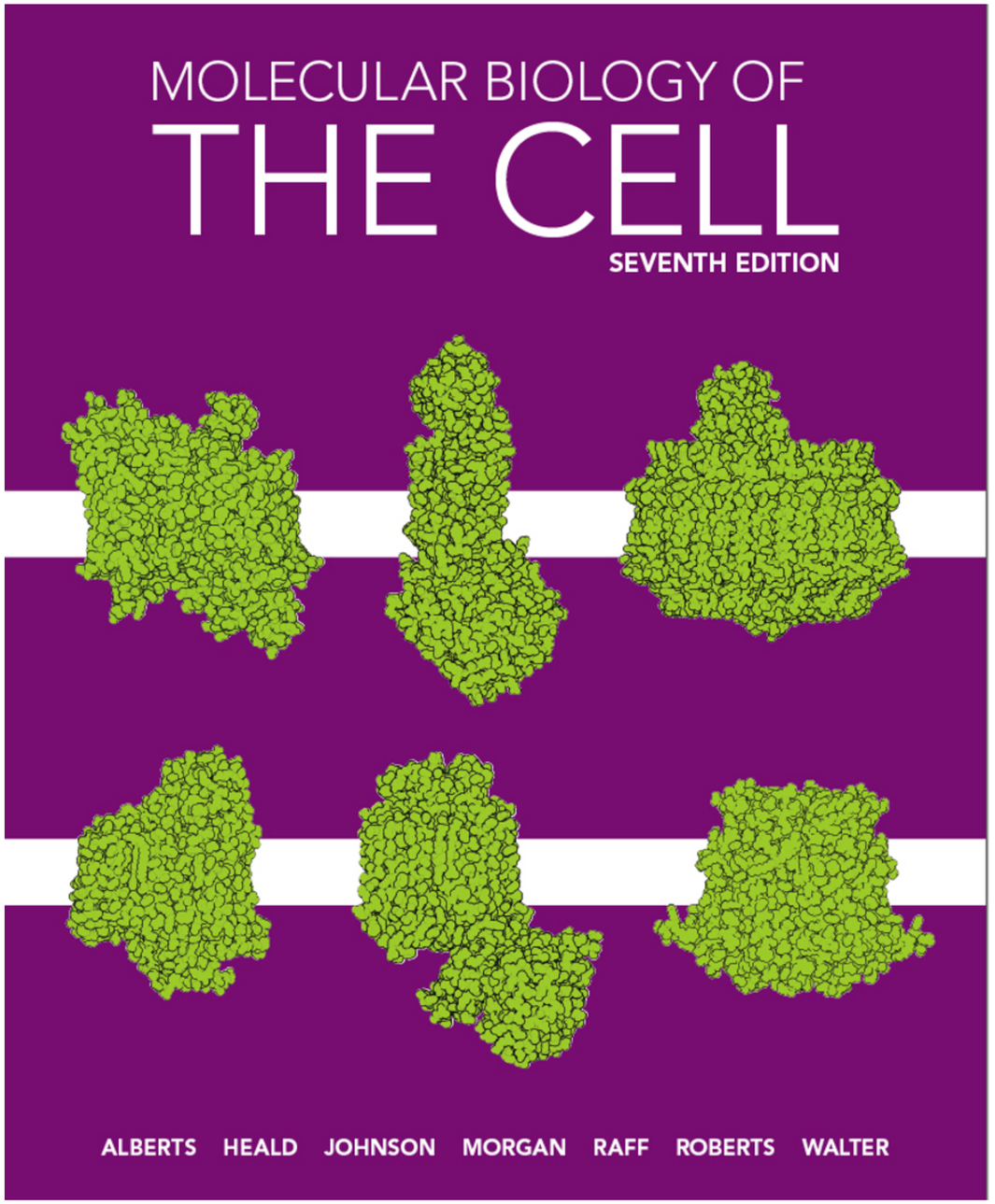
Cellular and Molecular Biology I

BIO-205-8

Camille Goemans - 2024



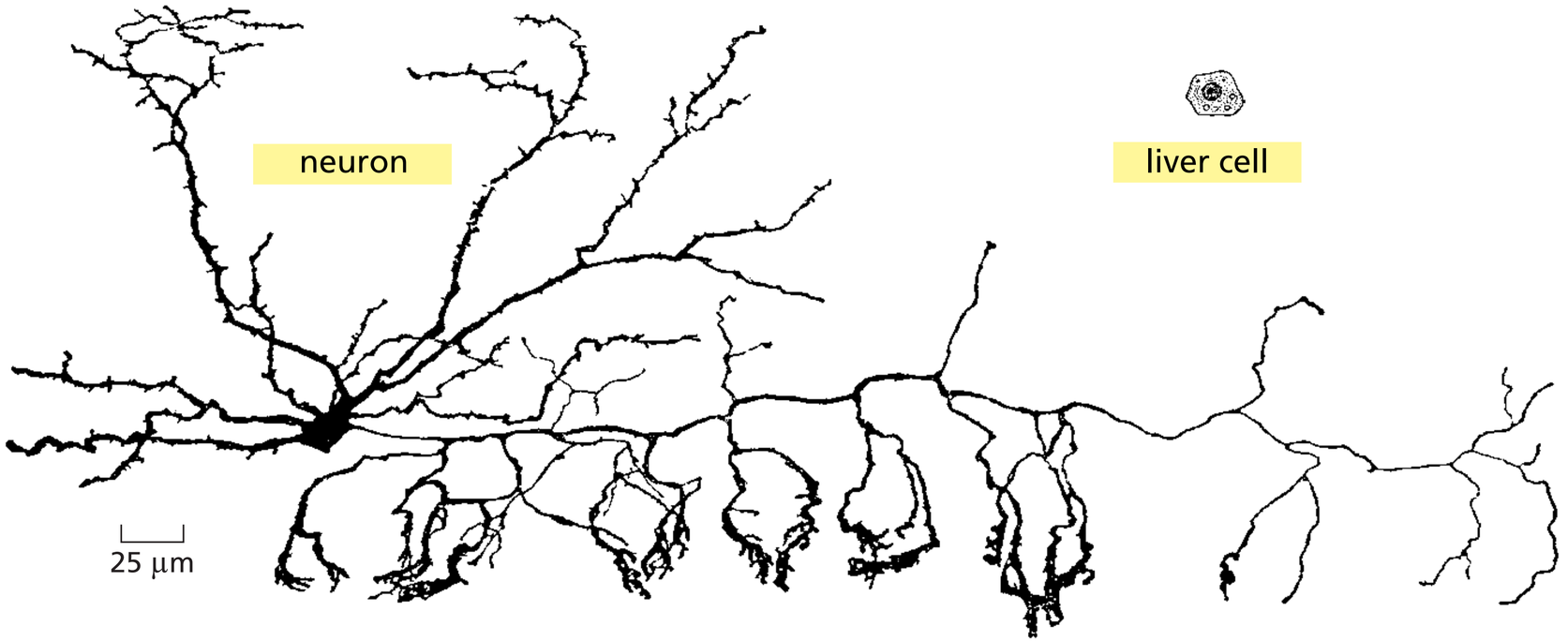
Chapter 7

Control of Gene Expression

Summary

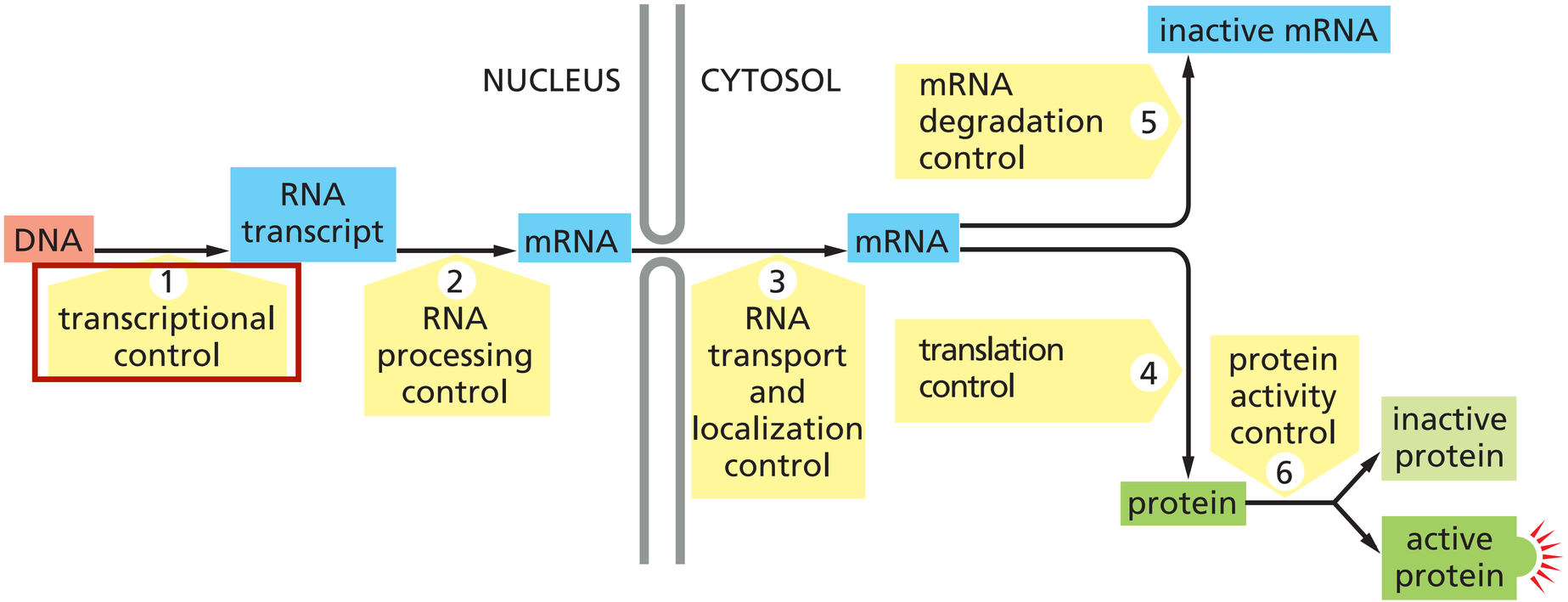
- Different cell types have the same DNA
- Transcriptional control
  - Transcriptional regulators
  - Activators
  - Repressors
  - Understanding other regulatory systems
  - **Combinatorial gene control and cell types**

Maintaining specialized cell types



Transcription regulators can cause each gene to be transcribed at the **right place and time**

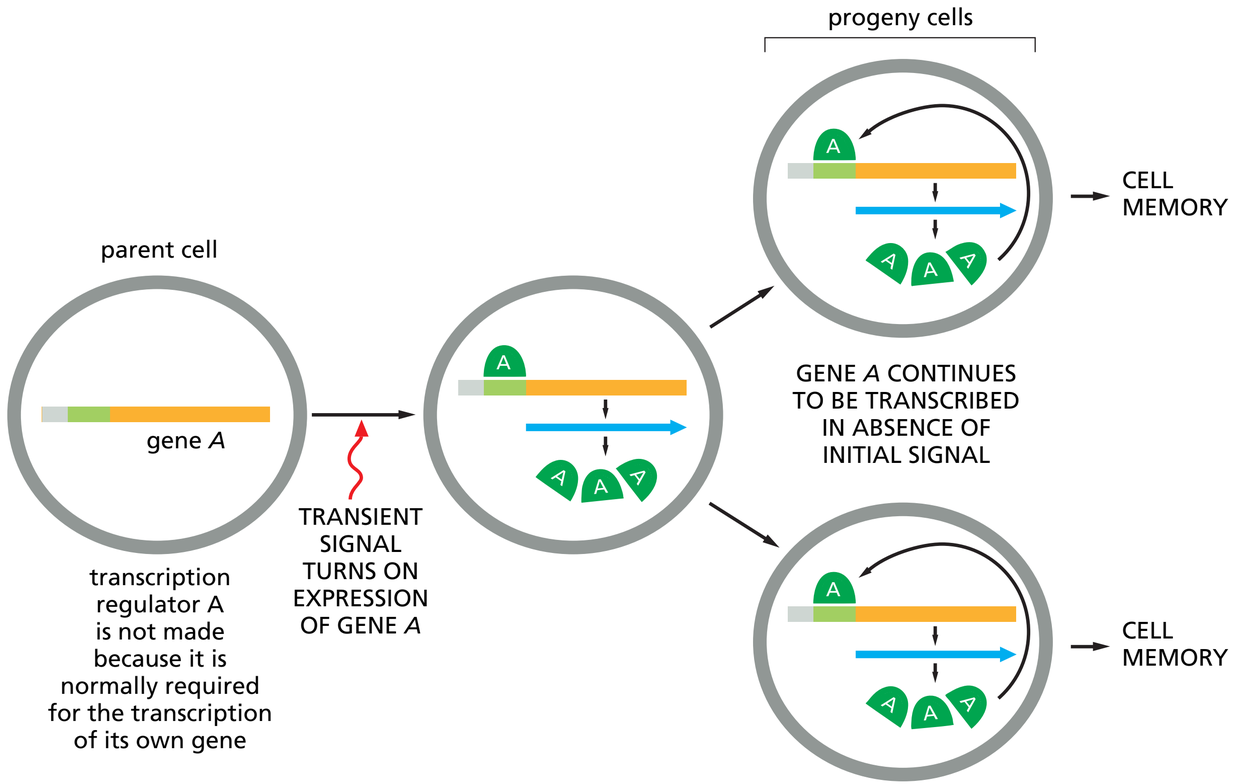
Quick recap


NUCLEUS
CYTOSOL
DNA
RNA transcript
mRNA
mRNA
inactive mRNA
mRNA degradation control
5
1
transcriptional control
2
RNA processing control
3
RNA transport and localization control
translation control
4
protein activity control
6
protein
inactive protein
active protein

Different cell types

Differentiated cells maintain **their identity**

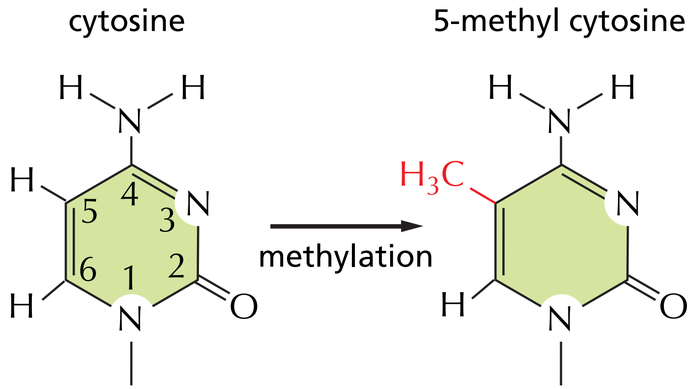
- Progeny will remain the **same cell type - *cell memory***
- Some are **terminally differentiated** (no further division) like neurons or skeletal muscle cells
- **Positive feedback loop** so a master transcription regulator activates transcription of its own gene

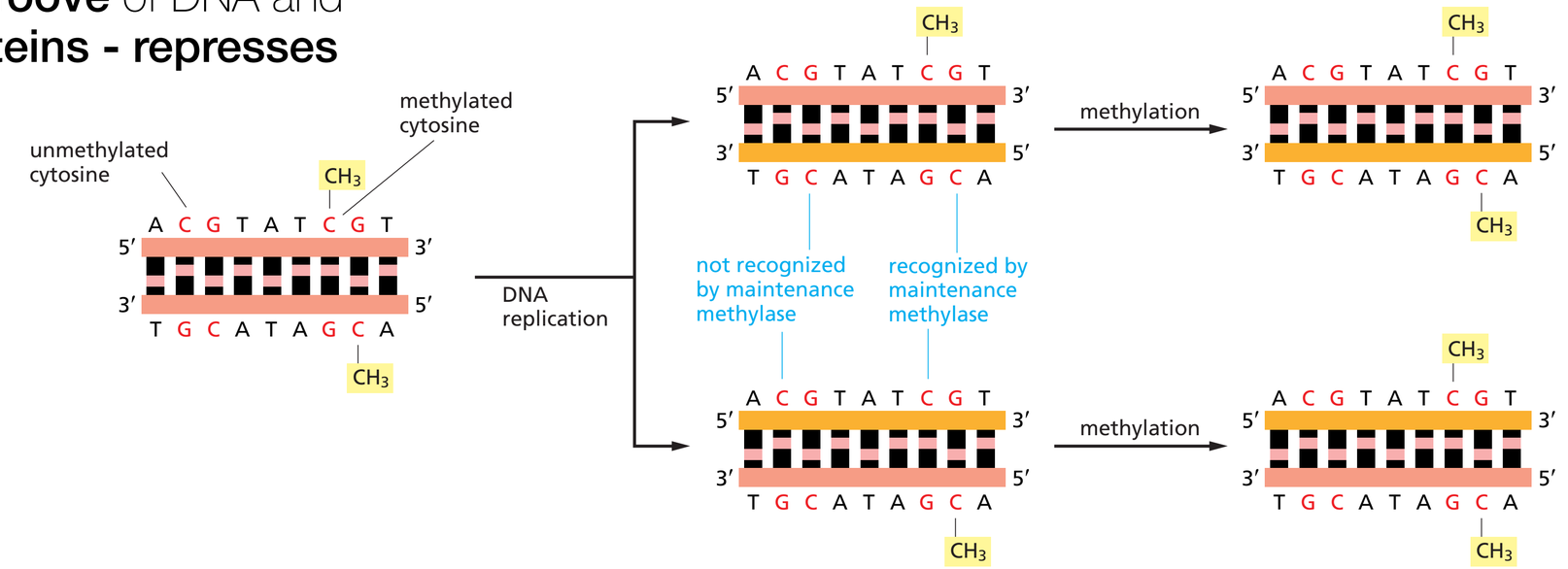


Cell memory

DNA methylation can be **inherited** when cells divide

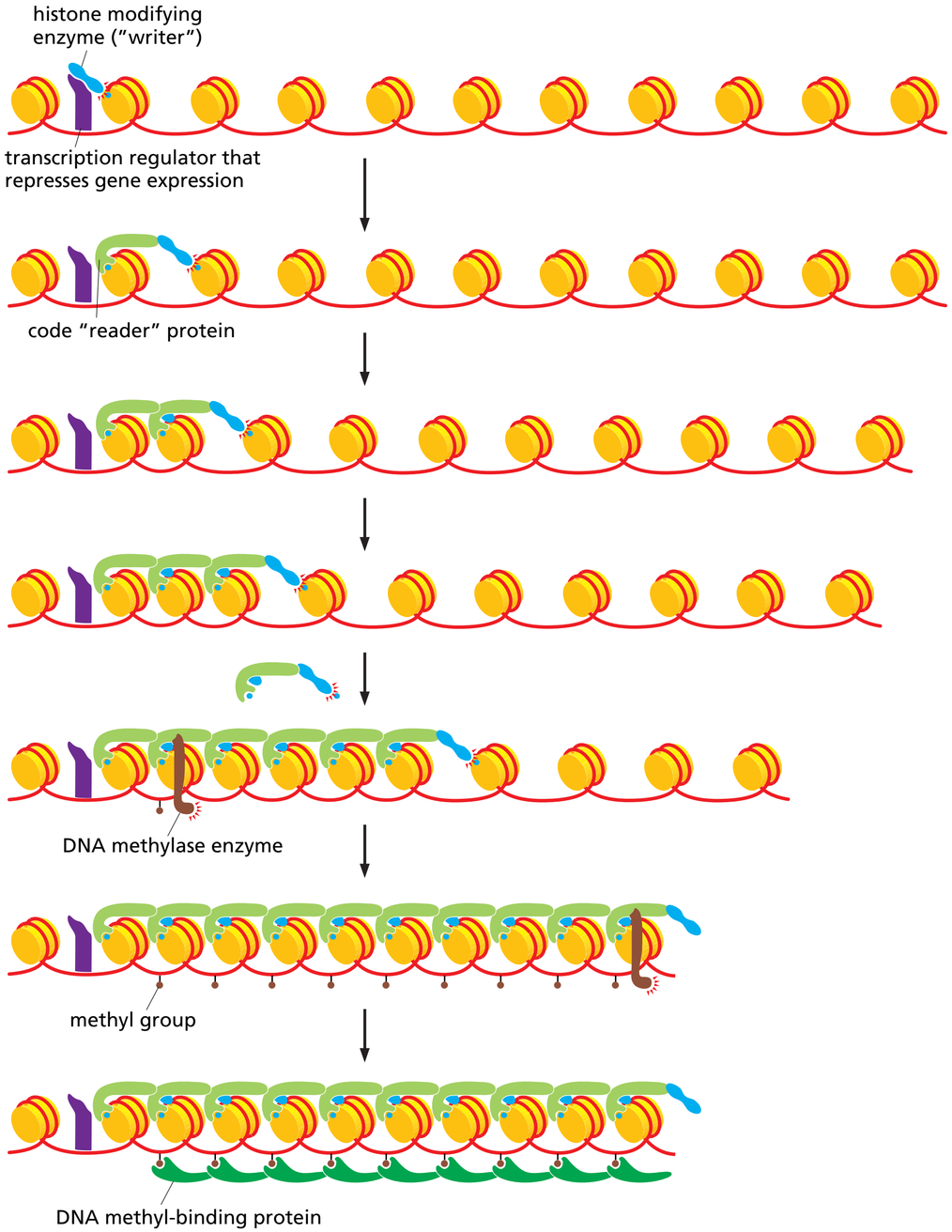
- **DNA methylation** occurs on **cytosine** (largely in sequence CG)
- **Maintenance methyl transferases** act on these newly-made CG sequences (paired with methylated CG sequences)
- Methyl group lies in the **major groove** of DNA and interfere with the **binding of proteins - represses transcription**





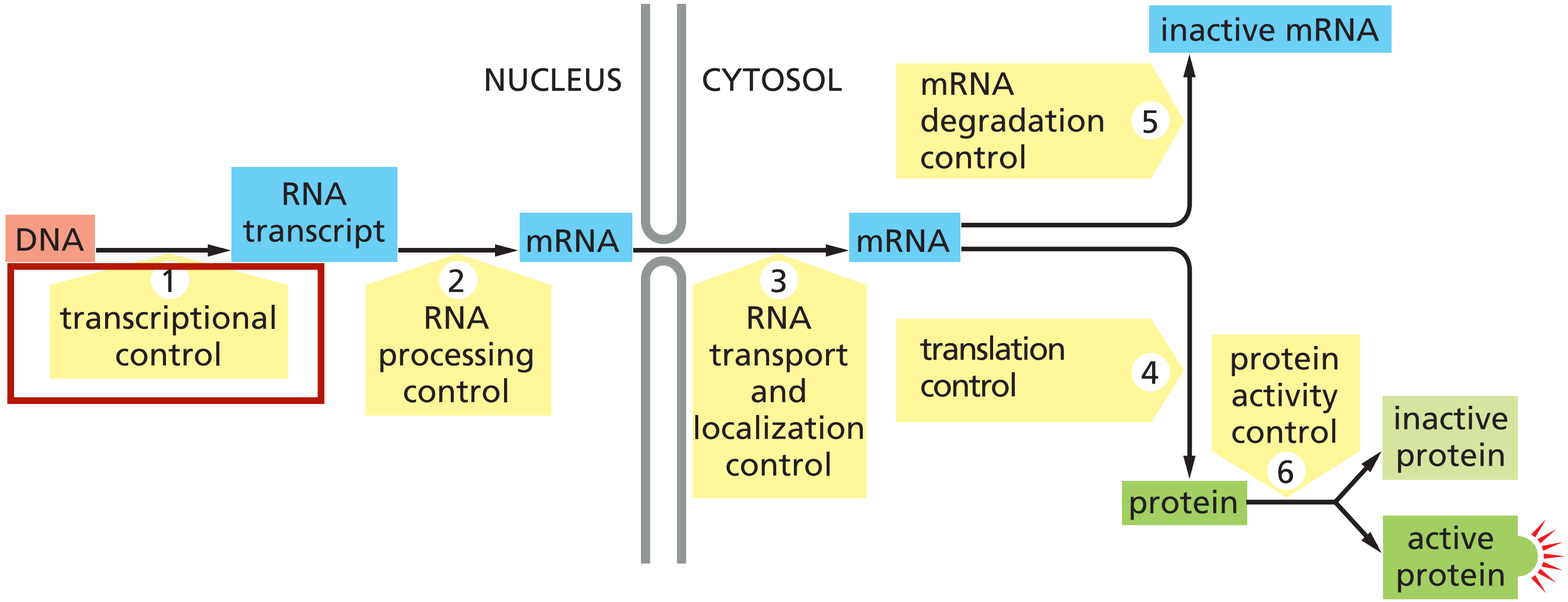
Cell memory

- Some proteins bind specifically to **methylated DNA**
- E.g. **histone modifying enzymes** leading to **repressive chromatin state**
- **Synergistic** action of chromatin structure and DNA methylation



Plan

- Transcriptional control
  - **Genomic imprinting**
  - X-chromosome inactivation
  - Epigenetic inheritance
- Post-transcriptional control
  - RNA processing
  - RNA export
  - Translational control
  - mRNA stability

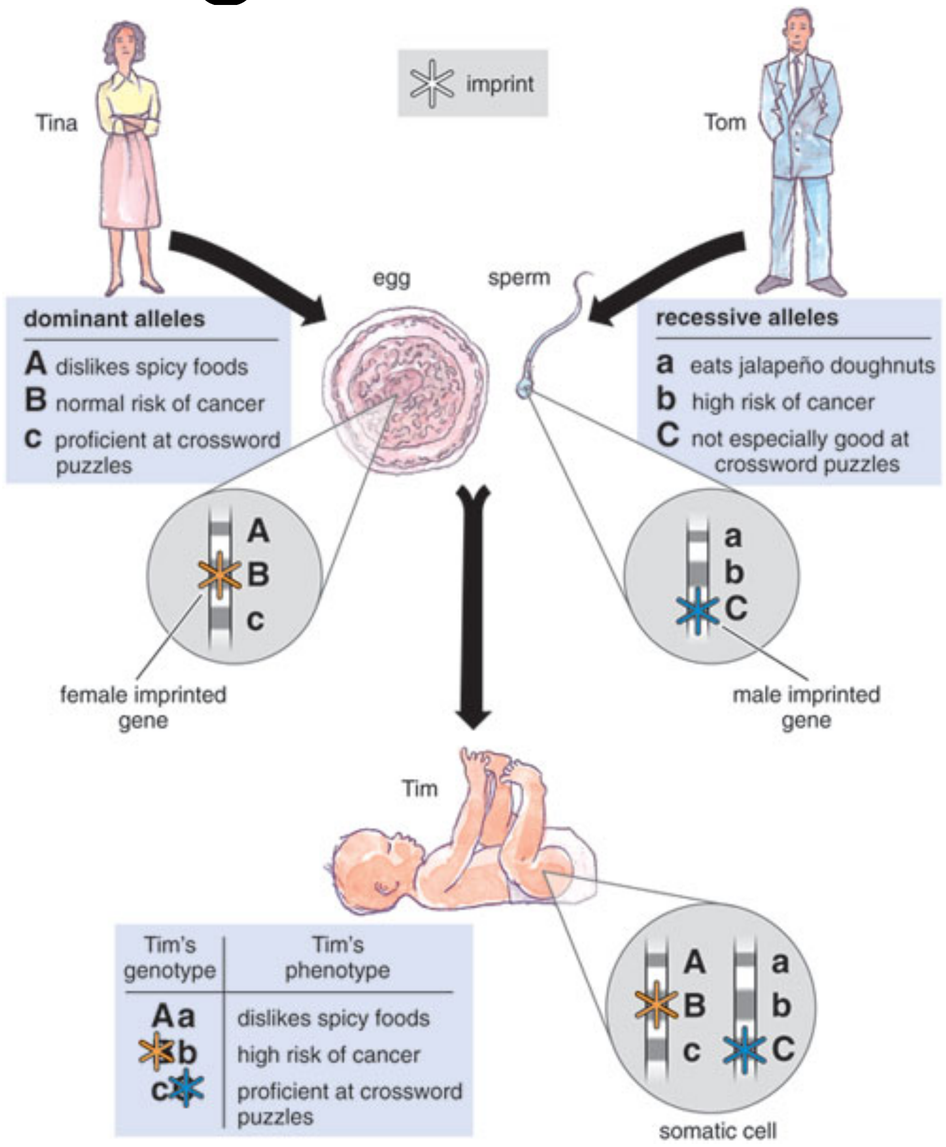


Genomic imprinting

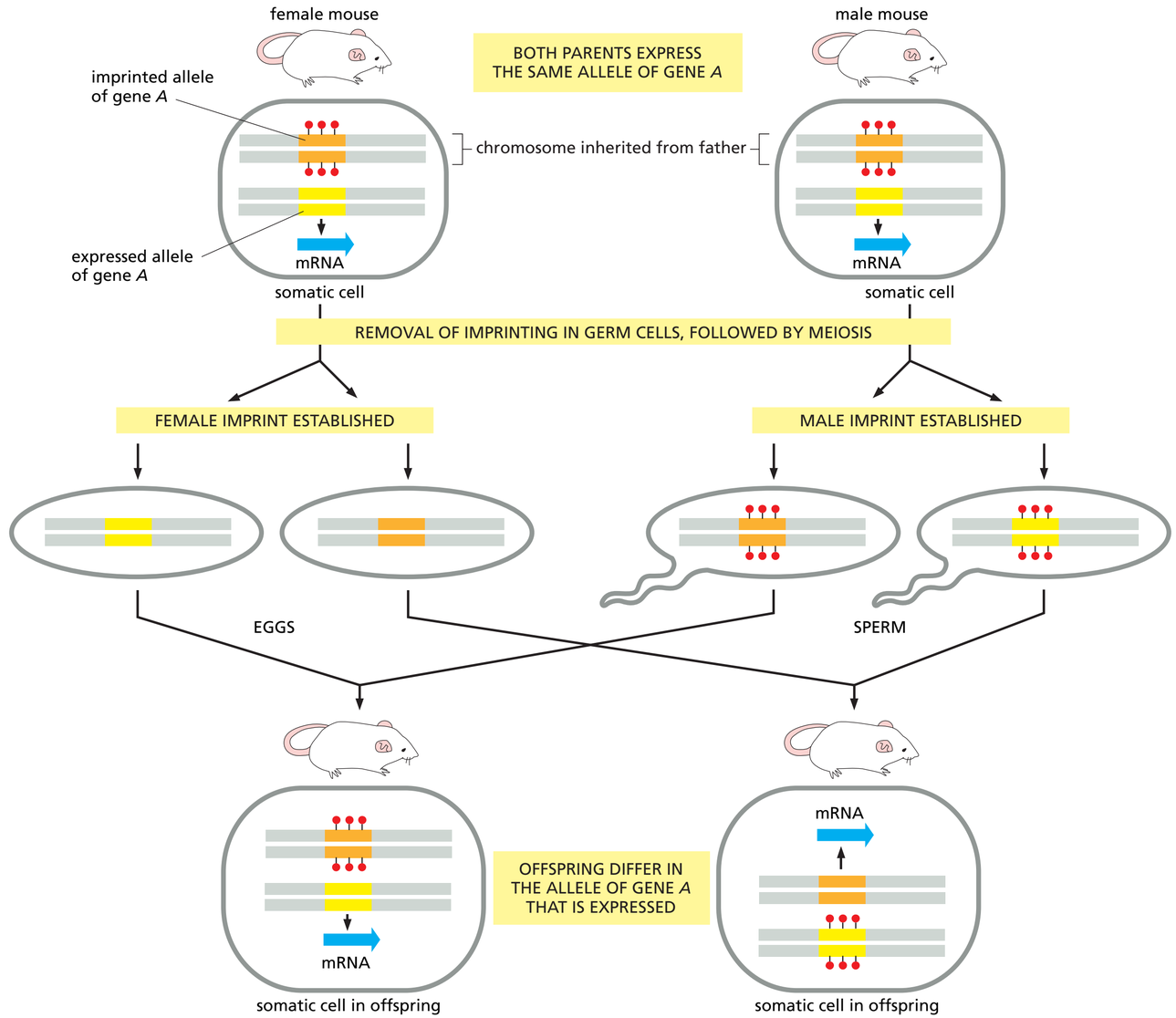
- Mammalian cells are **diploid** (one set of gene from the father and one from the mother)
- For a small subset of genes, expression depends on whether they have been inherited from the mother or the father - **when one copy is active, the other one is silent,** and *vice versa*
- This phenomenon is called **genomic imprinting**
- **300** genes in humans

Genomic imprinting is an epigenetic phenomenon that causes genes to be expressed or not, depending on whether they are inherited from the female or male parent.

Genomic imprinting

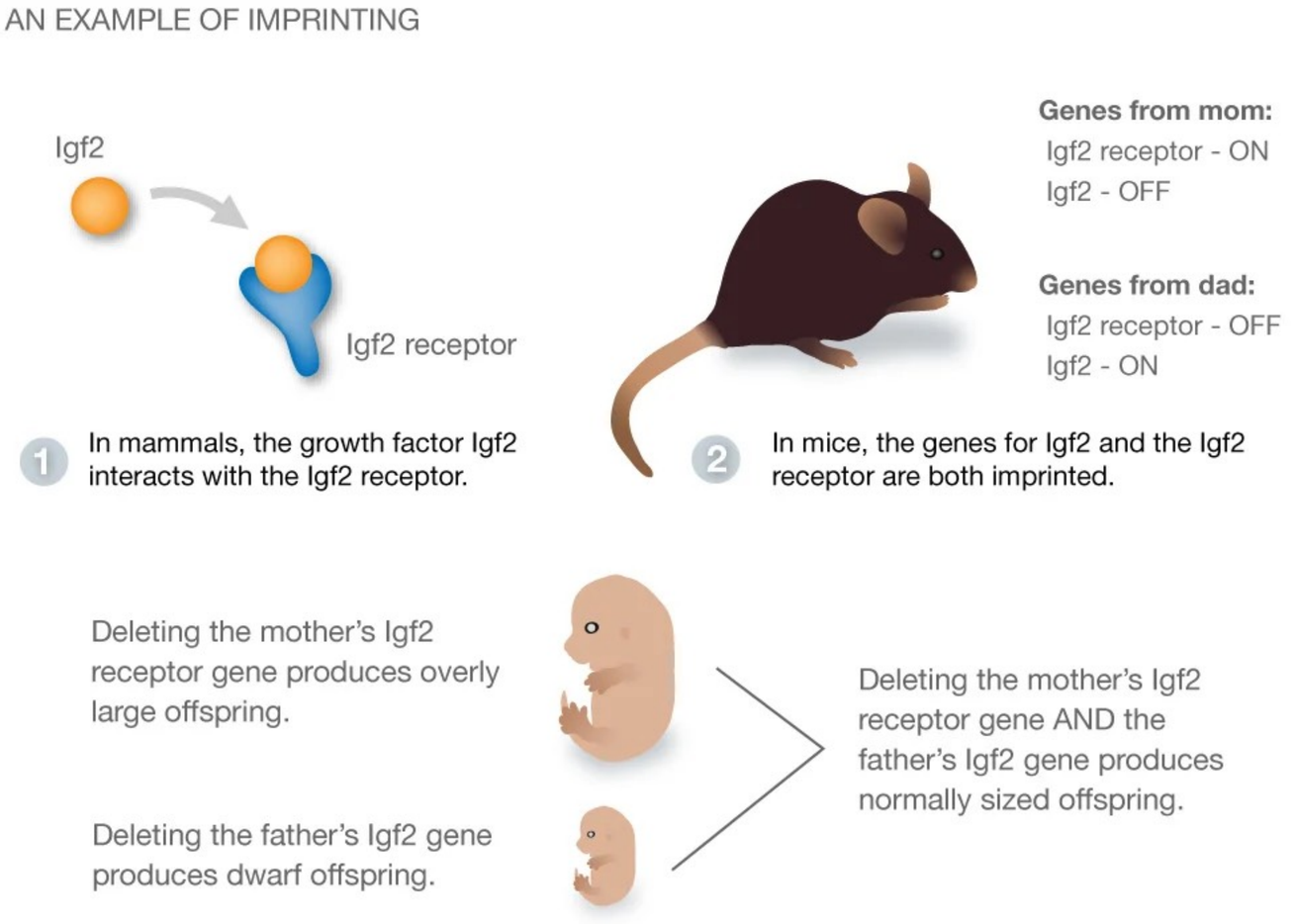


Genomic imprinting



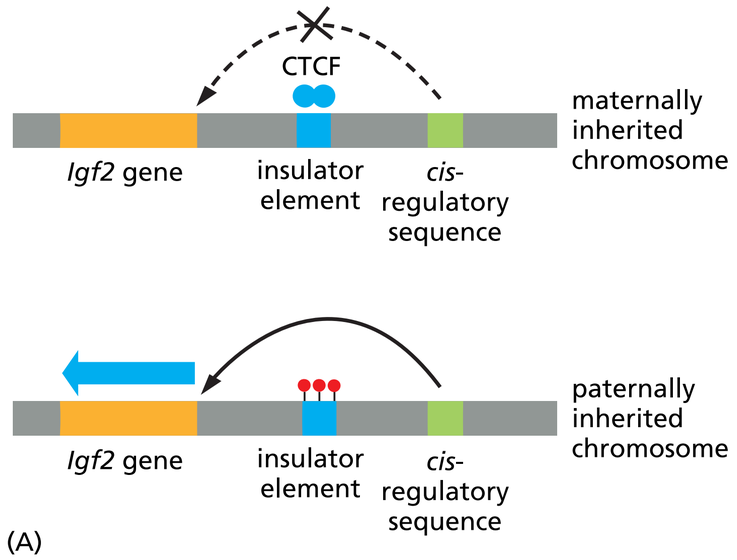
Genomic imprinting

- **Example:** Insulin-like growth factor-2 (Igf2) in mice
- Mice that do not express Ifg2 are smaller than normal mice
- Only the paternal copy of Igf2 is transcribed
- Mice with a mutated paternal gene are smaller whereas mice with a mutated maternal gene are normal
- In the embryo, these genes are marked by **methylation** according to whether they derive from egg or sperm chromosome
- DNA methylation is used to **distinguish** the 2 copies



Genomic imprinting

- Methyl imprint can silence or activate **nearby gene expression**
- For Igf2, methylation of an **insulator element** of the paternal chromosome blocks its function
- This allows a distant cis-regulatory element to **activate the transcription of Igf2**



(A)

Plan

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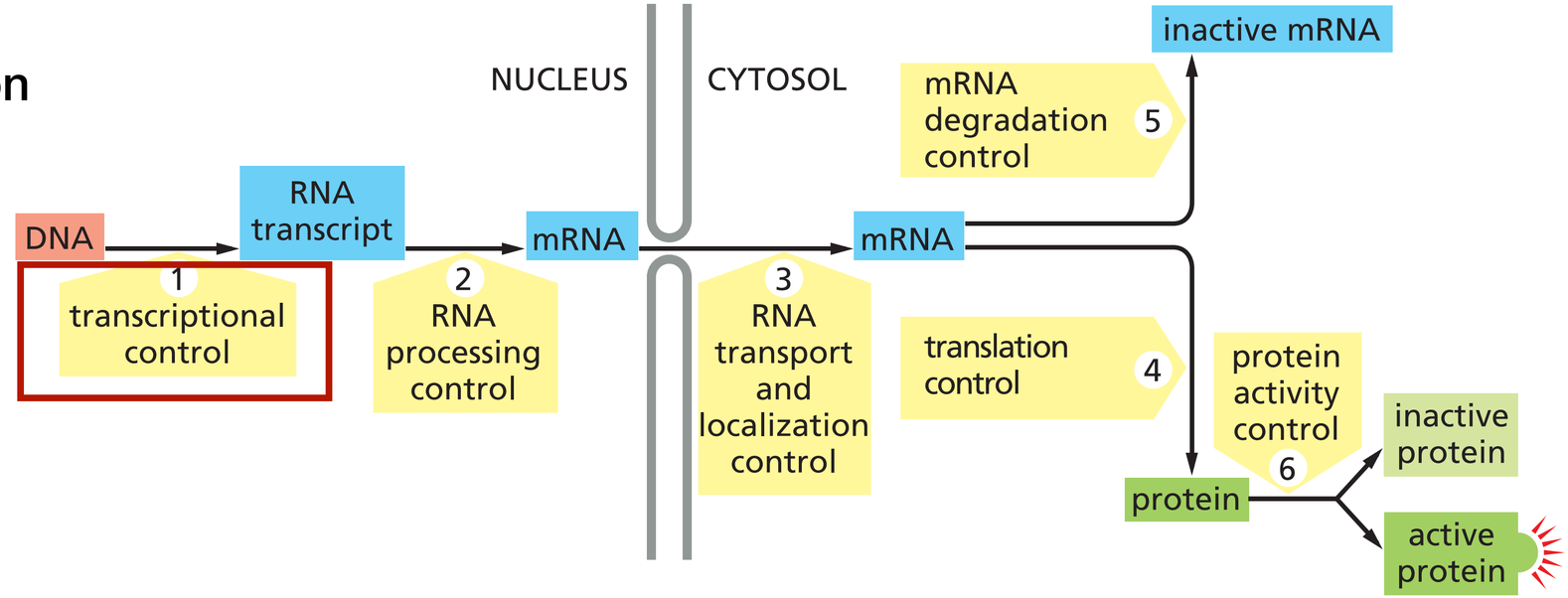
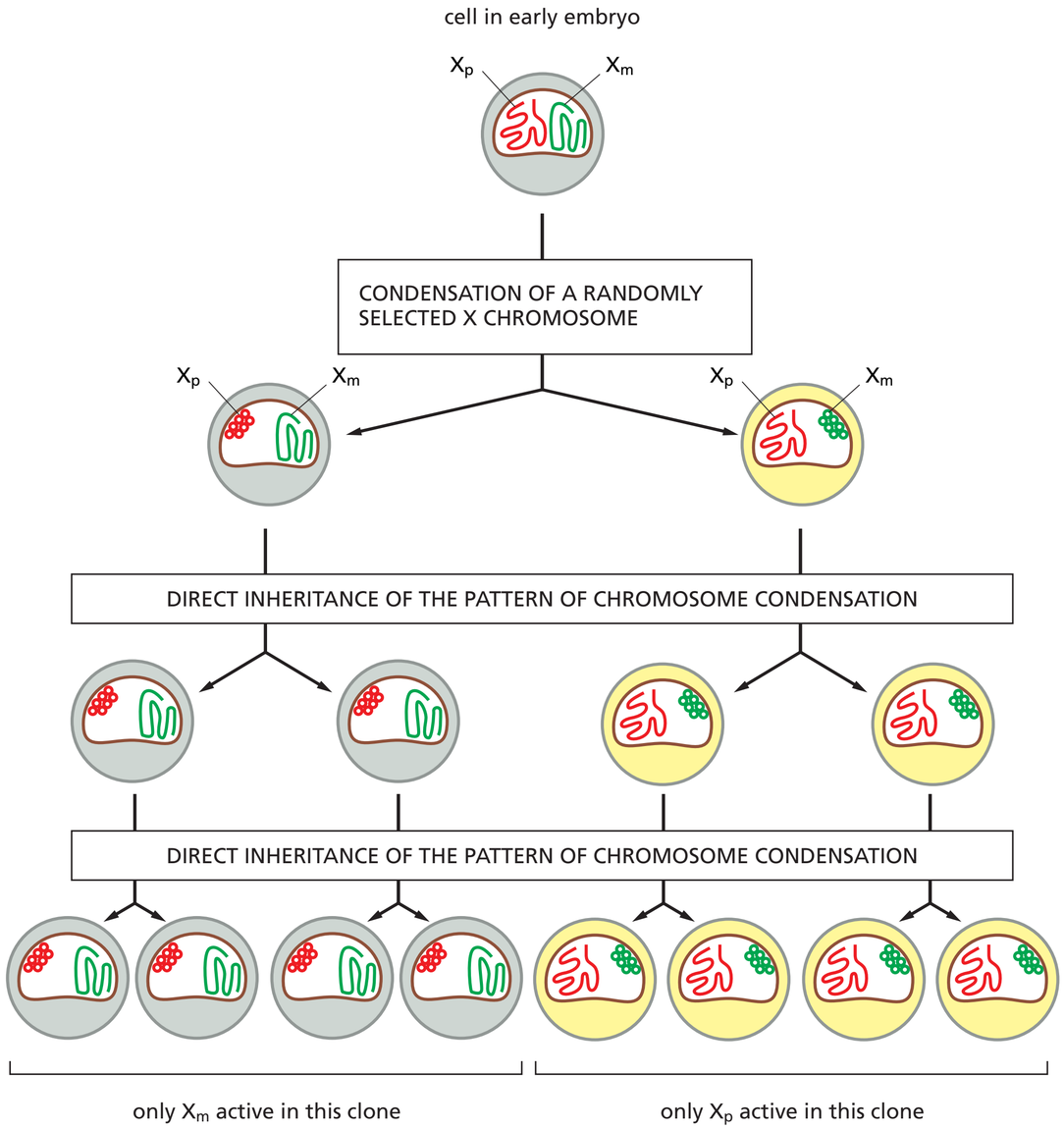


X chromosome inactivation

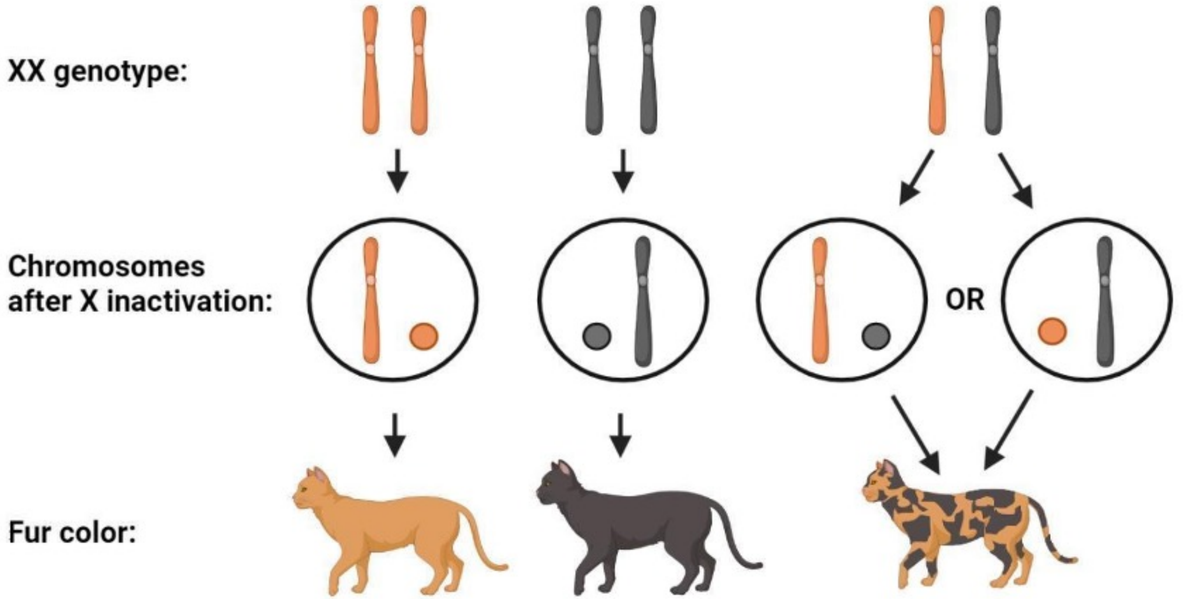
- Males and females differ in their **sex chromosomes** (XX and XY)
- Female cells contain **twice the amount** of X genes as do male cells
- The X chromosome contains **> 1000 genes**, whereas the Y **<100 genes**
- Mammals achieve dosage compensation by the transcriptional **inactivation of one of the two X chromosome in female somatic cells**

X chromosome inactivation



- In early embryo, one **X chromosome** (random) becomes highly **condensed**
- Once inactivated, it remains **silent** for all subsequent divisions
- It happens after several hundred cells are formed in the embryo, so **every female is a mosaic of clonal groups** of cells with either X chromosome silenced
- These clonal groups tend to remain **close together** during development
- Reversed during **germ-cell** formation

X chromosome inactivation

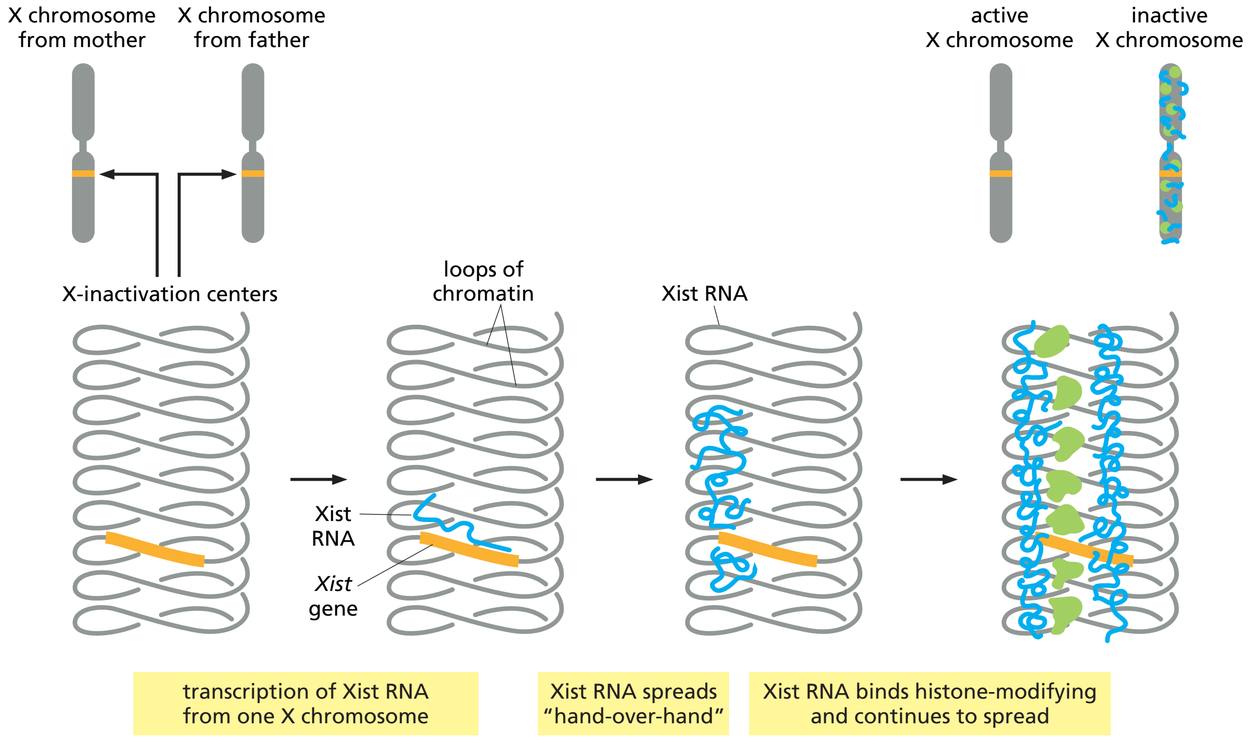


https://www.nature.com/scitable/blog/student-voices/

X chromosome inactivation

How is the **entire chromosome** inactivated?

- initiated from a single site near the middle of the X = the **X-inactivation center** (XIC)
- Within the XIC, a lncRNA (long non-coding RNA) is transcribed = **Xist**
- Xist **spreads over the chromosome** and silences genes
- Xist recruits **histone-modifying enzymes**
- 10% of the genes (including Xist) **escape** the silencing



Both imprinting and X-inactivation lead to **monoallelic gene expression** - only one of the two copies of a gene is expressed

Plan

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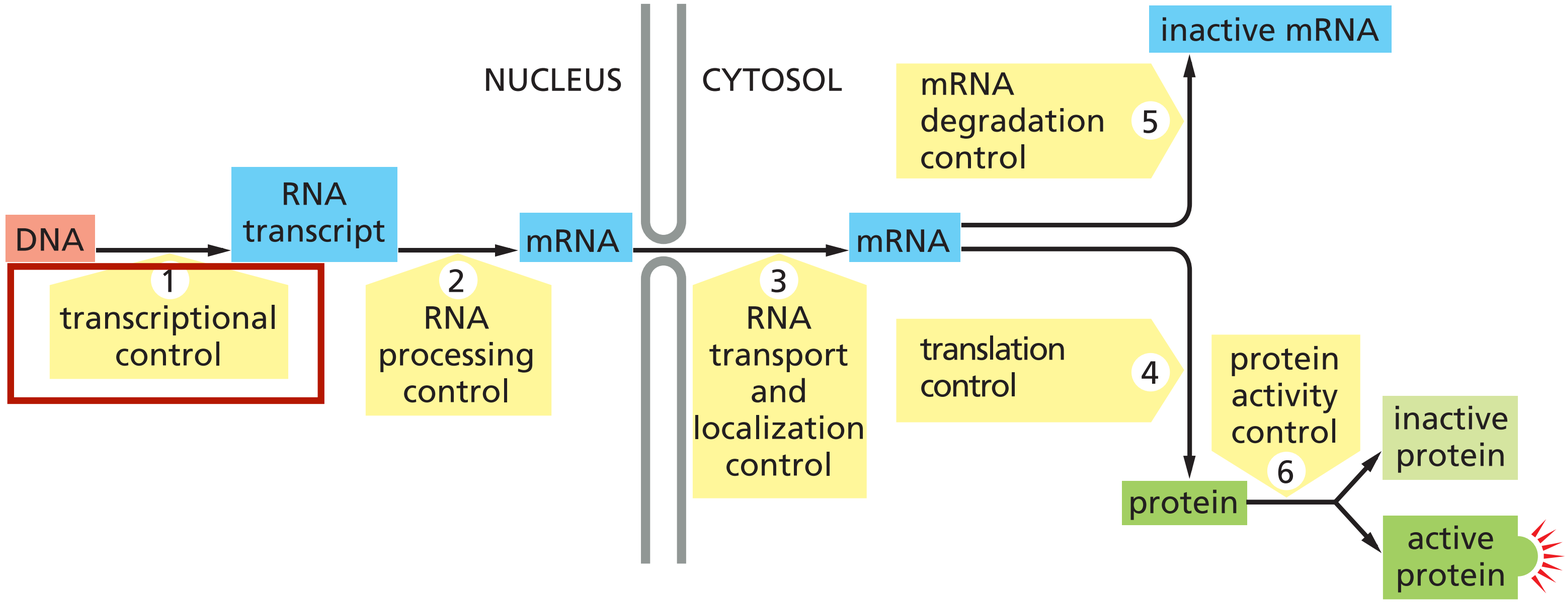
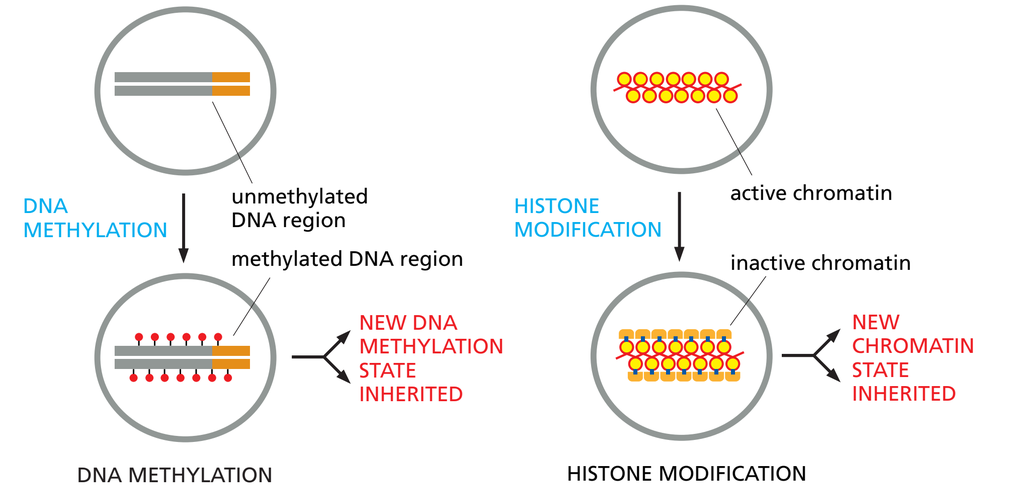


Epigenetic inheritance

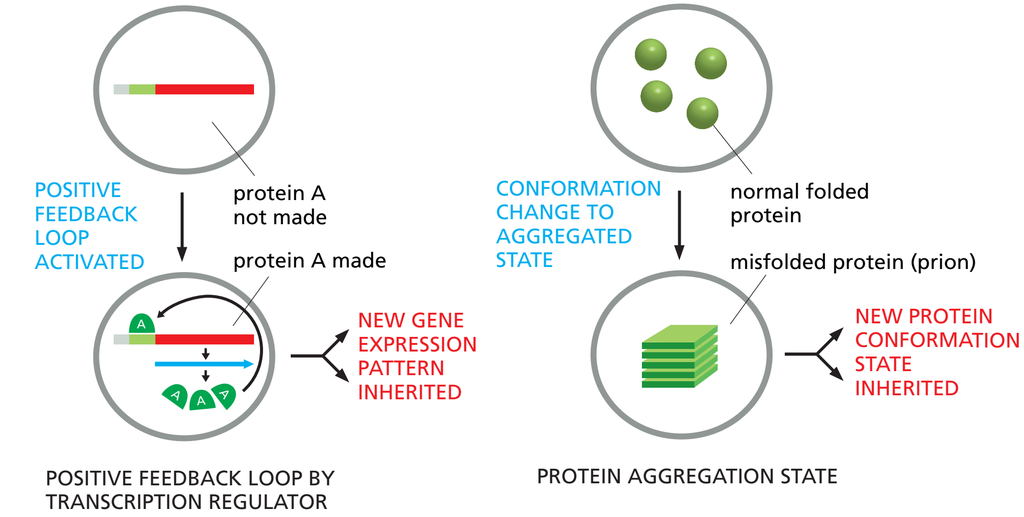
= The ability of a daughter cell to **retain a memory** of the gene expression patterns that were present in the parent cell

- Heritable alteration in a cell's **phenotype**
- Does not result from changes in the **sequence of DNA**
- **4 mechanisms** of epigenetic inheritance

Epigenetic inheritance



(A) EPIGENETIC MECHANISMS THAT ACT IN *CIS*



(B) EPIGENETIC MECHANISMS THAT ACT IN *TRANS*

Plan

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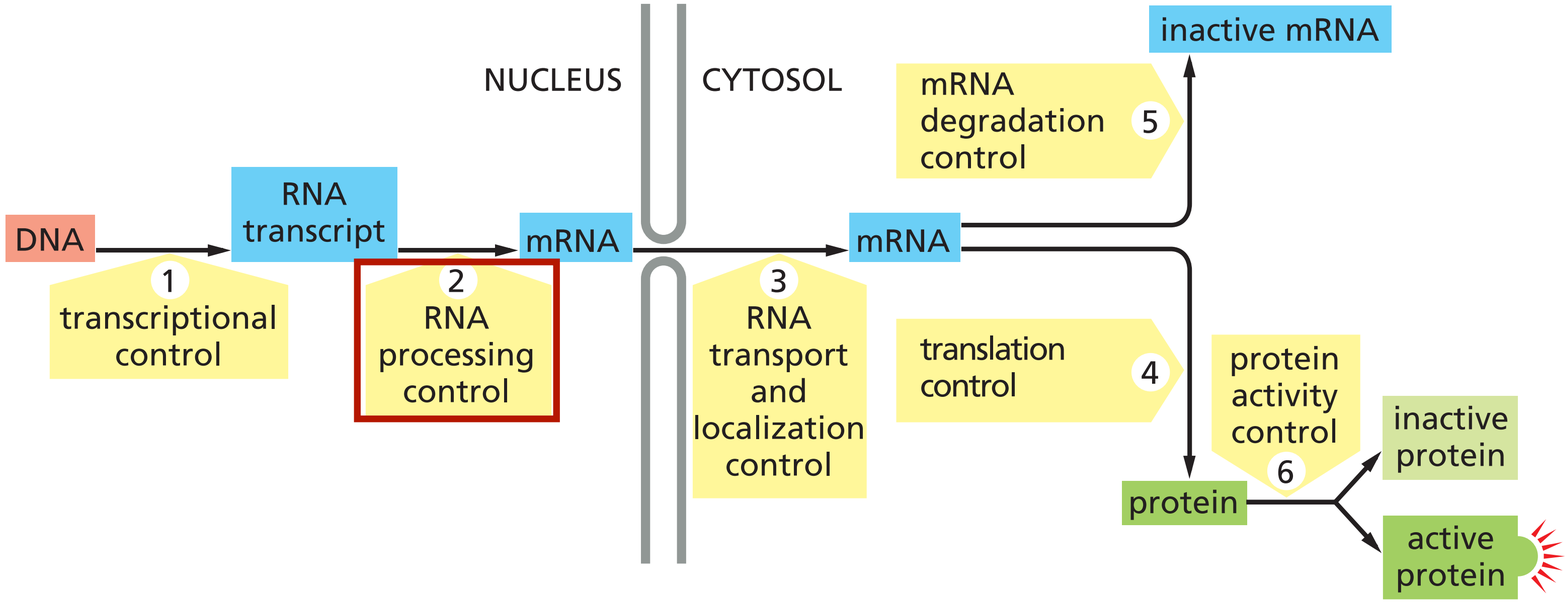
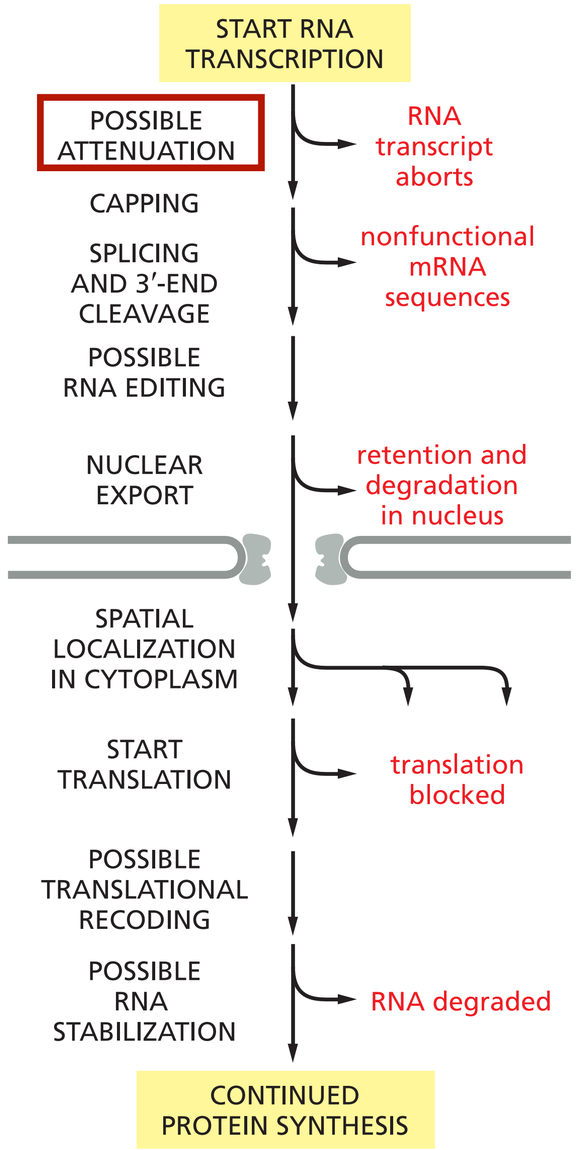


Post-transcriptional controls



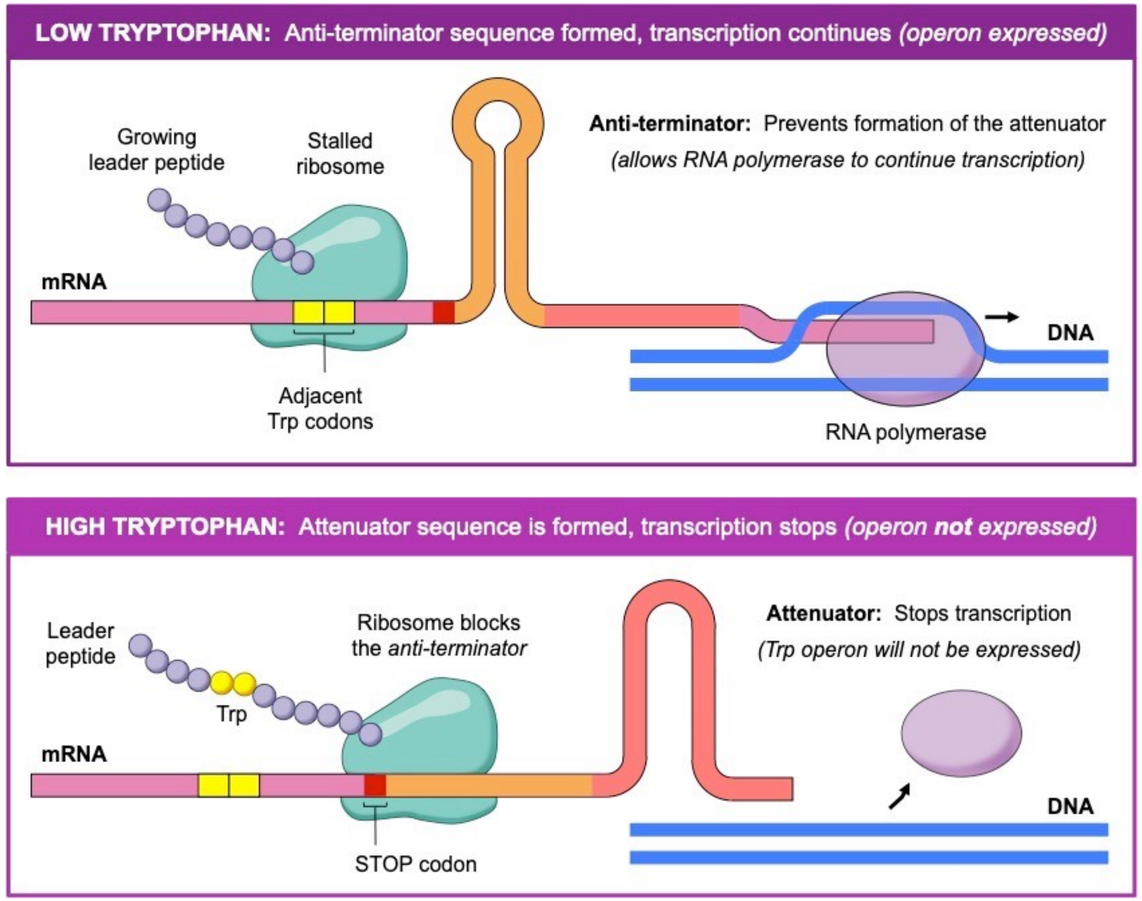
Transcription attenuation

The expression of some genes is inhibited by **premature termination of transcription**

- The nascent RNA chain can adopt a **structure** that causes it to interact with the **RNA polymerase**
- This leads to the **abortion of transcription**
- When the gene is needed, proteins bind to the RNA and **remove the attenuation**

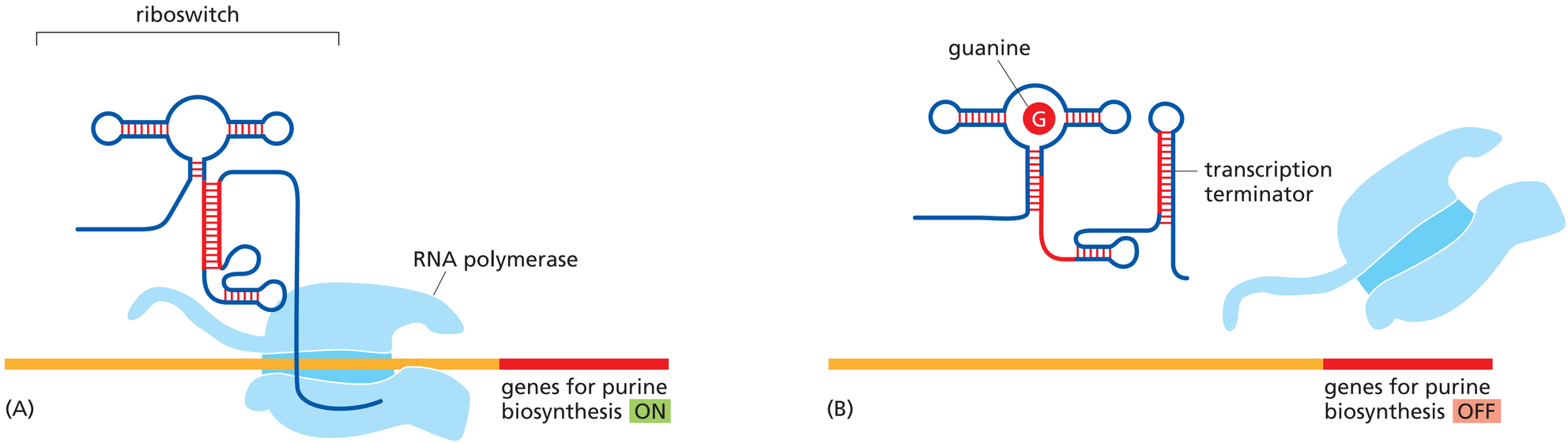
Transcription attenuation

The expression of some genes is inhibited by **premature termination of transcription**



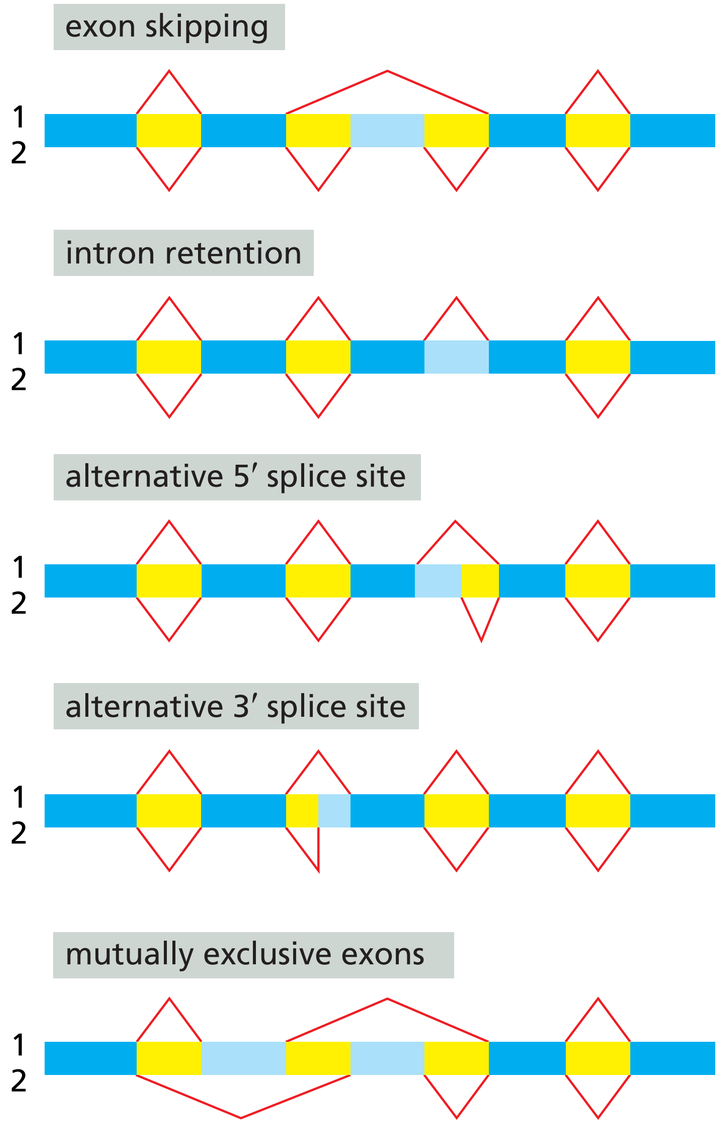
Riboswitches

- **Short sequences of RNA** that change conformation upon **metabolite binding**
- The **conformational change** regulates gene expression
- Often at **5’ end of mRNA**, folding during mRNA synthesis
- **Blocking or permitting** progress of the RNA polymerase



Alternative RNA splicing

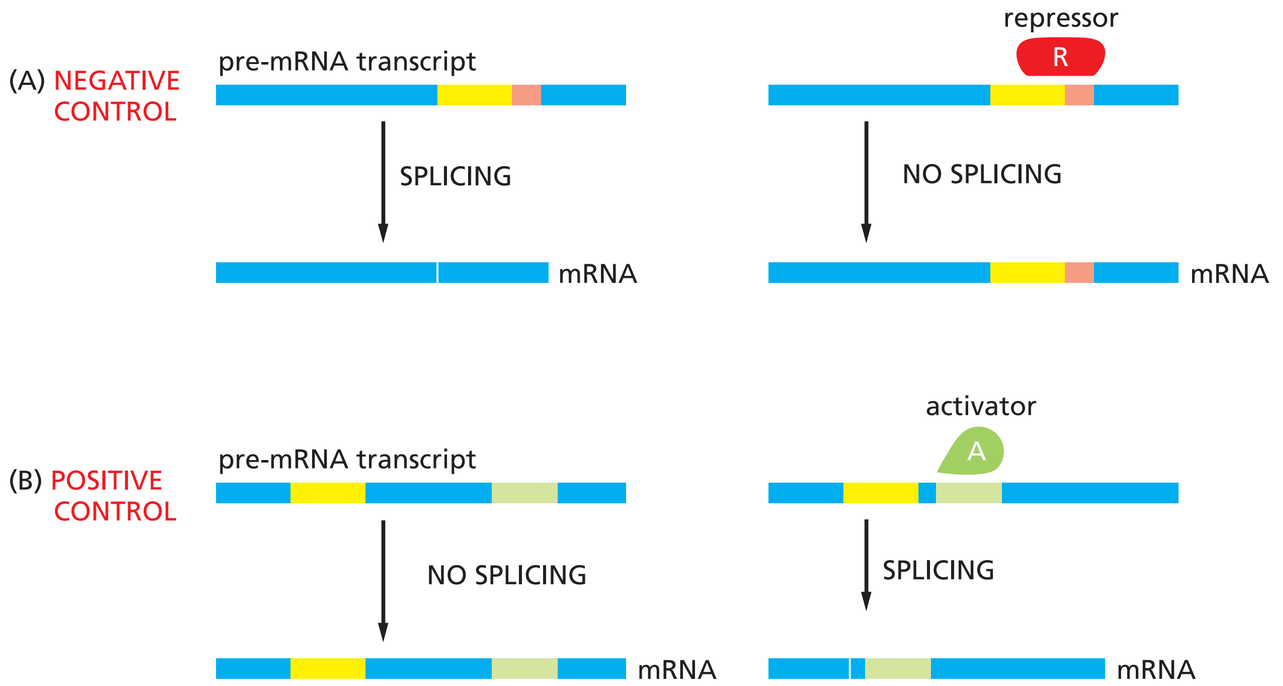
- **Splicing** = removes the introns from the mRNA precursor
- **Alternative splicing** allows to make different polypeptide chains from the same gene
- ~ **90% of human genes** produce multiple proteins this way



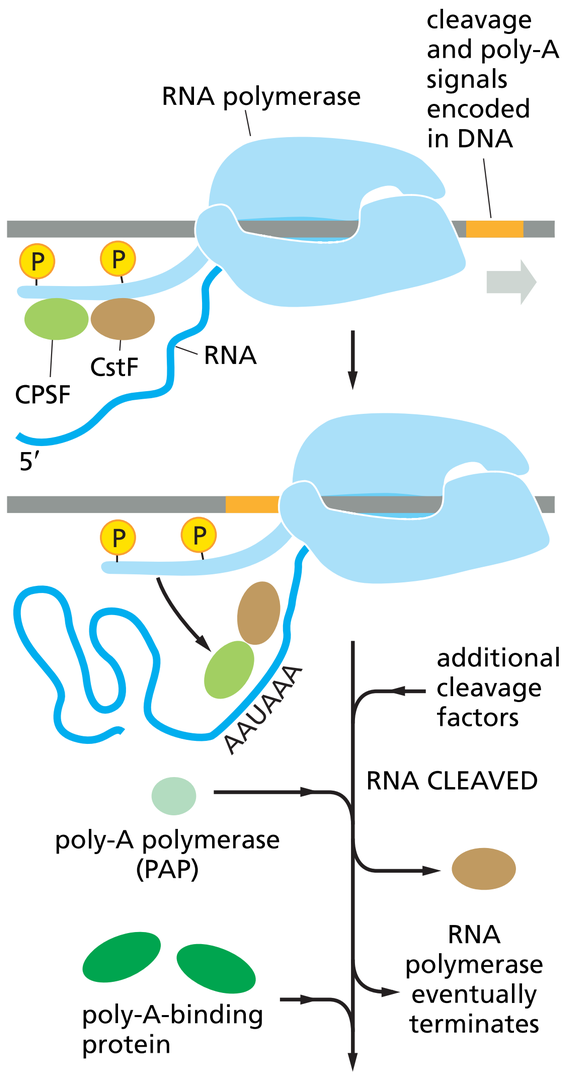
Alternative RNA splicing

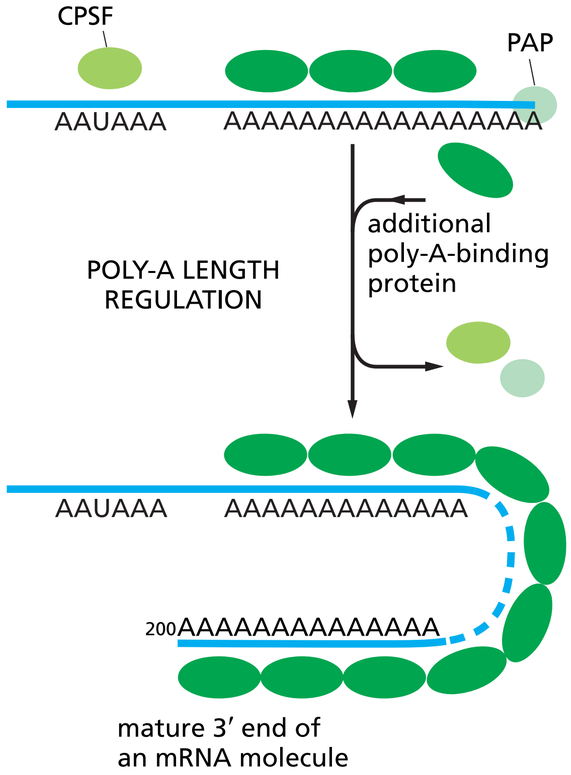
- RNA splicing can be regulated **negatively** (i.e. a regulatory molecule prevents the splicing machinery from accessing a site)

- RNA splicing can be regulated **positively** (i.e. a regulatory molecule helps direct the splicing machinery to a site)



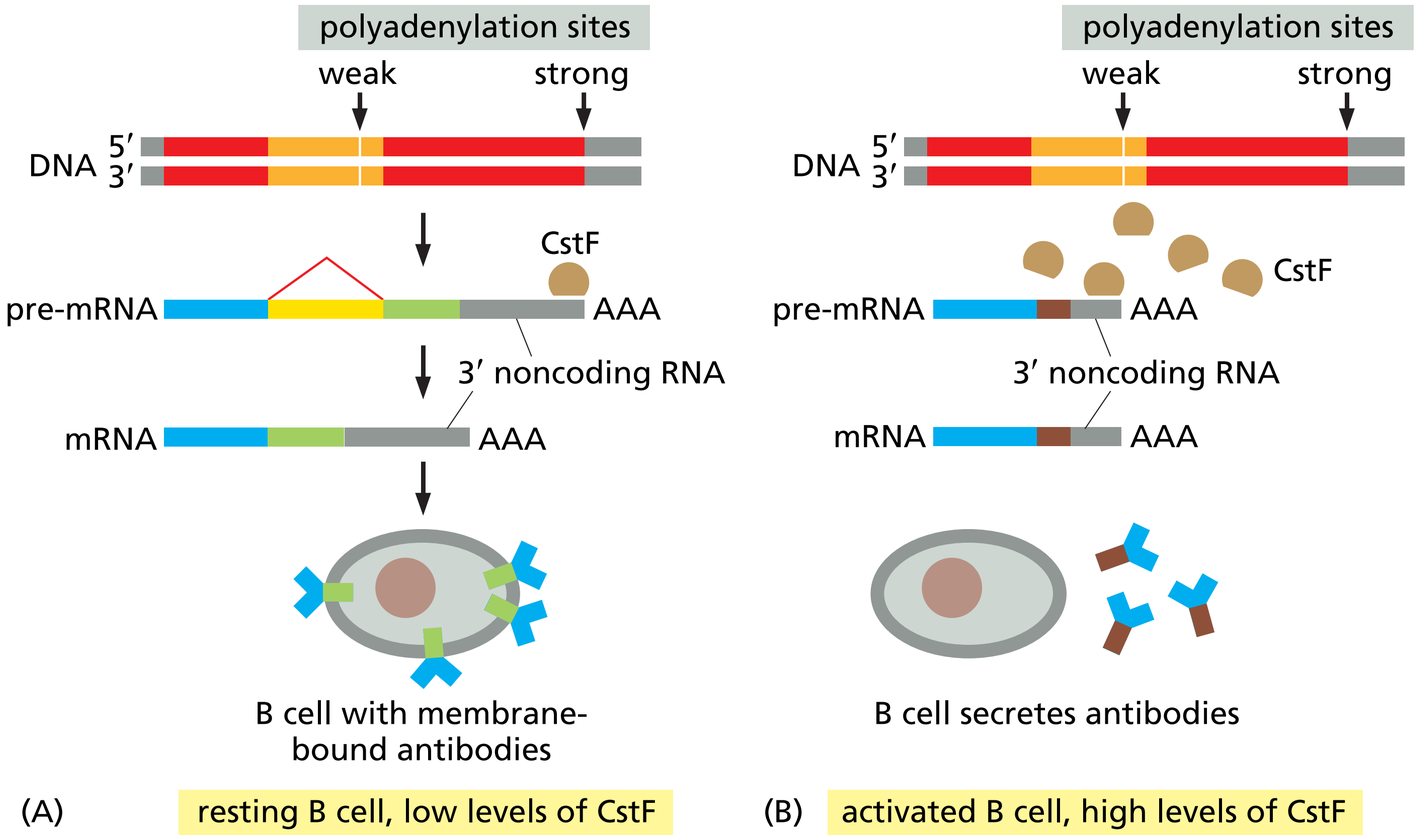
Transcript cleavage and polyA addition





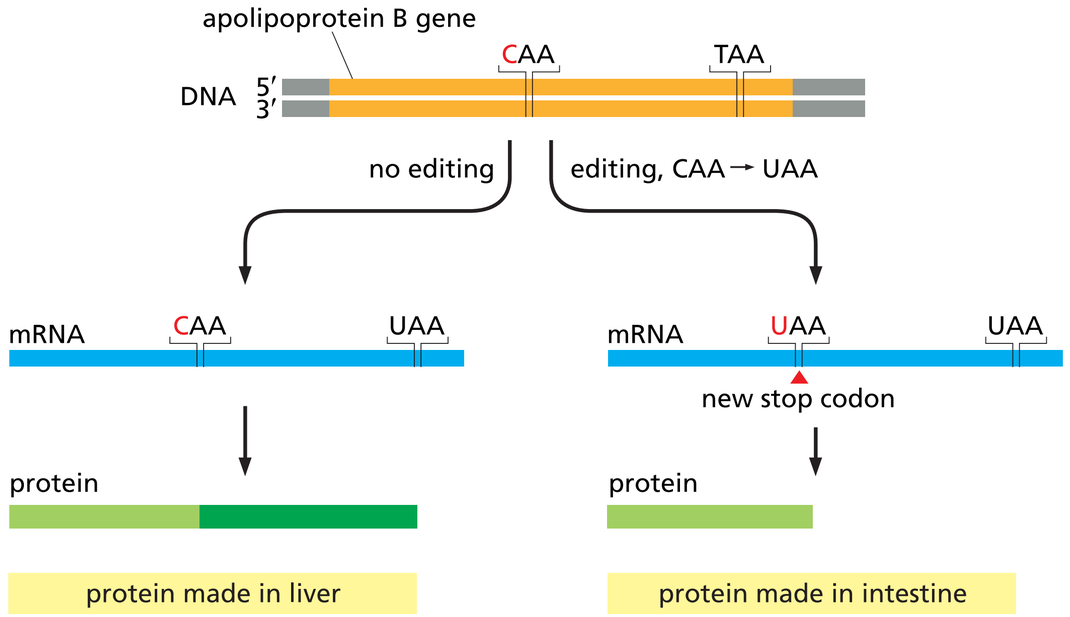
Transcript cleavage and polyA addition

- Cells can **control the site of cleavage and polyA** (leading to longer or shorter proteins)



RNA editing

- Alters the **nucleotide sequence** of RNA transcripts
  - **deamination of adenines** to produce inosine (A-to-I editing)
  - **deamination of cytosine** to produce uracil (C-to-U-editing)
- If it happens in a **coding region**, it may change the amino acid sequence of the protein
- If it happens **outside of a coding region**, it may affect splicing, transport of mRNA, efficiency of translation,…



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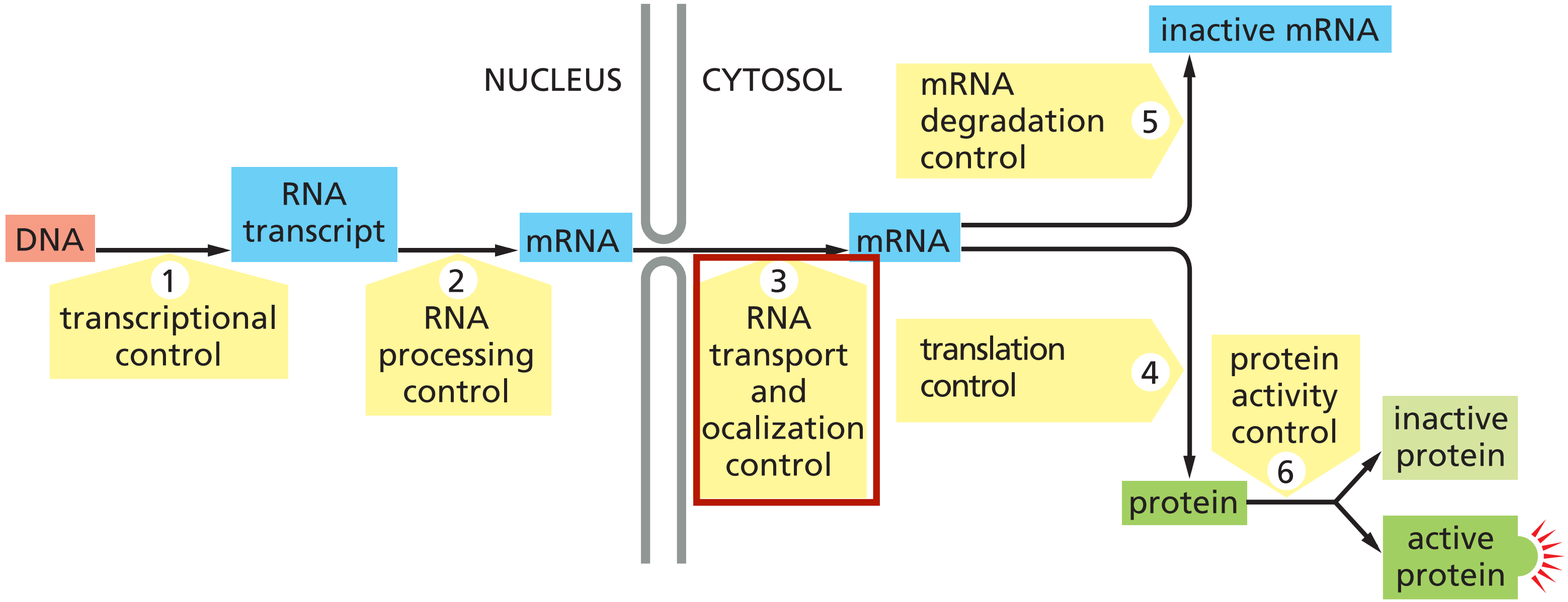
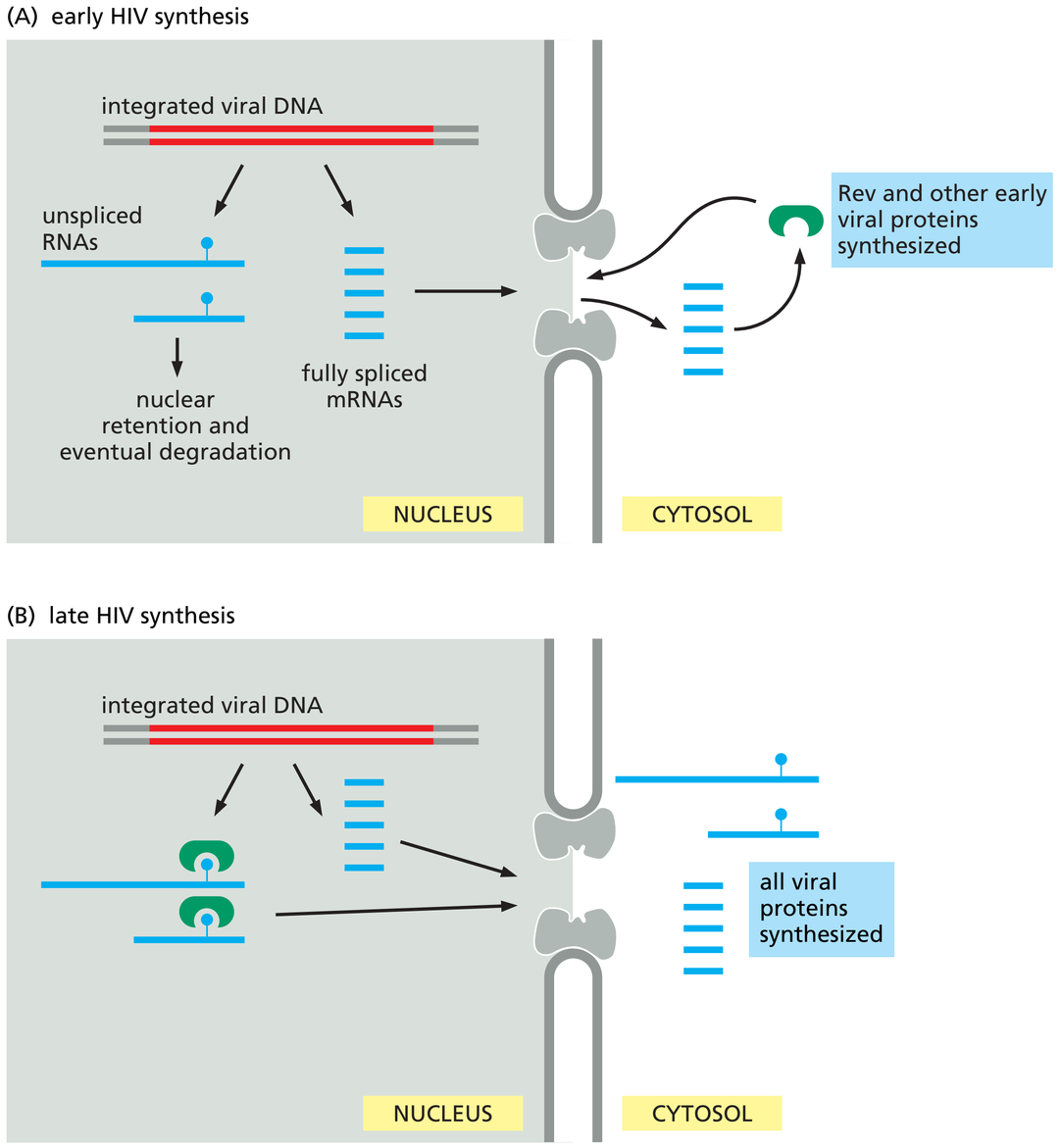


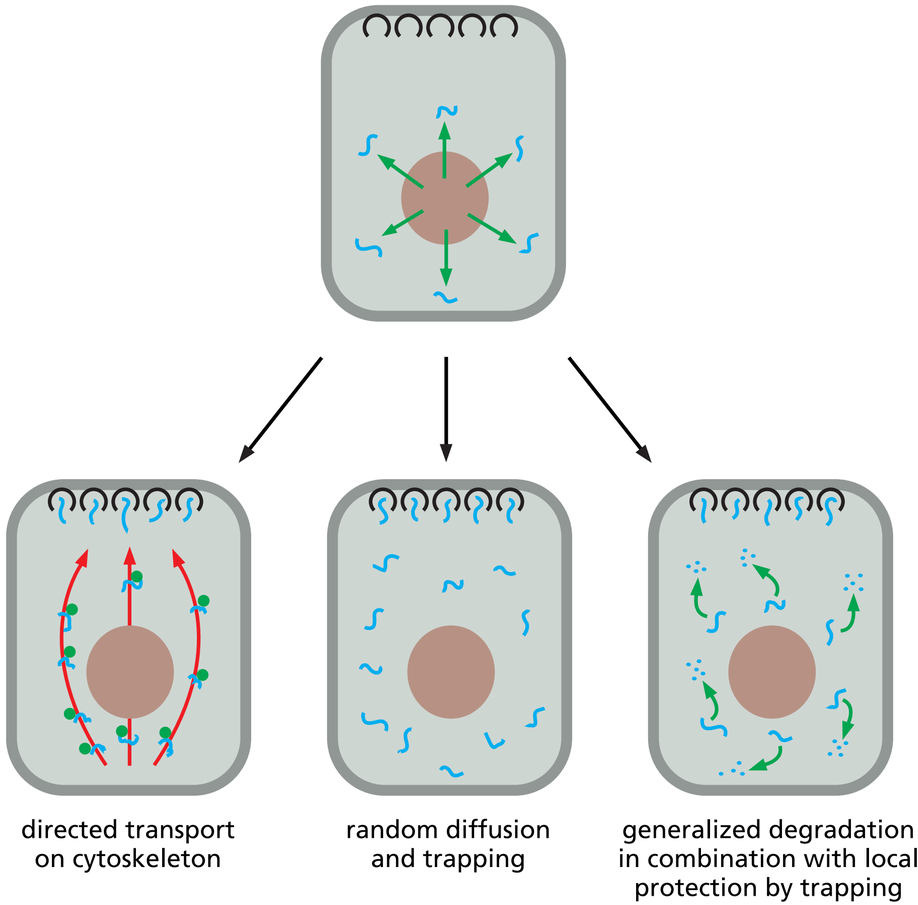
RNA transport

- Normally only happens once the **mRNA has been processed**
- In case of **RNA viruses** (e.g. HIV), the entire RNA has to come out of the nucleus to be packaged in new viral particles
- The HIV encodes **a protein (Rev)** that binds to a specific RNA sequence and interacts with a nuclear export receptor
- This **directs the movement** of RNA to the cytosol (despite the presence of introns)
- Importance of the **3'-UTR region** of the mRNA (beyond the stop codon)



RNA transport

- Once out of the nucleus, the mRNA can reach **different locations** in the cell
- For membrane or secreted proteins, the mRNA is targeted to the **endoplasmic reticulum**
- Many mRNAs are directed to **specific intracellular locations**, close to sites where the protein is needed
- This allows the establishment of **asymmetries** in the cytosol of the cell



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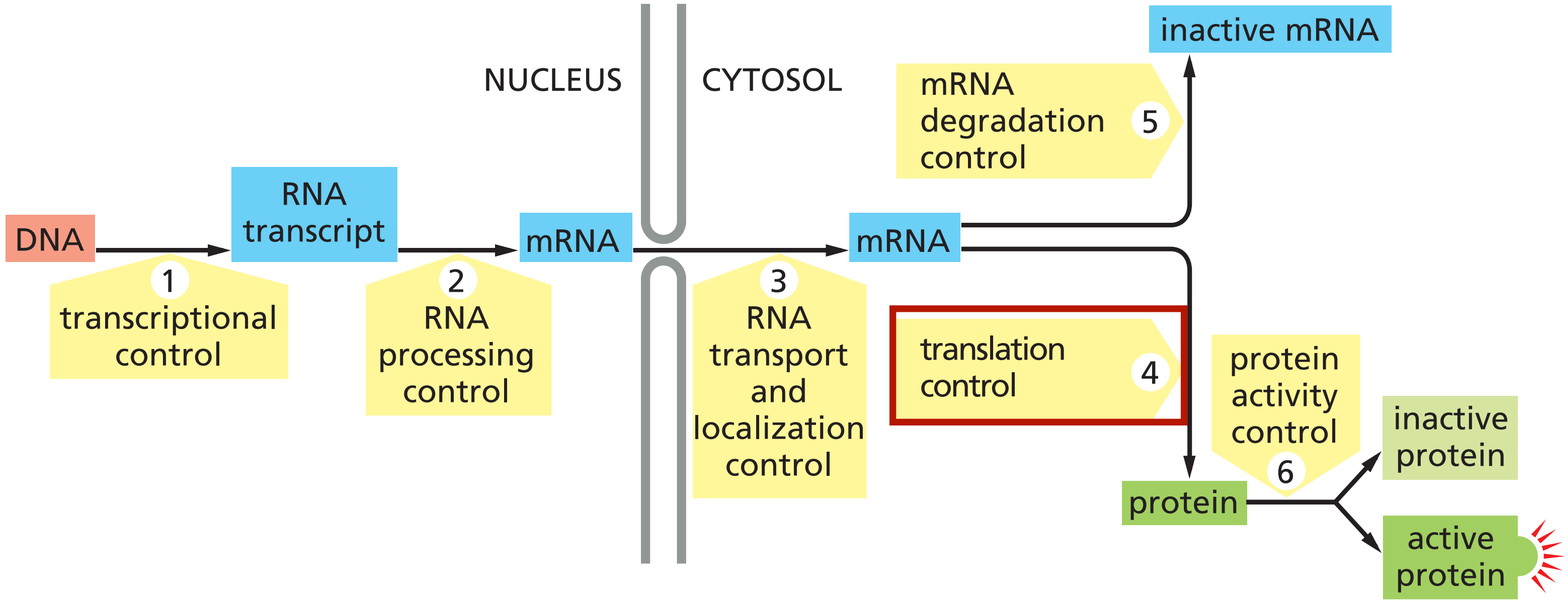
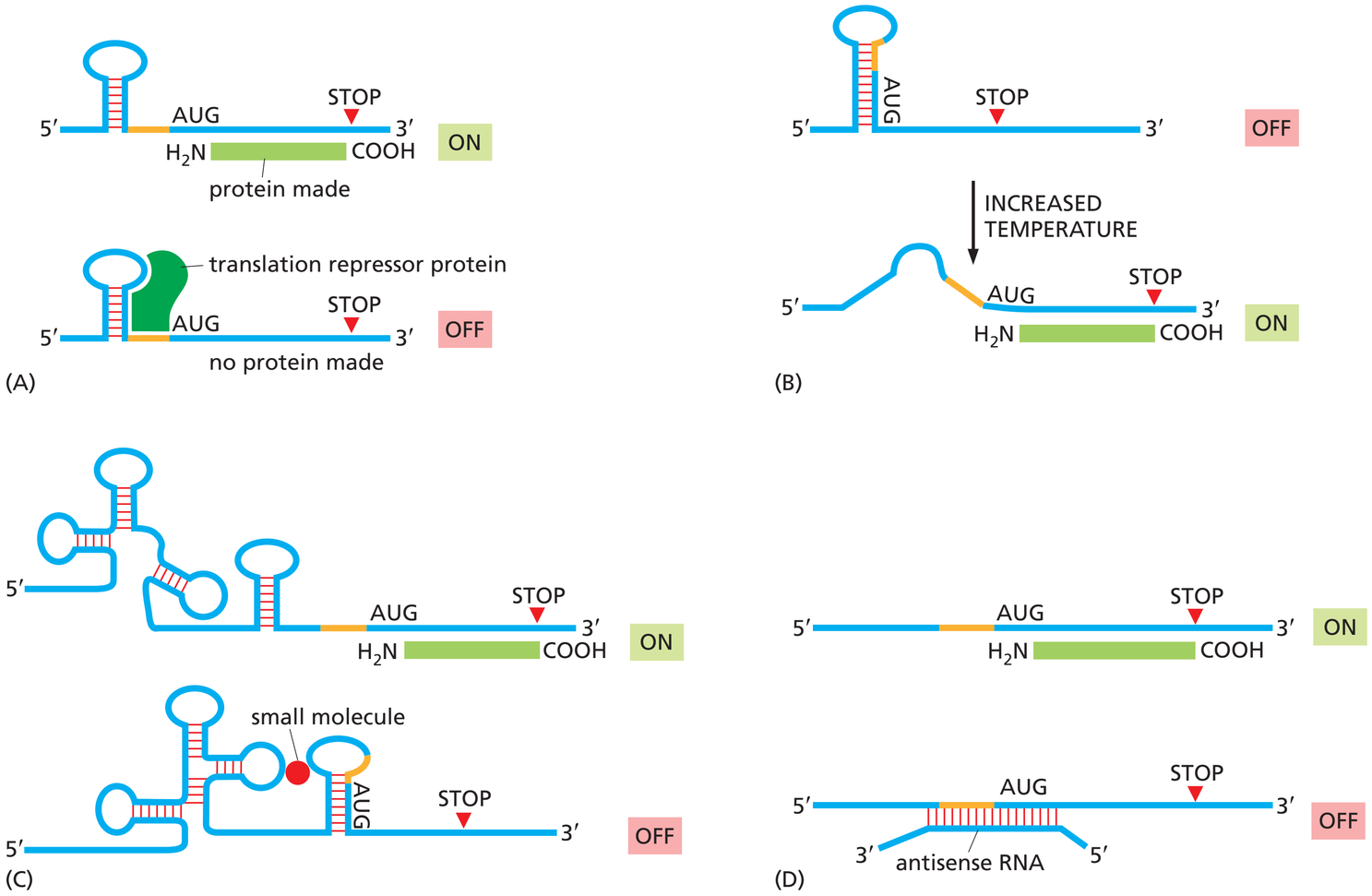


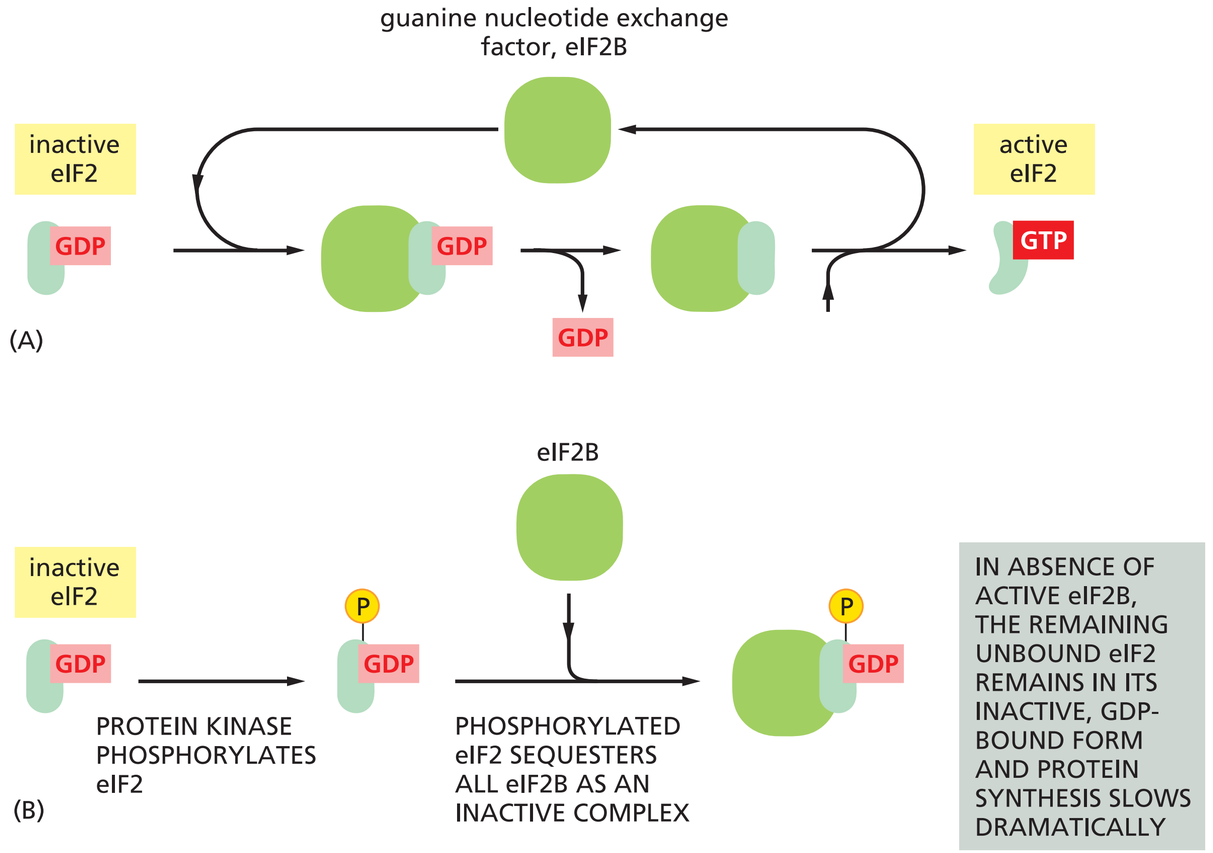
Translational control



- In bacteria, translational control happens by interfering with the **Shine-Dalgarno** sequence (upstream of the AUG codon)
- In Eukaryotes, translational repressors
  - bind to the **5’ of the mRNA**
  - bind to the **3’-UTR**
  - **miRNAs**

Translational control

- Cells decrease protein synthesis in response to **stresses** (low nutrients, infection, temperature increase, etc.)
- In Eukaryotes, this happens through the **phosphorylation** of the translation initiation factor eIF2



Translational control

- **Leaky scanning** (scanning ribosomal units ignore the first AUG codon) leads to proteins with different N-terminus and same C-terminus

- This can be used to produce proteins with different signal sequences, that will be sent to **different compartments**

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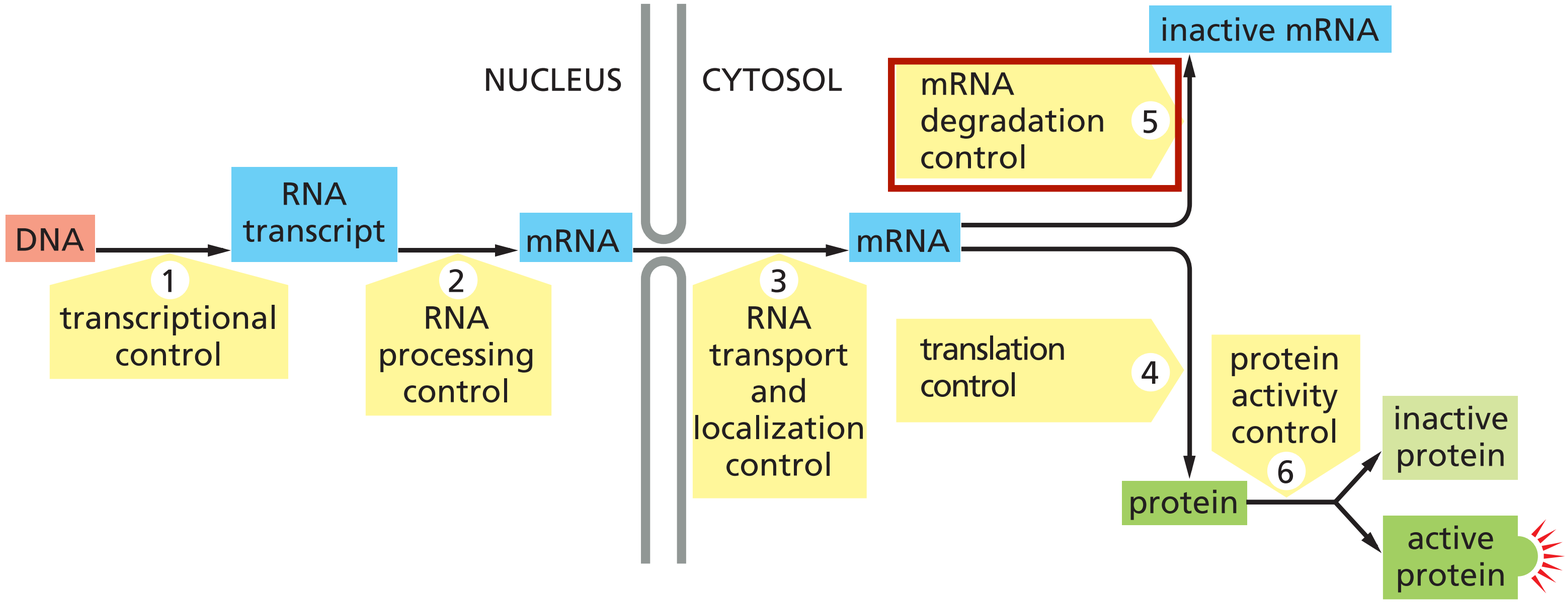


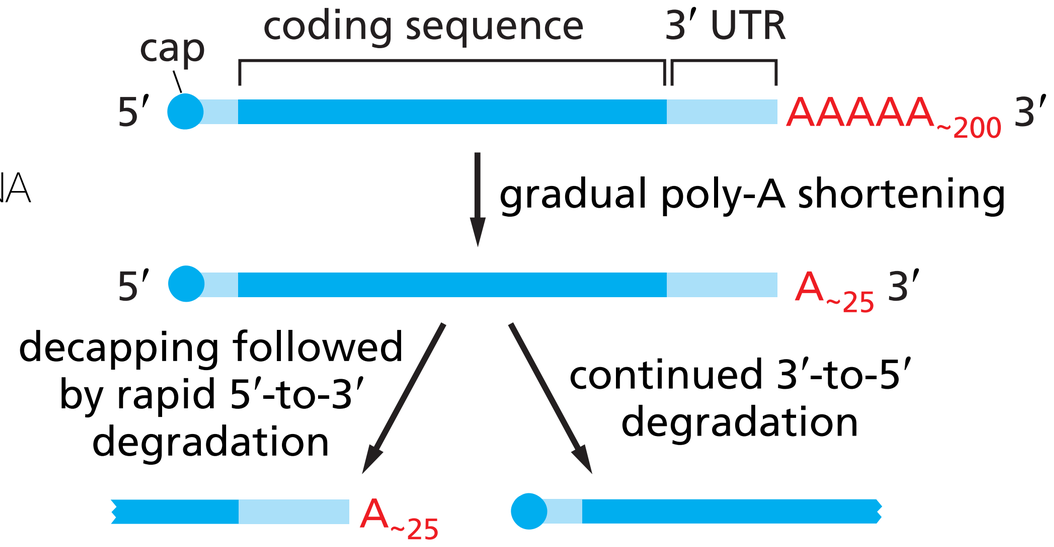
mRNA stability

In **bacteria**,

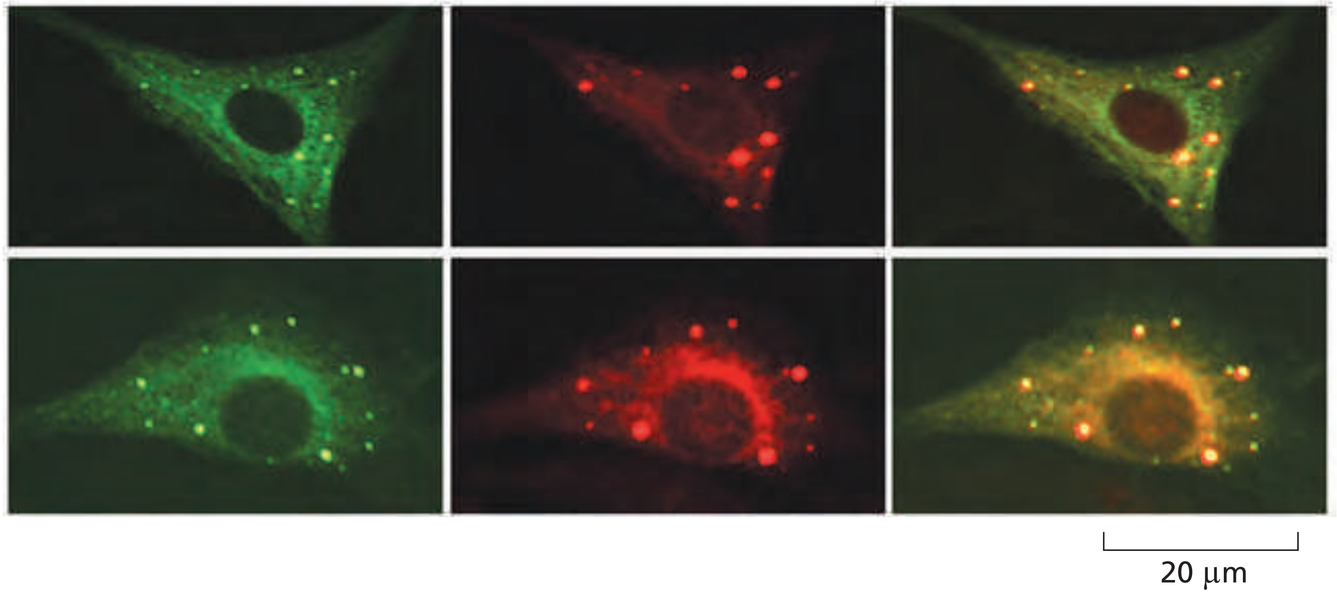
- Most mRNA are very **unstable** (half-life of <few minutes)
- **Exonucleases** (degrade from 3' to 5') are responsible for the degradation of mRNAs

In **Eukaryotes**,

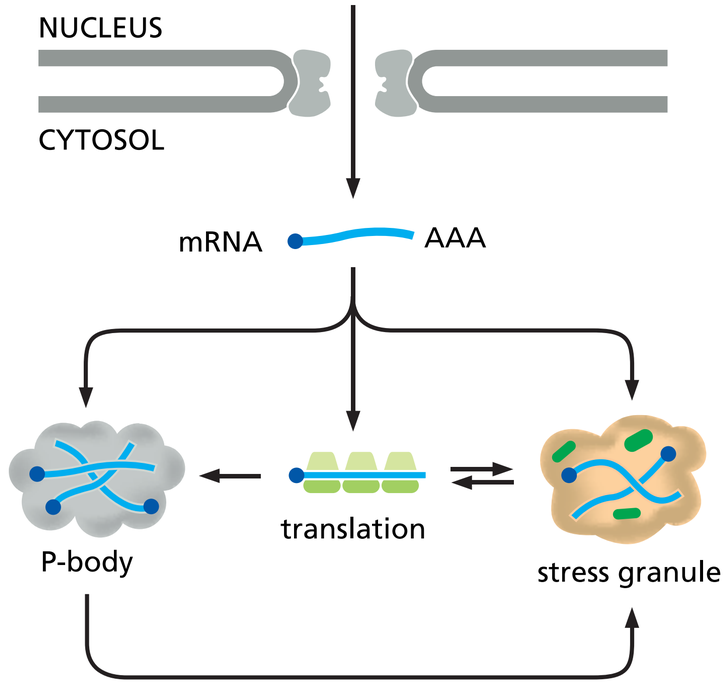
- mRNAs are more **stable** (half-lives from 30min to 10h)
- shortening of the **polyA tail by exonucleases** (count down of the mRNA half-live)
- when the **polyA reaches ~ 25 nt**
  - **cap** is removed and mRNA is degraded from 5'
  - mRNA continues to be degraded **from 3'**



P-bodies and stress granules

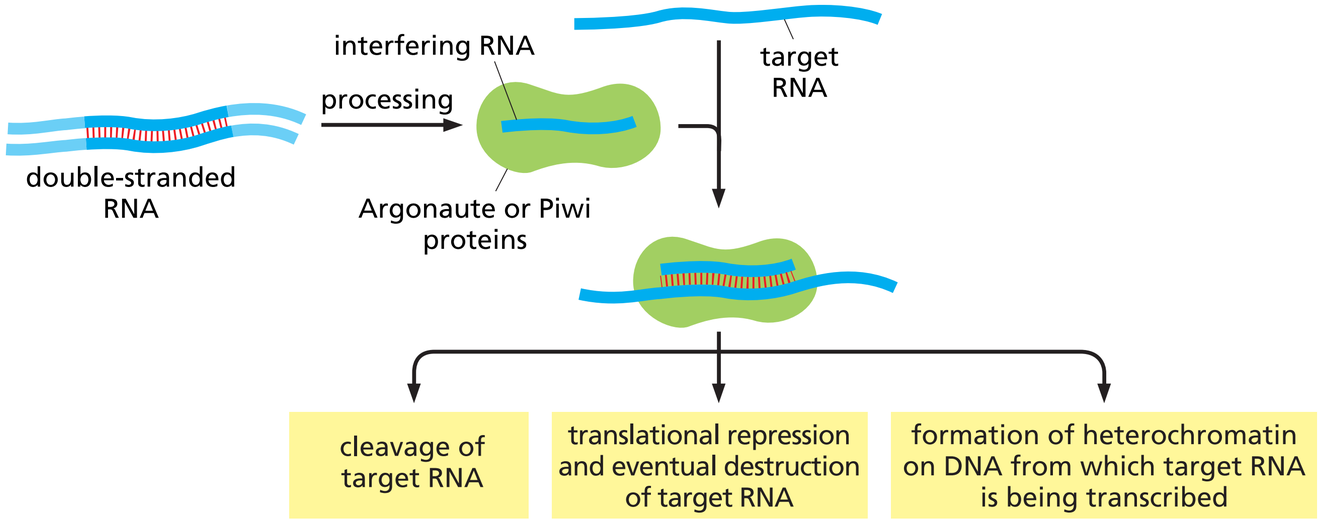


- **P-bodies** are large aggregates of proteins that work together and are held in close proximity, where mRNAs are degraded
- **Stress granules** keep the mRNA “translation-ready”



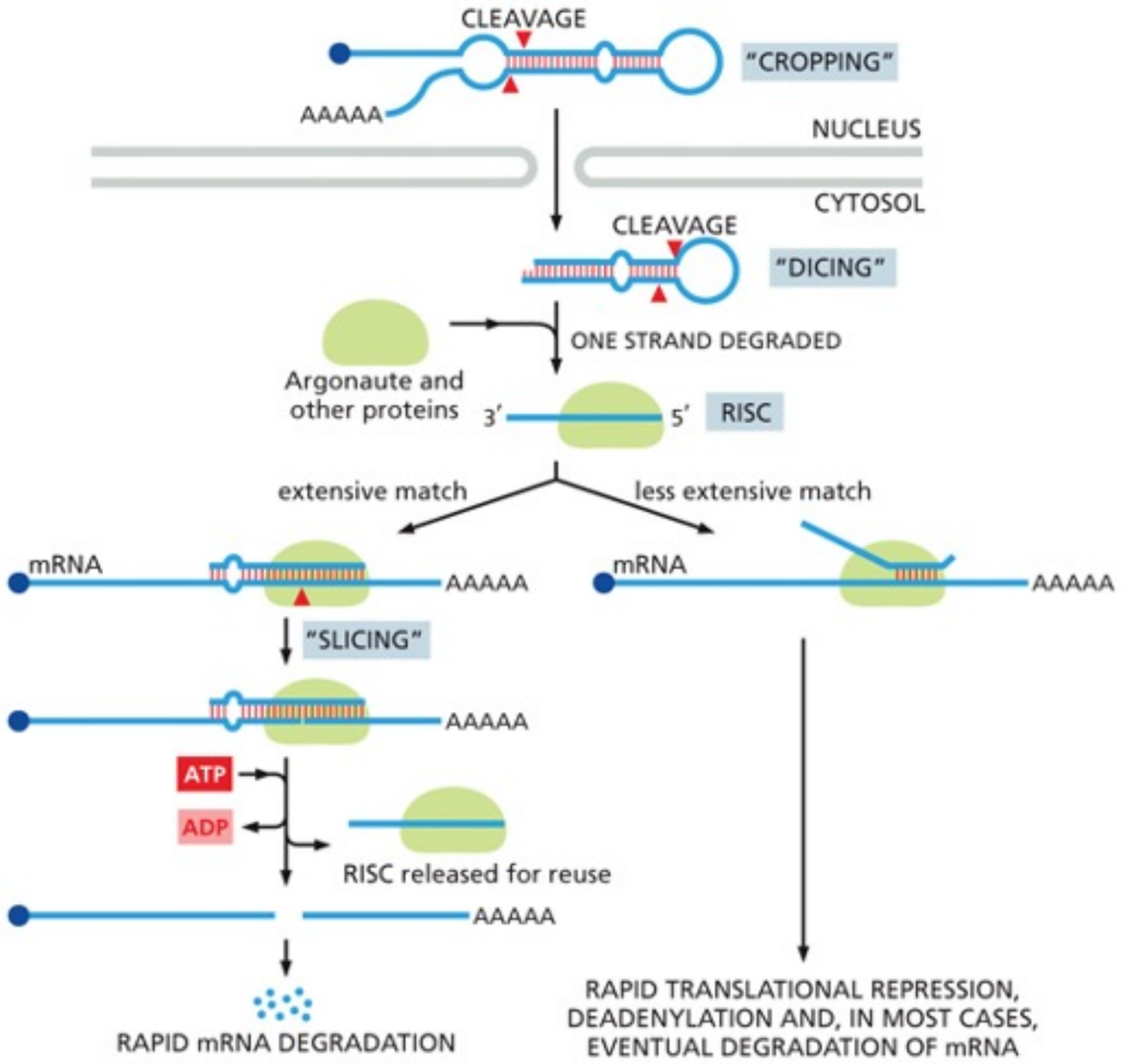
Noncoding RNAs - RNA interference (RNAi)

- **Short single-stranded RNAs** (20-30 nucleotides) serving as guide RNAs that bind other RNAs in the cell
- When the target is an **mRNA**, it **prevents** its translation or catalyzes its **degradation**
- When the target is an **RNA being transcribed**, it can direct the formation of **repressive chromatin**
- microRNAs (**miRNAs**), small interfering RNAs (**siRNAs**) and piwi-interacting RNAs (**piRNAs**)



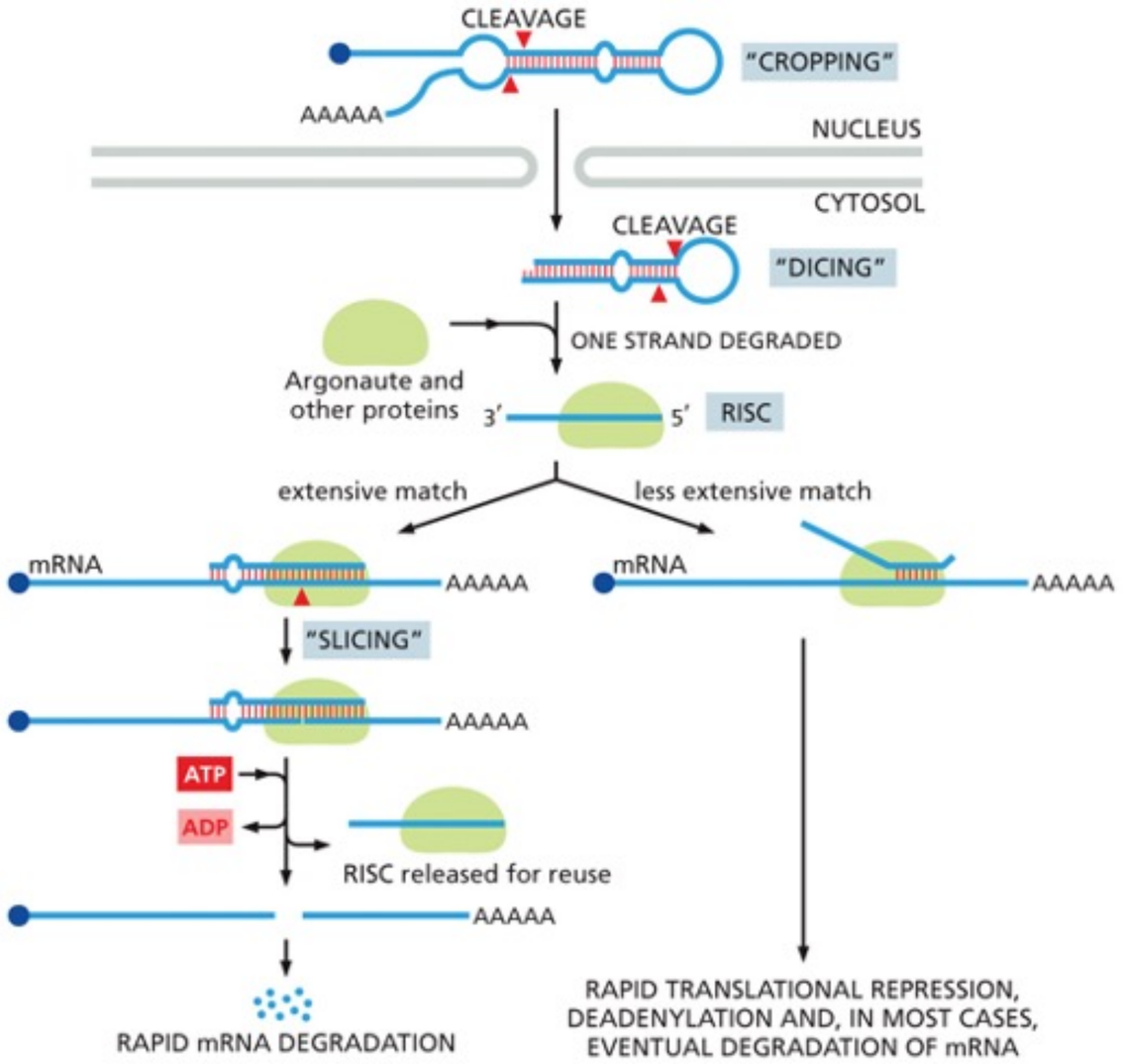
miRNAs regulate mRNA translation and stability

- **>1000** miRNAs in the human genome, controlling at least **1/2 of the coding genes**
- base-pair with **mRNAs** to fine-tune their **translation and stability**
- made by **RNA polymerase II**, **capped** and **poly-adenylated**
- special processing and assembly with a set of proteins forming the **RNA-induced silencing complex (RISC)**
- The complex searches for **complementary sequences**
- a single miRNA can regulate a **whole set of different mRNAs**

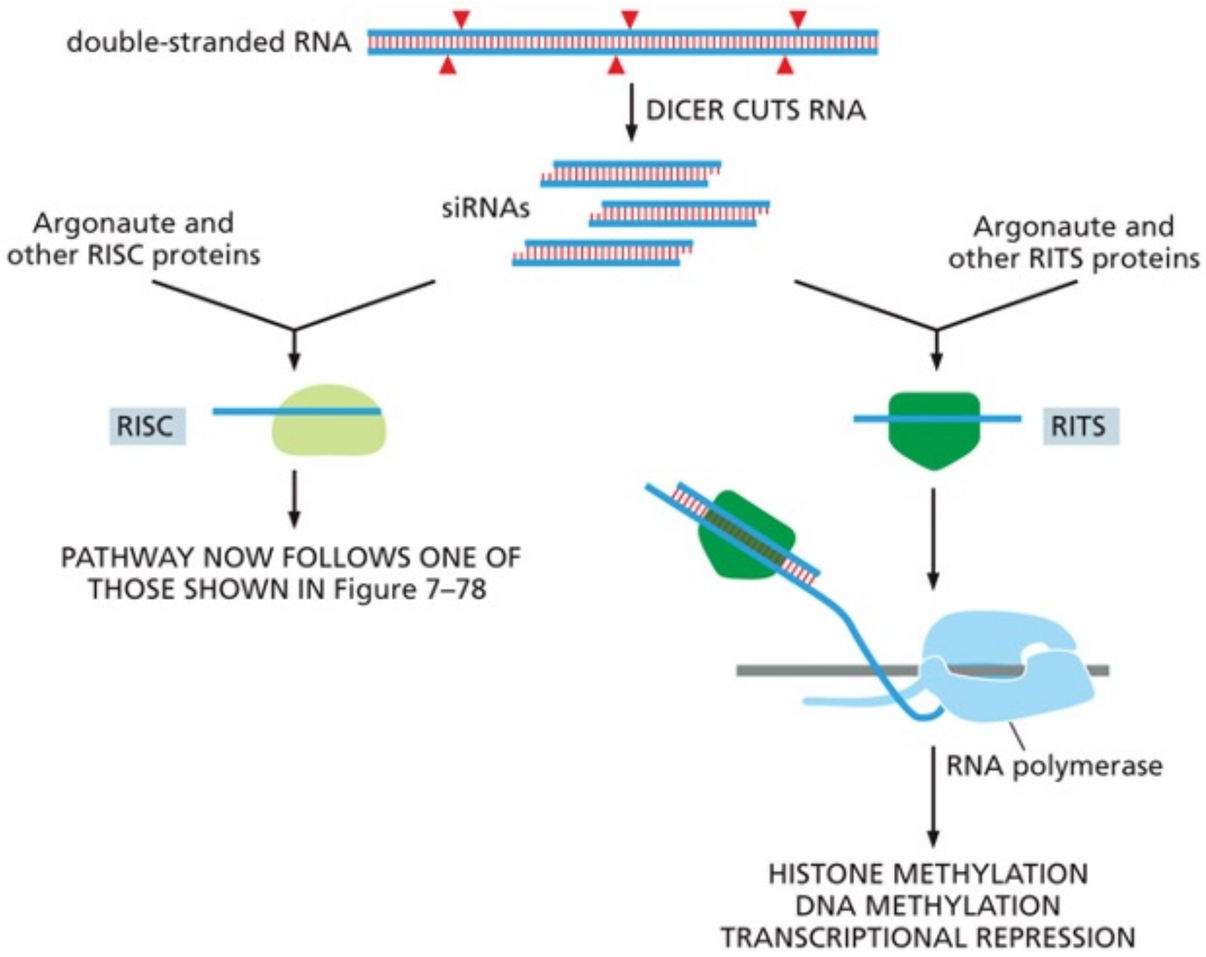


RNA interference as cell defense

- Degradation of **foreign RNA** (especially double-stranded RNA, as present in some viruses)
- The presence of dsRNA attract the protein complex containing **Dicer** (as for processing of miRNAs)
- This protein **cleaves dsRNA into small fragments** (~23 nt) called small interfering RNAs or **siRNAs**
- One strand is degraded and the other bound to the **RISC complex**
- The complex now targets and degrades **complementary RNA**



RNA interference and heterochromatin formation



- In some cases, RNAi shuts off **synthesis** of mRNA
- **RITS** = RNA-induced transcriptional silencing complex
- Causes the formation of **heterochromatin** (H3K9me3 mark)
- Maintains **transposable elements** in the silent form

piRNAs protect the germ-line from transposable elements

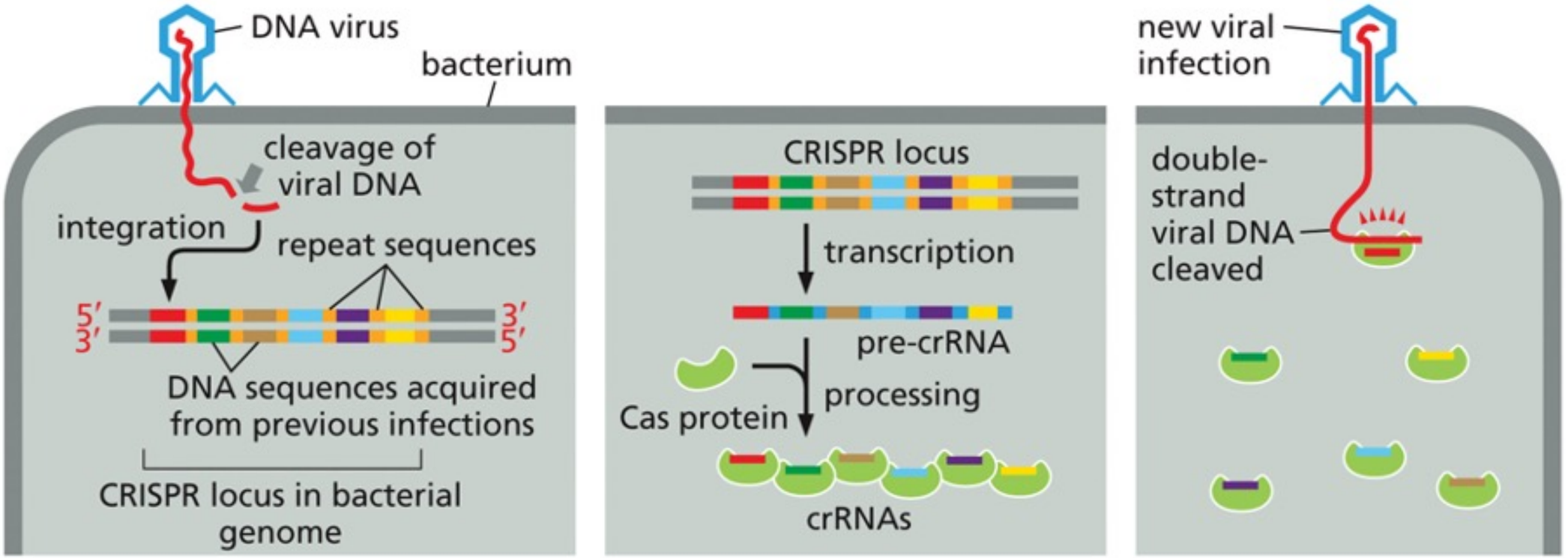
- piRNAs = **Piwi-interacting RNAs**, where Piwi is a class of proteins related to Agronaut
- In the germ-line, many **histone modifications are erased,** releasing **transposons** from their “normal contraints”
- piRNAs are transcribed from a specific cluster as **long single-stranded RNA**, that are further processed and assembled with **Piwi proteins**
- they then bind to complementary RNA, and both **cleave the RNAs** and package the **chromatin into repressive forms**

Arms race between cell's DNA and parasitic elements

- Even with our **defense mechanisms**, parasitic DNA (transposons, viruses, …) make up nearly **half of our DNA**
- **siRNA** and **piRNA** are surveillance systems (based on RNA-base pairing)
- Additional system with **sequence-specific DNA-binding proteins** (KRAB-ZPF proteins)
- They recognise viral or transposon **sequences**
- They recruit **histone writers** that place H3K9me3 marks on the nearby histones
- They recruit **DNA methylases** that methylate the surrounding DNA
- They recognise sequences that are **crucial** for the virus replication or transposon transposition

Bacteria use CRISPR to protect themselves from viruses

- CRISPR = clustered regularly interspersed short palindromic repeat



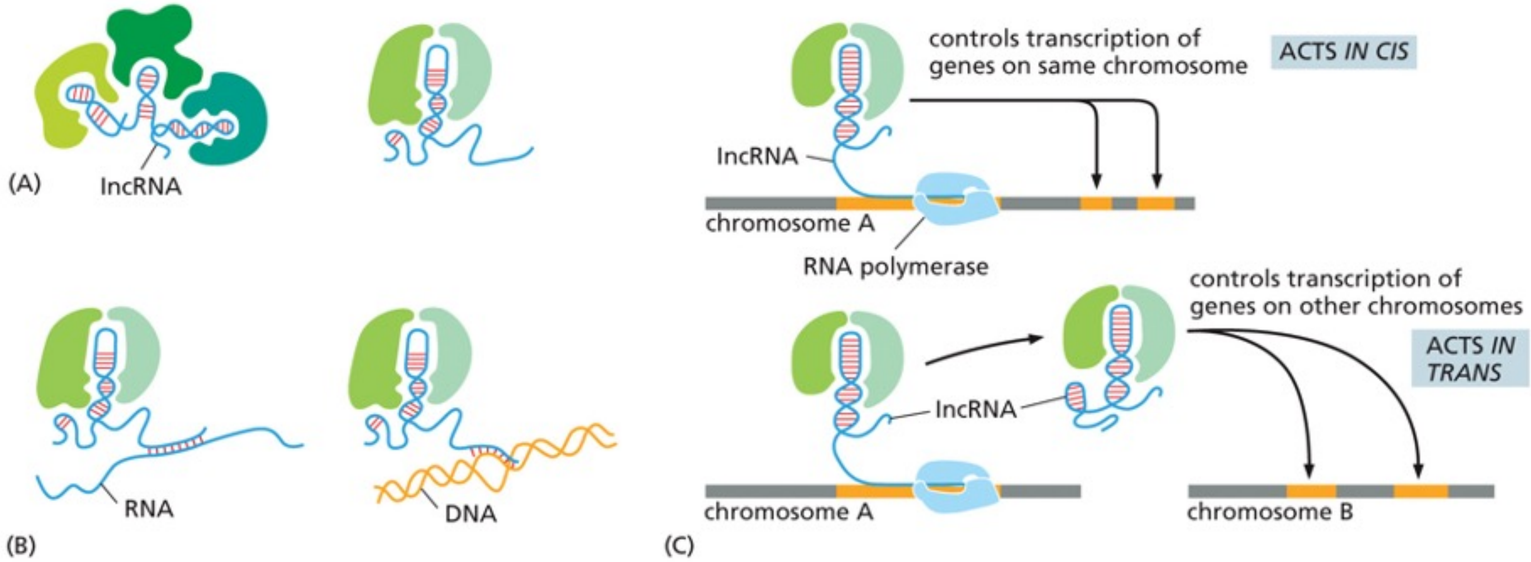
STEP 1: short viral DNA sequence is integrated into CRISPR locus

STEP 2: RNA is transcribed from CRISPR locus, processed, and bound to Cas protein

STEP 3: small crRNA in complex with Cas seeks out and destroys viral sequences

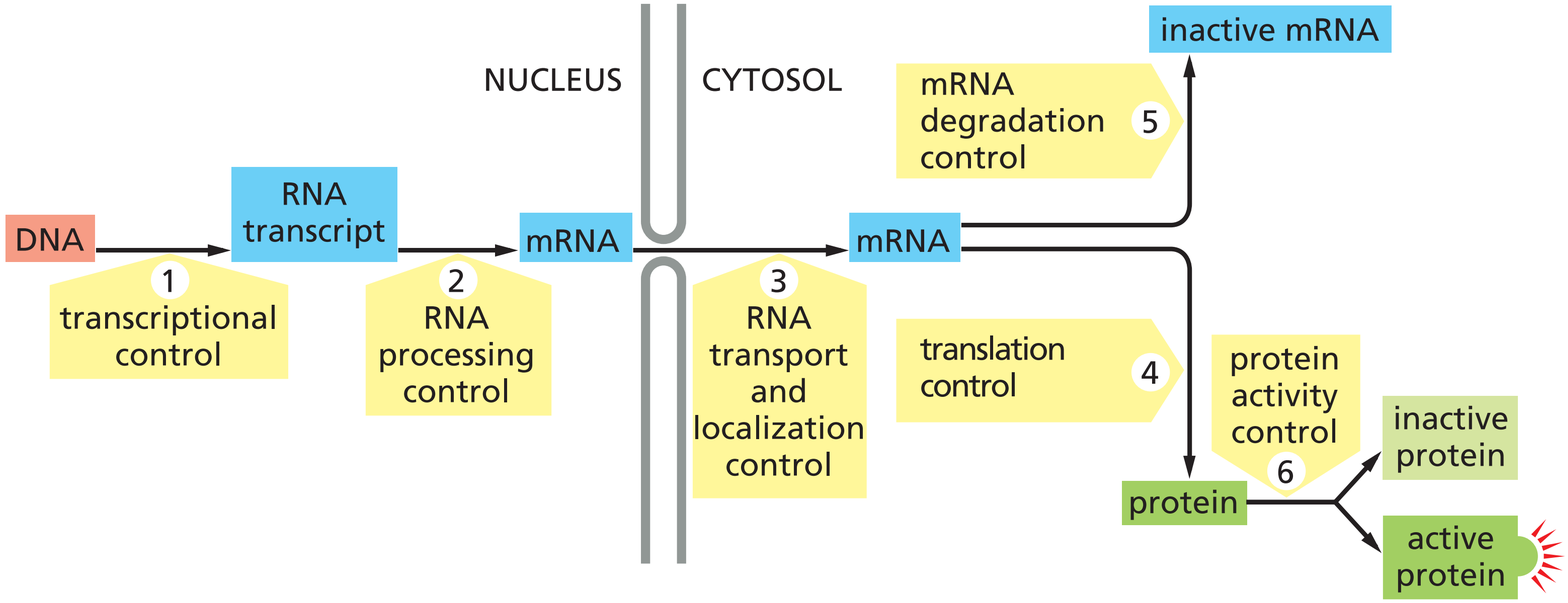
Long non-coding RNAs (lncRNA)

- >200 nt
- function mostly unknown
- > 5000 in the human genome
- Examples: RNA in telomerase, Xist RNA, RNA involved in imprinting
- They can work as **scaffold RNA molecules, guide sequences to bind specific RNA/DNA**, or **affect transcription**



Summary

- Transcriptional control
  - Genomic imprinting
  - X-chromosome inactivation
  - Epigenetic inheritance
- Post-transcriptional control
  - RNA processing
  - RNA export
  - Translational control
  - mRNA stability



Have a nice day!