Zoo-342 Molecular biology
Lecture 7

Prokaryotic transcription

- In prokaryotes, the enzyme **RNA polymerase** controls transcription of RNA.
- We previously discussed RNA polymerase during DNA replication.
- **Primase**, transcribed a short RNA primer using the DNA as the template.
- The RNA primer is extended in a 5′→3′ direction by DNA polymerase to synthesize the new DNA strand.
- Using DNA as a template, RNA polymerase adds ribonucleotides in a 5′→3′ direction.
- The *E. coli* **RNA polymerase** is composed of a **core enzyme** and a **sigma (σ) factor.**
- The core enzyme is composed of four subunits:
  - Two copies of an α-subunit.
  - One copy of beta (β) subunit.
  - One copy of beta prime (β′) subunit.

Process of prokaryotic transcription:

- The DNA region that RNA polymerase associates with immediately before beginning transcription is known as the **promoter**.
- Promoter is an important part of a gene for expression in both prokaryotes and eukaryotes with some differences.
- RNA polymerase **binds** to the promoter through the **σ-factor**.
- After transcription initiation, the σ-factor is **released** and RNA polymerase core enzymes (two α-subunits, one β-subunit, and one β’ -subunit) carries out RNA elongation.

1. Promoter sequences and transcription initiation:

- The bacterial RNA polymerase molecule binds a promoter region consisting of about **60 bp** of DNA.
- About **10** nucleotides before the **first transcribed base**, is a consensus sequence **5' TATAAT 3'.**
- This sequence is known as a **Pribnow box** (discovered by David Pribnow) (Figure 1).
- The nucleotides in the Pribnow box are **mostly adenines and thymines**, so the region is primarily held together by only two hydrogen bonds per base pair.
- The sequence of the **coding strand** can be used to directly determine the translated amino acid sequence.
- The **coding strand** has the same sequence as the **mRNA**, substituting **U for T** in the DNA.

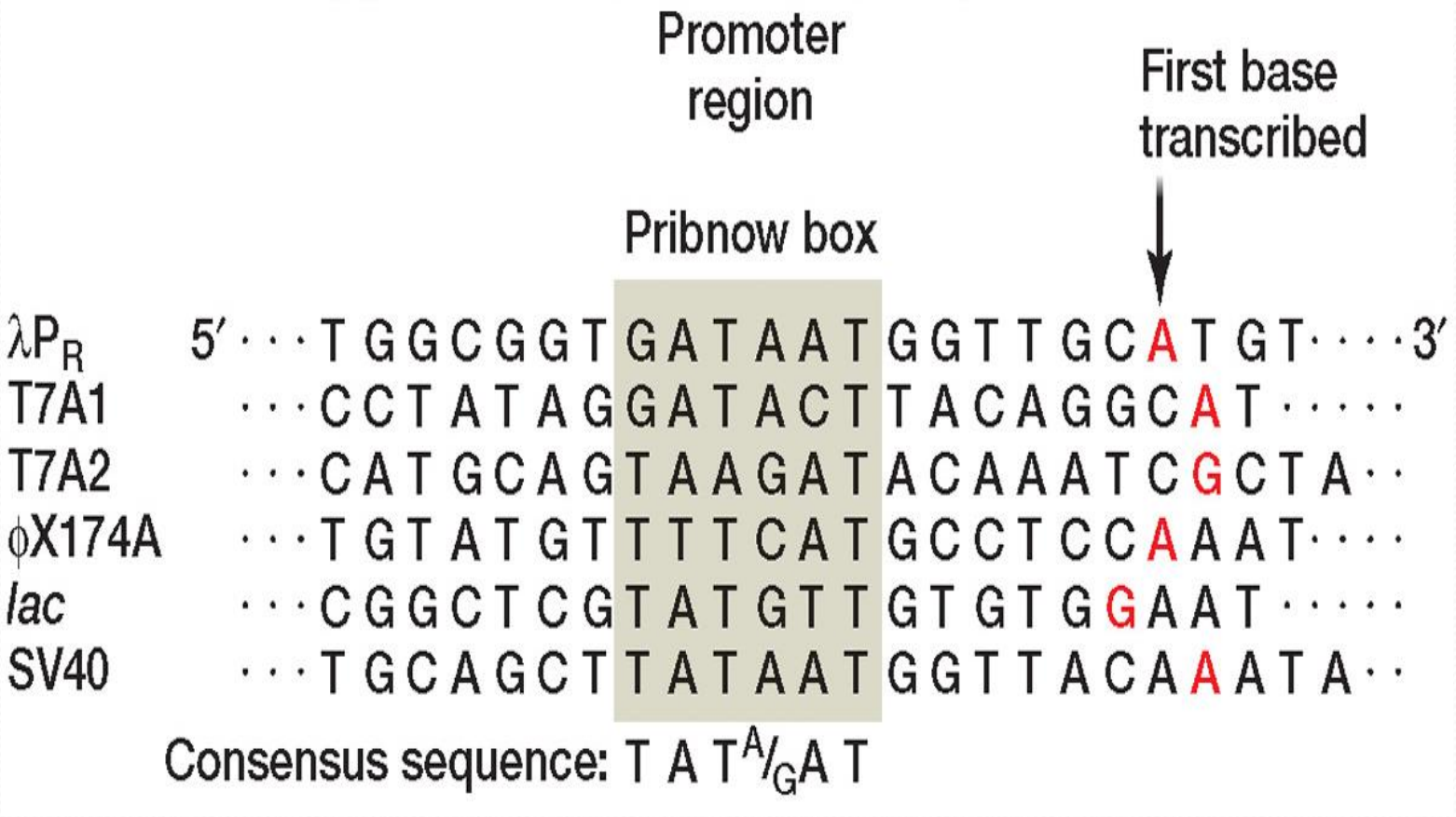

Figure 1: Nucleotide sequences of the nontemplate DNA strand for the bacterial-10 promoter region (Pribnow box). Lambda λ, T7 and ØX174A are bacteriophages. *Lac* is *E. coli* operon, and SV40 is an animal virus.

- Both the **DNA coding strand** and the **mRNA** are complementary to the **template strand** (Figure 2).
- The **first** transcribed base indicates by the number **+1**
- If the transcription is proceeding to the **right**, then the direction to the **right of +1** is called **downstream**.
- If the transcription is proceeding to the **left**, then the direction to the **left** of **negative numbers** is called **upstream**.
- The **Pribnow box** is often referred to as the **-10 sequence** (Figure 3).
- The second consensus sequence is **5' TTGTCA 3'** which is present in many *E. coli* promoter near position -35 and consequently is referred to as the **-35 sequence**.
- The **σ-factor recognizes** and **binds** both the **-35** and the **-10** sequences (Figure 4).
- Mutations in either the -10 or -35 sequences usually decreased the amount of transcription.

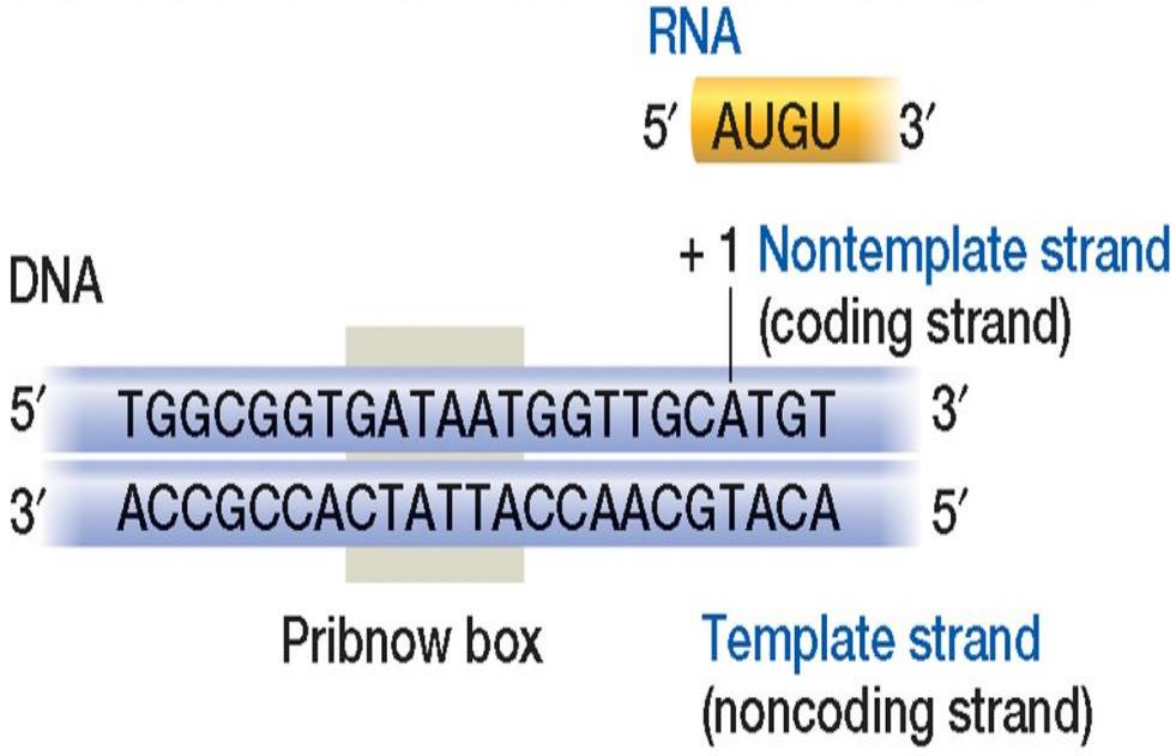

Figure 2: The relationship between the DNA strands and RNA

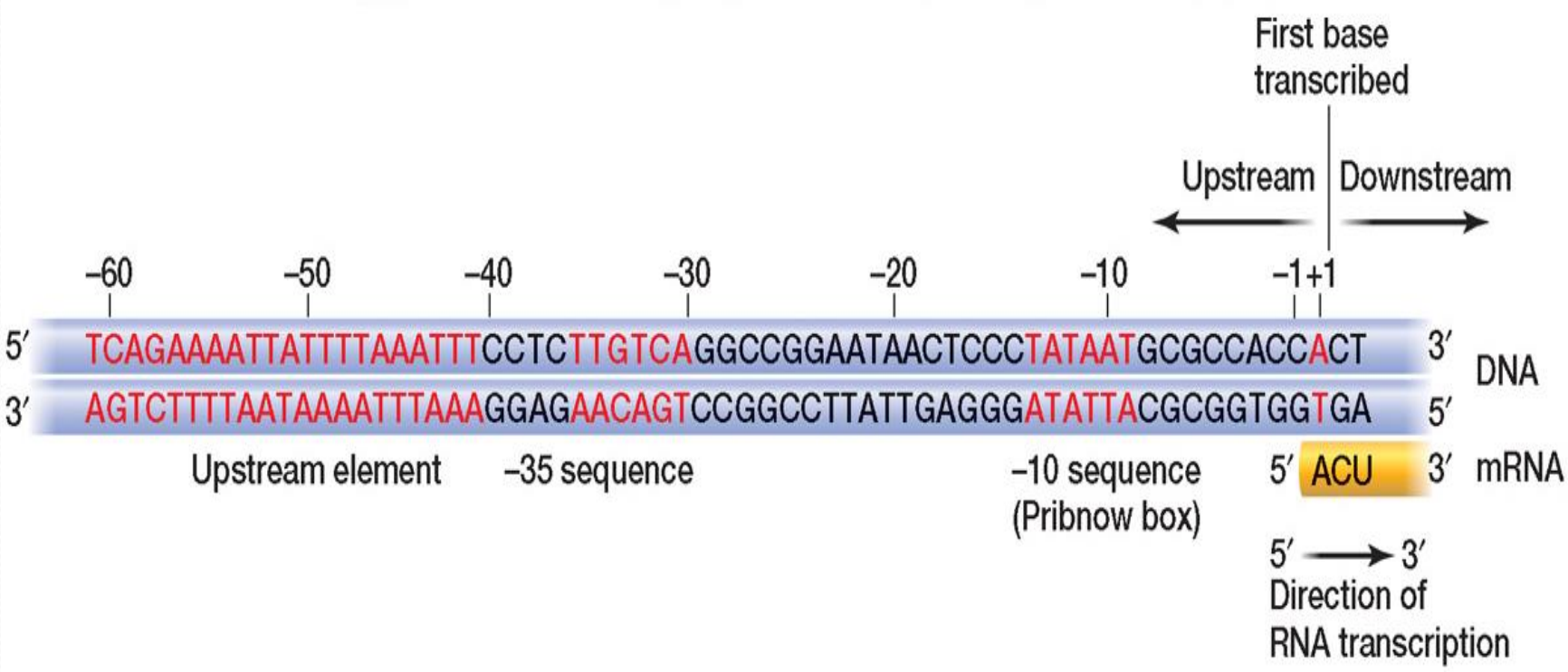

Figure 3: The promoter of the *Escherichia coli* rRNA gene

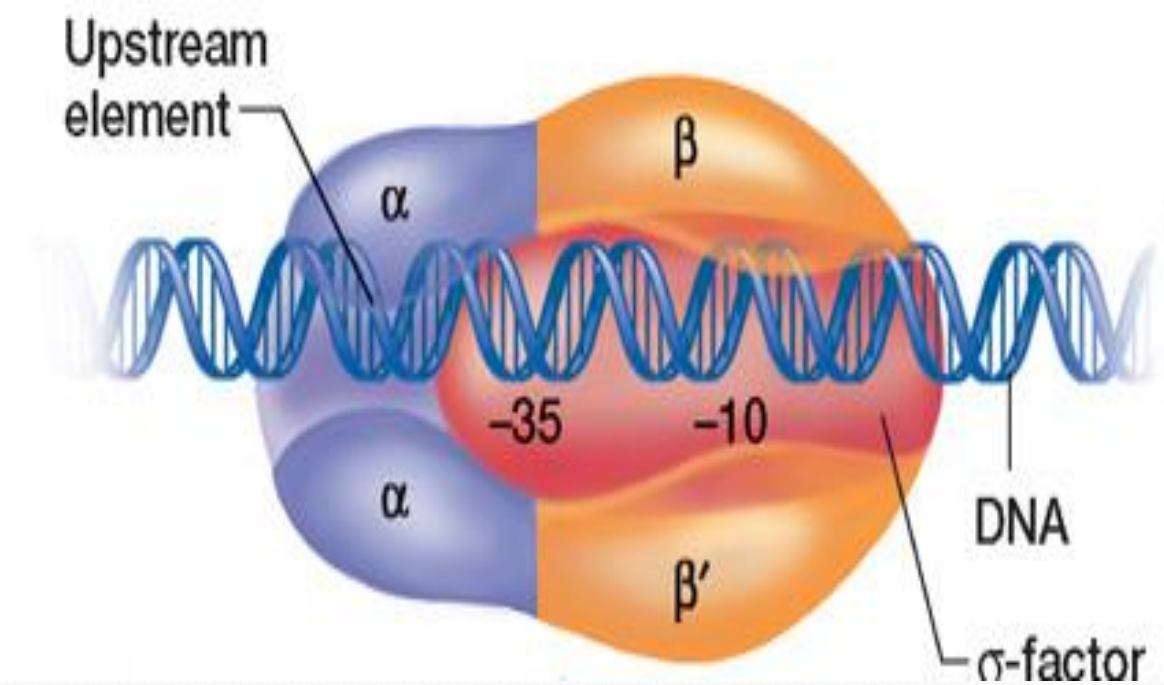

Figure 4: The composition of RNA polymerase

2- Transcription elongation:

- Transcription proceeds in the 5′→3′ direction. That is a single ribonucleotide is added a new to the 3′-OH free end of the RNA.
- Transcription proceeds along the DNA template in a 3′→5′ direction.
- RNA polymerase core enzymes (two α-subunits, one β-subunit, and one β′ -subunit) carries out RNA elongation.

3- Transcription termination:

- Transcription continues as RNA polymerase adds nucleotides to the growing RNA strand in a 5′→3′ direction according to the rules of complementary.
- The polymerase moves down the DNA until it reaches a stop signal or terminator sequence.