

1 **Chapter 6**

How Cells Read the Genome: From DNA to Protein

2 **Central Dogma of molecular biology**3

FROM DNA TO RNA

4 **Portions of DNA Sequence Are Transcribed into RNA**

- Transcription
 - DNA to RNA
 - Nucleotide sequence
 - RNA
 - Ribonucleotides
 - Uracil replaces thymine
 - Single stranded
 - Fold into 3D structures

5 **Transcription Produces RNA Complementary to One Strand of DNA**

- Template strand
 - Determines sequence of transcript
 - RNA displaced & DNA double helix reforms
- RNA polymerases
 - RNA chain grows 5' -3'
 - No primers required

6 **Cells Produce Several Types of RNA**

- Messenger RNA (mRNA)
 - Specify amino acid sequence
- Ribosomal RNA (rRNA)
 - Ribosome structure
- Transfer RNA (tRNA)
 - Between mRNA and amino acids
- Others
 - Splicing, modification, regulation
- Transcription units
 - Eukaryotic & prokaryotic

7 **Signals Encoded in DNA Tell RNA Polymerase Where to Start and Stop**

Prokaryotes

- Initiation
 - RNA polymerase holoenzyme
 - Polymerase & sigma (σ) factor
 - Promoter
 - Sequence triggers polymerase to bind to DNA
 - Sigma factor helps read sequence
 - ~ 10 nucleotides added at promoter (inefficient)
 - Elongation
 - Polymerase separates from promoter & sigma
 - Termination
 - Signal in DNA (AT string & hairpin)

- Template & RNA released

8 9 

Transcription Start and Stop Signals Are Heterogeneous in Nucleotide Sequence

- Consensus sequences
 - Determine strength (# of initiation events)
- Template strand varies

10 

Transcription Initiation in Eucaryotes Requires Many Proteins

- RNA polymerase I & III
 - tRNA, rRNA, others
- RNA polymerase II
 - Genes (proteins)
- Require many additional proteins
 - General transcription factors
 - Not just a single sigma
- Initiation must deal with DNA packing

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11 

RNA Polymerase II Requires General Transcription Factors

- Transcription factors for polymerase II
 - TFII
 - TFIIB, TFIID, TFIIF etc
 - Similar to function of sigma
 - TFIID (TBP)
 - TATA box
 - 25 nucleotides upstream of start site
 - Causes DNA distortion
 - TFIIH
 - DNA helicase
 - Phosphorylate C-terminal domain
 - Polymerase
 - Short strands before clearing promoter
 - TFIIH phosphorylase

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12 

Polymerase II Also Requires Activator, Mediator, and Chromatin-Modifying Proteins

- Transcriptional activators
 - Attract RNA polymerase II
- Mediator complex
 - Communication between polymerase & activator proteins
- Chromatin & histone remodeling complexes
- Activator proteins
 - Distant site (enhancer)

13 

- Transcription initiation
 - > 100 proteins
 - Assembly differs for each gene

14 **Transcription Elongation Produces Superhelical Tension in DNA**

- Elongation factors
 - Prevent premature dissociation of RNA polymerase
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15 **Transcription Elongation in Eucaryotes Is Tightly Coupled to RNA Processing**

- RNA modifications
 - 5' cap
 - 3' polyadenylation
 - RNA splicing
 - Remove introns
- All coupled to elongation
 - Begins with interactions on CTD (C-terminal domain)

16

17 **RNA Capping Is the First Modification of Eucaryotic Pre-mRNAs**

- ~25 nucleotides
 - Addition of modified guanine
- Enzymes bind to polymerase tail
 - Phosphatase
 - Removes 5' phosphate
 - Guanyl transferase
 - Adds GMP (5' to 5')
 - Methyl transferase
 - Adds methyl group
- Distinguishes mRNA from other types

18 **RNA Splicing Removes Intron Sequences from Newly Transcribed Pre-mRNAs**

- Exons
 - Expressed sequences
- Introns
 - Intervening sequences
 - Removed during RNA splicing
 - Two phosphoryl-transfer rxns (transesterifications)
 - Forms "lariat"
 - 5 RNA's & ~200 proteins
- Evolution of genes
- Alternative splicing

19 **Nucleotide Sequences Signal Where Splicing Occurs**

- Splicing recognition
 - 5' splice site
 - 3' splice site
 - Branch point

- Variable consensus sequences
 - Alternative splicing

20 **RNA Splicing Is Performed by the Spliceosome**

- Spliceosome
 - snRNP (small nuclear Ribonucleoproteins)
 - snRNA's (small nuclear RNAs)
 - Proteins

21 **RNA-Processing Enzymes Generate the 3' End of Eucaryotic mRNAs**

- 5' end
 - Capped as it emerges from RNA polymerase II
- 3' end
 - Addition of poly-A tail

22 **Mature Eucaryotic mRNAs Are Selectively Exported from the Nucleus**

- Mature mRNA
- Checked for proper processing
- Transported through nuclear pore

23 **The Nucleolus Is a Ribosome-Producing Factory**

- rRNA processing
- Ribosome assembly

24

FROM RNA TO PROTEIN

25 **An mRNA Sequence Is Decoded in Sets of Three Nucleotides**

- Translation
 - RNA Protein
 - Genetic code
 - Sequence of 3 nucleotides
 - Codon
 - mRNA

26 **tRNA Molecules Match Amino Acids to Codons in mRNA**

- Anti-codons
 - tRNA
 - Wobble position

27 **Specific Enzymes Couple Each Amino Acid to Its Appropriate tRNA Molecule**

- Aminoacyl-tRNA synthetases
 - Covalently attach AA to tRNA
 - Recognize specific tRNA sequence

28 **Amino Acids Are Added to the C-terminal End of a Growing Polypeptide Chain**

- Protein synthesis

- Stepwise from N-terminal to C-terminal

29 **The RNA Message Is Decoded in Ribosomes**

- Ribosome
 - rRNA
 - catalytic
 - Protein
 - Structural
 - Size variation

30

- Binding sites
 - E (exit site)
 - P (peptidyl-tRNA site)
 - A (aminoacyl-tRNA site)

31 **The Ribosome Is a Ribozyme**

- Ribozyme
 - Catalytic RNA

32 **Nucleotide Sequences in mRNA Signal Where to Start Protein Synthesis**

- Initiator tRNA
 - Methionine
- Eukaryotic loading
 - Eucaryotic initiation factors (eIF's)
 - Consensus sequence
 - 5'-ACCAUGG-3'
 - "leaky scanning"
 - Changes signal sequence

33 **Stop Codons Mark the End of Translation**

- UAA, UAG, UGA
 - No tRNA
- Release factors
 - Bind to A site
 - Adds water in place of aa
 - Frees carboxyl end from tRNA

34 **Proteins Are Made on Polyribosomes**

- Make more protein in a short amount of time

35 **There Are Minor Variations in the Standard Genetic Code**

- Different species & Organelles
- 21st aa selenocysteine
 - Selenium
 - UGA codon
 - Stem loop causes recoding event
- Translational frameshifting
 - Retroviruses
 - Caspid & reverse transcriptase