TRANSCRIPTOMICS

Course introduction

Day 01

https://tttorres.github.io/transcriptomics/

Transcriptomics

Course Introduction

Total workload: 32 hours (6 days)

Schedule	Time	Room
Friday, January 24	9 AM - 1 PM, 2 PM - 6 PM	411
Monday, January 27	9 AM - 1 PM, 2 PM - 6 PM	411
Tuesday, January 28	2 PM - 6 PM	411
Wednesday, January 29	2 PM - 6 PM	411
Thursday, January 30	9 AM - 1 PM	411
Friday, January 31	9 AM - 1 PM	411

Transcriptomics

Course Structure

1. **Lectures**
    - Fundamental concepts of transcriptomics
    - RNA-seq experimental design and workflows
2. **Hands-On Practice**
    - RNA-seq data analysis using open-source tools
    - Key steps: quality control, assembly, alignment, differential expression
3. **Discussion of Scientific Papers**
    - Real-world applications of RNA-seq
    - Critical evaluation of methodologies and results

Transcriptomics

Course objectives

1. **Study of transcriptomes**
    - Theory of RNA regulation.
    - Methods for studying RNA regulation.
2. **RNA-seq**
    - Learn to analyze your own RNA-seq data.
    - Develop a RNA-seq pipeline

Transcriptomics

Evaluation Criteria:

Weighting of each modality:

Modality	Percentage
1. Participation in article discussions	25%
2. Participation in theoretical discussions	25%
3. Final report based on results of practical analyses conducted during the course.	50%

Calculation of Final Grade:

Final grade = Average of 1 (25%) + 2 (25%) + 3 (50%)
Minimum passing grade: 14

TRANSCRIPTOMICS

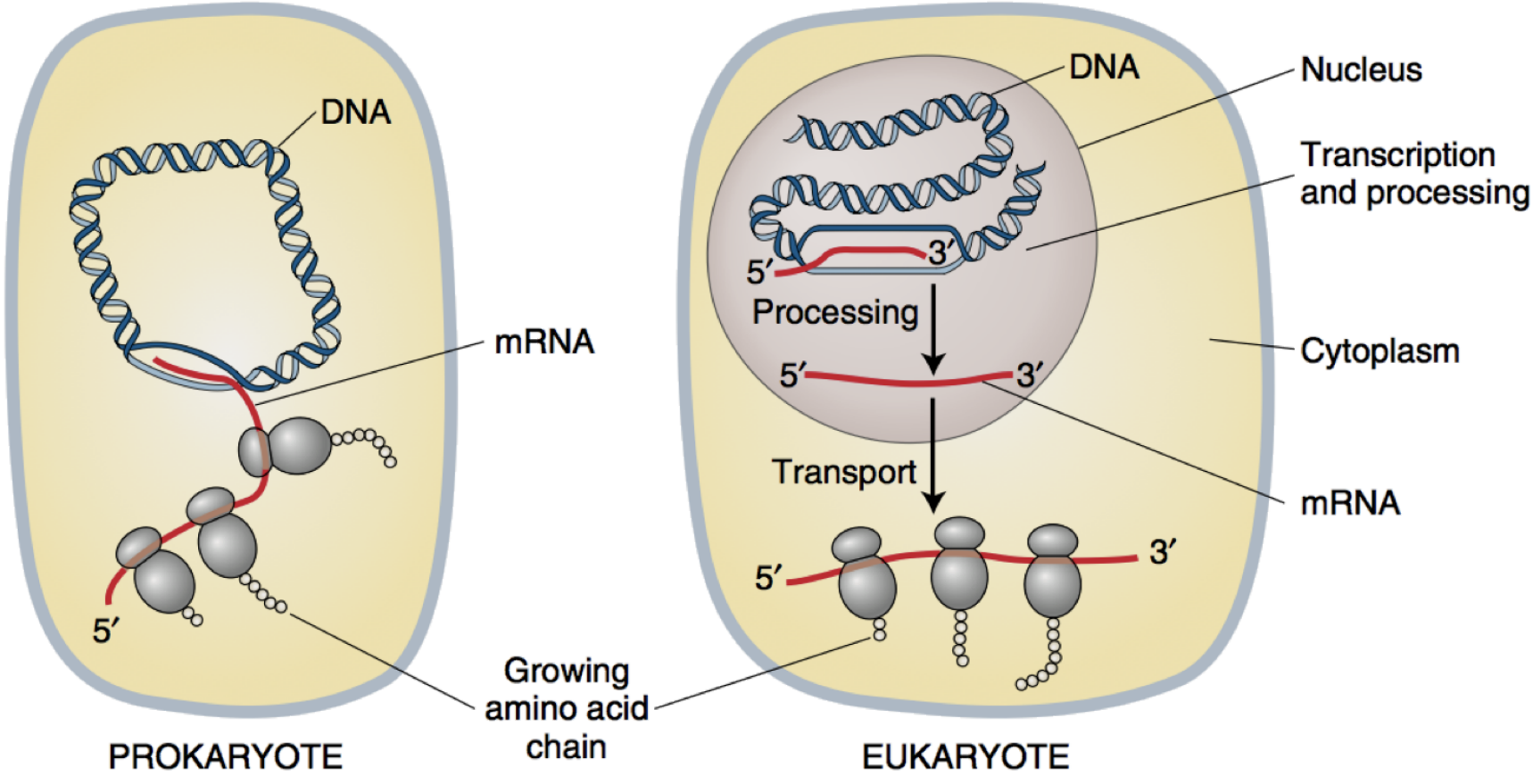
Regulation of Gene Expression

Day 01

https://tttorres.github.io/transcriptomics/

Regulation of transcription

Transcription



Transcription

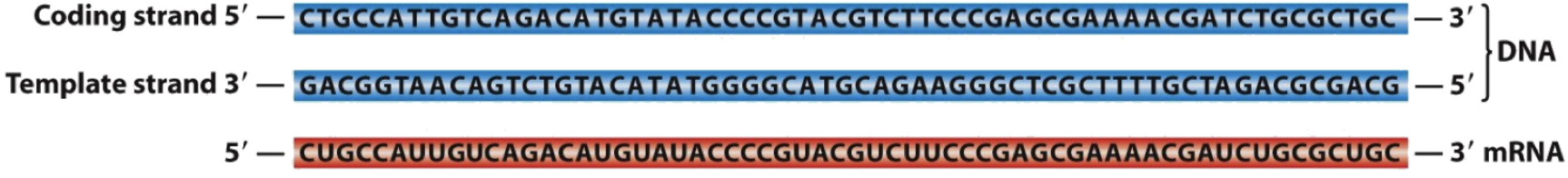
Classes of RNA

1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA)
4. Small nuclear RNA (snRNA)
5. MicroRNA (miRNA)
6. Small interfering RNA (siRNA)
7. Long non-coding RNA (lncRNA)
8. Piwi-interacting RNA (piRNA)
9. Small nucleolar RNA (snoRNA)

Transcription

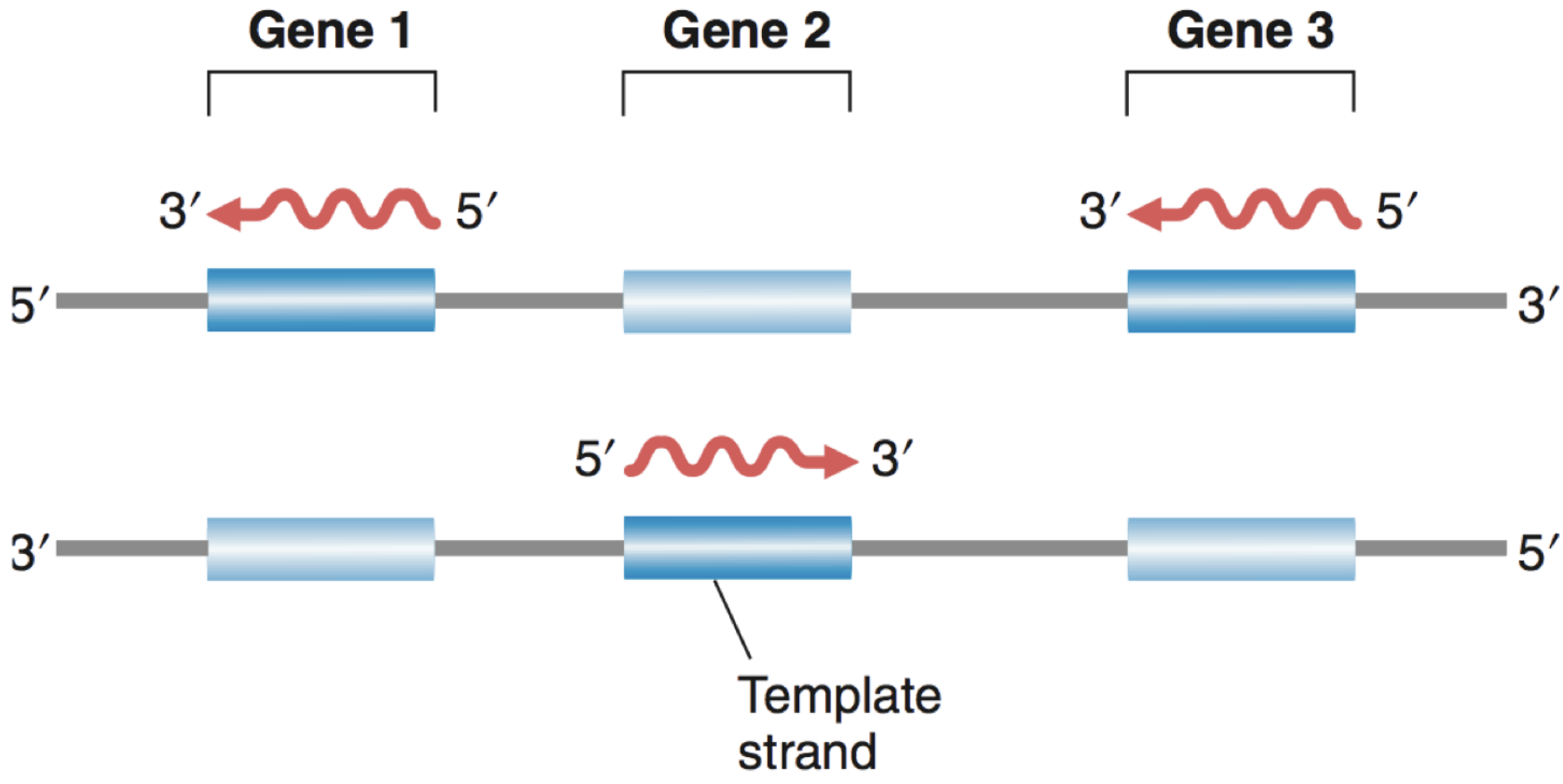
Some definitions

- Typically, the described strand is the coding strand, which corresponds to the mRNA sequence with U replaced by T.
- The template strand will be the reverse complement of the coding strand.



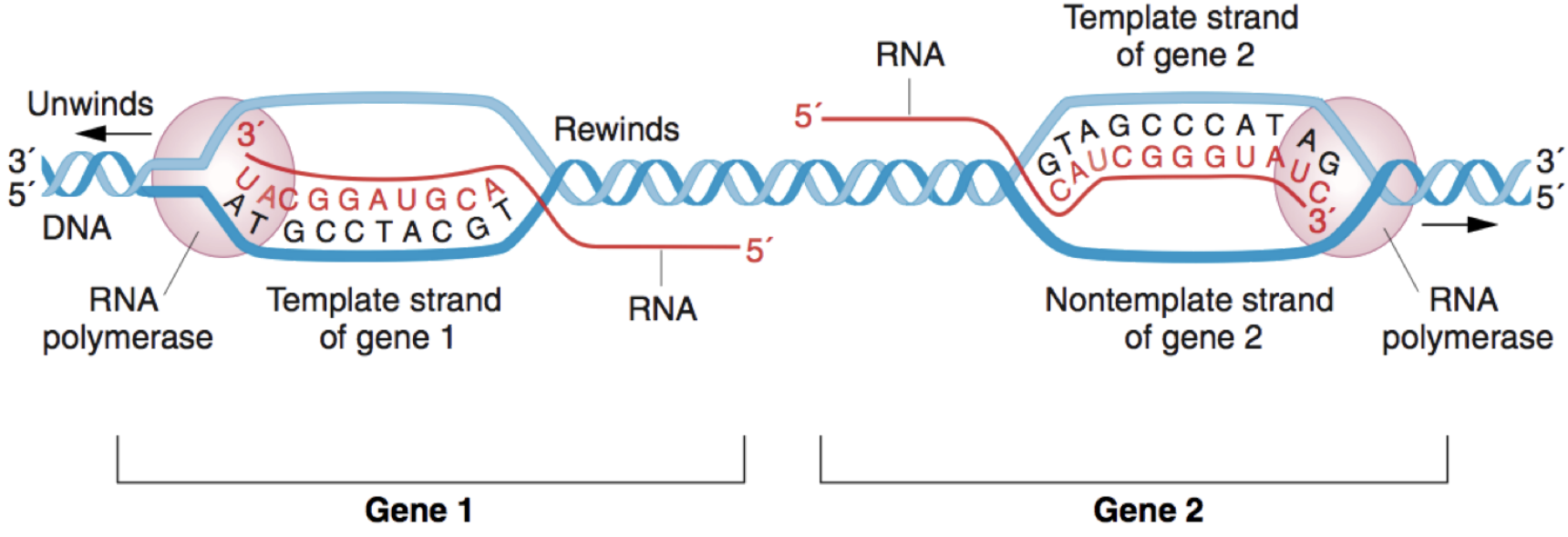
Transcription

DNA strands used as templates



Transcription

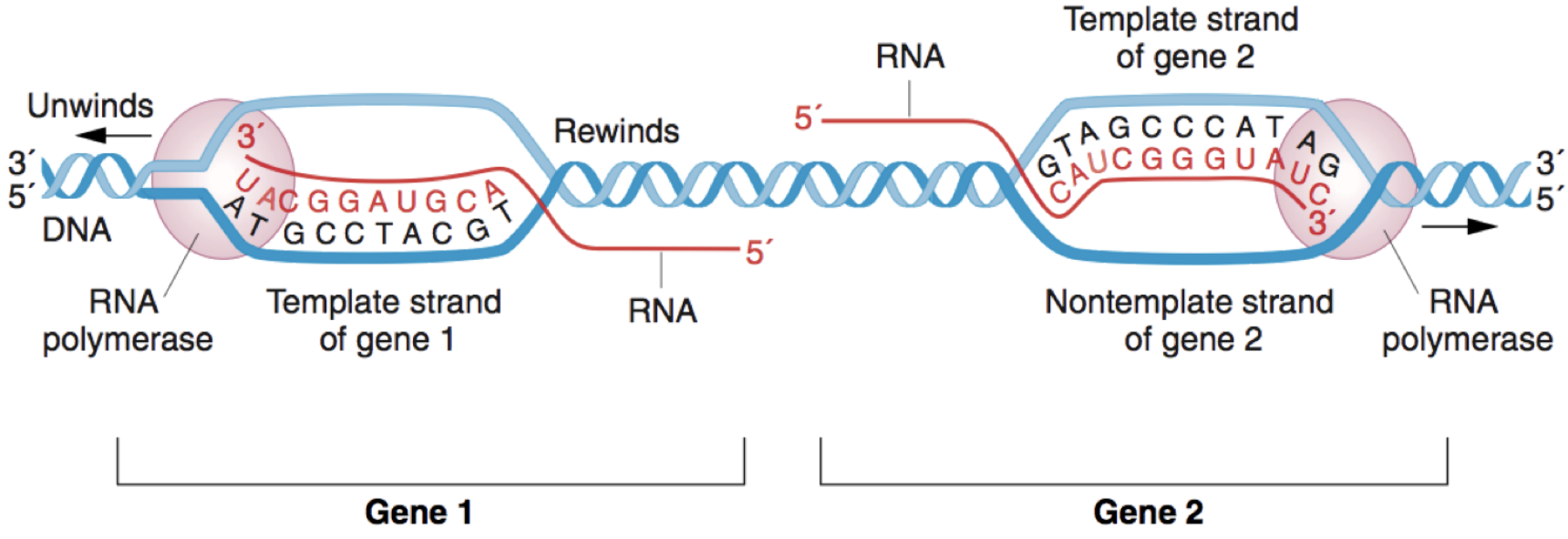
DNA strands used as templates



✏️ When DNA base sequences are cited in scientific literature, by convention it is the sequence of the nontemplate strand that is given, because this sequence is the same as that found in the RNA.

Transcription

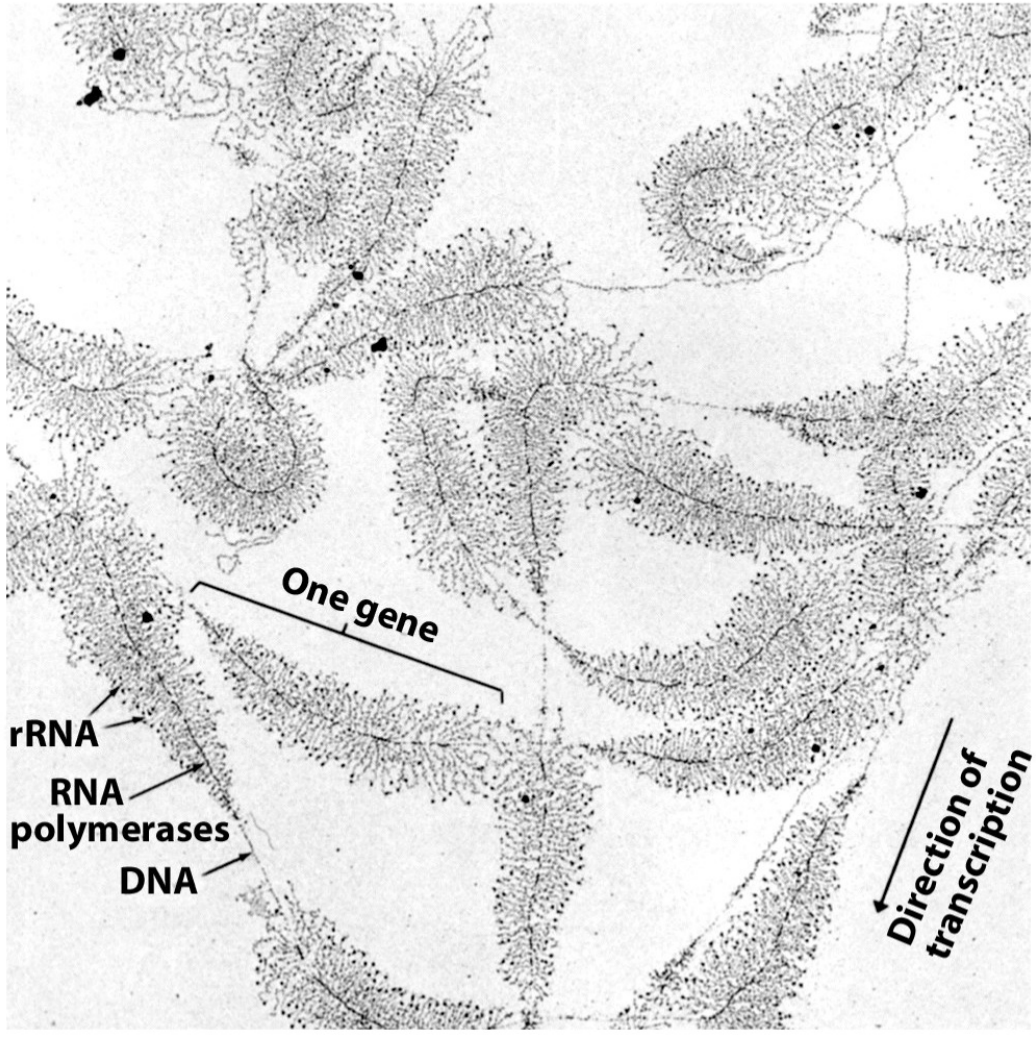
DNA strands used as templates



✏️ For this reason, the nontemplate strand of the DNA is referred to as the coding strand.

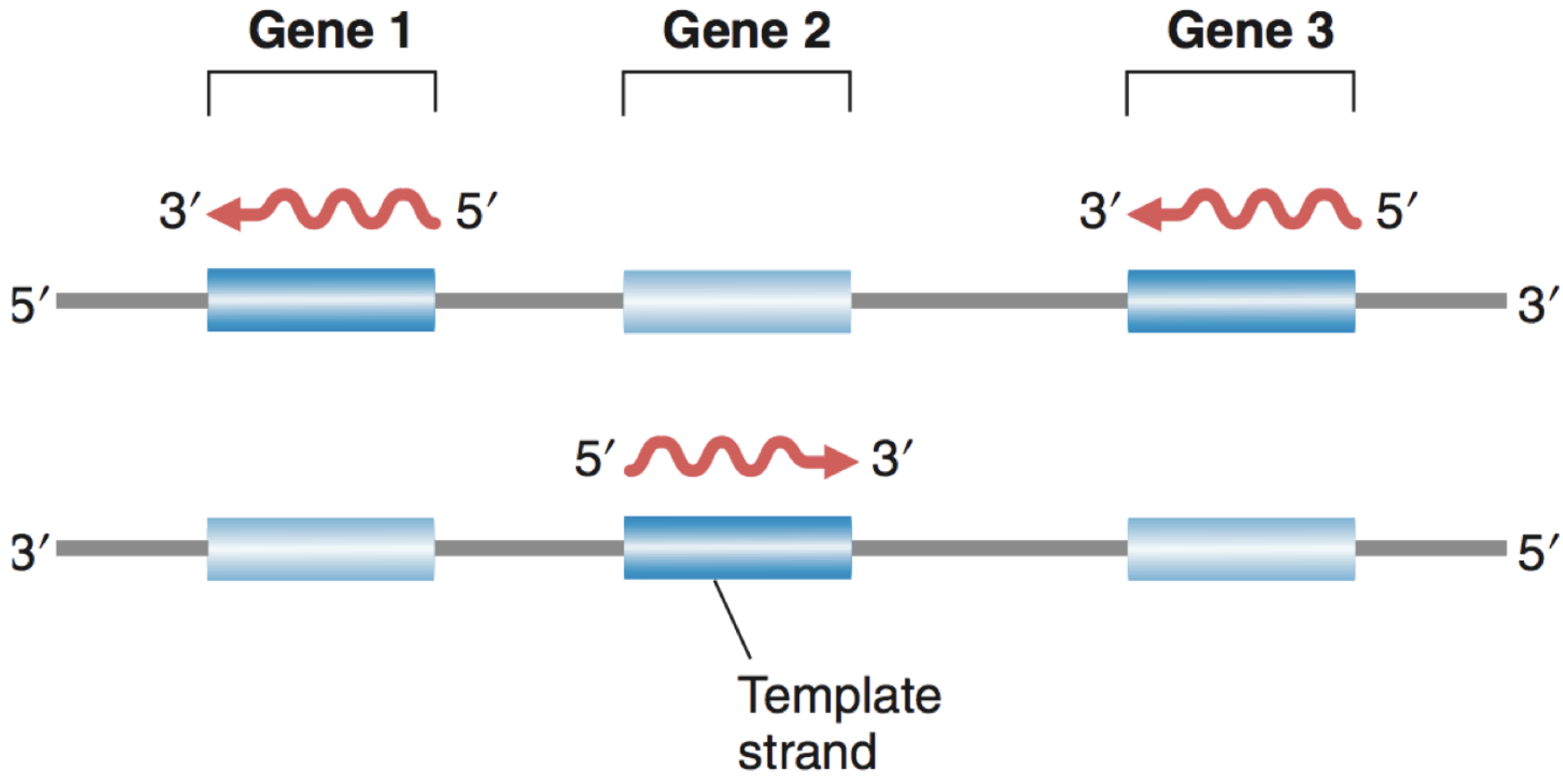
Transcription

DNA strands used as templates



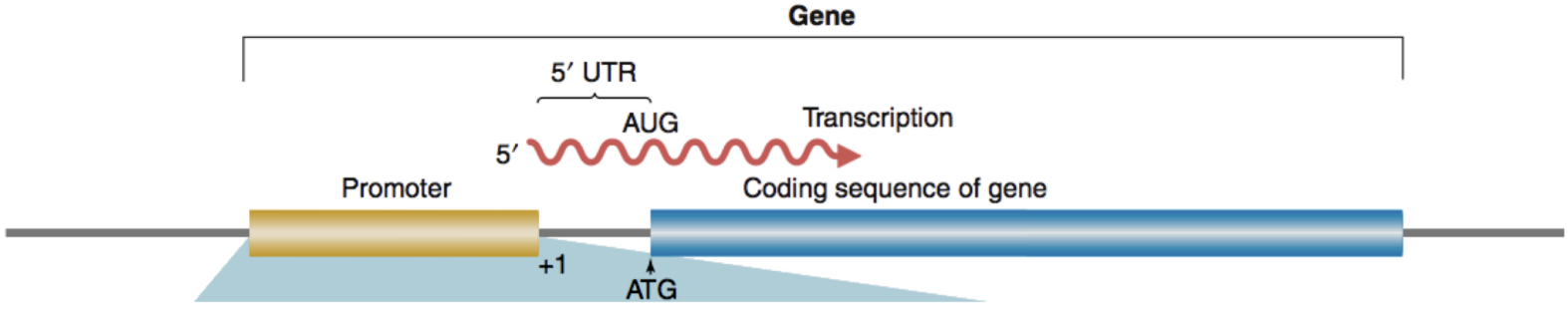
Transcription

Finding genes in chromosomes



Transcription in Prokaryotes

Finding genes in chromosomes



Transcription in Prokaryotes

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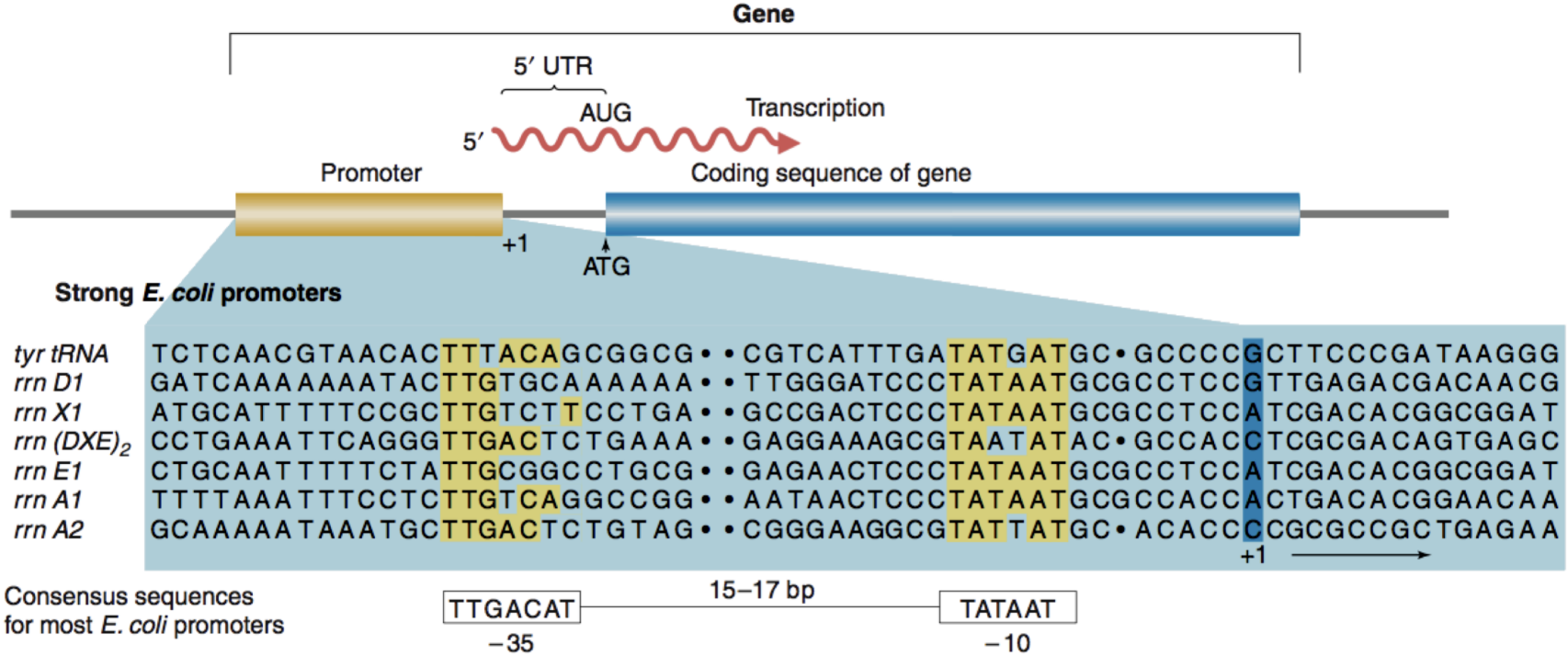
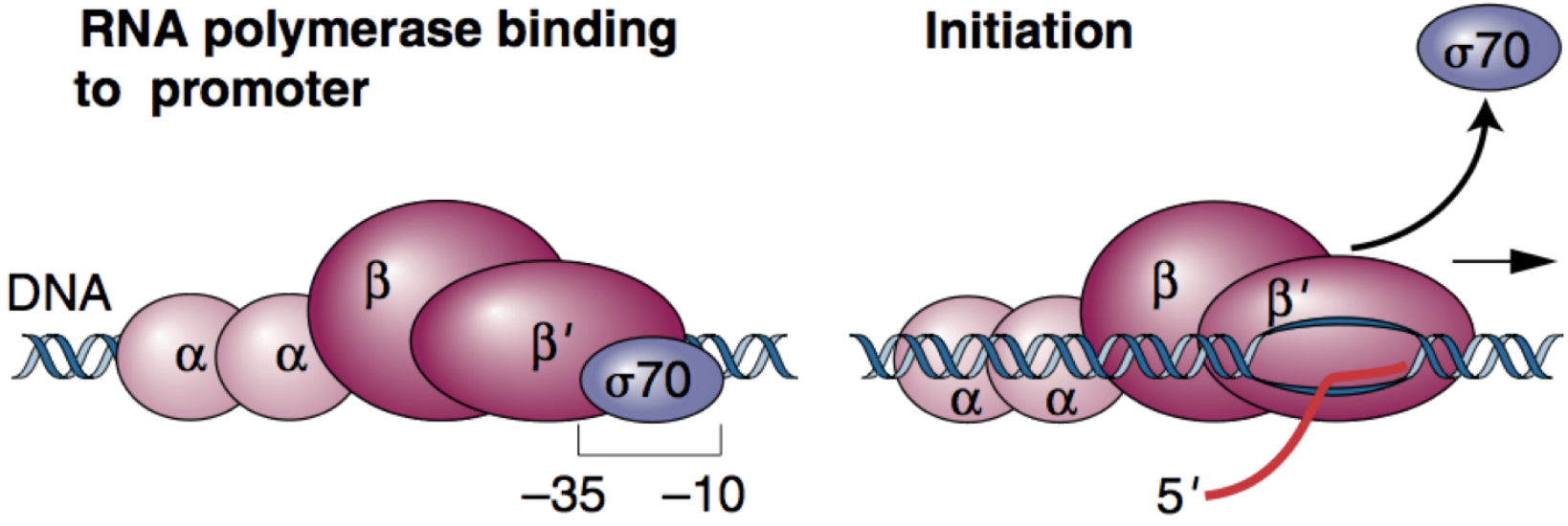


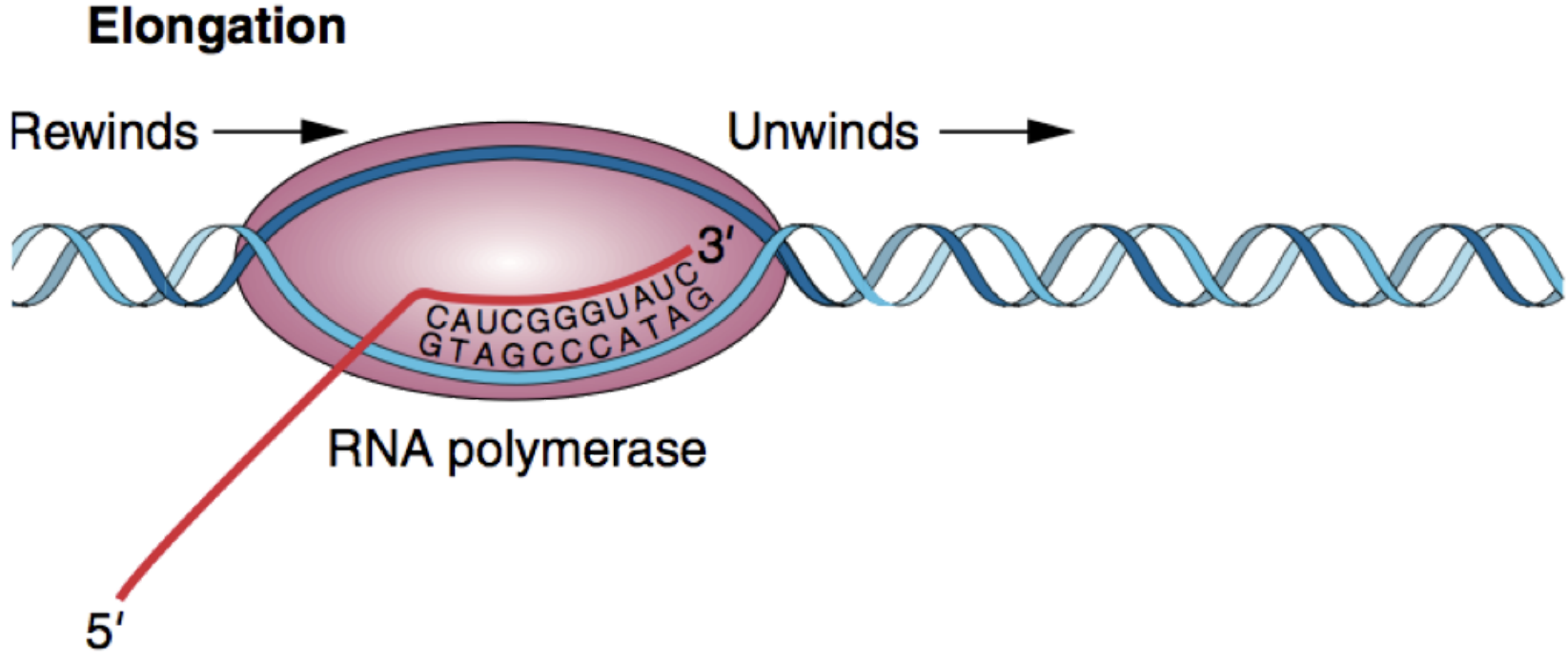
Transcription in Prokaryotes

Transcription initiation



Transcription in Prokaryotes

Transcription elongation and termination



Transcription in Prokaryotes

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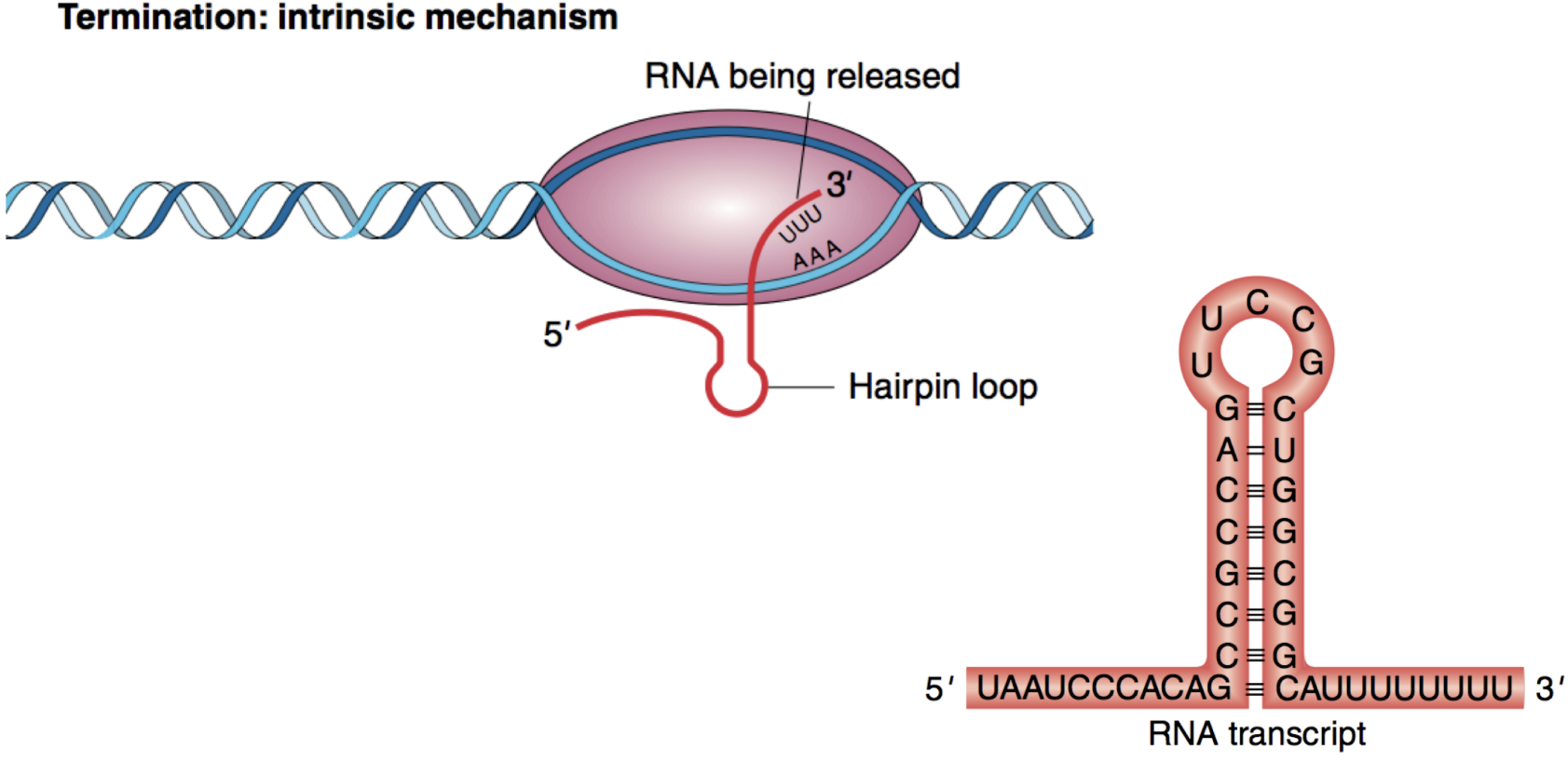


Transcription in Eukaryotes

Eukaryotes *vs.* Prokaryotes

1. **More genes, more dispersed**: in eukaryotes, genes are typically larger, interspersed with introns, and regulated individually.
2. **Presence of a nuclear membrane**: in eukaryotes, transcription occurs in the nucleus, and mRNA must be transported to the cytoplasm for translation.
3. **DNA is organized into chromatin**: in eukaryotes, DNA is tightly wrapped around histones, requiring chromatin remodeling for transcription to occur.

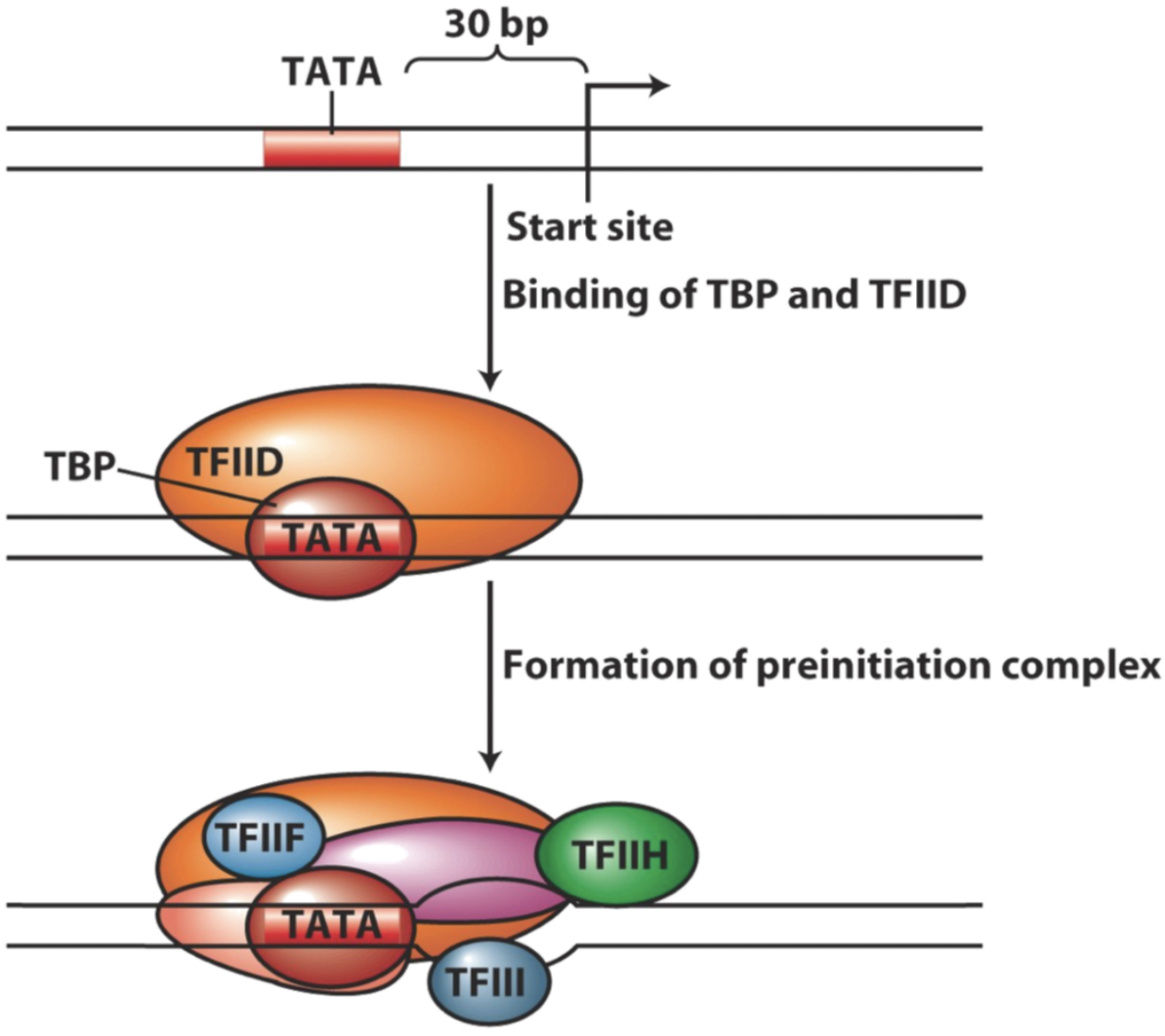
Transcription in Eukaryotes

Eukaryotes *vs.* Prokaryotes

4. **RNA polymerases**: eukaryotes have multiple RNA polymerases (I, II, III), each specialized for different types of RNA.
5. **mRNA processing**: mRNA undergoes extensive processing in eukaryotes, including 5' capping, splicing, and 3' polyadenylation.
6. **Regulation of transcription**: complex regulation involving enhancers, silencers, and multiple transcription factors is observed in eukaryotes.

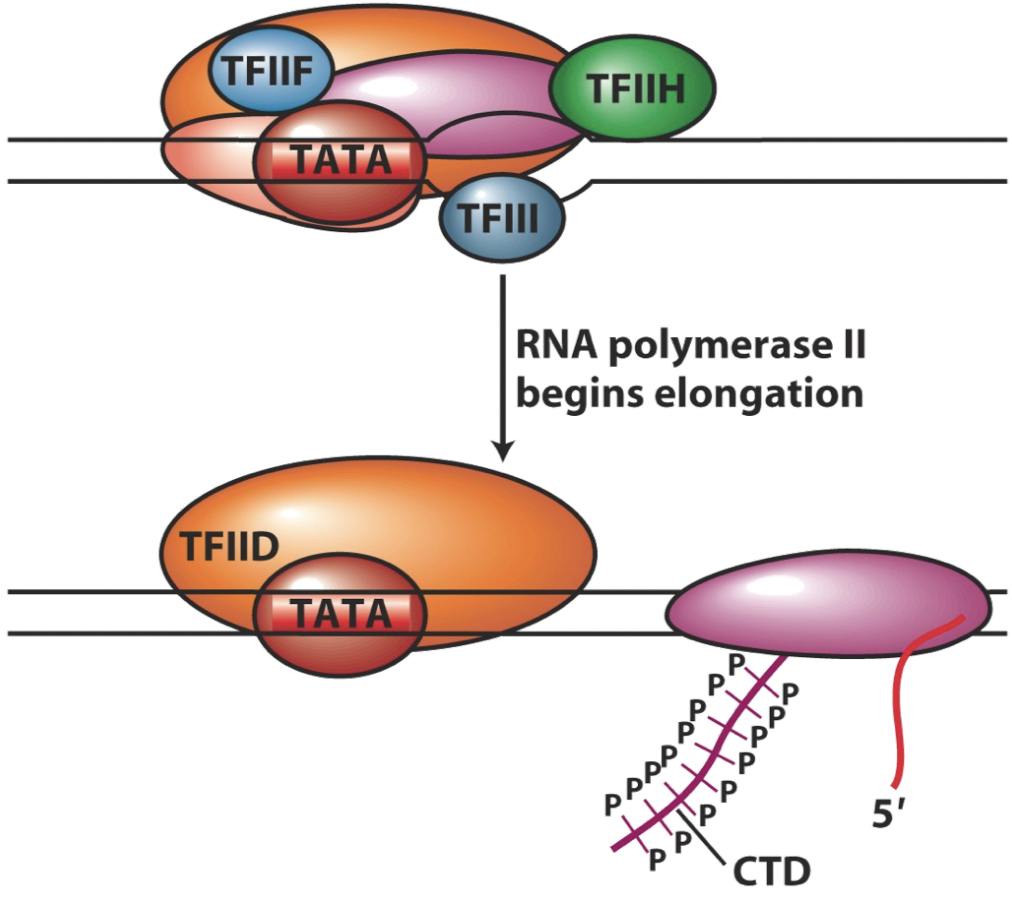
Transcription in Eukaryotes

Initiation



Transcription in Eukaryotes

Initiation and elongation

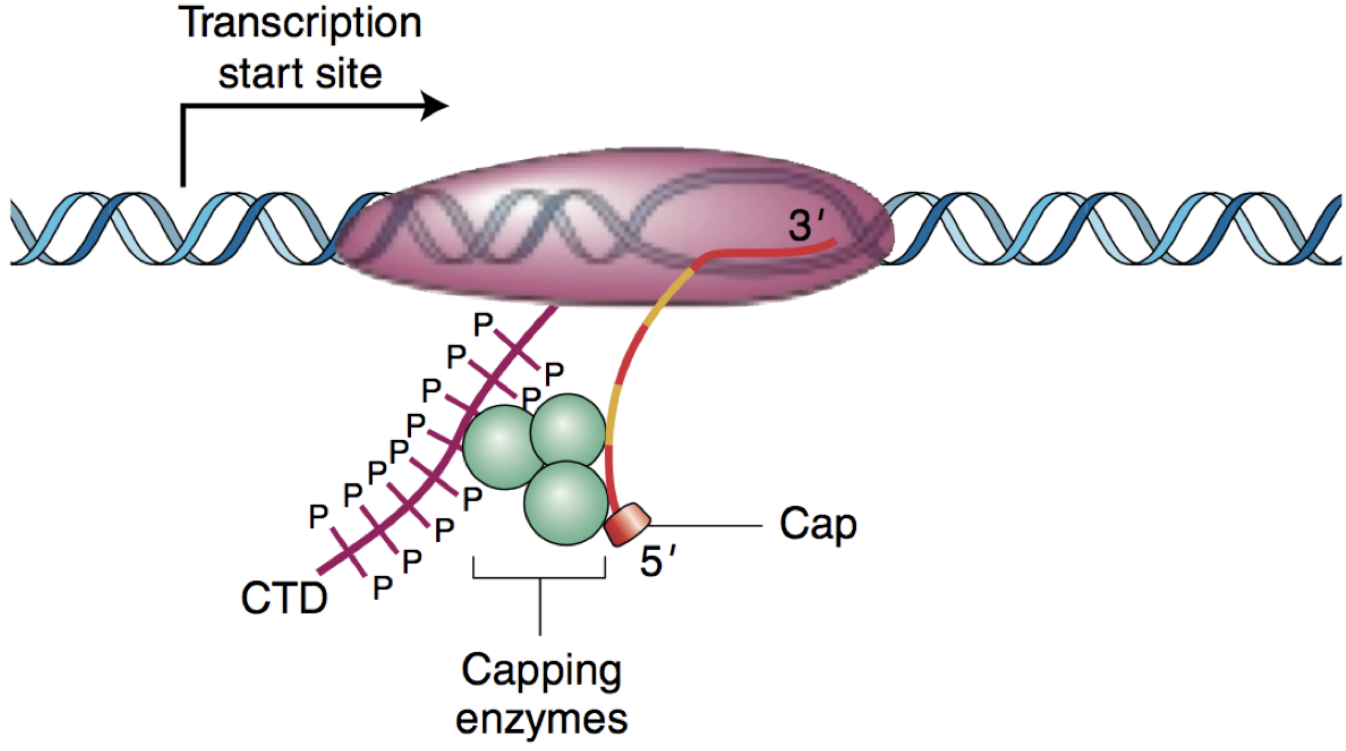


CTD = Carboxi Terminal Domain

Transcription in Eukaryotes

Cotranscriptional processing of RNA

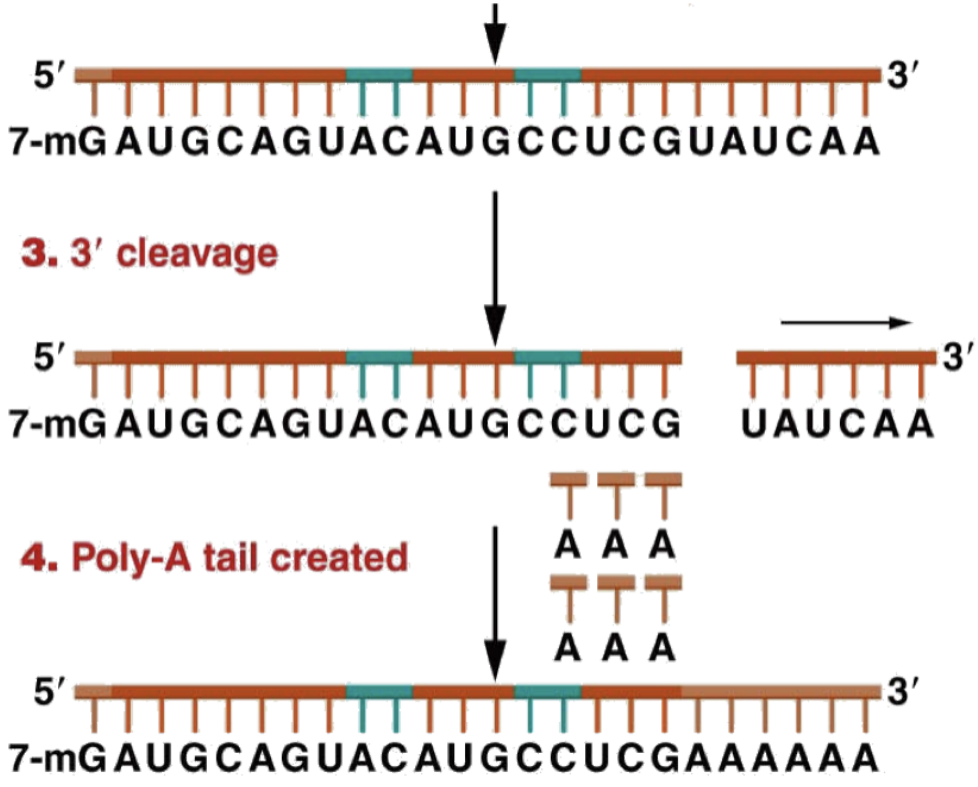
1. **Addition of the 5' cap** – Attachment of a 7-methylguanosine (a modified guanine nucleotide) to the 5' end of the nascent mRNA.



Transcription in Eukaryotes

Posttranscriptional RNA processing

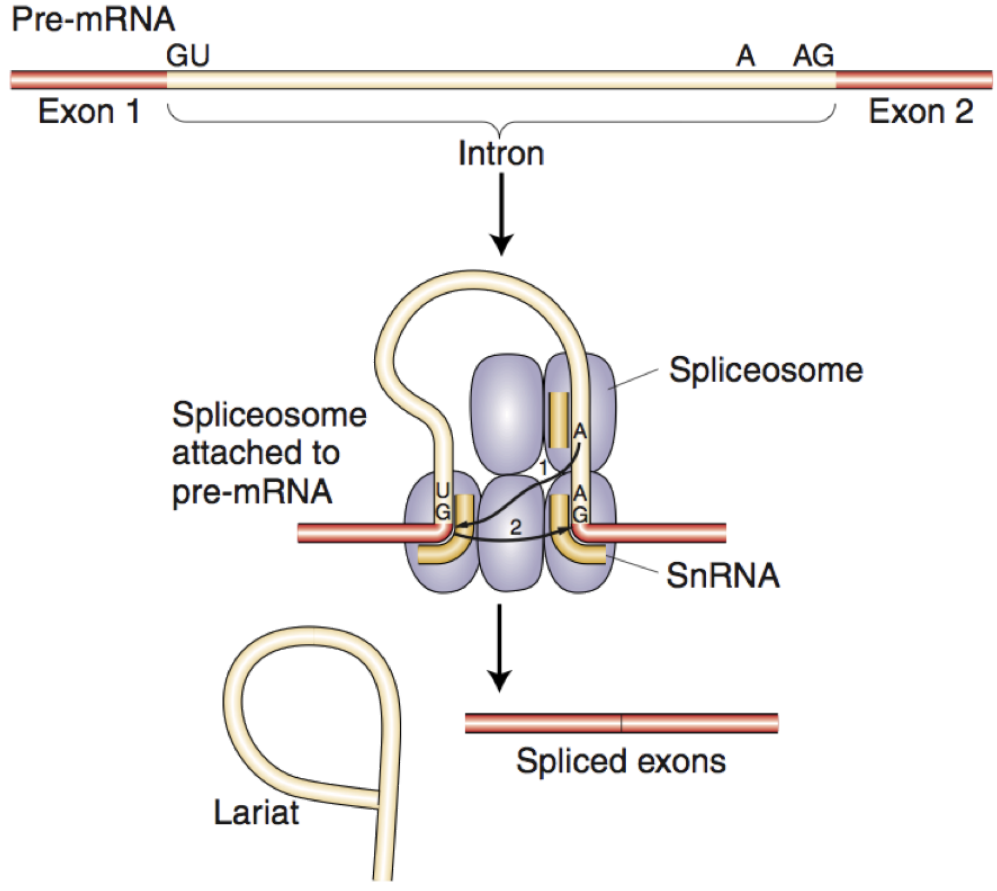
2. **Polyadenylation** – tis reached, An enzyme recognizes the conserved sequence AAUAAA or AUUAAA and cuts off approximately 20 bases farther down. Then a poly(A) tail added.



Transcription in Eukaryotes

Posttranscriptional RNA processing

2. **Intron splicing** – sequences within the hnRNA (heterogeneous nuclear RNA, the term used for pre-mRNA) are removed



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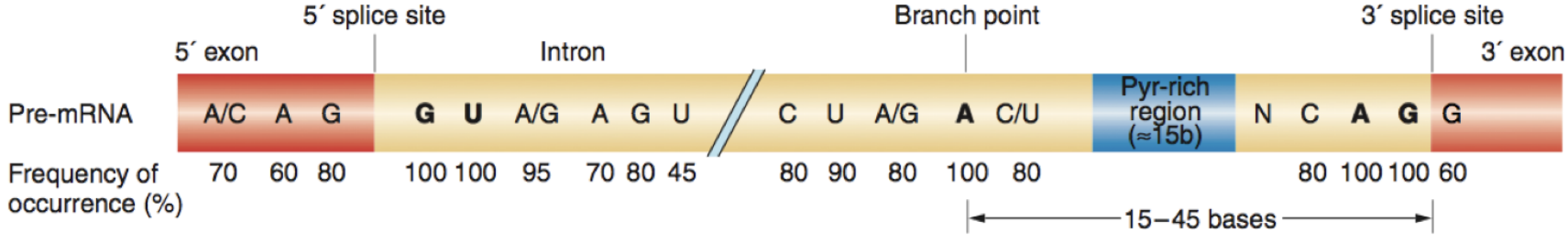

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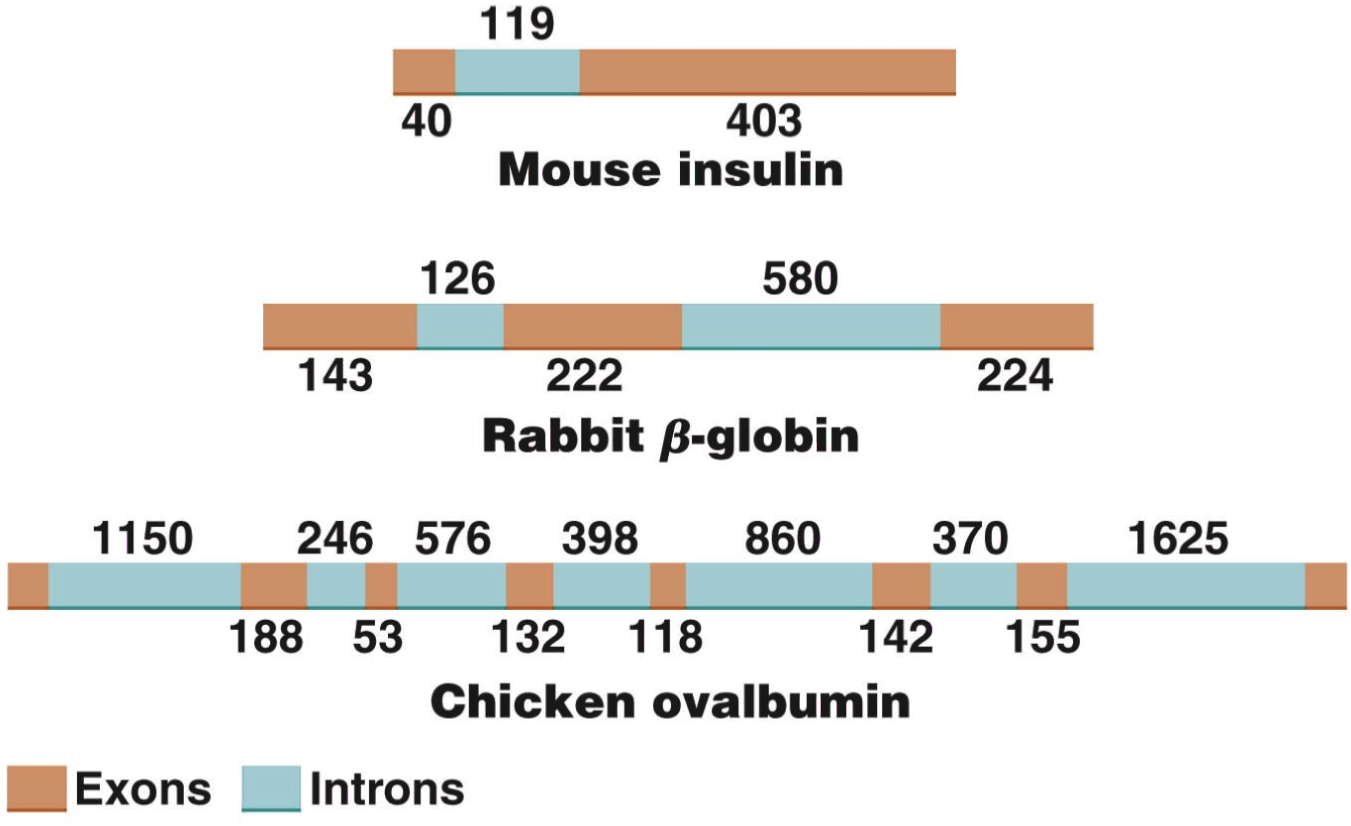
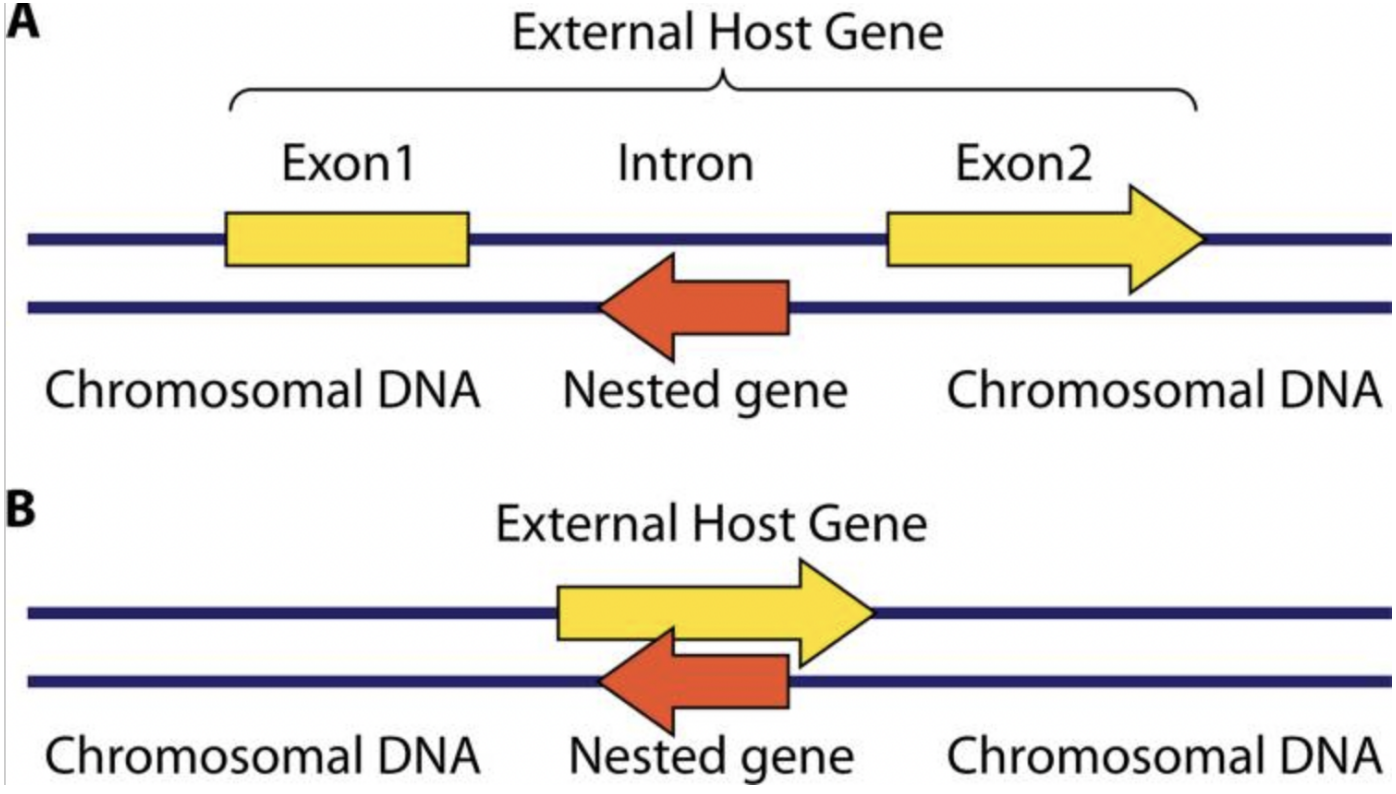


Transcription in Eukaryotes

Nested genes



Kumar (2009) Eukaryot Cell, 8, 1321–9.

Transcription in Eukaryotes

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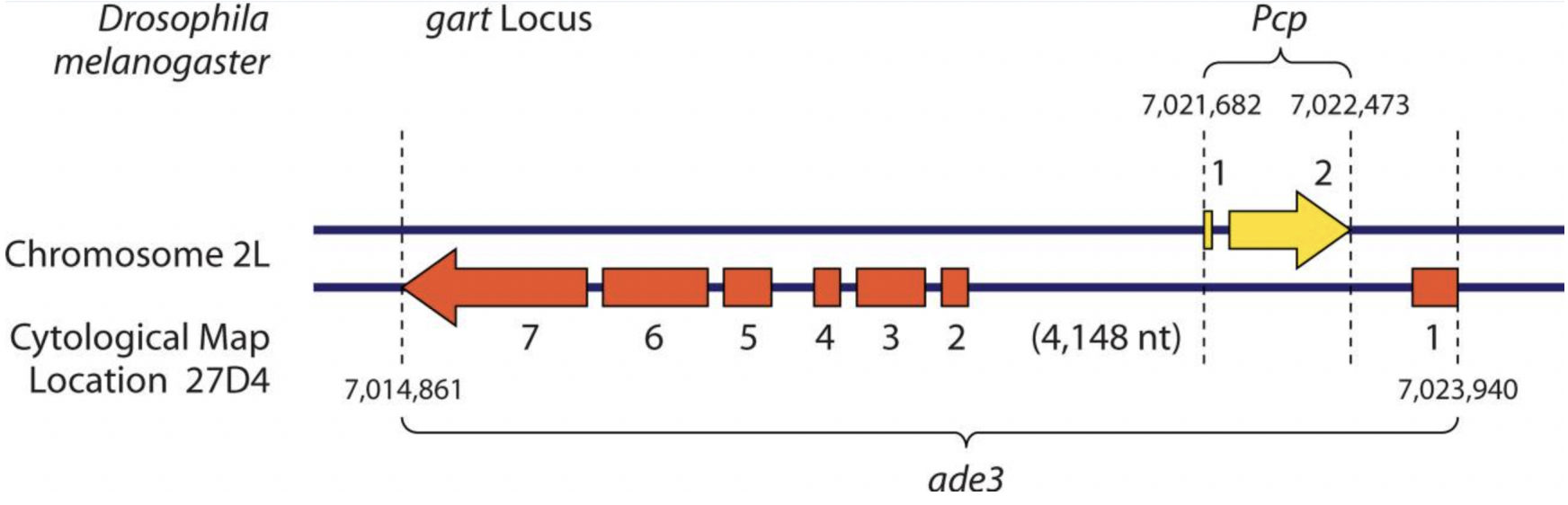

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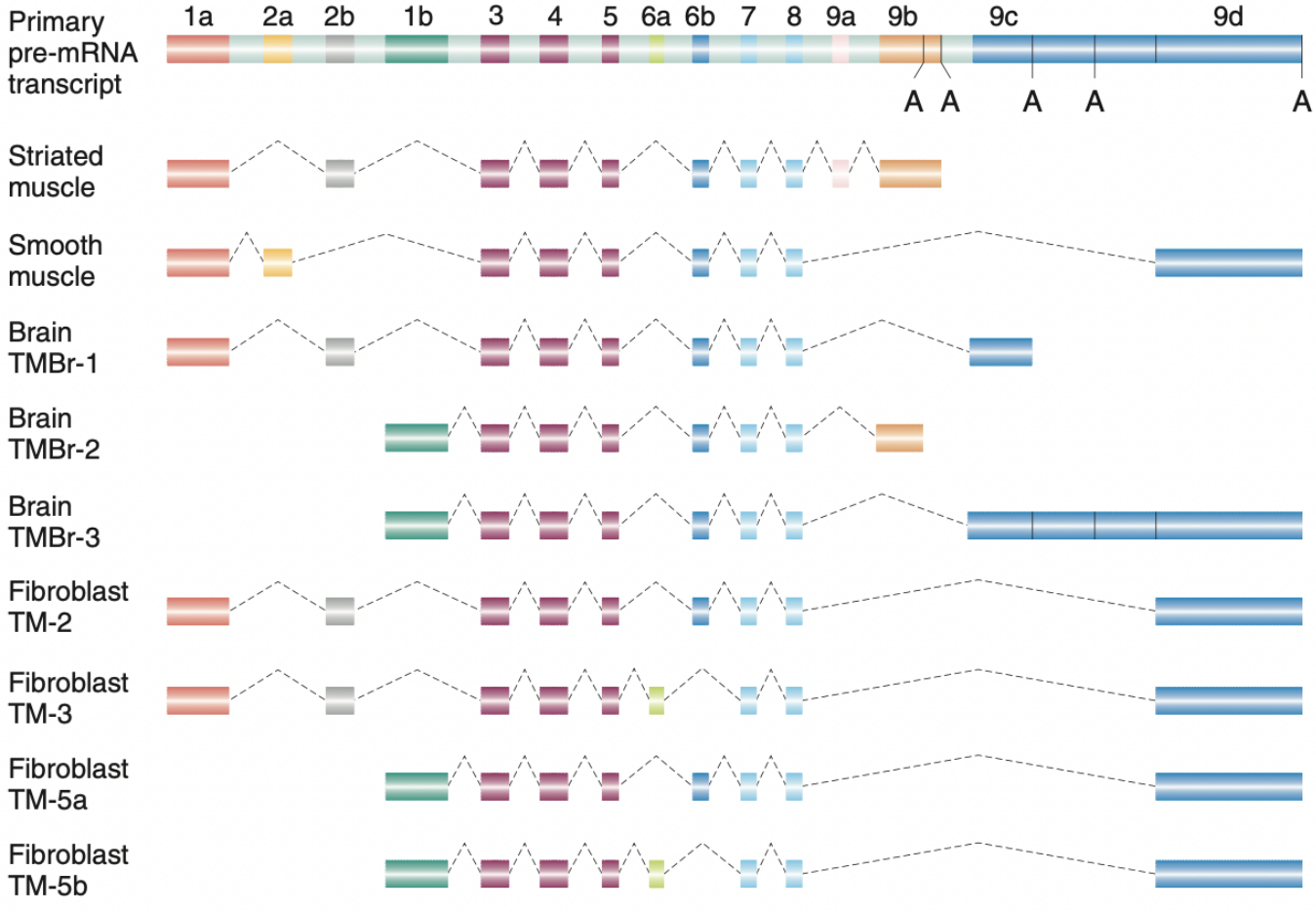
Transcription in Eukaryotes

Alternative Splicing (alpha-tropomyosin)



Transcription in Eukaryotes

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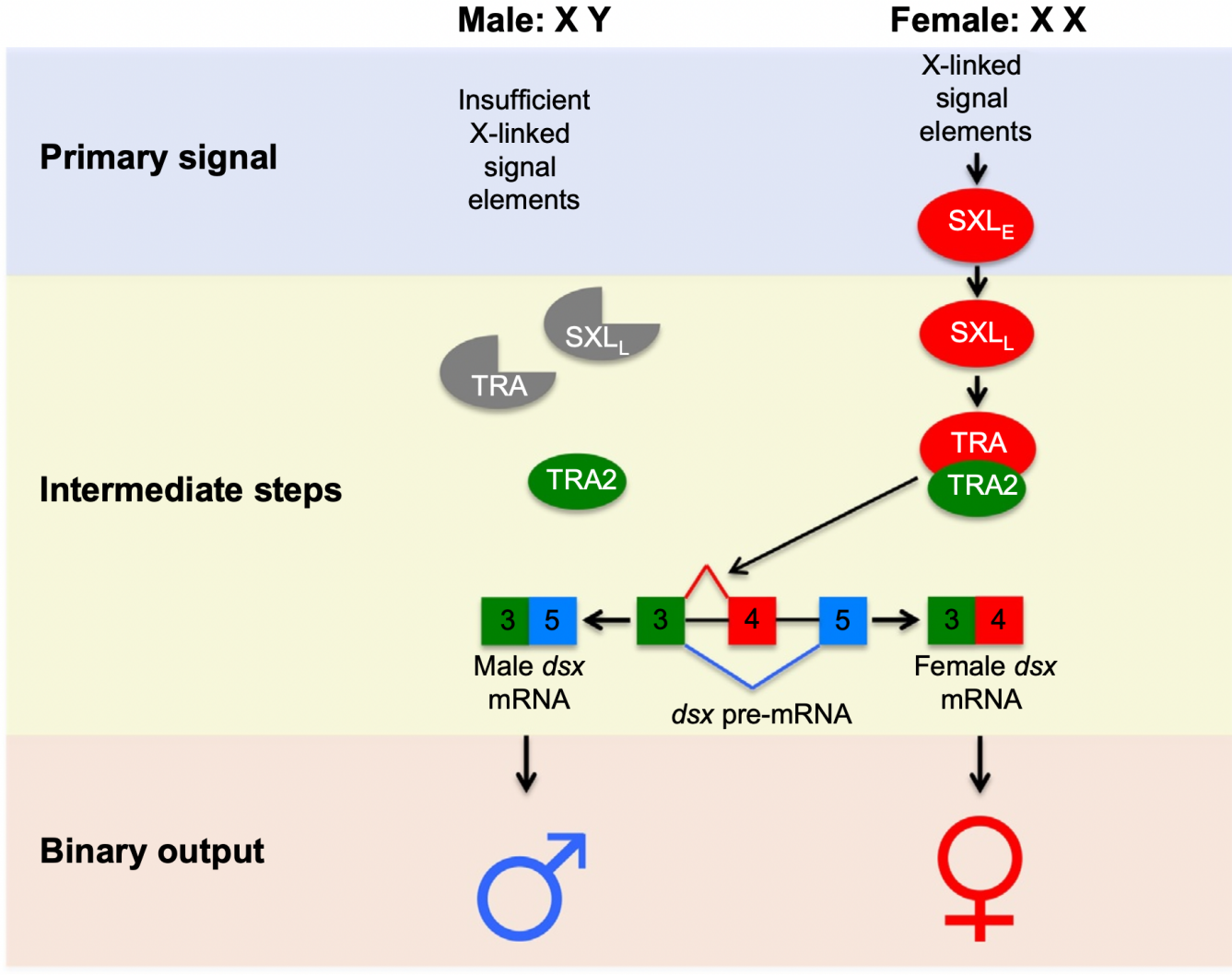
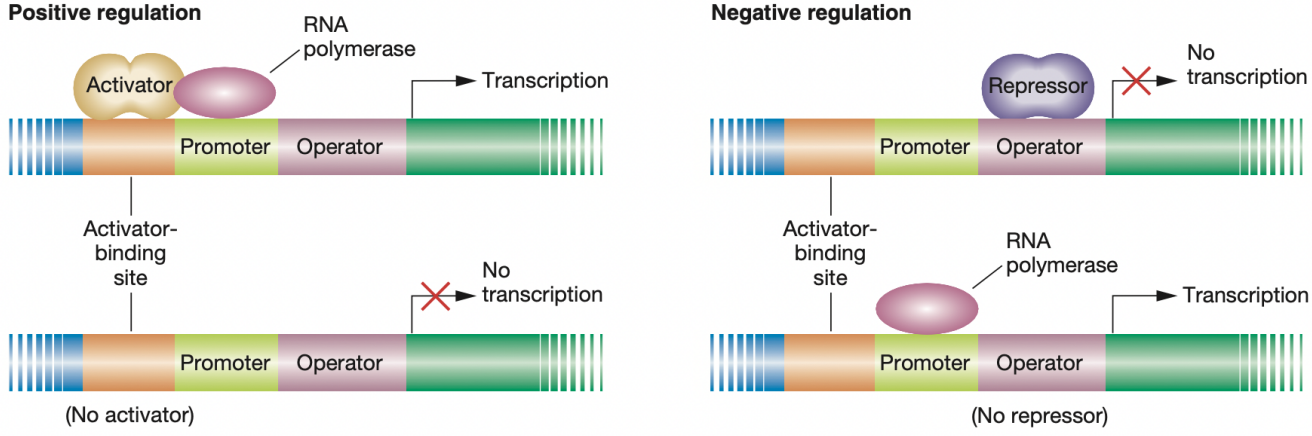


Regulation of Transcription in Prokaryotes

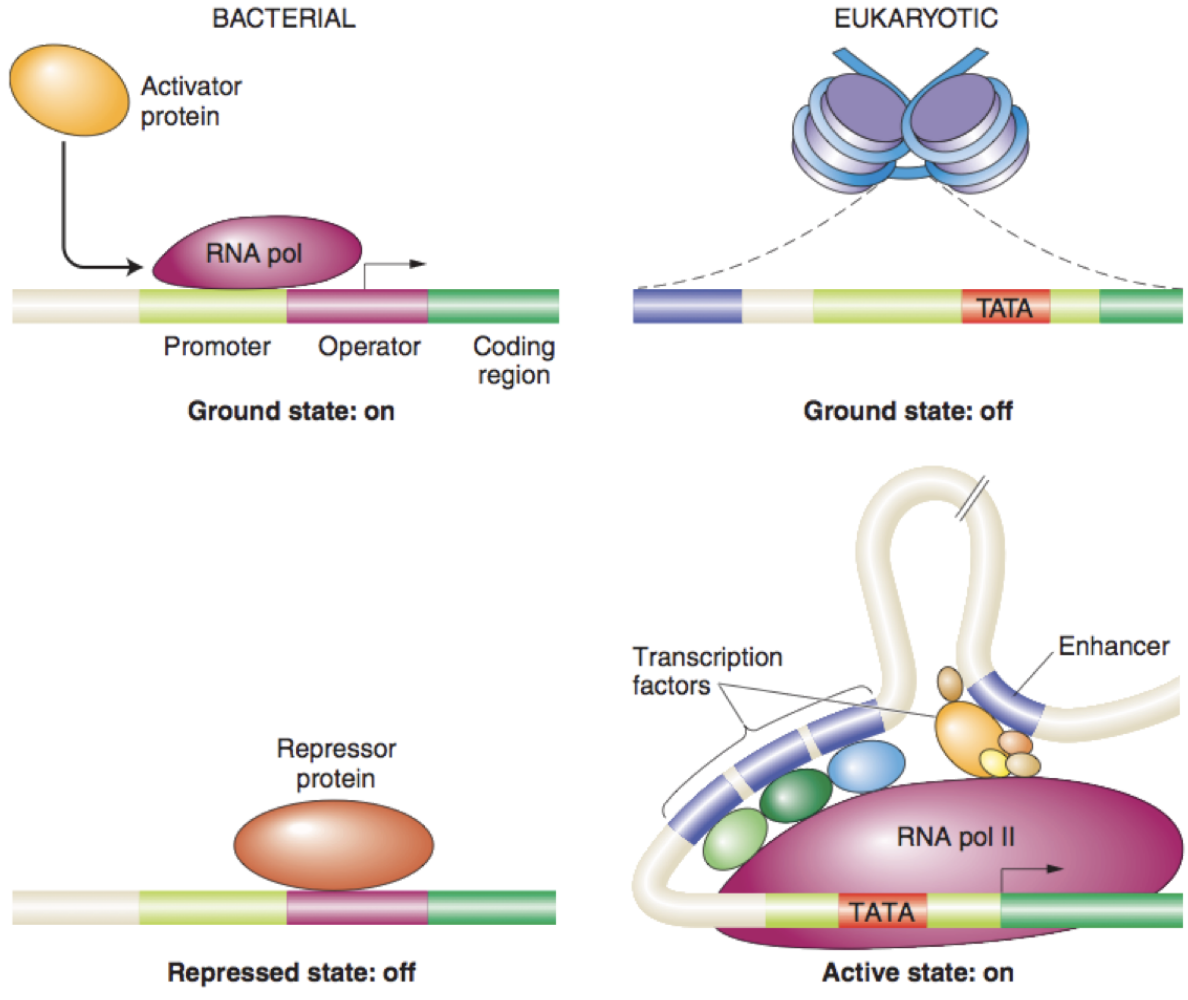
Genetic switches

1. Recognize environmental conditions in which they should activate or repress the transcription of the relevant genes;
2. Toggle on or off, like a switch, the transcription of each specific gene or group of genes.



Regulation of Transcription in Eukaryotes

Eukaryotes *vs.* Prokaryotes

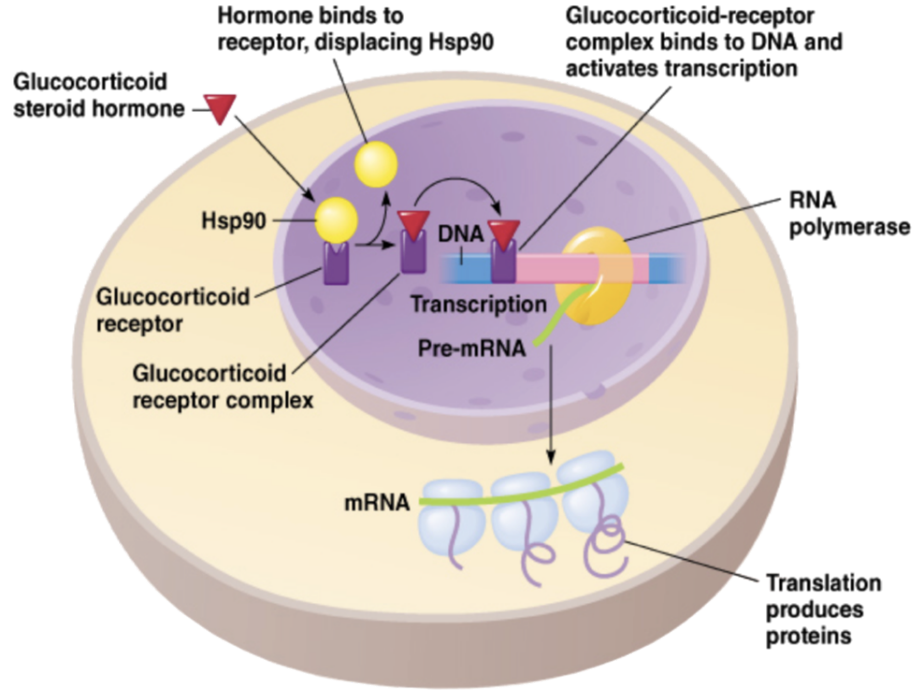


Regulation of Transcription in Eukaryotes

Two categories

➡️ **Short-term - genes are quickly turned on or off in response to the environment and demands of the cell.**

➡️ Long-term - genes for development and differentiation.



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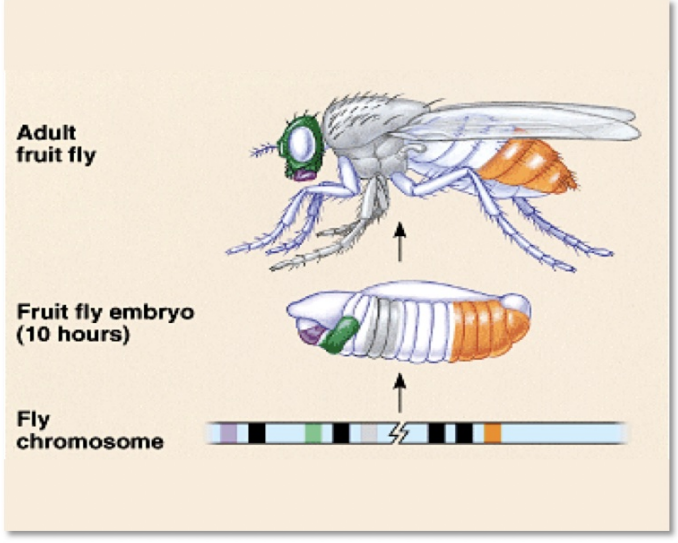
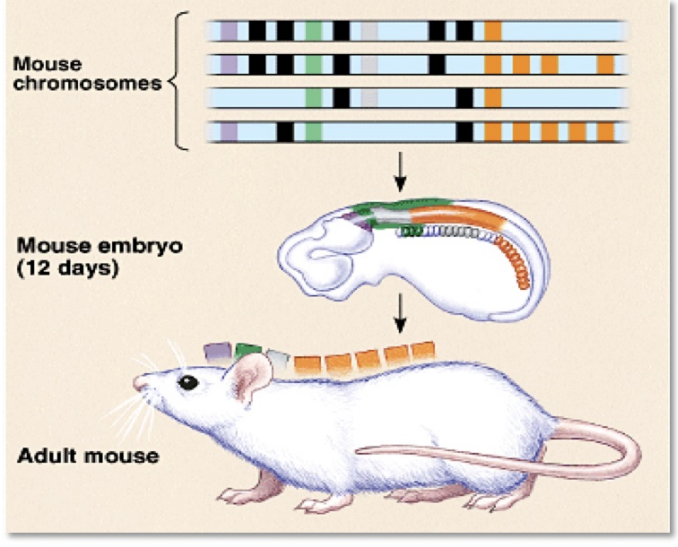
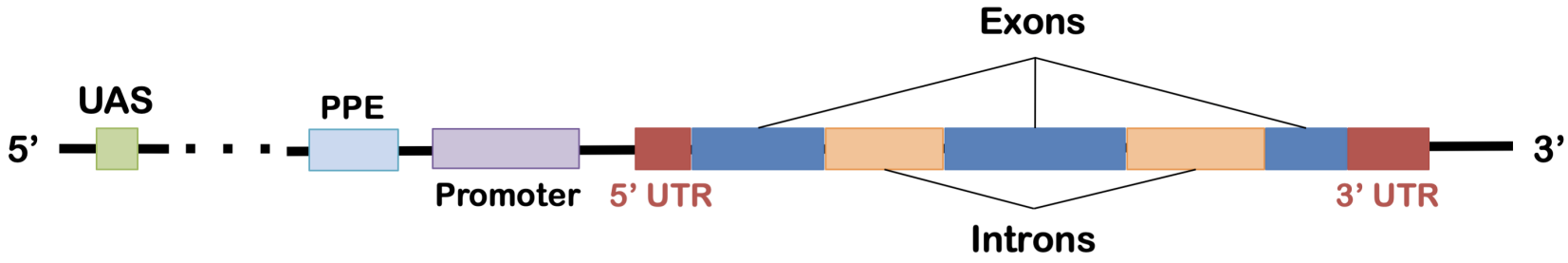




Regulation of Transcription in Eukaryotes

Cannonical gene structure



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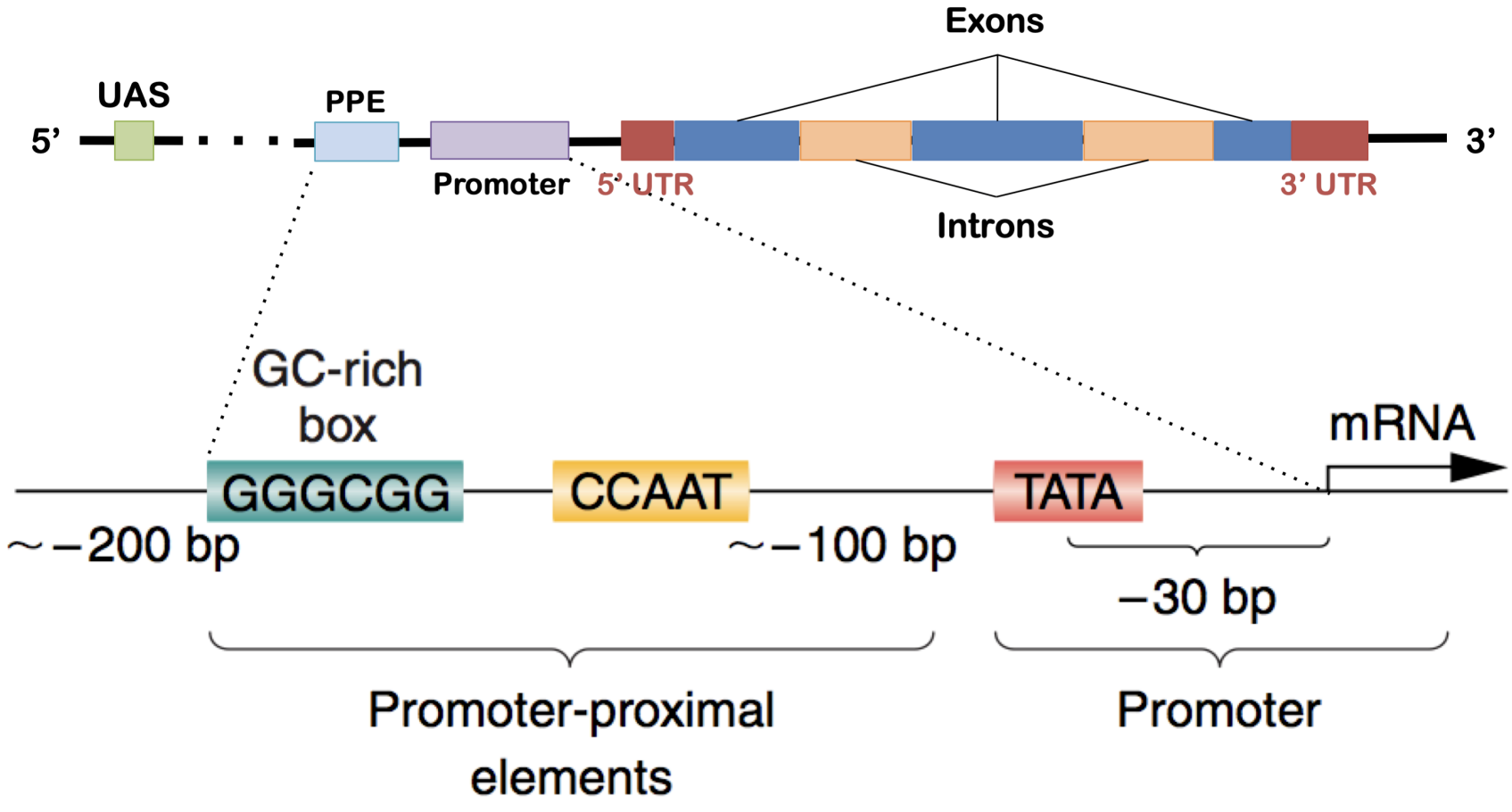
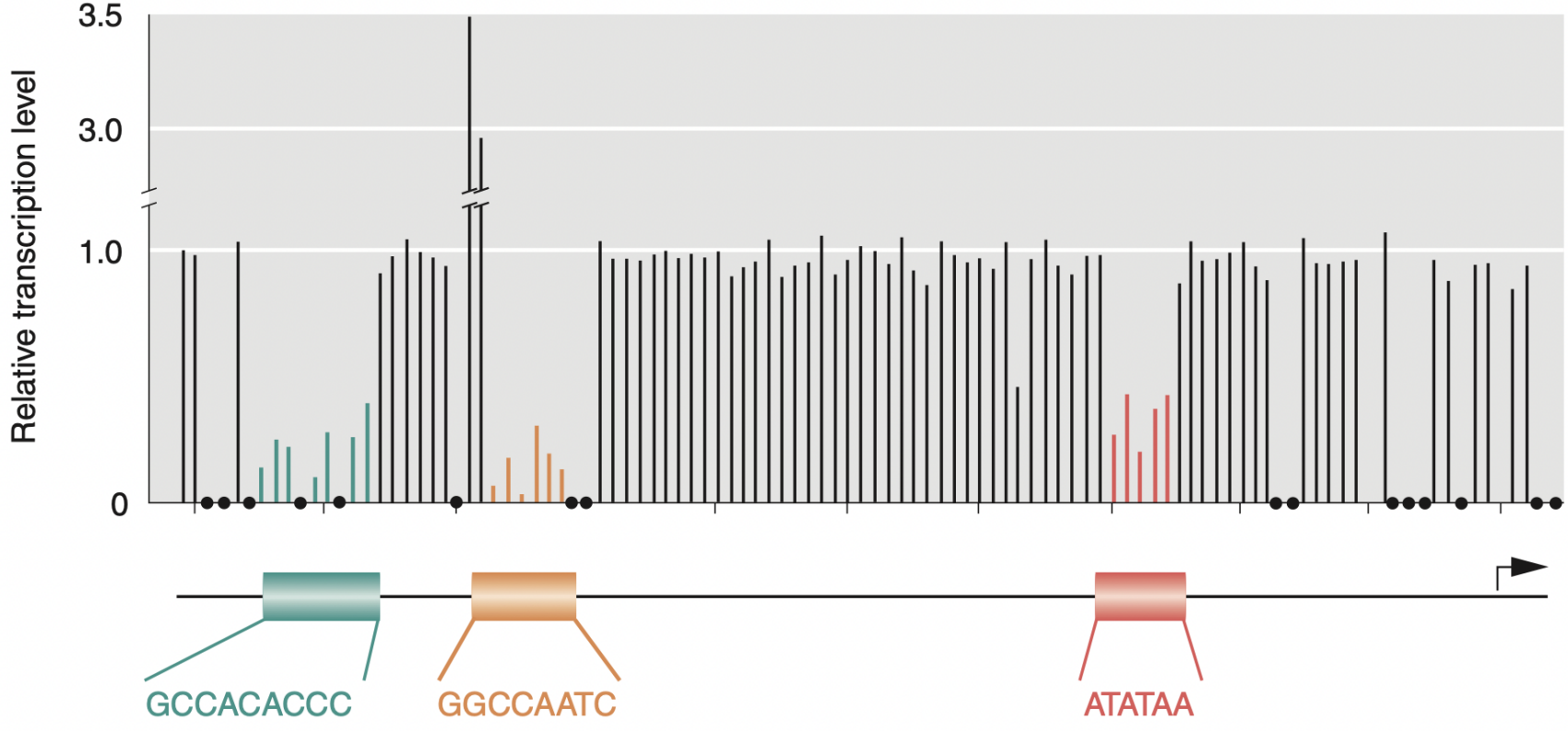


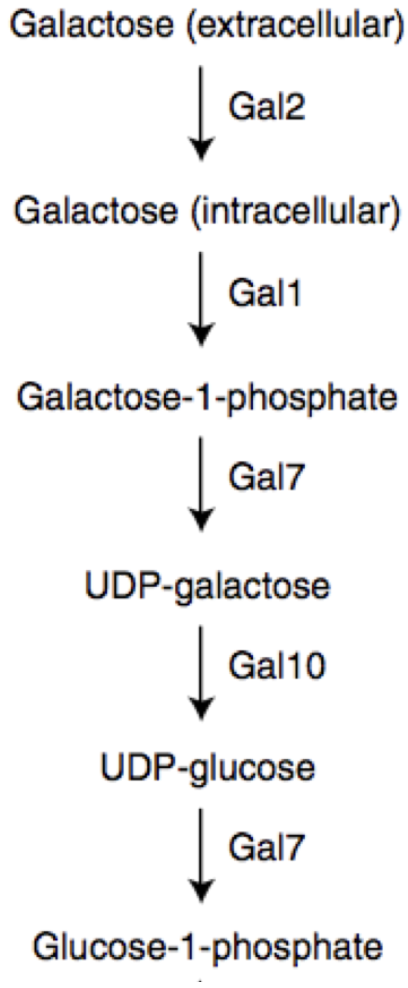
Regulation of Transcription in Eukaryotes

Promoter-proximal elements



Regulation of Transcription in Eukaryotes

The GAL System in yeast



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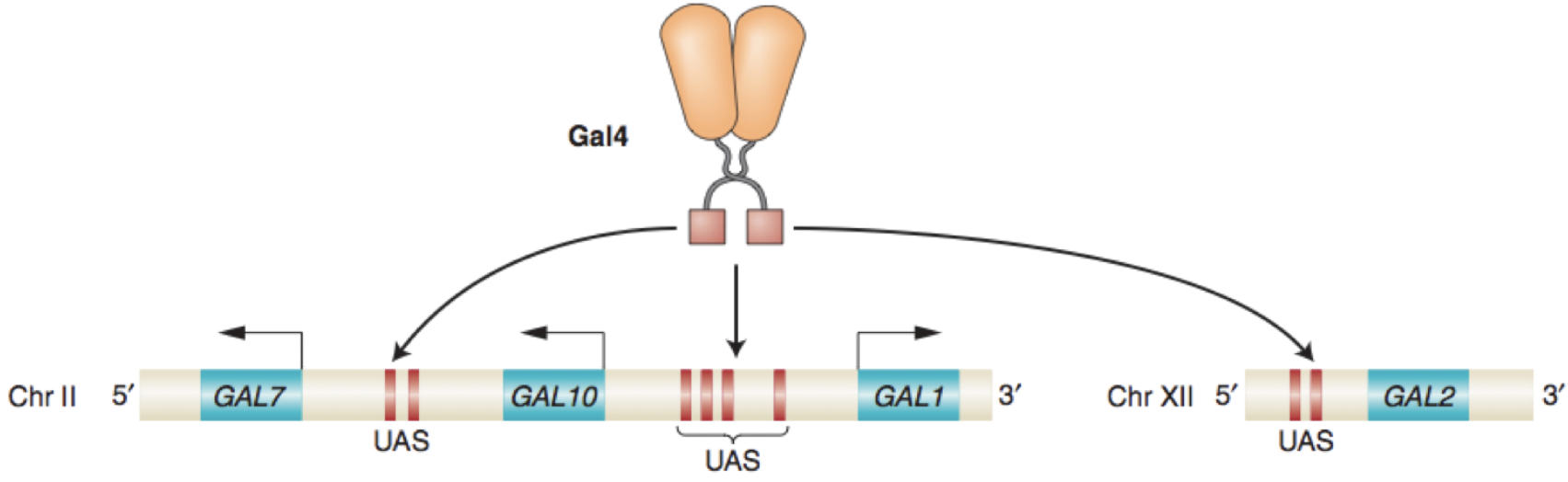

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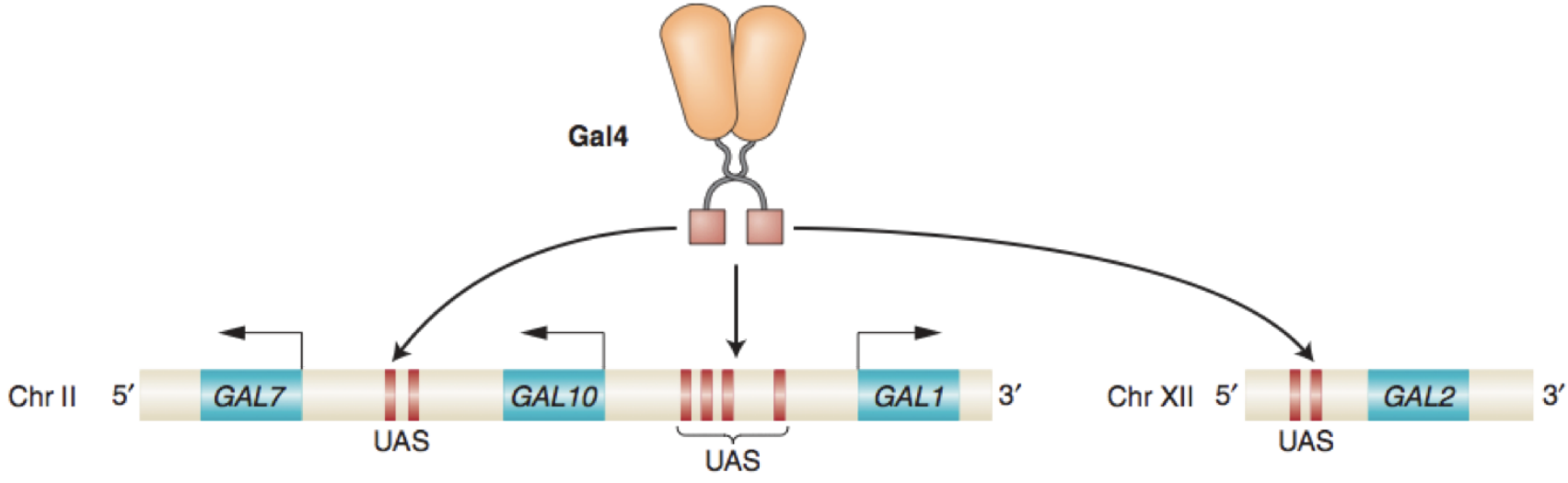

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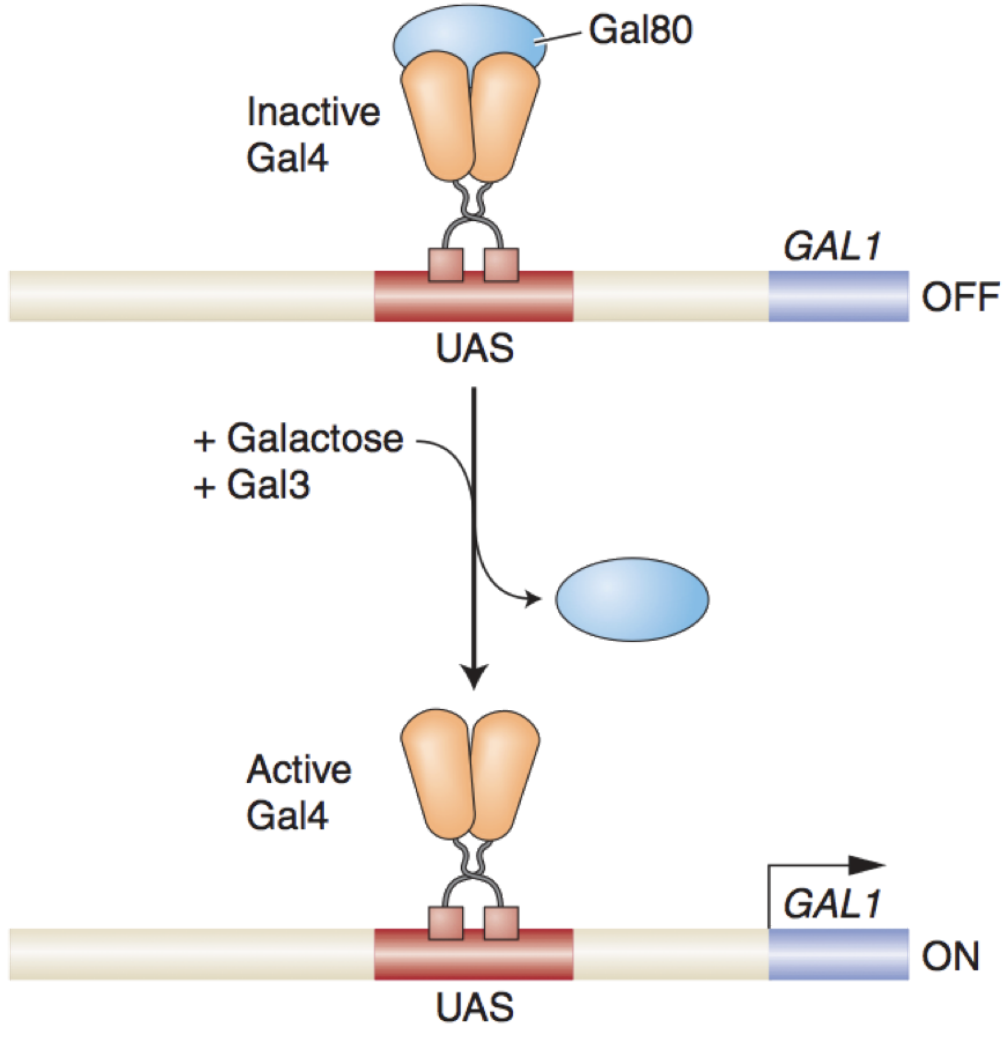

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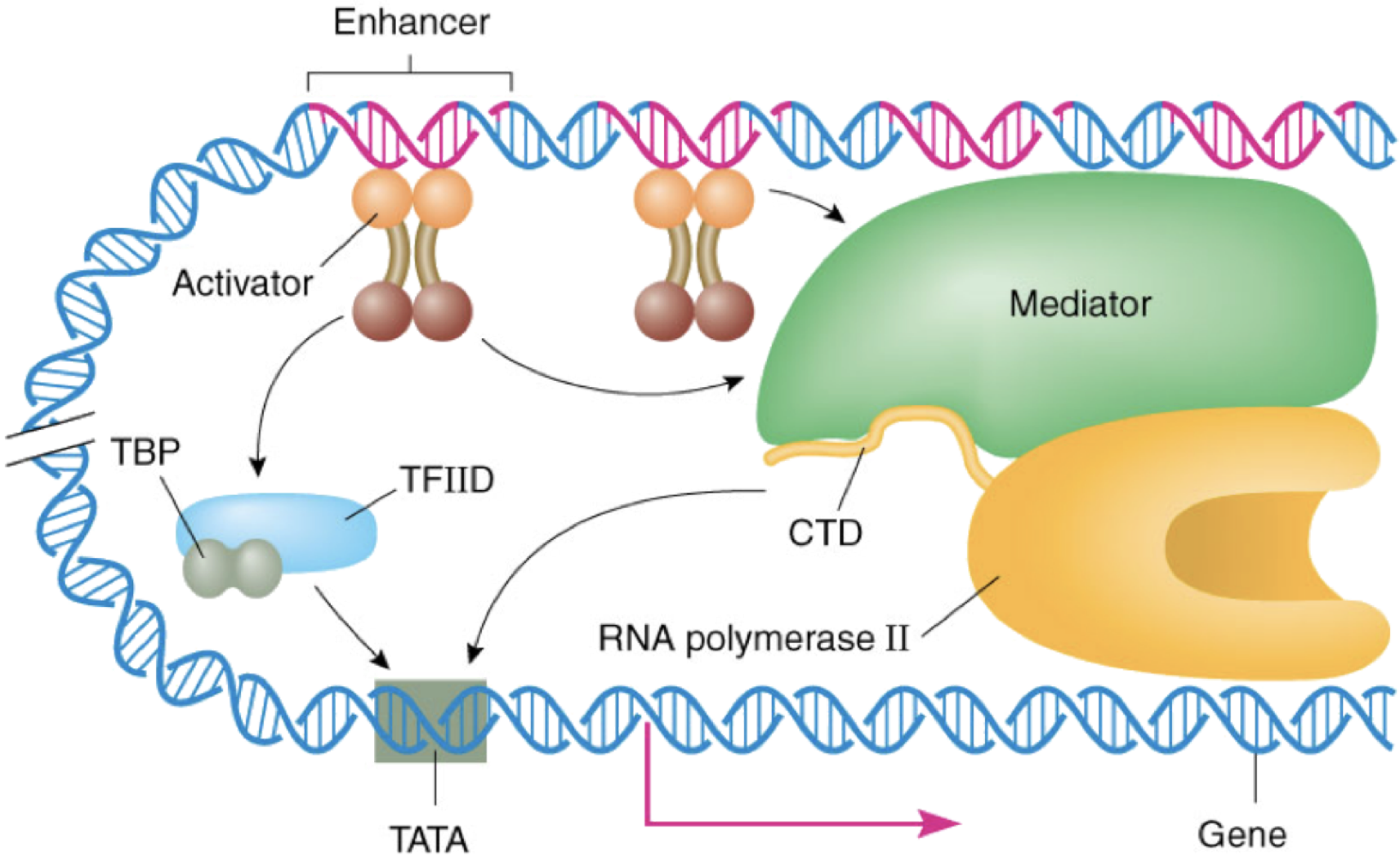
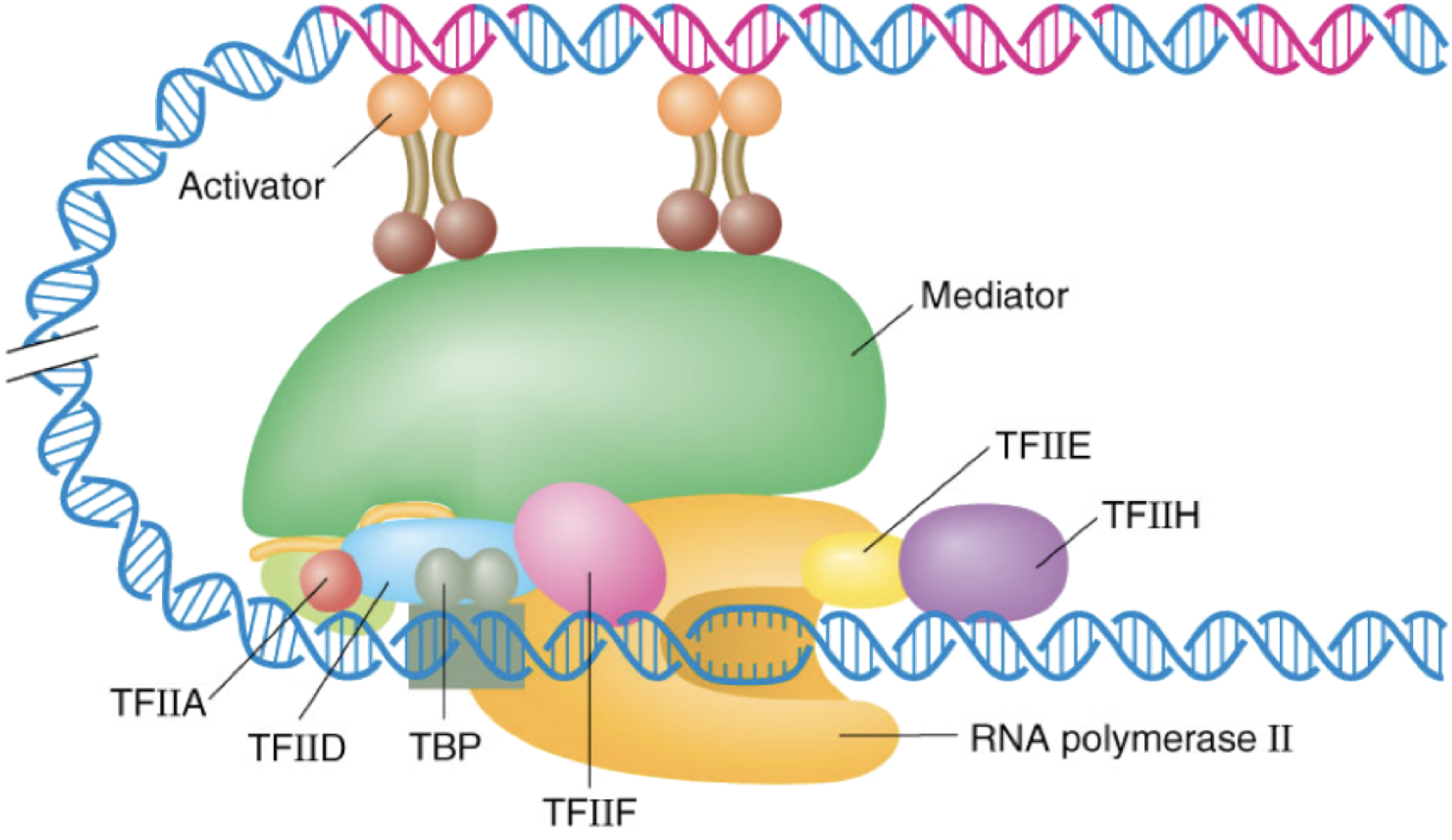


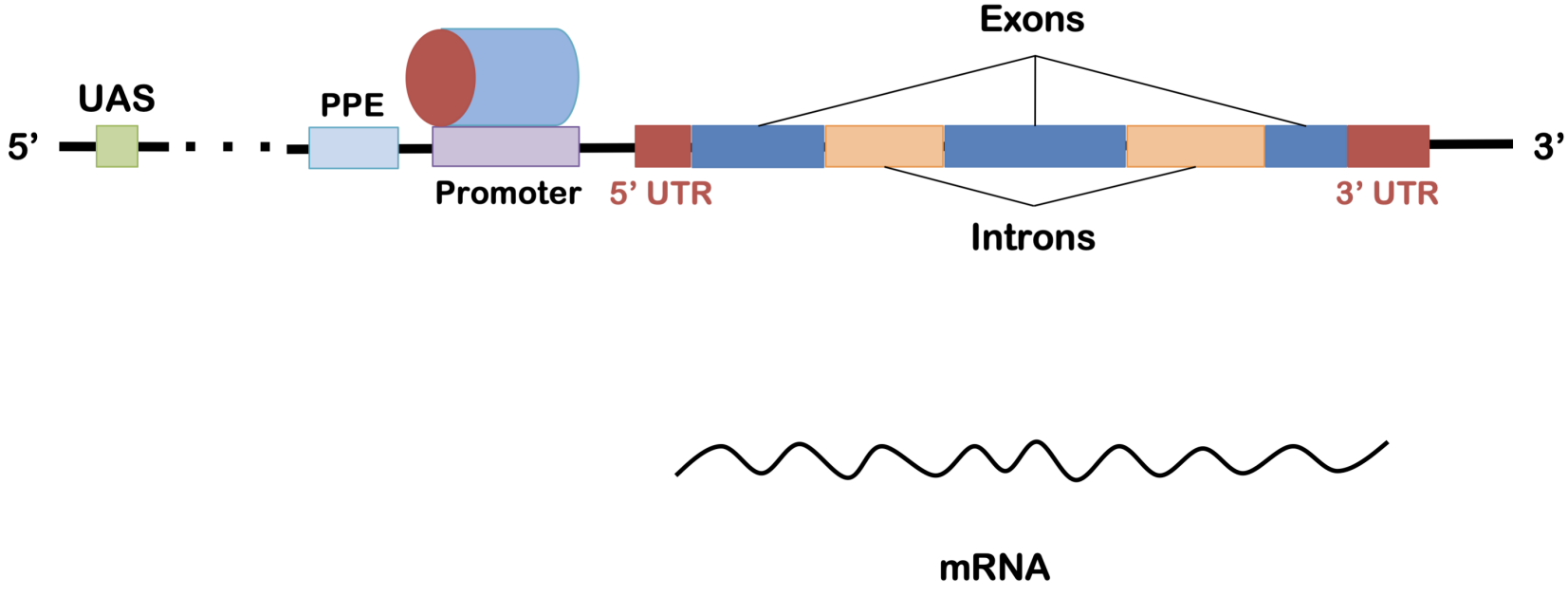
Regulation of Transcription in Eukaryotes

Eukaryotic transcriptional machinery



Regulation of Transcription in Eukaryotes

Eukaryotic transcriptional machinery



Regulation of Transcription in Eukaryotes

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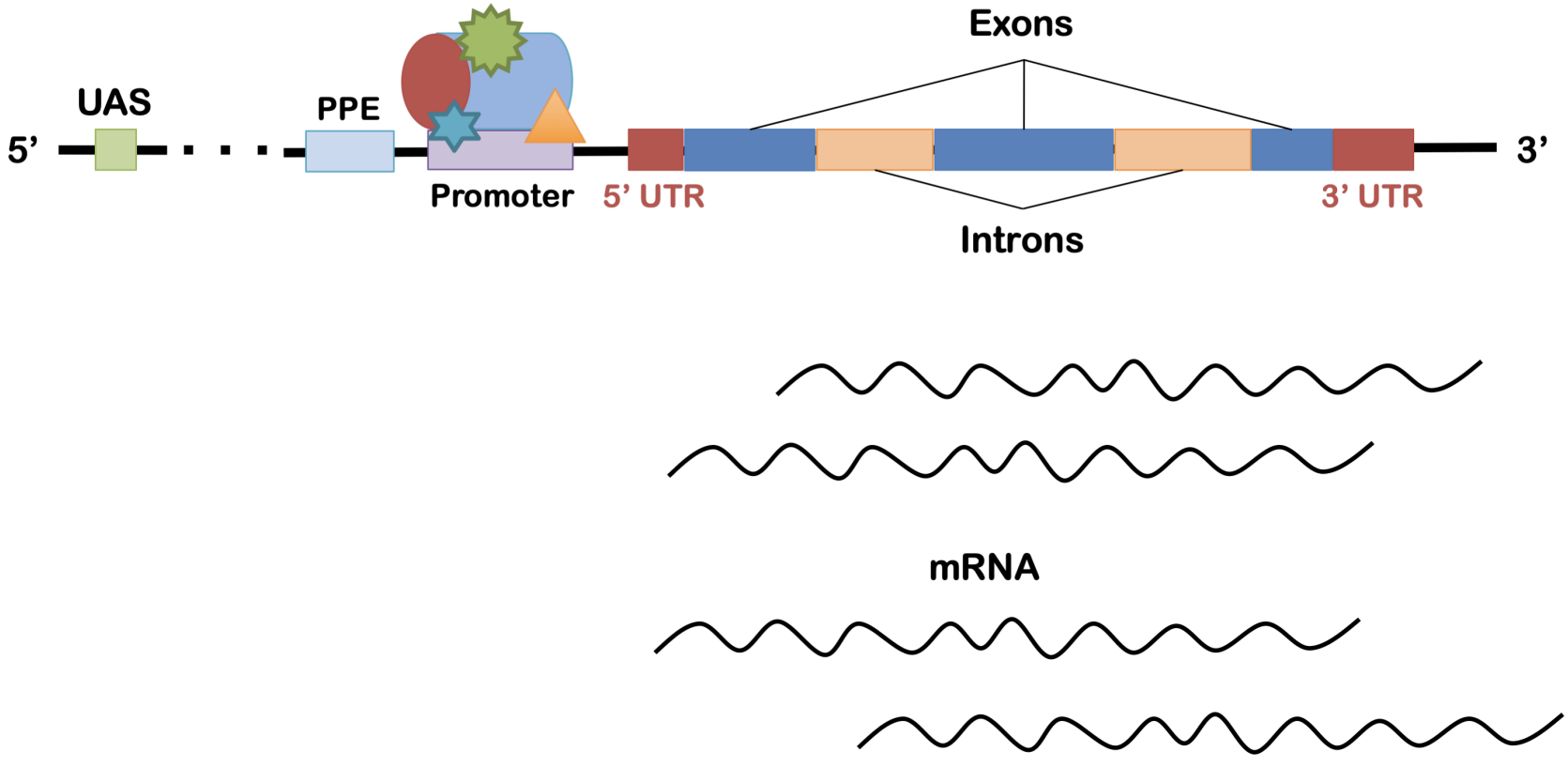
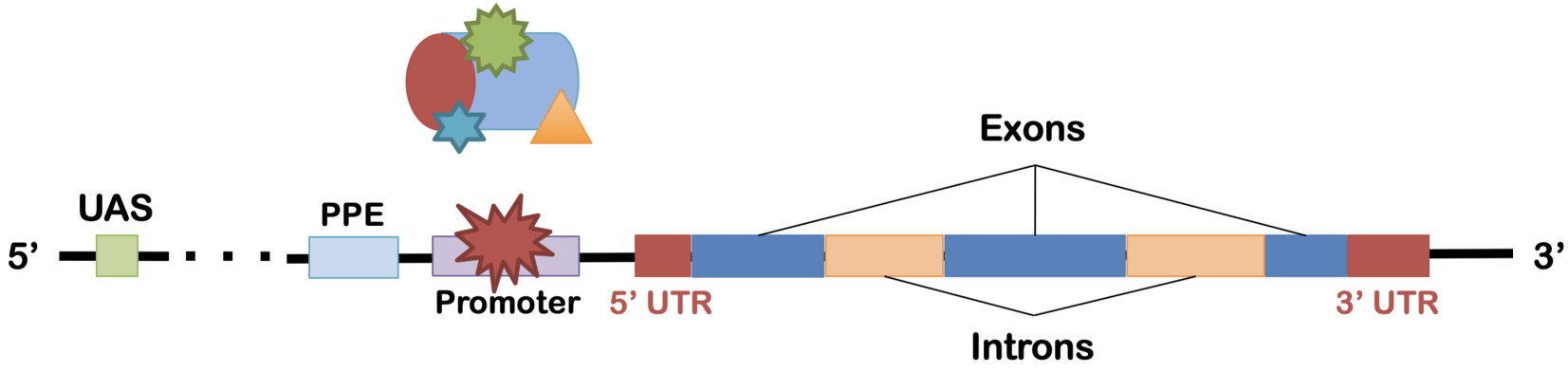


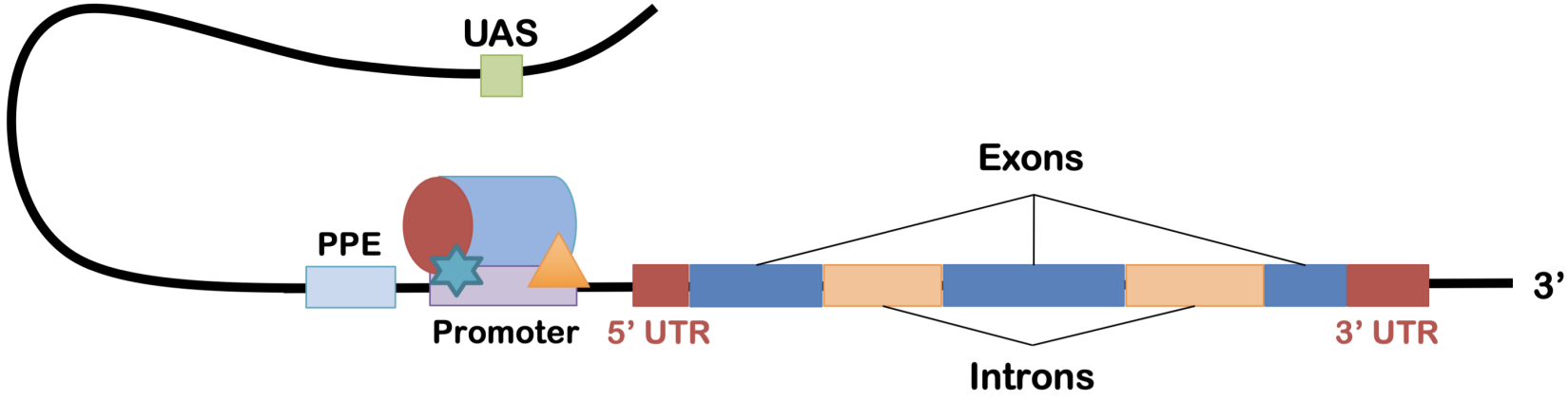
Regulation of Transcription in Eukaryotes

Eukaryotic transcriptional machinery



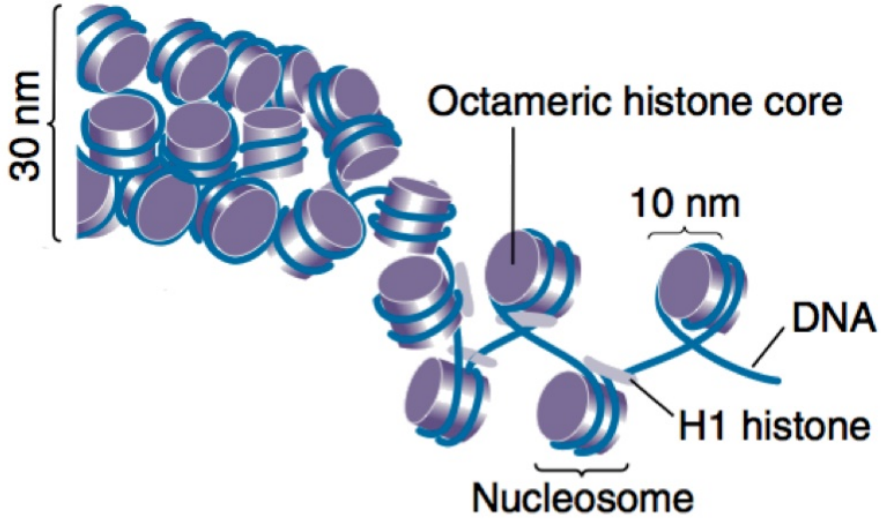
Regulation of Transcription in Eukaryotes

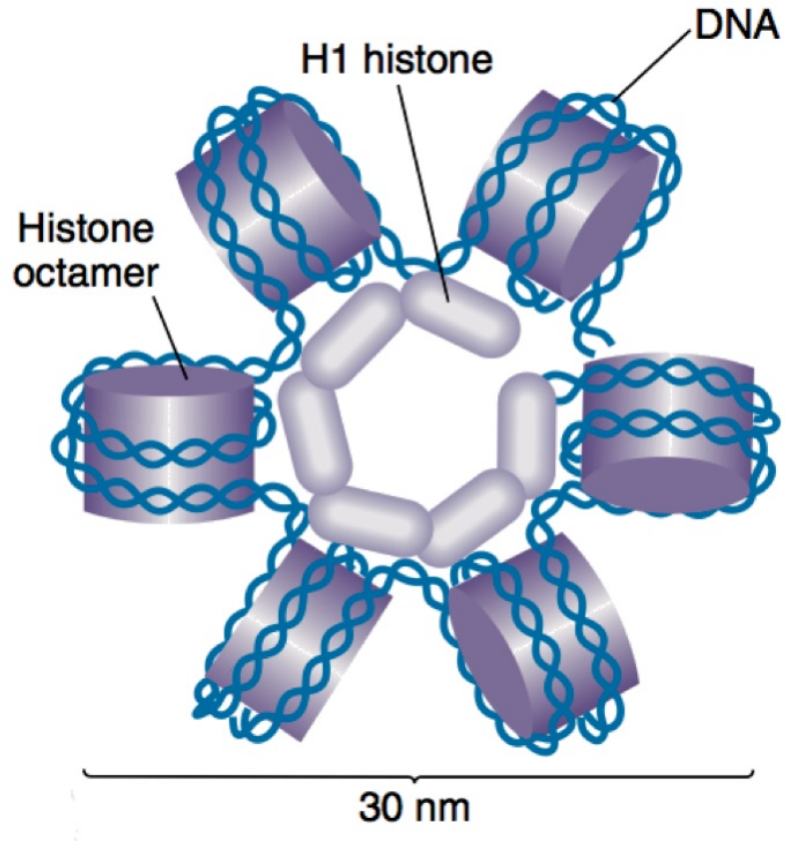
Eukaryotic transcriptional machinery



Regulation of Transcription in Eukaryotes

Chromatin structure: nucleosomes





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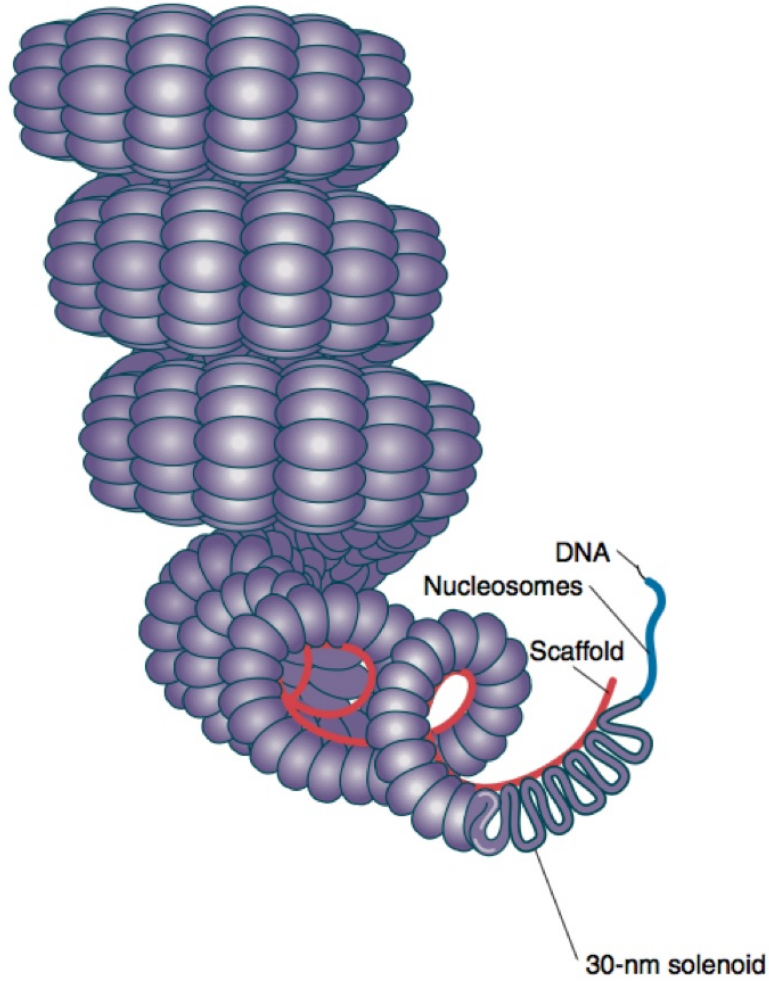
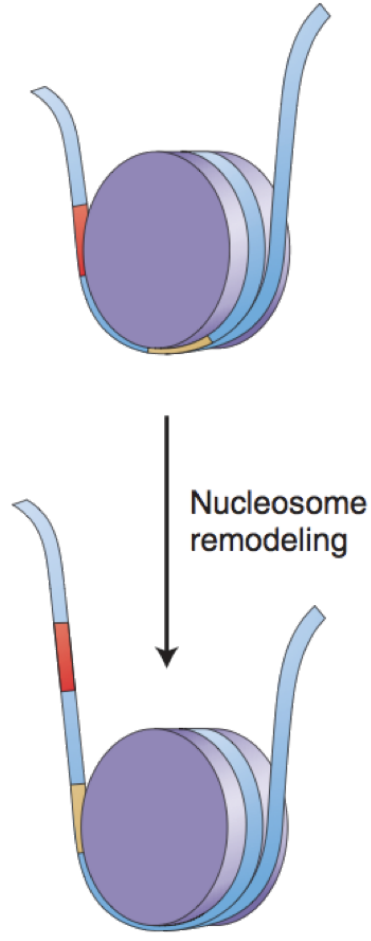


Regulation of Transcription in Eukaryotes

Chromatin structure: nucleosomes



Regulation of Transcription in Eukaryotes

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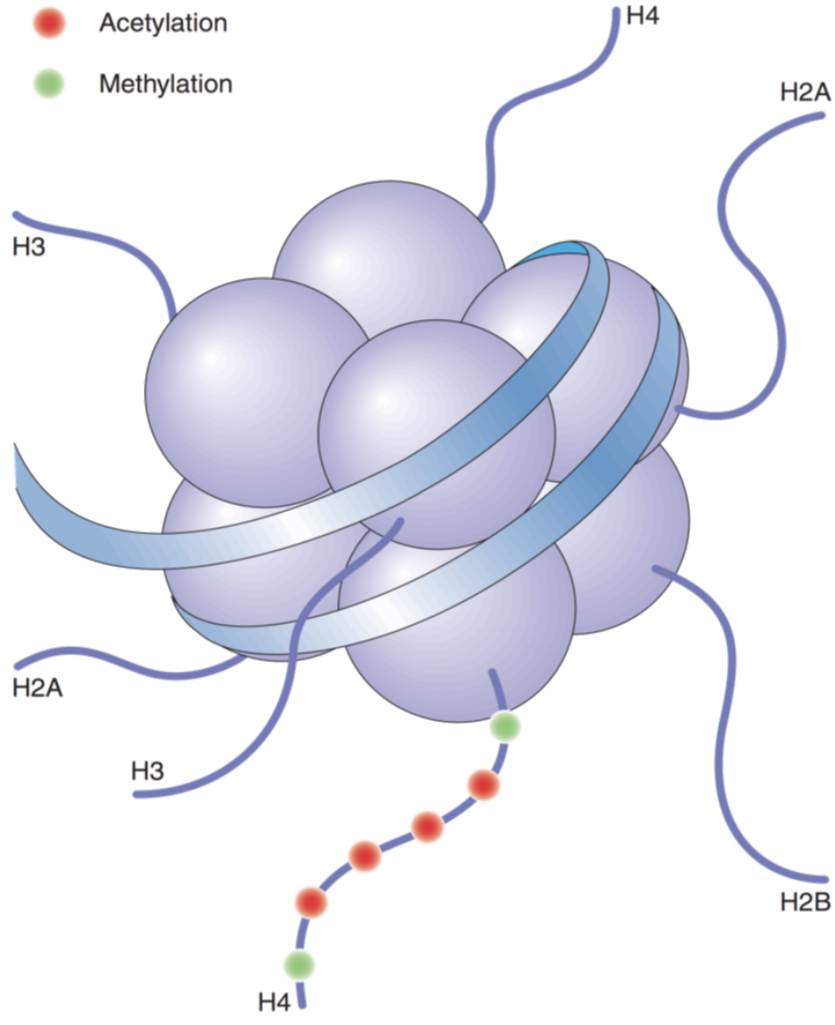

Regulation of Transcription in Eukaryotes

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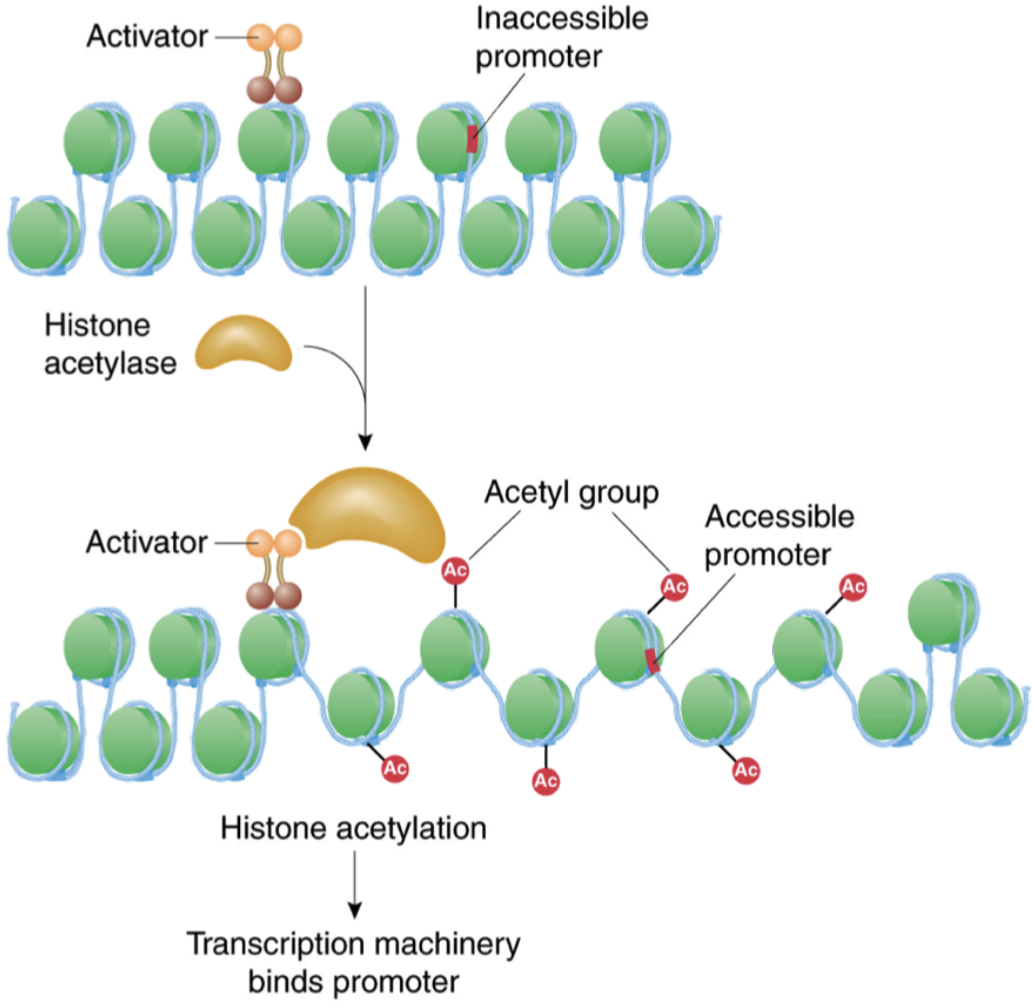
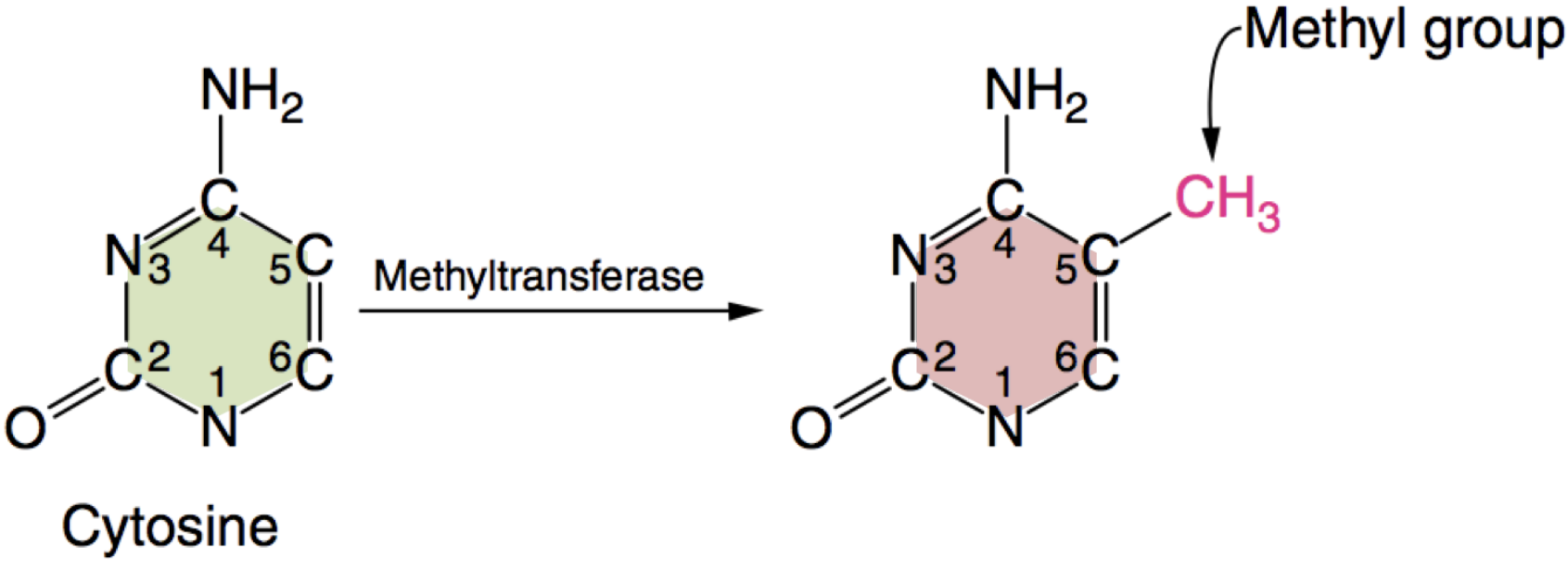


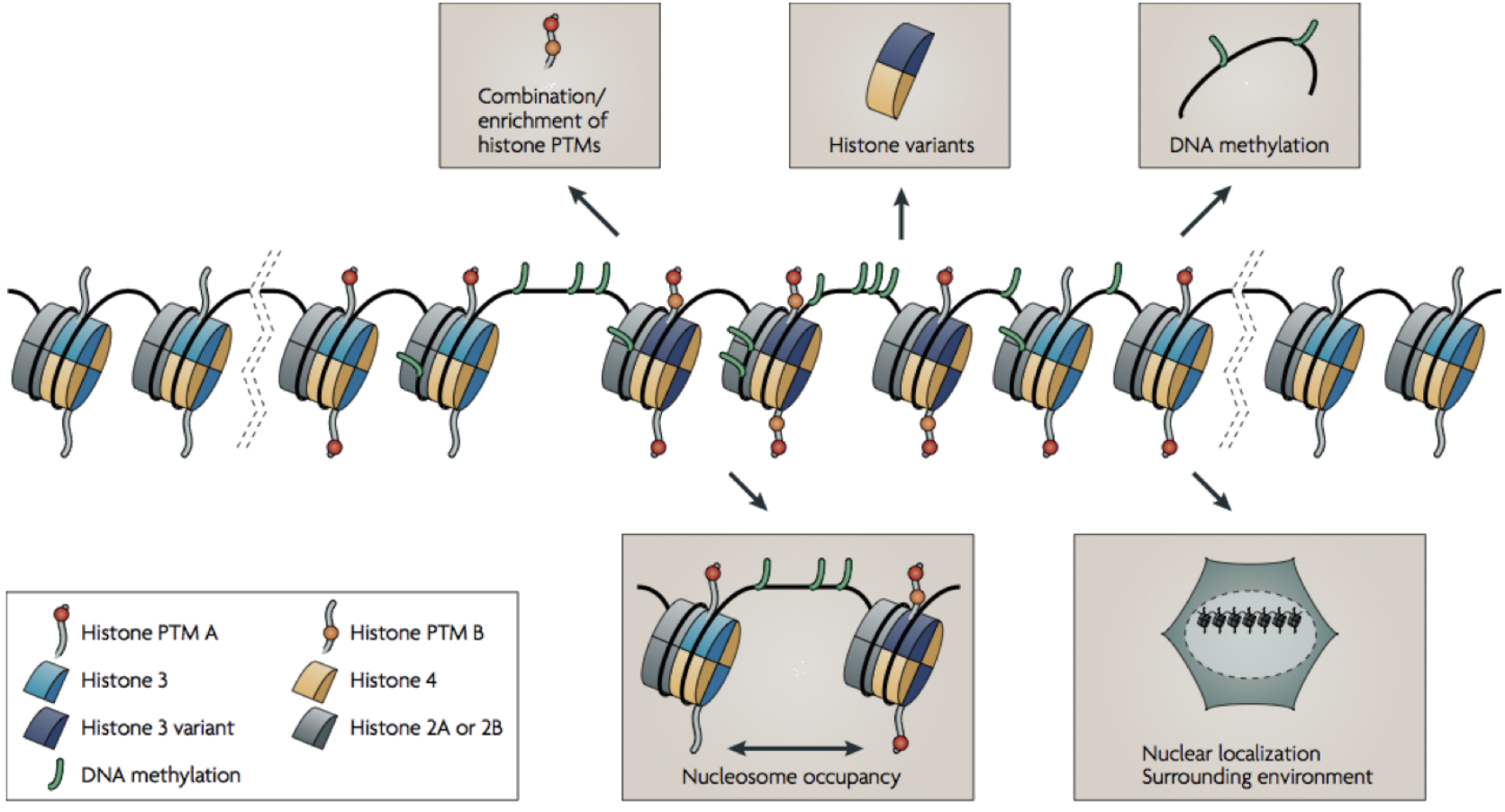
Regulation of Transcription in Eukaryotes

Chromatin structure: DNA methylation



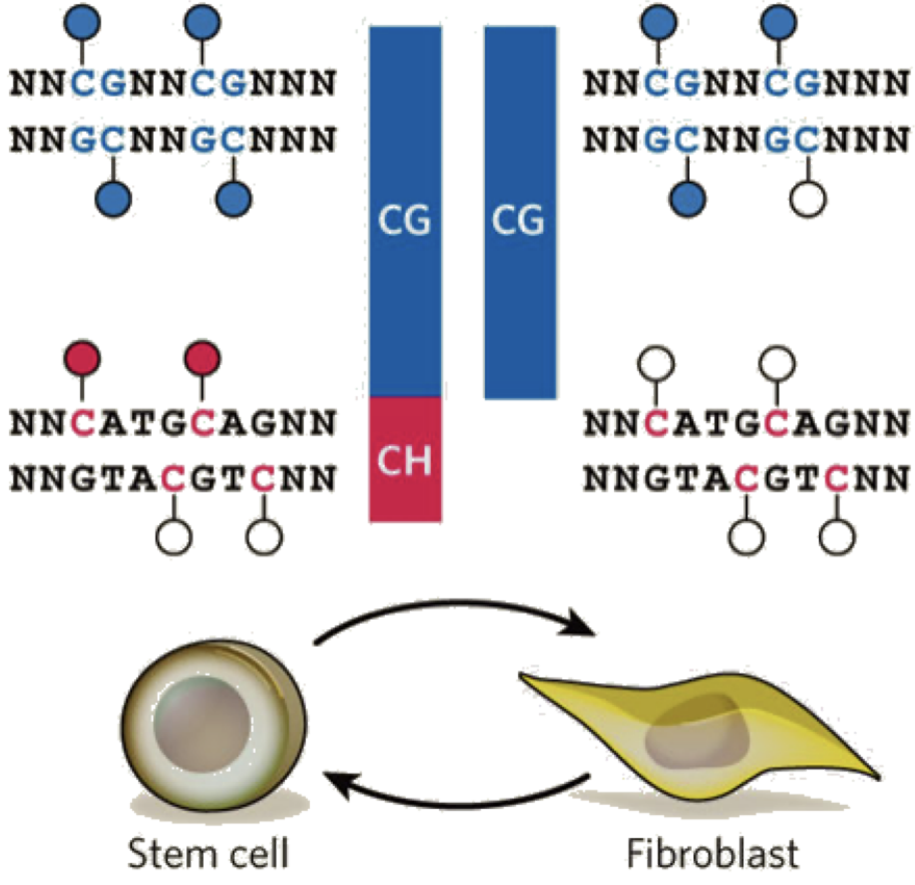
Regulation of Transcription in Eukaryotes

Epigenetic marks



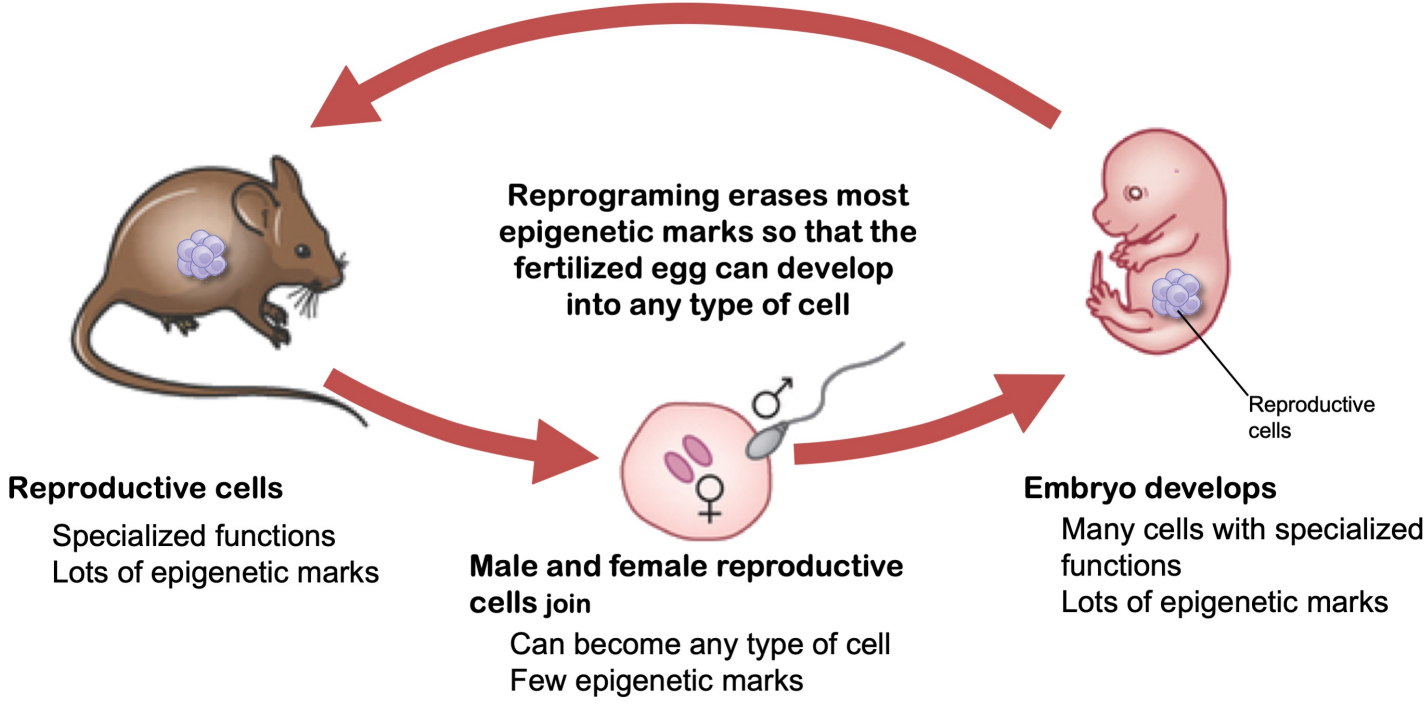
Regulation of Transcription in Eukaryotes

Epigenetic: cell differentiation



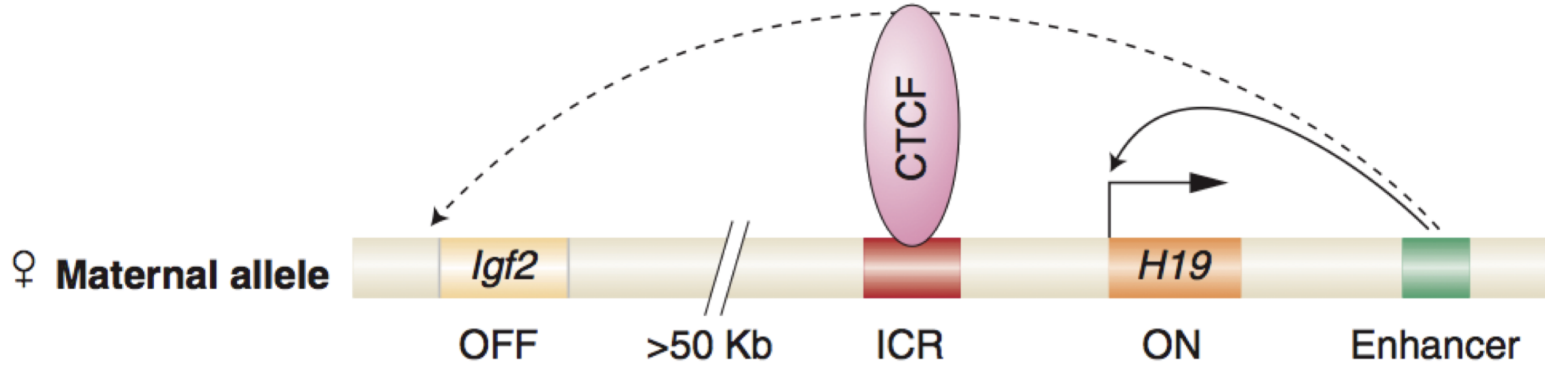
Regulation of Transcription in Eukaryotes

Epigenetic: cell differentiation



Regulation of Transcription in Eukaryotes

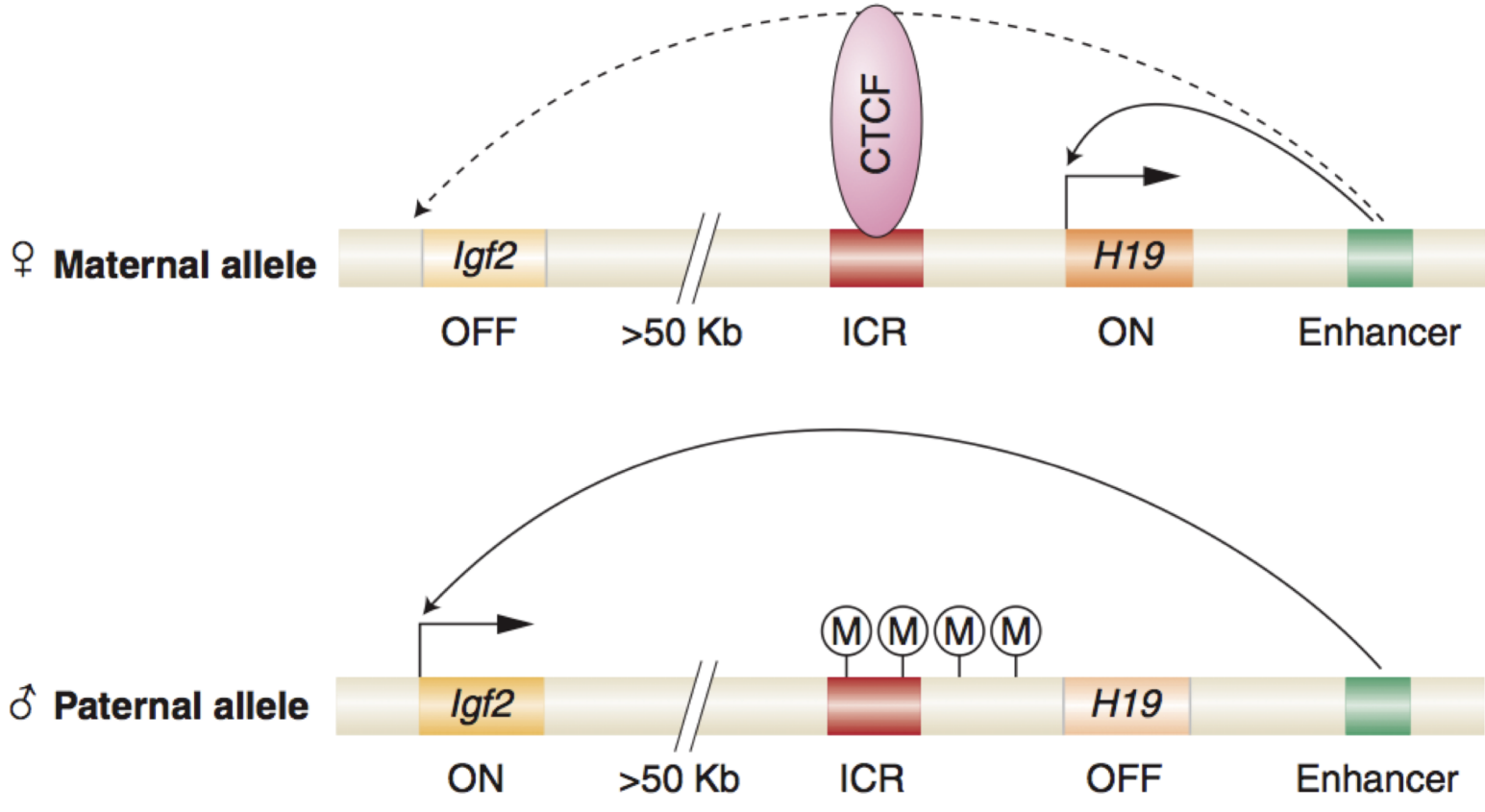
Epigenetic: genomic imprinting



Insulin-like Growth Factor, *Igf2*

Regulation of Transcription in Eukaryotes

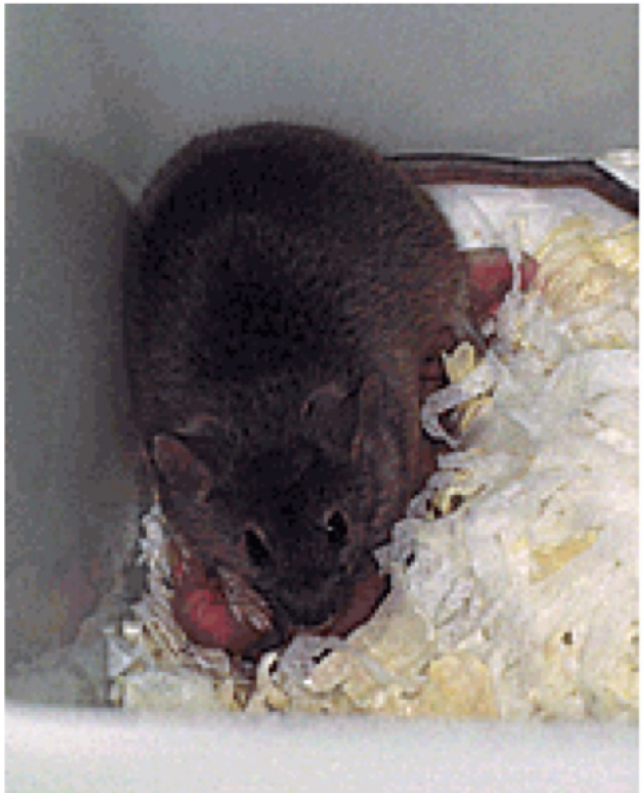
Epigenetic: genomic imprinting

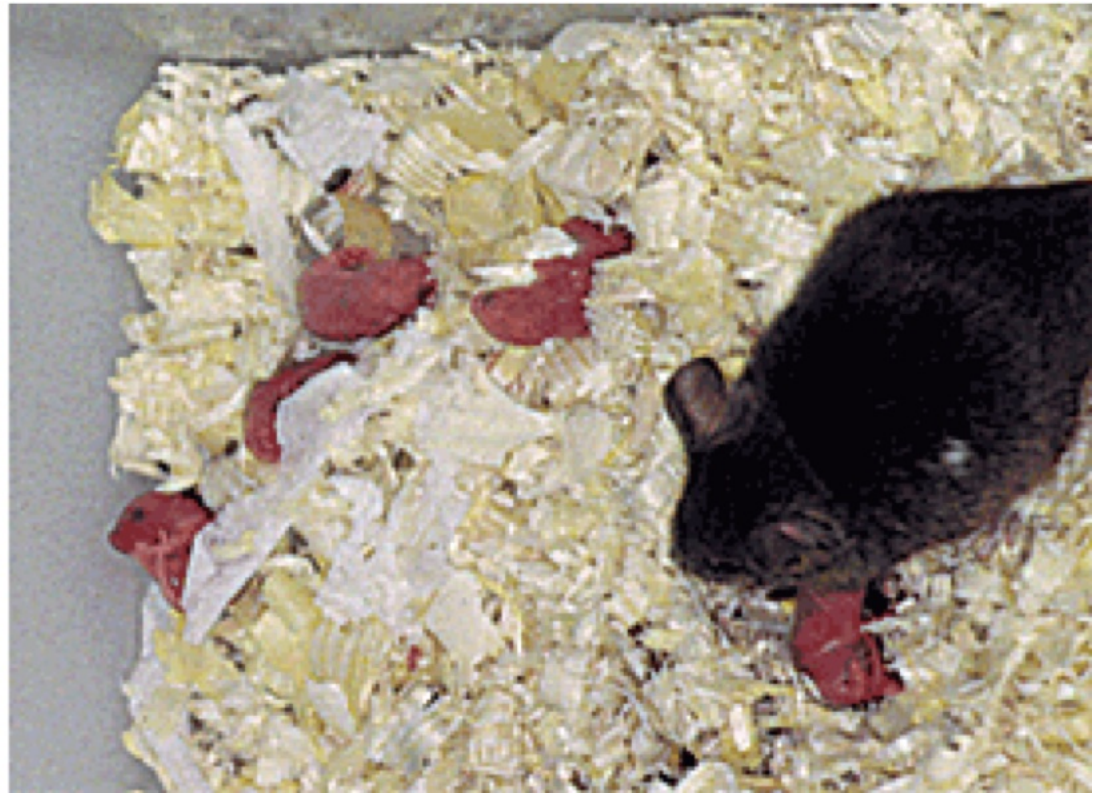


Insulin-like Growth Factor, *Igf2*

Regulation of Transcription in Eukaryotes

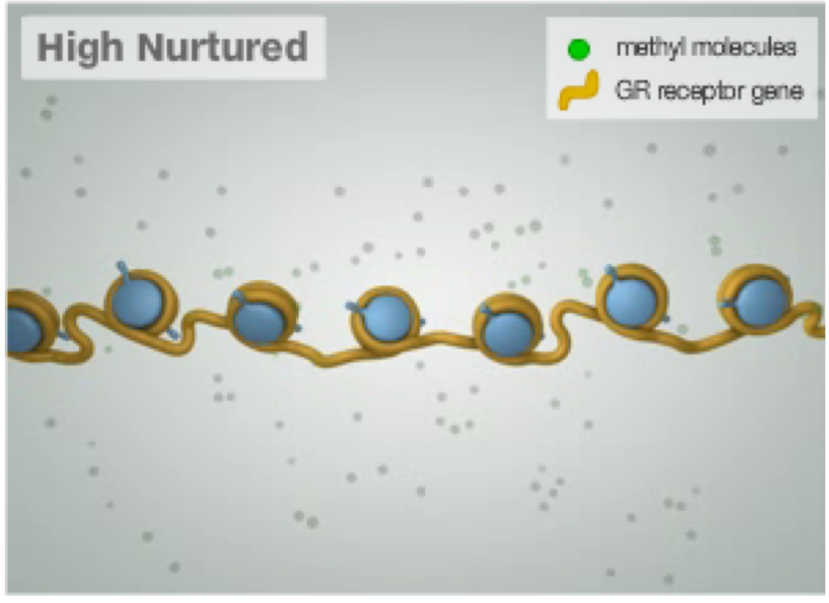
Epigenetic: epigenetics inheritance

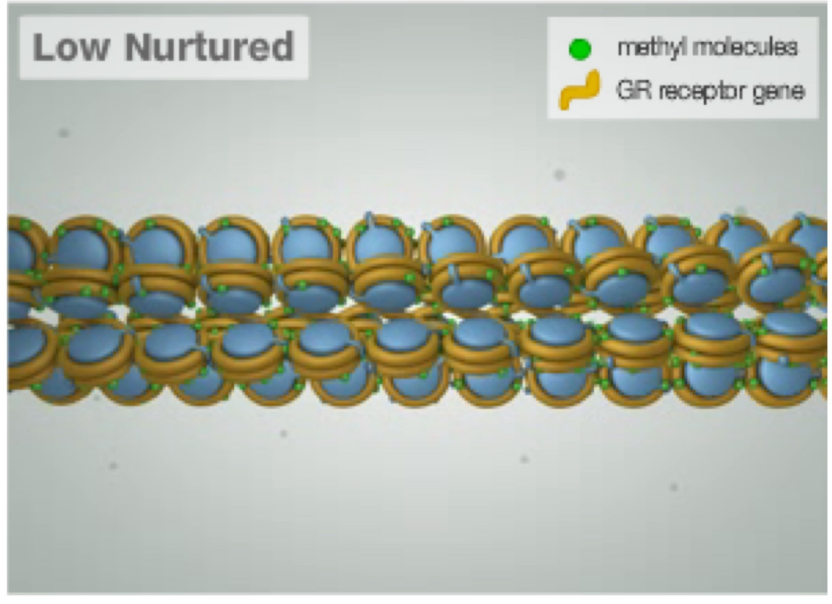




Regulation of Transcription in Eukaryotes

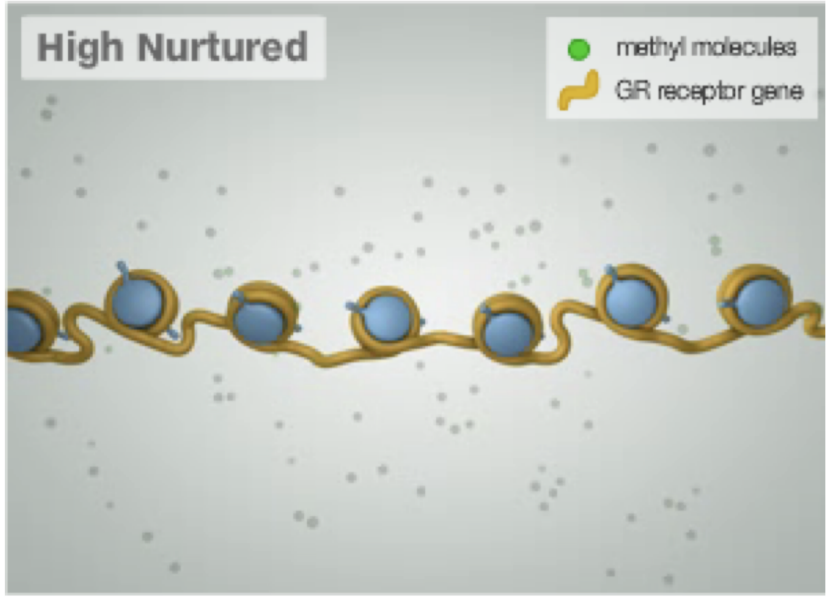
Epigenetic: epigenetics inheritance

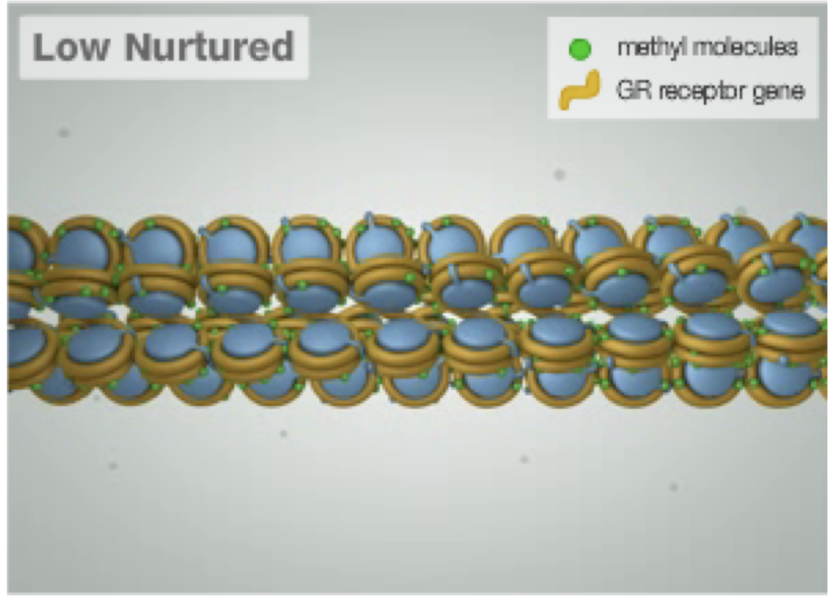




Regulation of Transcription in Eukaryotes

Epigenetic: epigenetics inheritance





Regulation of Transcription in Eukaryotes

RNA processing control

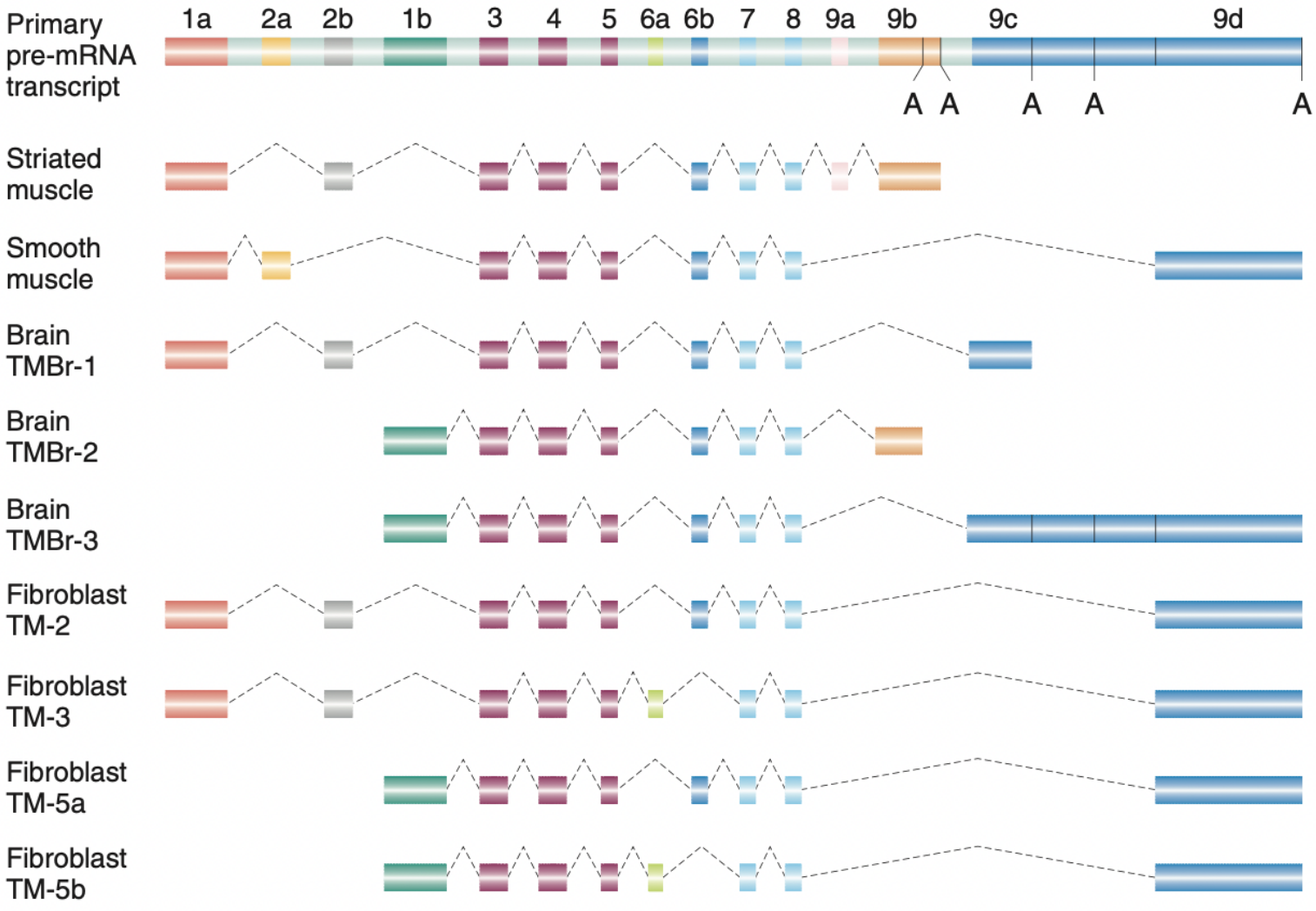
Two independent regulatory mechanisms occur:

- Alternative polyadenylation = where the polyA tail is added
- Alternative splicing = which exons are spliced

> Alternative polyadenylation and splicing can occur together.

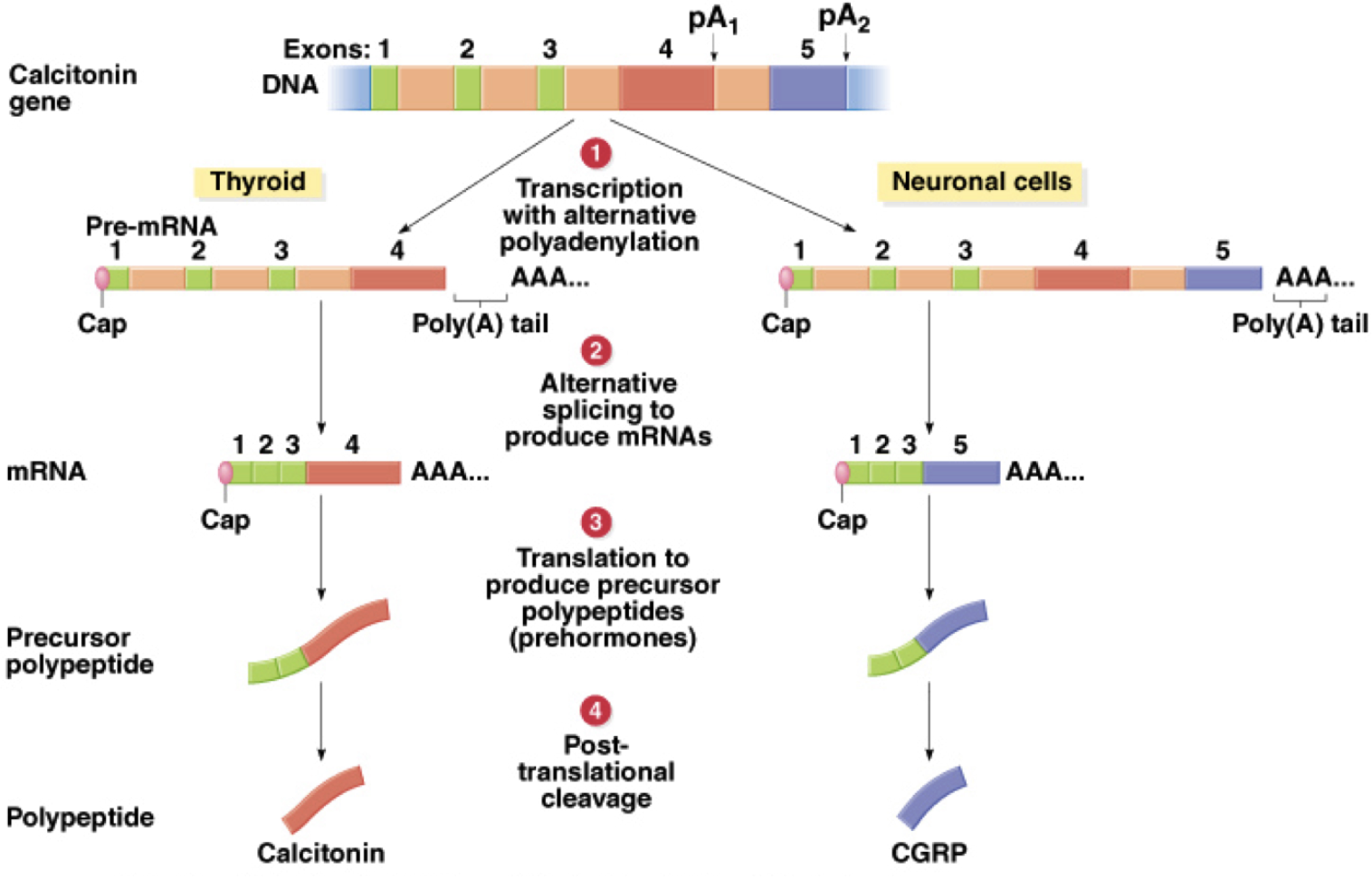
Regulation of Transcription in Eukaryotes

RNA processing control



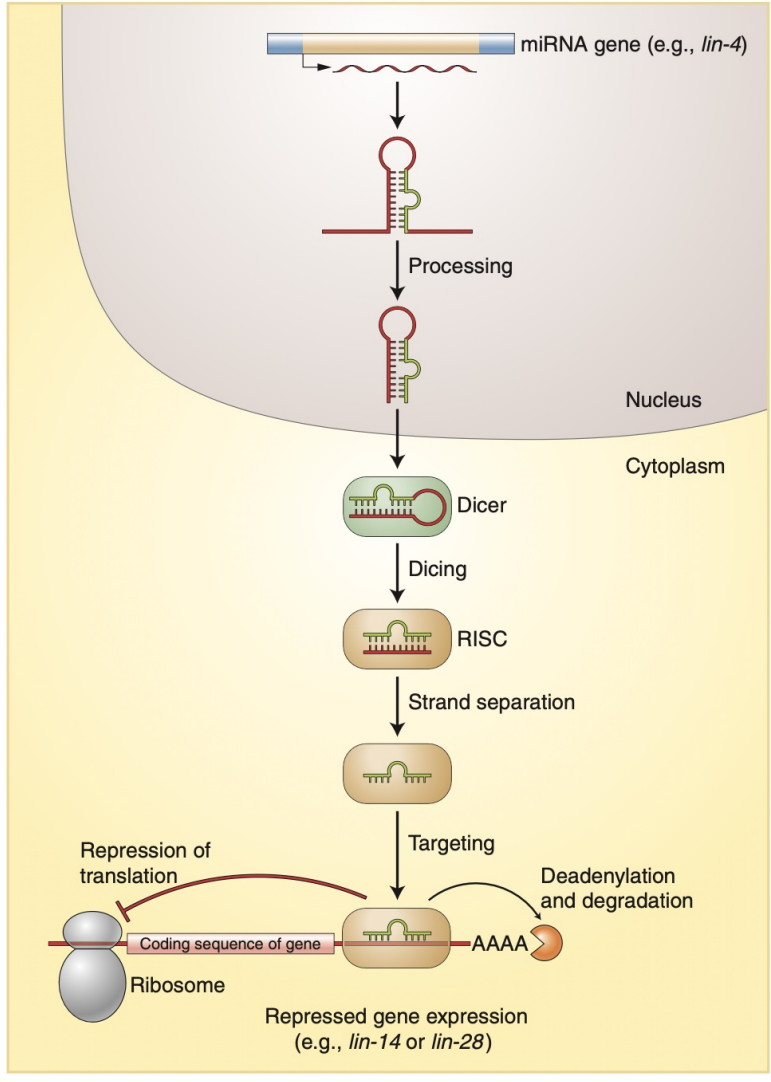
Regulation of Transcription in Eukaryotes

RNA processing control



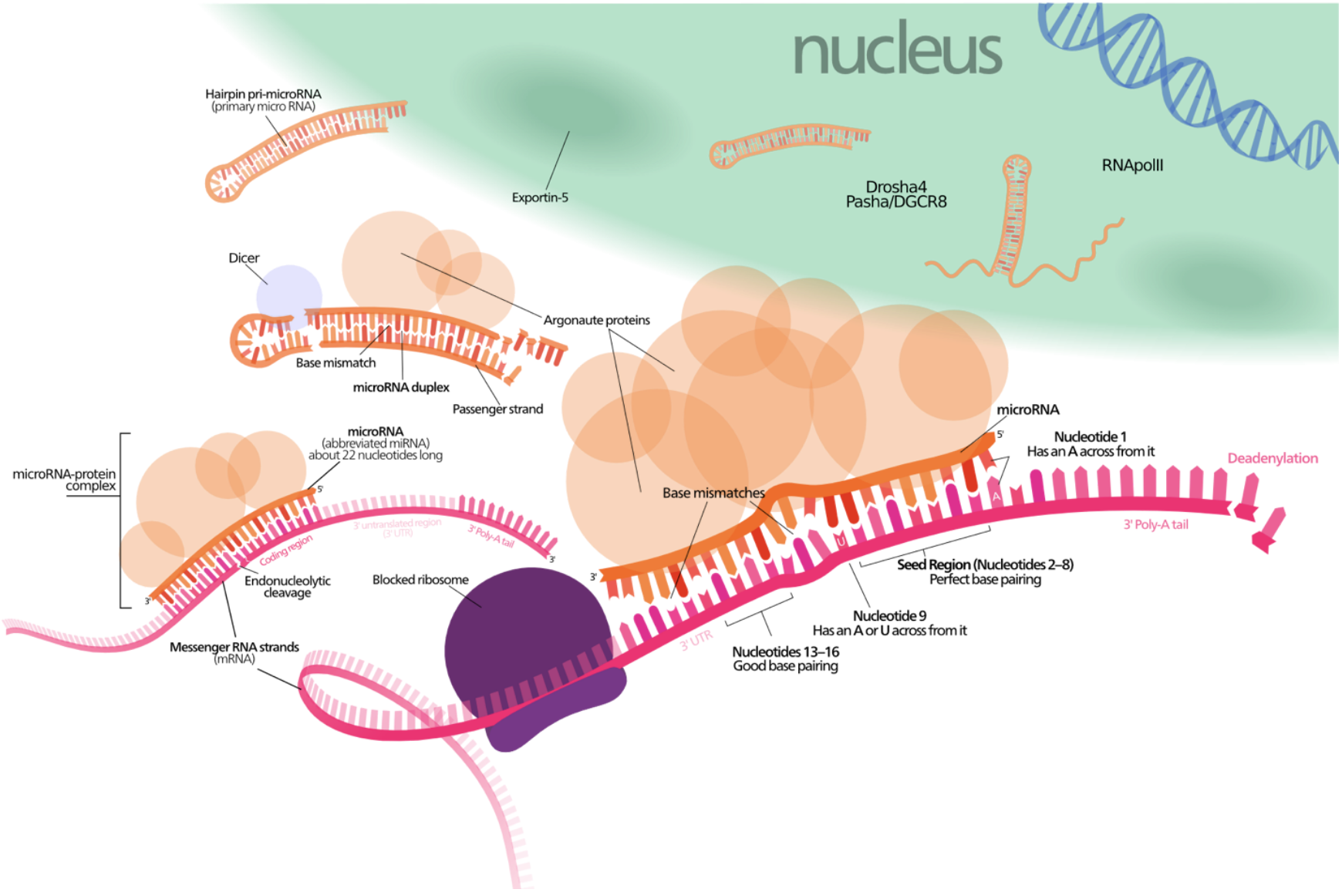
Regulation of Transcription in Eukaryotes

micro RNAs (miRNAs)



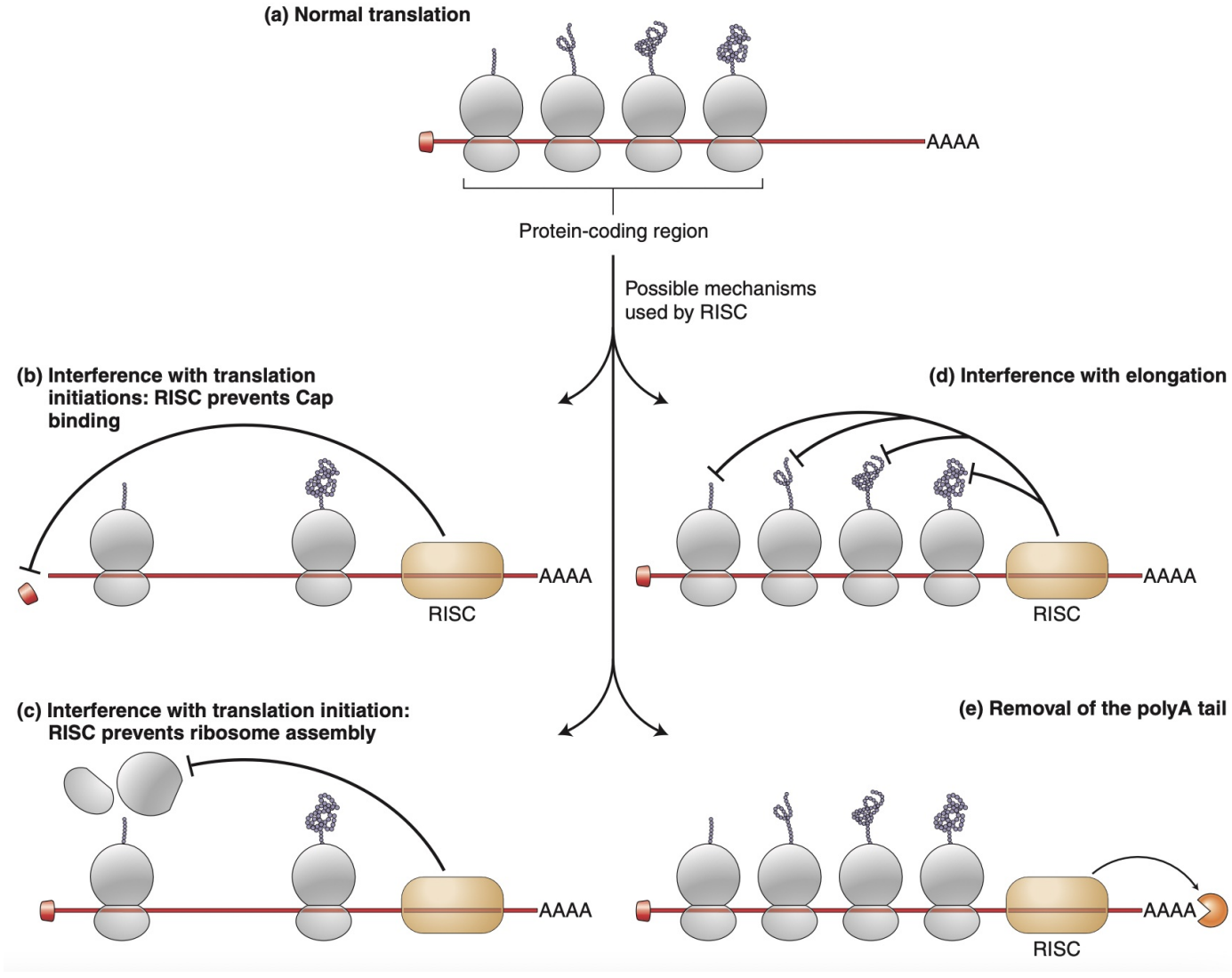
Regulation of Transcription in Eukaryotes

micro RNAs (miRNAs)



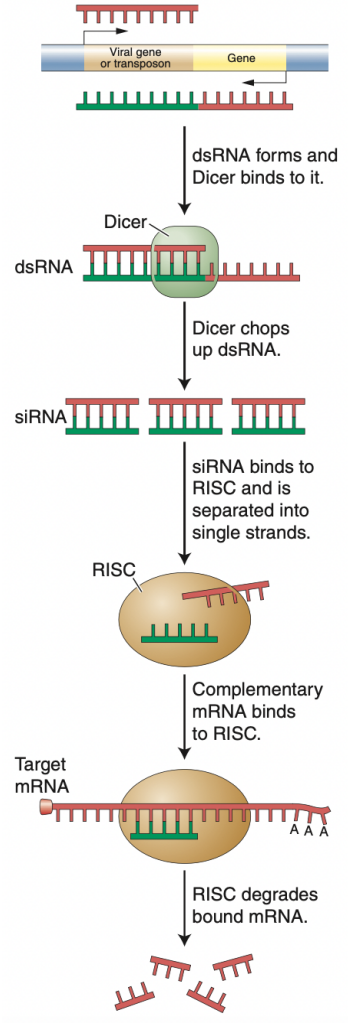
Regulation of Transcription in Eukaryotes

Models for the repression of translation by miRNA



Regulation of Transcription in Eukaryotes

small interfering RNAs (siRNAs)



Regulation of Transcription in Eukaryotes

Summary

Control of Gene Expression in Prokaryotes:

- Transcription

Control of Gene Expression in Eukaryotes:

- Transcription
- RNA Processing
- RNA Transport
- RNA Translation
- RNA Degradation
- Protein Degradation