Summary of Chapter 25

1. Terminology
   - Replication: DNA → DNA
   - Transcription: DNA → RNA
   - Translation: RNA → Protein
   - mRNA, rRNA, tRNA
   - Template strand = Coding strand = Antisense strand
   - Non-template strand = Noncoding strand = Sense strand
   - “-10-bp” means the 10 bp upstream from the initiation site.
   - Promoter --- Initial RNA polymerase and factor binding region on the template DNA.

2. \textit{E. coli} RNA polymerase
   - Multi-subunit enzyme, and \( \sigma^{70} \) subunit dissociates from the core polymerase after binding to DNA. \( \alpha_2\beta\beta'\sigma^{70} \) (holoenzyme) \( \leftrightarrow \alpha \beta \beta' \) (core) + \( \sigma^{70} \) (\( \sigma \)-factor)
   - RNA synthesis is three steps: Initiation → Elongation → Termination
   - Initiation sites have unique DNA sequences called promoter which is recognized by \( \sigma \).
     1. -10-bp (Pribnow box) has TATAAT consensus sequence.
     2. -35-bp region has TTGACA consensus sequence.
     3. The initiation sites are either A or G (purine sequence).
   - Elongation process
     1. Holoenzyme binds very tightly (\( K_d = 10^{-12} \) M) to the promoter.
     2. RNA synthesis is immediately started (Elongation direction is 5’→3’ direction). The substrates are ATP, GTP, CTP, and UTP, and PP\(_i\) is one of products. Substrate hydrolysis provides synthesis energy.
     3. After 10 nucleotides are added, \( \sigma \)-factor is dissociated from the enzyme.
     4. RNA polymerase under- and over-winds DNA at the behind and ahead of transcription bubble, respectively. Thus the nascent RNA is straight line. Topoisomerase I and II relax the supercoiled DNA.
   - \textbf{Rifamycin} binds to RNA polymerase and inhibits its activity, \textbf{cardycepin} incorporated into RNA and inhibits the chain elongation, \textbf{actinomycin} binds to DNA and inhibits the RNA polymerase activity.
   - Termination
     1. Sequence termination: GC rich palindrome sequence followed by 4~10 T sequence in DNA.
        The palindrome section of RNA forms a hairpin structure which induces the conformational changes in RNA polymerase. Thus, the RNA is released.
     2. \( \rho \)-factor termination: Protein \( \rho \)-factor travels with RNA polymerase, and at the pause site (GC -rich palindrome sequence which allow the RNA to form a hairpin structure), \( \rho \)-factor induces termination of the synthesis.

3. Eukaryotic RNA polymerase
   - Three types of RNA polymerases (RNAPs)
     1. RNA polymerase I: present in nucleolus, makes pre-rRNA, \( \alpha \)-amanitin resistant.
     2. RNA polymerase II: present in nucleoplasm, makes hnRNA, \( \alpha \)-amanitin sensitive.
     3. RNA polymerase III: present in nucleoplasm, makes pre-tRNA, 5S rRNA, \( \alpha \)-amanitin
less sensitive.

- The core structure is $\alpha_2\beta\beta'$, but no $\sigma$-factor.
- RNA polymerases require a promoter, enhancers, and transcription factors.
  - RNAPI promoters:
    - are species specific, each RNAPI recognizes a specific promoter.
    - are located -186 to +6 on the DNA template strand.
  - RNAPII promoters:
    - The constitutive genes have GC box (GGGCGG consensus sequence) in their promoters
    - The structural genes have TATA box (TATATAATA sequence) in their promoters.
    - are located -25 to -30 on the DNA template strand.
  - RNAPIII promoters:
    - are located at downstream, +40 to +80 on the DNA template strand.
- Enhancers:
  - are inducible transcription factor binding sites on the DNA template.
  - have specific sequences.
  - Without enhancer, RNA synthesis rate is reduced as much as $10^9$ times.
- Transcription factors (TFs)
  - There are three classes of TFs for RNAPII.
    1. General TFs --- recognize the promoter (initiation site) and deliver RNAPII to it. They form a pre-initiation complex (PIC) with RNAPII.
    2. Upstream TFs --- bind to specific DNA sequences at upstream of the initiation site, and stimulate or repress the transcription initiation (PIC). They recognize both DNA and PIC.
    3. Inducible TFs --- are activated by phosphorylation or by a specific ligand binding, and then bind to specific DNA sequences at upstream of the initiation site, and stimulate or repress the transcription initiation (PIC). Example: steroid receptors.
- Transcription factors have unique structural motifs.
  1. Zinc finger DNA-binding motifs --- Amino acid sequence contains (-Cys-Cys----His-His--)$_n$ repeats. A Zn$^{2+}$ is coordinated by 2 Cys and 2 His.
  2. Leucine zippers --- Amino acid sequence contains 7-residue pseudo-repeat (a-b-c-d-e-f-g)$_n$, in which a and d are hydrophobic and Lue residue, respectively. These sections form $\alpha$-helices and a and d residues of two parallel $\alpha$-helices interact with each other as a zipper.
- Posttranscriptional processing
  - The just transcribed mRNA and tRNA, called “primary transcripts”, are not biologically active. They need specific modifications.
    1. Appending nucleotide sequences to their 3’- and 5’-ends.
    2. Removal of polynucleotide segments by exo and endonucleases.
    3. Modification of specific nucleotides.
- Eukaryotic mRNA processing
  1. The 5’-end is capped by 7-methylguanosine via 5’-5’ triphosphate bridge.
  2. A poly(A)$_n$, ($n = 20$~$50$) tail is appended to the 3’-end. The poly(A) tail protects the RNA against degradation by RNases.
  3. Removal of intervening sequences. mRNA primary transcripts are composed of intron and exon regions. The intron sections must be removed to form a mature mRNA by
splicing reactions.

5. Splicing reaction

- mRNA
  1. Introns contain consensus sequences for splicing.
     5'-GU···CURAY···AG-3' where R (purine) = A or G; Y (pyrimidine) = C or U.
  2. The 2’-OH of A in CURAY attacks 5’-phosphate of 5’-G to form 2’,5’-lariat structure.
  3. The liberated 3’-OH of the exon attacks the phosphate of the 5’-terminal residue of the
     next exon. The intron is cut off, and two exons are connected to each other.
- snRNPs (small nuclear RNA complexed with proteins) facilitate the RNA splicing
  reactions.
- Ribosomal RNA (rRNA)
  - No intron in prokaryotes and very few intron in eukaryotes.
  - Prokaryotes:
    1. One primary transcript contains 23S, 16S, 5S rRNAs, and some tRNAs with spacers.
    2. These RNAs are cut out by various RNases.
  - Eukaryotes:
    1. The primary transcript, 45S rRNA, contains 18S, 5.8S, and 28S rRNAs with spacers.
    2. rRNAs are cut out by RNases, and introns are eliminated by self-splicing mechanism.
    3. Self-splicing mechanism:
       - The 3’-OH of a free guanosine cleaves the phosphodiester bond of the 3’-end of left exon.
       - The liberated 3’-OH of the left exon attacks the 5’-end phosphate of the right exon. The
         intron is cleaved, and the two exons are connected.
       - The cleaved intron is further spliced into two pieces.
- Ribozymes
  - Self-splicing function of RNA indicates that some RNAs have an enzyme activity.
  - Small hammerhead RNAs have indeed nuclease activities that cleave a single strand DNA. These RNAs are called ribozymes.
- tRNA processing
  - Prokaryotic tRNAs
    1. Primary transcript (pre-tRNA) contains two introns at both 5’- and 3’-ends.
    2. After folding into a cloverleaf structure, the introns are cleaved by RNase P and F, respectively.
    3. The accepting arm 3’-ACC is attached to the 3’-end.
    4. Base modifications take place after splicing.
  - Eukaryotic tRNAs
    1. Pre-tRNA is immediately folded into a cloverleaf structure.
    2. 19-nucleotides at 5’-end are cleaved.
    3. The accepting arm 3’-ACC is attached to the 3’-end.
    4. 14-nucleotides intervening sequence in the anticodon arm is removed and ligated to yield a
       mature tRNA.
- Mature tRNA structure
  - tRNA has a cloverleaf secondary structure, which contains three stem loops (called D arm,
    anticodon arm, and TψC arm), a small arm (variable arm), and accepting stem.
- The tip of the anticodon arm has an anticodon (XYZ), and the 3’-terminal is a single strand with sequence of -CCA-OH where an amino acid is attached.