DNA Structure and Organization

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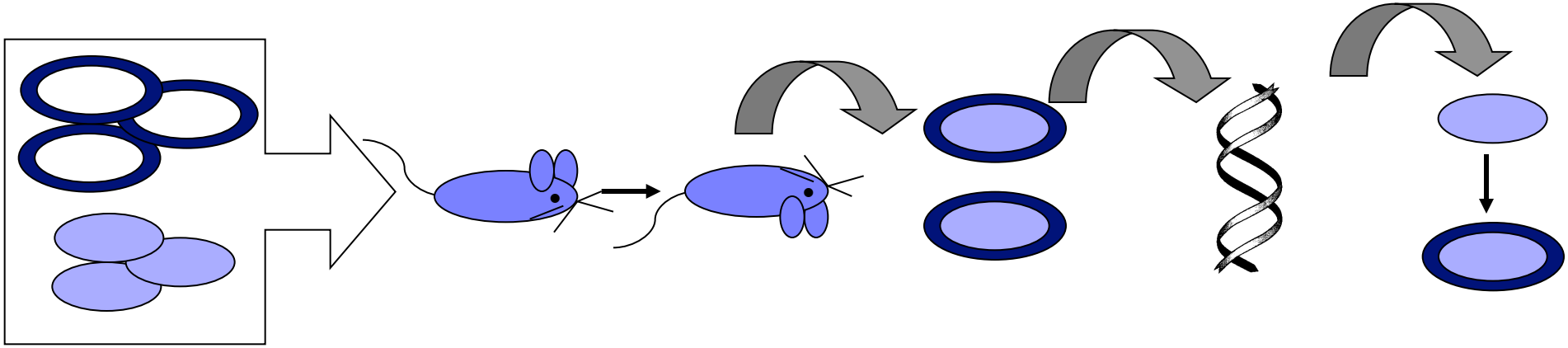
DNA is the Chemical Basis of Heredity.

[Avery, MacLeod, and McCarthy, 1944]

Wild-type *Pneumonococcus* (*Smooth* phenotype –S), Virulent

Heat-killed *Pneumonococcus* (*Smooth* phenotype-S), Avirulent

Mutant strain of *Pneumonococcus* (*Rough* phenotype –R), Avirulent



Infect mouse with mixture of heat-killed S-strain and live R-strain of *Pneumonococcus*. None of them can kill mice on their own.

Mouse dies

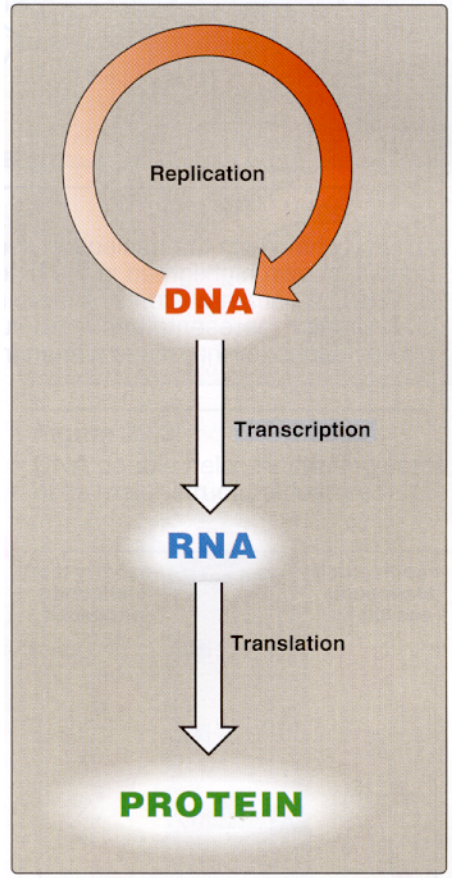
Dead mouse has live, smooth bacteria

Extract DNA and add to R-type bacteria

DNA **transforms** R-bacteria to S-bacteria

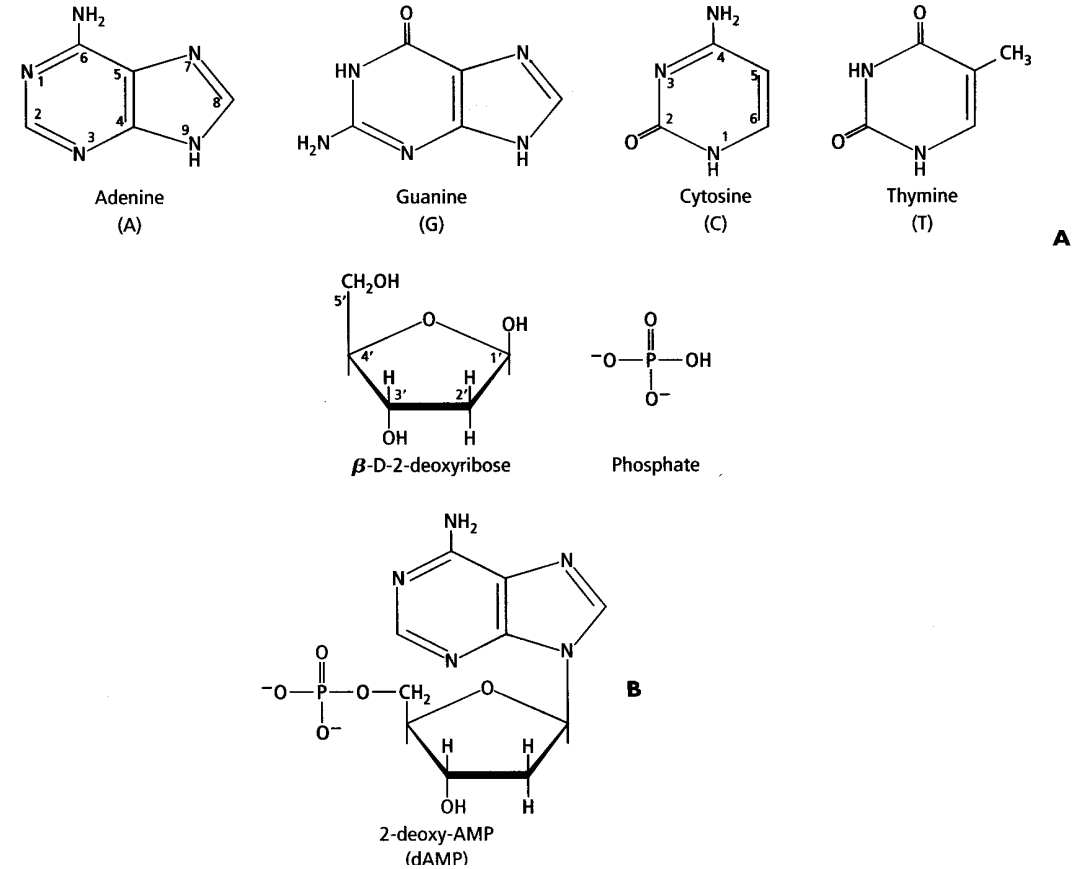
Nucleic acids are required for the storage and expression of genetic information.

- All visible properties and functions of a cell (**phenotype)** are determined by proteins.
- The structure (and therefore the function) of proteins is determined by their primary amino acid sequence.
- The amino acid sequence of the proteins is determined by the nucleotide sequence of DNA.



Chemical structure of the DNA molecule: the building blocks of DNA

- DNA is a polymer of deoxyribonucleoside monophosphates
- Each deoxyribnucleoside monophosphate contains:
  - A pentose sugar (2-deoxyribose)
  - Phosphate
  - One heterocyclic base, which can be either a purine (adenine or guanine), or a pyrimidine (thymine or cytocine)
- The C atoms of the 2- deoxyribtose are numbered with a prime (‘) in order to be distinguished from the C or N atoms of the bases.



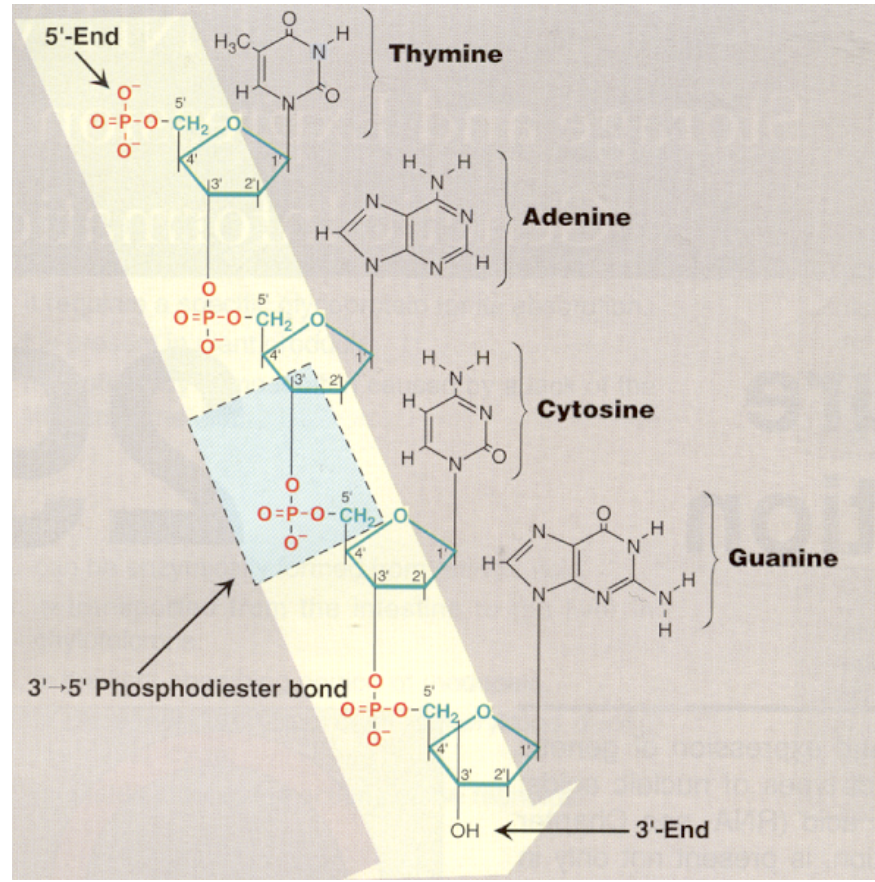
Chemical structure of the DNA molecule: the primary structure

The backbone of the DNA molecule is formed by alternating phosphate and 2-deoxyribose residues held by phosphodiester bonds.

The DNA molecule has polarity. By convention, the 5’ -terminus is written at the left end of the sequence and the 3’ -terminus at the right end.

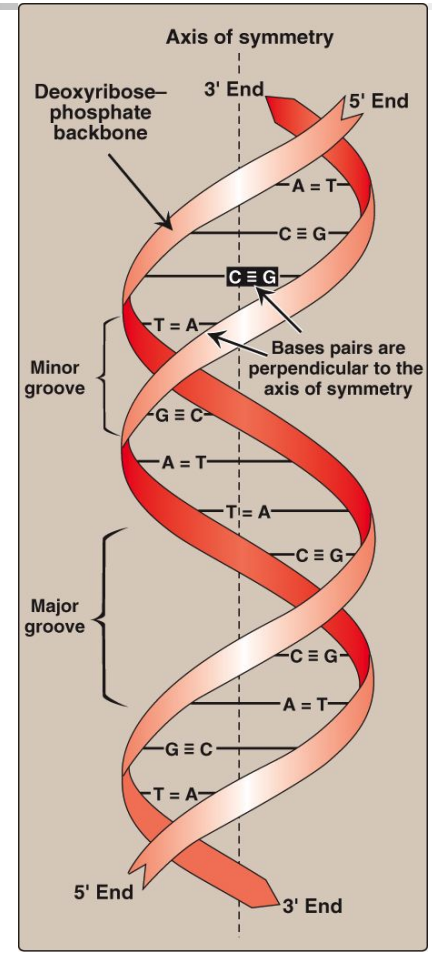
Variability of DNA structure: 4^n sequence possibilities exist for a sequence of n nucleotides.

Deoxyribonucleases (endonucleases and exonucleases hydrolyticaly cleave the phosphodiester bonds)

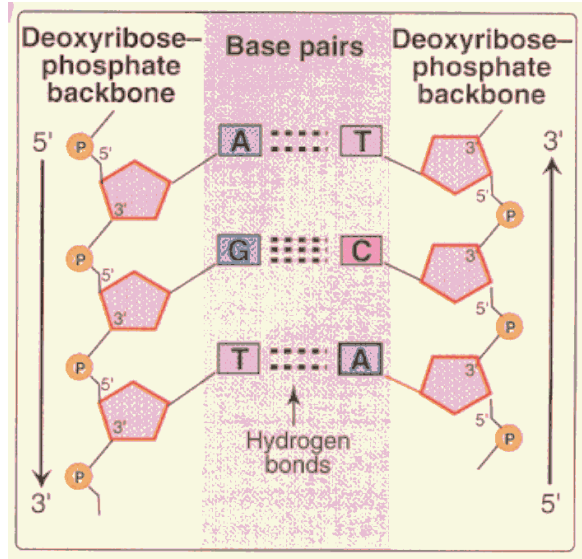


Secondary structure of the DNA molecule: The Watson-Crick double helix.

- The two strands of the double helix have opposite polarity.
- The hydrophilic deoxyribose-phosphate backbones of the two DNA strands form two ridges on the surface of the molecule. Phosphate groups have a negative charge at physiologic pH.
- Bases in opposite strands interact by hydrogen bonds.
- The double-helix structure is stabilized by the hydrogen bonds plus the hydrophobic interactions between the stacked bases.
- The hydrophobic bases are stacked flat on top of each other inside the molecule, creating a major and a minor groove.
- These grooves provide binding to regulatory proteins.
- Anticancer drugs (dactinomycin) intercalate into the minor groove interfering with DNA or RNA synthesis.



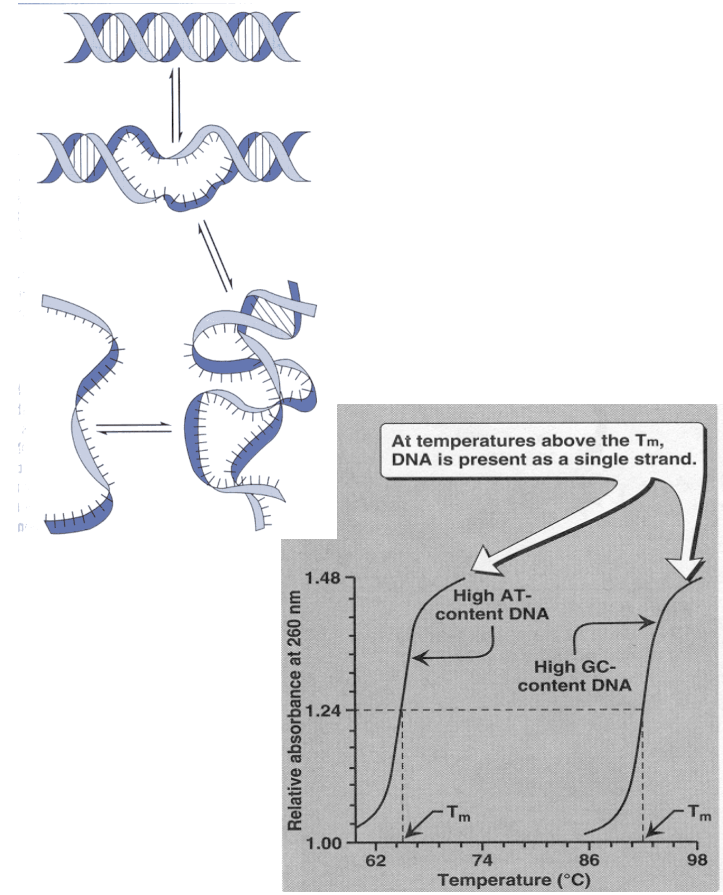
Base Pairing



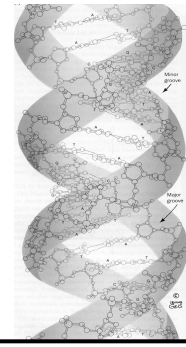
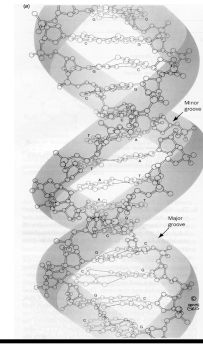
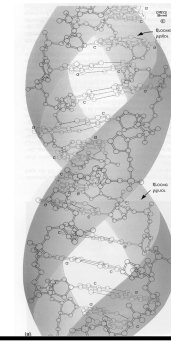
- The interactions between the bases in opposite DNA strands are highly specific:
  - Adenine always pairs with thymine; guanine always pairs with cytosine.
- Each A-T base pair is held together by two hydrogen bonds; each C-G pair by three.
- Double-stranded DNA molecules always contain equal molar amounts of purines (A and T), and equal molar amounts of pyrimidines (G and C) (“Chargaff Rule”).
- Each polynucleotide chain of the DNA double–helix is always the complement of the other.

Denaturation of the double-helix DNA molecule

- The two DNA strands can be separated when the hydrogen bonds between the paired bases are disrupted.
- Denaturation of DNA can occur under the following conditions:
  - Increased temperature
  - Changes in the pH
  - Decreased salt concentration
  - Presence of denaturing agents, such as formamide
- Denaturation begins in areas with high A-T content. G-C pairs (three hydrogen bonds) are more difficult to separate than A-T pairs (two hydrogen bonds).
- Single-stranded DNA has higher (~37%) absorbance at OD 260nm than double-stranded DNA, due to loss of the base stacking interactions.
- Renaturation (annealing) of the DNA molecule occurs spontaneously when cooled down slowly (5°C to 20° C below the Tm).



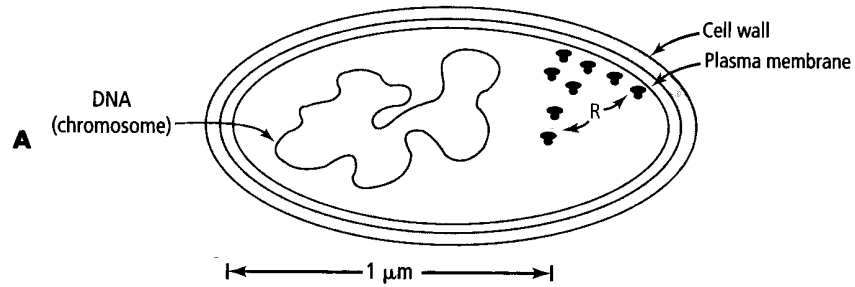
Structural forms of the double-helix

Property	***A-DNA***	***B-DNA***	***Z-DNA***
Helix sense	Right	Right	Left
Base pairs/turn	11	10	12
Rise/base pair*	2.6 Å	3.4 Å	3.7 Å
Helix pitch*	28.6 Å	34 Å	44.4 Å
Widest diameter	25.5 Å	23.7 Å	18.4 Å
Major groove	Narrow, very deep	Wide and deep	Wide and flat
Minor groove	Wide and shallow	Narrow and deep	Very narrow

*One angstrom (Å) is 0.1 nm or 10^{-10} m

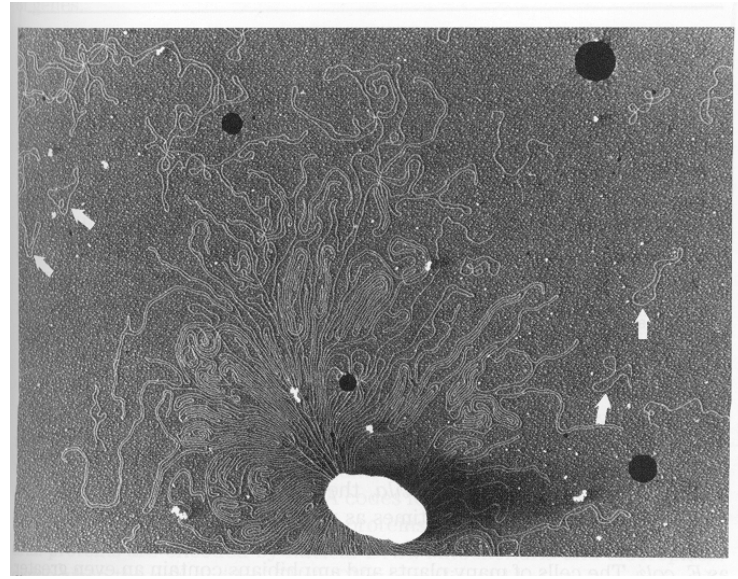
The bacterial chromosome



- The chromosome of a typical bacterium consists of a single, double-stranded DNA molecule (*E. coli*: 4,6 x 10^6 bp), usually associated with proteins.
- The bacterial chromosome contains only one copy of each gene (**haploid**) (*E. coli*: 4,300 genes encoding proteins and 115 genes for RNA).
- Every gene is precisely colinear with the amino acid sequence (or RNA) it encodes.
- Many bacteria additionally contain small, circular extrachromosomal DNA molecules, called **plasmids**.

Plasmids

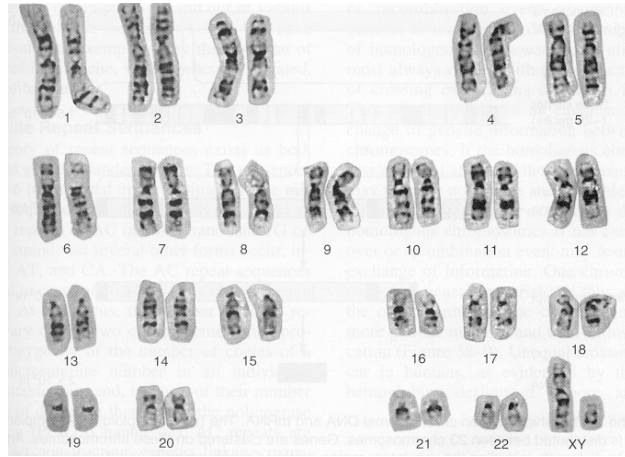
- Plasmids are small, circular, extrachromosomal DNA molecules, present in bacteria and some lower eukaryotes.
- Plasmid size can vary from 2,000 to 200,000 bp.
- Copy number may vary
- Genes carried on plasmids are usually not essential for the survival of the cells.
- Plasmids are self-replicating entities. Their replication may or may not be synchronized to chromosomal division.



Biological importance of plasmids

- Gene products encoded by plasmids are usually **antibiotic resistance** determinants (R factors), toxin production, ability to degrade unusual substrates.
- Plasmids can be transferred from one cell to an other, some of them independently via conjugation (eg the F “fertility” factor of *E.coli*).
- For these reasons, plasmids are involved in the development of bacterial strains that are resistant to particular classes of antibiotics.
- Frequent prescription of low doses of antibiotics, if this is not strictly indicated, provides the selective pressure for the emergence of resistant strains and should be avoided.
- Plasmids are also important tools in molecular biology and the recombinant technology, because they can serve as **vectors** for gene cloning.

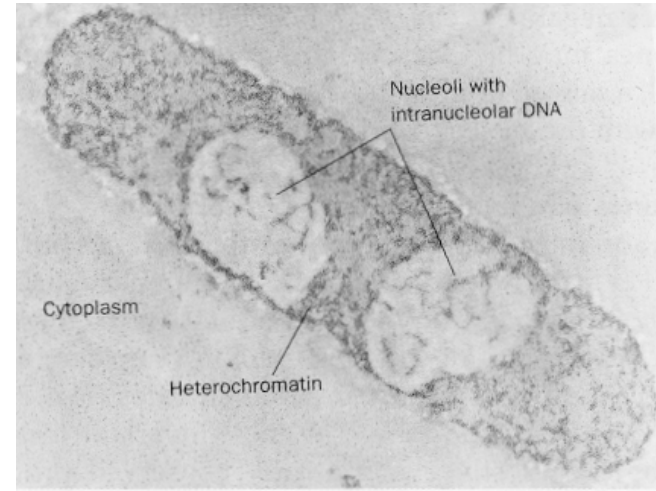
Organization of the Human Genome



- The haploid human genome:
  - contains ~3.9 billion base pairs
  - Has a stretched-out length of 130 cm
  - Is packaged in 23 chromosomes
- Each chromosome contains one, linear, double stranded DNA molecule, which is several cm long, and contains 50,000-100,000 genes, plus non-coding DNA.

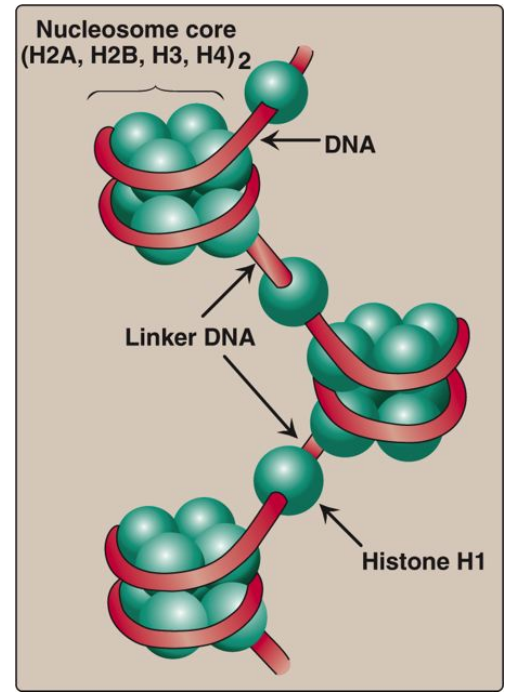
The eukaryotic chromosome

- In a nondividing eukaryotic cell the chromosomal material **(chromatine)** appears to be amorphous, and dispersed throughout the nucleus.
- Chromatin is a nucleoprotein complex consisting of:
  - DNA (~50%)
  - Histones
  - Non- histone proteins (proteins involved in DNA replication and in transcription)
  - Small amounts of RNA
- There are two types of chromatin:
  - Euchromatin, less densely packed, genetically expressed
  - Heterochromatin, more condensed, deeper stained, not genetically expressed



Histones and the formation of nucleosomes

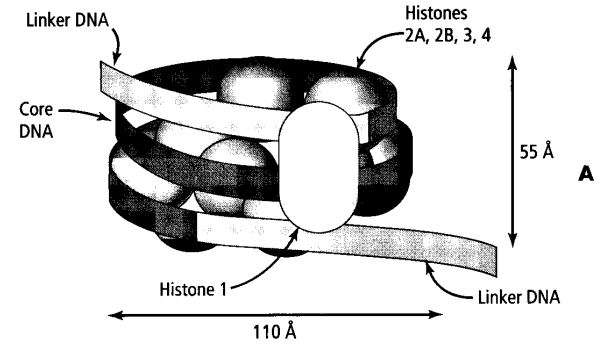
- Histones are small basic proteins which associate with the negatively charged DNA via electrostatic interactions. Their function is to condense the DNA..
- There are five types of histones in eukaryotic cells:
  - H2A and H2B: significantly conserved among species. Lysine-rich. Associate to form dimers (H2A-H2B).
  - H3 and H4: highly conserved. Arginine-rich. They form tetramers containing two molecules of each: $(H3\text{-}H4)_2$.
  - H1: not very conserved among species (tissue-specific and species-specific. Do not bind to chromatin tightly. Help packing the nucleosomes into tighter structures.
- Nucleosome formation:
  - Histones form octamers consisting of one $(H3\text{-}H4)_2$ tetramer plus two (H2A-H2B) dimers.
    DNA (about 146 bp) wraps around the histone octamer, making 1.75 superhelical turns

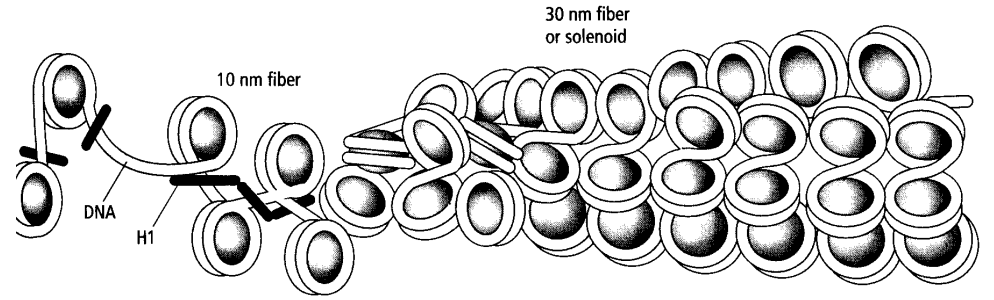


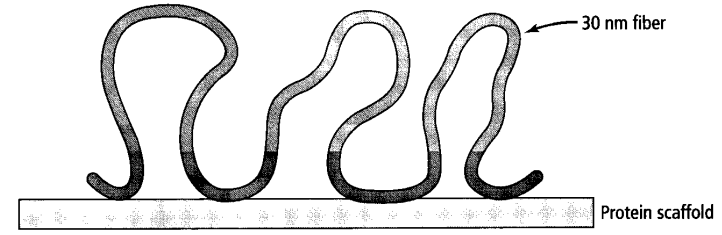
Nucleosomes are connected by a variable length of DNA (50-60 bp) which is associated with a single H1.

Higher order structures contribute to the further compaction of chromatin

- **10-nm fibril**: nucleosomes arranged with their flat surfaces parallel to the fibril axis.
- **30-nm chromatin fiber**: 6 to 7 nucleosomes per turn, with their flat surfaces parallel to each other. Stabilized by H1.
- Chromatin fibers form **loops (domains)** (30,000 to 100,000 pb) anchored in a supporting matrix (scaffold). Each loop may correspond to a separate genetic function.

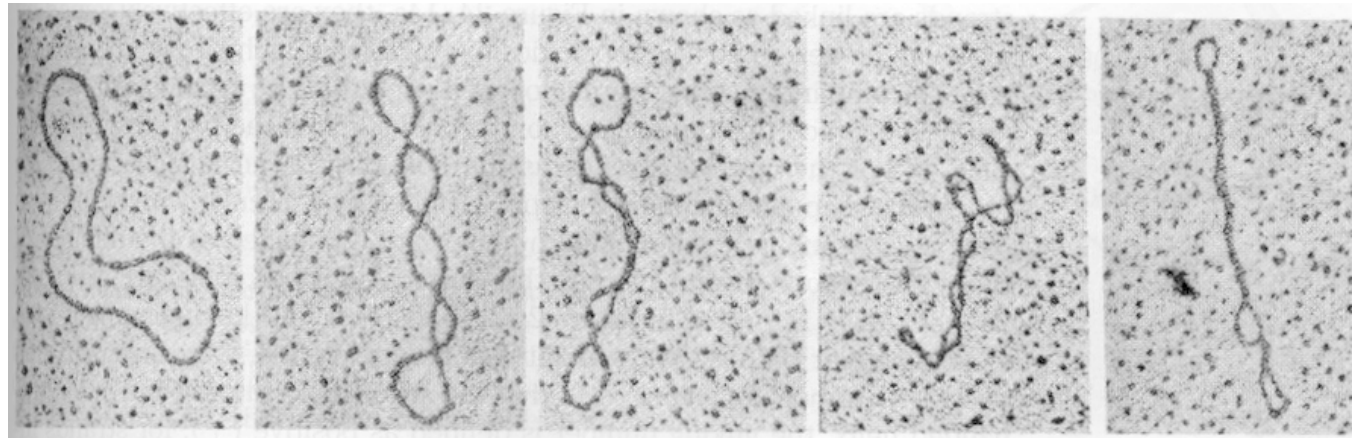




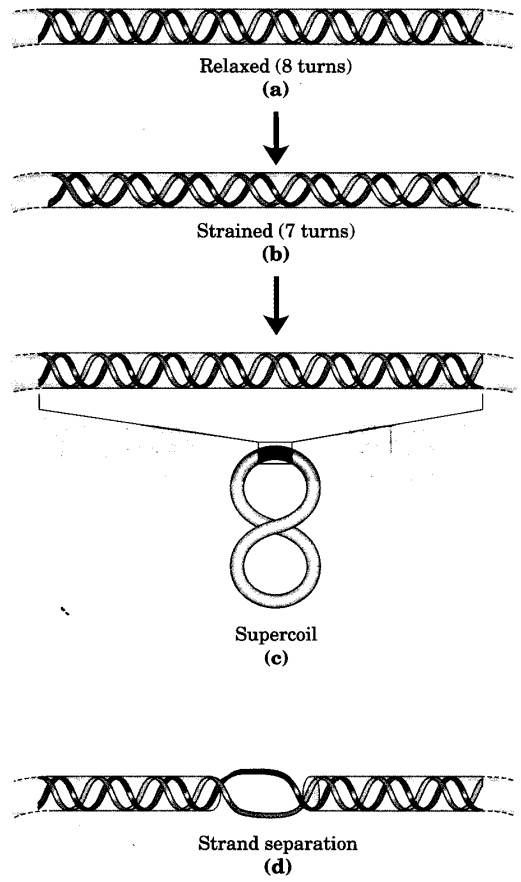


DNA Supercoiling

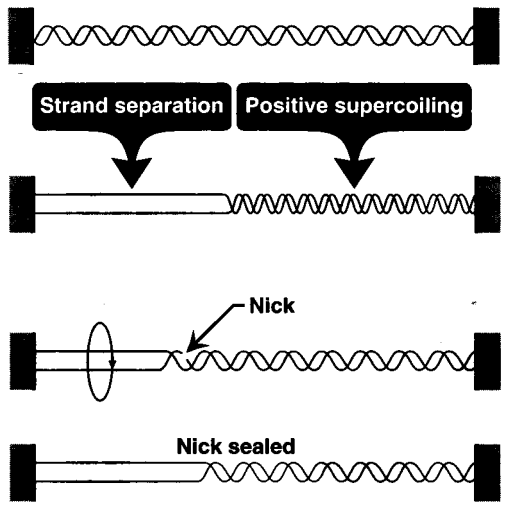
- Supercoiling is an important intrinsic property of the DNA structure which allows the high compaction of the DNA. It is a highly regulated process.
- DNA is coiled in the form of a double helix, in which both strands of the DNA coil around an axis. The further coiling of that axis upon itself produces DNA supercoiling.
- When there is no bending of the DNA axis upon itself, the DNA is said to be in a **relaxed** state.



Effects of negative supercoiling (underwining)



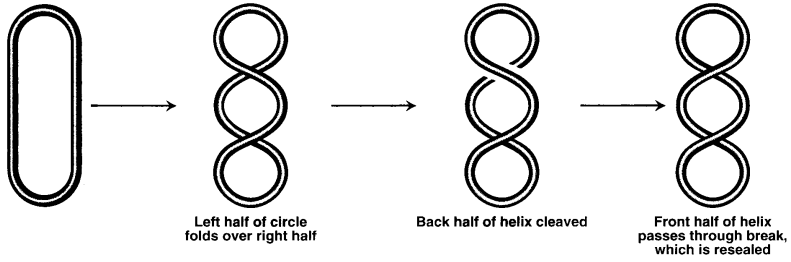
Topoisomerases increase or decrease the extend of DNA underwinding.



Mechanism of action of Type I topoisomerase

Type I topoisomerases:
- cut a single strand of the double helix
- have both nuclease and ligase activities
- do not require ATP
- relaxes negative supercoils in bacteria, negative and positive supercoils in eukaryotic cells.

Type II topoisomerases:
- bind to both strands of the double helix
- relaxes both negative and positive supercoils in prokaryotic and eukaryotic cells
- required for the separation of the interlocked molecules of DNA following chromosomal replication
- do not require ATP

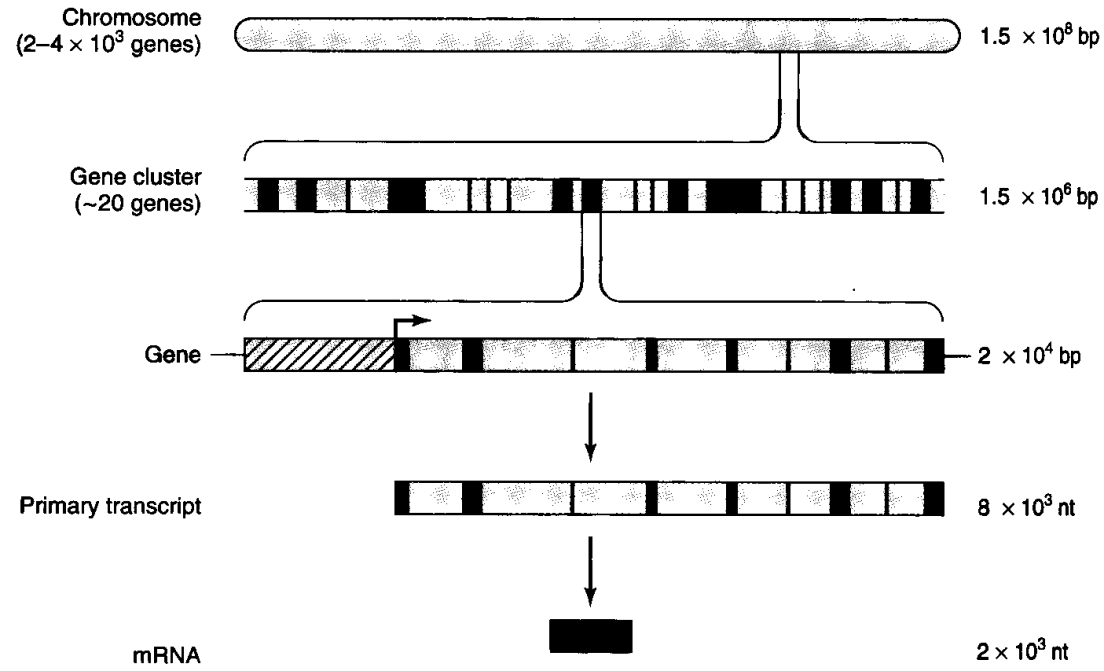


Mechanism of action of Type II topoisomerase

DNA gyrase:
-Unusual type II topoisomerase found in *E. coli*.
-introduces negative supercoils in to resting circular DNA, facilitating future replication
- requires ATP
- inactivated by quinolones

Many eukaryotic genes contain intervening non-transcribed sequences

- The coding regions of the eukaryotic genom (**exons**) are interrupted by large intervening sequences of non-coding DNA (**introns**).
- Introns may be important in the **evolution** of biological function, because they separate functional domains of coding information allowing genetic rearrangements to occur more rapidly through recombination.
- Only ~ 100,000 proteins are expressed in human cells…that implies that much of the human DNA is not expressed.



Significant portion of eukaryotic DNA consists of Repetitive Sequences

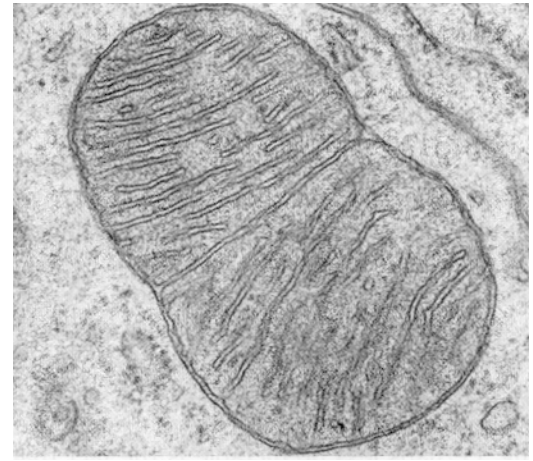
- Highly repetitive sequences (simple-sequence DNA or "satellite" DNA
  - 5-500 bp long, repeated millions of times per cell
  - Usually clustered in the centromeres and telomeres
  - Transcriptionally inactive (may play structural role)
- Moderately repetitive sequences
  - Repeated less than 10^6 times
  - Not clustered, usually transcribed
  - Most arise from **transposition (retroposons)**
    - **Long interspersed repeat sequences (LINEs)**
      - Species specific, 6-7 kbp long, 20-50 thousand copies in mamalian genomes
    - **Short interspersed repeat sequences (SINEs)**
      - 70-300 bp long, may be repeated 100,000 times
      - Best characterized moderately repetitive sequence in humans is the "***Alu* family**". Comprises 1-3% of the total DNA. Most of them are "mobile" elements.

Single Nucleotide Polymorphisms (SNPs, "snips")

- DNA sequence variations that oc
- cur when a single nucleotide in the genome sequence is altered (eg: AAGGCTAA to ATGGCTAA)
- Must occur in at least 1% of the population
- Occur every 100 to 300 bases in the human genome.
- May occur in both coding and noncoding regions of the genome
- Each persons genetic material contains a unique SNP pattern .
- Most of SNPs have no effect on cell function, but others could predispose to disease or influence response to a drug.

Mitochondrial DNA

- In typical somatic cells, mitochondria contain less than 0.1% of the cell's DNA.
- It encodes for mitochondrial tRNAs, rRNAs, and few mitochondrial proteins (less than 5% of them).
- Mitochondrial DNA gets replicated before each mitochondrial division, which occurs before cell devision.
- Mitochondria may have evolved from ancient bacteria, which gained access to the cytoplasm of host cells
- A number of diseases, including some myopathies, neurologic disorders and some cases of diabetes melitus are due to mutations in mt DNA..



Viral genome

- Viruses contain generally much less DNA than cells, because they use the resources of the host cell for their propagation.
- Many viruses, including almost all the ones that infect plants, have single-stranded, RNA genomes.
- The shape of the viral genome changes during replication within the host: many linear DNAs become circular and and all single-stranded DNAs become double-stranded