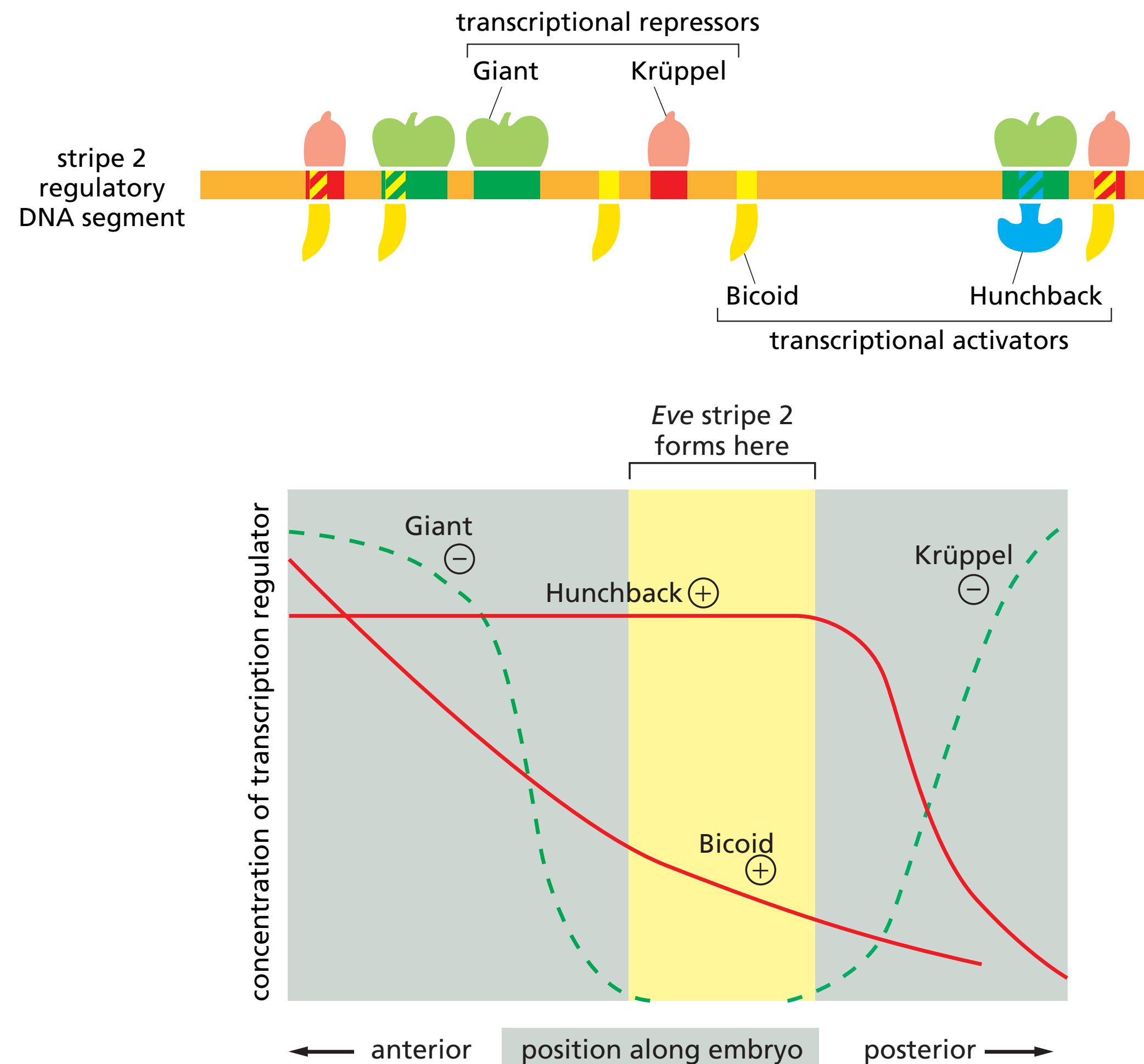
Drosophila Even-skipped gene

- How did we link the DNA regulatory sequence to its function?

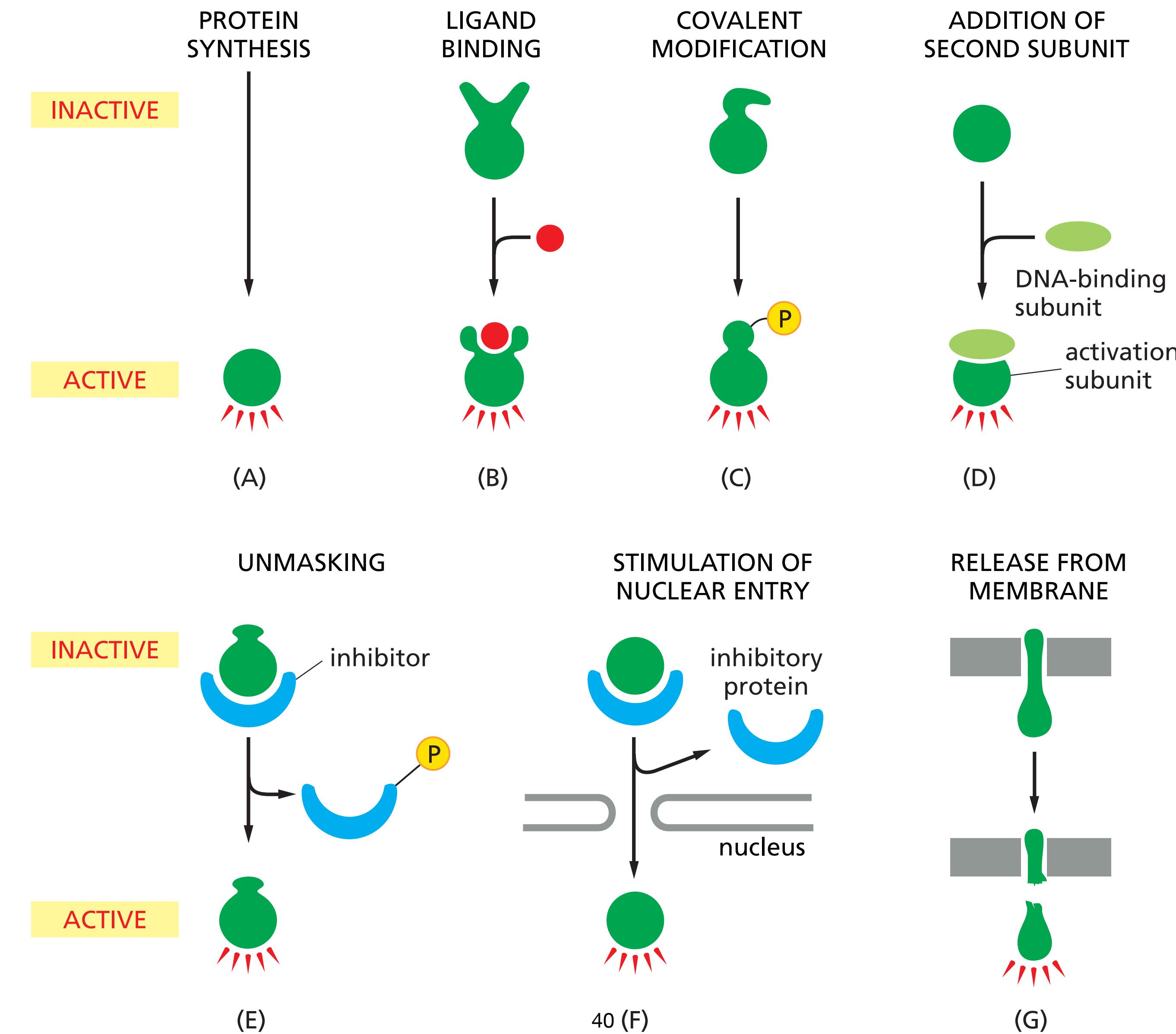


Drosophila Even-skipped gene

- How is the positioning in each stripe controlled?



Modulation of the activity of transcription regulators



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- **Understanding other regulatory systems**
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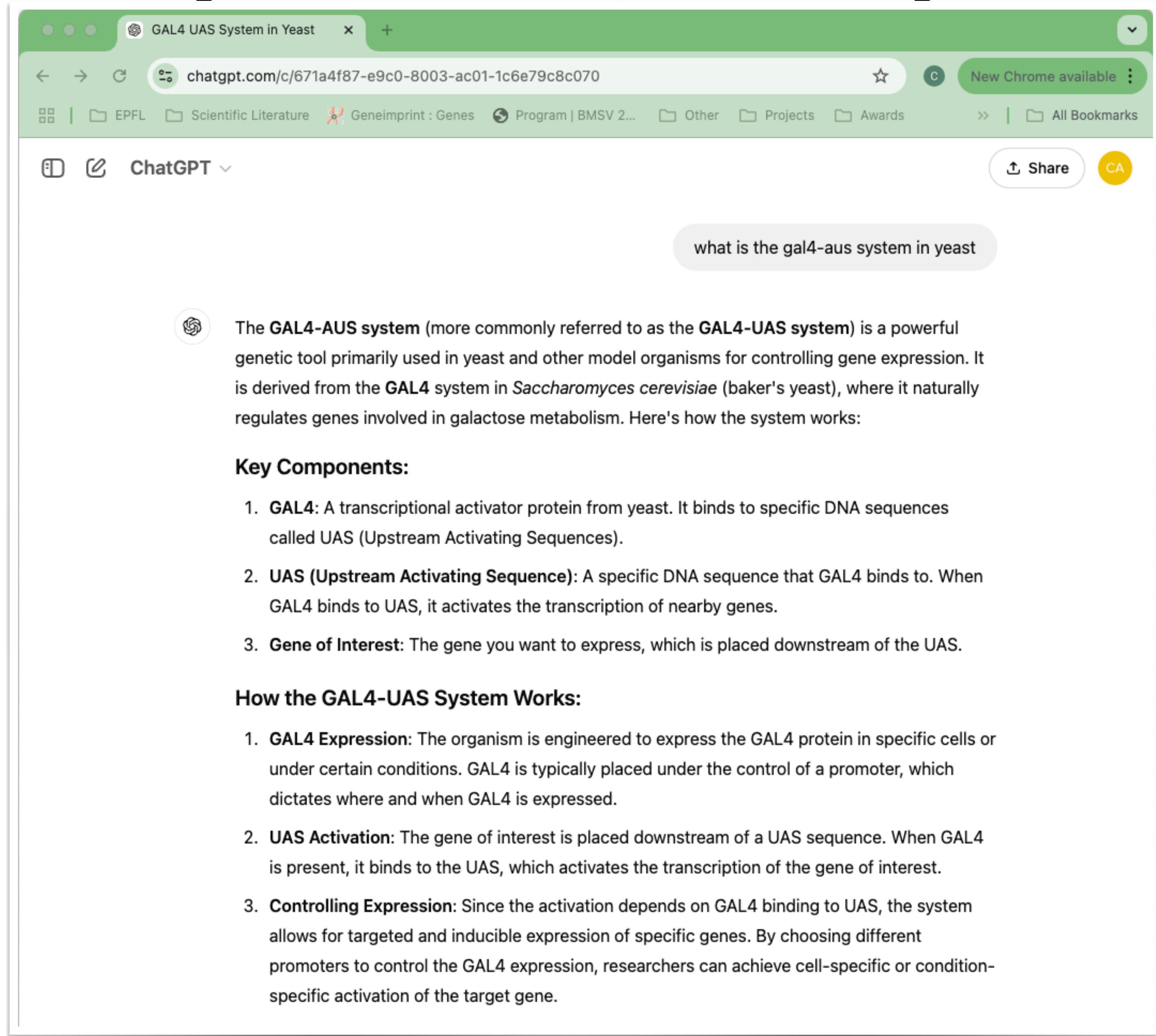
Understanding other systems

- Take a few minutes to search online and figure out the mechanism
- How does it work? who are the players?
- Why is it useful in labs?

1. Gal4-UAS in yeast

2. Arabinose operon in bacteria

How does the yeast Gal4-AUS system work?



The screenshot shows a web browser window with a green header bar. The title bar reads "GAL4 UAS System in Yeast". The address bar shows the URL "chatgpt.com/c/671a4f87-e9c0-8003-ac01-1c6e79c8c070". Below the address bar is a navigation bar with links to "EPFL", "Scientific Literature", "Geneimprint : Genes", "Program | BMSV 2...", "Other", "Projects", "Awards", and "All Bookmarks". The main content area is titled "ChatGPT" and contains a question "what is the gal4-aus system in yeast". The response starts with a paragraph about the GAL4-AUS system, followed by sections on "Key Components" and "How the GAL4-UAS System Works", each with a numbered list of points.

The **GAL4-AUS system** (more commonly referred to as the **GAL4-UAS system**) is a powerful genetic tool primarily used in yeast and other model organisms for controlling gene expression. It is derived from the **GAL4** system in *Saccharomyces cerevisiae* (baker's yeast), where it naturally regulates genes involved in galactose metabolism. Here's how the system works:

Key Components:

1. **GAL4**: A transcriptional activator protein from yeast. It binds to specific DNA sequences called UAS (Upstream Activating Sequences).
2. **UAS (Upstream Activating Sequence)**: A specific DNA sequence that GAL4 binds to. When GAL4 binds to UAS, it activates the transcription of nearby genes.
3. **Gene of Interest**: The gene you want to express, which is placed downstream of the UAS.

How the GAL4-UAS System Works:

1. **GAL4 Expression**: The organism is engineered to express the GAL4 protein in specific cells or under certain conditions. GAL4 is typically placed under the control of a promoter, which dictates where and when GAL4 is expressed.
2. **UAS Activation**: The gene of interest is placed downstream of a UAS sequence. When GAL4 is present, it binds to the UAS, which activates the transcription of the gene of interest.
3. **Controlling Expression**: Since the activation depends on GAL4 binding to UAS, the system allows for targeted and inducible expression of specific genes. By choosing different promoters to control the GAL4 expression, researchers can achieve cell-specific or condition-specific activation of the target gene.

How does the yeast Gal4-UAS system work?

Applications:

- **Targeted Gene Expression:** The GAL4-UAS system is widely used for driving the expression of specific genes in particular tissues, cells, or developmental stages.
- **Conditional Expression:** Researchers can make gene expression conditional by using promoters responsive to environmental factors (e.g., temperature, drugs, or other metabolites like galactose).
- **Genetic Screens:** It's often employed in functional genomic screens to overexpress or silence genes, helping to identify gene functions or interactions.

Why It's Popular in Yeast:

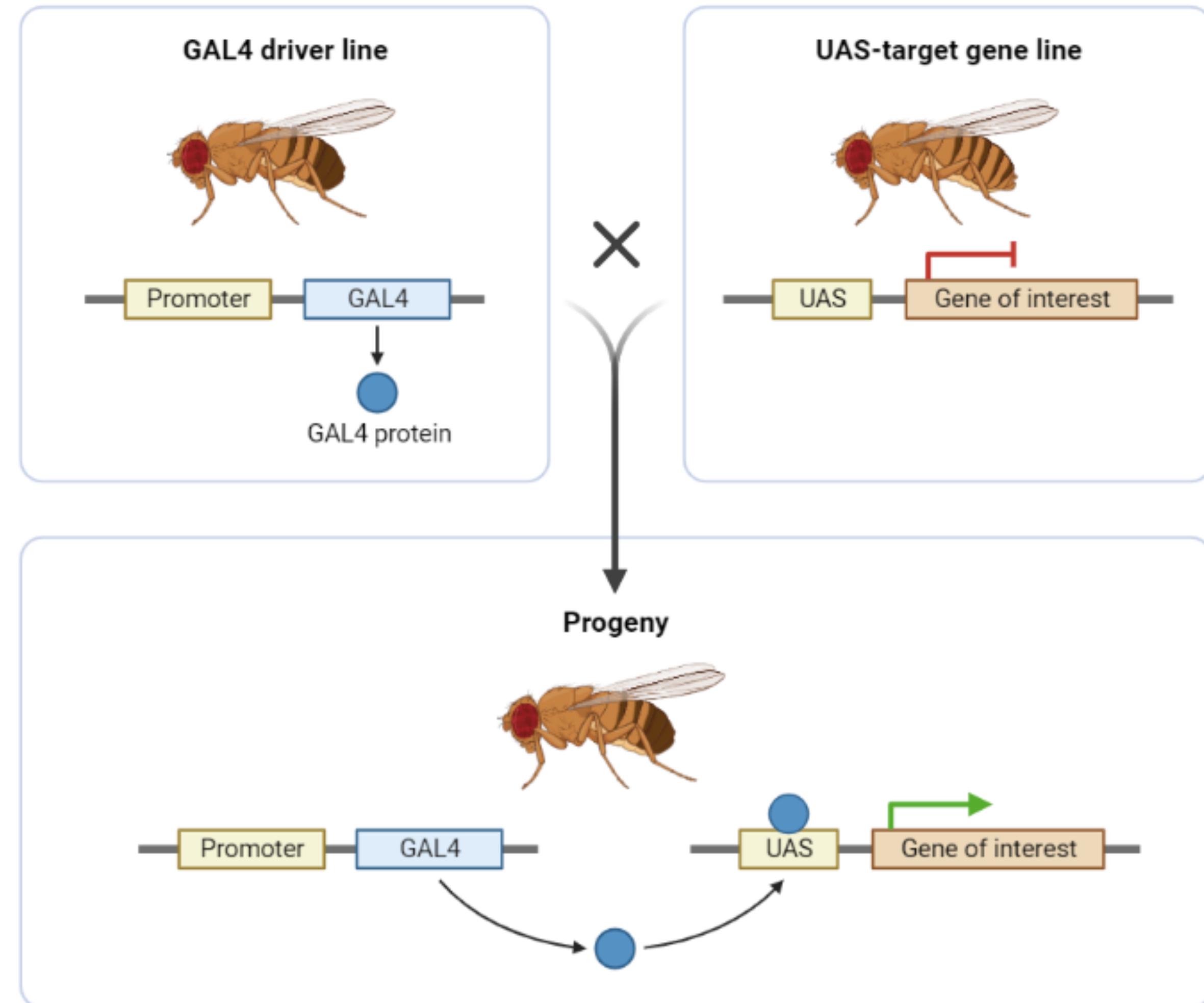
- In yeast, this system is native to the regulation of genes involved in galactose metabolism. In a natural setting, when galactose is present, the GAL4 protein activates the transcription of genes needed to metabolize galactose by binding to UAS sequences upstream of those genes.
- The GAL4-UAS system is modular and easily manipulated in yeast, making it useful for studying gene function, regulatory elements, and metabolic pathways.

In summary, the **GAL4-UAS** system allows for precise control of gene expression in yeast and other organisms, making it a versatile tool in molecular biology.



How does the yeast Gal4-AUS system work?

- Why is it useful?



How does the ara (pBAD) system work?

how does the arabinose promoter work



The **arabinose promoter** (commonly referred to as **P_BAD**) is a regulated promoter derived from the **ara operon** in *Escherichia coli* (E. coli) that controls the expression of genes involved in the metabolism of arabinose, a sugar. This promoter is widely used in genetic engineering to control gene expression because it can be turned on or off depending on the presence of arabinose in the growth medium.

Key Components:

1. **P_BAD (arabinose promoter):** The promoter sequence that controls the transcription of genes involved in arabinose metabolism.
2. **AraC protein:** A regulatory protein that both activates and represses the arabinose promoter depending on the presence or absence of arabinose.
3. **Arabinose:** A sugar molecule that serves as the inducer, activating the promoter by interacting with AraC.

How the Arabinose Promoter (P_BAD) Works:

1. In the Absence of Arabinose (Repression):

- When arabinose is not present, the AraC protein binds to two regions within the operon, **O2** and **I1**. This creates a loop in the DNA that blocks the RNA polymerase from binding to the **P_BAD** promoter.
- This looping prevents transcription of the downstream gene, effectively repressing gene expression.

How does the ara (pBAD) system work?

2. In the Presence of Arabinose (Activation):

- When arabinose is added to the environment, it binds to the AraC protein. This binding causes a conformational change in AraC, preventing the DNA loop from forming.
- AraC now binds to different sites, **I1** and **I2**, opening up the promoter and allowing RNA polymerase to access the **P_BAD** promoter.
- Transcription of the gene downstream of the **P_BAD** promoter is initiated, and the gene is expressed.

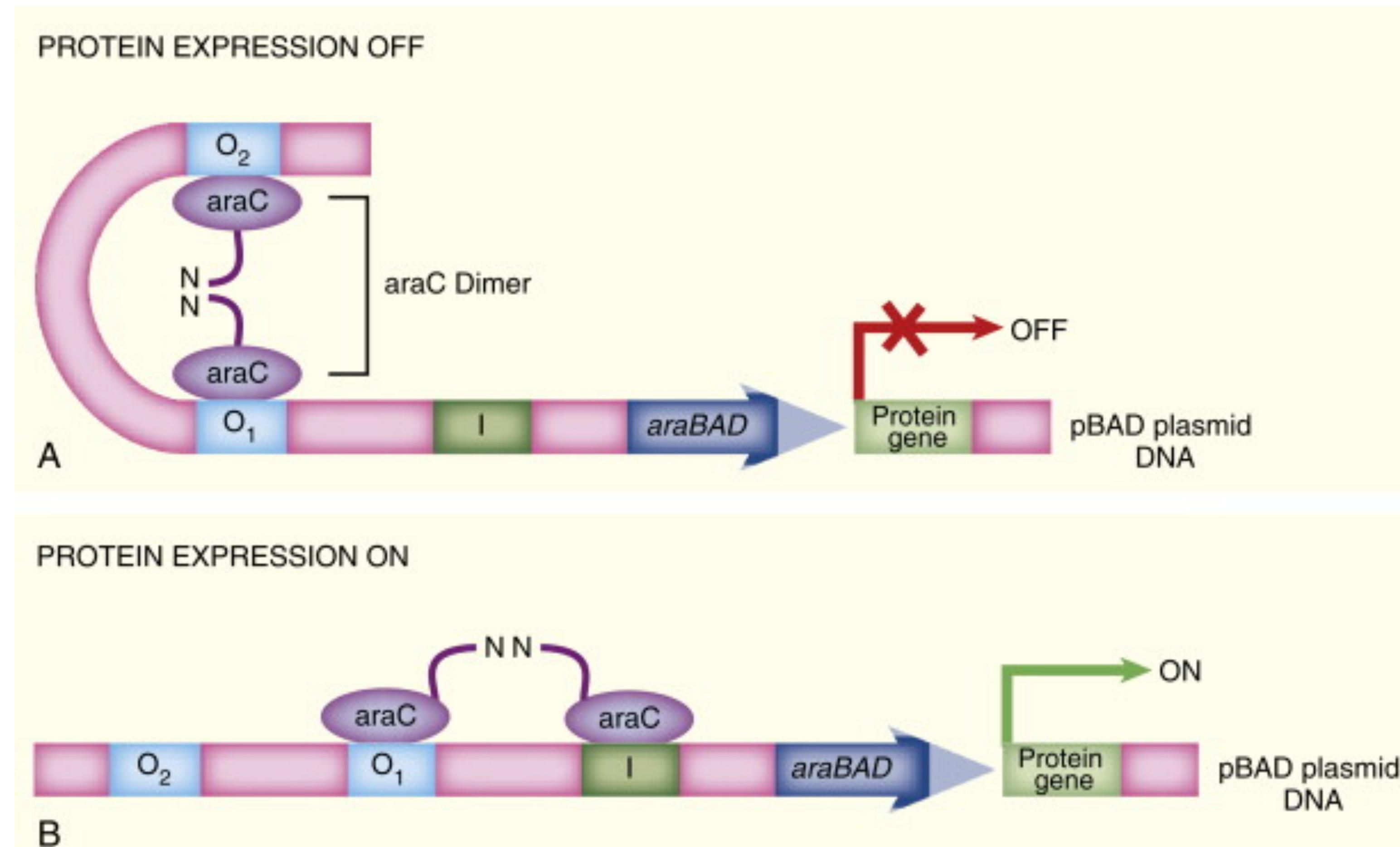
Strength of Expression:

- The strength of expression from the arabinose promoter can be fine-tuned by varying the concentration of arabinose in the medium. This gives researchers precise control over the level of gene expression.
- The system is **tightly regulated**—in the absence of arabinose, expression levels are very low, and when arabinose is present, the system can be fully activated.

Applications in Biotechnology:

1. **Inducible Gene Expression:** The arabinose promoter is often used to control the expression of genes in bacteria. By adding arabinose, the researcher can induce the expression of the target gene, and by removing it, the gene can be repressed.
2. **Protein Production:** This system is commonly used for expressing recombinant proteins. By controlling when the protein is expressed, researchers can optimize growth conditions before inducing protein production.
3. **Genetic Studies:** It is used in functional genomics to study gene function by conditionally expressing or silencing genes in response to arabinose.

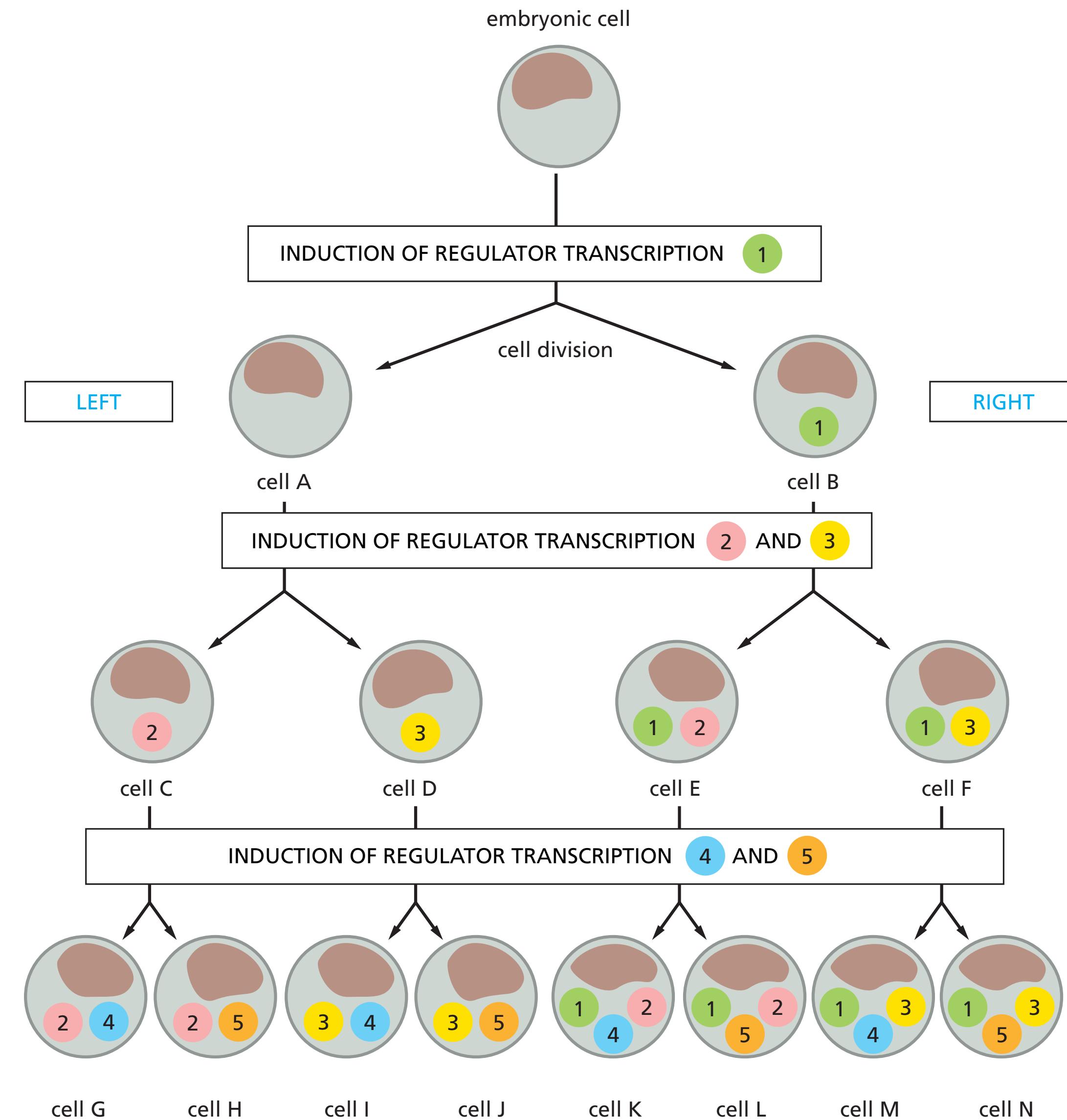
How does the ara (pBAD) system work?



Plan

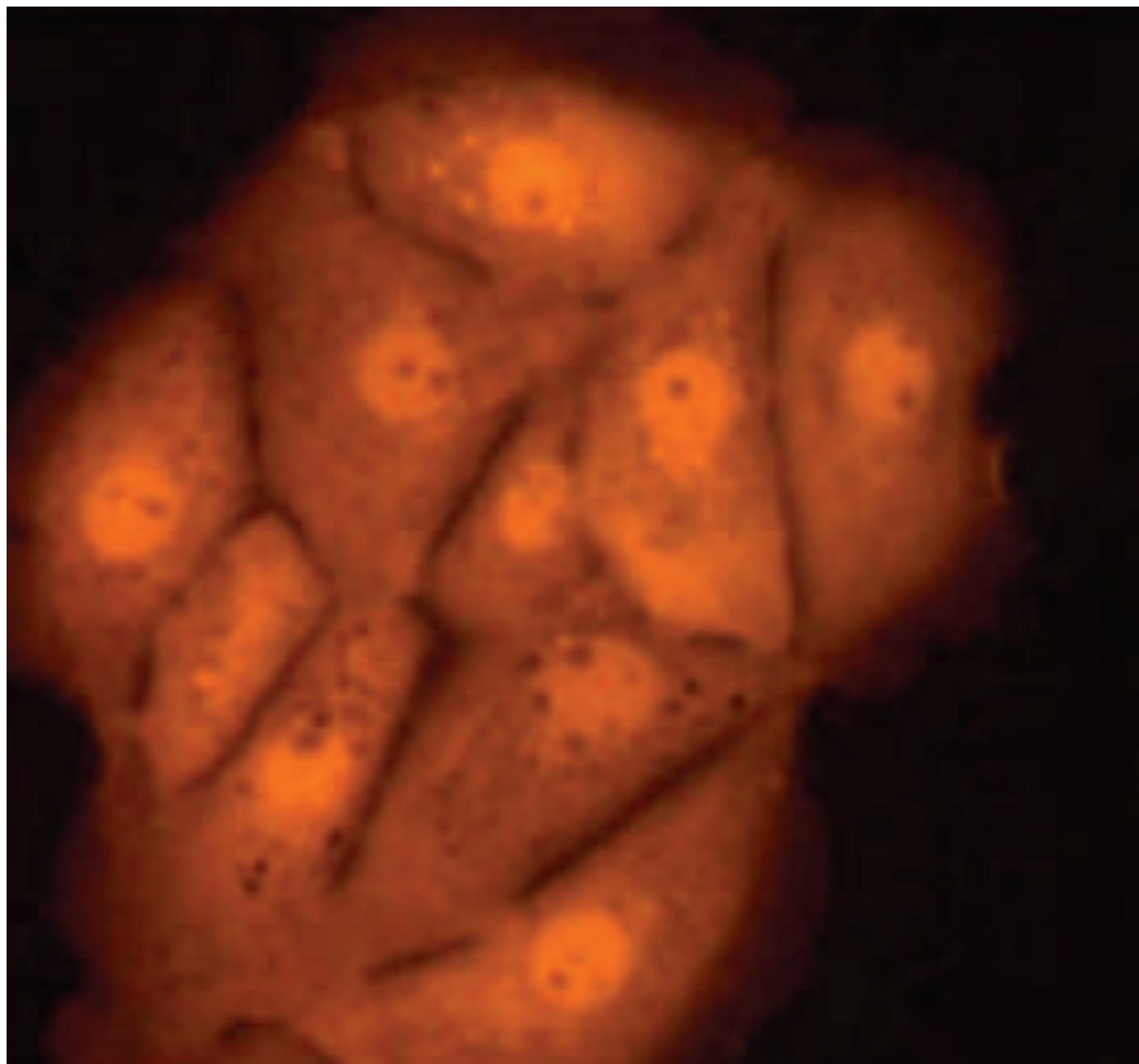
- Different cell types have the same DNA
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 - **Combinatorial gene control and cell types**

Combinatorial gene control creates cell types



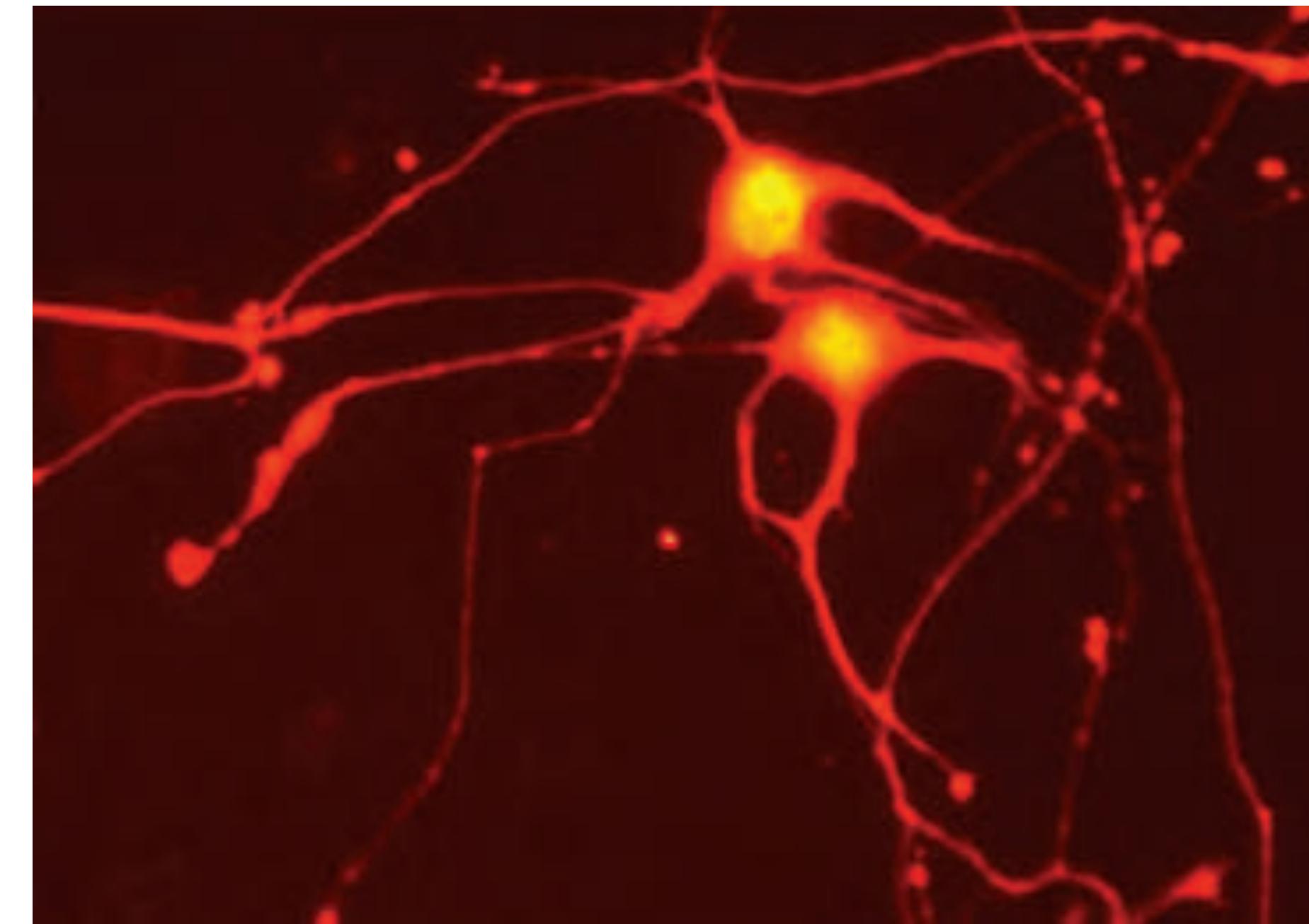
Combinatorial gene control creates cell types

- Artificial expression of 3 neuron-specific transcription regulators in liver cells converts them in nerve cells



(A)

50 μm

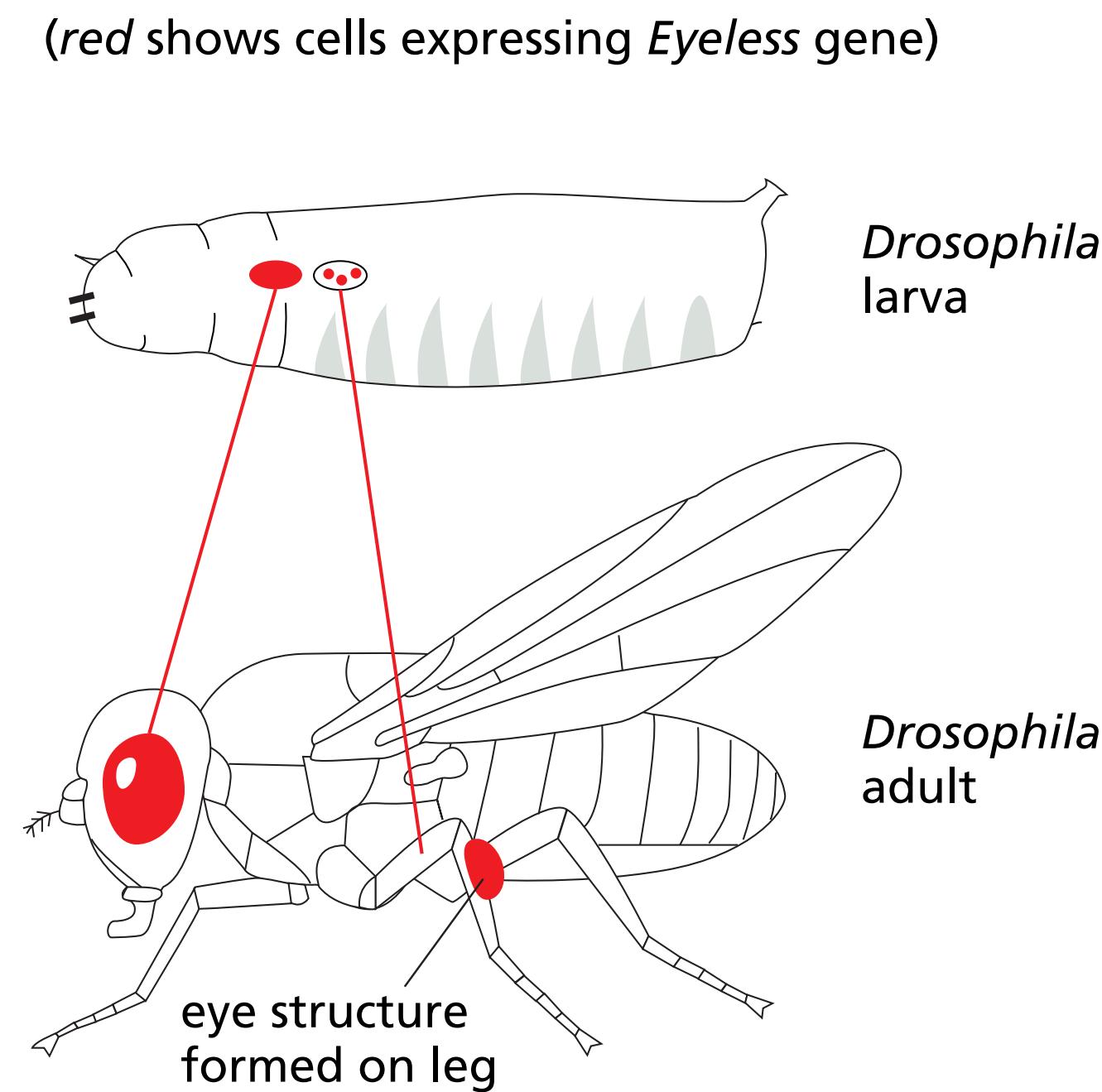
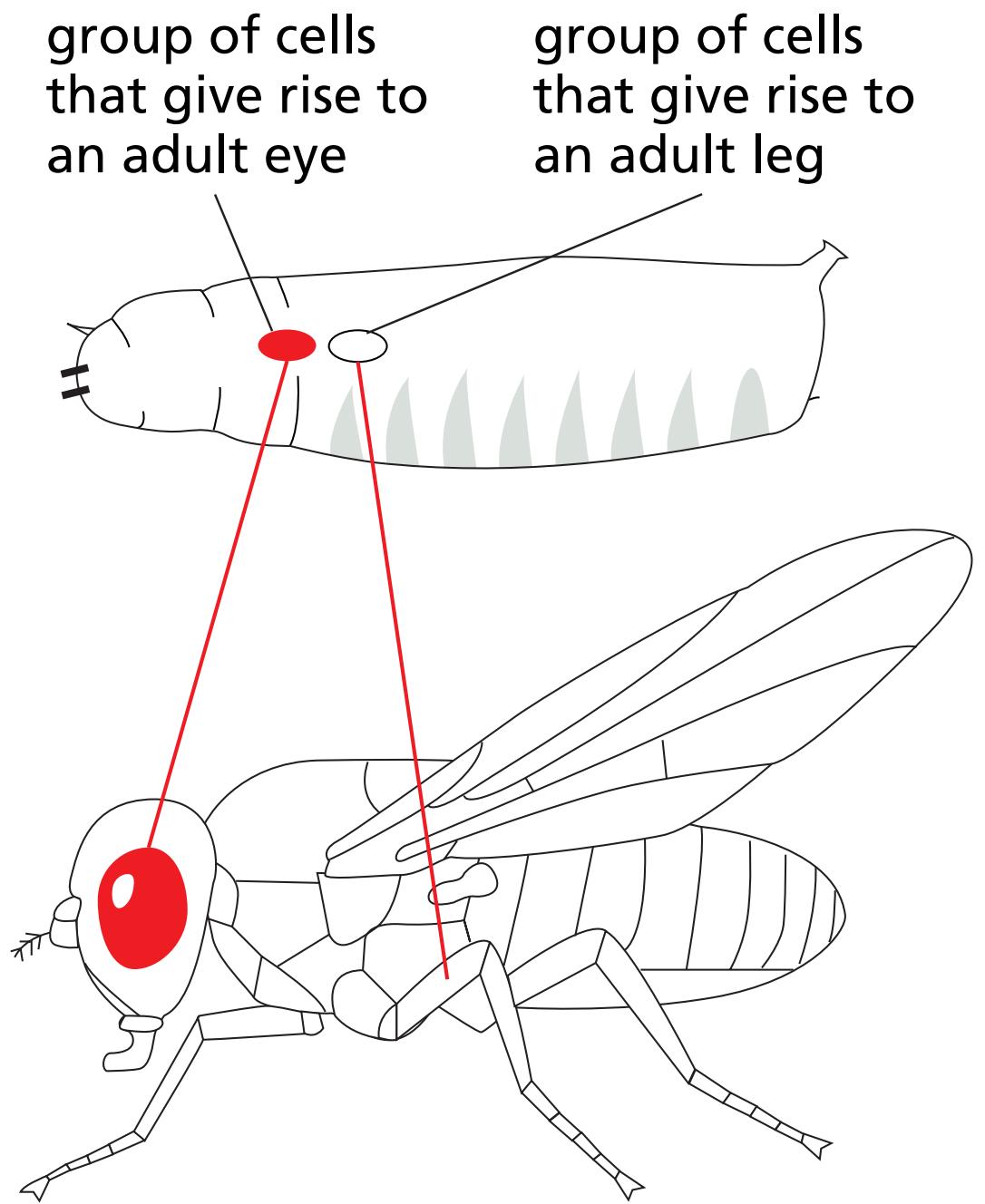


(B)

50 μm

Combinatorial gene control creates cell types

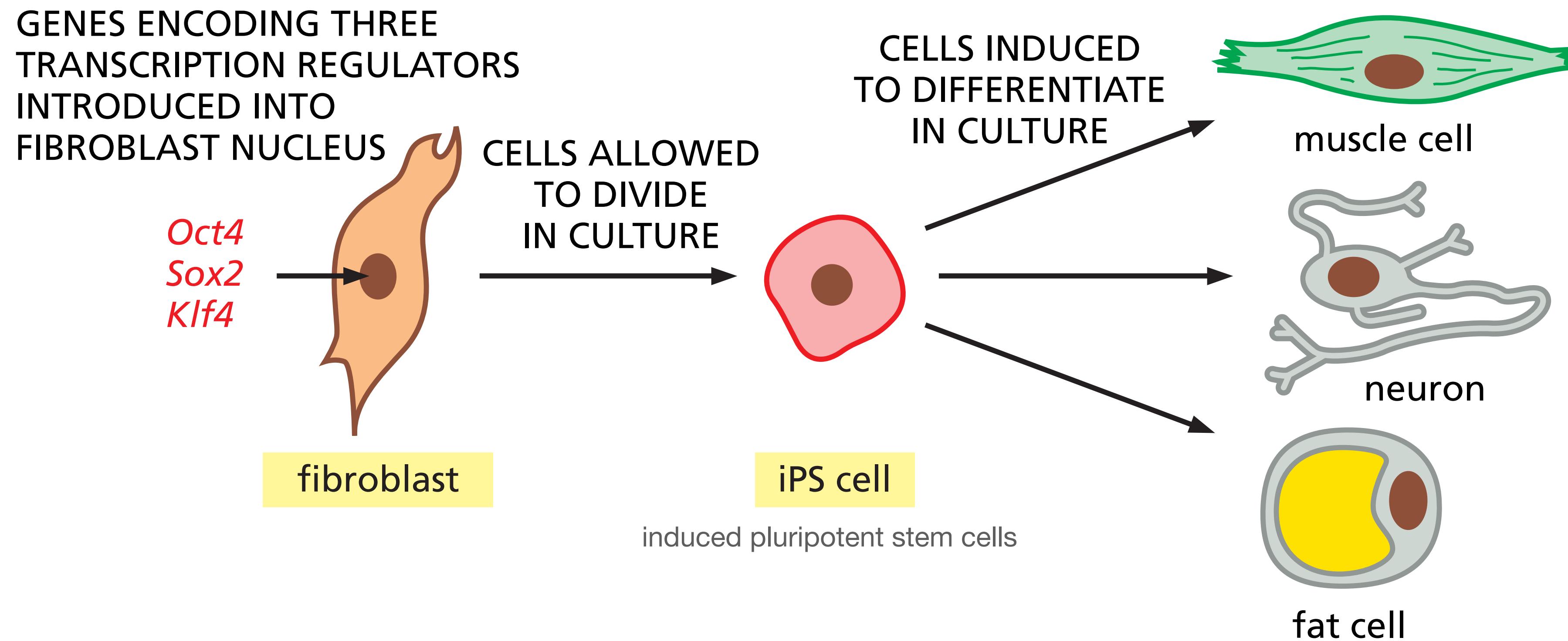
- Artificial expression of 1 transcription regulator from the eye region to the leg region



(A)

Pluripotent stem cells

- Manipulation of transcription regulators to de-differentiate cells



Different cell types

- Different cell types express different proteins
- Controlled by **master transcription regulators**

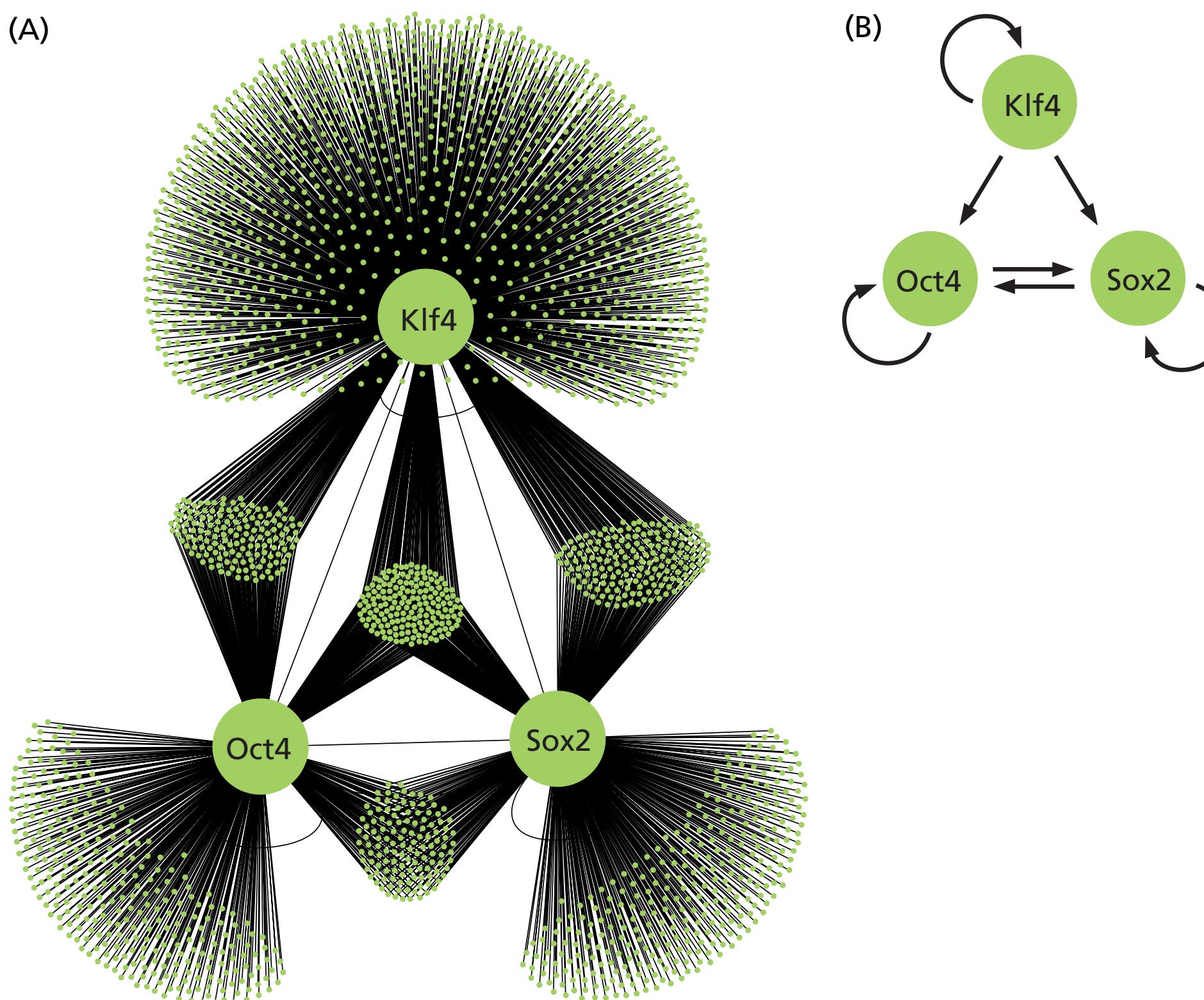
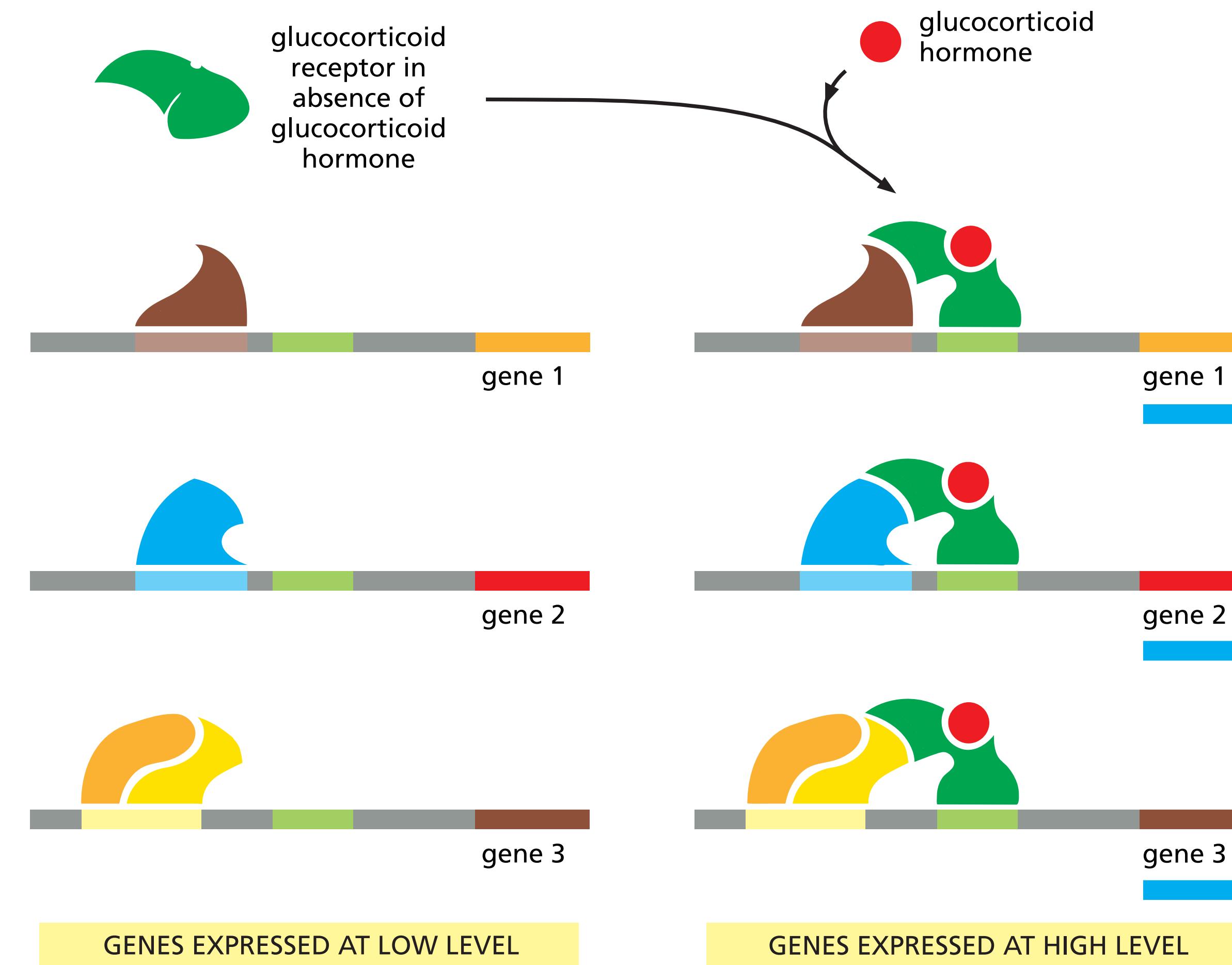


Figure 7–37 A portion of the transcription network specifying embryonic stem cells. (A) The three master transcription regulators in Figure 7–36 are shown as large circles. Genes whose *cis*-regulatory sequences are bound by each regulator in embryonic stem cells are indicated by a small dot (representing the gene) connected by a thin line (representing the binding reaction). Note that many of the target genes are bound by more than one of the regulators. (B) The master regulators control their own expression. As shown here, the three transcriptional regulators bind to their own control regions (indicated by feedback loops), as well as those of the other master regulators (indicated by straight arrows). (Courtesy of Trevor Sorrells, based on data from J. Kim et al., *Cell* 132:1049–1061, 2008.)

Different cell types

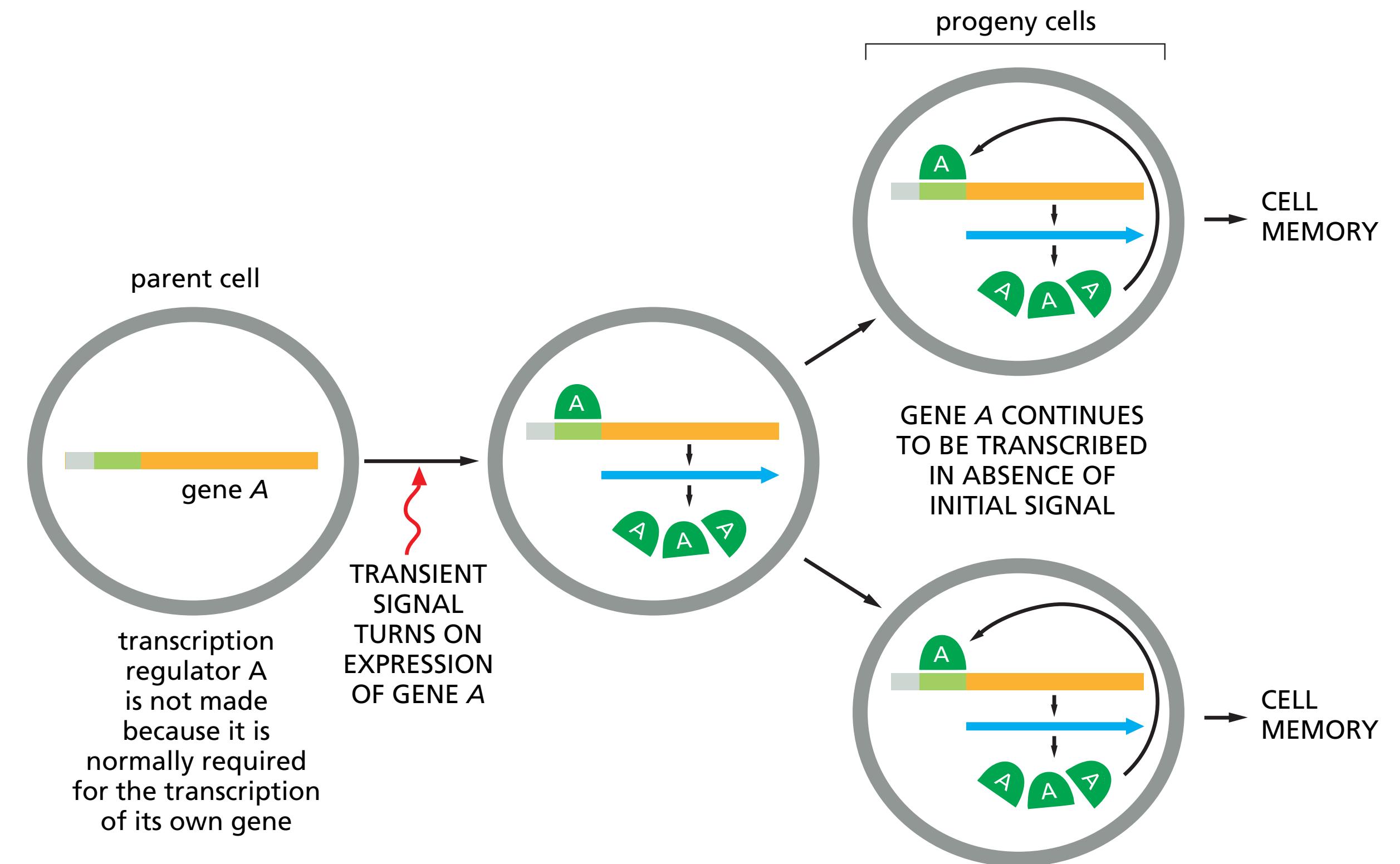
- Specialised cells rapidly turn genes on and off



Different cell types

Differentiated cells maintain **their identity**

- Progeny will remain the **same cell type - cell memory**
- Some are **terminally differentiated** (no further division) like neurons or skeletal muscle cells
- **Positive feedback loop** so a master transcription regulator activates transcription of its own gene



Summary

- Different cell types have the same DNA
- Transcriptional control
 - Transcriptional regulators
 - Activators
 - Repressors
 - Understanding other regulatory systems
 - Combinatorial gene control and cell types

Have a nice day!