

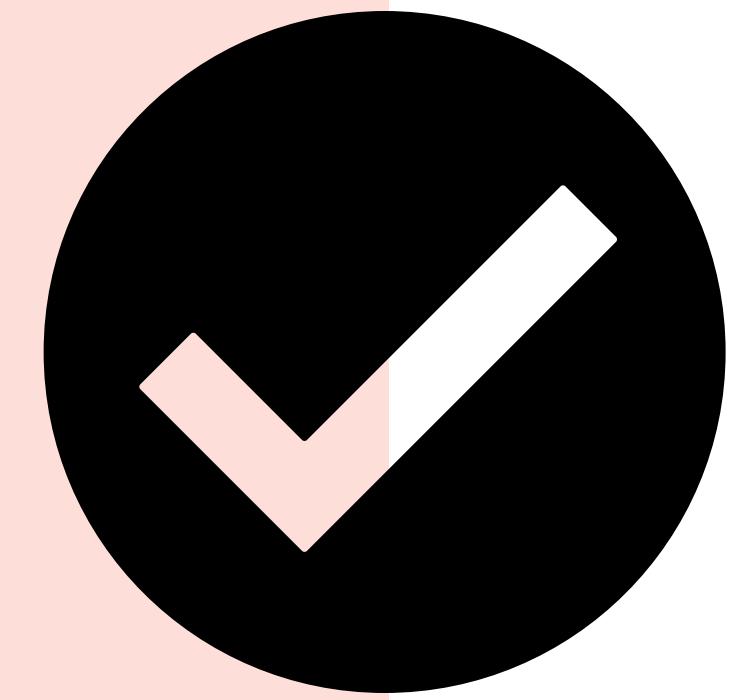
Cellular and Molecular Biology I

BIO-205-5

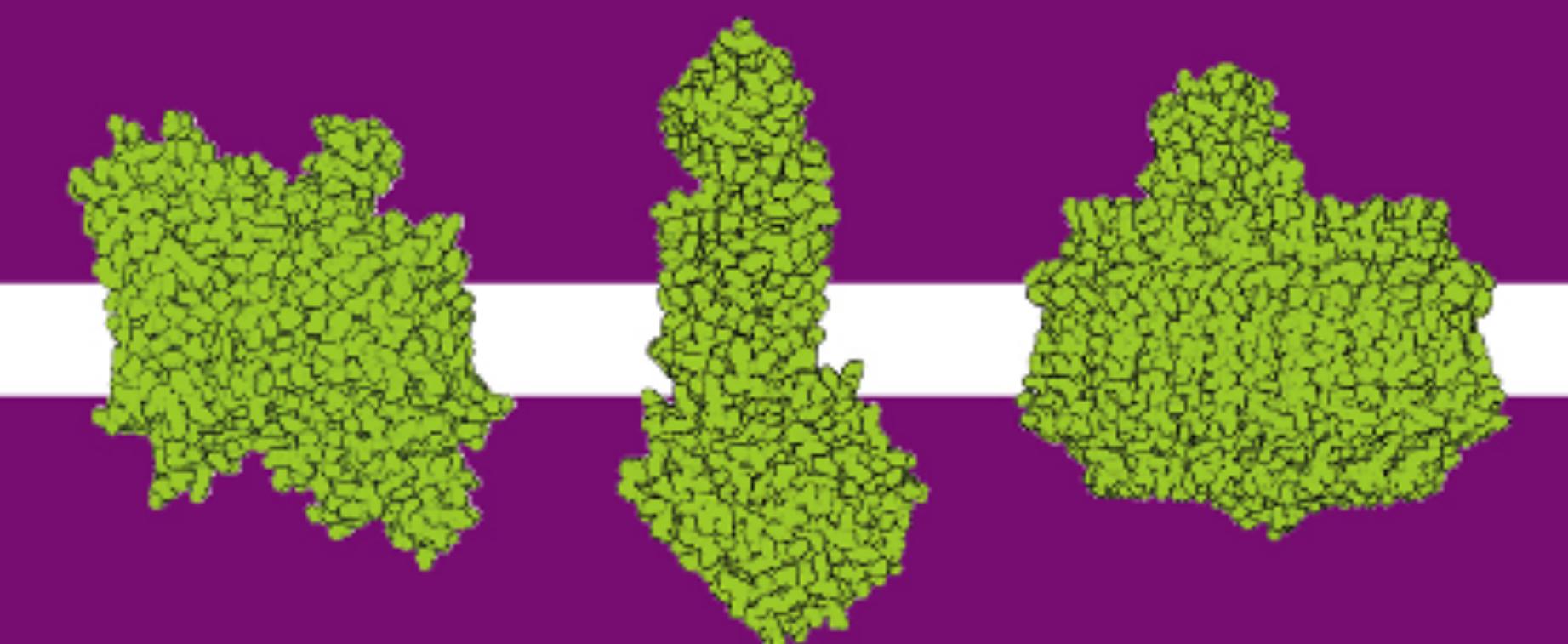
Camille Goemans - 2024

Quick recap

- Lecture 1 - Structure and packing of DNA
- Lecture 2 - Structure of chromosomes and how genomes evolve
- Lecture 3 - Mobile genetic elements and DNA replication
- Lecture 4 - DNA replication and DNA repair



MOLECULAR BIOLOGY OF
THE CELL
SEVENTH EDITION



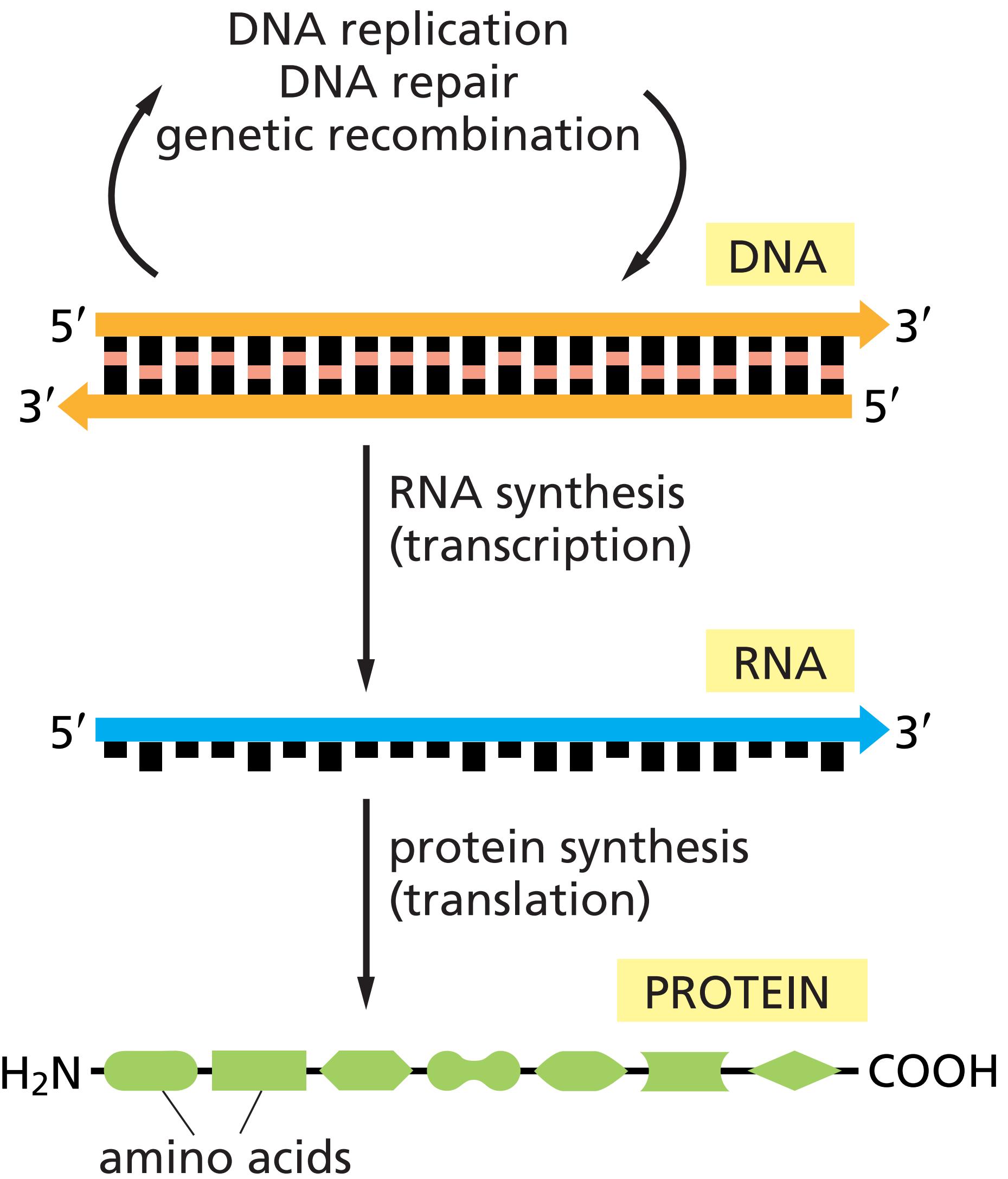
ALBERTS HEALD JOHNSON MORGAN RAFF ROBERTS WALTER

Chapter 6

How Cells Read the Genome: From DNA to Protein

Plan

- RNA
- Transcription
- Transcription initiation
- RNA processing
- Non-coding RNAs

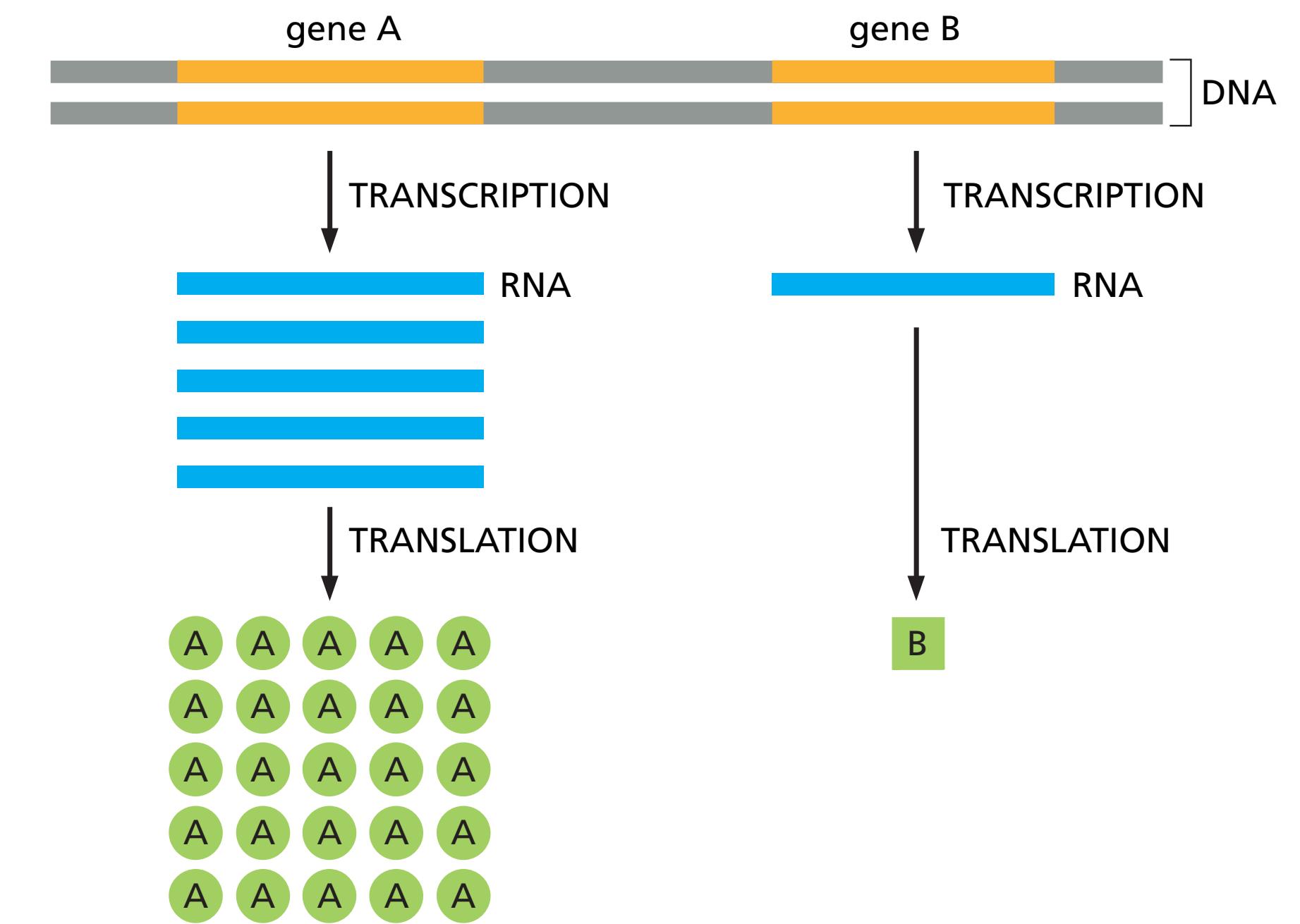


Central dogma of molecular biology

- **all cells** express their genetic information the same way
- important **variations** between organisms (e.g. RNA transcripts in eukaryotes are subject to a series of processing steps in the nucleus)
- some **RNAs are final products**: some with 3D structure and catalytic function in the cell, other as regulators of gene expression (next chapter)
- roles of many **non-coding RNAs** are unknown

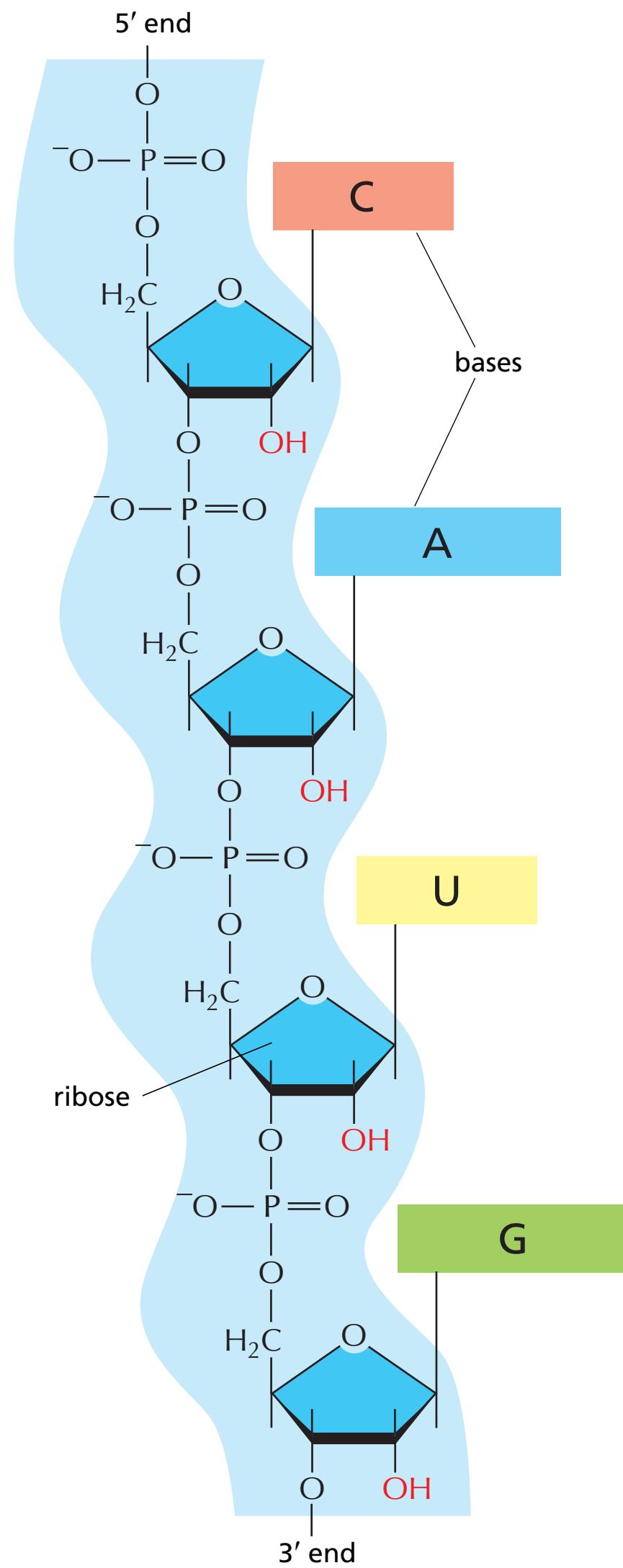
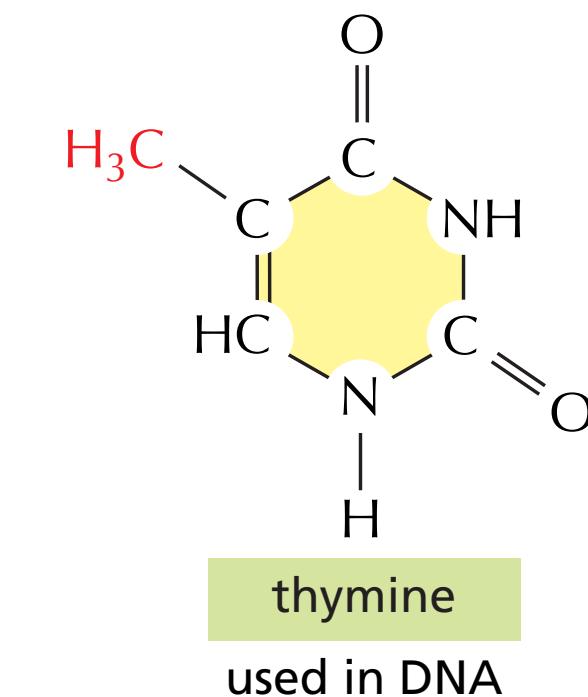
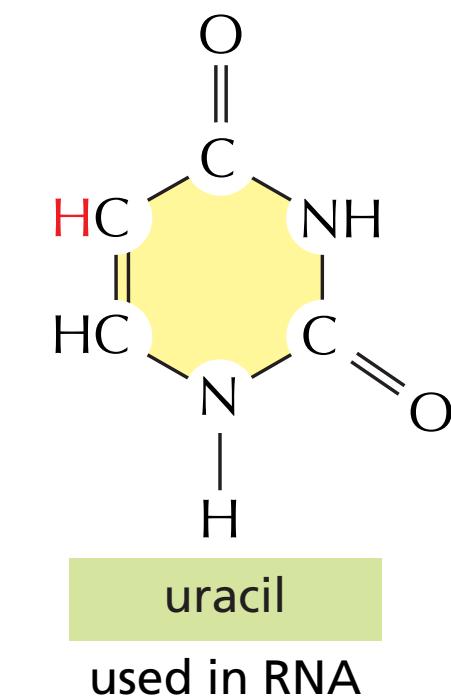
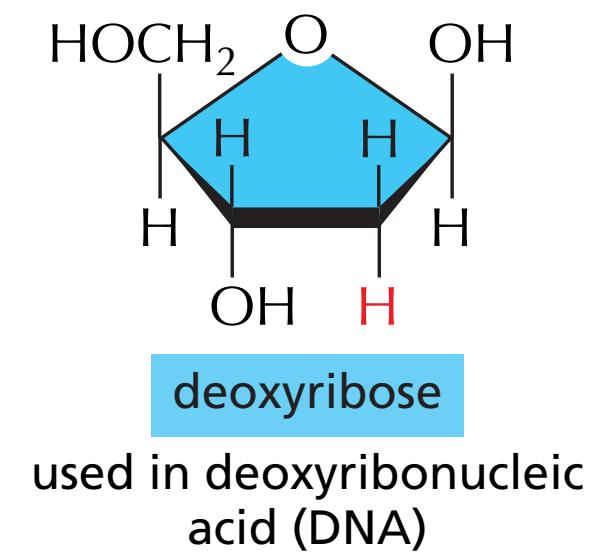
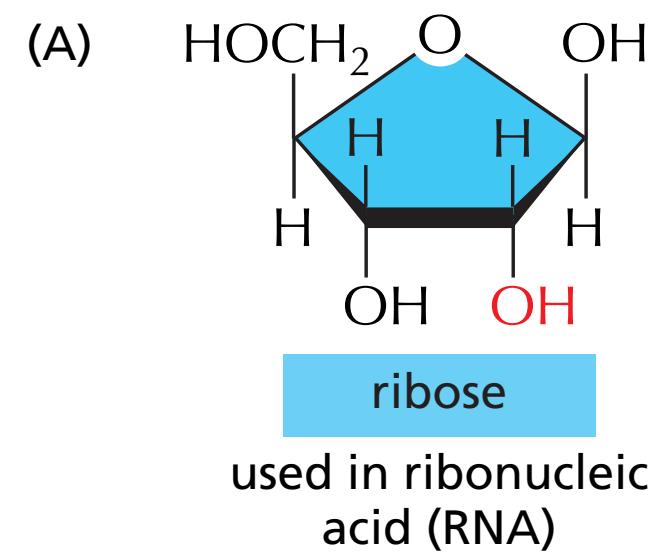
From DNA to RNA

- **Many identical RNA molecules** can be made from the same gene
- Genes can be transcribed and translated with **different efficiencies**
- Cells can **regulate** the expression of each gene depending on **its needs**



RNA molecules are single-stranded

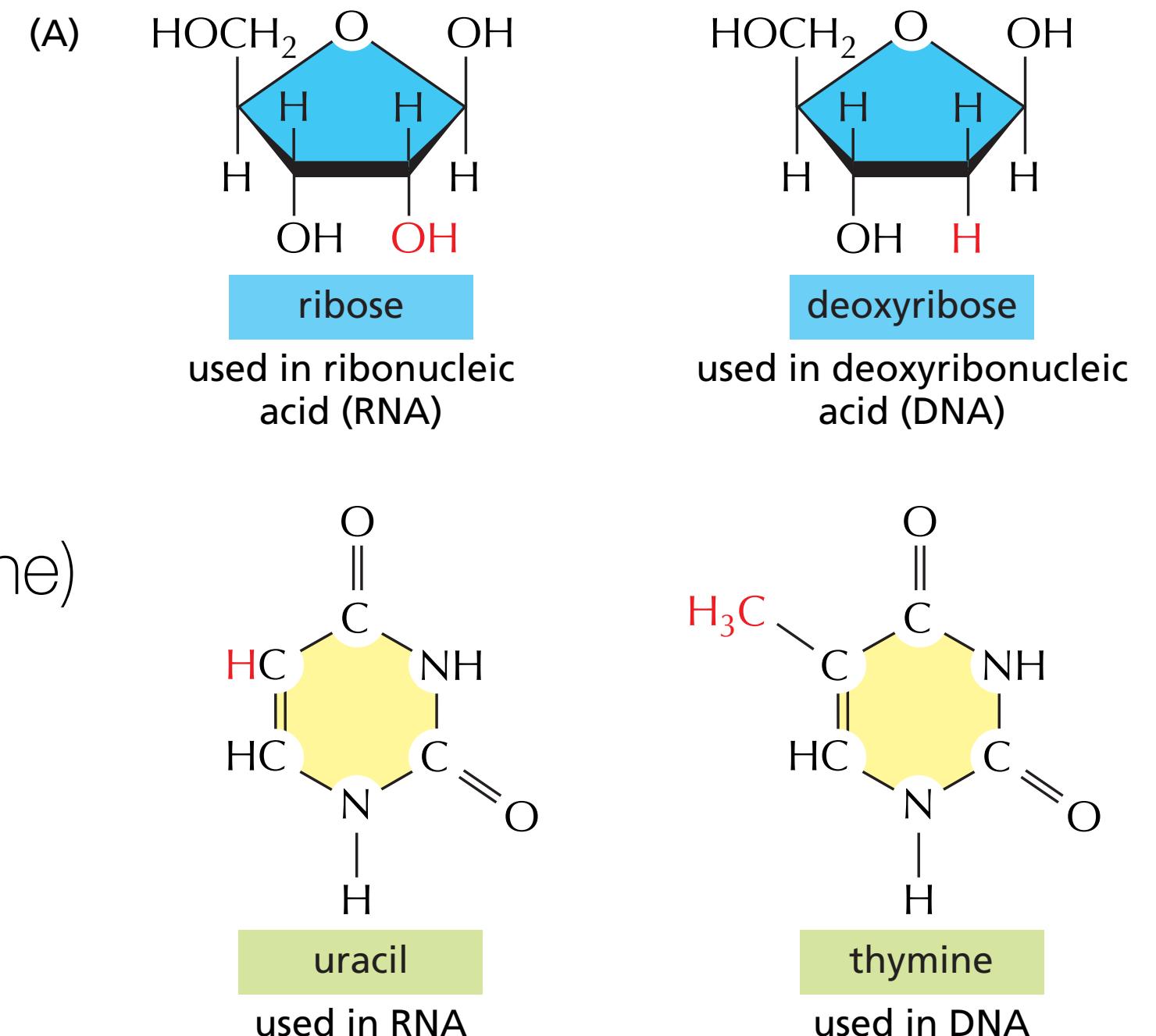
- The cell copies a portion of its DNA into an RNA nucleotide sequence = **transcription**
- RNA is a linear polymer made of **4 nucleotides** linked by **phosphodiester bonds**



RNA molecules are single-stranded

- RNA is **different than DNA** in 2 aspects:

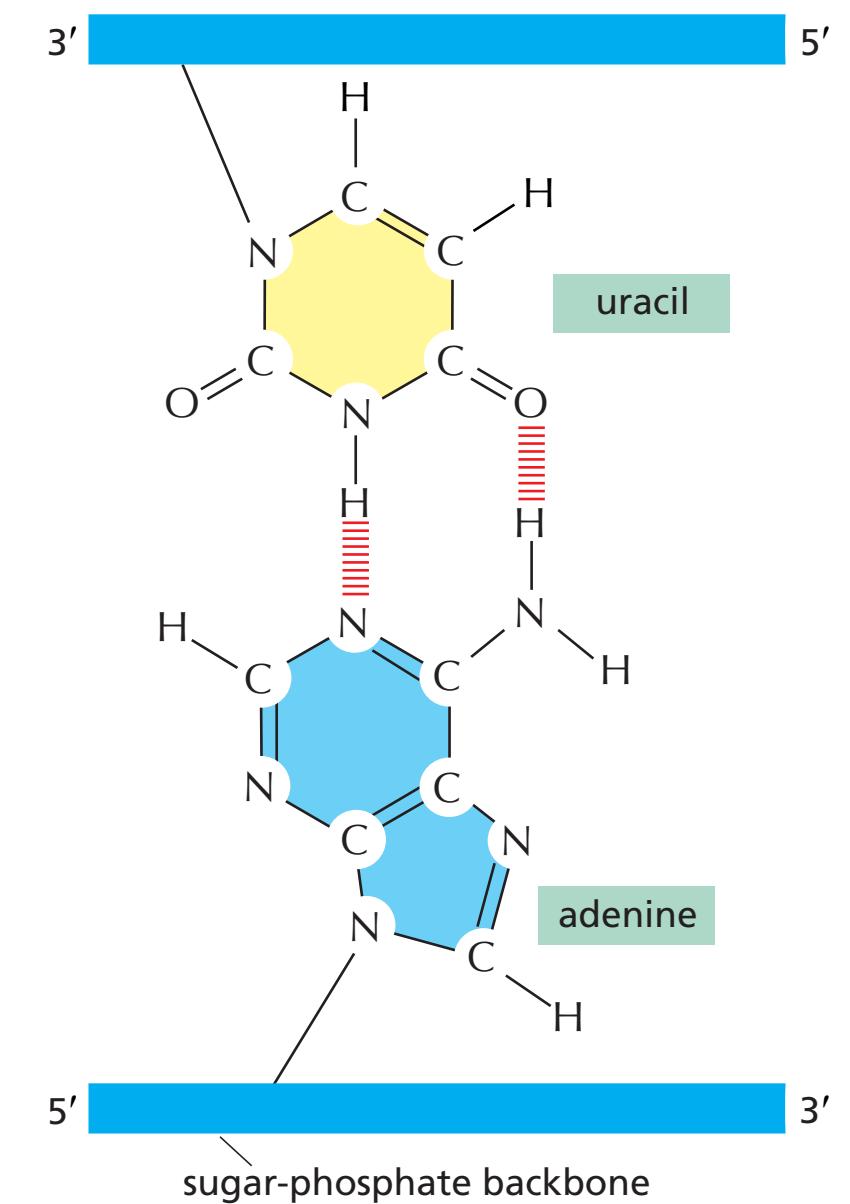
- nucleotides are **ribonucleotides** rather than deoxyribonucleotides
- contains **uracil** instead of thymine base (which also forms H-bonds with adenine)



RNA molecules are single-stranded

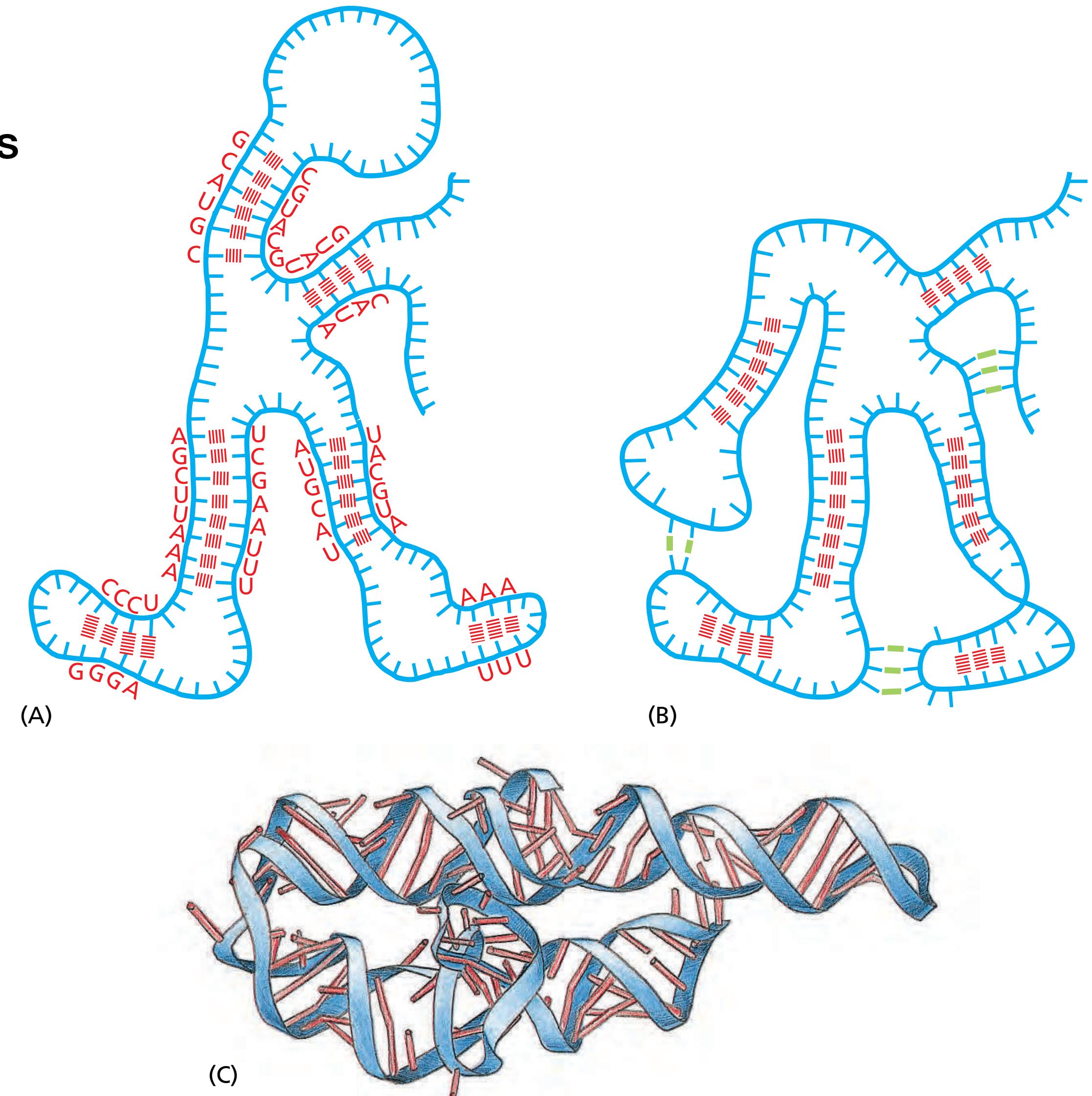
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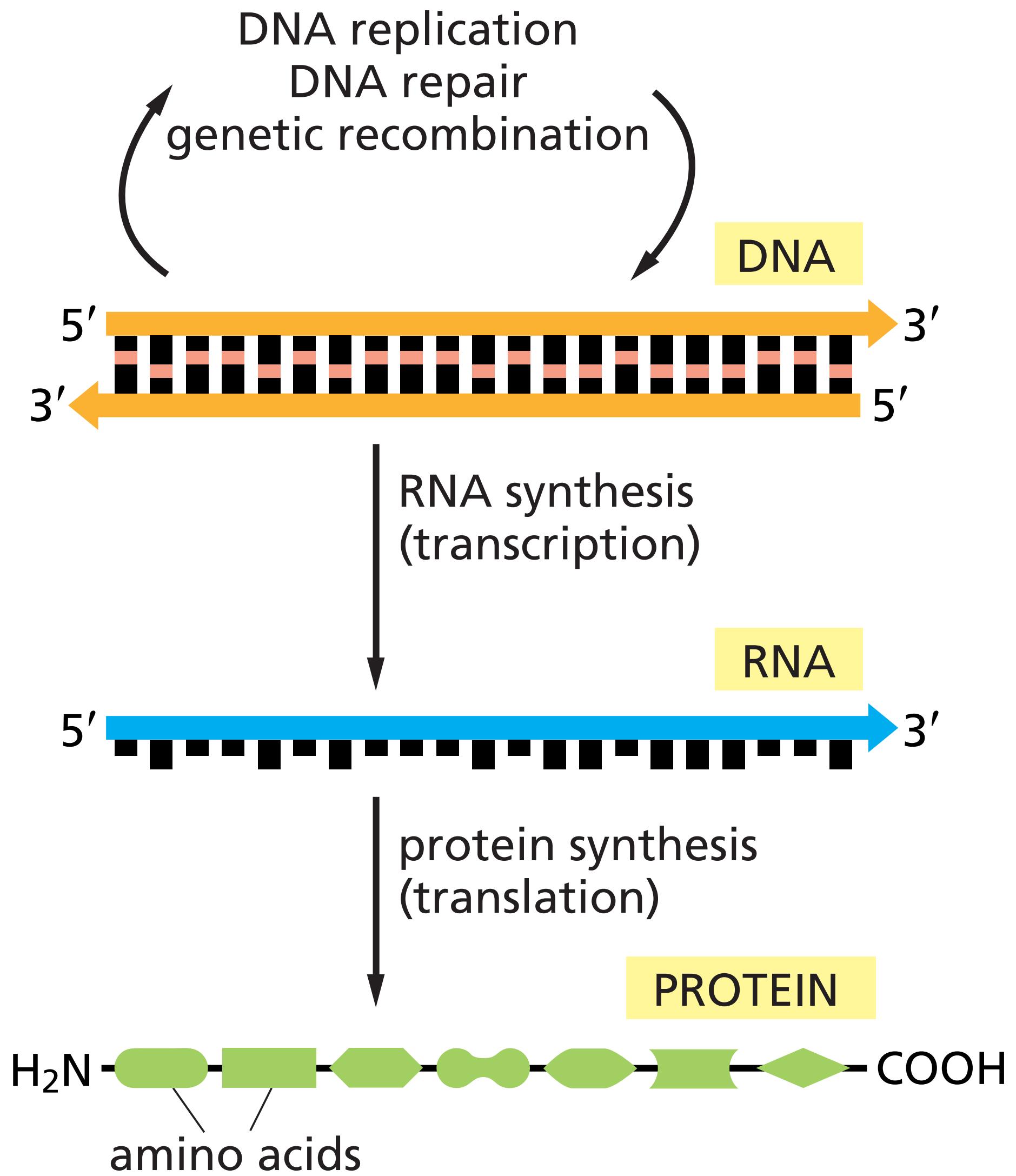
RNA molecules are single-stranded

- RNA is single-stranded and **folds into particular shapes**



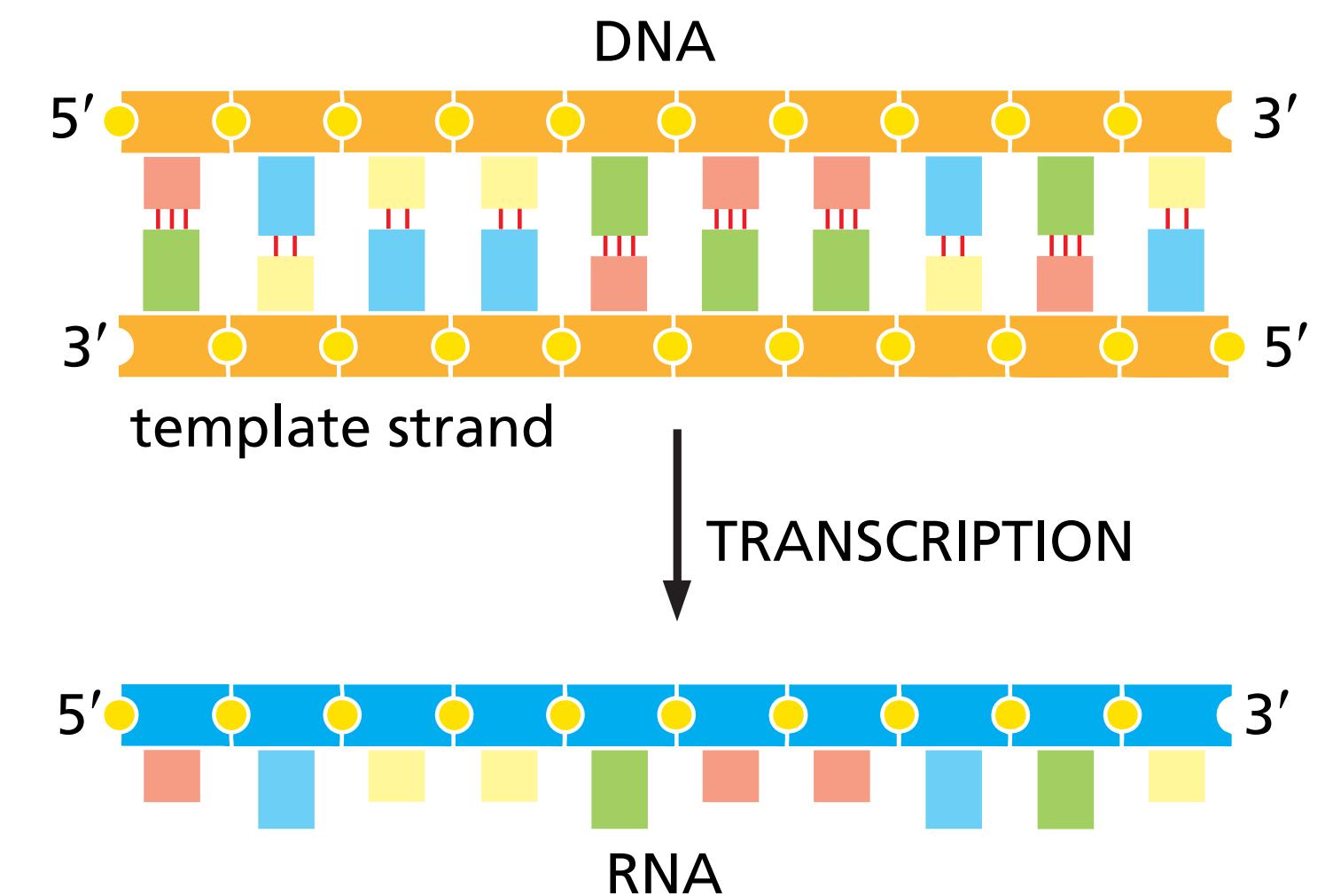
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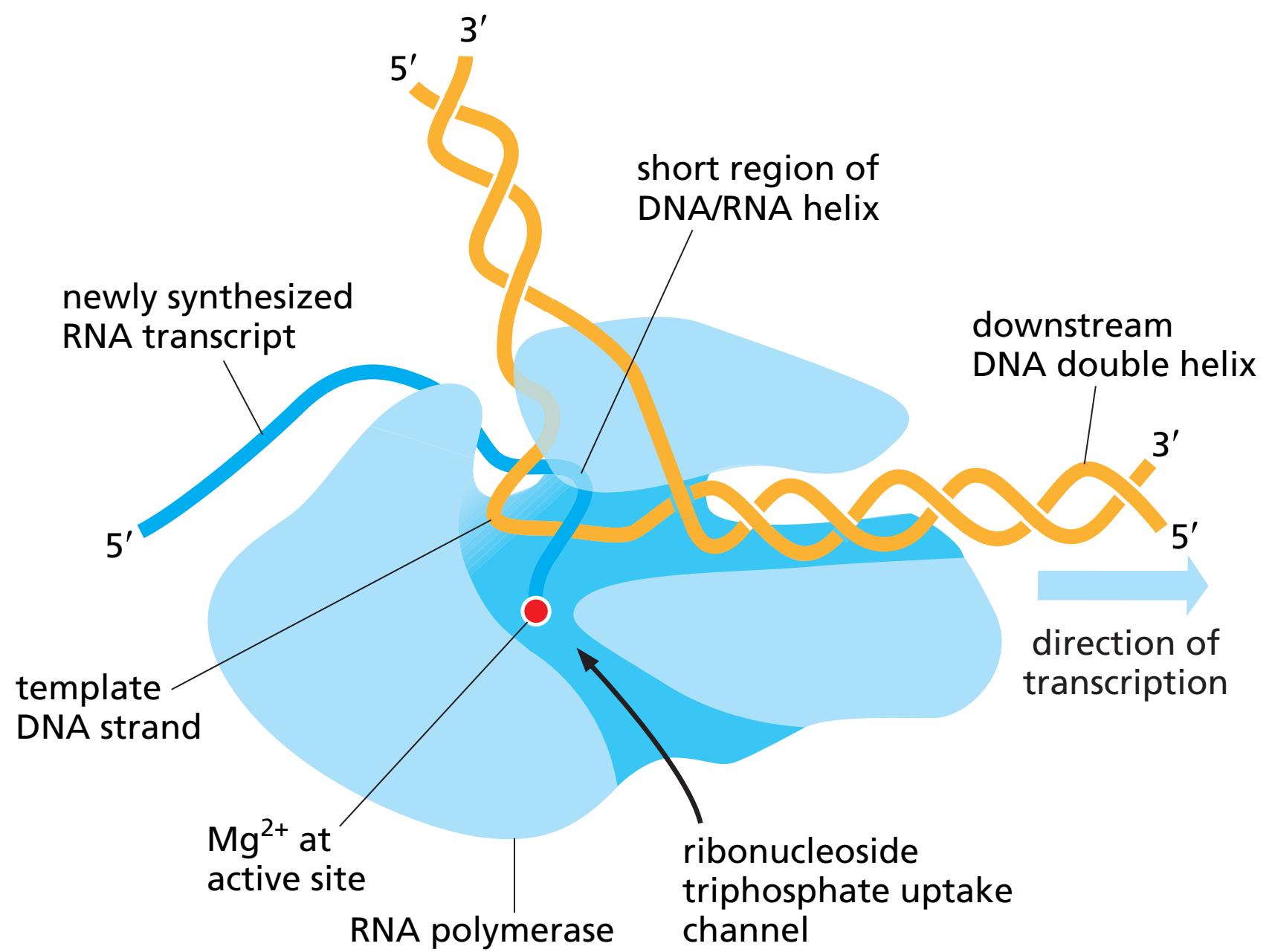


Transcription

- RNA **complementary** to one strand of DNA
- Starts with the **opening and unwinding of DNA** to expose the bases of each strand
- One of the strands then acts as a **template** for synthesis of an RNA molecule (complementary base-pairing)
- When a good match is made, the **ribonucleotide is covalently** linked to the growing RNA = transcript



Transcription



- The growing RNA is extended one nucleotide at a time **from 5' to 3'**
- The substrates are **ribonucleoside triphosphates** (ATP, CTP, UTP, GTP)

Transcription

Transcription

Example: ribosomal RNAs



- **Many RNA copies** from the same gene can be made in a short time
- New molecules can be made **before the previous one is completed**

RNA polymerase vs. DNA polymerase

- **RNA polymerase** catalyzes essentially the same chemical reaction as **DNA polymerase**
- Differences between the enzymes:
 - links **ribonucleotides** and not **deoxyribonucleotides**
 - can start an RNA chain without a **primer** (less accurate)
 - makes 1 **mistake** every 10^4 nucleotide (as compared to 10^7 for DNA polymerase)
 - the RNA polymerase that begins an RNA molecule finishes it **without dissociating** from the DNA
- **RNA polymerase** have a modest proof-reading mechanism: if an incorrect ribonucleotide is added, the active site can perform an excision reaction and the nucleoside is released
- DNA- and RNA-polymerase are quite **different structurally**, such enzymes have arisen twice during early evolution of the cells

Cells produce different categories of RNAs

TABLE 6–1 Principal Types of RNAs Produced in Cells

| Type of RNA | Function |
|-------------|--|
| mRNAs | Messenger RNAs, code for proteins |
| rRNAs | Ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis |
| tRNAs | Transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids |
| snRNAs | Small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA |
| snoRNAs | Small nucleolar RNAs, help to process and chemically modify rRNAs |
| miRNAs | MicroRNAs, regulate gene expression by blocking translation of specific mRNAs and cause their degradation |
| siRNAs | Small interfering RNAs, turn off gene expression by directing the degradation of selective mRNAs and the establishment of compact chromatin structures |
| piRNAs | Piwi-interacting RNAs, bind to piwi proteins and protect the germ line from transposable elements |
| lncRNAs | Long noncoding RNAs, many of which serve as scaffolds; they regulate diverse cell processes, including X-chromosome inactivation |

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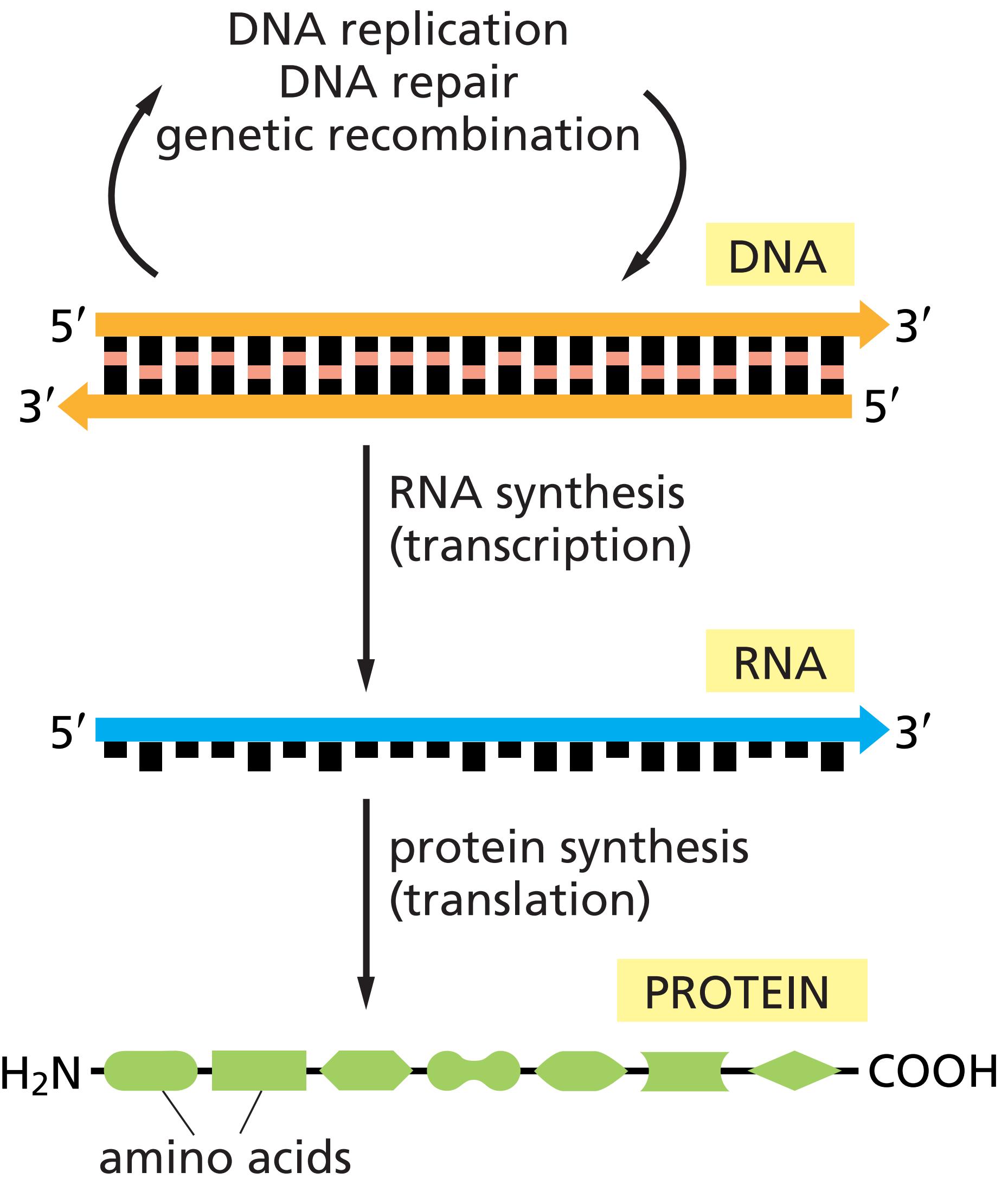
- >10.000 in humans
- enzymatic, structural or regulatory function
- many with mysterious function

Transcription unit

- Each transcribed segment of DNA is a **transcription unit**
- In Eukaryotes it typically carries the information of **one gene**
- In bacteria, a set of **adjacent genes** is often transcribed together, the mRNA carries the information for several distinct proteins

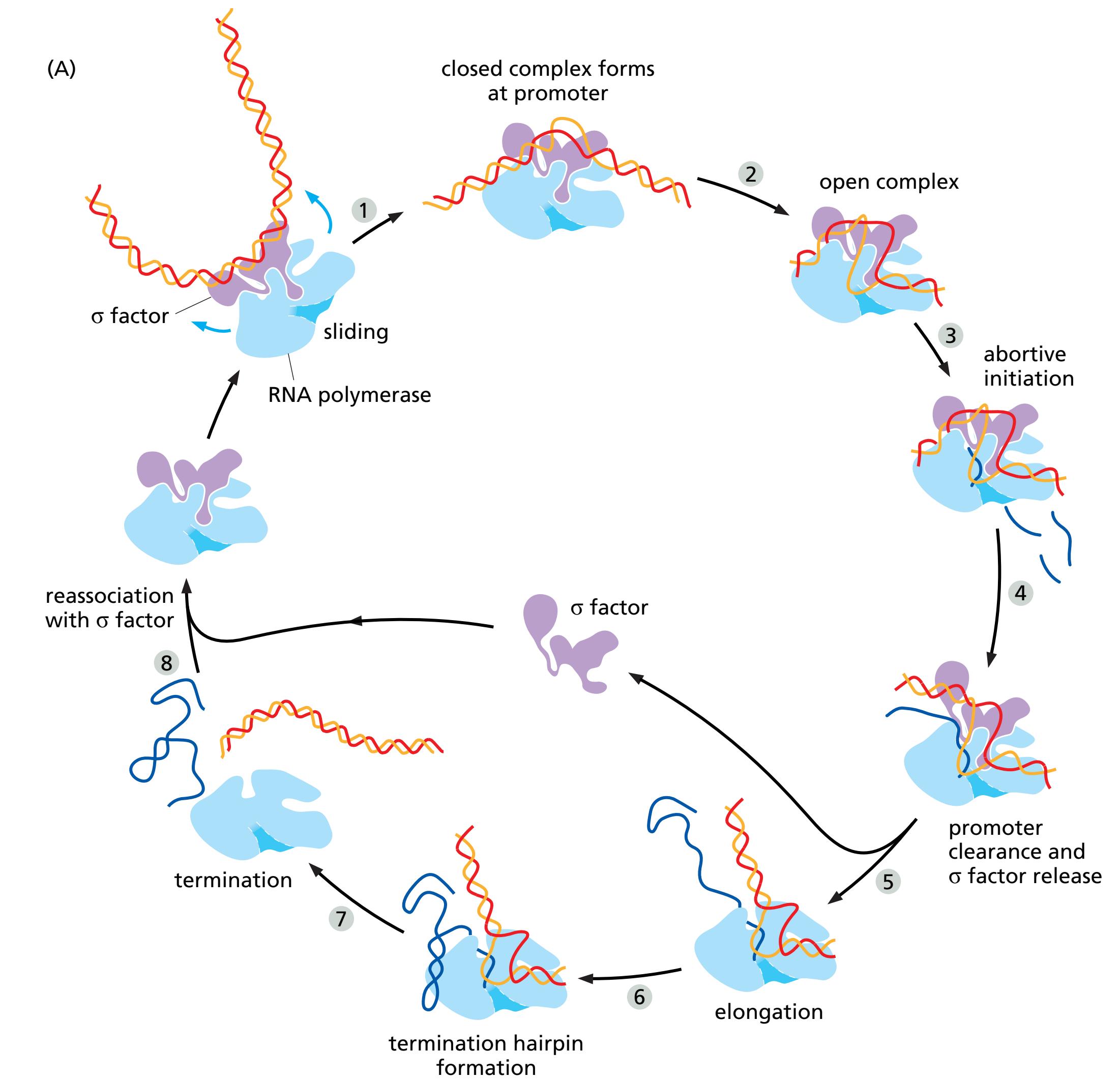
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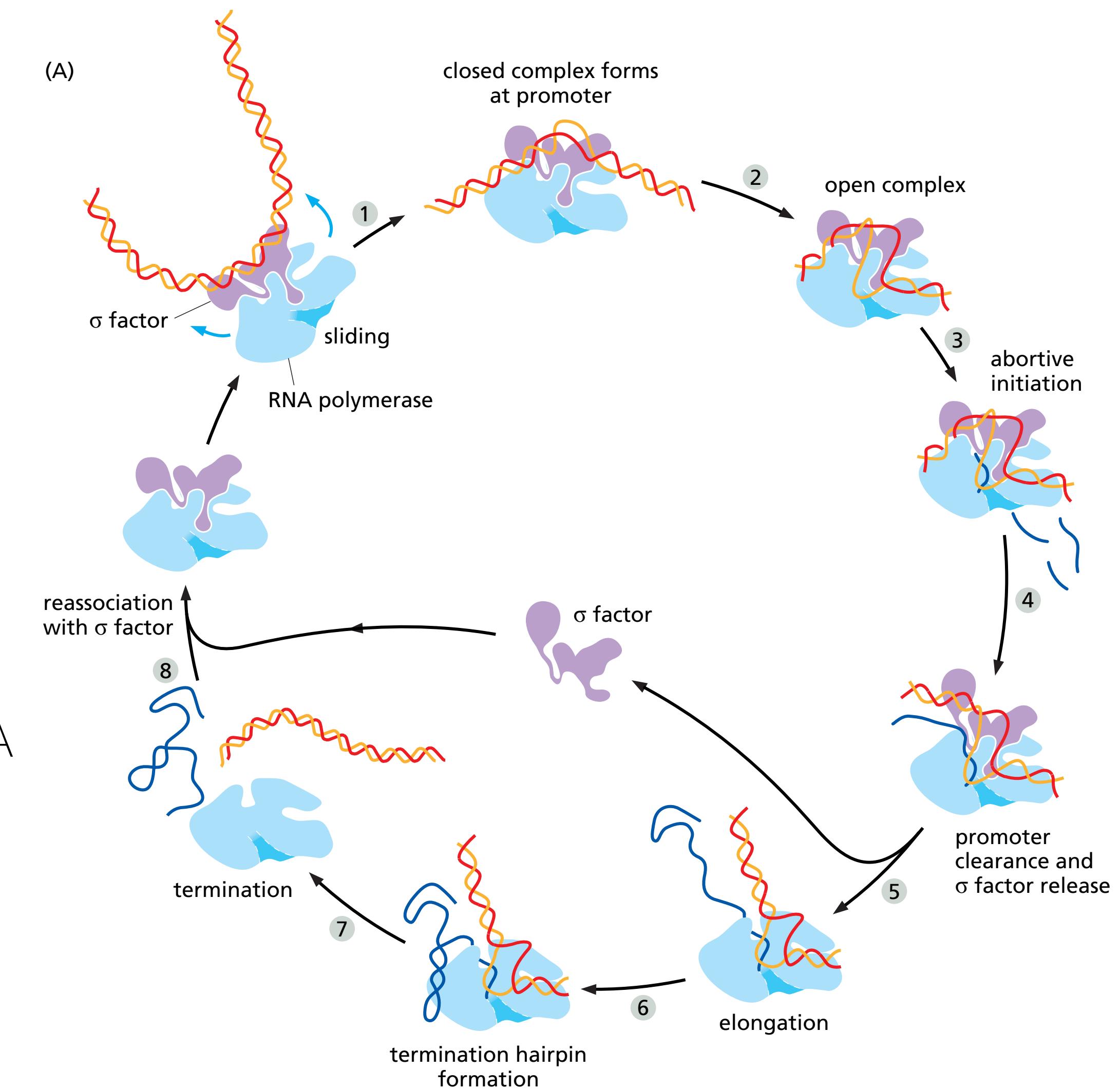
Where to start?

- The RNA polymerase has to know where to start and where to end
- In **bacteria**:
 - **sigma factor** (additional subunit of the polymerase)
 - when they find a **promoter** (=starting point of RNA synthesis), the RNA polymerase binds tightly
 - opens up the double helix =transcription bubble
 - sigma factor stabilises one of the strand and the other serves as template



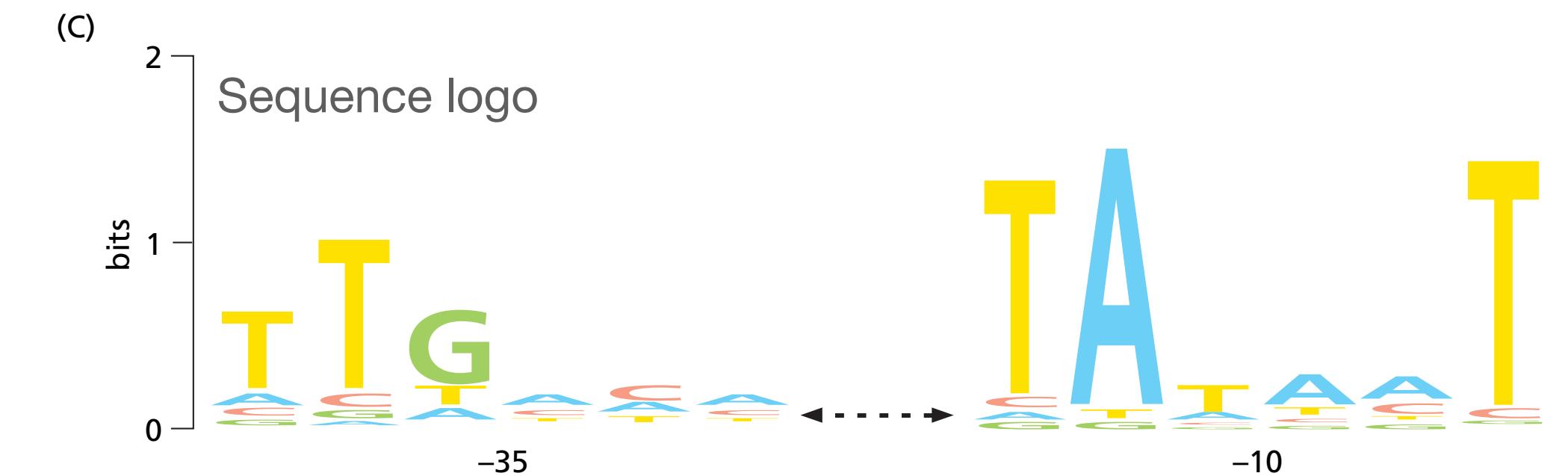
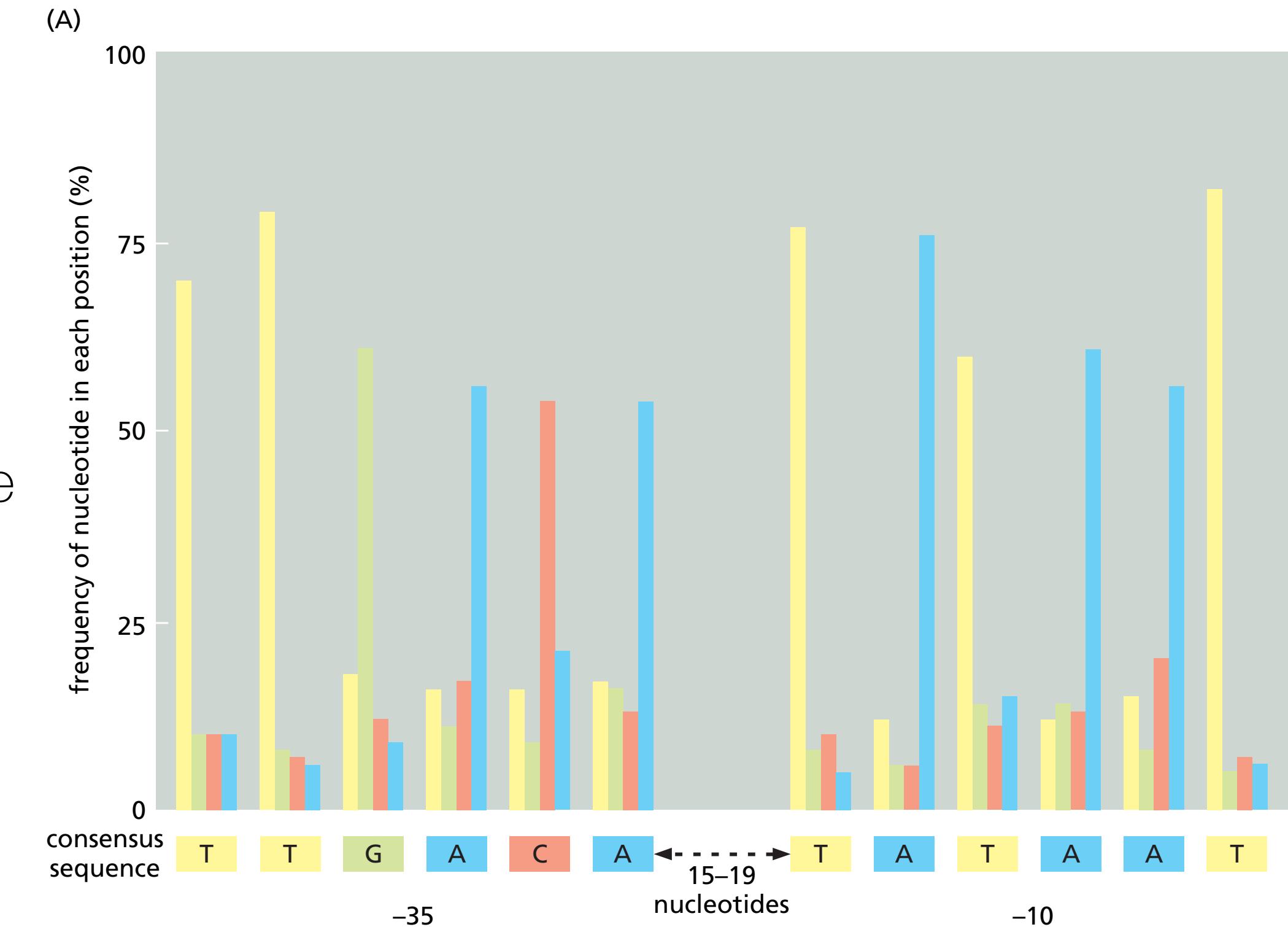
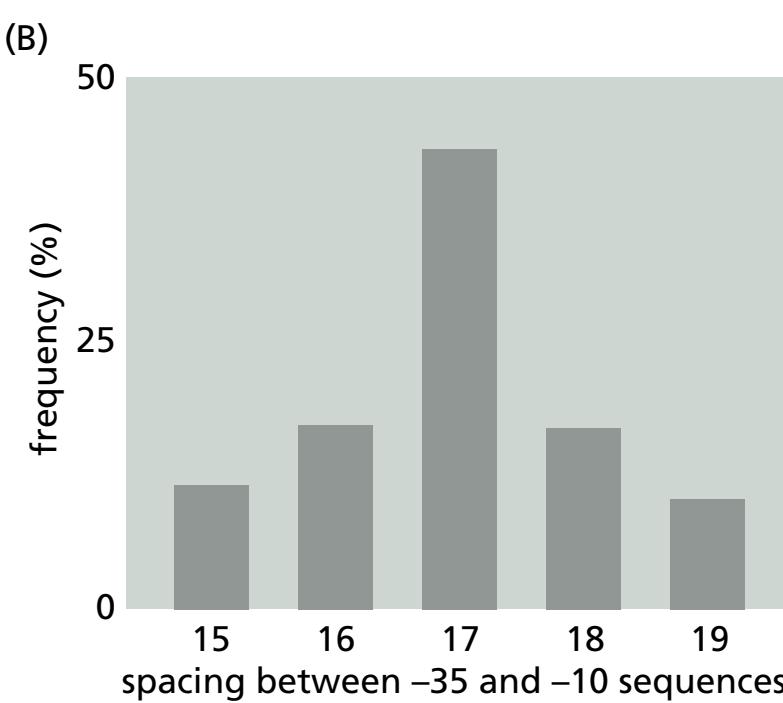
Where to start?

- In bacteria:
 - **abortive initiation** when the RNA polymerase remains bound to the promoter, which creates a stress
 - RNA polymerase **releases** itself from the promoter and discards the sigma factor
 - RNA polymerase **moves down along DNA** and synthesizes RNA
 - RNA polymerase encounters a **terminator**, it releases the RNA molecule and the DNA template
 - In bacteria, a **terminator** is a string of A-T pairs preceded by a twofold symmetric DNA sequence which forms a hairpin when transcribed into RNA



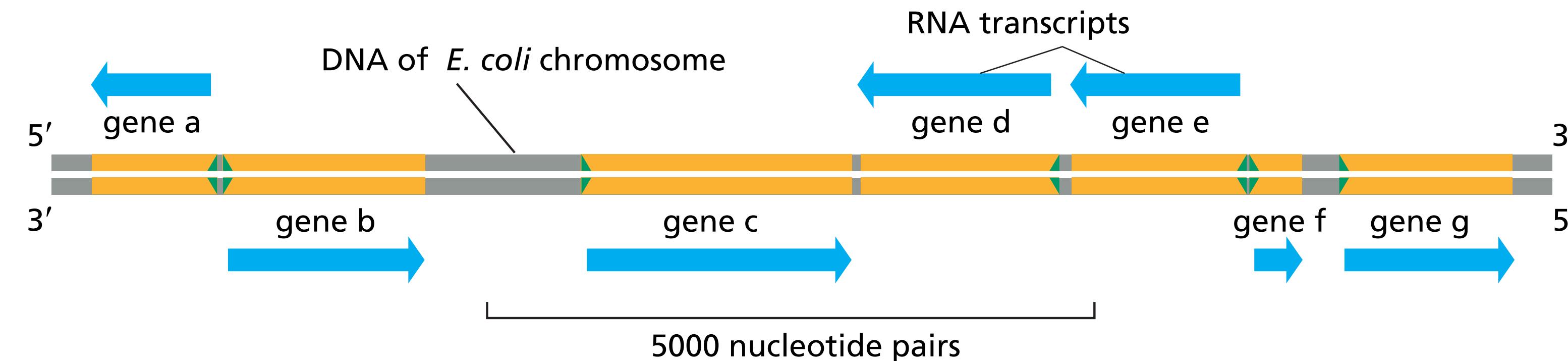
Where to start?

- **Initiation and termination signals** are sometimes difficult to recognise
- Comparison of many **bacterial promoters** shows a high degree of variation although they are recognised by the sigma factor (-10 and -35 sequences)
- **Consensus sequence** = common features



Where to start?

- **Promoter sequences are asymmetric** so the RNA polymerase can only bind in one direction
- The RNA polymerase only synthesises RNA in the **5' to 3' direction**
- The promoter specifies which **strand** to take as template



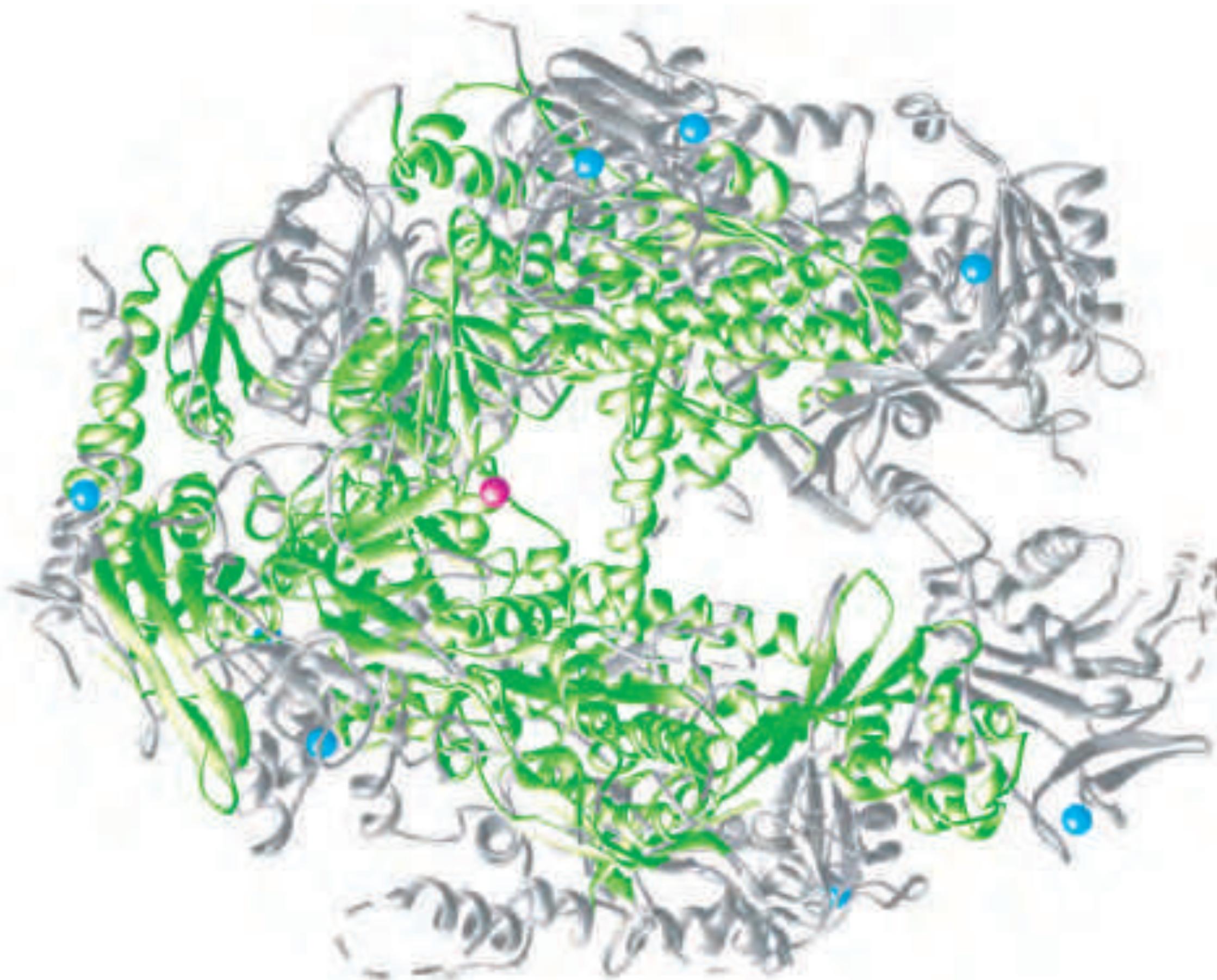
And in eukaryotes?

- **Three types** of RNA polymerase (I, II and III)
- **Structurally** similar but transcribe different **categories of genes**

TABLE 6–2 The Three RNA Polymerases in Eukaryotic Cells

| Type of polymerase | Genes transcribed |
|--|---|
| RNA polymerase I | 5.8S, 18S, and 28S rRNA genes |
| RNA polymerase II | All protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, lncRNA genes, and most snRNA genes |
| RNA polymerase III | tRNA genes, 5S rRNA genes, some snRNA genes, and genes for other small RNAs |
| The rRNAs were named according to their “S” values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA. | |

And in eukaryotes?



- **Structurally** similar to bacterial RNA polymerase
 - BUT require many initiation factors (not just a sigma factor) = **general transcription factors**
 - BUT transcription initiation takes place on DNA that is **packaged into nucleosomes and chromatin**

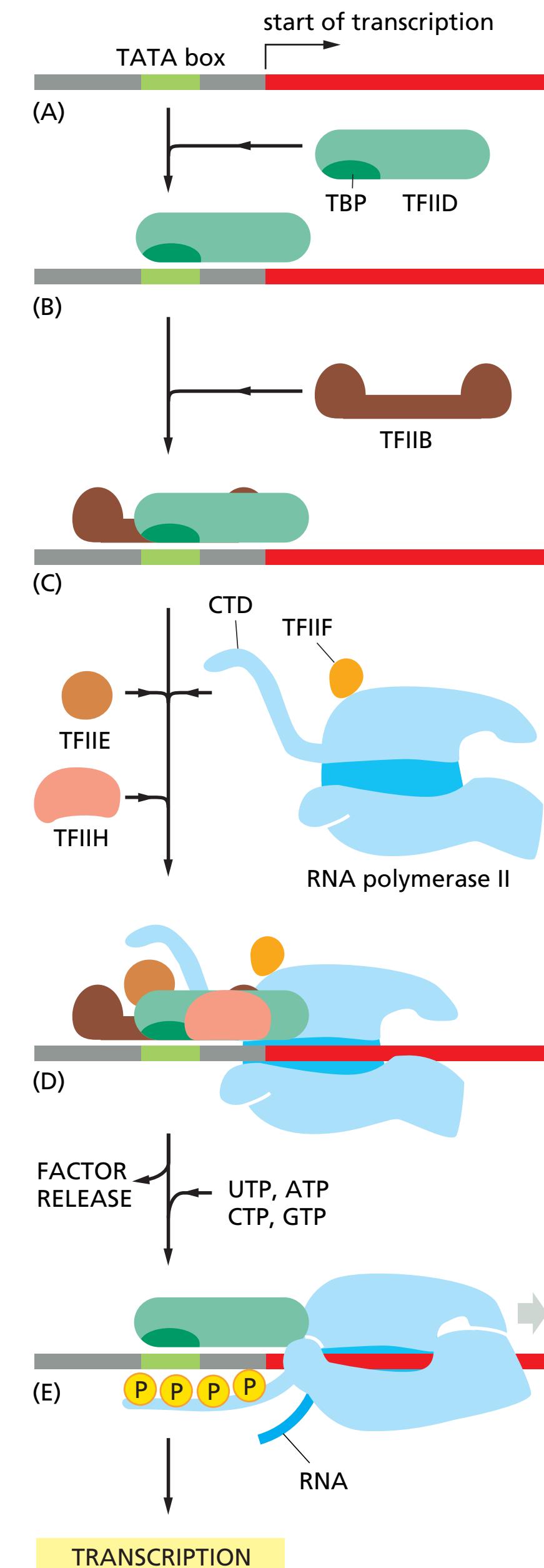
General transcription factors

- Help **position** eukaryotic RNA polymerase correctly at the promoter
- Aid in **pulling the two strands** of DNA apart
- **Release** the RNA polymerase from the promoter to start its elongation mode
- Are needed at **nearly all promoters** used by RNA polymerase II
- Denoted TFIIA, B, C, ... (for transcription factor for polymerase II)

Transcription initiation

TABLE 6–3 The General Transcription Factors Needed for Transcription Initiation by Eukaryotic RNA Polymerase II

| Name | Number of subunits | Roles in transition initiation |
|--|--------------------|--|
| TFIID TBP subunit TAF subunits | 1 ~11 | Recognizes TATA box Recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP |
| TFIIB | 1 | Recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription |
| TFIIF | 3 | Stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH |
| TFIIE | 2 | Attracts and regulates TFIIH |
| TFIIH | 9 | Unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter |
| TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain. | | |



Transcription initiation

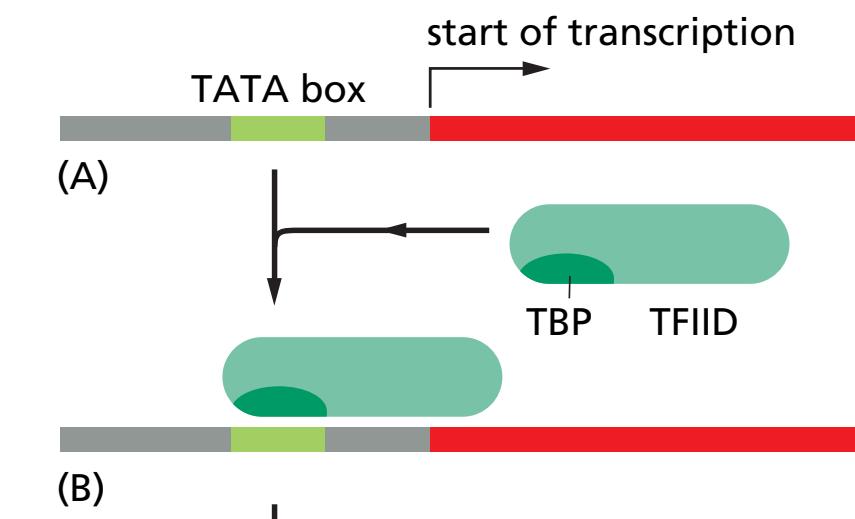
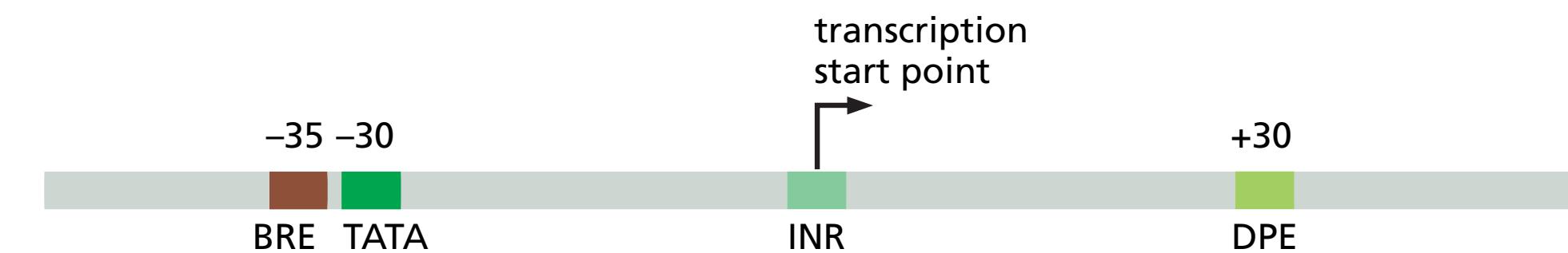


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| TAF subunits | ~11 | Recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP |



- **TATA box:** DNA sequence of T and A; typically 25 nt upstream of the transcription start
- **most important one** for RNA polymerase II

| element | consensus sequence | general transcription factor |
|---------|-------------------------|------------------------------|
| BRE | G/C G/C G/A C G C C | TFIIB |
| TATA | T A T A A/T A A/T | TBP subunit of TFIID |
| INR | C/T C/T A N T/A C/T C/T | TFIID |
| DPE | A/G G A/T C G T G | TFIID |

Transcription initiation

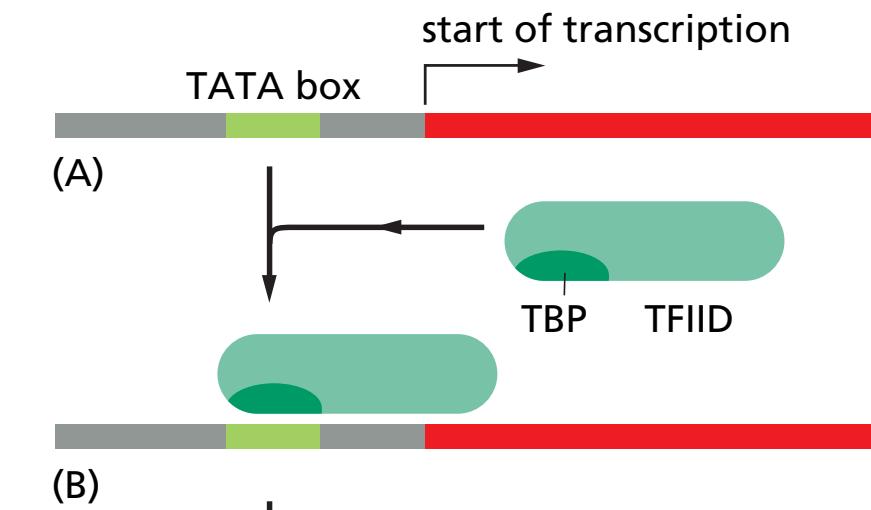
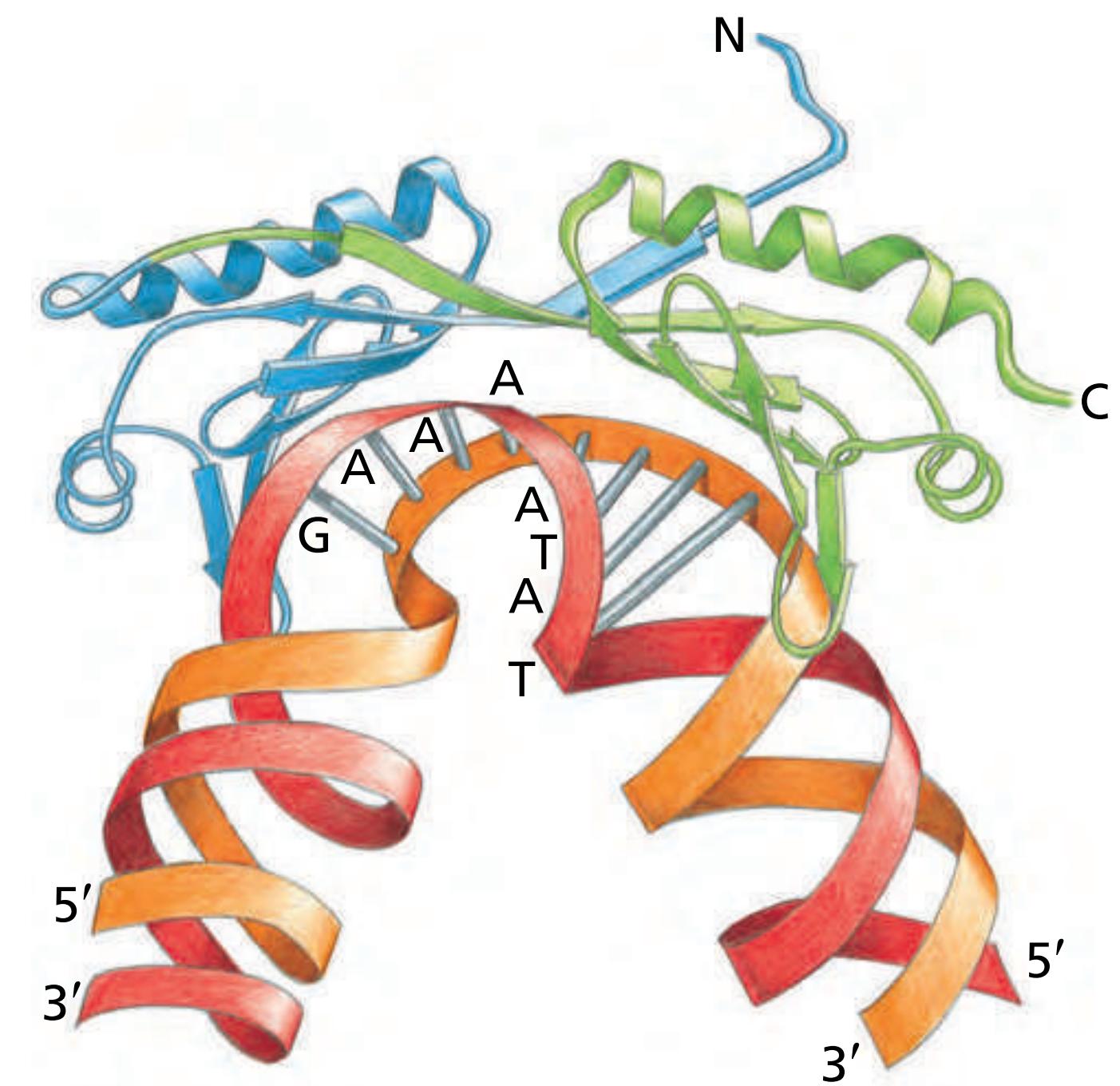


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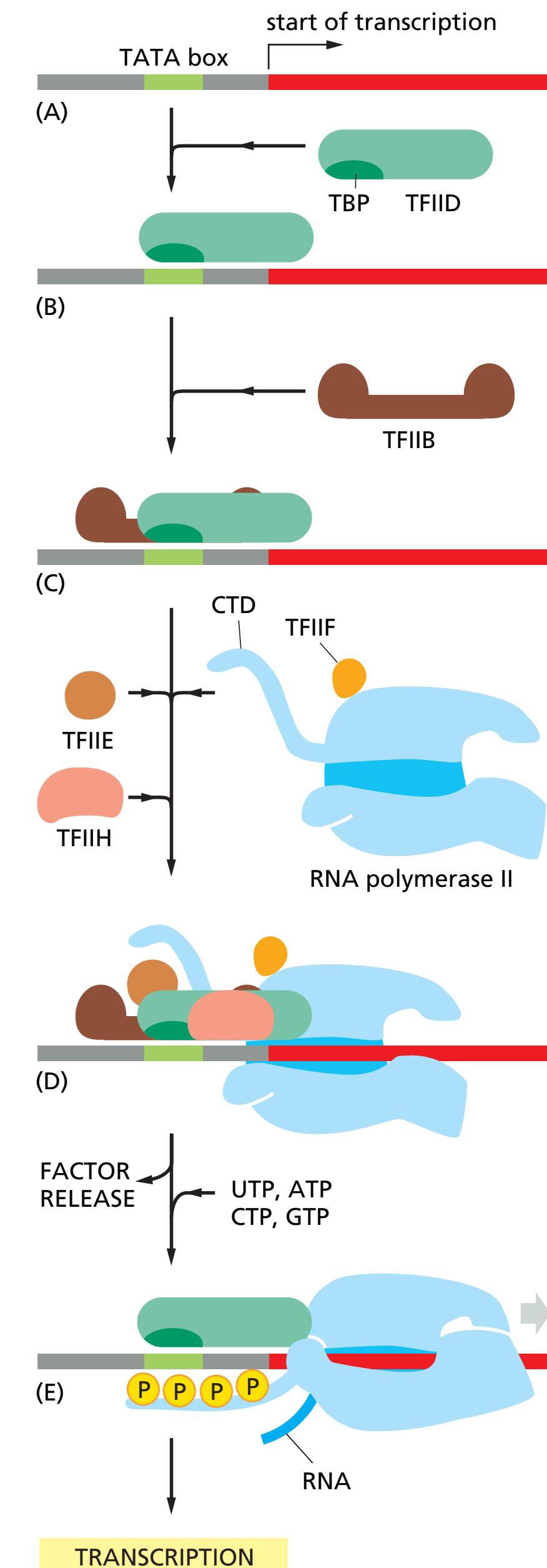


- TBP binding creates a **distortion in the DNA** of the TATA box, which allows the assembly of other factors (to form a complete **transcription initiation complex**)

Transcription initiation

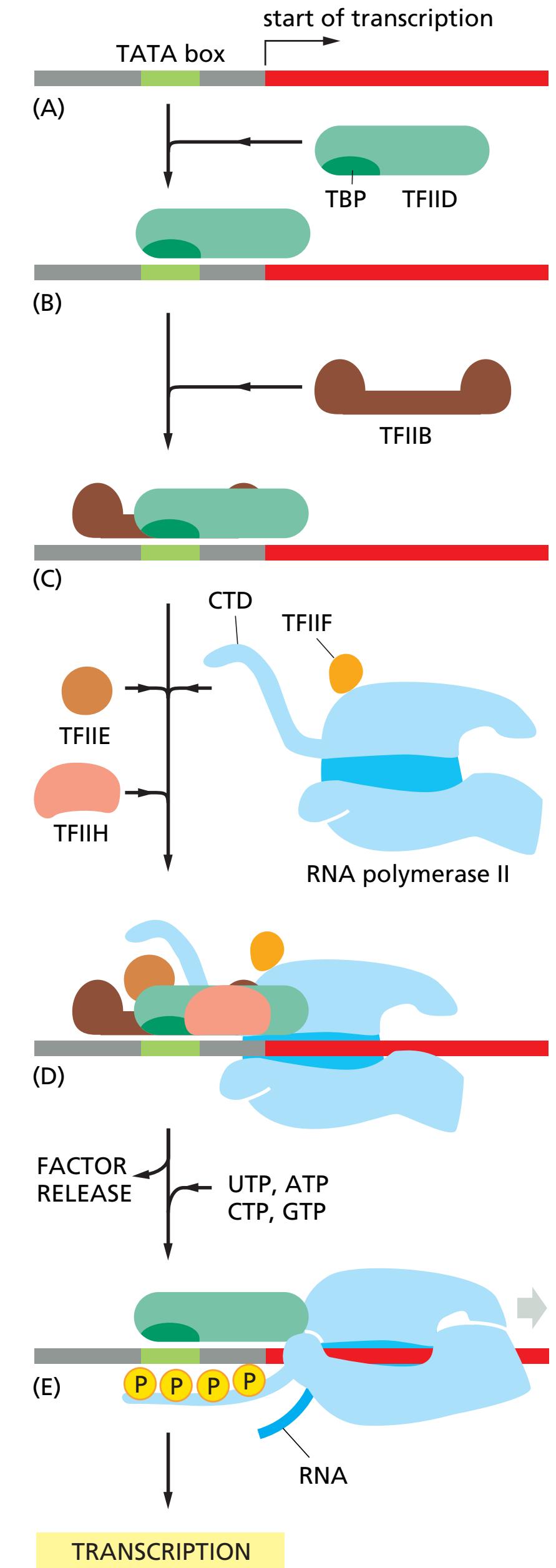
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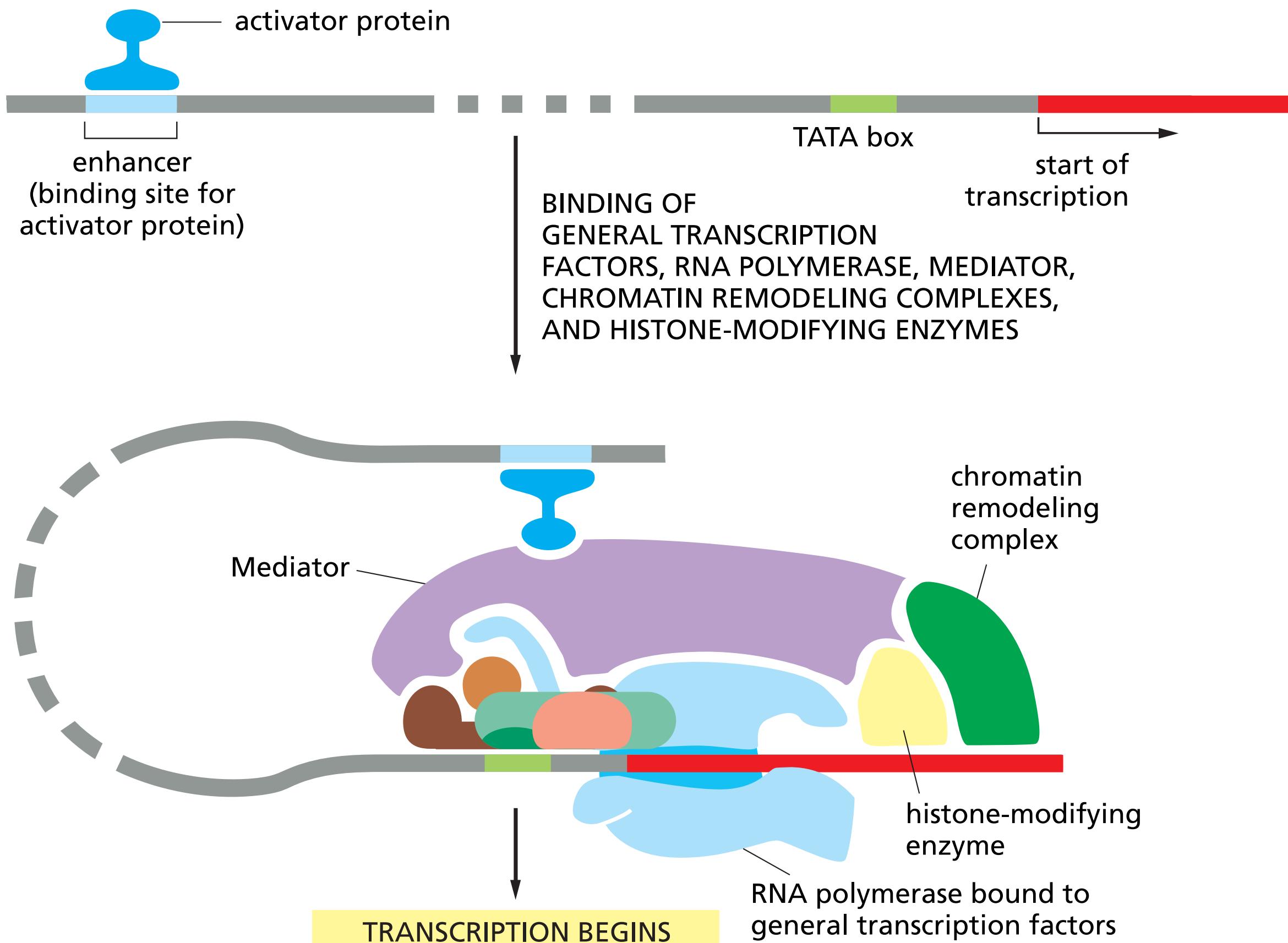
Transcription initiation

- RNA polymerase II must have **access to the template strand** at the transcription start point
- TFIIB contains a DNA helicase, which hydrolyzes ATP and unwinds DNA
- Like for bacteria, RNA polymerase II remains at the **promoter**, synthesizing short RNAs until it goes through **conformational changes** and starts the **elongation phase** of transcription
- A key step in this transition is the addition of **phosphate groups** to the tail of the RNA polymerase (C-terminal domain)
- Once the RNA polymerase II starts elongation, most **general transcription factors are released**



Transcription

Activator, mediator and chromatin-modifying proteins



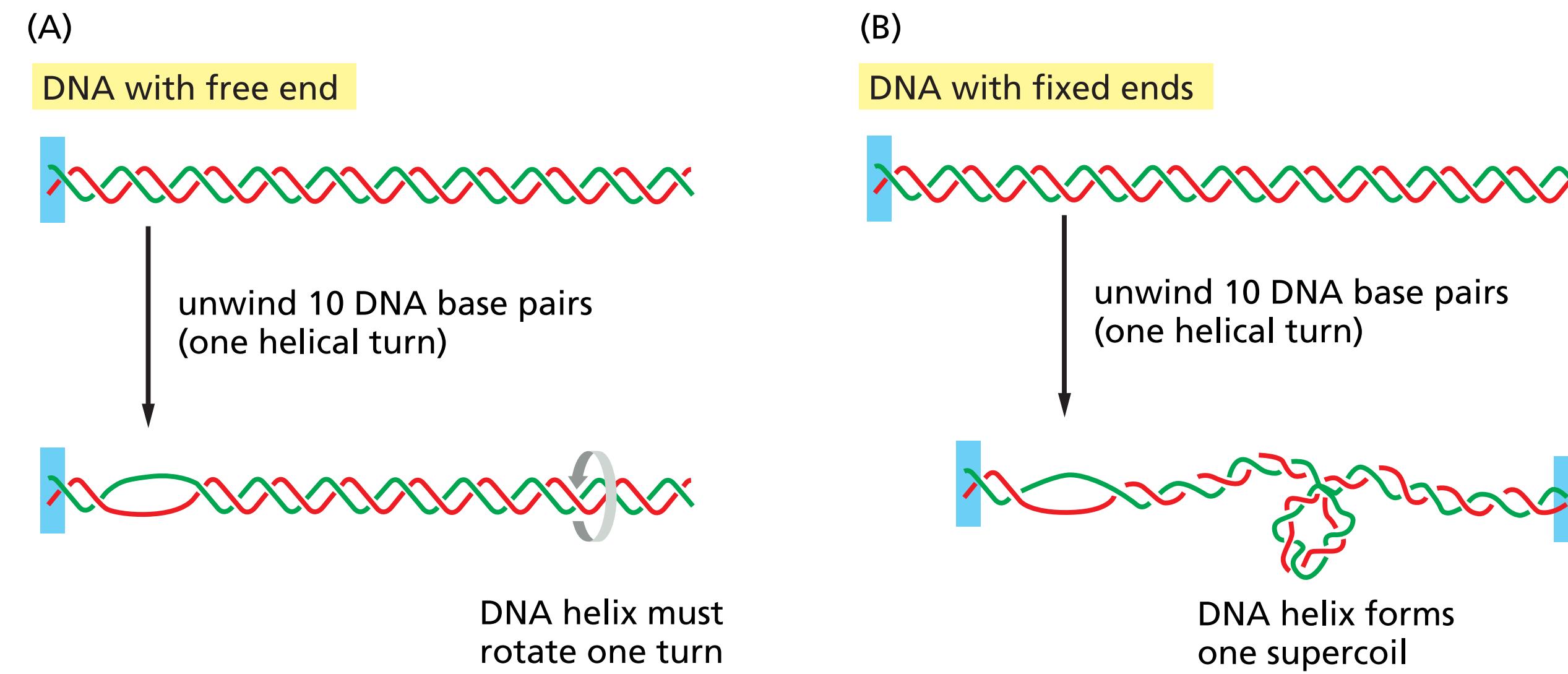
- DNA in eukaryotic cells is **packaged into nucleosomes and complex chromatin structure**
- Need of **additional proteins**
 1. **Transcriptional activators** are proteins that bind to specific DNA sequences called **enhancers** that help attract the RNA polymerase (discussed later)
 2. **Mediator** is a large protein complex that allows the **communication** between the activator and RNA polymerase complex
 3. **Chromatin-remodelling complex** and **histone-modifying enzymes**

Elongation factors

- Once it starts elongating, RNA polymerases are associated with a series of **elongation factors** (both in bacteria and eukaryotic cells)
- These factors are proteins that **decrease the likelihood that RNA polymerase dissociates** from the DNA before it reaches the end of the gene
- Some of these proteins also help **with chromatin remodelling and histone modifications** in eukaryotes

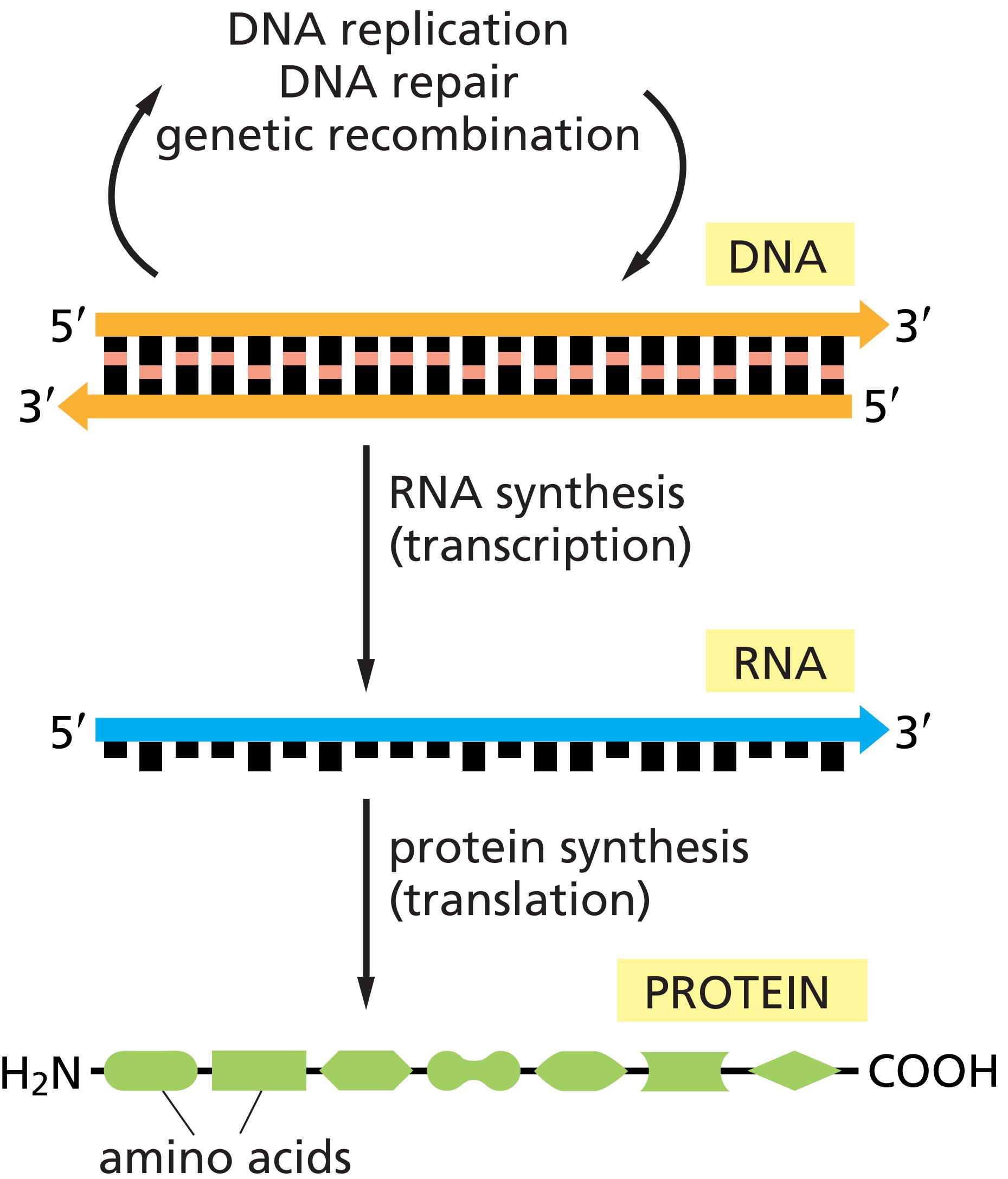
Superhelical tension

- **DNA supercoiling** is the conformation that DNA adopts in response to superhelical tension
- RNA polymerase creates superhelical tension as the ends of the stretch of DNA are anchored
- In eukaryotes, this might help with **DNA unwrapping** from nucleosome
- Resolved by **DNA topoisomerases (DNA gyrase)**



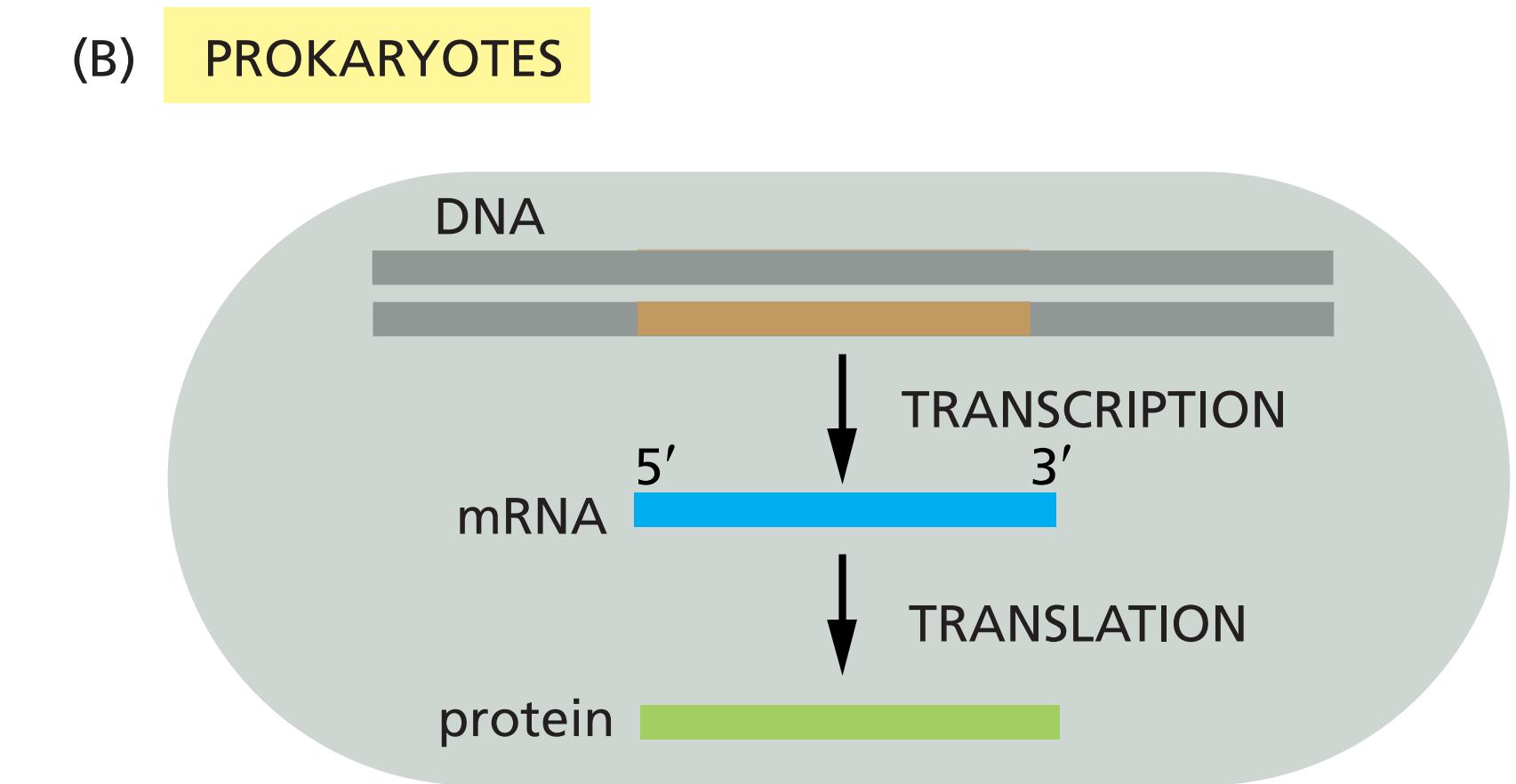
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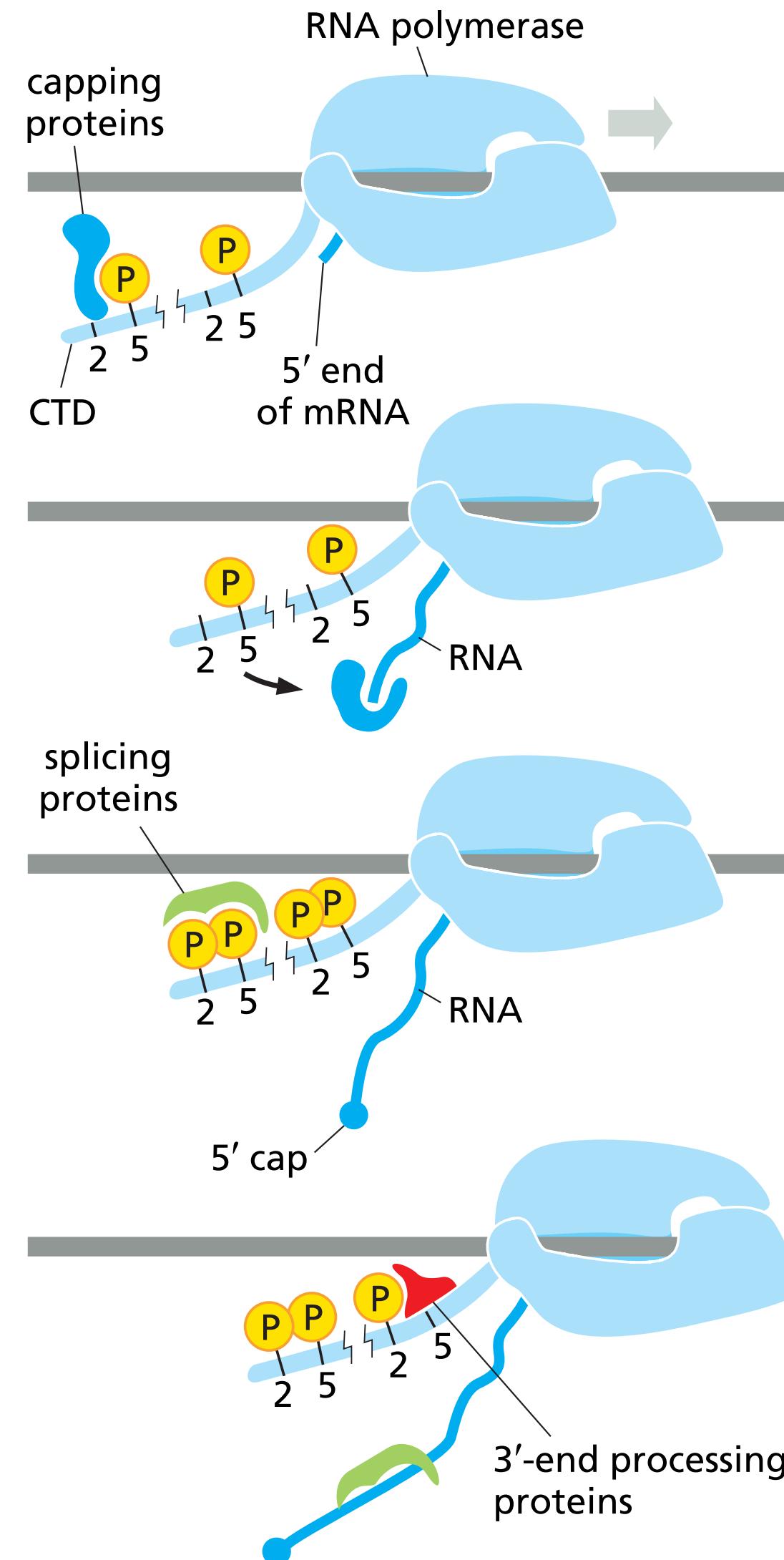


RNA processing

- In **bacteria**, mRNAs are synthesized by the RNA polymerase starting and stopping at specific spots

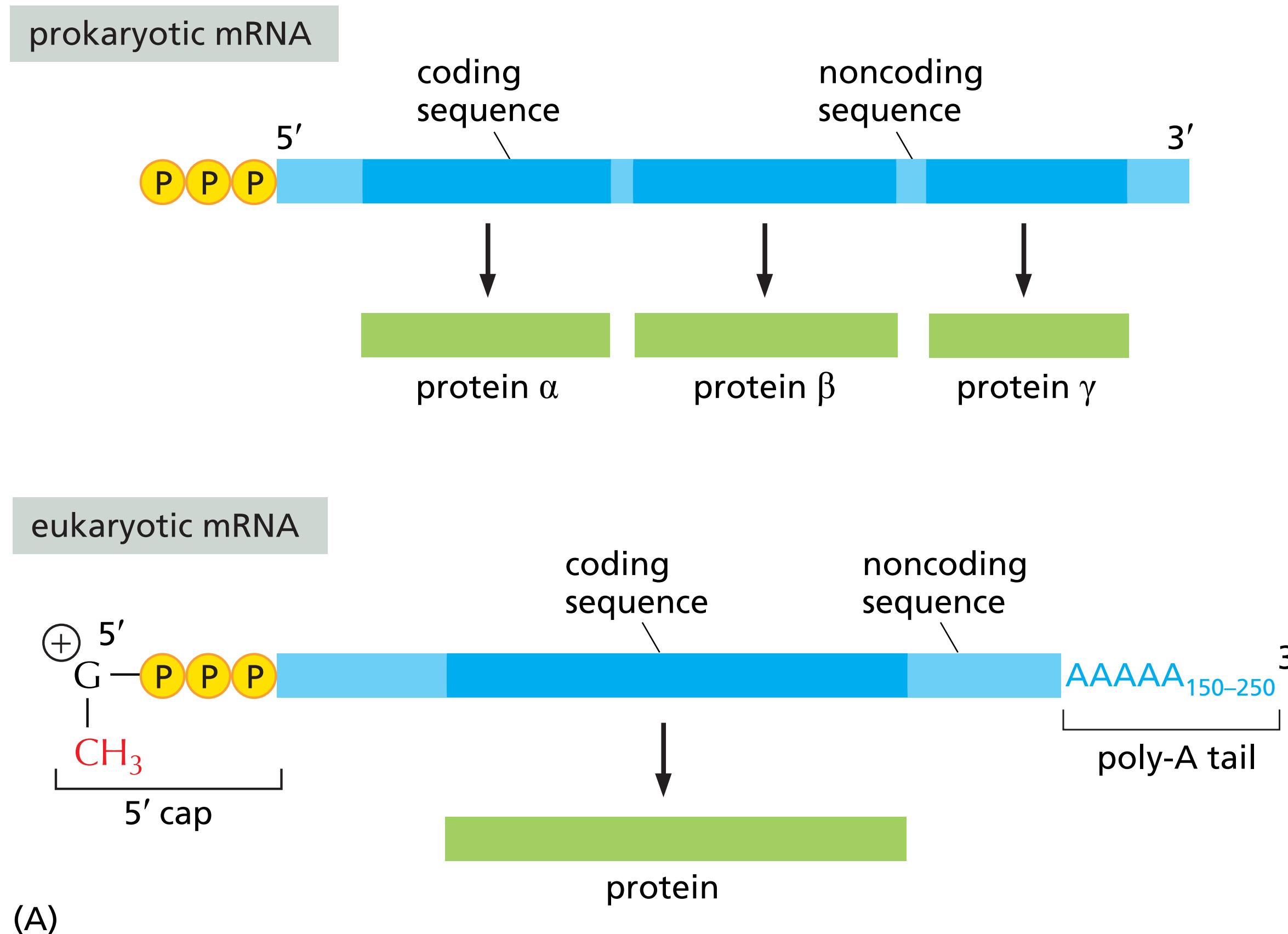


RNA processing



- In **Eukaryotes**, transcription is only the **first step** of producing a mature mRNA
- Other steps are **modification of the ends of the RNA** and the removal of introns sequences (**RNA splicing**)
- All **coupled** with transcription elongation
- During transcription initiation, the polymerase tail is **phosphorylated** at its C-t domain
- This allows the association of a **new set of proteins** to the RNA polymerase

RNA ends modifications



- **Both ends** are modified
 - **capping** of the 5' end
 - **polyadenylation** of the 3' end
- Allows the cell to assess if both ends are there and the **mRNA is intact** before being exported out of the nucleus for translation

RNA capping

- **First modification** of eukaryotic pre-mRNAs

- After ~25 nt of RNA are produced, the 5' end of the RNA is modified by addition of a **cap**

- The cap consists of a **modified guanine nucleotide**

- **3 enzymes:**

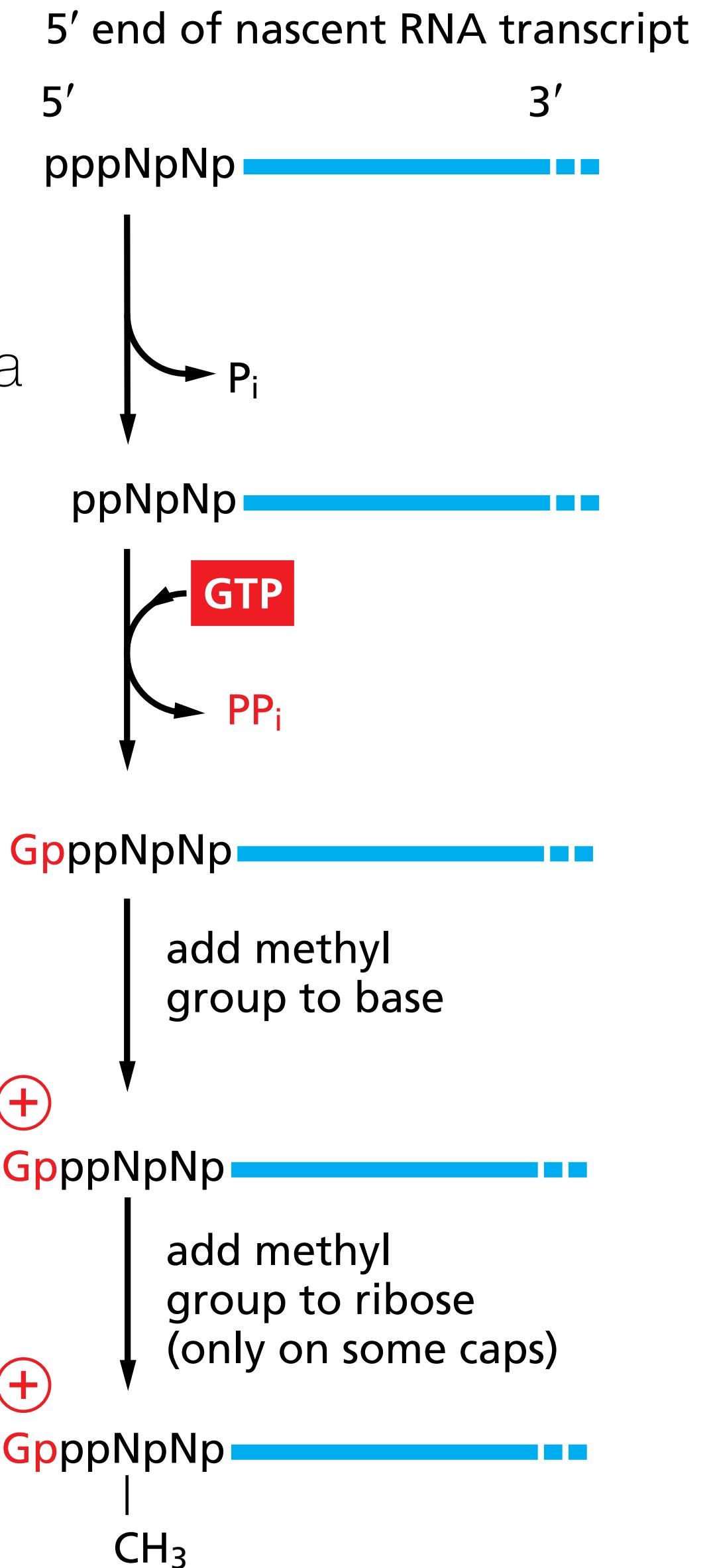
1. **Phosphatase** removes a phosphate from the 5' end of the RNA

2. A **guanyl transferase** adds a GMP in a reverse linkage (5' ot 5')

3. A **methyl transferase** adds a methyl group to the guanosine

- All bind to the **RNA polymerase phosphorylated at Ser5** (a modification that happens during transcription initiation)

- The cap helps the cell to **distinguish** between mRNAs and other RNAs



Have a nice day!