vi. Structure, function and metabolisms of nucleic acids, DNA and RNA

Introduction to Nucleic Acids

1. **Definition and Importance**
    - **Nucleic acids** (DNA and RNA) are polymers of nucleotides, serving as the repositories of genetic information (DNA) and mediators of gene expression (RNA).
    - Underpin virtually all aspects of cell function—inheritance, protein synthesis, gene regulation.
2. **Nucleotide Composition**
    - Each nucleotide consists of a **nitrogenous base** (purine or pyrimidine), a **five-carbon sugar** (ribose or deoxyribose), and one or more **phosphate groups**.
    - **Purines**: Adenine (A), Guanine (G).
    - **Pyrimidines**: Cytosine (C), Thymine (T; in DNA), Uracil (U; in RNA).
3. **Types of Nucleic Acids**
    - **Deoxyribonucleic Acid (DNA)**: Typically double-stranded, long-term information storage.
    - **Ribonucleic Acid (RNA)**: Usually single-stranded, diverse roles in protein synthesis (mRNA, rRNA, tRNA) and regulation (miRNA, lncRNA, etc.).

DNA: Structure and Function

1. **Double Helix Model**
    - **Watson–Crick Structure** (B-form DNA): Two antiparallel polynucleotide strands twisted into a right-handed helix.
    - Bases pair via hydrogen bonds (A–T with 2 H-bonds, G–C with 3 H-bonds).
    - **Major and Minor Grooves**: Binding sites for proteins (transcription factors, enzymes).
2. **Forms of DNA**
    - **B-DNA**: Most common physiological form.
    - **A-DNA**: More compact, right-handed, often in dehydrated samples or RNA–DNA hybrids.
    - **Z-DNA**: Left-handed helix, occurs in certain GC-rich regions; implicated in regulatory roles.
3. **DNA Packaging**
    - **Prokaryotes**: Single circular chromosome supercoiled by **DNA gyrase**, packaged in the nucleoid.
    - **Eukaryotes**: Multiple linear chromosomes wound around histones, forming **nucleosomes**; further coiling yields higher-order chromatin structures.
4. **DNA Functions**
    - **Genetic Information Storage**: Encodes genes, regulatory sequences.
    - **Transmission and Replication**: Ensures faithful inheritance.
    - **Long-Term Stability**: Double-stranded structure and repair mechanisms protect genetic content over generations.

DNA Metabolism

DNA Replication

1. **Semi-Conservative Mechanism**
    - Each daughter molecule contains one parental strand and one newly synthesized strand (Meselson–Stahl experiment).
    - **Bidirectional** from **origins of replication**; **forks** proceed in opposite directions.
2. **Key Enzymes and Proteins**
    - **Helicase**: Unwinds the double helix.
    - **Single-Strand Binding Proteins (SSBs)**: Stabilize unwound template strands.
    - **DNA Polymerase**: Catalyzes nucleotide addition in the 5′→3′ direction. Different polymerases in prokaryotes (Pol I, III) vs. eukaryotes (Pol α, δ, ε).
    - **Primase**: Synthesizes short RNA primers.
    - **Ligase**: Joins Okazaki fragments on the lagging strand.

3. **Leading vs. Lagging Strands**
    - **Leading Strand**: Synthesized continuously.
    - **Lagging Strand**: Discontinuous synthesis forms **Okazaki fragments**, later joined by DNA ligase.
4. **High Fidelity and Proofreading**
    - DNA polymerases often have **3′→5′ exonuclease** activity to correct misincorporated nucleotides.
    - The **Mismatch Repair** system further enhances accuracy.

DNA Repair

1. **Types of Damage**
    - **Spontaneous** (depurination, deamination), **UV-induced** (thymine dimers), **chemical mutagens**, **ionizing radiation** (strand breaks).
2. **Major Repair Pathways**
    - **Base Excision Repair (BER)**: Removes damaged bases (e.g., uracil in DNA) via DNA glycosylases, followed by endonuclease and polymerase fill-in.
    - **Nucleotide Excision Repair (NER)**: Fixes bulky lesions (thymine dimers).
    - **Mismatch Repair (MMR)**: Corrects replication errors (mismatched bases).
    - **Double-Strand Break Repair**: Non-homologous end joining (NHEJ) or homologous recombination (HR).

RNA: Structure and Types

1. **RNA Structure**
    - Generally single-stranded with **ribose** sugar (2′-OH) and **uracil** instead of thymine.
    - Can form **secondary structures** (hairpins, stem-loops) and **tertiary structures** (tRNA cloverleaf, ribozymes).
2. **Classes of RNA**
3. **Messenger RNA (mRNA)**
    - Encodes protein sequences. In eukaryotes, typically **monocistronic**; in prokaryotes, often **polycistronic**.
    - Eukaryotic mRNAs have a **5′ cap** and **3′ poly(A) tail**.
4. **Ribosomal RNA (rRNA)**
    - Major structural and catalytic component of **ribosomes**.
    - In eukaryotes, 28S, 18S, 5.8S, 5S rRNAs. In prokaryotes, 23S, 16S, 5S rRNAs.
5. **Transfer RNA (tRNA)**
    - Adaptor molecules, each carrying a specific amino acid to the ribosome.
    - Anticodon loop pairs with mRNA codon, ensuring correct amino acid incorporation.
6. **Non-Coding Regulatory RNAs**
    - **MicroRNAs (miRNAs)**, **small interfering RNAs (siRNAs)**: Gene silencing via mRNA cleavage or translational repression.
    - **Long noncoding RNAs (lncRNAs)**: Chromatin remodeling, transcriptional regulation.
    - **snRNA, snoRNA**: Involved in splicing (snRNPs) and rRNA modification (snoRNPs).

RNA Metabolism

Transcription

1. **Prokaryotic Transcription**
    - Single **RNA polymerase** (σ factor confers promoter specificity).
    - **Promoters**: –35 and –10 (Pribnow box) regions.
    - **Termination**: Rho-dependent or Rho-independent (hairpin loop + U-tract).
2. **Eukaryotic Transcription**
    - **Three RNA Polymerases**:
        - **Pol I**: rRNA.
        - **Pol II**: mRNA, some snRNA.
        - **Pol III**: tRNA, 5S rRNA, small RNAs.
    - **Promoters and Enhancers**: TATA box, GC box, etc.
    - **General Transcription Factors (TFIIX)** assemble into a preinitiation complex.

- **Termination**: Pol II transcripts cleaved past poly(A) signal; coupled with polyadenylation.

Post-Transcriptional Modifications

1. **5′ Capping**
    - Addition of 7-methylguanosine cap to 5′ end of nascent mRNA.
    - Protects mRNA from degradation, aids ribosome binding.
2. **3′ Polyadenylation**
    - Poly(A) polymerase adds ~50–250 adenines to 3′ end.
    - Stabilizes mRNA, facilitates nuclear export.
3. **Splicing**
    - Removes **introns**, ligates, exons. Mediated by the **spliceosome** (snRNPs U1, U2, U4, U5, U6).
    - **Alternative Splicing**: Creates multiple protein isoforms from a single gene.

RNA Degradation and Turnover

- **Exoribonucleases** degrade RNA from 5′→3′ or 3′→5′ directions.
- **RNA interference** pathways (RISC, Dicer) can target specific mRNAs for degradation.

Nucleotide Biosynthesis and Degradation

Purine and Pyrimidine Synthesis

1. **De Novo Pathways**
    - **Purine Synthesis**: Built on a ribose phosphate scaffold (PRPP → IMP → AMP/GMP). Key regulatory enzyme is **glutamine-PRPP amidotransferase**.
    - **Pyrimidine Synthesis**: Carbamoyl phosphate + aspartate → orotate → UMP → UTP → CTP. The first enzyme is **carbamoyl phosphate synthetase II** (cytosolic).
2. **Salvage Pathways**
    - Recycling free bases (hypoxanthine, guanine, adenine) via **HGPRT** or adenine phosphoribosyltransferase.
    - Lesch–Nyhan syndrome (HGPRT deficiency) exemplifies salvage pathway failure.

Nucleotide Degradation

1. **Purine Degradation**
    - Ultimately forms **uric acid**. Excess → gout (hyperuricemia, crystal deposition in joints).
2. **Pyrimidine Degradation**
    - Broken down to simpler molecules (β-alanine, β-aminoisobutyrate), less clinically problematic.
3. **Regulation**
    - Feedback inhibition of key enzymes ensures balanced purine/pyrimidine pools.

Overall Biological Significance

1. **Genetic Information Flow**
    - **Central Dogma**: DNA → RNA → Protein. Nucleic acids coordinate heredity and phenotype expression.
    - **Transcriptional and Post-Transcriptional Regulation**: Vital for cell differentiation, adaptation, disease states (e.g., cancer).
2. **Clinical Applications**
    - **Nucleic Acid Therapies**: Antisense oligonucleotides, RNAi-based drugs, mRNA vaccines.
    - **Diagnostic Tools**: PCR, qRT-PCR, sequencing, microarrays.
    - **Inherited Disorders**: E.g., **Splice site mutations** causing β-thalassemia, or defects in DNA repair leading to cancer predisposition.
3. **Pharmacological Targets**
    - Many antibiotics (e.g., rifampin, fluoroquinolones) target bacterial DNA/RNA metabolism.
    - Anticancer drugs (e.g., 5-FU, methotrexate) inhibit nucleotide biosynthesis.

Concluding Remarks

Nucleic acids—DNA as the stable genetic blueprint and RNA in its myriad functional forms—are central to life's molecular processes. Their **structures** (helixes, base pairing, secondary/tertiary folds) govern how genetic information is stored, replicated, transcribed, and translated. The cell's **metabolic** pathways for nucleotides (de novo and salvage synthesis, catabolism) enable dynamic regulation of nucleotide pools, ensuring fidelity and adaptability under changing conditions.

The sophisticated regulation of **DNA replication**, **repair**, **transcription**, and **RNA processing** reflects evolution's solutions to preserve genomic integrity while facilitating complexity in gene expression. Understanding these core biochemistry and molecular biology concepts is paramount for fields as diverse as **genetics, medicine, pharmacology, and biotechnology**, all harnessing nucleic acids for diagnostics, therapeutics, and the quest to unravel life's molecular foundations.