Genome and its organization

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The Syllabus

MLB-G-CC-3-1

Concepts of Molecular Biology (4+2 credits)

MLB-G-CC-3-1-TH (4 credits/50 marks)

Basic Concepts of genome and its organisation (20 hrs)

Nucleic acid as the genetic material (Griffith's experiment, Avery, MacLeod and McCarty's experiment, Hershey-Chase experiment), Importance of Molecular Biology, Central Dogma of Molecular Biology, Model organisms for studying Molecular Biology. (5 hrs)

Structure and functions of Nucleic acids: Nucleosides & Nucleotides, purines and pyrimidines. Biologically important nucleotides, (5 hrs)

Watson and Crick model of DNA structure, A, B & Z forms of DNA, Supercoiled and relaxed DNA, denaturation and renaturation of DNA, melting temperature (Tm), hyperchromic effect.(5 hrs)

Genome and its organisation : (idea about gene, coding sequence, regulatory sequence, intron, exon, Nucleosome structure and packaging of DNA into higher order structures, brief idea of chloroplast DNA and Mitochondrial DNA).(5 hrs)

The Organization of DNA in Chromosomes

1. Cellular DNA is organized into chromosomes. A **genome** is the chromosome or set of chromosomes that contains all the DNA of an organism.

2. In prokaryotes the genome is usually a single circular chromsome. In eukaryotes, the genome is one complete haploid set of nuclear chromosomes; mitochondrial and chloroplast DNA are not included.

Viral Chromosomes

1. A virus is nucleic acid surrounded by a protein coat. The nucleic acid may be dsDNA, ssDNA, dsRNA or ssRNA, and it may be linear or circular, a single molecule or several segments.

2. Bacteriophages are viruses that infect bacteria. Three different types that infect E. coli are good examples of the variety of chromosome structure found in viruses.

The T-even phages (T2, T4 and T6) have similar structures; all have dsDNA genomes composed of a one linear DNA molecule

ΦX174 is a small, simple virus with one short ssDNA chromosome

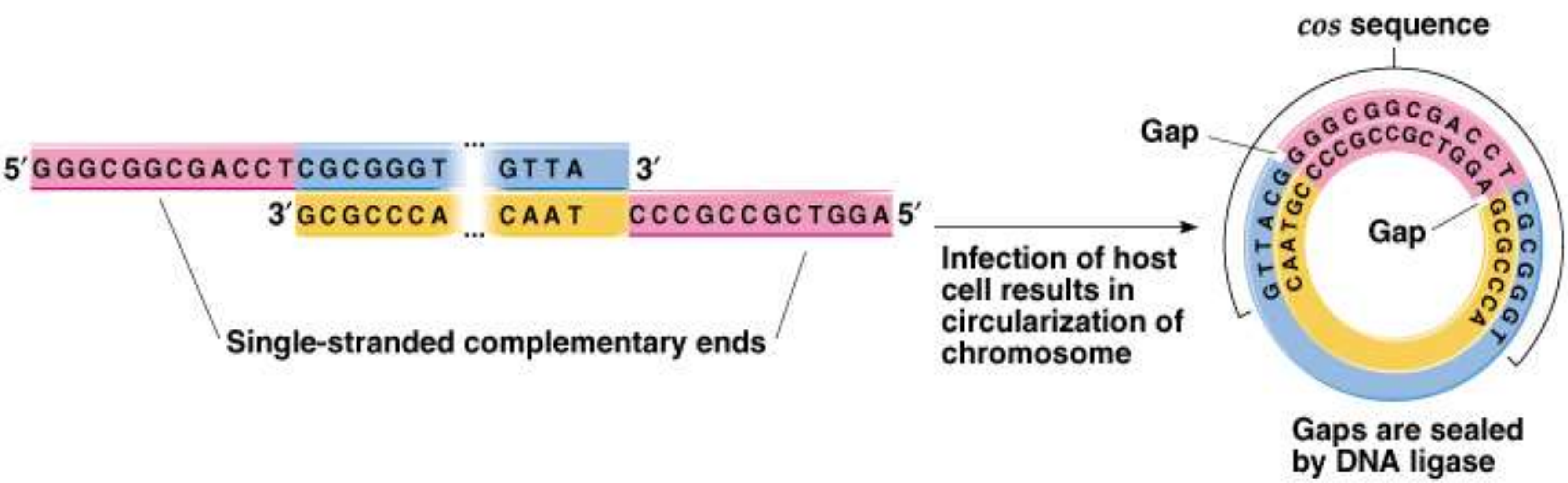
Bacteriophage λ

λ phage

Bacteriophage λ is somewhat like the T-even phages in structure.

However, its chromosome is a linear molecule of dsDNA when packaged inside the protein head.

After the virus infects its host and injects its DNA inside the host cell, the viral chromosome becomes circular due to base-pairing of complementary 12-base single-stranded regions (cos sites) at the ends of the linear molecule

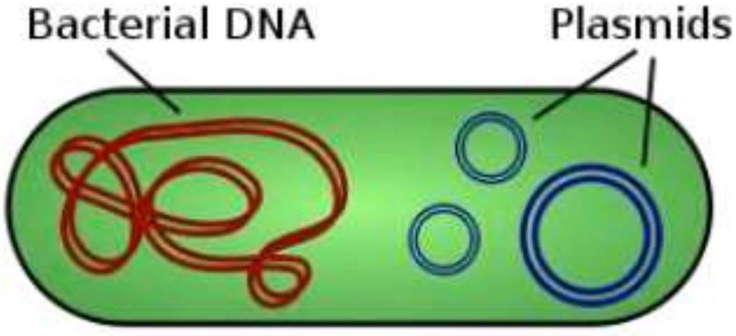


Prokaryotic Chromosomes

1. The typical prokaryotic genome is one circular dsDNA chromosome, but some prokaryotes are more exotic, with a main chromosome and one or more smaller ones. When a minor chromosome is dispensable to the life of the cell, it is called a plasmid. Some examples:

   a. *Borrelia burgdorferi* (Lyme disease in humans) has a 0.91-Mb linear chromosome, plus an additional 0.53-Mb of DNA in 17 different linear and circular molecules.

   b. *Agrobacterium tumefaciens* (crown gall disease of plants) has a 3.0-Mb circular chromosome and a 2.1-Mb linear one.

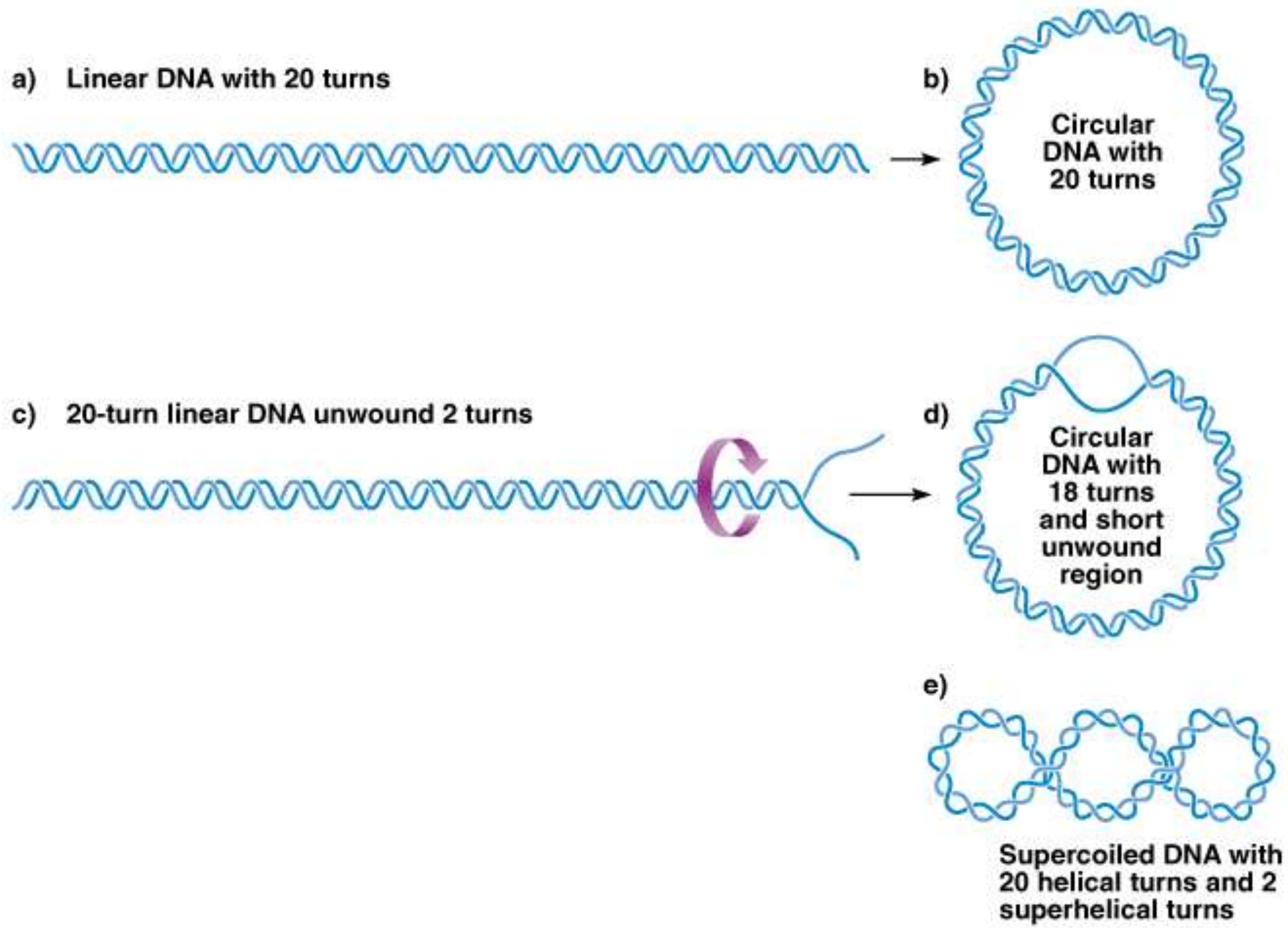


Important Facts

Both Eubacteria and Archaebacteria lack a membrane-bounded nucleus, hence their classification as prokaryotes. Their DNA is densely arranged in a cytoplasmic region called the *nucleoid*.

In an experiment where *E. coli* is gently lysed, it releases one 4.6-Mb circular chromosome, highly supercoiled (Figures 2.19 and 2.20). A 4.6-Mb double helix is about 1mm in length, about 10^3 times longer than an *E. coli* cell. DNA supercoiling helps it fit into the cell.

DNA supercoiling



Important Facts…contd

A molecule of B-DNA, with 10bp/turn of the helix, is in relaxed conformation. However, when the molecule circularizes, it will form superhelical.

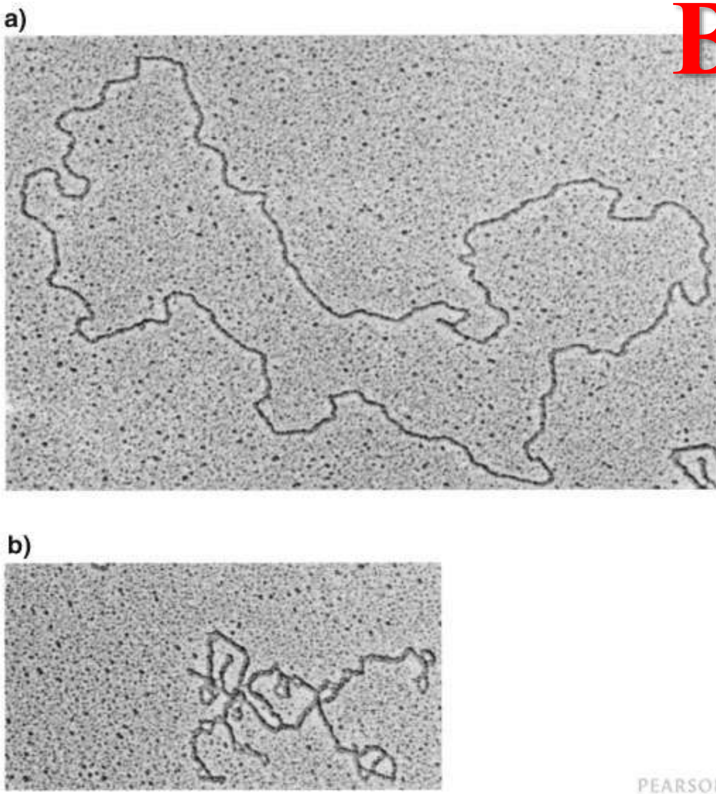
Either overwinding or underwinding DNA will create a structure where 10bp/turn of the helix is not the most energetically favored conformation, and supercoils will be induced. Both positive and negative supercoils will condense the DNA.

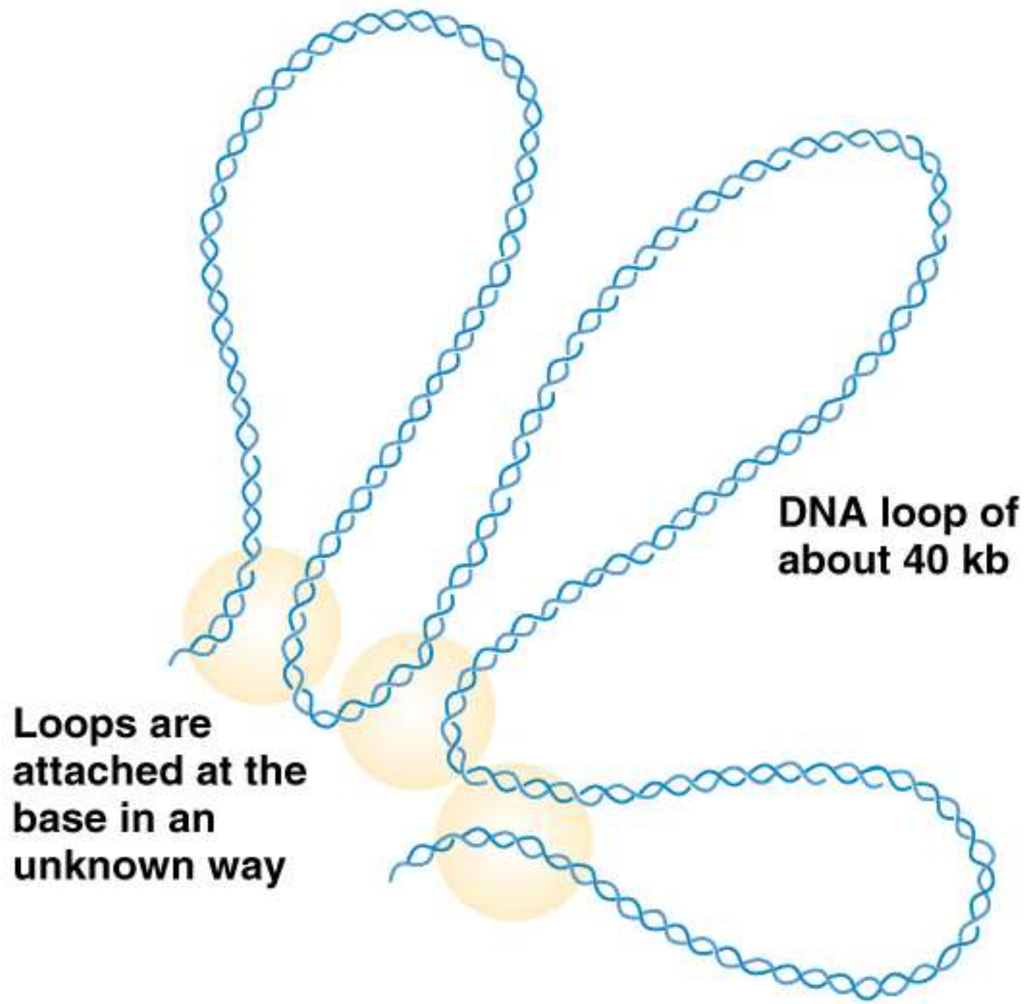
All organisms contain topoisomerase enzymes to supercoil their DNA.

Prokaryotes also organize their DNA into looped domains, with the ends of the domains held so that each is supercoiled independently.

The compaction factor for looped domains is about 10-fold. In *E. coli* there are about 100 domains of about 40kb each.

Model for the Structure of Bacterial Chromosome





Eukaryotic Chromosomes

The genome of most prokaryotes consists of one chromosome, while most eukaryotes have a diploid number of chromosomes.

A genome is the information in one complete haploid chromosome set. The total amount of DNA in the haploid genome of a species is called its C value.

The structural complexity and the C value of an organism are not related, creating the C value paradox.

More about genome size

C value = total amount of DNA in the haploid (1N) genome

Varies widely from species to species and shows no simple relationship to structural or organizational complexity.

Examples	C value (bp)
λ	48,502
T4	168,900
HIV-1	9,750
E. Coli	4,639,221
Lilium formosanum	36,000,000,000
Zea mays	5,000,000,000
Amoeba proteus	290,000,000,000
Drosophila melanogaster	180,000,000
Mus musculus	3,454,200,000
Canis familiaris	3,355,500,000
Equus caballus	3,311,000,000
Homo sapiens	3,400,000,000

Chromatin Structure

1. Both **histones and non-histone proteins** are involved in physical structure of the chromosome.

2. Histones are abundant, small proteins with a net (+) charge. **The five main types are H1, H2A, H2B, H3 and H4**. By weight, chromosomes have equal amounts of DNA and histones.

3. Histones are highly **conserved** between species (H1 less than the others).

4. Histones organize DNA, condensing it and preparing it for further condensation by nonhistone proteins. This compaction is necessary to fit large amounts of DNA (2m/6.5ft in humans) into the nucleus of a cell.

5. **Non-histone is a general name for other proteins associated with DNA.** This is a big group, with some structural proteins, and some that bind only transiently. Non-histone proteins vary widely, even in different cells from the same organism. Most have a net (-) charge, and bind by attaching to histones.

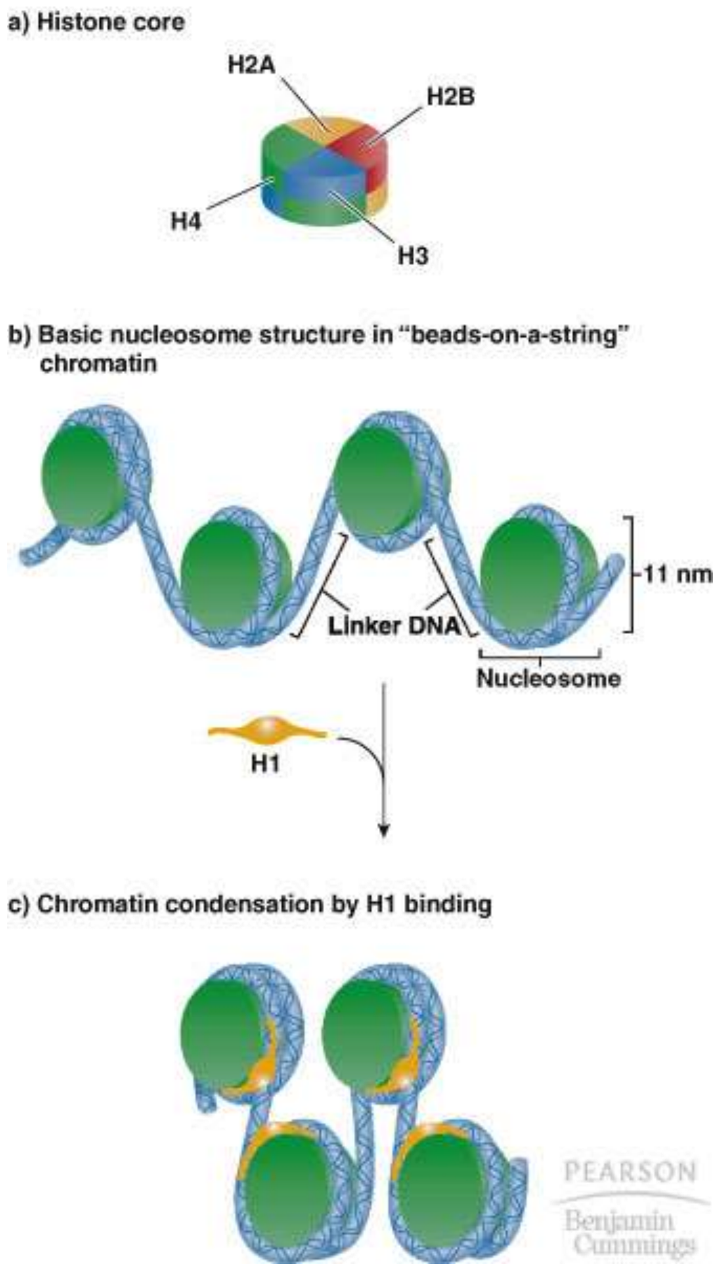
Chromatin Structure…contd

6. Chromatin formation involves histones, and condenses the DNA so it will fit into the cell. Chromatin formation has two components:

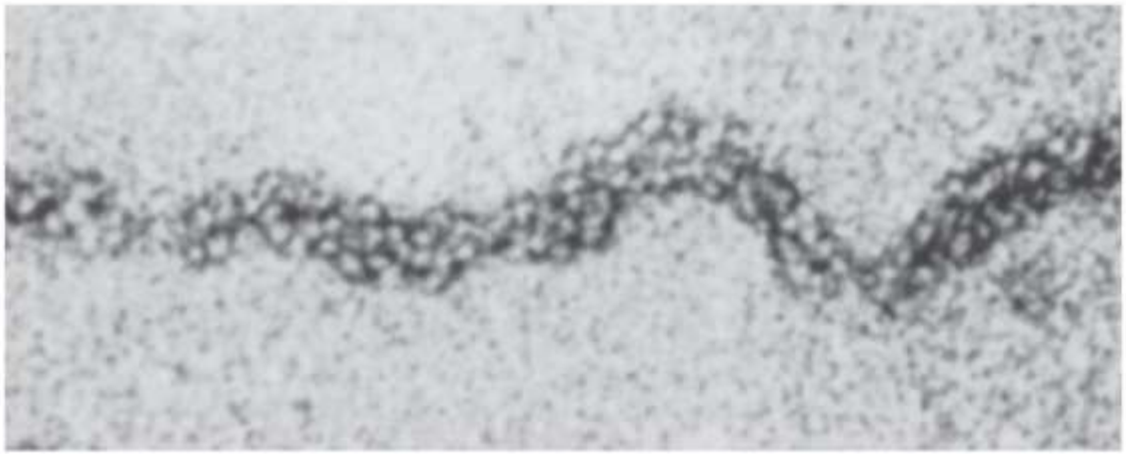
7. Two molecules each of histones H2A, H2B, H3 and H4 associate to form a **nucleosome core**, and DNA wraps around it **1 3⁄4 times** for a 7-fold condensation factor. Nucleosome cores are about **11 nm in diameter**

8. H1 further condenses the DNA by connecting nucleosomes to create **chromatin with a diameter of 30nm**, for an additional 6-fold condensation. The solenoid model proposes that the nucleosomes form a spiral with 6 nucleosomes per turn.

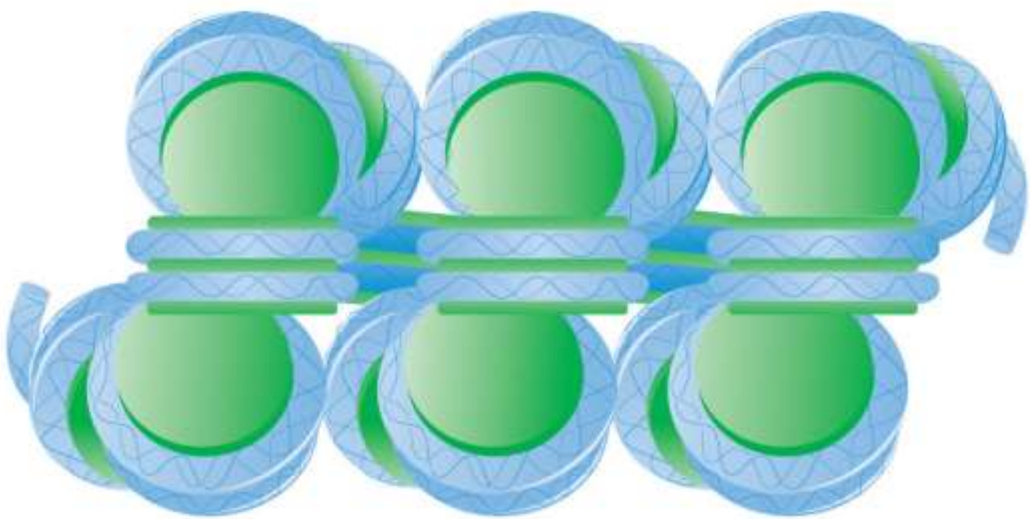
Basic Eukaryotic Chromatin Structure



a) Electron micrograph of 30-nm chromatin fiber

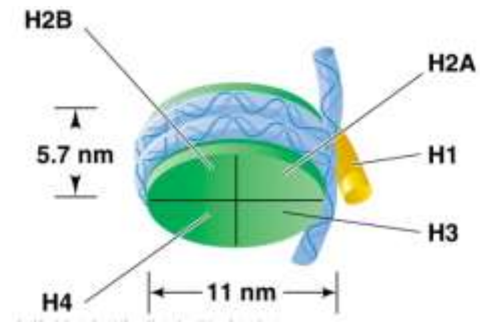


b) Solenoid model for nucleosome packaging in the 30-nm chromatin fiber (H1 is not shown)



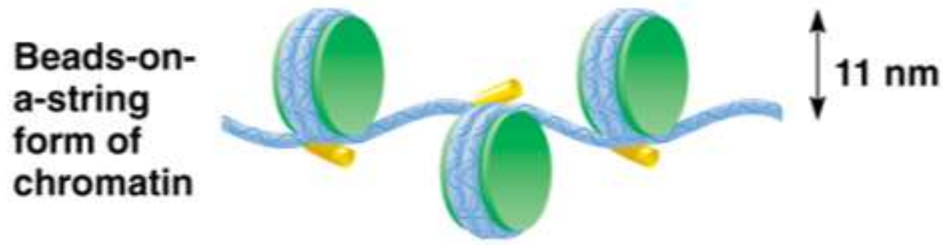
Packing of DNA into chromosomes

Level 1: Winding of DNA around <u>histones</u> to create a <u>nucleosome</u> structure.

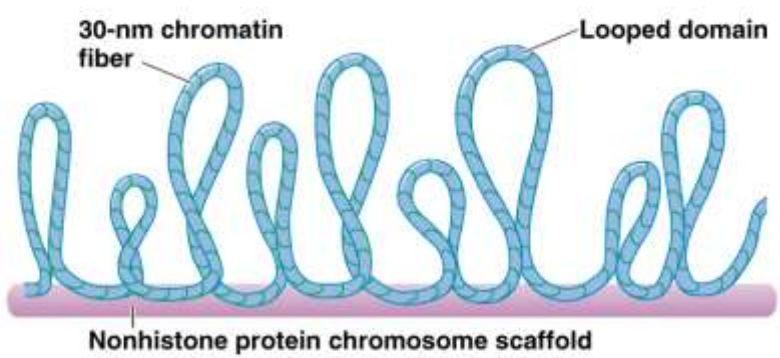


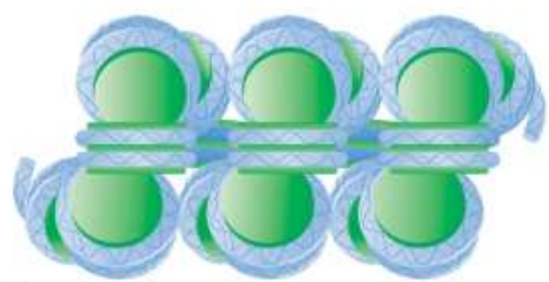
Level 2 : Nucleosomes connected by strands of linker DNA like <u>beads on a string</u>.

Level 3: Packaging of nucleosomes into 30-nm <u>chromatin fiber</u>.



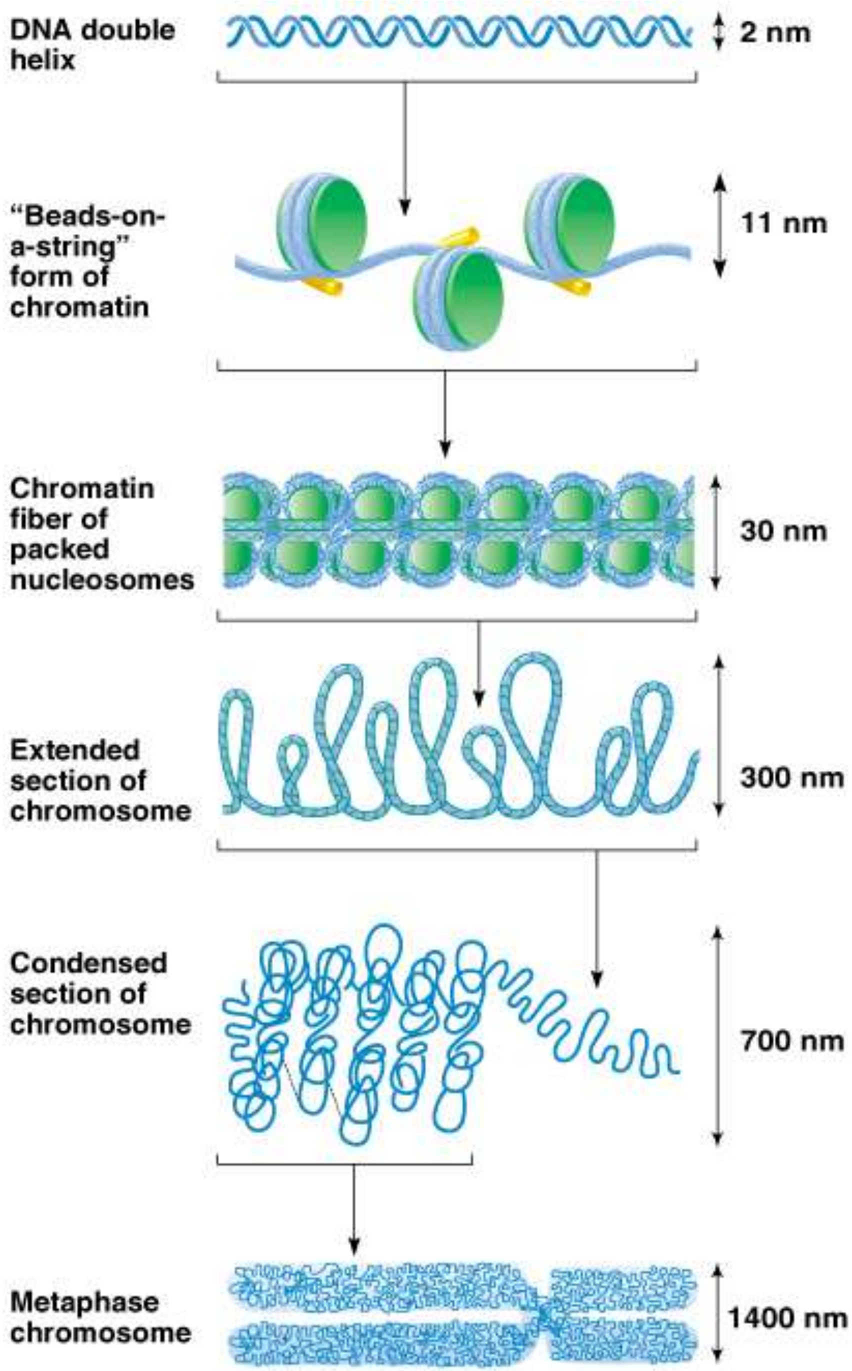
Level 4: Formation of looped domains.

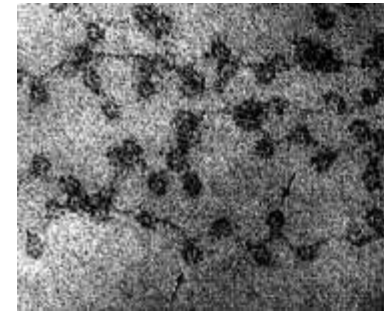




b)

Packing of DNA into chromosomes

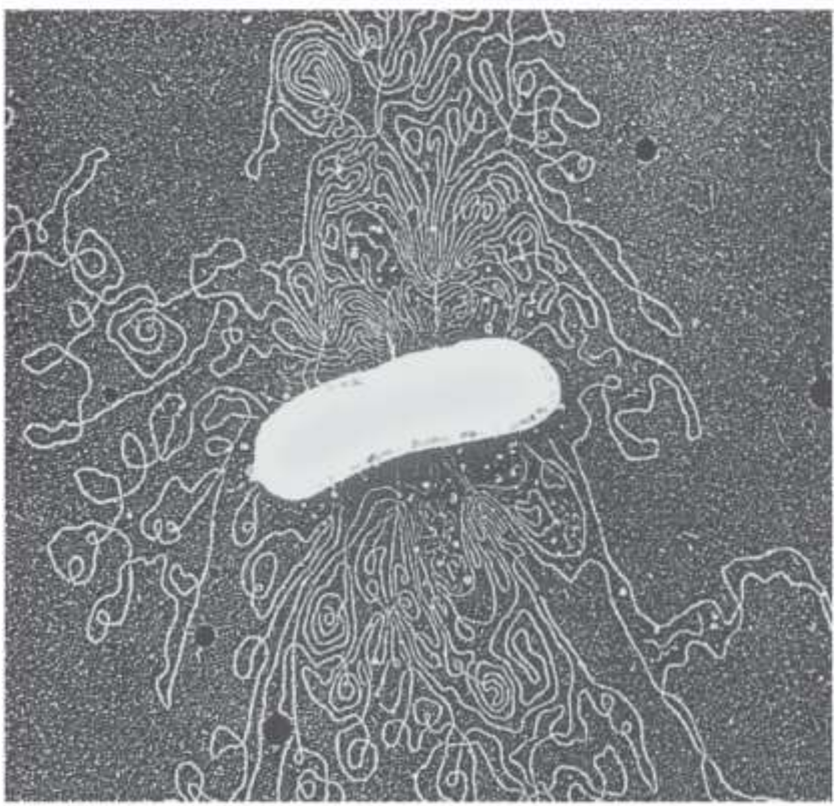




Some Important Facts

Measured linearly, the *Escherichia coli* genome (4.6 Mb) would be 1,000 times longer than the *E. coli* cell.

The human genome (3.4 Gb) would be 2.3 m long if stretched linearly.



Chromosome released from lysed *E. coli* cell.

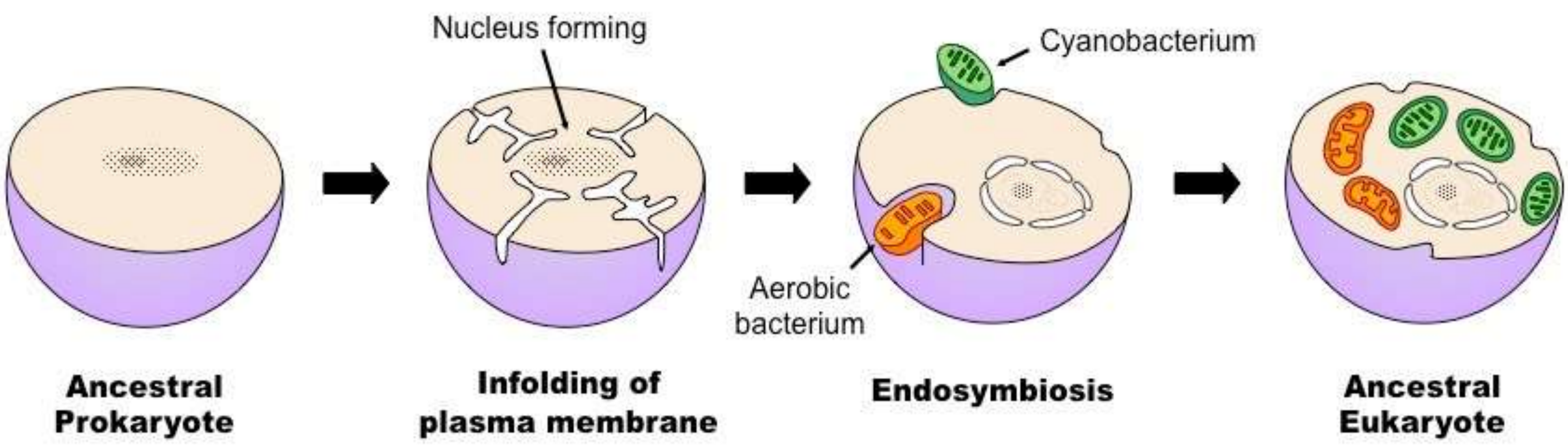
The Endosymbiotic theory

An endosymbiont is a cell which lives inside another cell with mutual benefit.

Eukaryotic cells are believed to have evolved from early prokaryotes that engulfed other cells by phagocytosis.

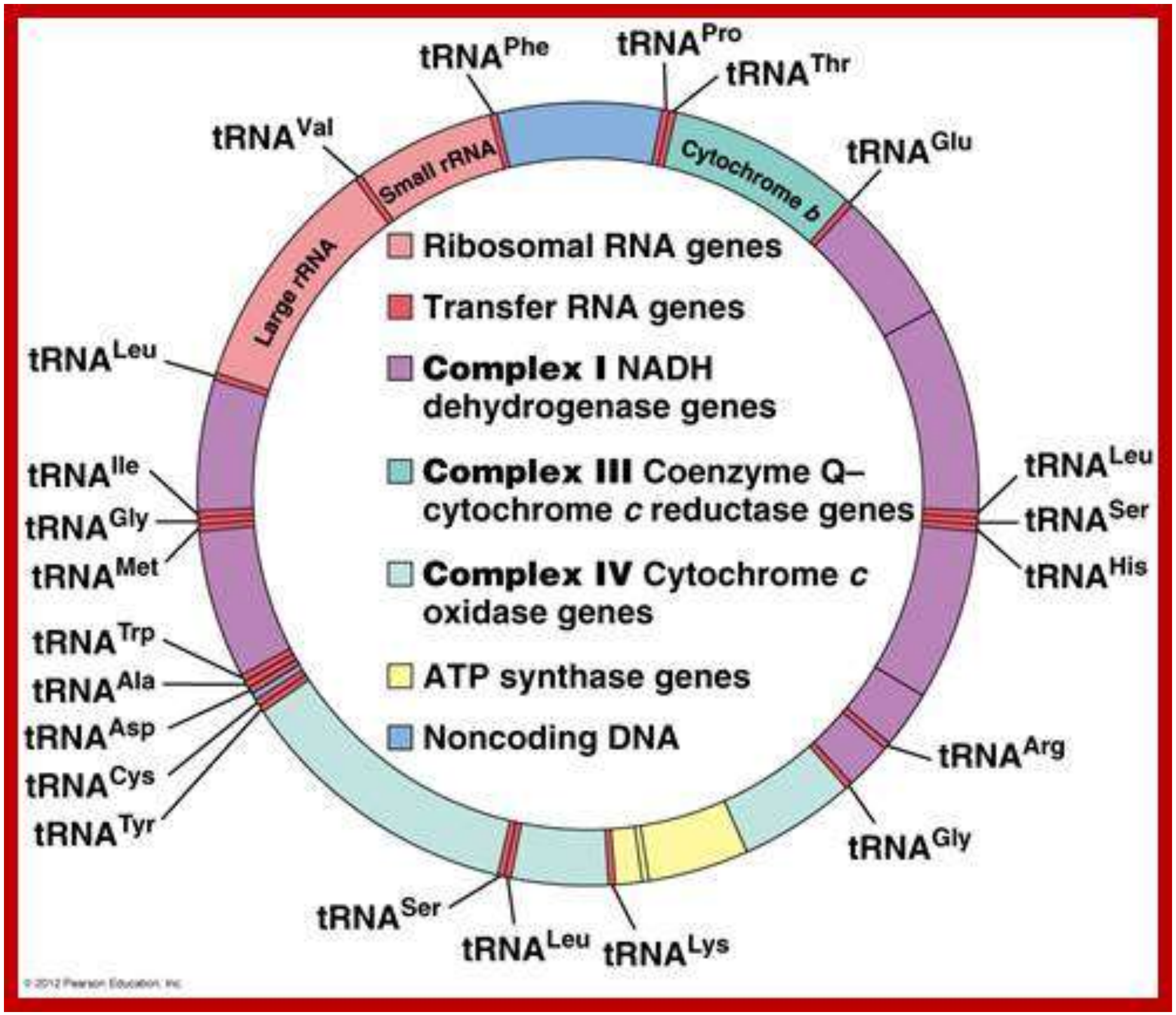
The engulfed prokaryotic cell remained undigested as it contributed new functionality to the engulfing cell (e.g. photosynthesis).

Over generations, the engulfed cell lost some of its independent utility and became a supplemental organelle



Chloroplast DNA

Chloroplasts have their own DNA.(cpDNA) It is also known as the plastome . Its existence was first proved in 1962, and first sequenced in 1986



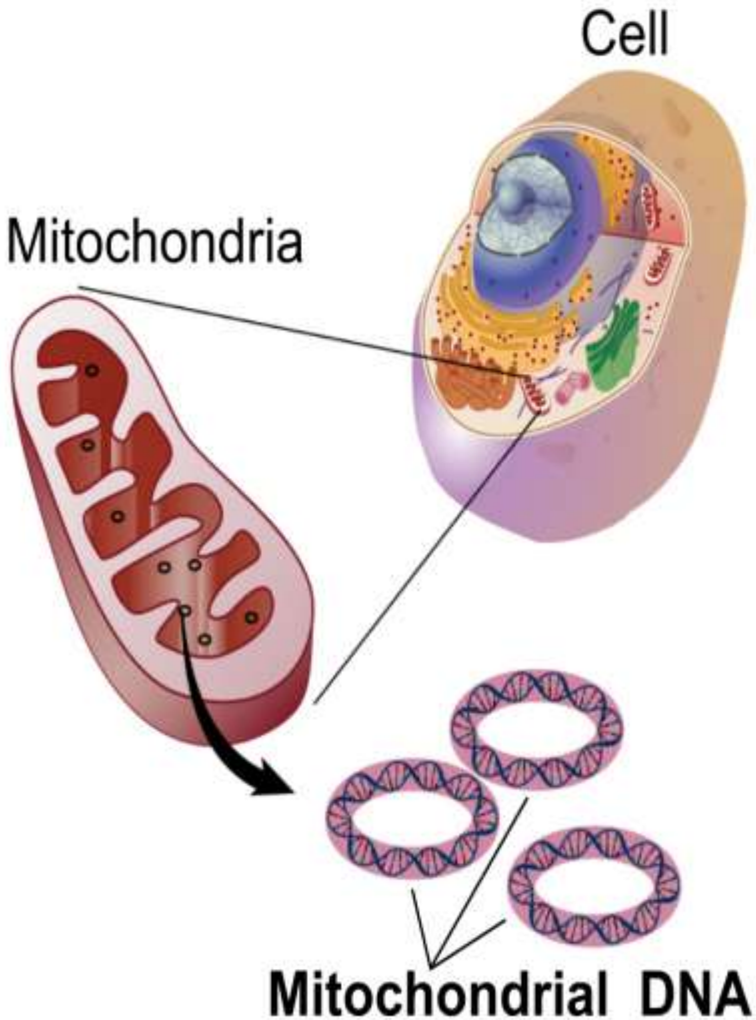
Usually single large circular.

Present in multiple copies.

About 120,000 to 170,000 bp long

Mitochondrial DNA

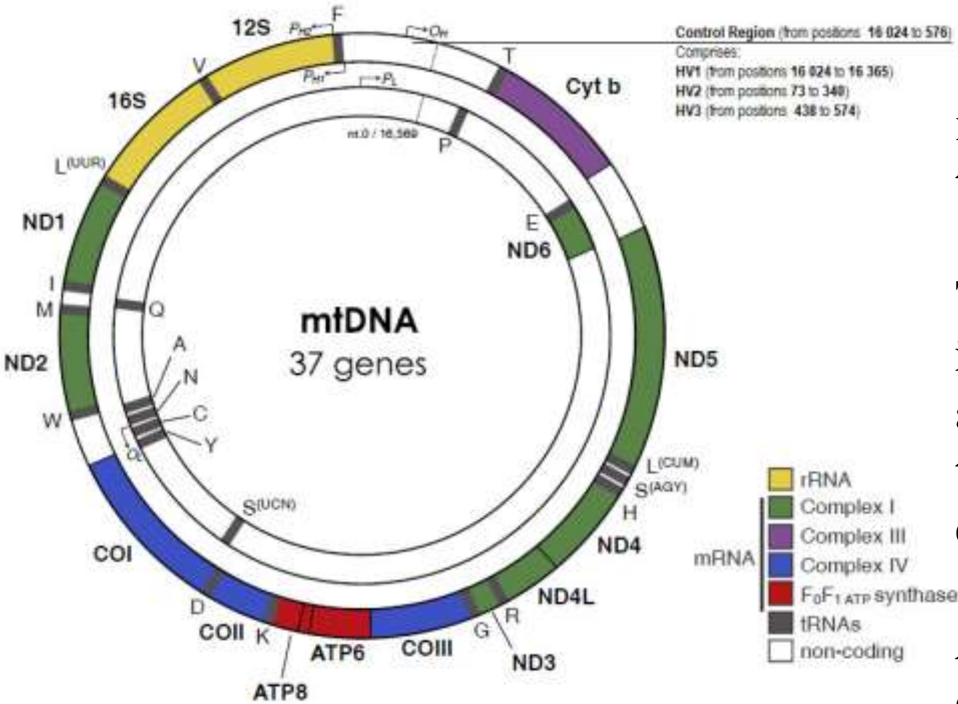
The mitochondrial DNA (mtDNA) was first identified and isolated by Margit Nass and Sylvan Nass in 1963



The mtDNA is a histone-free circular double-stranded DNA molecule, with around 16 569 base-pairs and weighting 10^7 Daltons

Mitochondrial DNA (mtDNA) presents several characteristics useful for forensic studies, especially related to the lack of recombination, to a high copy number, and to matrilineal inheritance.

Mitochondrial DNA



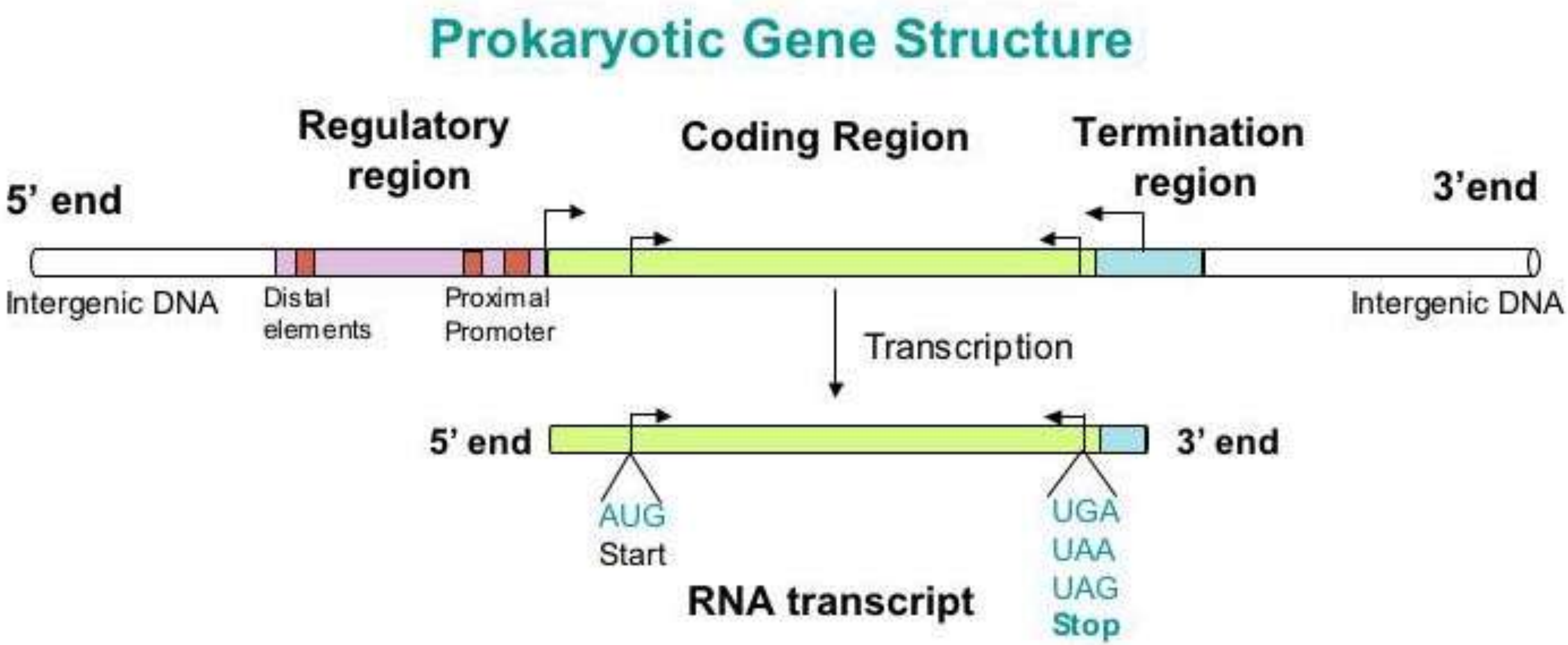
mtDNA strands have different densities due to different G+T base composition.

The heavy (H) strand encodes more information, with genes for two rRNAs (12S and 16S), twelve polypeptides and fourteen tRNAs, while the light (L) strand encodes eight tRNAs and one polypeptide.

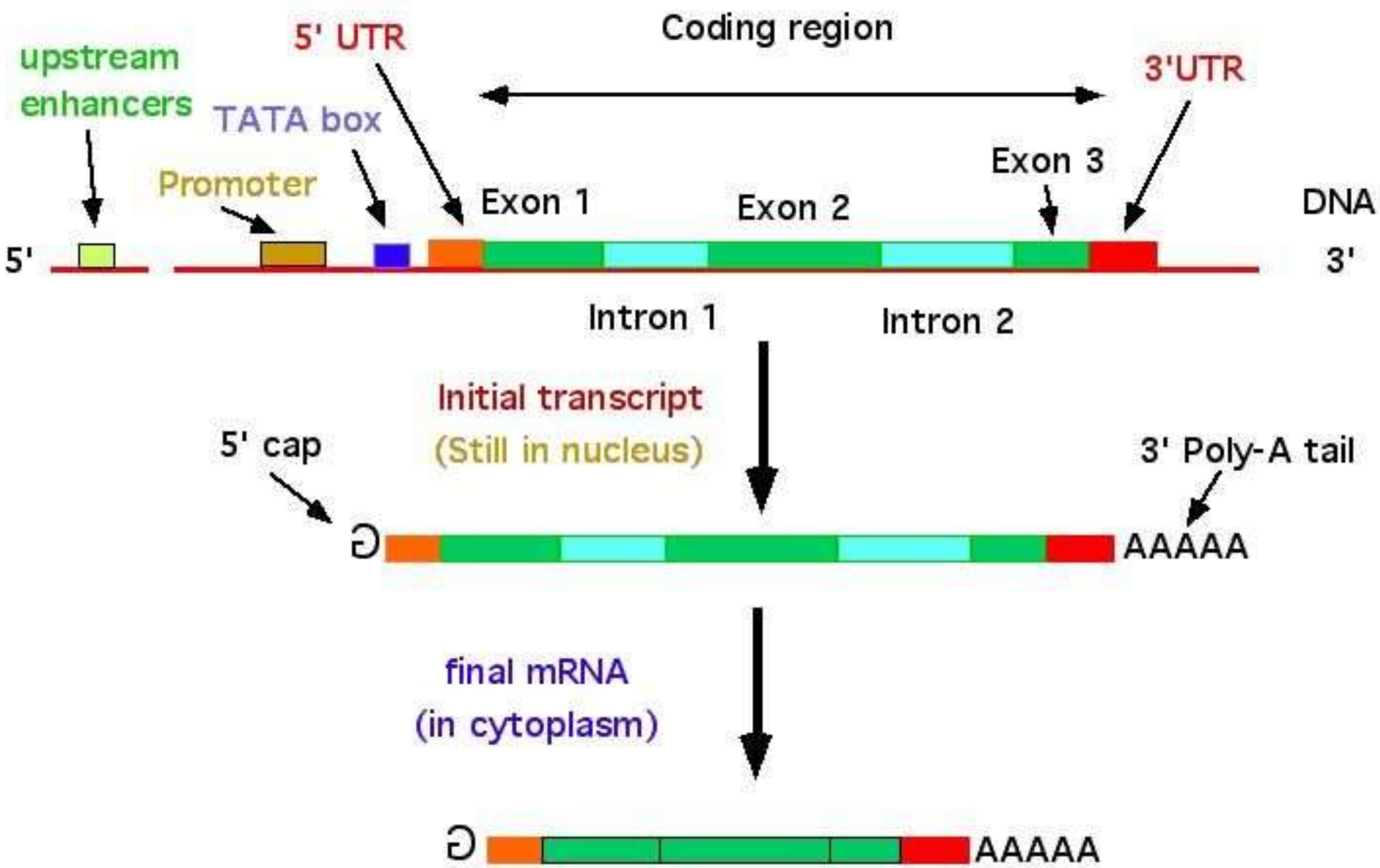
All the 13 protein products are part of the enzyme complexes that constitute the oxidative phosphorylation system.

Other characteristic features of the mtDNA are the intronless genes and the limited, or even absent, intergenic sequences, except in one regulatory region.

Gene, Coding sequence and Regulatory elements



Split Genes in Eukaryotes




Thank You