Nucleotides and Nucleic Acids

gene = chromosomal segment that codes for a functional polypeptide chain or RNA molecule

1. **Composition of Nucleic Acids**
   (RNA --> N base + 5 C ribose sugar + P_i)
   (DNA --> N base + 5 C deoxyribose sugar + P_i)
   - Nucleotides as building blocks: Nucleotide = N base + ribose sugar + phosphate
     ribonucleotides (RNAs ; ribose sugar)
     deoxyribonucleotides (DNA ; deoxyribose sugar)

2. **N bases (Pyrimidines and Purines) / tautomeric forms (**know structures**)**
   - Pyrimidines : U = uracil ; T = Thymine ; C = Cytosine
   - Purines : A = Adenine ; G = Guanine

3. **Nucleosides (deoxynucleosides)**
   - N base + ribose sugar (or deoxyribose sugar)
   – β-N-glycosidic bonds (C1' to N1 of pyrimidine or N9 of purine)
   - Nomenclature : **Adenosine** / deoxyadenosine (dA)
     Guanosine Uridine Thymidine Cytidine
   - Conformations of nucleosides - syn / anti
   - Other nucleosides : AZT (3'-azido-2',3'-dideoxythymidine)

4. **Nucleotides (deoxynucleotides)**
   - Nucleotides = nucleoside + 5' phosphates
   - AMP / ADP (ppA) / ATP (pppA)
   - Other nucleotides : dideoxynucleotides (DNA ladder sequencing)

5. **Roles of Nucleotides**
   - Building blocks of Nucleic Acids (--> RNA ; --> DNA)
     - 3',5' phosphodiester bonds (direction to sugar-phosphate backbone)
   - Nucleotide Derivatives used in Metabolic Cosubstrates
     - Glycogen synthesis : UDP-Glucose (hemiacetal phos.)
     - Lipid biosynthesis : CDP-ethanolamine / CDP-choline
   - Energy currency : ATP/ADP; GTP/GDP
   - Nucleotides as Regulatory Molecules: cAMP / G proteins

6. **Nucleic Acids - Primary Structure**:
   - polymers: two types: **DNA** = deoxyribonucleic acid
     RNA = ribonucleic acid
   - oligonucleotides--short pieces of DNA or RNA
   - monomer units connected by covalent, **phosphodiester** bond
     - directionality--phosphodiester linkage is 3' to 5'
       with a 5' phosphate and a 3' OH
   - nucleotide sequence = genetic information is stored in the primary sequence
   - sequence is written 5' to 3' : 5' pAGCTAAGGCCTTTACTAG OH 3'

7. Nucleic Acid Structure: The Double Helix-(1953) - B-DNA

-Composition: Chargaff (1950) - %A = %T; %G = %C implying that the bases must be paired, A to T and G to C.

- X-ray diffraction of DNA by Rosiland Franklin
- ***Double helix*** of Watson and Crick (1953)
  - DNA model of two ***antiparallel*** strands in a ***double helix*** with the ***sugar-phosphate*** backbone on the outside and complimentary ***base pairs*** on the inside. Single stranded RNAs can also fold back on themselves to create short double helical segments (rRNA, mRNA, tRNA, small RNA).
  - Complimentary ***base pairing*** of A = T and G ≡ C arises due to the complimentary hydrogen bonds made between these purine-pyrimidine pairs.
  - The double helix (B-DNA) has ~10 bp/turn with a helical repeat of ~34Å or 3.4 nm.
  - The antiparallel strands create "major" and "minor" groves which can bind various proteins to form nucleoprotein complexes.
  - DNA in other conformations (A-DNA, Z-DNA)
  - Human DNA consists of about 5,800,000,000 nucleotides arranged on 23 chromosomes.
- The double helix of DNA permits us to understand the processes of replication and protein synthesis.

8. Other DNA Structures

- B-DNA: 0.34nm spacing, 10 bp repeat, major & minor grooves, Watson Crick pairing
- A-DNA: low humidity, 11 bp repeat, major~minor, similar to structure seen in RNA
- Z-DNA: left-handed helix, "zig-zag" backbone, uses "syn" conformation, GCGCGC
- "**palindromic** sequences - inverted repeats are self-complementary (hairpins / cruciforms)
- DNA supercoiling (coiling of the coil - supercoiling)
  - Topoisomerases are enzymes that can increase or decrease DNA supercoiling
- Chromatin: chromosomes are composed of chromatin (DNA + histone proteins)

9. RNAs - RNAs of three basic types of single-stranded nucleic acid.

- ***Messenger RNA (mRNA)*** contains the "***codons.***" Codons are 3-base triplets that code for the amino acid sequence of the protein to be synthesized.
- ***Ribosomal RNA (rRNA)*** is the most abundant type of RNA in the cell and represents the major components of the ***ribosome*** which is the site of protein synthesis. The X-ray structures of the large and small ribosomal subunits reveal the structures of the rRNAs and show that the ribosome is a ribozyme!!
- ***Transfer RNAs (tRNA)*** are low molecular weight nucleic acid molecules which can contain about 90 nucleotides and transport activated amino acids to the ribosome. The X-ray structures of several tRNAs have been determined. Most tRNAs are "L" shaped having the 3-base "anticodon" at one end and the attachment site for the amino acid at the other (3') end.

10. Nucleic Acid Chemistry

- Denatuted DNA: - heat denaturation of DNA is called "melting," this usually occurs at a rather sharp temperature (Tm) which indicates a cooperative process.
  (Tm increases with higher G≡C content)
- Modified bases: deamination / depurination / thymine dimers
- Alkaline hydrolysis of RNA: role of 2' -OH
- Nucleases: exonucleases vs. endonucleases: "Restriction Enzymes" - sequence specific DNA nucleases, generates "restriction fragments" used in genetic engineering

11. DNA Sequencing Techniques

- Maxam-Gilbert: uses chemical cleavages to generate four sets of labeled fragments
- Sanger Method: uses dideoxynucleotides for chain termination to generate fragments
    - Separation and detection of DNA fragments
        - Gel Electrophoresis - separation base on size to charge ratios
        - Detection methods - radioisotopes / fluorescence
- sequences are always read in the 5' $\rightarrow$ 3' direction.

    For example: 5' - AGGTCTCAAGCTATAAGCCATCATC - 3'
- The human genome project seeks to determine the primary structure of human DNA estimated to consist of ~3 billion base pairs and is nearly completed. The sequence of the yeast genome of ~13 million base pairs was completed in 1996 and several other genomic sequences are also now available (see NCBI web site).
- **Recombinant DNA** refers to the splicing together of DNA from different species. It is possible using recombinant DNA technology to insert a gene (from a human or synthesized to order) into a **plasmid**, or circular piece of DNA, and then into a bacterium in such a way that the bacterial cell will now produce the corresponding protein of the new gene.
    - ***Restriction enzymes*** (endonucleases) are used to cut out the gene of interest and open up the plasmid. There are over 100 restriction enzymes available, each one is specific for cutting at a particular DNA sequence.
    - Restriction sites are "**palindromic** sequences."