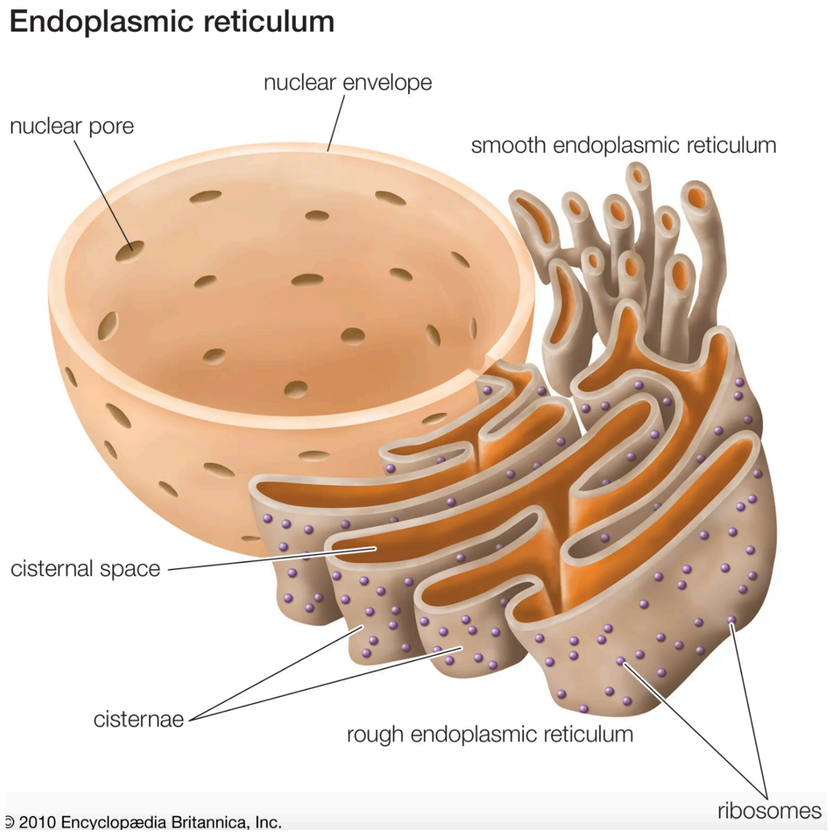
Cellular and Molecular Biology I

BIO-205-7

Camille Goemans - 2024

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

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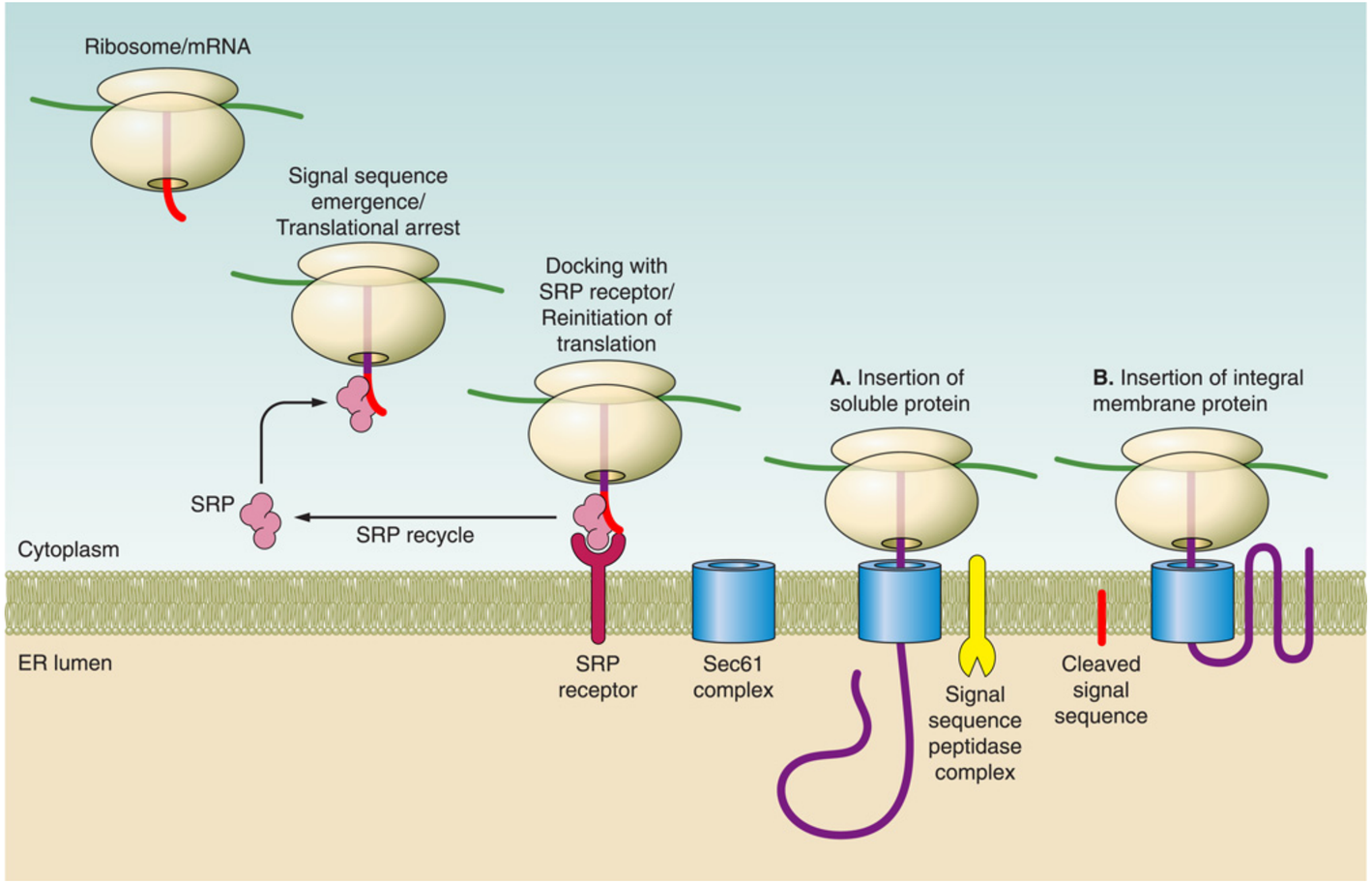
- How are ribosomes located on the surface of the endoplasmic reticulum?



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A few things to discuss

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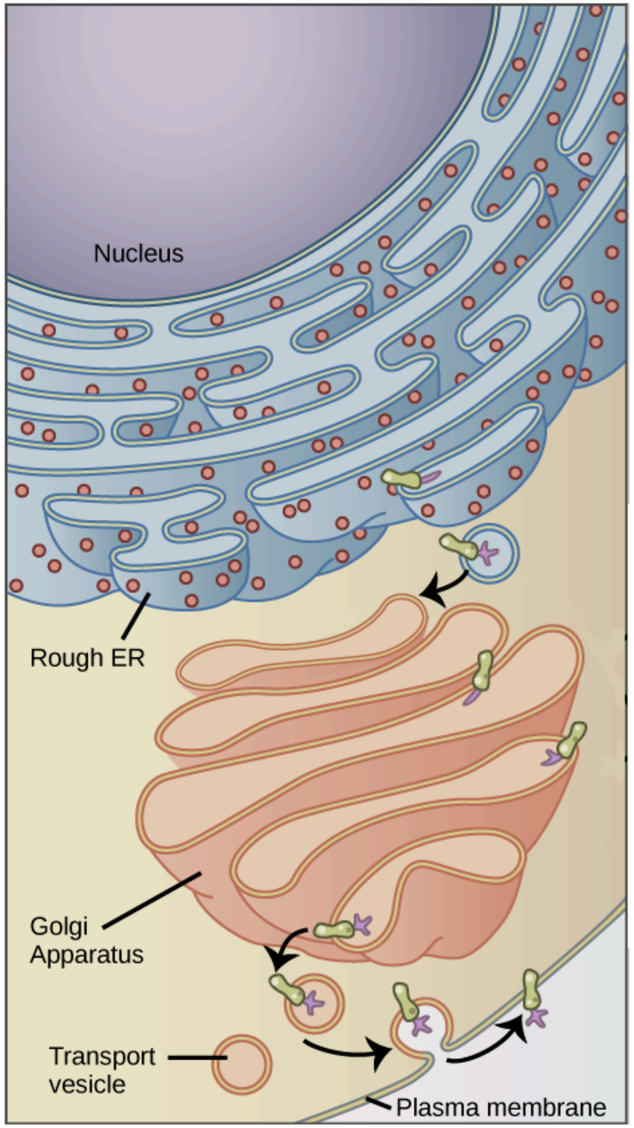
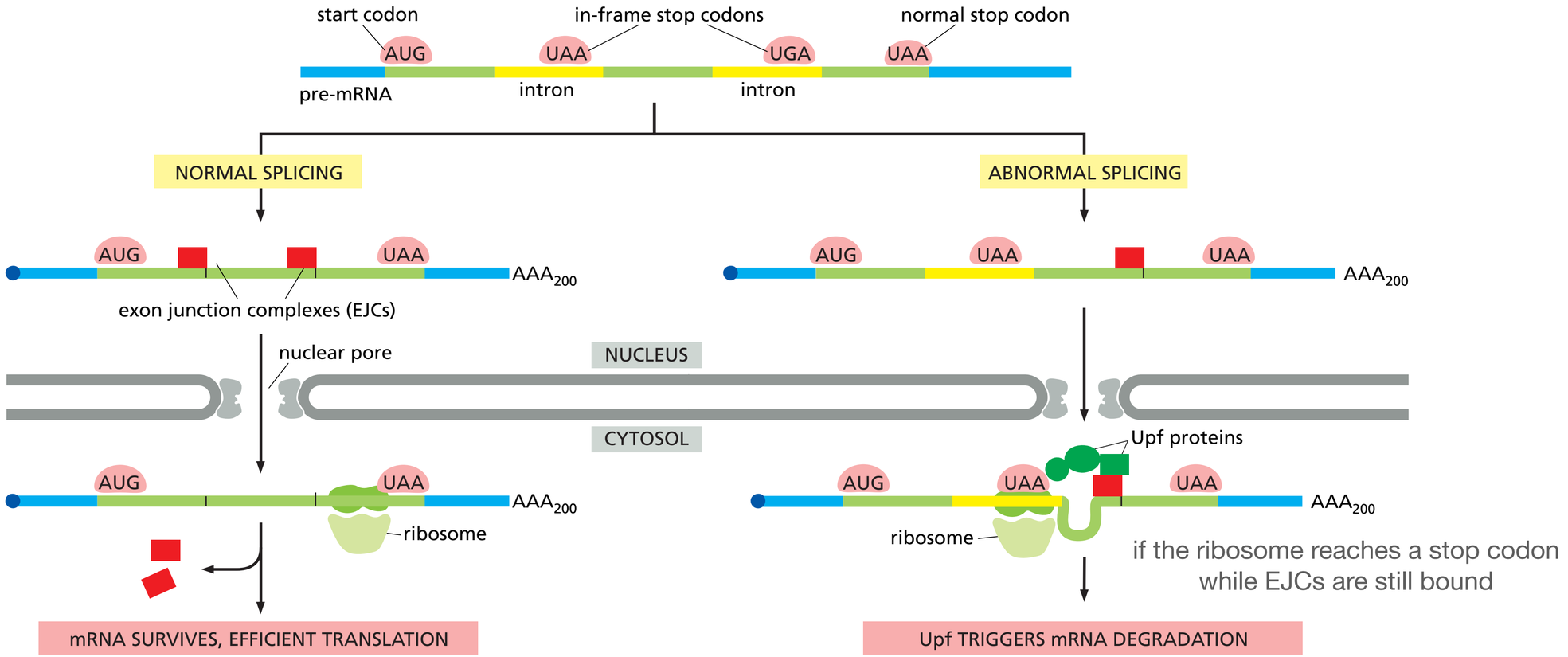


https://www.khanacademy.org/science/biology/gene-expression-central-dogma/translation-polypeptides/a/protein-targeting-and-traffic

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



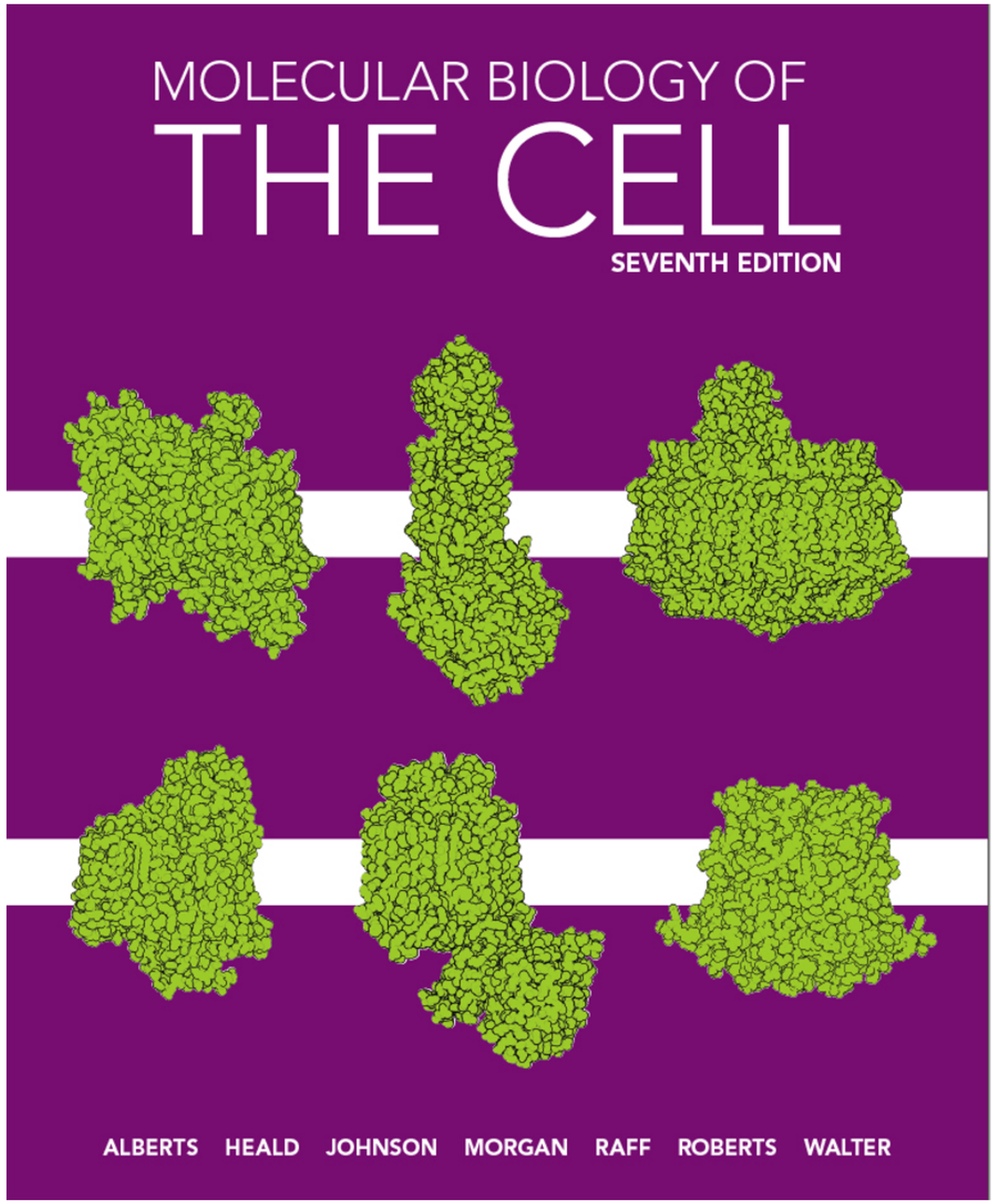
Quality control mechanism: erratum

- 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.



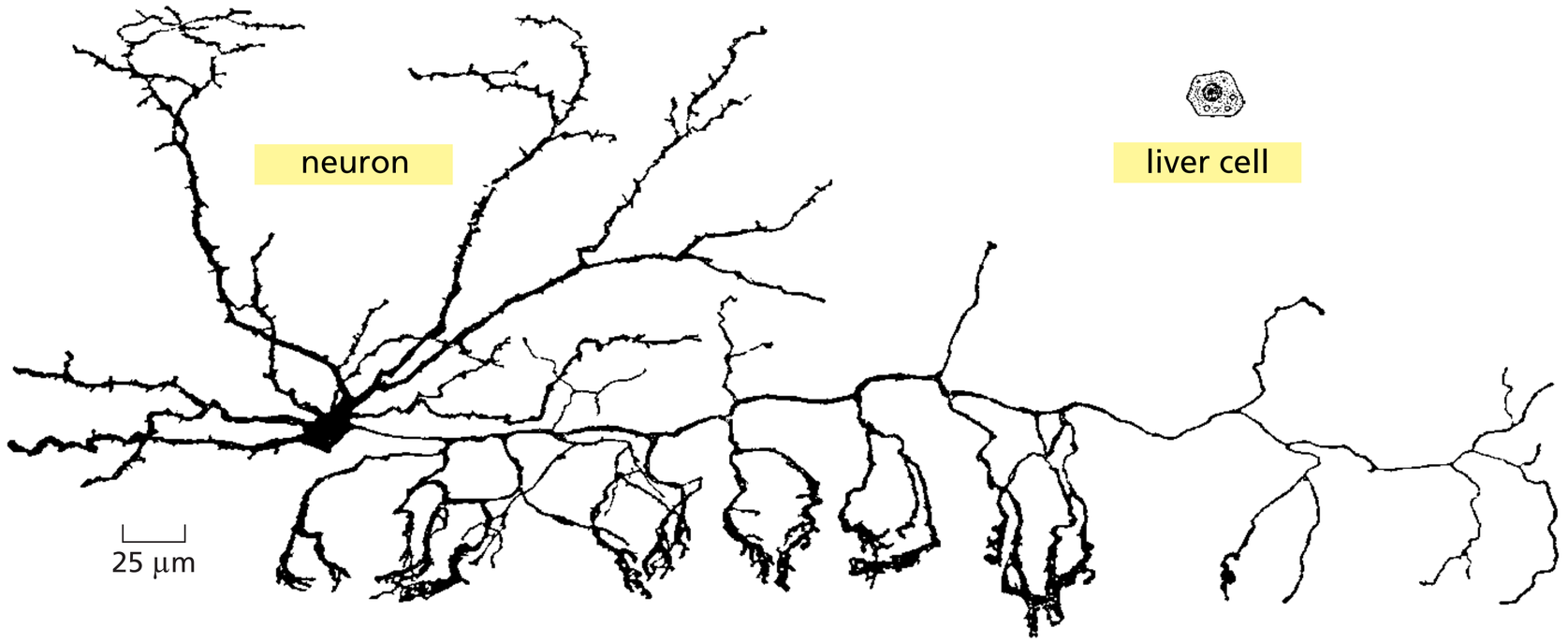
Chapter 7

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

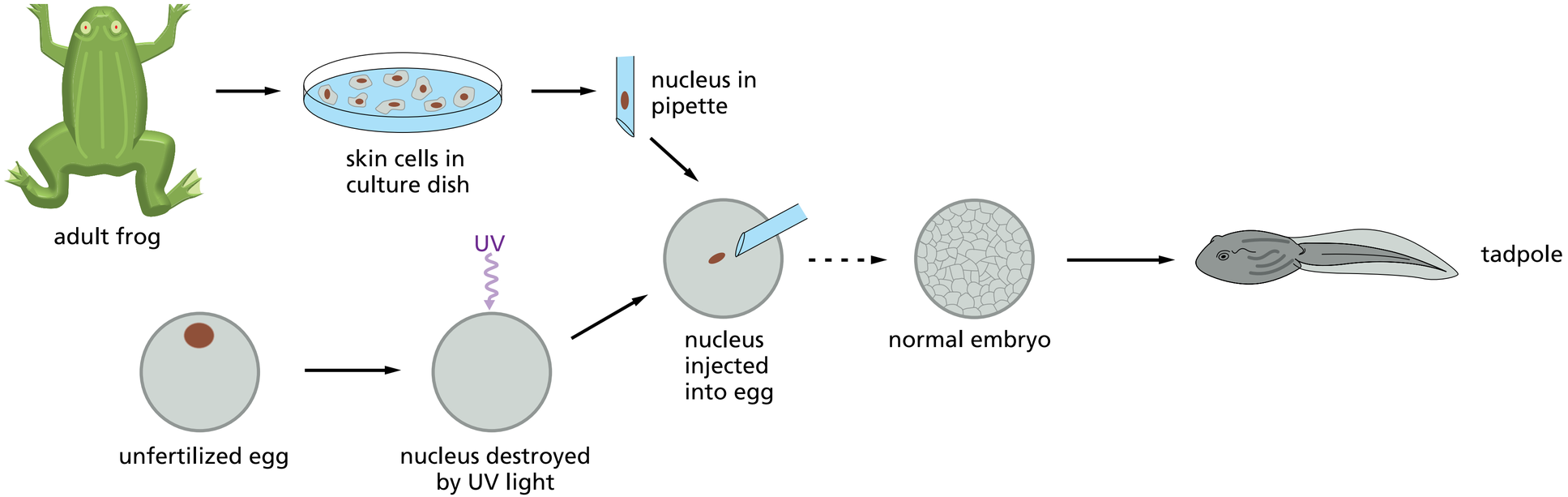
Different cell types contain the same DNA



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
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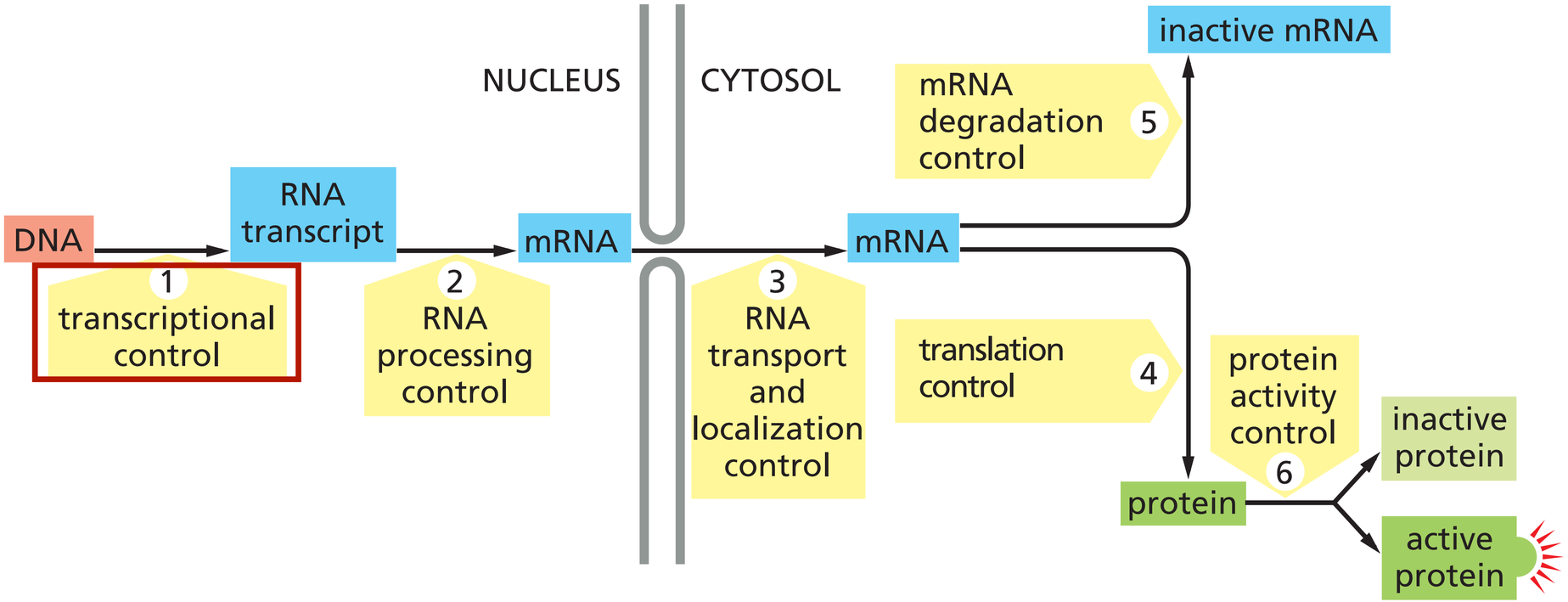
Levels of gene expression regulation



Plan

- Different cell types have the same DNA
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  - **Transcriptional regulators**
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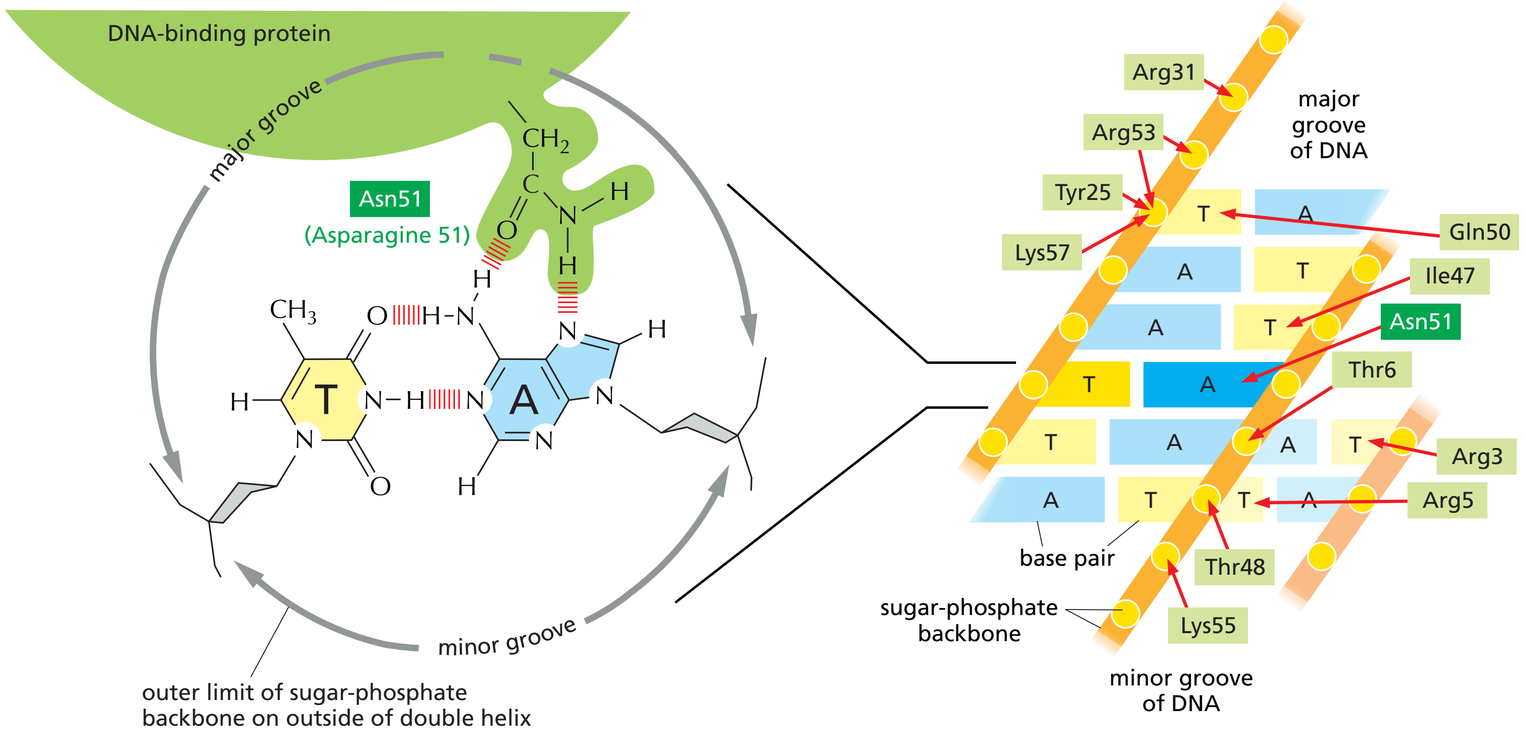
Control of transcription by DNA-binding proteins

- 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?

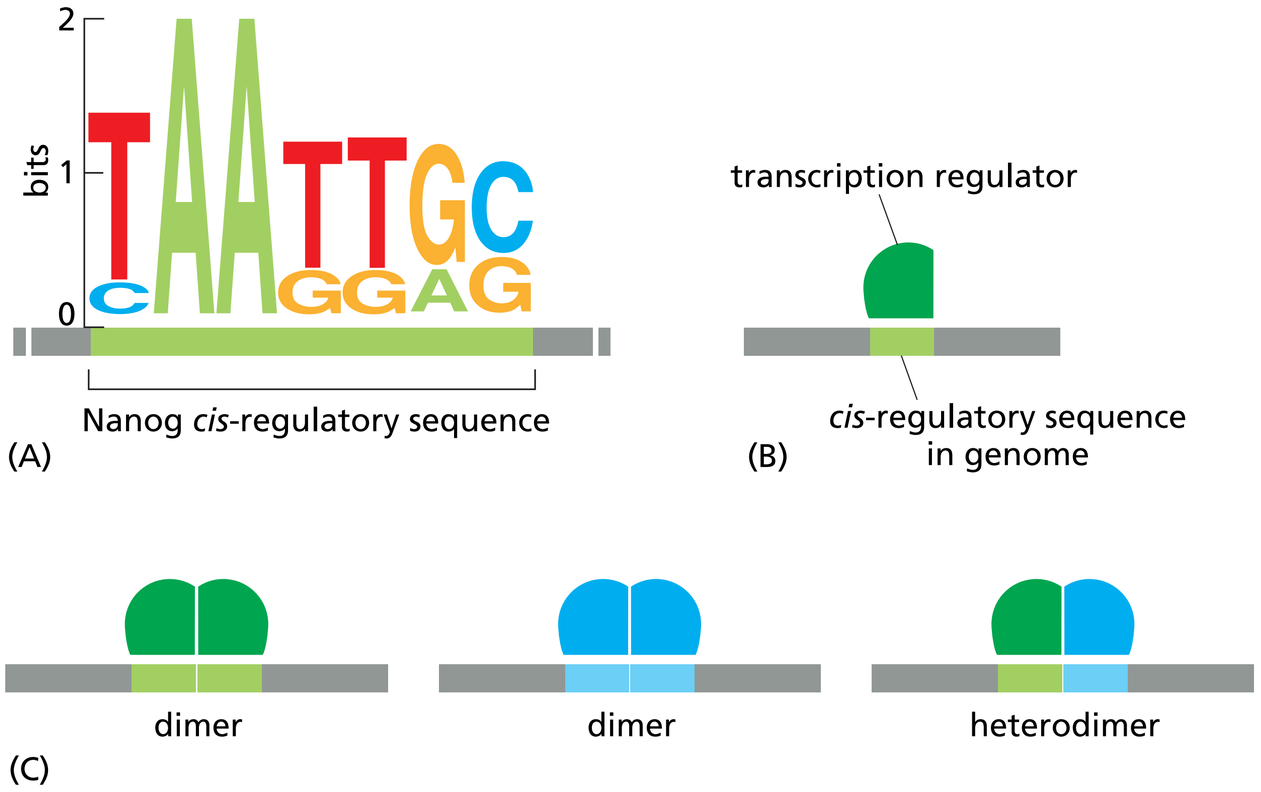
- the surface of the protein is **complementary** to the special surface features of a portion of DNA
- contacts involve **hydrogen bonds, ionic bonds and hydrophobic interactions**



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

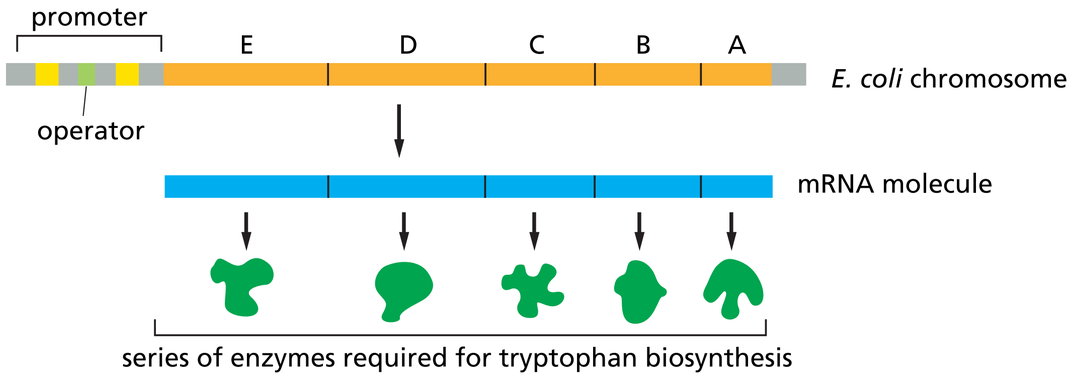


Control of transcription by DNA-binding proteins

Transcription regulators **switch genes on and off**

- The tryptophan repressor (bacteria)

The tryptophan operon

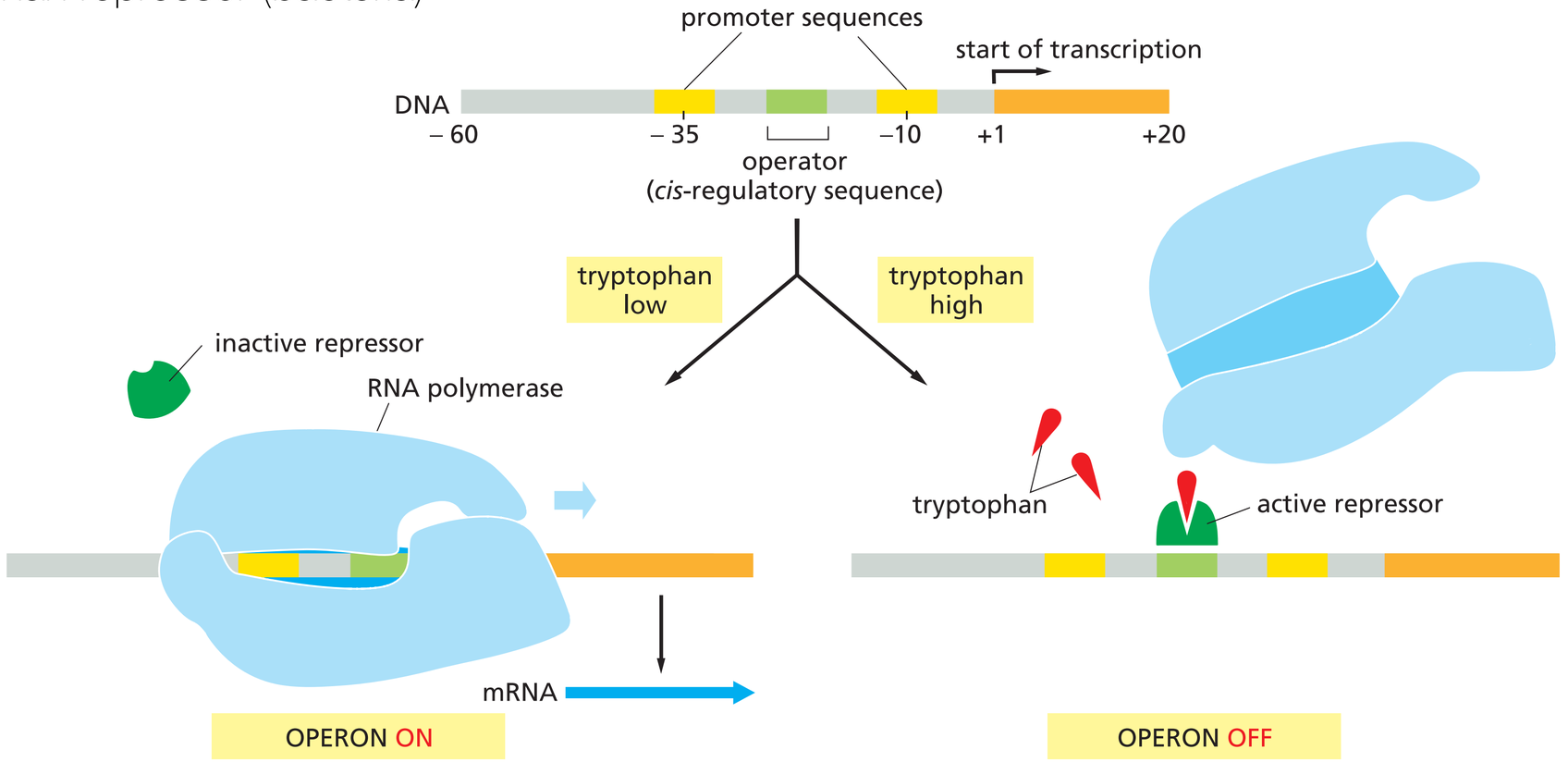


- When tryptophan levels are low, the operon is transcribed
- When tryptophan levels are high, the operon is not transcribed

Control of transcription by DNA-binding proteins

Transcription regulators **switch genes on and off**

- The tryptophan repressor (bacteria)



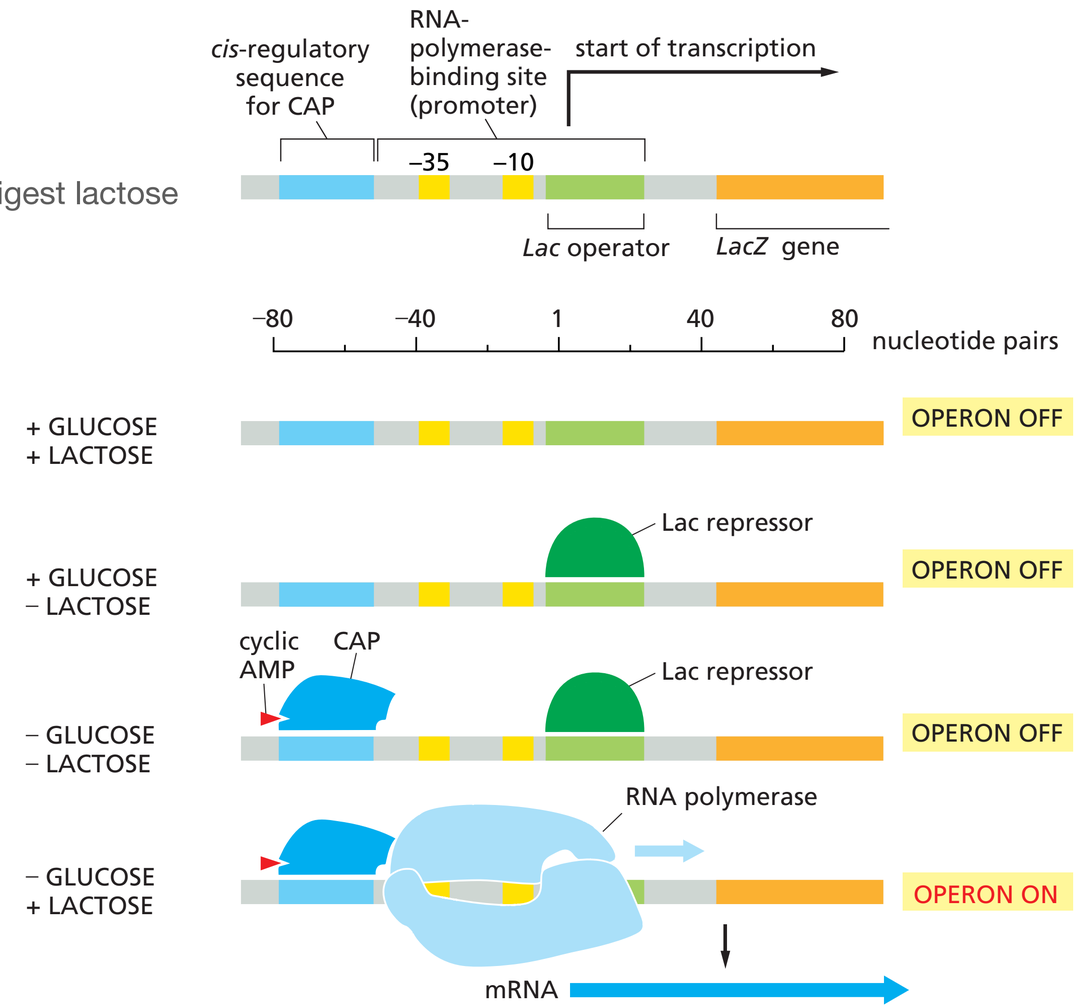
Transcriptional **repressors** turn genes off, transcriptional **activators** turn genes on

Control of transcription by DNA-binding proteins

Transcription regulators **switch genes on and off**

- The lac operon (bacteria)

Import and digest lactose

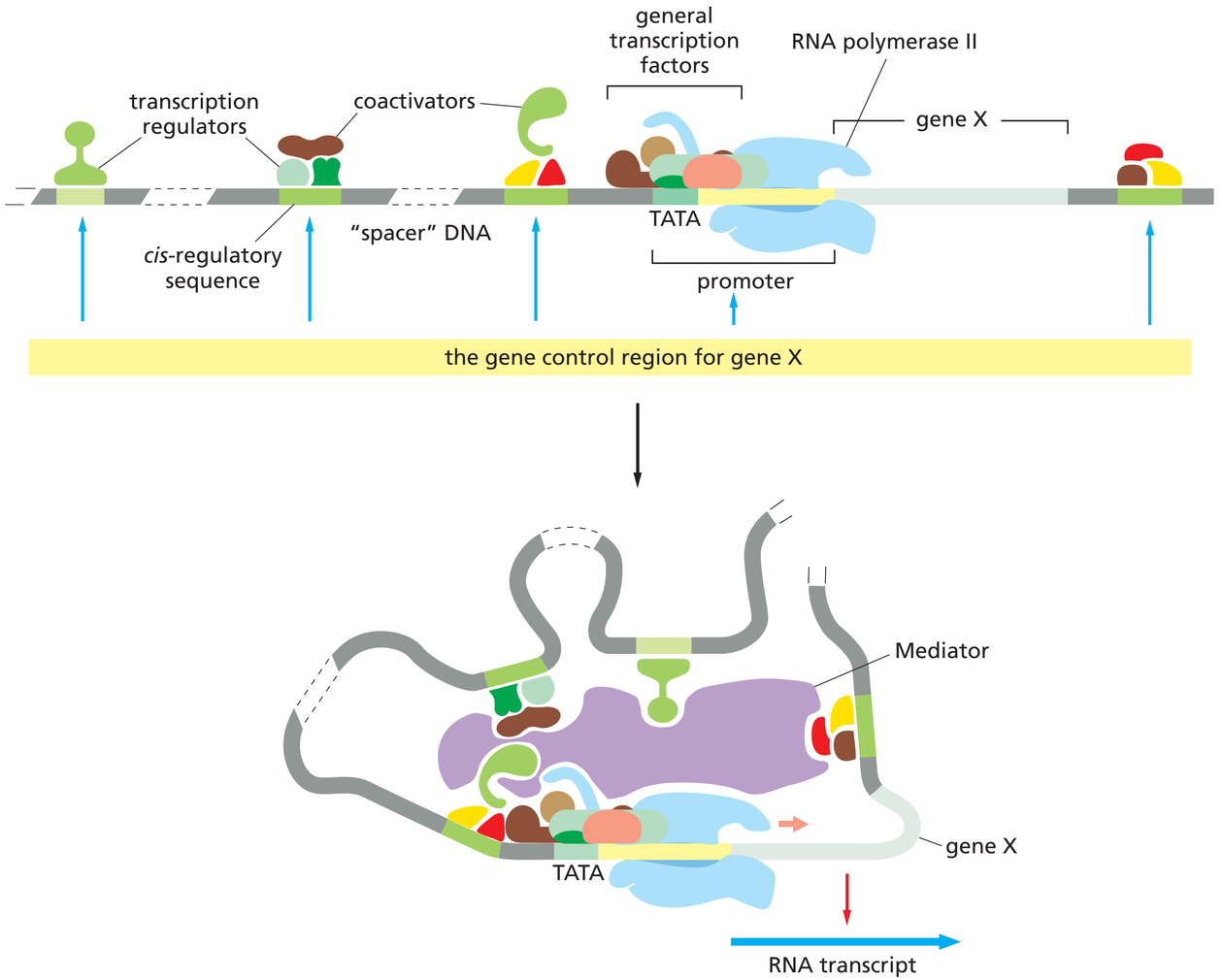


CAP binding allows to use other carbon sources

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

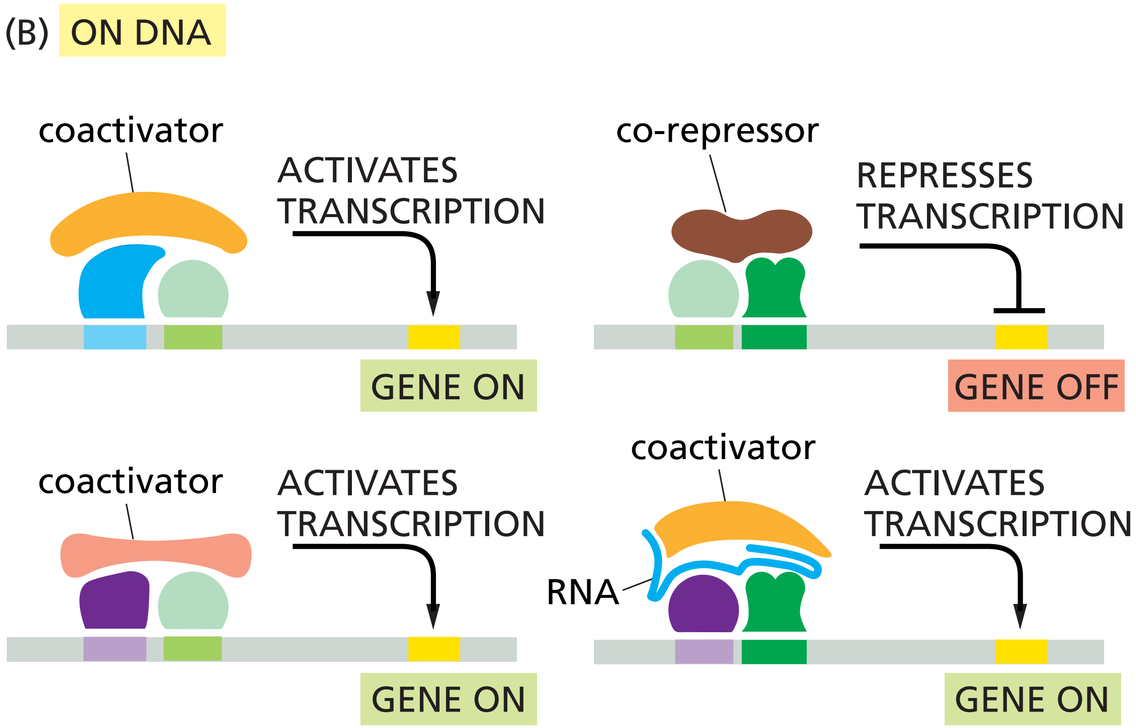
Control of transcription by DNA-binding proteins

- 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

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- Transcriptional control
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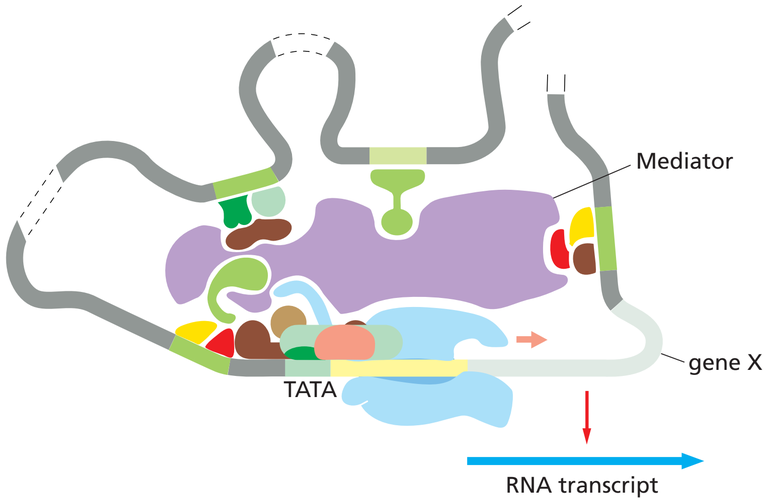
  - **Activators**
  - Repressors
  - Understanding other regulatory systems
  - Combinatorial gene control and cell types

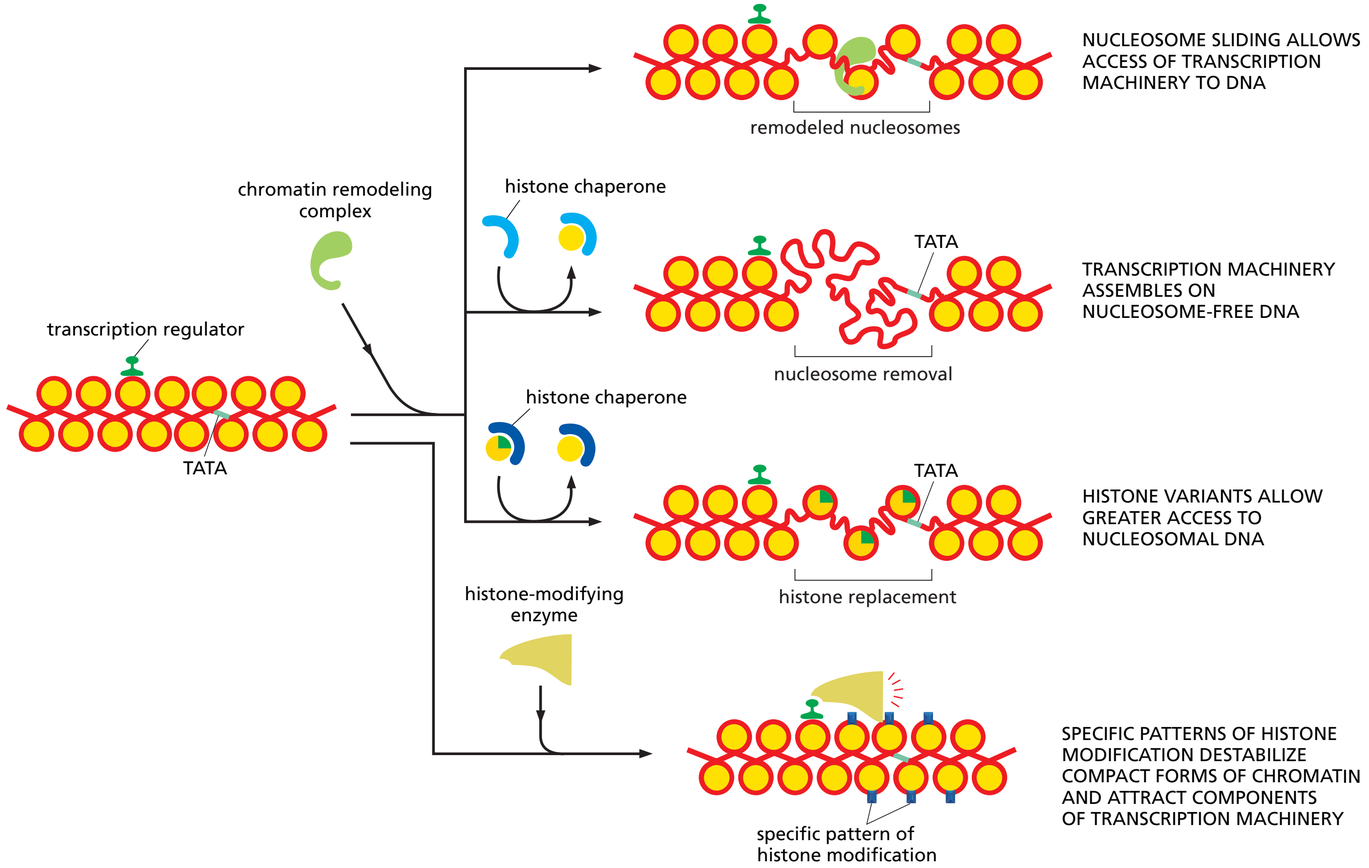
Activator proteins

- 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



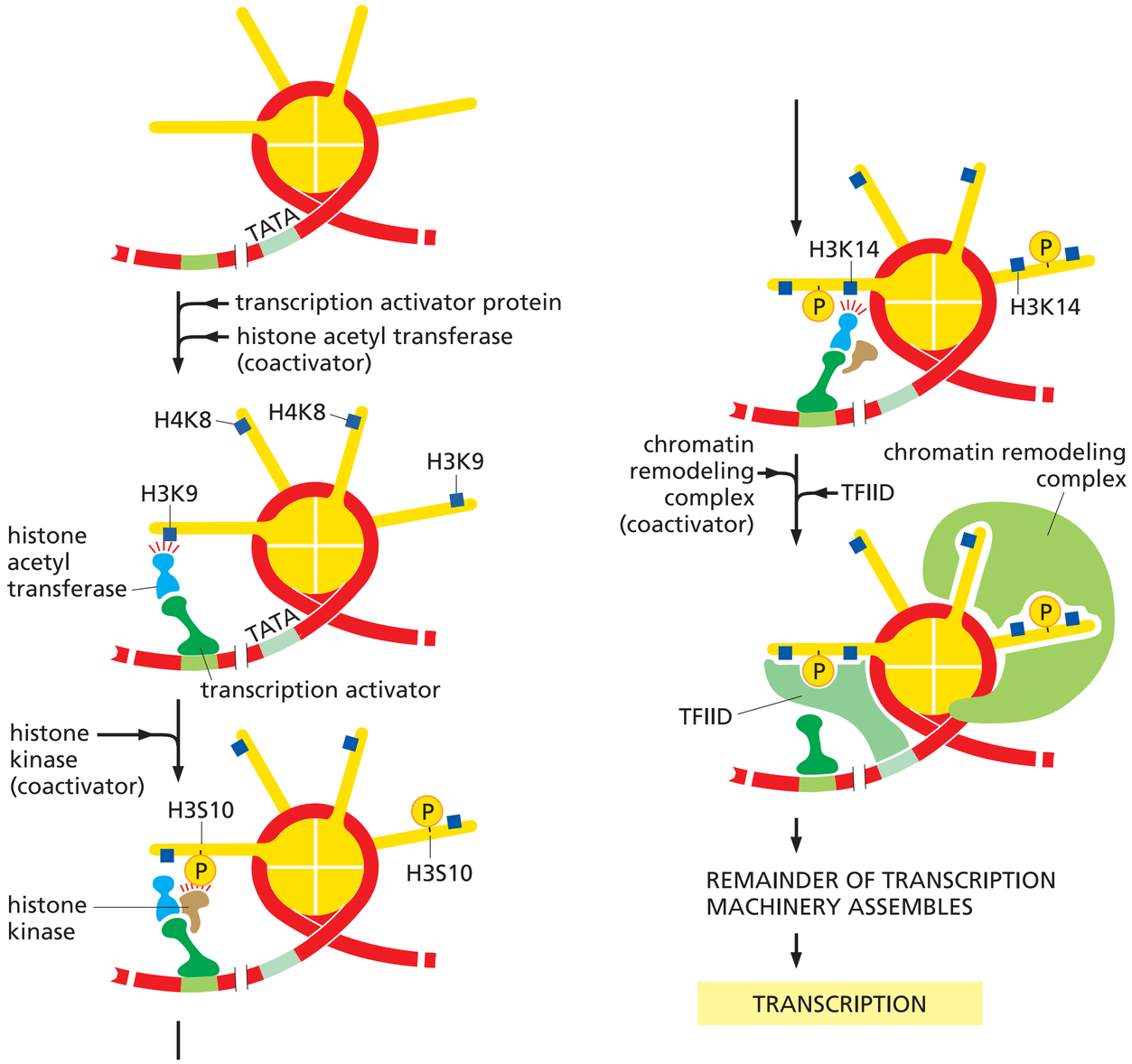
Activator proteins

- 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)

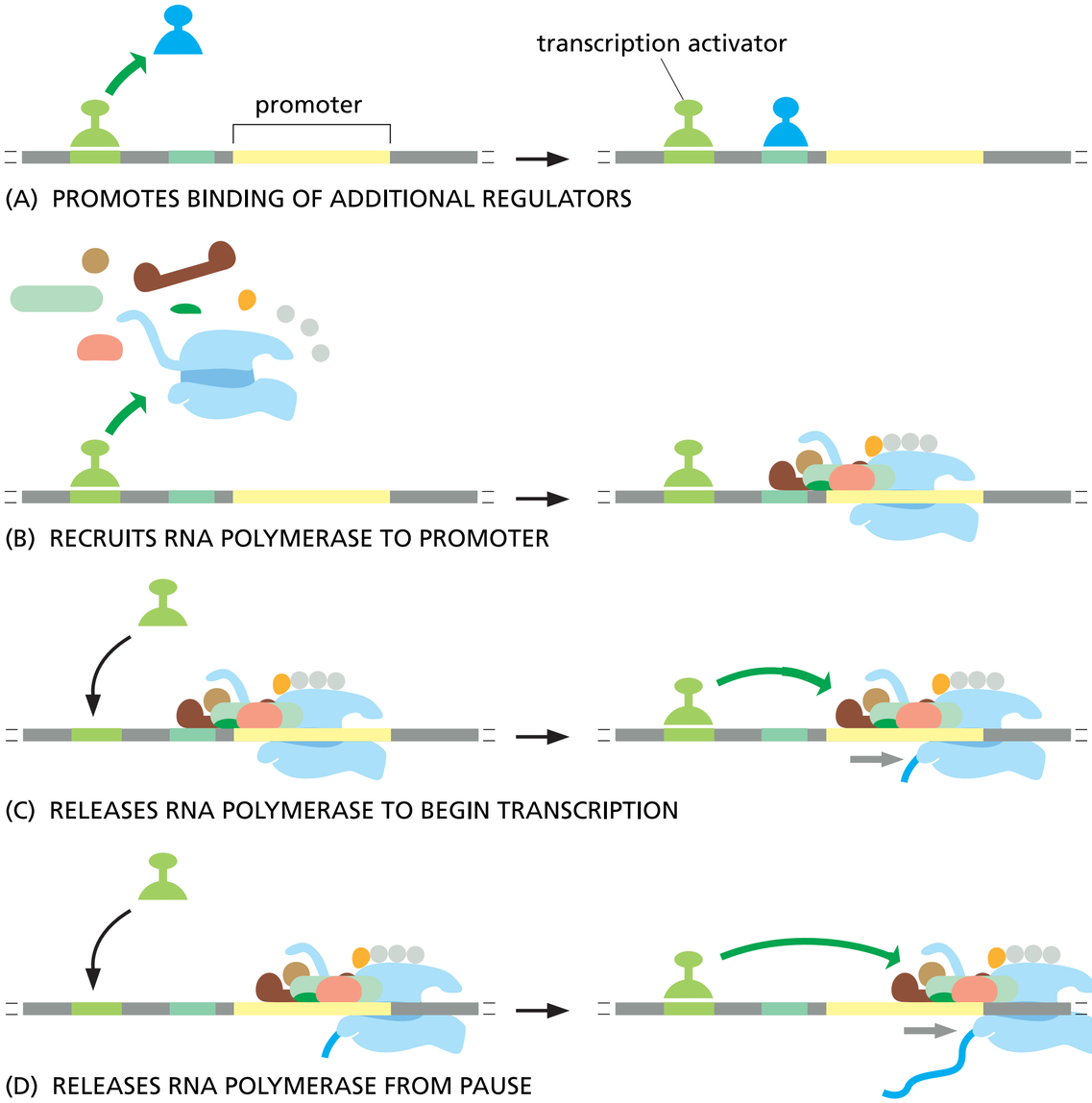


Activator proteins

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



Activator proteins



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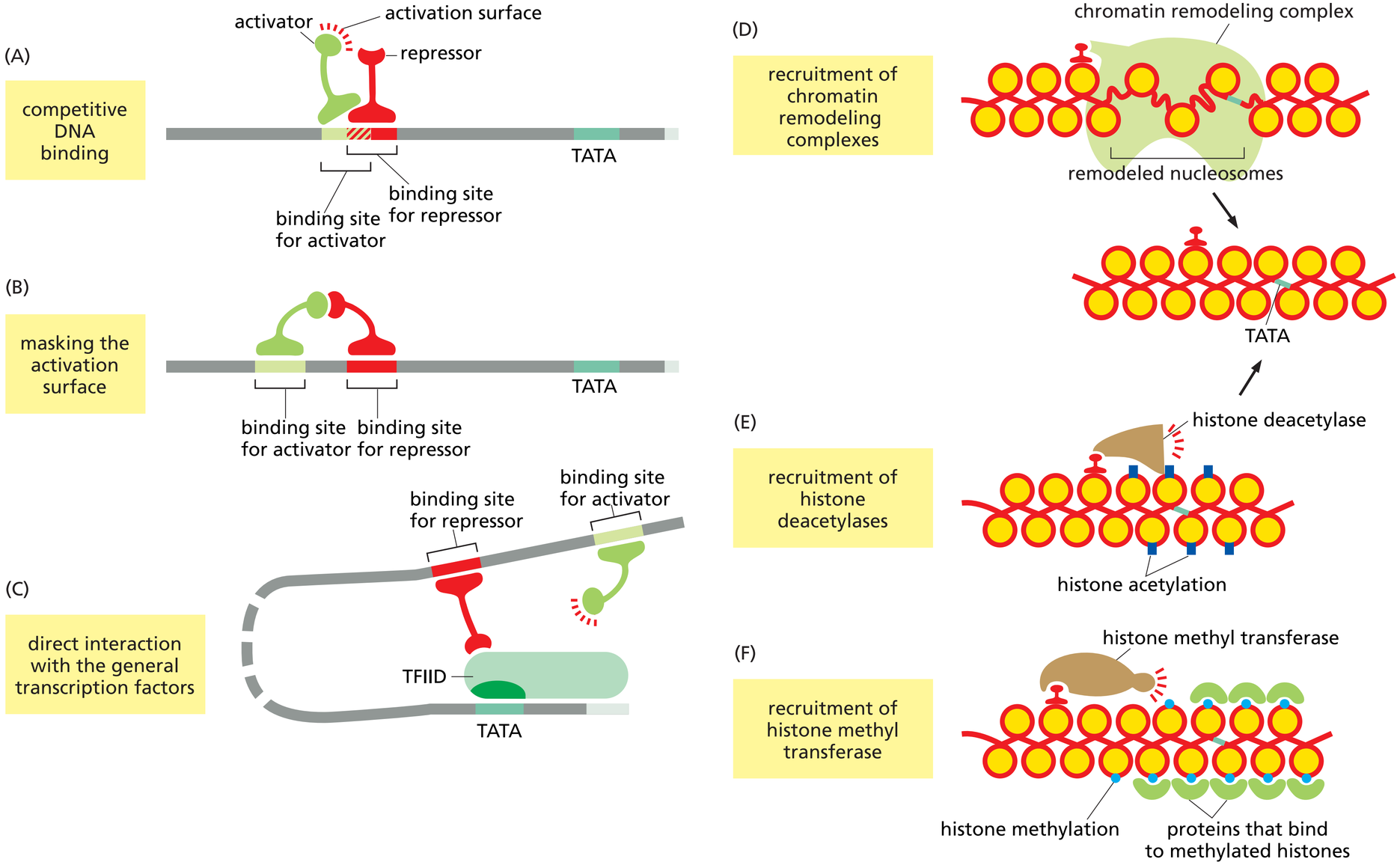
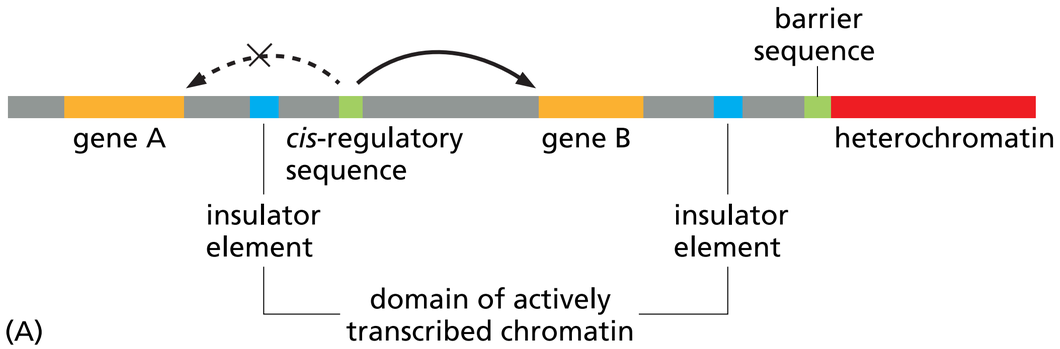
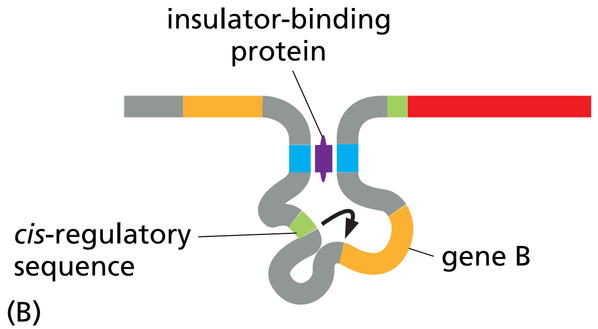
Repressor proteins



Insulator DNA sequences

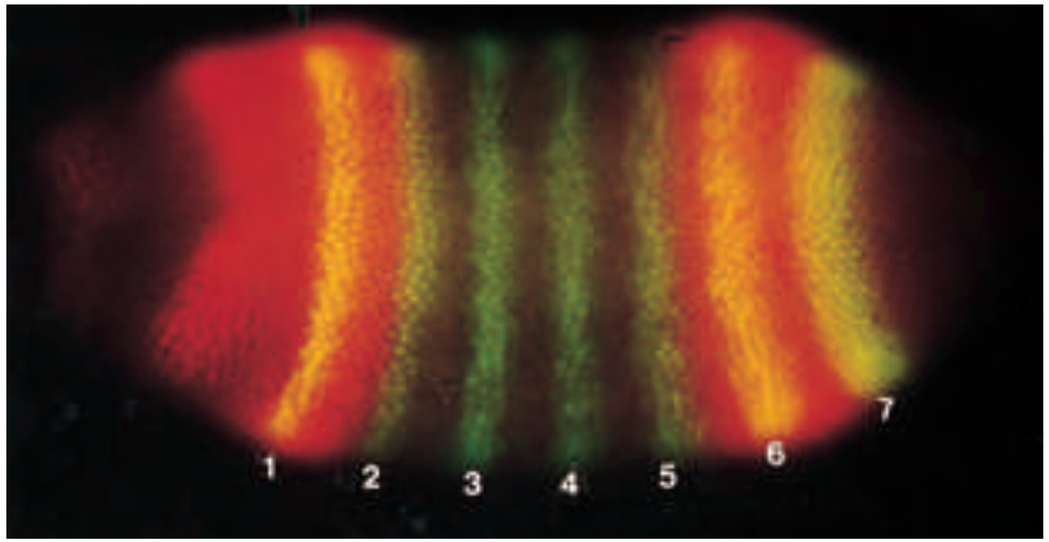
- Prevent cis-regulatory elements to **activate inappropriate** genes





Drosophila Even-skipped gene

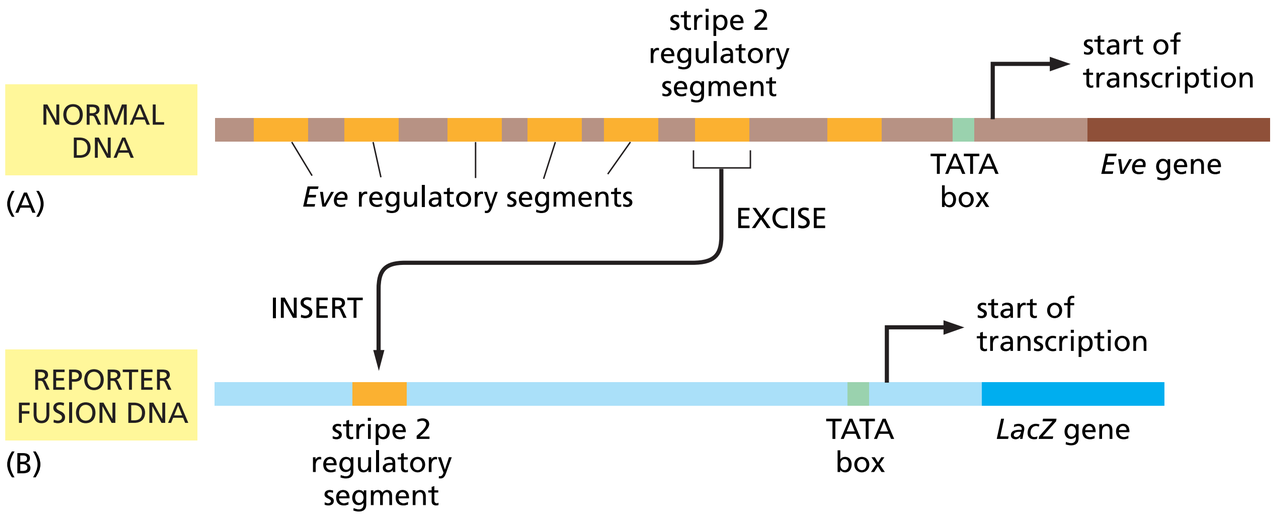
- Plays an important role in Drosophila embryo
- The embryo is a single giant cell with multiple nuclei in a giant cytoplasm with a mixture of transcription factors
- Those are distributed unevenly
- The nuclei rapidly start to express different genes because they are exposed to different transcription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

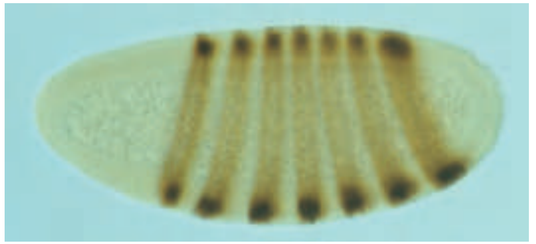


Eve is expressed in 7 precisely positioned stripes

Drosophila Even-skipped gene

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



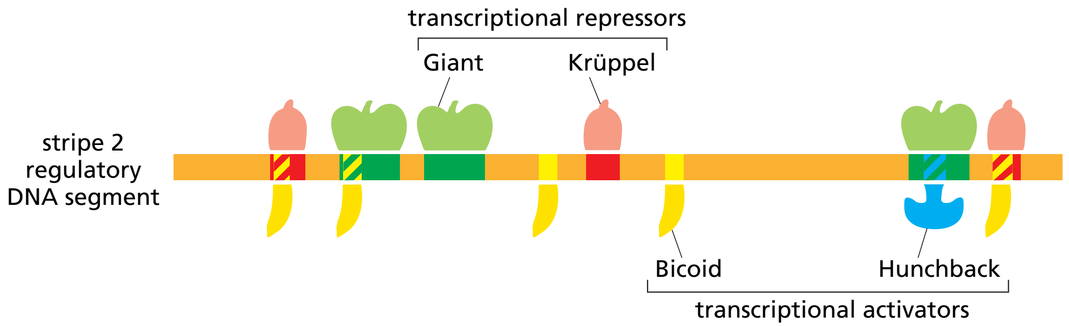


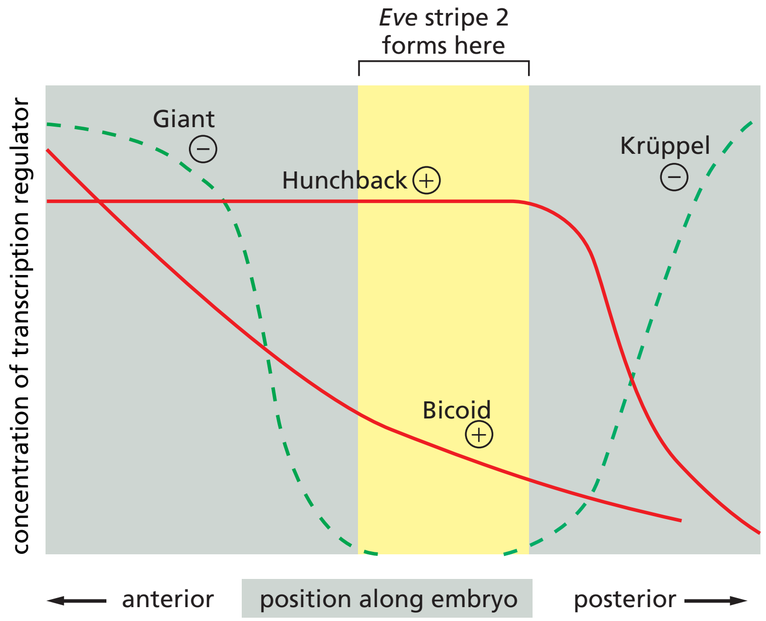
(C)

(D)

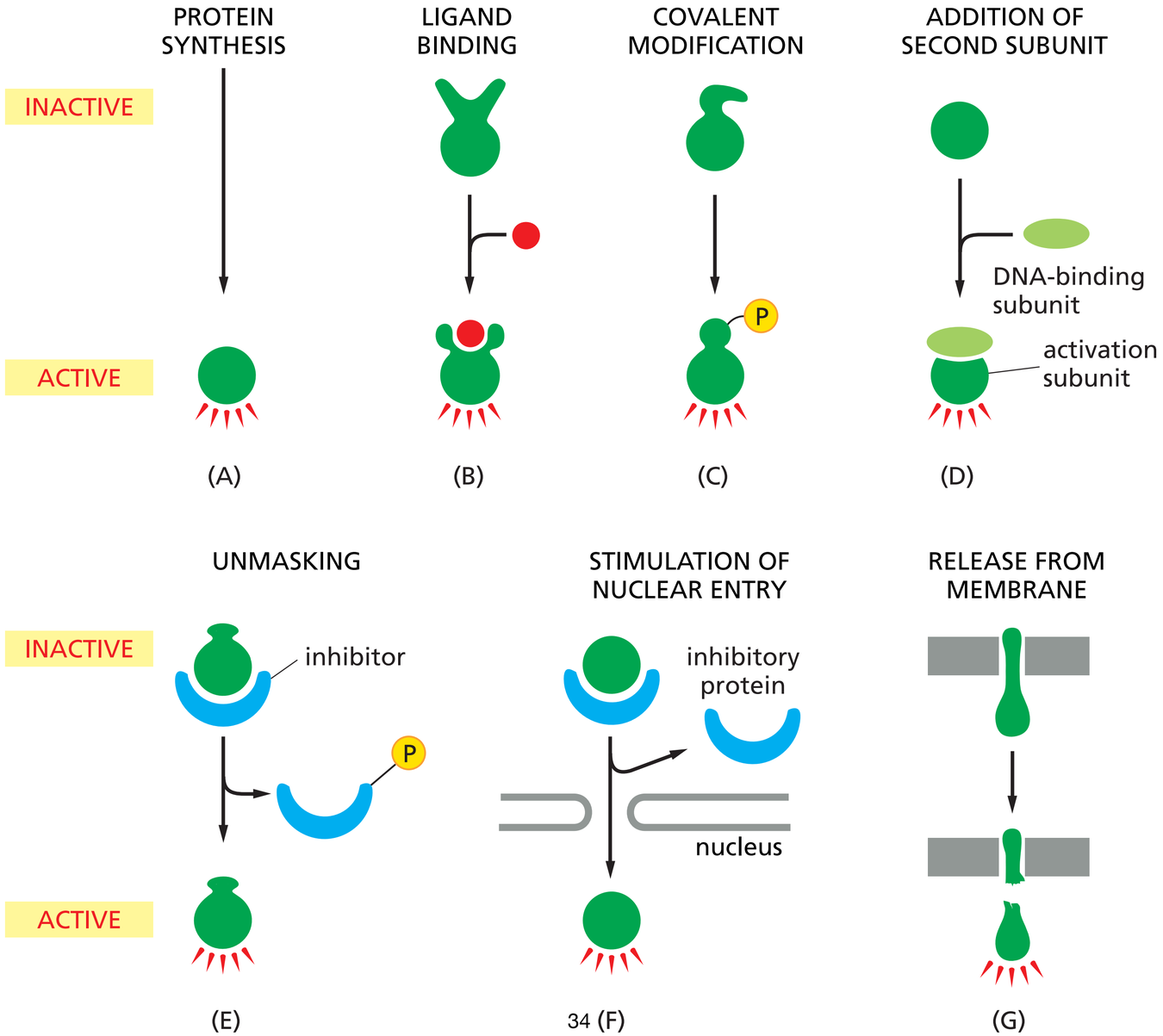
Drosophila Even-skipped gene

- How is the positioning in each stripe controlled?





Modulation of the activity of transcription regulators



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- Transcriptional control
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  - Repressors
  - **Understanding other regulatory systems**
  - Combinatorial gene control and cell types

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

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- Different cell types have the same DNA
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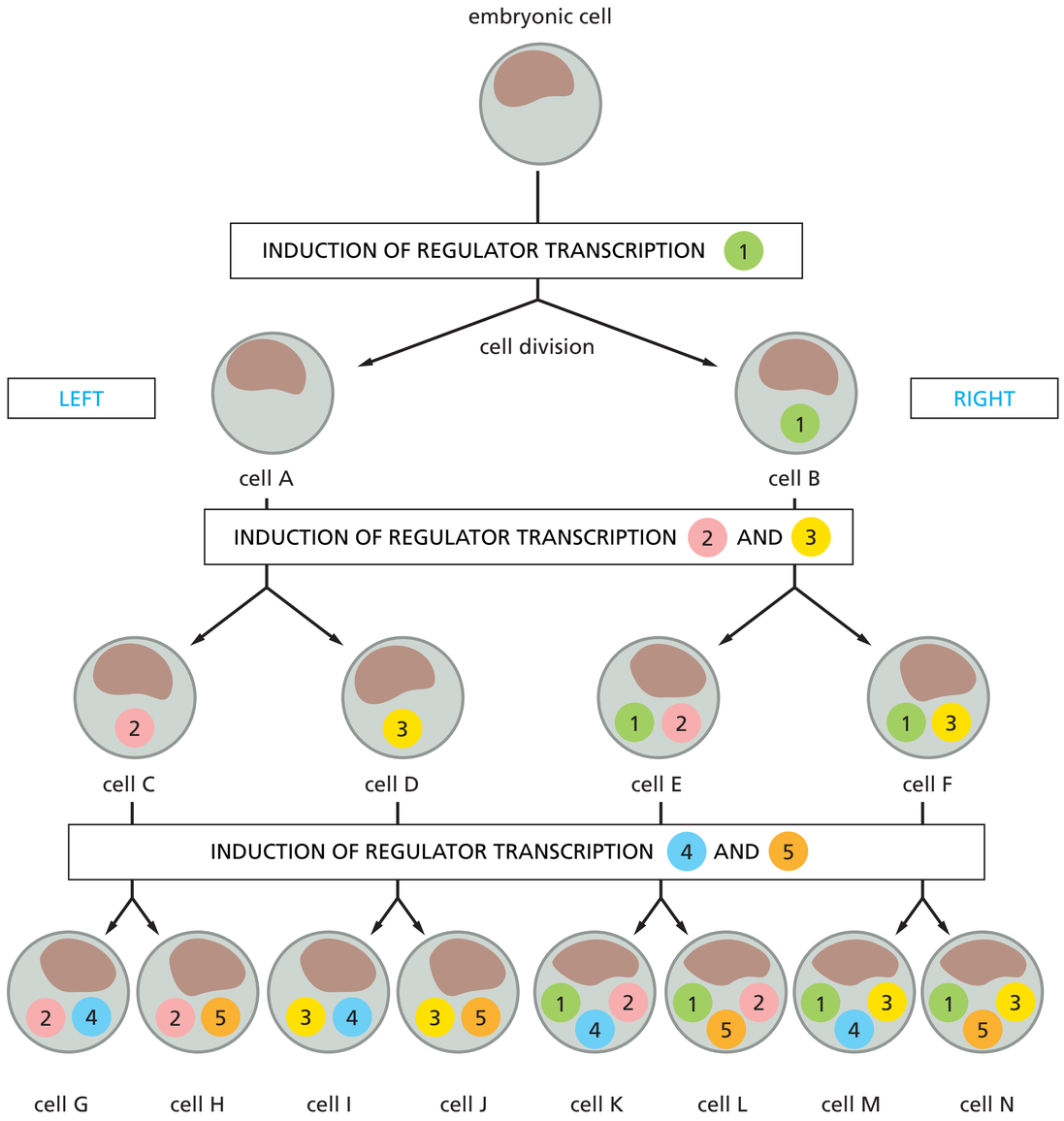
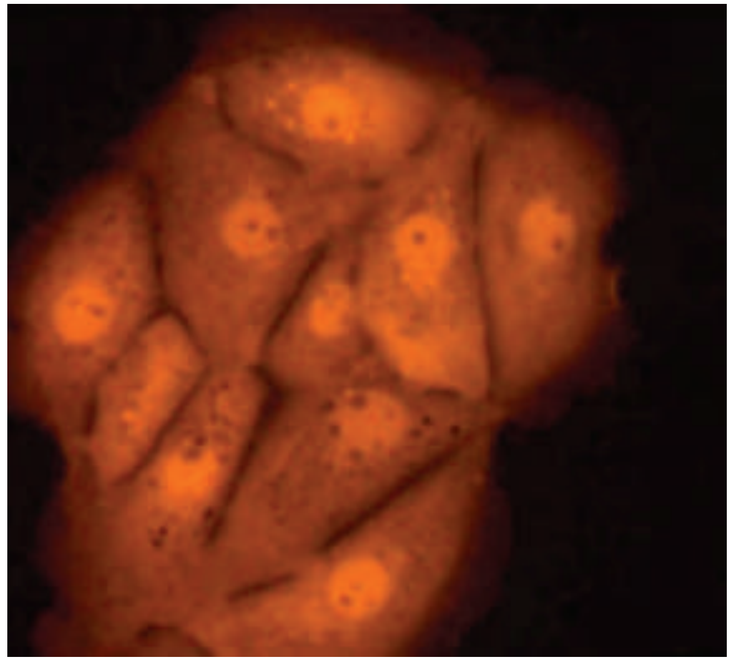
Combinatorial gene control creates cell types



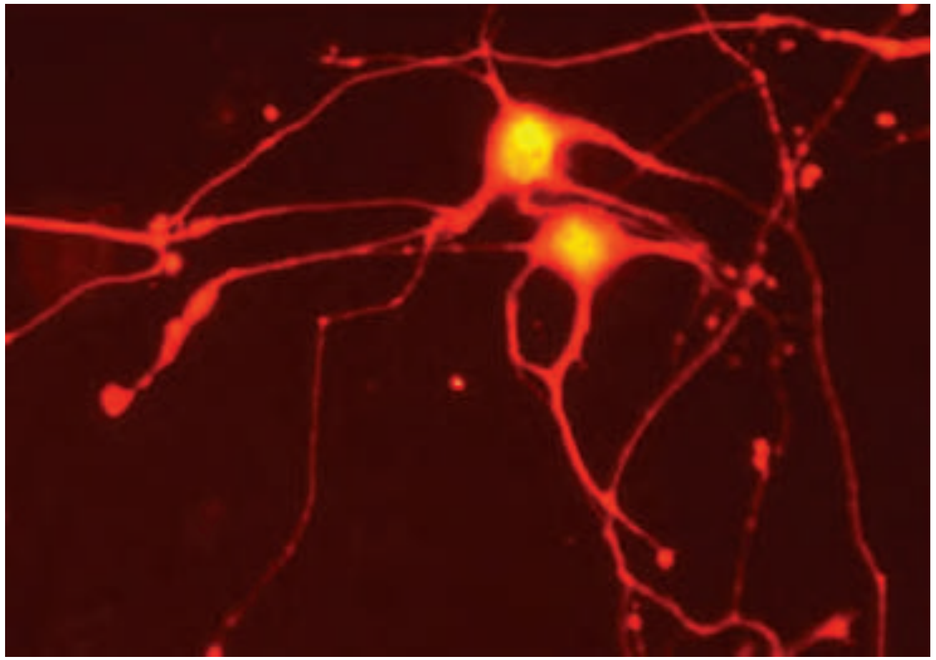
Combinatorial gene control creates cell types

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



(A)

50 μm

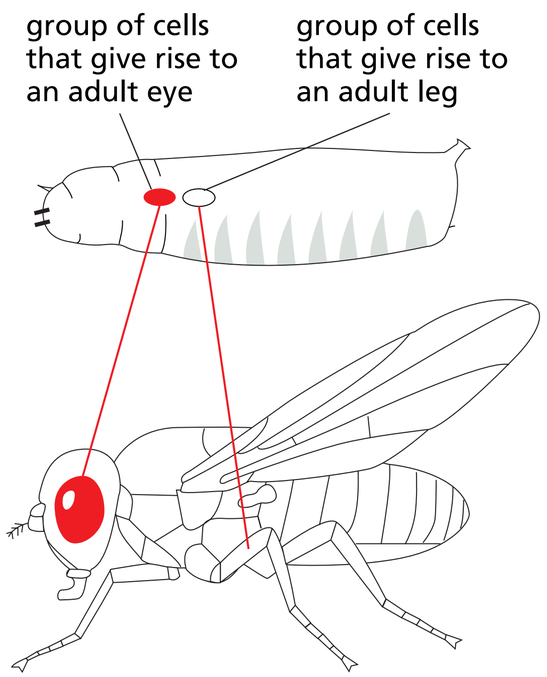


(B)

50 μm

Combinatorial gene control creates cell types

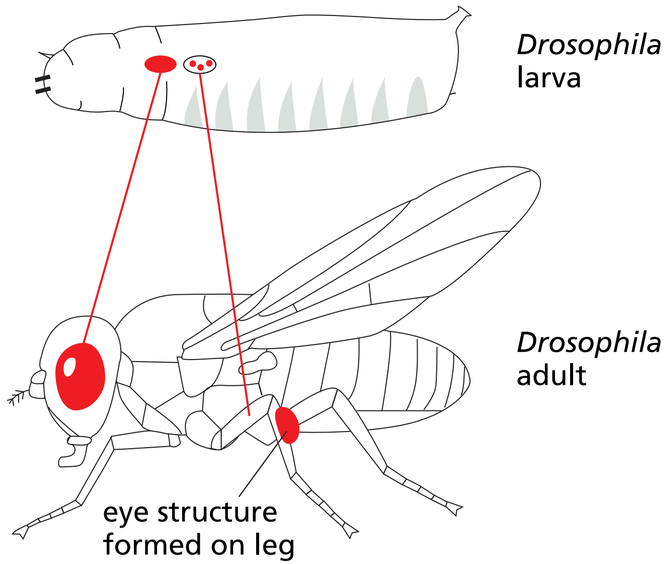
- Artificial expression of 1 transcription regulator from the eye region to the leg region



normal fly

(A)

(*red* shows cells expressing *Eyeless* gene)

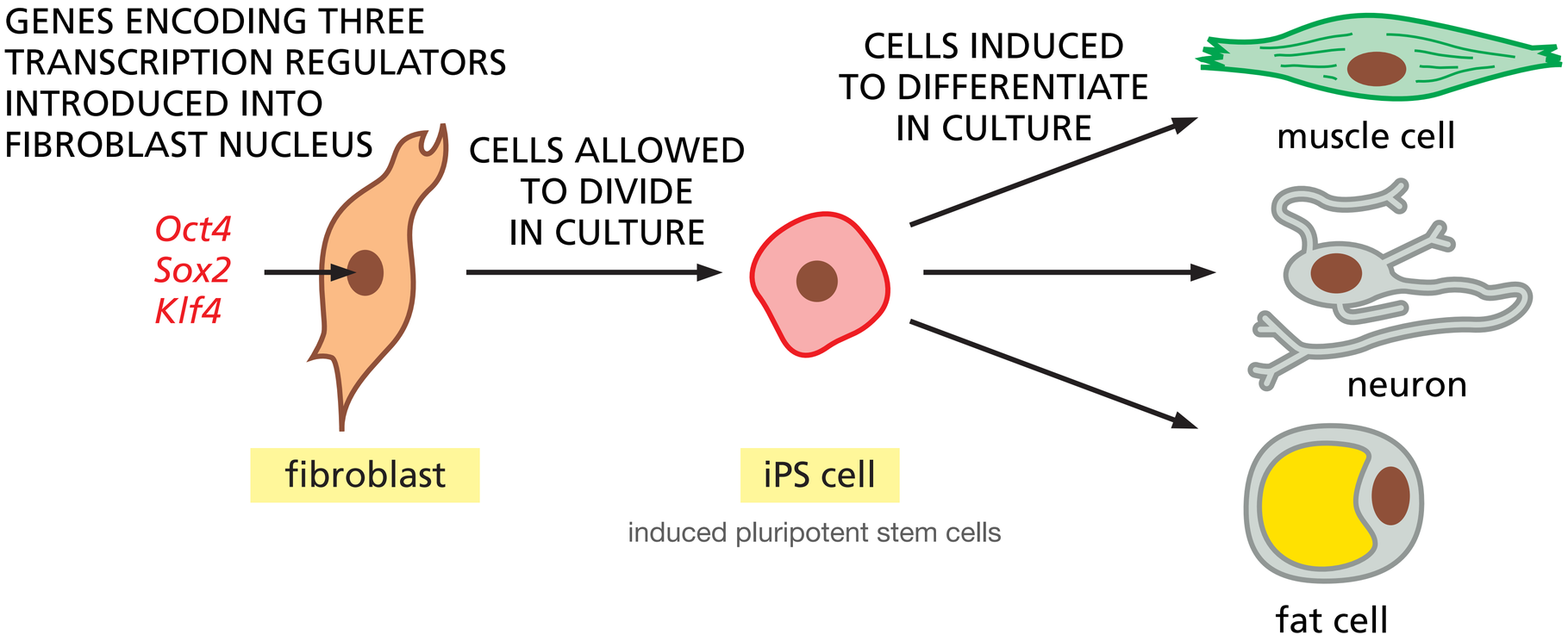


fly with *Eyeless* gene artificially
expressed in leg precursor cells

(B)

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**

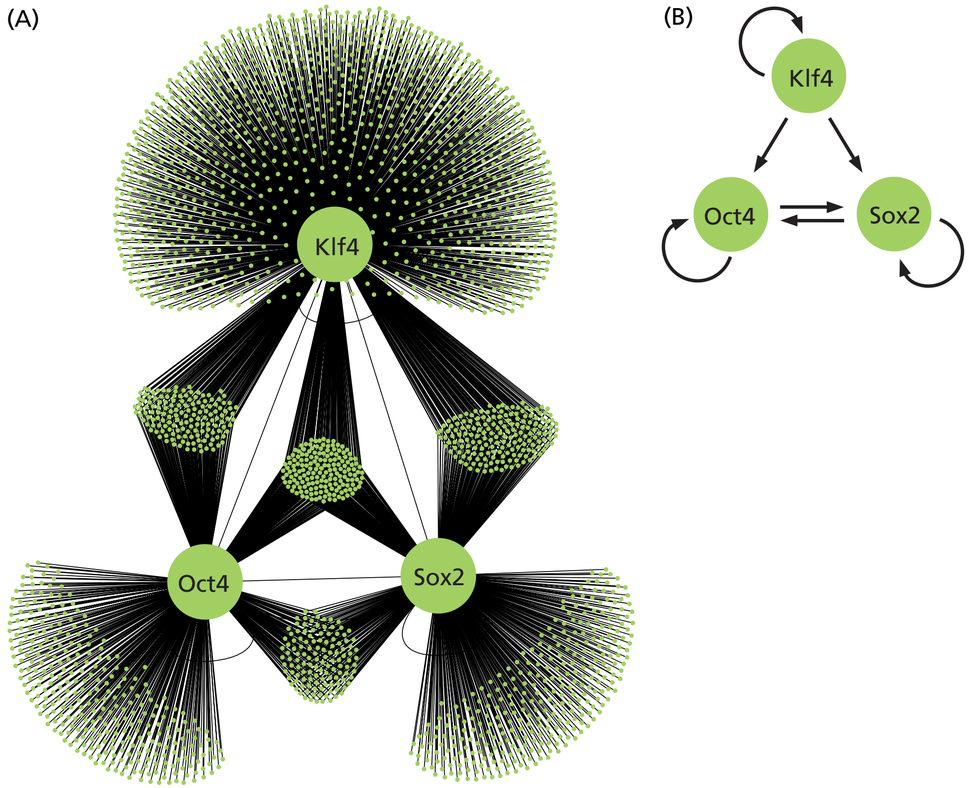
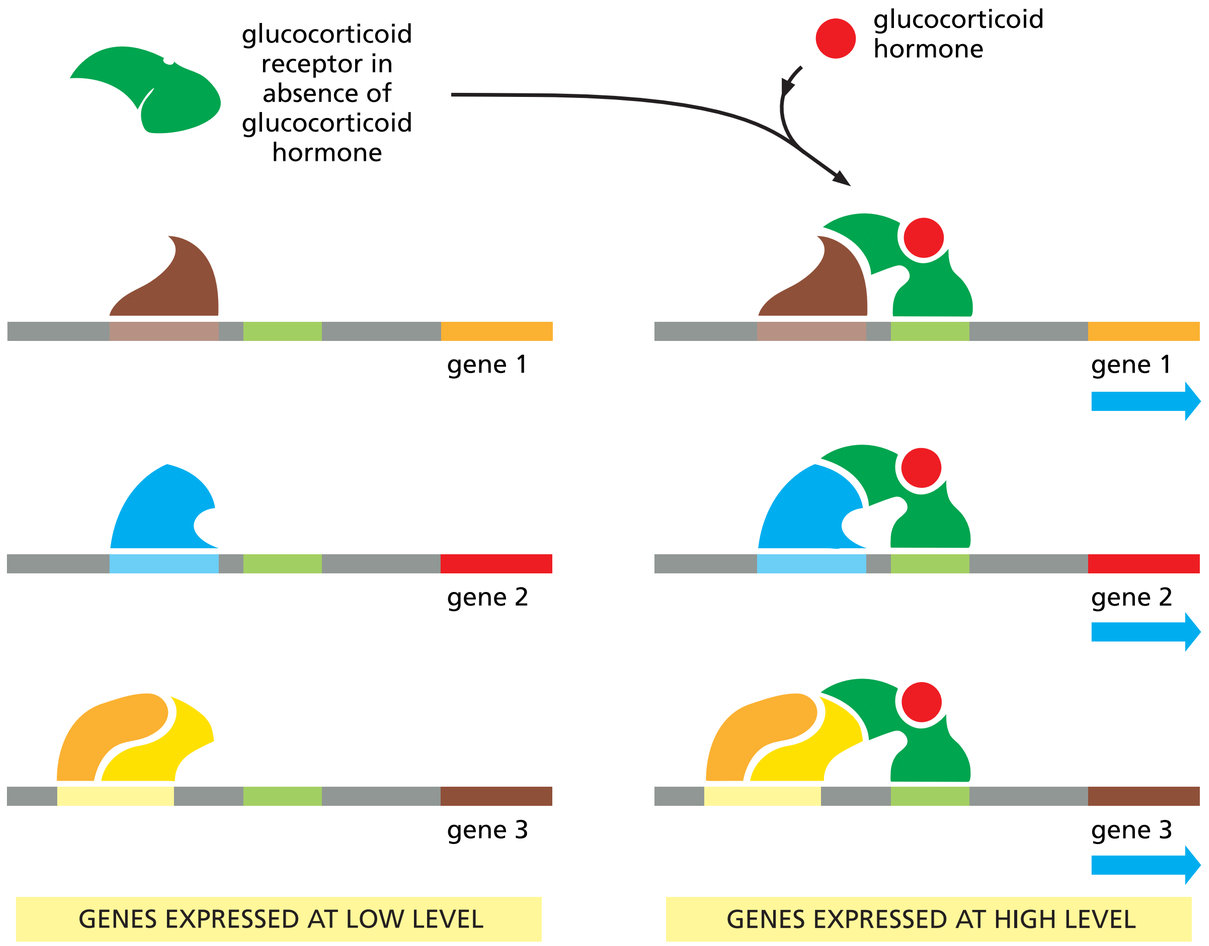


Figure 7–37 A portion of the transcription network specifying embryonic stem cells. (A) The three master transcription regulators in Figure 7–36 are shown as large circles. Genes whose *cis*-regulatory sequences are bound by each regulator in embryonic stem cells are indicated by a small dot (representing 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

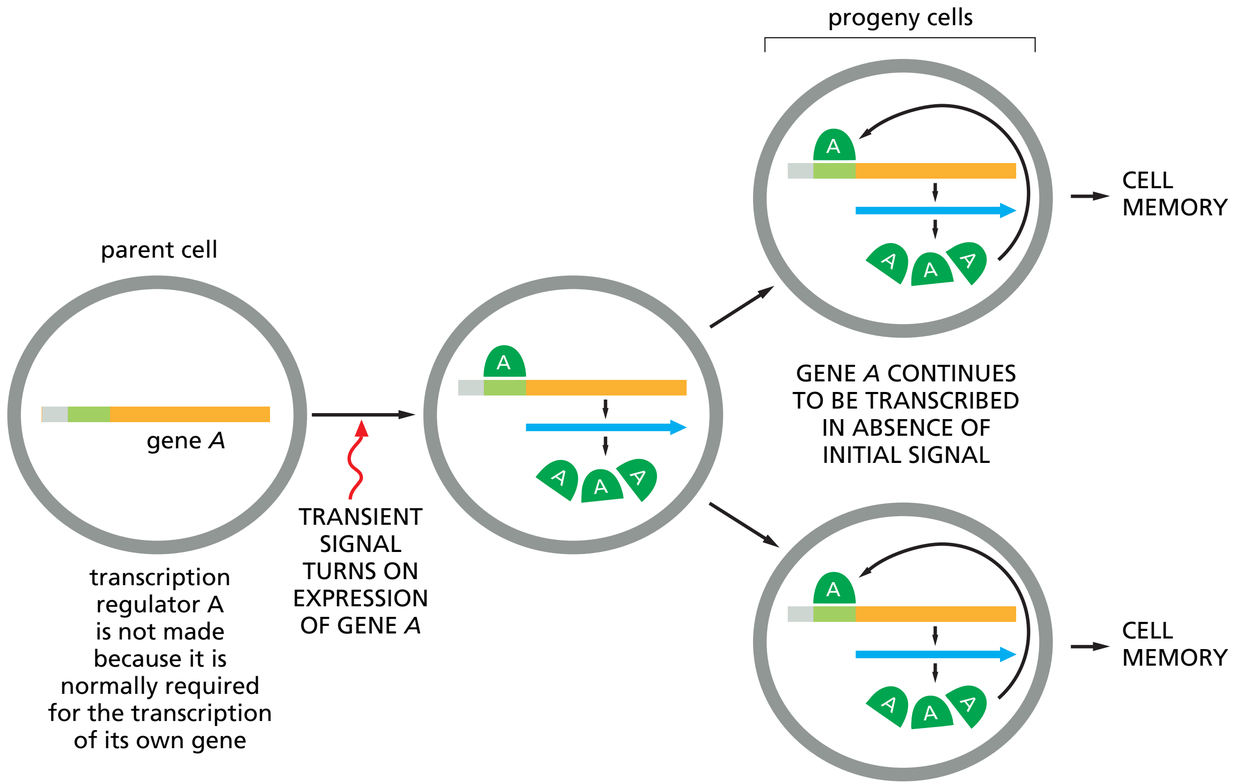
- Specialised cells rapidly turn genes on and off



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
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Have a nice day!