



FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

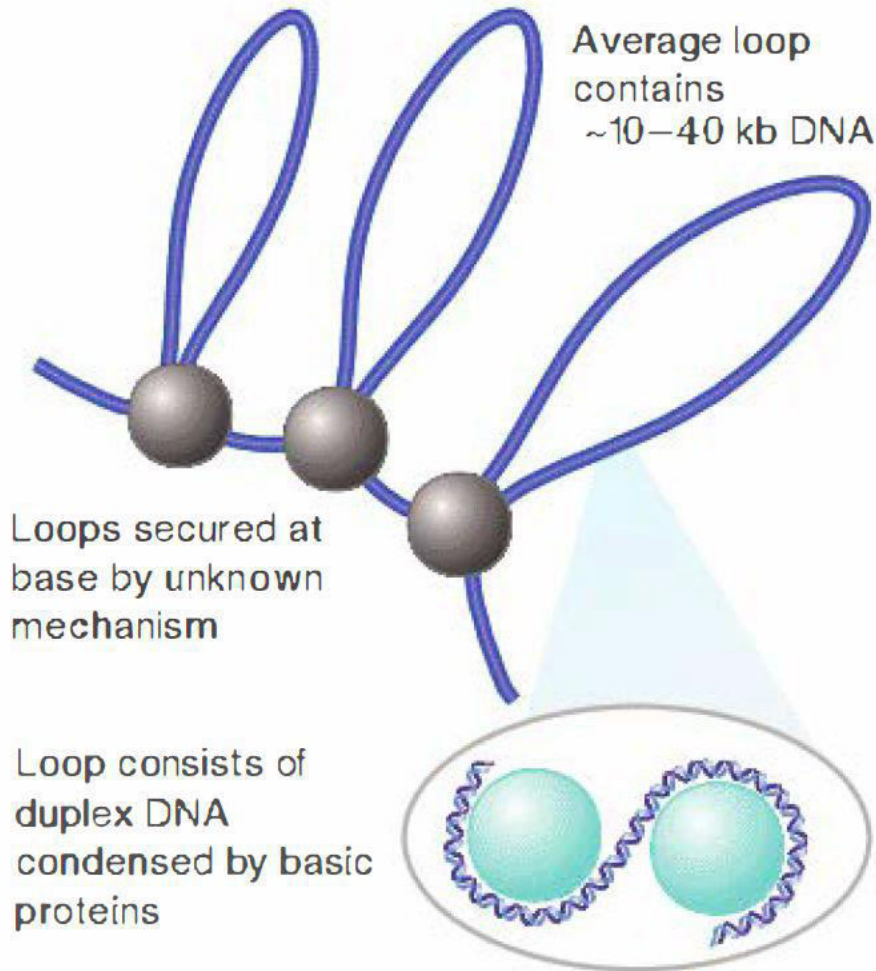
Dr. Simranjit Singh
Assistant Professor
Department of Biotechnology
Rama University, Kanpur

Fundamental facts about the prokaryotic nuclear genome

- Each bacterial chromosome is made by a single circular DNA molecule (rarely linear).
- Usually each cell contain one single copy of each chromosome.
- The genetic material can be seen as a fairly compact clump (or series of clumps) that occupies about a third of the volume of the cell named NUCLEOID.
- The DNA of these loops is not found in the extended form of a free duplex, but instead is compacted by association with proteins.



Structure organization of genome in Prokaryotes



The nucleoid has 400 independent negatively supercoiled domains. The average density of supercoiling is 1 turn/100 bp. Each domain consists of a loop of DNA, the ends of which are secured in some (unknown) way that does not allow rotational events to propagate from one domain to another. (nick caused by Ethidium Bromide)

FIGURE 9.7 The bacterial genome consists of a large number of loops of duplex DNA (in the form of a fiber), each of which is secured at the base to form an independent structural domain.

Structure organization of genome in Prokaryotes

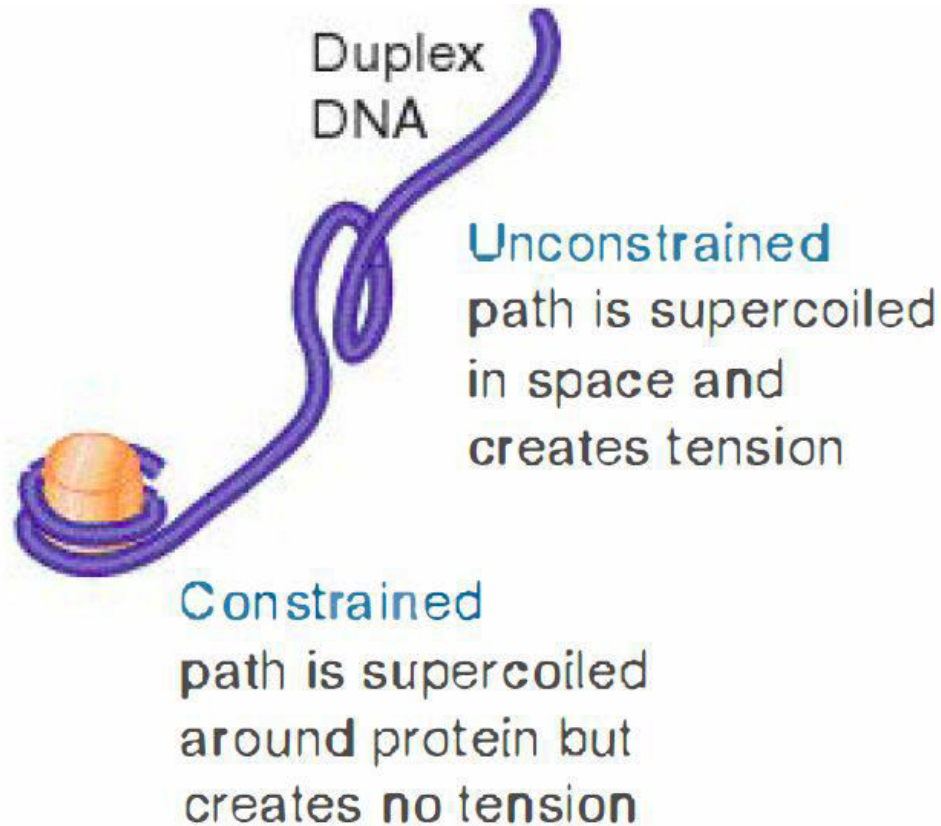


FIGURE 9.8 An unrestrained supercoil in the DNA path creates tension, but no tension is transmitted along DNA when a supercoil is restrained by protein binding.

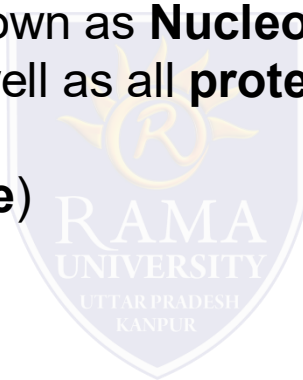
Structure organization of genome in Prokaryotes

The Escherichia. coli chromosome

A single closed-circular DNA, 4.6Mp

The DNA packaged into a region known as **Nucleoid** that contains high concentration of **DNA** (up to 30-50 mg/ml) as well as all **proteins** associated with DNA.

Continuous replication (**no cell cycle**)

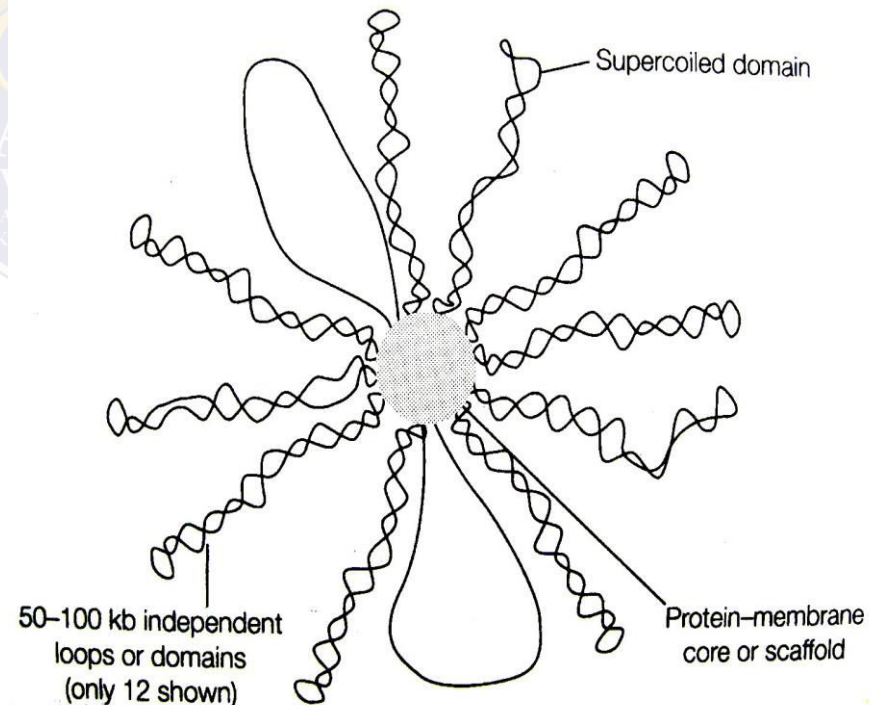


Structure organization of genome in Prokaryotes

DNA domains

- Observed under electron microscope
- **50-100 domains** or loops per *E. coli* chromosome, with **50-100 kb/loop**

• **The ends of loops** are constrained by binding to a structure which probably consists of **proteins** attach to part of the **cell membrane**.



Structure organization of genome in Prokaryotes

Supercoiling of the genome

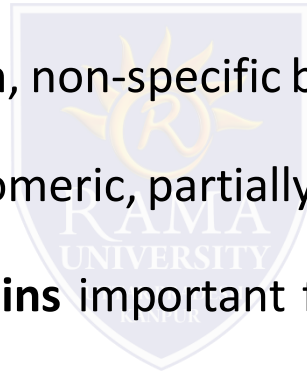
- E. coli chromosome as a whole is **negatively supercoiled**.
- **Individual domains** may be **supercoiled independently** (topological independent).
- Direct **biochemical evidence** is lacking for different level of **supercoiling** in **different domains**.



Structure organization of genome in Prokaryotes

DNA-binding proteins

- **Histone-like proteins** essential for DNA packaging to **stabilize and constrain** the supercoiling.
- **HU**: a small basic dimeric protein, non-specific binding to DNA, most abundant.
- **H-NS (protein H1)**: neutral monomeric, partially non-specific binding
- **Site-specific DNA binding proteins** important for organization of DNA domains (*RNA polymerases, IHF etc*).



Fundamental facts about the eukaryotic nuclear genome

- It is linear, as opposed to the typically circular DNA of bacterial cells.
- It conforms to the Watson-Crick double-helix structural model.
- It is embedded in nucleosomes—complex DNA-protein structures that pack together to form chromosomes.
- Eukaryotic genomes vary dramatically in terms of size and gene counts.
- Genome size and the number of genes present in an organism reveal little about that organism's complexity

Structure organization of genome in Eukaryotes

- Two features of eukaryotic genomes present a major information-processing challenge.
- First, the typical multicellular eukaryotic genome is much larger than that of a prokaryotic cell.
- Second, cell specialization limits the expression of many genes to specific cells.
- The estimated 25,000 genes in the human genome include an enormous amount of DNA that does not code for RNA or protein.
- This DNA is elaborately organized. ° Not only is the DNA associated with protein, but also this DNA-protein complex called chromatin is organized into higher structural levels than the DNA-protein complex in prokaryotes.



Structure organization of genome in Eukaryotes

- When compared with prokaryotic cell, Eukaryotic cell is having the large amount of DNA in its nucleus.
- The DNA is found associated with proteins.
- Chromatin is the complex basis of DNA and protein that makes up chromosomes consists of Linear unbroken double stranded DNA. Chromatin is of two types

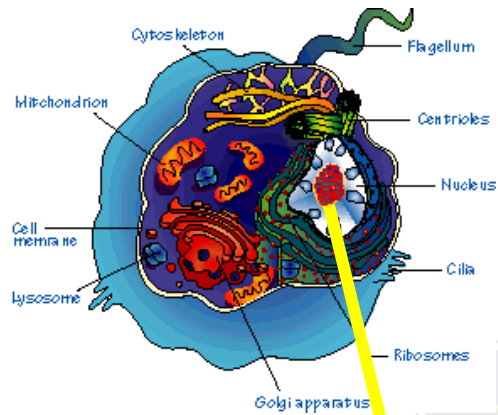


a. Euchromatin, and b. Heterochromatin

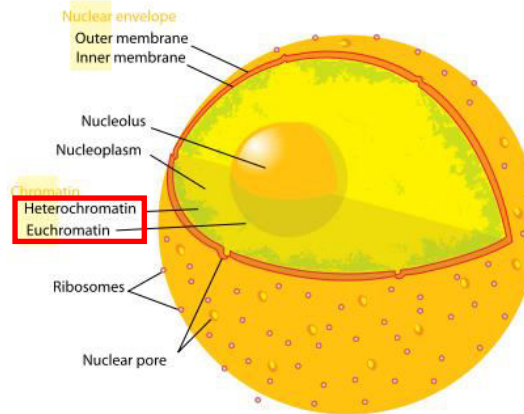
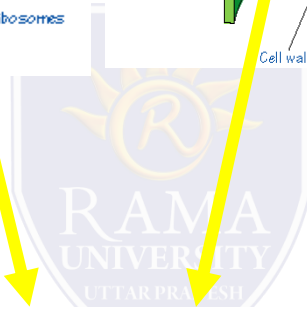
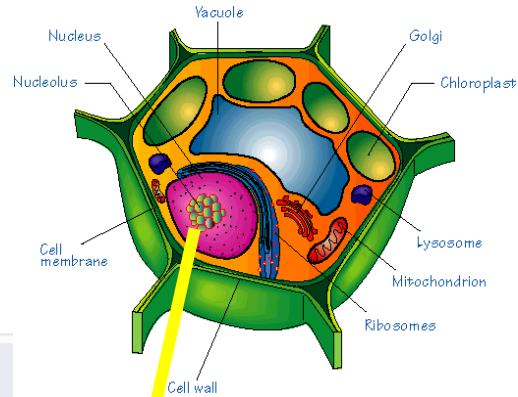
- a. Euchromatin:** type of chromatin which is genetically active. It consists of all functional genes which are expressive, stains very lightly.
- b. Heterochromatin:** It is genetically inactive, the genes are not expressive. heterochromatin stains darkly because the chromatin region here is highly condensed.

Structure organization of genome in Eukaryotes

Animal cells



Plant cells

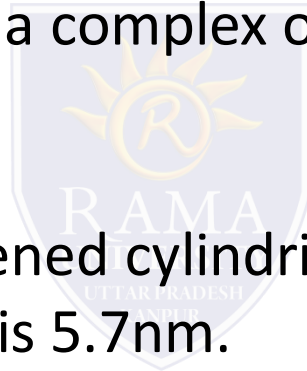


Structure organization of genome in Eukaryotes

- Histones are the chief protein components of chromatin they act as spools around which DNA winds and play a role gene regulation.
- Five major histone classes are present which are associated with eukaryotic genome i.e., H1, H2A, H2B, H3 & H4.
- These are basic proteins which have a positive charge at normal pH which facilitates the binding of negatively charged DNA

Structure organization of genome in Eukaryotes

- The simplest level of packing of DNA which is found winding around Histones is known as Nucleosome.
- There are two molecules of each of four types of Histones namely H2A, H2B, H3& H4. this give rise to a complex of 8 proteins named as “Histone octomer”
- This Histone octomer is flattened cylindrical particle of about 11 nm and thickness of nucleosome is 5.7nm.

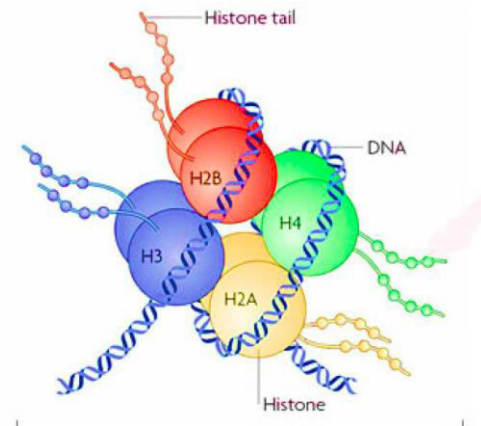


Structure organization of genome in Eukaryotes

- The H1 protein is present only in single set. These nucleosomes are attached to each other by means of a thin naked DNA which is known as Linker DNA (the H1 is associated with Linker DNA)
- Higher order organization is seen further where this nucleofilament has the appearance of beads on a string at 11 nm
- The complexity in the organization may be represented further by the packing of chromosomes in a highly compact fashion giving rise to 30 nm, then 300 nm, 700 nm and finally 1400 nm in thickness which are able to see as rod like chromosome at metaphase of cell division.

Structure organization of genome in Eukaryotes

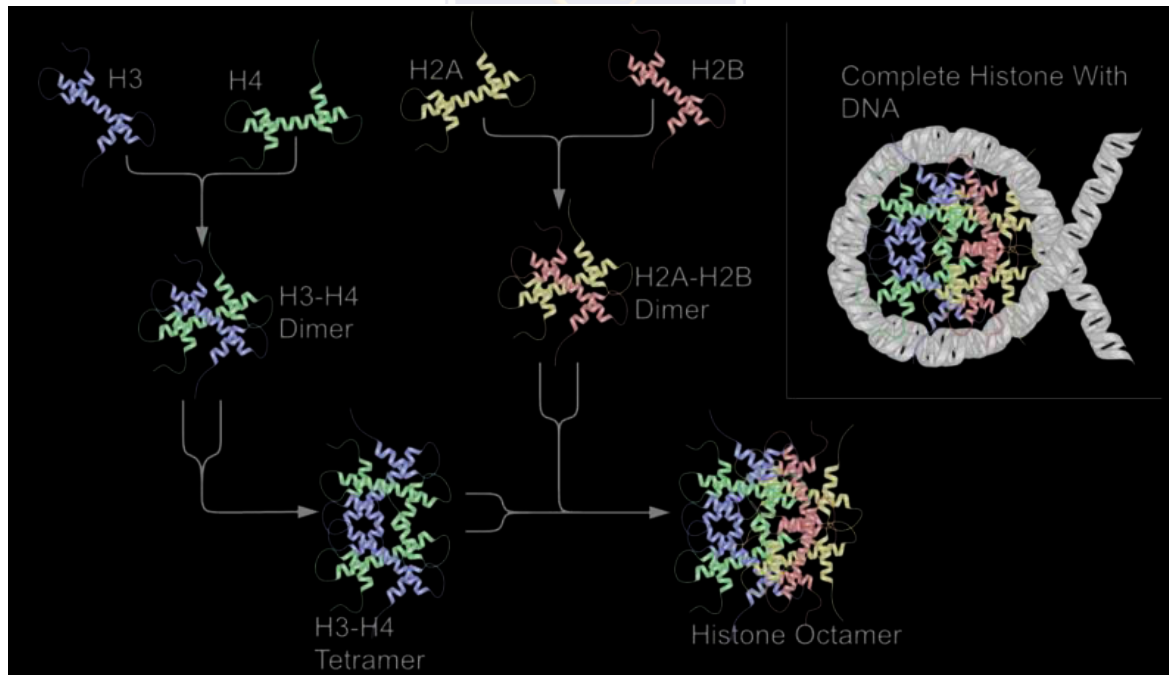
1. The simplest level is chromatin: a double stranded structure of DNA
2. This DNA forms a complex with proteins called “ histone proteins”
3. This histone-protein complex is known as nucleosome
4. These histone proteins are H1, H2A, H2B, H3 and H4
5. Each nucleosome consists of eight histone proteins, (two of each H2A, H2B, H3 and H4 histone proteins)
6. These histone proteins forms a core of nucleosome
7. DNA wrapped around these core by 1.65 times.
(less than 2 rounds)



Structure organization of genome in Eukaryotes

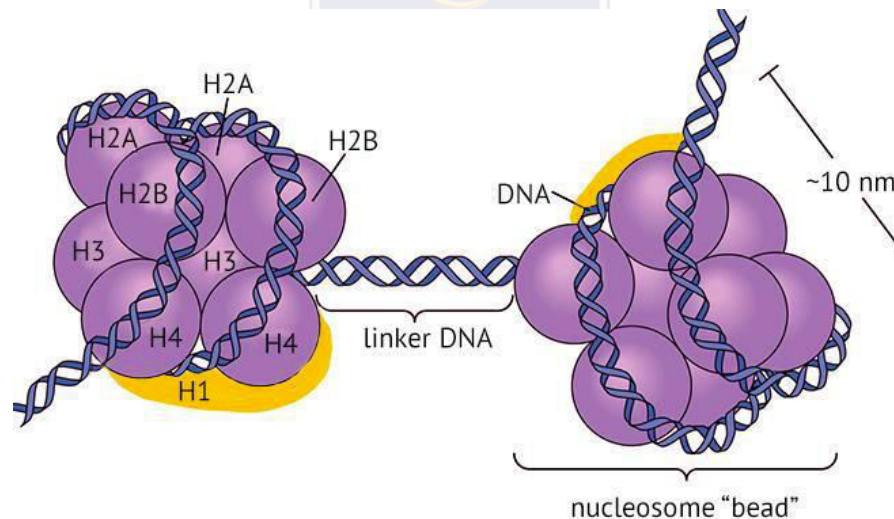
The major protein components of chromatin.

- Four families of core histone: H2A, H2B, H3 and H4, An additional non-core histone H1.
- Small, 10 kDa for core histones and 23 kDa for H1.
- Basic (rich in lysine and arginine) and tightly binds to DNA.



Structure organization of genome in Eukaryotes

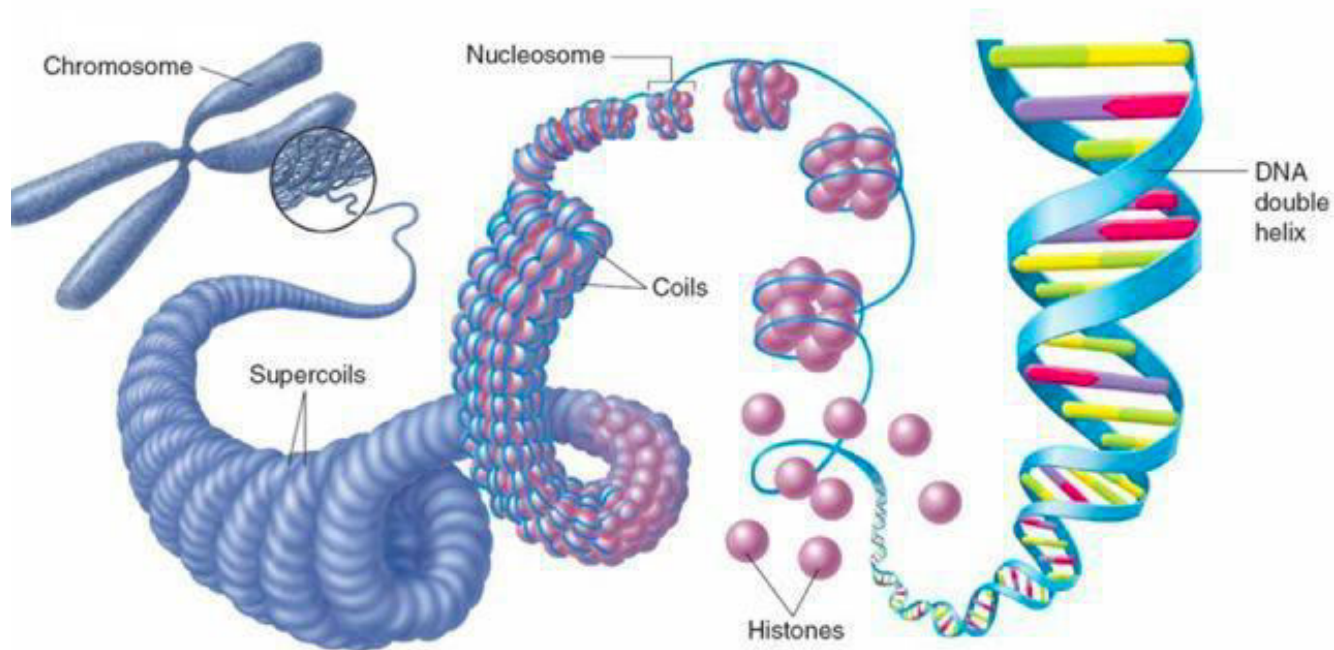
- These nucleosomes form a bead-like structure
- Two nucleosome beads are attached to each other through linker DNA regions
- A nucleosome with H1 protein is a chromosome



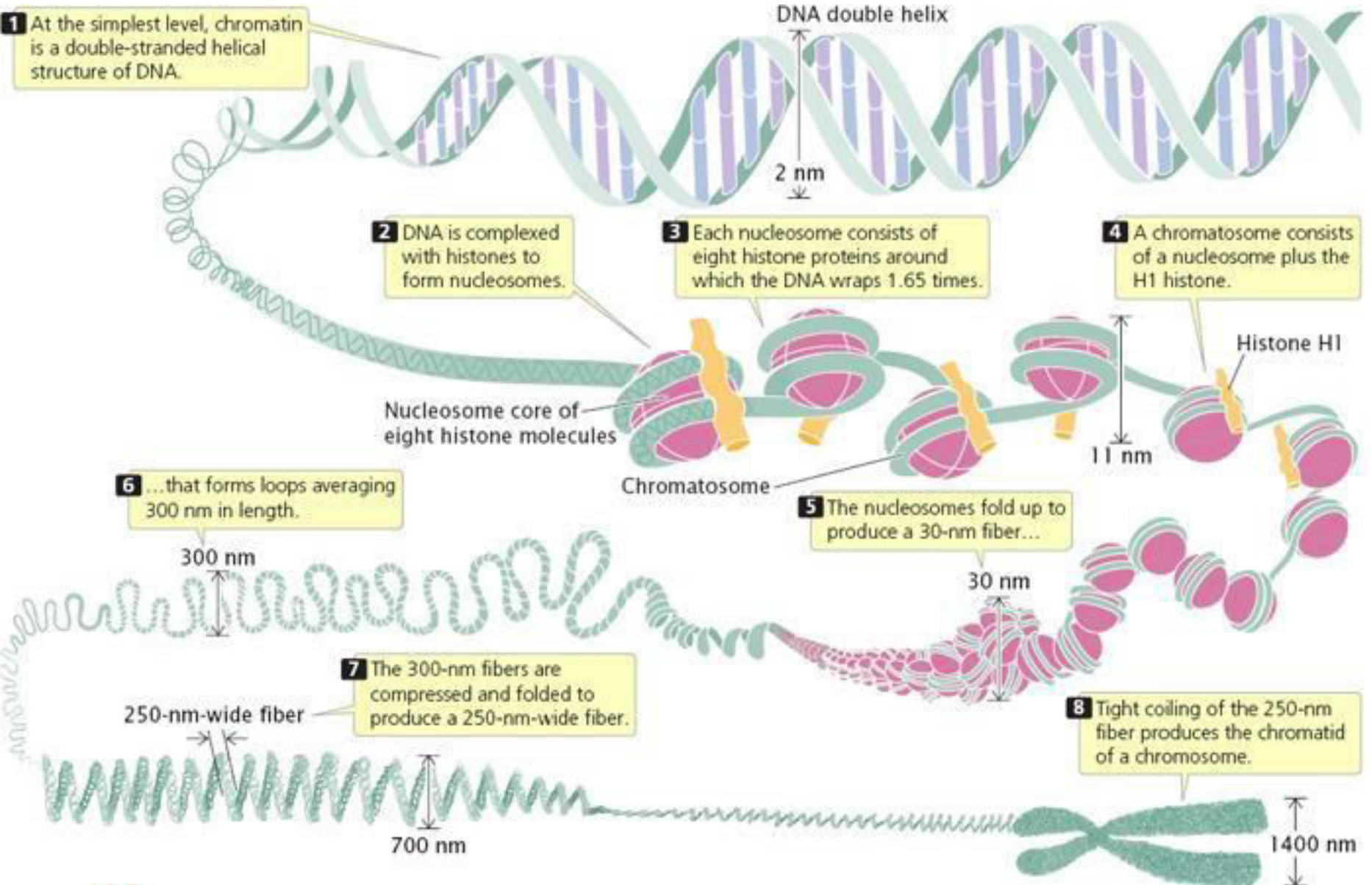
Structure organization of genome in Eukaryotes

DNA and Chromosomes

■ Eukaryotic Chromosome Structure

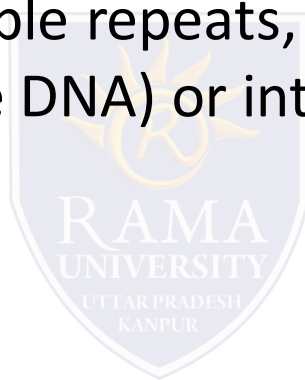


Structure organization of genome in Eukaryotes



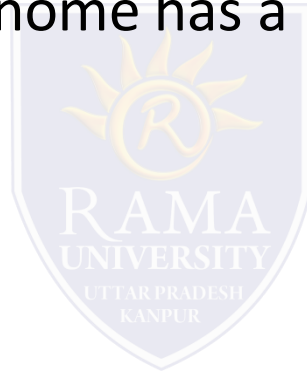
Genome complexity — *Noncoding DNA*

- DNA sequence that does not code for protein or RNA, including
- Introns (unique sequence) in genes
- DNA consisting of multiple repeats, can be tandemly repeated sequences (e.g. satellite DNA) or interspersed repeats (e.g. Alu element) etc.



Genome complexity — *Unique sequence DNA*

- The slowest to reassociate
- Corresponds to coding regions of genes occurring in one or a few copies/haploid genome
- All the DNA in *E. coli* genome has a unique sequence.



Genome complexity — *Tandem gene clusters*

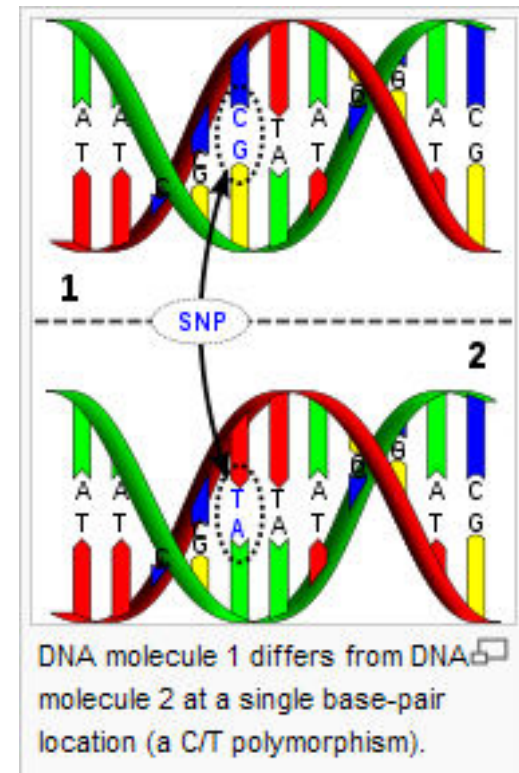
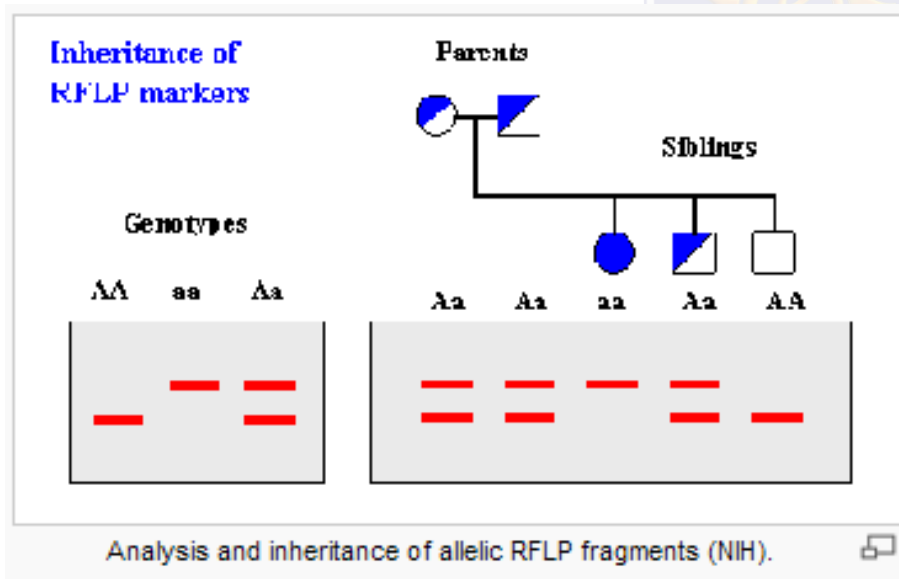
- Tandem gene clusters:
 - (1) moderately repetitive DNA consists of a number of types of repeated sequence.
 - (2) genes whose products are required in unusually large quantities, e.g. there are 10-10000 copies of rDNA encoding 45S precursor and X100 copies of histone genes.

Genome complexity — Dispersed repetitive DNA

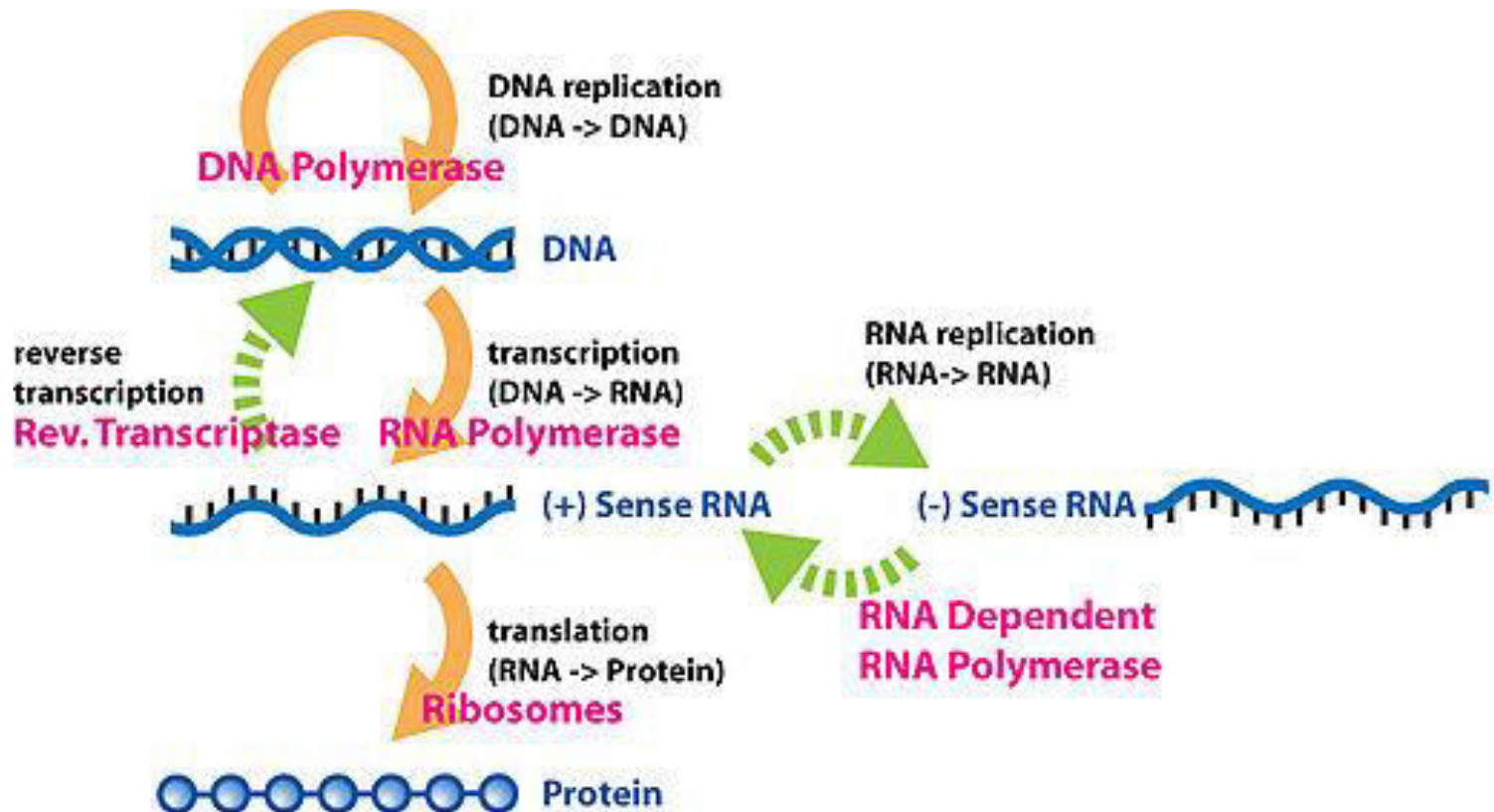
- Moderately repetitive (x100- x1000 copies)
- Scattered throughout the genome
- Human Alu elements: 300bp, 300 000 –500 000 copies of 80-90% identity
- Human L1 element
- Alu + L1= ~ 10% of human genome.
- Functions of these repetitive DNA: largely unknown

Genome complexity — Genetic polymorphism

1. Single-nucleotide polymorphism
2. Simple sequence length polymorphism
3. Restriction fragment length polymorphism
4. Single strand conformation polymorphism



The flow of genetic information — *The central dogma*



The flow of genetic information — *The central dogma*

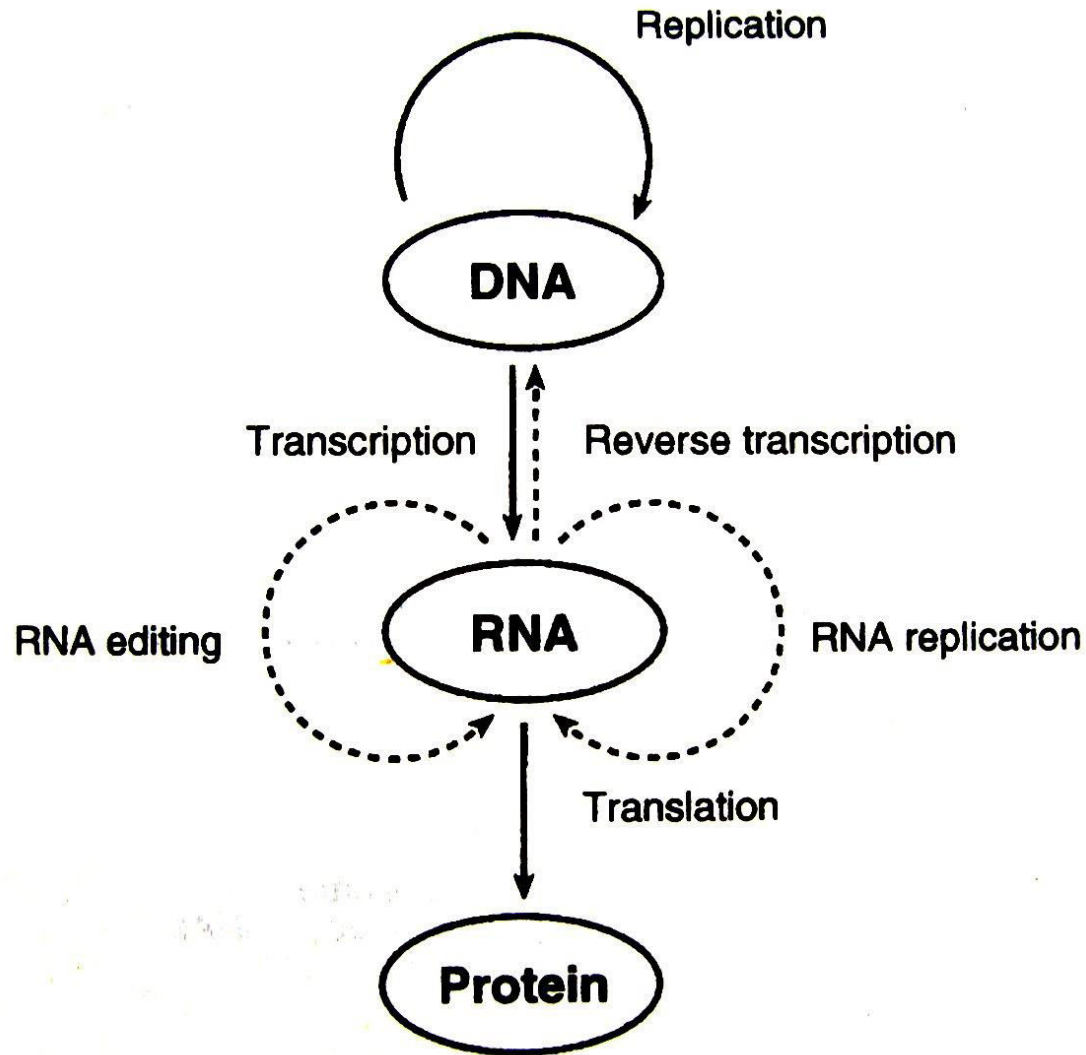
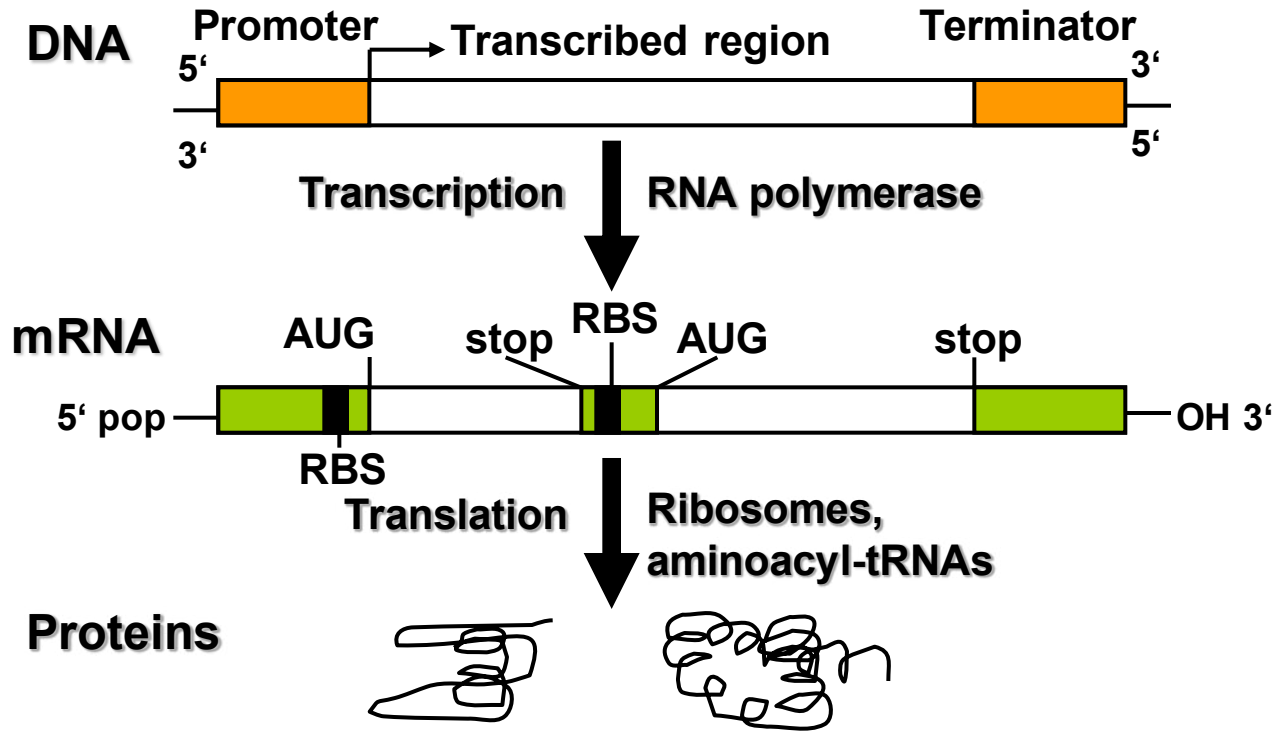
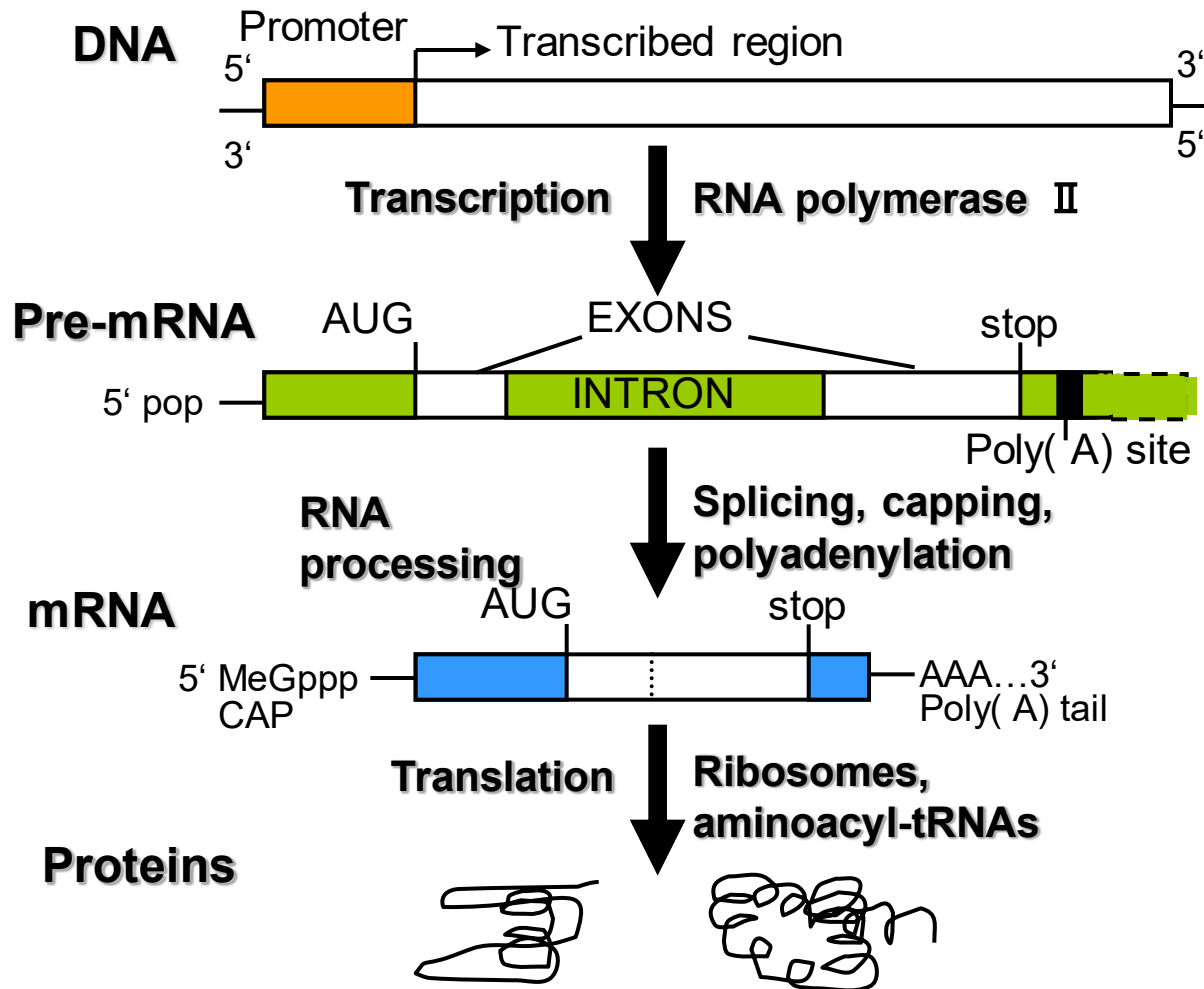


Fig. 1. The flow of genetic information.

The flow of genetic information – *Prokaryotic gene expression*



The flow of genetic information – *Eukaryotic gene expression*



Thank you Very Much

