Ch 31: RNA Structure, Synthesis, and Processing

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I. Overview

- Central role of RNA
    - DNA = genetic master plan.
    - RNA = "working copies" used to express genetic information.
- Transcription
    - DNA strand serves as template for RNA synthesis.
    - Produces:
        - mRNA → translated into protein.
        - rRNA, tRNA, and other ncRNAs → structural, catalytic, regulatory roles (not translated).
- Gene expression products
    - Final product may be RNA or protein depending on gene.
    - Only ~2% of genome encodes proteins.
- Key features of transcription

    - Highly selective (unlike replication, which is "all-or-none").
    - Selectivity guided by:
        - DNA-embedded signals → define start/stop points & transcription frequency.
        - Regulatory proteins → modulate transcription.
    - Basis of biochemical differentiation of tissues.

- RNA modifications

    - Primary transcripts often altered post-synthesis:
        - Terminal additions, base modifications, trimming, internal segment removal.
    - Convert inactive precursors → functional RNA molecules.

- Transcriptome

    - The complete set of RNA transcripts expressed by a genome.

II. RNA Structure

- General properties (vs DNA)

    - RNA = unbranched polymer of nucleoside monophosphates (3′→5′ phosphodiester bonds).
    - Key differences from DNA:
        - Smaller.
        - Ribose instead of deoxyribose.
        - Uracil (U) instead of thymine (T).
        - Single-stranded, capable of folding into complex structures.

- Major types of RNA (for protein synthesis)

    - rRNA
    - tRNA
    - mRNA

- Other small ncRNAs (eukaryotic)

    - snoRNA → nucleolus.
    - snRNA → nucleus.
    - miRNA → cytoplasm.

A. Ribosomal RNA (rRNA)

- Function: structural + catalytic component of ribosomes (sites of protein synthesis).

- Species:
    - Prokaryotes: 23S, 16S, 5S.
    - Eukaryotes (nuclear): 28S, 18S, 5.8S, 5S.
    - Mitochondrial (mtDNA): 12S, 16S.
- Abundance: ~80% of total cellular RNA.
- Note: Some rRNA act as ribozymes (catalytic RNA).

B. Transfer RNA (tRNA)

- Smallest RNA species (4S).
- Function: adaptor between amino acid & mRNA codon.
    - Each tRNA carries its specific amino acid covalently linked at 3′ end.
    - Recognizes codon on mRNA to ensure correct amino acid incorporation.
- Abundance: ~15% of total RNA.
- Structural features:
    - High % of unusual bases (e.g., dihydrouracil).

    - Extensive intrachain base pairing → cloverleaf secondary structure → folded tertiary structure.

- Genetics:

    - Encoded in both nuclear & mitochondrial DNA.
    - Human mitochondrial genome encodes 22 tRNAs.

- Clinical relevance:

    - Mutations in mitochondrial tRNA genes cause diseases:
        - tRNALys mutation → MERRF (myoclonic epilepsy with ragged red fibers), mitochondrial encephalomyopathy.
        - tRNALeu mutation → MELAS (mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes).

C. Messenger RNA (mRNA)

- Smallest fraction by mass (~5% of total RNA) but most heterogeneous in sequence & size.

- Function: coding RNA → carries genetic info from DNA to ribosomes.

- Organization:
    - Prokaryotes, mitochondria, chloroplasts, some viruses: polycistronic mRNA (multiple genes per transcript).
    - Eukaryotes: monocistronic mRNA (one gene per transcript).

- Structure:
    - Protein-coding regions → translated.
    - Untranslated regions (UTRs) → at both 5′ and 3′ ends (regulatory).
    - Special features in eukaryotes (not in prokaryotes):
        - 5′ cap: 7-methylguanosine linked via unusual 5′-to-5′ triphosphate bond.
        - 3′ poly-A tail: long stretch of adenines.

III. Prokaryotic Gene Transcription

General Features

- RNA Polymerase (RNA pol) in prokaryotes:

- Single enzyme synthesizes all RNAs (mRNA, tRNA, rRNA).
- Exception: Short RNA primers for DNA replication are made by primase (specialized monomeric enzyme).

- Basic mechanism:
  - Recognizes promoter region → binds → makes complementary RNA → stops at termination region.
  - RNA synthesized 5′ → 3′, antiparallel to DNA template.
  - Base-pairing rules:
    - G → C, C → G, T → A, A → U.
  - RNA is complementary to template (antisense strand) and identical to coding (sense strand) except U replaces T.

- Template strand choice:
  - For each gene, only one strand serves as template.
  - Determined by promoter location.

A. Prokaryotic RNA Polymerase

1. Core Enzyme

- Subunits: 2α, 1β, $1\beta'$, 1Ω.
- Functions:
  - α, $\Omega \rightarrow$ assembly of enzyme.
  - $\beta' \rightarrow$ template binding.
  - $\beta \rightarrow$ polymerase activity ($5' \rightarrow 3'$).
- Called core enzyme, but lacks specificity (cannot recognize promoter).

2. Holoenzyme

- Core enzyme + σ (sigma) factor.
- Function: σ factor provides specificity $\rightarrow$ allows recognition of promoter regions.
- Types: Different σ factors recognize different sets of genes.
  - Example: σ^{70} = predominant sigma factor.

B. Steps in RNA Synthesis

1. Initiation

- Promoter binding: RNA pol holoenzyme binds to promoter (non-transcribed region).
- Consensus sequences in promoter:
  - −35 sequence (5′-TTGACA-3′): Initial contact point for holoenzyme → forms closed complex.
  - −10 sequence (Pribnow box) (5′-TATAAT-3′): Site of DNA unwinding (~14 bp) → forms open complex (transcription bubble).
- Clinical note: Mutations in −35 or −10 reduce transcription efficiency.

2. Elongation

- Unwinding: RNA pol unwinds DNA helix (supercoils relieved by topoisomerases).
- Transcript formation:
  - Begins with short RNAs (discarded).
  - True elongation starts once transcript >10 nucleotides.
  - σ factor released → core enzyme continues

processively.

- Mechanism:
    - Uses NTPs as substrates.
    - Releases pyrophosphate for each nucleotide added.
    - Always 5′ → 3′ direction.
- Differences from DNA pol:
    - No primer needed.
    - No 3′→5′ proofreading exonuclease.
    - Higher error rate, but can backtrack, cleave, and restart.

3. Termination

a. Rho-Independent Termination

- Requires formation of hairpin loop:
    - Self-complementary GC-rich sequence in RNA folds back.
    - Stabilized by H-bonds → forms stem-loop structure.
- Followed by poly-U sequence at RNA 3′ end.

- Weak A–U pairing → destabilizes RNA–DNA hybrid → RNA released.

b. Rho-Dependent Termination

- Requires ρ (rho) factor:
    - Hexameric ATPase with helicase activity.
    - Binds rut (rho utilization) site (C-rich) on RNA near 5′ end.
    - Moves along RNA using ATP.
    - When it reaches RNA pol (paused at termination site), helicase separates RNA–DNA hybrid → transcript released.

4. Antibiotics Affecting Prokaryotic Transcription

- Rifampin (rifampicin):
    - Binds β subunit of RNA pol.
    - Blocks initiation → prevents chain growth beyond 3 nucleotides.
    - Used in tuberculosis treatment.

- Dactinomycin (Actinomycin D):

    - Intercalates between DNA bases.
    - Inhibits both initiation and elongation.
    - First antibiotic used in tumor chemotherapy.

IV. Eukaryotic Gene Transcription

General Features

- More complex than prokaryotic transcription.

- Uses three separate RNA polymerases (I, II, III) for different RNAs.

- Requires numerous transcription factors (TFs):

    - Bind to core promoter, proximal elements, or distal elements (enhancers/silencers).
    - Essential for assembling the transcription initiation complex and deciding which genes are transcribed.

- Chromatin accessibility is critical:

    - DNA must be decondensed for TFs and RNA pol to access promoter regions.

A. Chromatin Structure and Gene Expression

- Euchromatin: Loosely packed, transcriptionally active.
- Heterochromatin: Highly condensed, transcriptionally inactive.
- Chromatin remodeling: Reversible conversion between euchromatin and heterochromatin.
- Histone modifications:
    - Histone acetylation (via HATs) → removes + charge from lysine → weakens histone–DNA interaction → transcription ↑.
    - Histone deacetylation (via HDACs) → restores + charge → tighter DNA–histone binding → transcription ↓.
- ATP-dependent nucleosome repositioning also required for DNA access.

B. Nuclear RNA Polymerases

1. RNA Polymerase I

- Location: Nucleolus.

- Function: Synthesizes precursor of 28S, 18S, 5.8S rRNAs.

2. RNA Polymerase II

- Location: Nucleoplasm.
- Function: Synthesizes:
    - Pre-mRNA (hnRNA) → processed into mature mRNA.
    - Certain small ncRNAs: snoRNA, snRNA, miRNA.

a. Promoters for RNA Pol II

- TATA (Hogness) box: Consensus TATAAA, ~25 bp upstream of transcription start.
- Alternative elements: Initiator (Inr), DPE (if TATA box absent).
- All are cis-acting elements (same DNA molecule as gene).
- Function: Binding sites for general transcription factors (GTFs).

b. General Transcription Factors (GTFs)

- Minimum requirements for promoter recognition, RNA pol II recruitment, preinitiation complex formation, and basal transcription.

- Encoded by separate genes → trans-acting.

- Key players:

    - TFIID = contains TATA-binding protein (TBP) + TATA-associated factors (binds TATA box).
    - TFIIF = recruits RNA pol II to promoter.
    - TFIIH = helicase activity (unwinds DNA) + kinase activity (phosphorylates RNA pol II, enabling promoter clearance).

- Note: Unlike prokaryotes, RNA pol II does not directly recognize promoter.

c. Regulatory Elements and Specific Transcription Factors (STFs)

- Proximal elements (~200 bp upstream): e.g., CAAT box, GC box.

- Distal elements: enhancers or silencers, can act thousands of bp away.

- STFs:
    - Bind regulatory elements.
    - Control transcription frequency and gene expression timing (e.g., hormone response).
    - Have two domains: DNA-binding domain + activation domain.
    - Recruit GTFs and coactivators (e.g., HATs for chromatin remodeling).
- Mediator complex: multisubunit coactivator linking RNA pol II, GTFs, and STFs.

d. Enhancers and Silencers

- Enhancers:
    - Increase transcription initiation.
    - Location flexible: upstream, downstream, near, or far from promoter; can act in either DNA strand.
    - Contain response elements (binding sites for STFs).
    - Work by DNA looping, bringing STFs into contact with Mediator + promoter TFs.
- Silencers: Similar to enhancers, but reduce gene expression.

e. Inhibition of RNA Pol II

- α-Amanitin (toxin from *Amanita phalloides*, "death cap" mushroom):
    - Binds RNA pol II tightly.
    - Blocks mRNA synthesis → lethal.

3. RNA Polymerase III

- Function: Synthesizes tRNA, 5S rRNA, some snRNA and snoRNA.

V. Posttranscriptional Modification of RNA

- Primary transcript
    - Initial, linear RNA copy of a transcription unit (DNA region between initiation and termination sequences).
    - Modified differently depending on RNA type (tRNA, rRNA, mRNA).

A. Ribosomal RNA (rRNA)

- Source: Generated from long precursor molecules

(pre-rRNA).

- Prokaryotes: Single pre-rRNA → 23S, 16S, and 5S rRNA.
- Eukaryotes: Single pre-rRNA → 28S, 18S, and 5.8S rRNA.
    - Exception: 5S rRNA synthesized separately by RNA polymerase III.
- Processing:
    - Cleavage by ribonucleases → intermediate rRNA pieces.
    - Further trimming by exonucleases.
    - Base & ribose modifications.
- Location in eukaryotes:
    - rRNA genes found in tandem arrays.
    - Synthesis & processing occur in the nucleolus.
    - snoRNA (small nucleolar RNA) facilitates base/sugar modifications.

B. Transfer RNA (tRNA)

- Produced from precursor molecules (both prokaryotic

& eukaryotic).

- Posttranscriptional modifications:
    - Removal of sequences at both ends.
    - Removal of introns (if present) from anticodon loop by nucleases.
    - Addition of –CCA sequence at 3′ end by nucleotidyltransferase.
    - Modification of bases at specific positions → generates unusual bases (unique identity for each tRNA).

C. Eukaryotic Messenger RNA (mRNA)

- Primary transcripts synthesized by RNA polymerase II = heterogeneous nuclear RNA (hnRNA).

- Undergoes co- and posttranscriptional modifications → mature mRNA.

- Key modifications:

1. Addition of 5′ cap

- Structure: 7-methylguanosine attached to 5′ end via

5′→5′ triphosphate linkage (resistant to nucleases).

- Process:
  - γ-phosphoryl group removed from 5′ triphosphate.
  - Guanylyltransferase adds GMP.
  - Methylation by guanine-7-methyltransferase (cytosol).
  - Methyl donor: S-adenosylmethionine (SAM).
- Functions:
  - Stabilizes mRNA.
  - Permits efficient initiation of translation.

2. Addition of 3′ poly-A tail

- Structure: 40–250 adenylates added to 3′ end.
- Process:
  - Pre-mRNA cleaved downstream of AAUAAA consensus sequence (polyadenylation signal).
  - Poly-A tail added by polyadenylate polymerase using ATP.

- Functions:
    - Terminates transcription.
    - Stabilizes mRNA.
    - Facilitates nuclear export.
    - Aids in translation.
    - Gradually shortened in cytosol.
- Exception: Histone mRNAs lack poly-A tails.

3. Splicing

- Definition: Removal of noncoding sequences (introns) & joining of coding sequences (exons).
- Spliceosome: Large complex carrying out splicing.

a. Role of small nuclear RNA (snRNA)

- snRNA + proteins = snRNP ("snurp") → U1, U2, U4, U5, U6.
- Recognize intron boundaries via base pairing with consensus sequences.
- Clinical note: SLE patients produce antibodies against

snRNP.

b. Mechanism of splicing

- Branch site adenine (A) within intron initiates reaction.
- Steps:
    1. 2′-OH of branch A attacks 5′ splice donor site → unusual 2′→5′ bond forms → lariat structure.
    2. 3′-OH of exon 1 attacks 5′ phosphate of exon 2 → phosphodiester bond → exons joined.
    3. Lariat intron excised → degraded or used as precursor for ncRNA (e.g., snoRNA).
- Invariant sequences: GU at 5′ end, AG at 3′ end of introns.

c. Effect of splice site mutations

- Consequences:
    - Skipping of exons.
    - Retention of introns.
    - Activation of cryptic splice sites.

- Clinical example: β-thalassemia → defective β-globin due to abnormal splicing.

- General: ~20% of genetic diseases linked to splicing errors.

4. Alternative splicing

    - 90% of human pre-mRNAs undergo alternative splicing.

- Produces multiple mRNA variants → multiple protein isoforms from single gene.

- Example: Tropomyosin (TM) → tissue-specific isoforms via alternative splicing.