UNIT -IV

Cell biology

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Introduction:

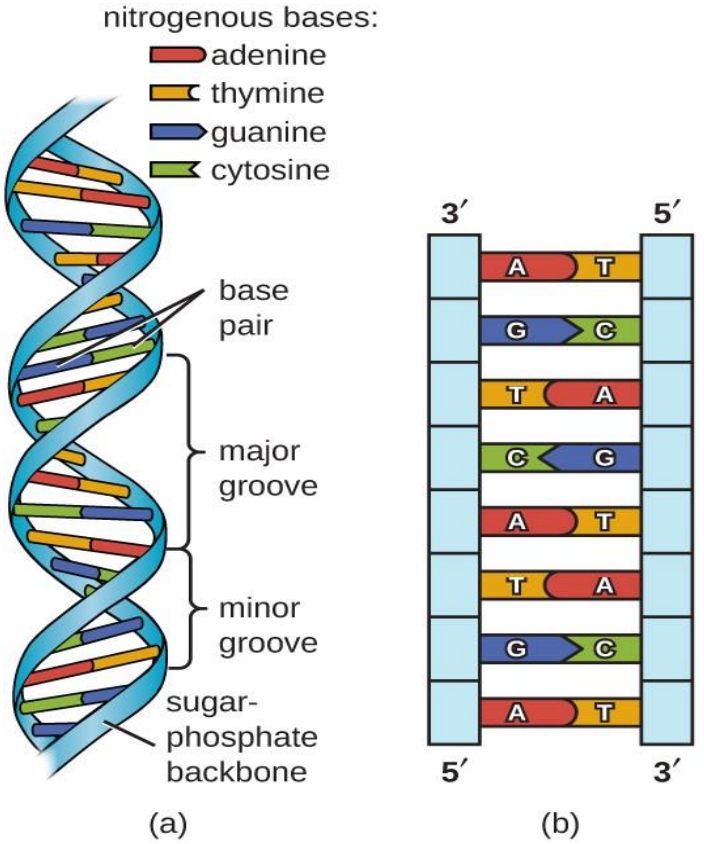
- **DNA** stands for Deoxyribonucleic Acid which is a molecule that contains the instructions an organism needs to develop, live and reproduce.
- These instructions are found inside every cell and are passed down from parents to their children.
- It is a nucleic acid and is one of the four major types of macromolecules that are known to be essential for all forms of life.
- DNA is found in the nucleus, with a small amount of DNA also present in mitochondria in the eukaryotes.

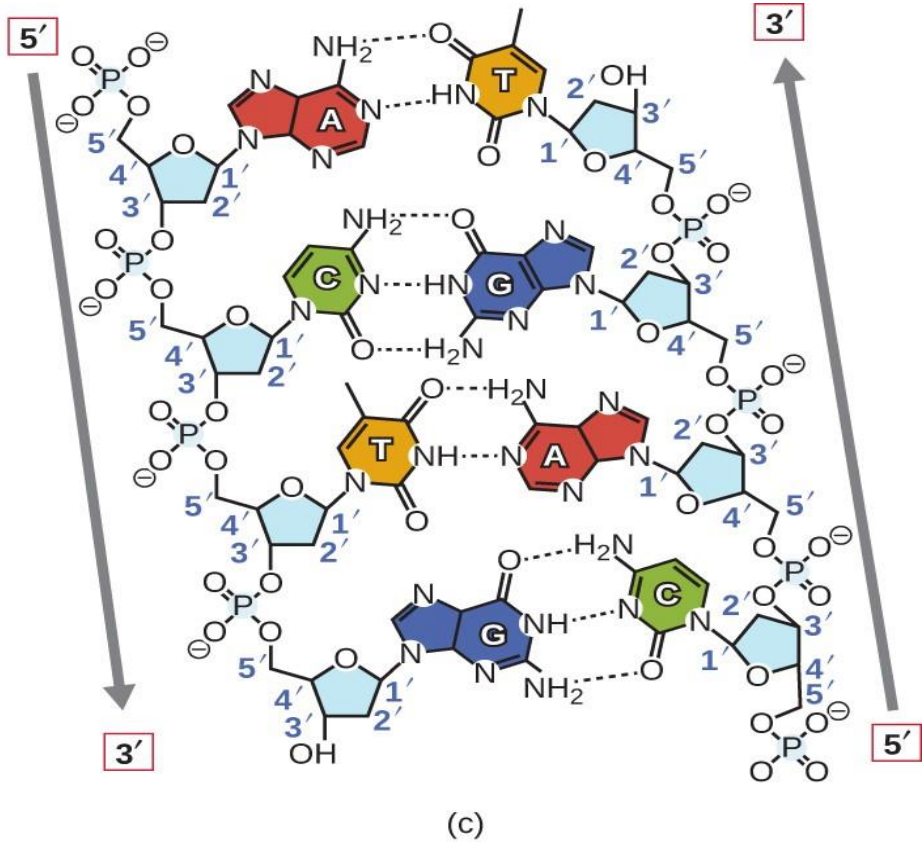
Watson and Crick proposed the double helix model for DNA.

(a) The sugar-phosphate backbones are on the outside of the double helix and purines and pyrimidines form the “rungs” of the DNA helix ladder.

(b) The two DNA strands are antiparallel to each other.

(c) The direction of each strand is identified by numbering the carbons (1 through 5) in each sugar molecule. The 5' end is the one where carbon #5 is not bound to another nucleotide; the 3' end is the one where carbon #3 is not bound to another nucleotide





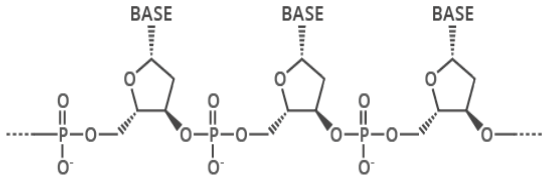
- In 1953, James Watson and Francis Crick discovered the **structure of DNA**
- The works of Rosalind Franklin lead to Watson and Crick's discovery.
- Franklin first had pointed out that the DNA is made up of two spirals.
- The structure of DNA is a double helix structure because it looks like a twisted ladder.
- The sides of the ladder are made of alternating sugar (deoxyribose) and phosphate molecules while the steps of the ladder are made up of a pair of nitrogen bases.
- There are 4 types of nitrogen bases Adenine (A) Thymine (T) Guanine (G) Cytosine (C)

DNA Pairing.

- The nitrogen bases have a specific pairing pattern.
- This pairing pattern occurs because the amount of adenine equals the amount of thymine (A=T)
- the amount of guanine equals the amount of cytosine. The pairs are held together by hydrogen bonds (G=C)

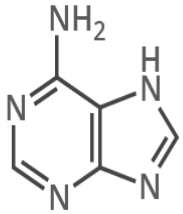
THE CHEMICAL STRUCTURE OF DNA

THE SUGAR PHOSPHATE 'BACKBONE'

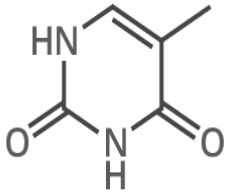


DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

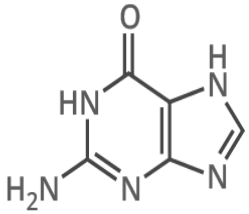
A ADENINE



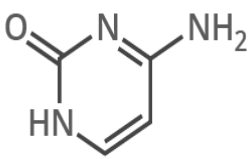
T THYMINE



G GUANINE

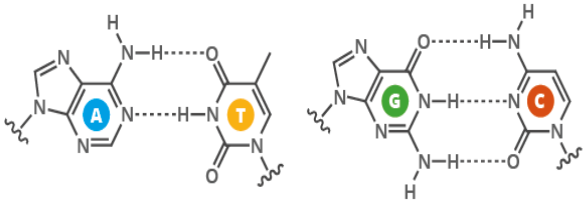


C CYTOSINE



WHAT HOLDS DNA STRANDS TOGETHER?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.



FROM DNA TO PROTEINS

The bases on a single strand of DNA act as a code. The letters form three letter codons, which code for amino acids - the building blocks of proteins.

An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.

- DNA is a double-stranded helix. That is each DNA molecule is comprised of two biopolymer strands coiling around each other to form a double helix structure.
- These two DNA strands are called polynucleotides, as they are made of simpler monomer units called nucleotides.
- Each strand has a 5′end (with a phosphate group) and a 3′end (with a hydroxyl group).
- The strands are antiparallel, meaning that one strand runs in a 5′to 3′direction, while the other strand runs in a 3′ to 5′ direction.
- The two strands are held together by hydrogen bonds and are complimentary to each other.

- Basically, the DNA is composed of deoxyribonucleotides.

- The deoxyribonucleotides are linked together by 3′ – 5′phosphodiester bonds.
- The nitrogenous bases that compose the deoxyribonucleotides include adenine, cytosine, thymine, and guanine.
- The complimentary of the strands are due to the nature of the nitrogenous bases.
- The base adenine always interacts with a thymine (A-T) on the opposite strand via two hydrogen bonds
- cytosine always interacts with guanine (C-G) via three hydrogen bonds on the opposite strand.
- The shape of the helix is stabilized by hydrogen bonding and hydrophobic interactions between bases.

- The diameter of double helix is 2nm and the double helical structure repeats at an interval of 3.4nm which corresponds to ten base pairs.

Major and Minor Grooves of the DNA

- As a result of the double helical nature of DNA, the molecule has two asymmetric grooves. One groove is smaller than the other.
- This asymmetry is a result of the geometrical configuration of the bonds between the phosphate, sugar, and base groups that forces the base groups to attach at 120 degree angles instead of 180 degree.
- The larger groove is called the major groove, occurs when the backbones are far apart; while the smaller one is called the minor groove, occurs when they are close together.
- Since the major and minor grooves expose the edges of the bases, the grooves can be used to tell the base sequence of a specific DNA molecule.
- The possibility for such recognition is critical, since proteins must be able to recognize specific DNA sequences on which to bind in order for the proper functions of the body and cell to be carried out.

Properties of DNA

- DNA helices can be right handed or left handed. But the B – conformation of DNA having the right handed helices is the most stable.
- On heating the two strands of DNA separate from each other and on cooling these again hybridize.
- The temperature at which the two strands separate completely is known as melting temperature (Tm). Melting temperature is specific for each specific sequence.
- The B sample of DNA having higher melting point must have more C-G content because C-G pair has 3 hydrogen bonds.
- The sequence of bases along the DNA molecule encodes for the sequence of amino acids in every protein in all organisms.

Types of DNA

Eukaryotic organisms such as animals, plants and fungi, store the majority of their DNA inside the cell nucleus and some of their DNA in organelles such as mitochondria.

Based on the location DNA may be:

1. Nuclear DNA

- Located within the nucleus of eukaryote cells.
- Usually has two copies per cell.
- The structure of nuclear DNA chromosomes is linear with open ends and includes 46 chromosomes containing 3 billion nucleotides.
- Nuclear DNA is diploid, ordinarily inheriting the DNA from two parents. The mutation rate for nuclear DNA is less than 0.3%.

2. Mitochondrial DNA

- Mitochondrial DNA is located in the mitochondria.
- Contains 100-1,000 copies per cell.
- Mitochondrial DNA chromosomes usually have closed, circular structures, and contain for example 16,569 nucleotides in human.
- Mitochondrial DNA is haploid, coming only from the mother.
- The mutation rate for mitochondrial DNA is generally higher than nuclear DNA.

Forms of DNA

- Most of the DNA is in the classic Watson-Crick model simply called as B-DNA or B-form DNA.
- In certain condition, different forms of DNAs are found to be appeared like A-DNA,Z-DNA,C- DNA,D-DNA,E-DNA.
- This deviation in forms are based on their structural diversity.

1.B-DNA - Most common, originally deduced from X-ray diffraction of sodium salt of DNA fibres at 92% relative humidity.

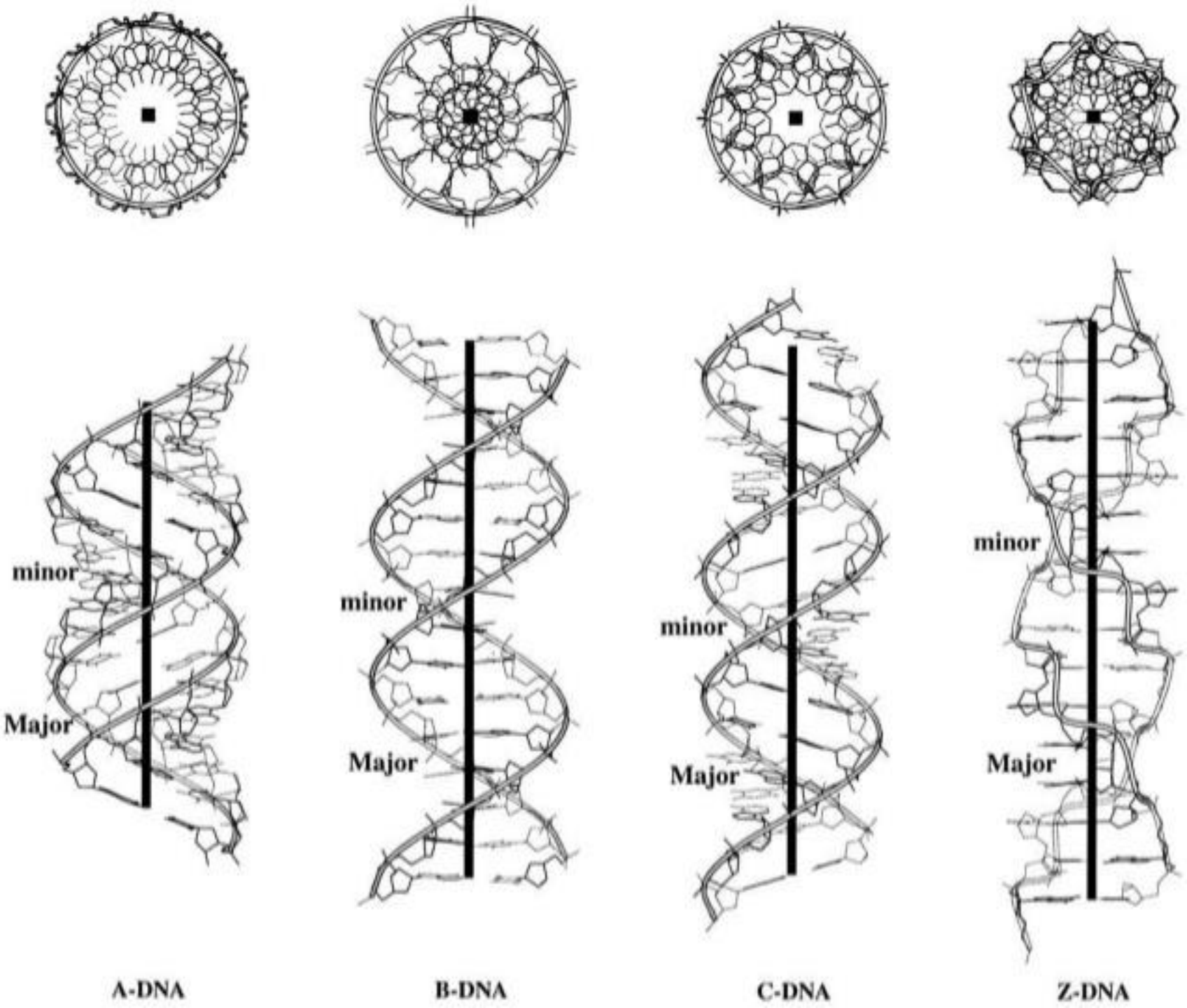
2.A-DNA - Originally identified by X-ray diffraction of analysis of DNA fibres at 75% relative humidity.

3.Z-DNA - Left handed double helical structure winds to the left in a zig- zag pattern.

4.C-DNA - Formed at 66% relative humidity and in presence of Li+ and Mg2+ ions.

5.D-DNA - Rare variant with 8 base pairs per helical turn, form in structure devoid of guanine .

6.E- DNA - Extended or eccentric DNA.


minor
Major
minor
Major
minor
Major
minor
Major
A-DNA
B-DNA
C-DNA
Z-DNA

Functions of DNA

- DNA has a crucial role as genetic material in most living organisms.
- It carries genetic information from cell to cell and from generation to generation.

- Thus its major functions include:

✓ Storing genetic information
✓ Directing protein synthesis
✓ Determining genetic coding
✓ Directly responsible for metabolic activities, evolution, heredity, and differentiation.
✓ It is a stable molecule and holds more complex information for longer periods of time

Introduction:

- RNA or ribonucleic acid is a polymer of nucleotides which is made up of a ribose sugar, a phosphate, and bases such as adenine, guanine, cytosine, and uracil.
- It is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes.

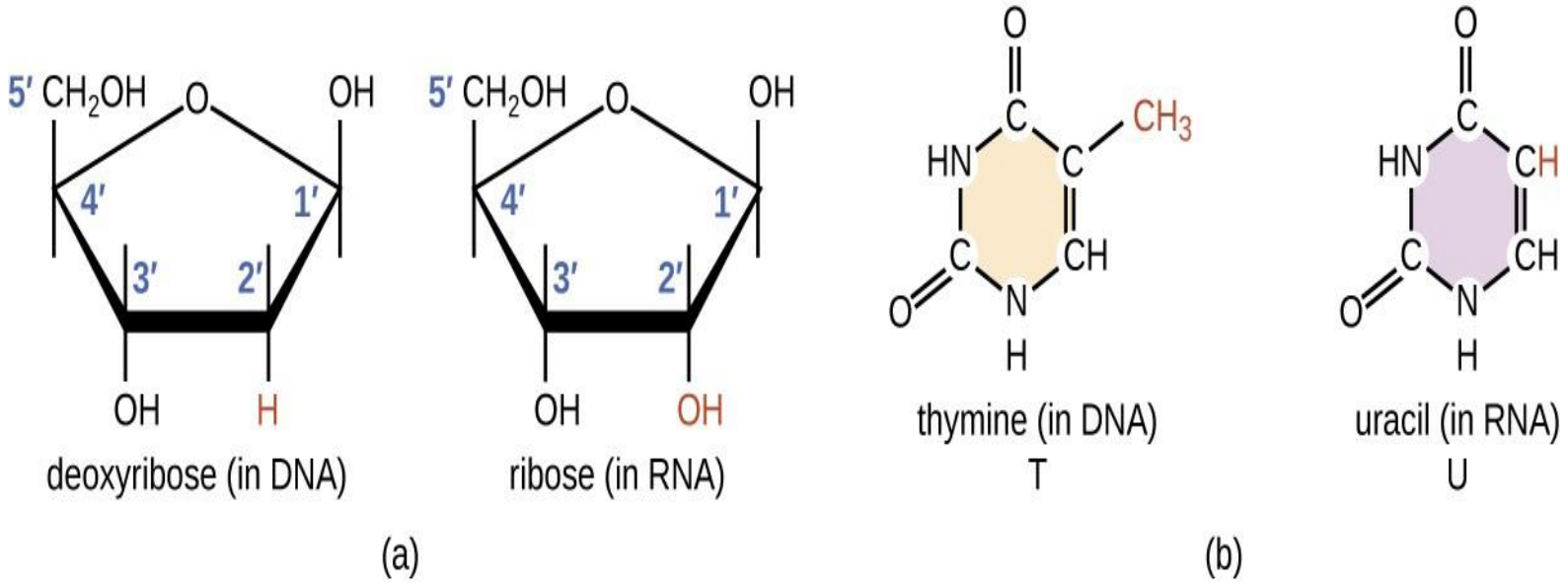
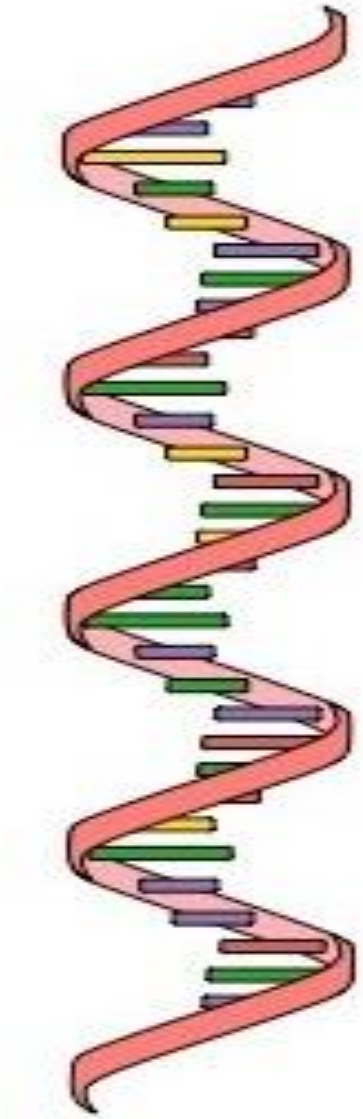
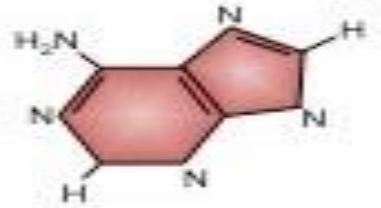


Figure: (a) Ribonucleotides contain the pentose sugar ribose instead of the deoxyribose found in deoxyribonucleotides.
(b) RNA contains the pyrimidine uracil in place of thymine found in DNA.



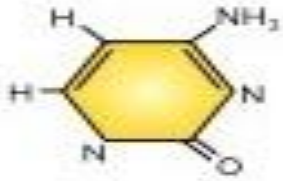
RNA

Adenine

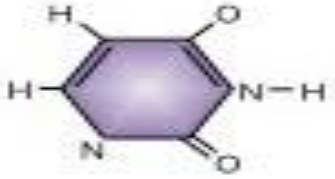


Guanine

Cytosine



Uracil

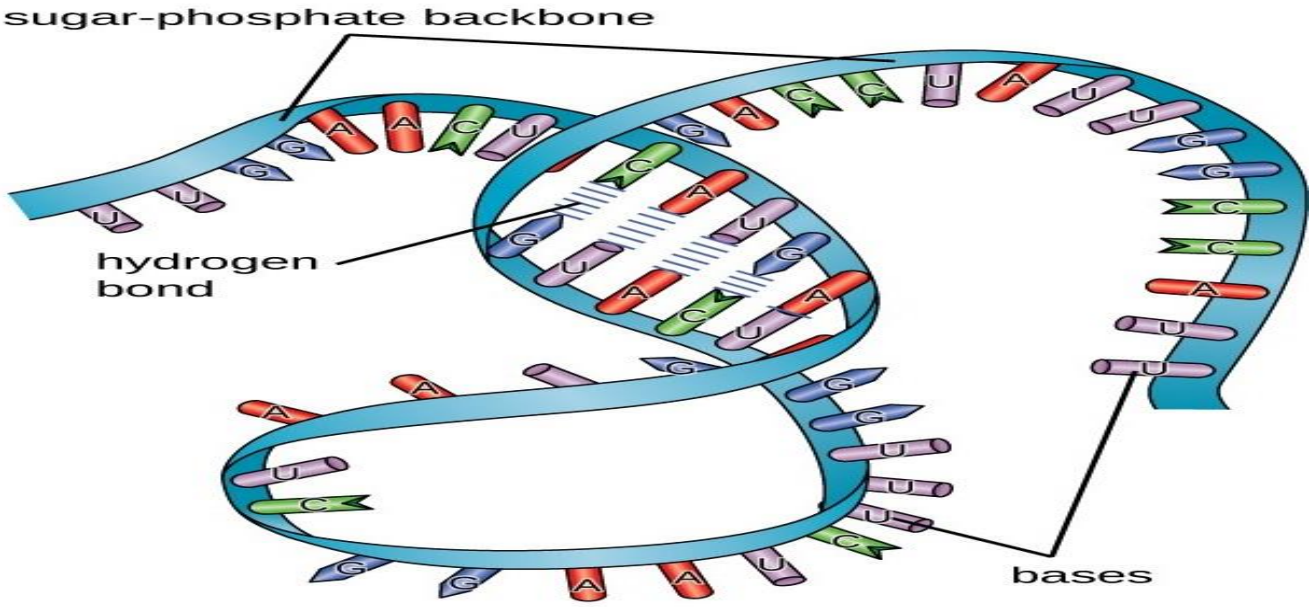


- Like **DNA**, RNA is a long polymer consisting of nucleotides.
- RNA is a single-stranded helix.
- The strand has a 5′end (with a phosphate group) and a 3′end (with a hydroxyl group).
- It is composed of ribonucleotides.
- The ribonucleotides are linked together by 3′ –> 5′ phosphodiester bonds.
- The nitrogenous bases that compose the ribonucleotides include adenine, cytosine, uracil, and guanine.
- Thus, the difference in the structure of RNA from that of DNA include:
- The bases in RNA are adenine (abbreviated A), guanine (G), uracil (U) andcytosine (C).
- Thus thymine in DNA is replaced by uracil in RNA, a different pyrimidine. However, like thymine, uracil can form base pairs with adenine.

- The sugar in RNA is ribose rather than deoxyribose as in DNA.

- The corresponding ribonucleosides are adenosine, guanosine, cytidine and uridine. The corresponding ribonucleotides are adenosine 5’-triphosphate (ATP), guanosine 5’-triphosphate (GTP), cytidine 5’-triphosphate (CTP) and uridine 5’-triphosphate (UTP).

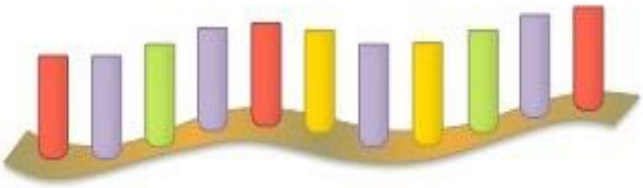
RNA Secondary Structure



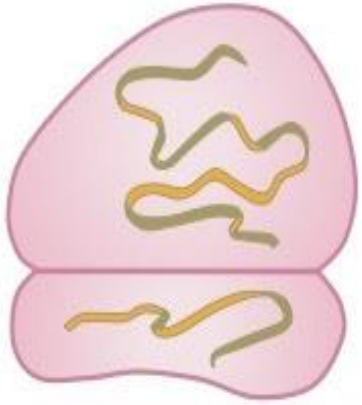
- Most RNA molecules are single-stranded but an RNA molecule may contain regions which can form complementary base pairing where the RNA strand loops back on itself.
- If so, the RNA will have some double-stranded regions.
- Ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) exhibit substantial secondary structure, as do some messenger RNAs (mRNAs).

Types of RNA

- In both prokaryotes and eukaryotes, there are three main types of RNA –
- **rRNA (ribosomal)**
- **tRNA (transfer)**
- **mRNA (messenger)**



Messenger RNA (mRNA)



Ribosomal RNA (rRNA)

Transfer RNA (tRNA)

Messenger RNA (mRNA)

- Accounts for about 5% of the total RNA in the cell.
- Most heterogeneous of the 3 types of RNA in terms of both base sequence and size.
- It carries the genetic code copied from the DNA during transcription in the form of triplets of nucleotides called codons.
- As part of post-transcriptional processing in eukaryotes, the 5' end of mRNA is capped with a guanosine triphosphate nucleotide, which helps in mRNA recognition during translation or protein synthesis.
- Similarly, the 3' end of an mRNA has a poly A tail or multiple adenylate residues added to it, which prevent enzymatic degradation of mRNA. Both 5' and 3' end of an mRNA imparts stability to the mRNA.

Function

- mRNA transcribes the genetic code from DNA into a form that can be read and used to make proteins. mRNA carries genetic information from the nucleus to the cytoplasm of a cell.

Ribosomal RNA (rRNA)

- Found in the ribosomes and account for 80% of the total RNA present in the cell.
- Ribosomes consist of two major components: the small ribosomal subunits, which read the RNA, and the large subunits, which join amino acids to form a polypeptide chain. Each subunit comprises one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins (r-protein or rProtein).
- Different rRNAs present in the ribosomes include small rRNAs and large rRNAs, which denote their presence in the small and large subunits of the ribosome.
- rRNAs combine with proteins in the cytoplasm to form ribosomes, which act as the site of protein synthesis and has the enzymes needed for the process.
- These complex structures travel along the mRNA molecule during translation and facilitate the assembly of amino acids to form a polypeptide chain. They bind to tRNAs and other molecules that are crucial for protein synthesis.
- **Function**
- rRNA directs the translation of mRNA into proteins.

tRNA

- t-RNA (transfer RNA) is also named as S-RNA (soluble or supernatant RNA) and adaptor RNA.
- t-RNA is a family of nearly 60 small sized ribonucleic acids.
- 10 – 15% of total cellular RNA is t-RNA.
- t-RNAs are small molecules with about 74 – 95 ribonucleotides.
- Sedimentation constant – 3.8S
- Molecular weight – nearly 25,000 – 30,000 Dalton
- t-RNAs are made up of a single stranded polynucleotide chain

Unique feature of tRNA

- **Presence of un usual base pairs**

- In addition to usual N-bases (A,U,G,C) tRNA contains number of unusual bases.
- These unusual bases are important as they **protect t-RNA molecules from dehydration by Rnase**, when tRNAs are floating freely in cytoplasm.
- **Protects mainly by methylation**

❖Inosine (I) – Adenine

❖Pseudouracil (ψ) - Uracil

❖Dihydroxyuridine- urdine

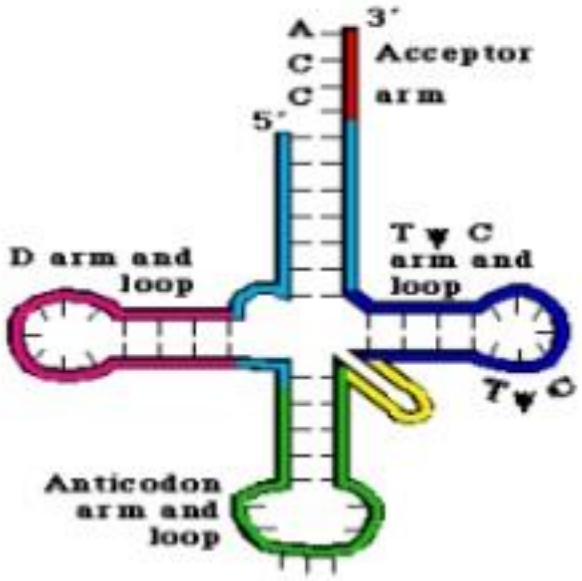
Structure of tRNA

- **Primary structure**- linear sequence of nucleotides
- **Secondary structure**-Clover leaf model
- **Tertiary structure**- 3-D structure of tRNA , L shape, Helix stacking

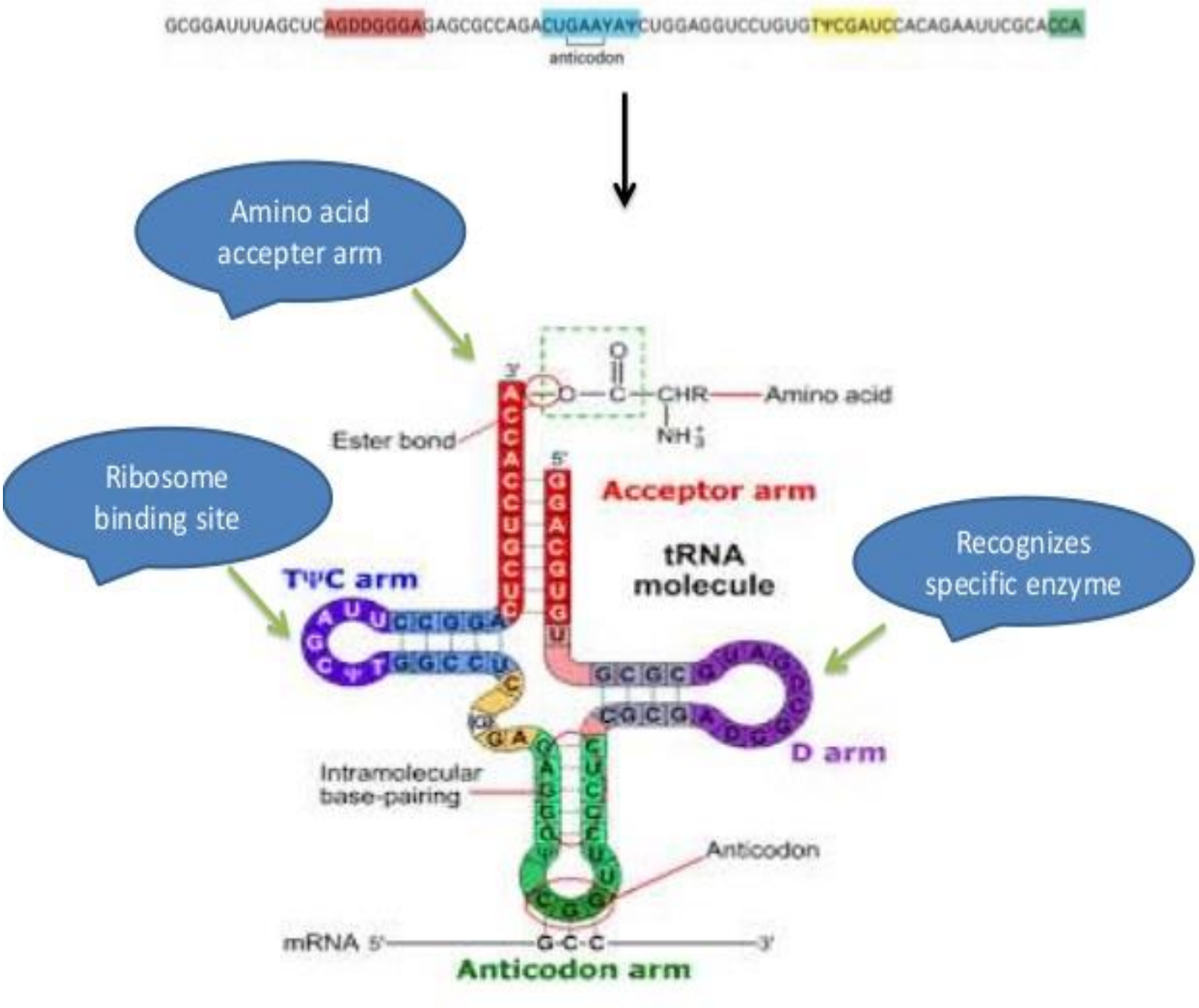
Primary Structure

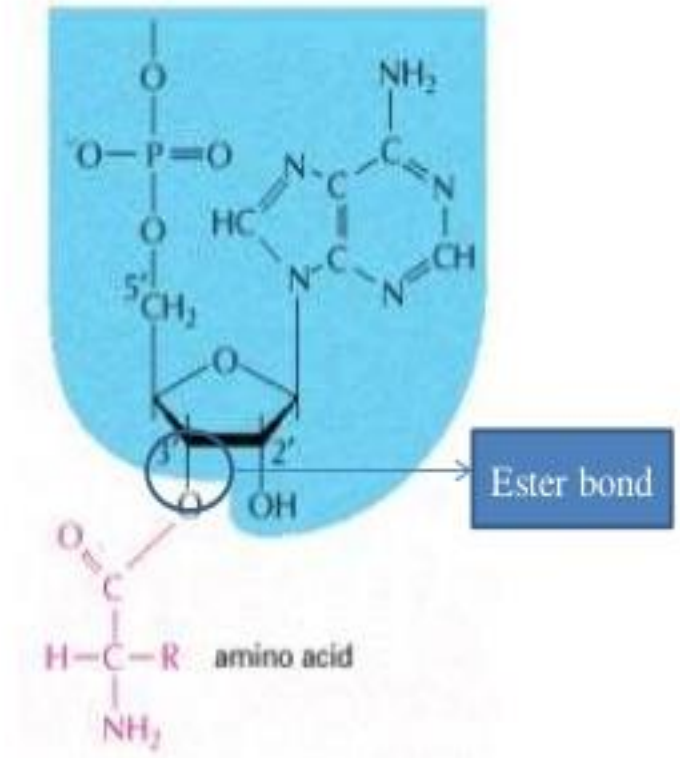
- Linear sequence of nucleotides is 60-90 in nt long but most commonly 76
- Many **modified bases,** sometimes accounting for **20%** of the total bases in any one tRNA molecules
- All of them are created post transcriptionally.

Secondary structure/ clover leaf model



- Robert Holley proposed clover leaf model for the first time in 1968.
- It is a two dimensional description of the t-RNA.


GCGGAUUUAGCUCAGDDGGGAGAGCGCCAGACUGAAYAYCUGGAGGUCCUGUGTΨCGAUCCACAGAAUUCGCACCA
anticodon
Amino acid accepter arm
Ribosome binding site
Recognizes specific enzyme
Ester bond
CHR
Amino acid
NH3+
Acceptor arm
tRNA molecule
TΨC arm
D arm
Intramolecular base-pairing
Anticodon
mRNA 5'
G-C-C
3'
Anticodon arm

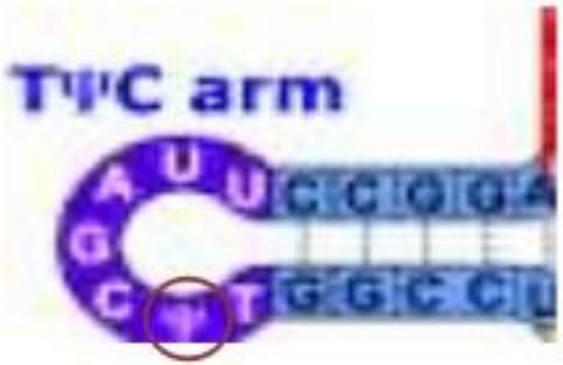


Amino acid acceptor arm

- Double helical (both 5' and 3' ends of tRNA)
- 7 base pairs unpaired
- At 3' end, **5'CCA3'** protrudes with –OH at the tip
- Site for **attachment of amino acid**
- -COOH of specific amino acid joins with –OH of A in CCA to form amino acyl tRNA

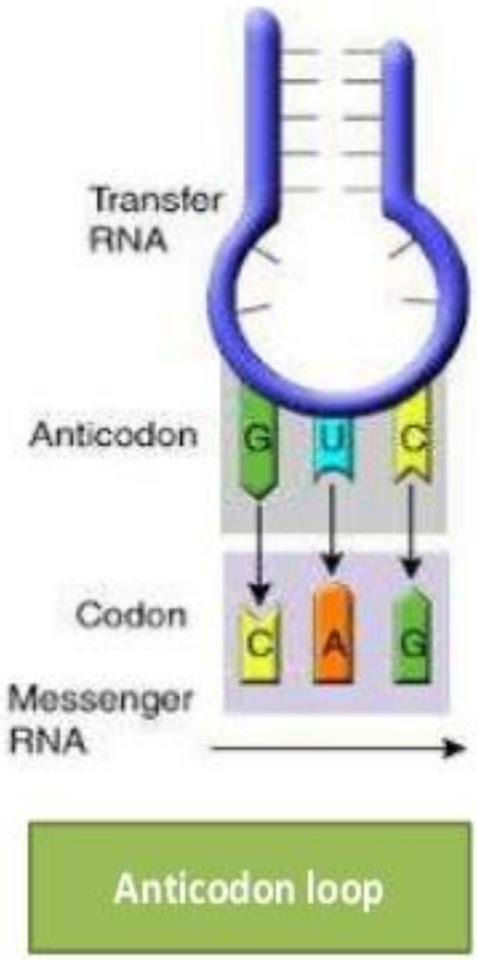
D arm

- **DHU or D arm** – This arm consists of stem and loop with unusual pyrimidine nucleotide **dihydrouracil**.
- 4 bp stem with a loop contain dihydrouridine
- Recognition site for the **specific enzyme aminoacyl-tRNA synthetase** that activate the amino acid
- Play a important role in the stabilization of the tRNA's tertiary structure.



T ψ C arm

- TψC arm is named for the presence of sequence TψC (thymine – pseudouridine (ψ) – cytosine), where pseudouridine is unusual base.
- This arm also consists of stem and loop.
- Stem contains 5 base pairs; outermost of these pairs is C-G. Loop contains 7 unpaired nucleotides
- This loop contains a ribosome recognition site.



- This arm also contains stem and loop.
- Stem consists of 5 base pairs and loop (called as anticodon loop or loop II) contains 7 unpaired nucleotides.
- Out of these 7 unpaired nucleotides the middle three form anticodon.
- Anticodon recognizes and codon of mRNA and binds to it.

- The original wobble pairing rules, as proposed by Crick. Watson-Crick base pairs are shown in **bold**, wobble base pairs in *italic*:

- The thermodynamic stability of a wobble base pair is comparable to that of a Watson-Crick base pair

tRNA 5' anticodon base	mRNA 3' codon base
A	**U**
C	**G**
G	**C** or *U*
U	**A** or *G*
I	*A* or *C* or *U*

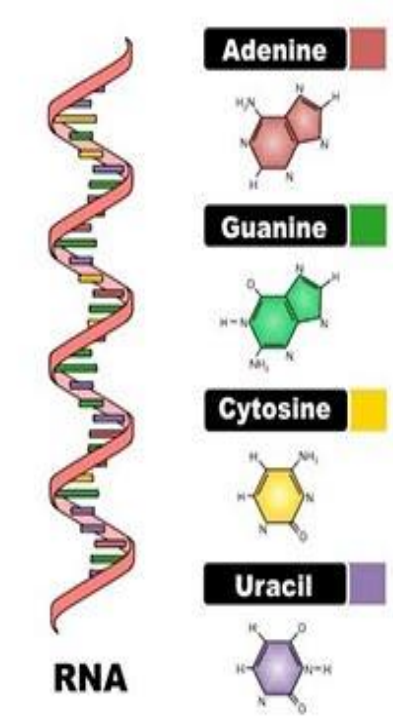
Variable arm

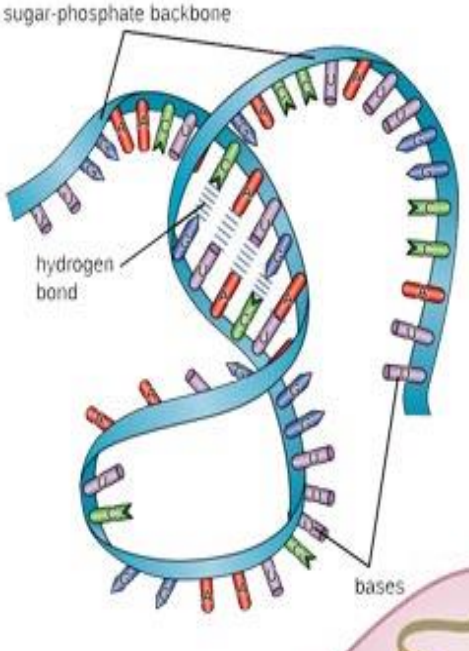
- The variable arm has between 3 and 21 nucleotides, depending on which amino acid the tRNA encodes.
- Between anticodon loop and TΨU loop
- This tRNA's variable arm is very short so it looks quite different from the other arms of the molecule.
- May present or absent, it depends on species.
- The length of the variable arm is important in the recognition of the aminoacyl tRNA synthetase for the tRNA.
- Variable arm helps is stability of tRNA
- tRNAs are called class 1 if they lack it, and class 2 if they have it.

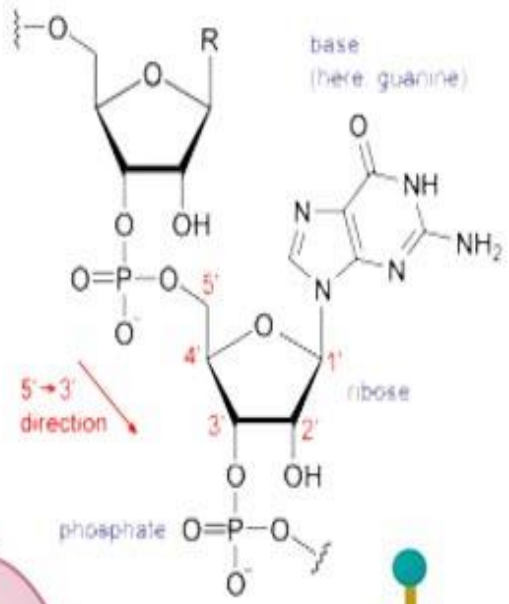
Functions of tRNA

- Help in the recognition of *Aminoacyl tRNA synthetase* enzyme
- Picks up specific amino acid from cytoplasm and carries to site of protein synthesis
- Attaches itself to ribosome in accordance with sequence specified by mRNA
- Transmits amino acid to polypeptide chain
- **Participate in non protein synthetic processess such as a primer during reverse transcription in retrovirus life cycles**

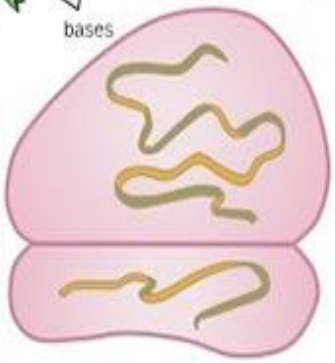
Other Properties of RNA







Messenger RNA (mRNA)



Ribosomal RNA (rRNA)

Transfer RNA (tRNA)

- RNA forms in the nucleolus, and then moves to specialized regions of the cytoplasm depending on the type of RNA formed.
- RNA, containing a ribose sugar, is more reactive than DNA and is not stable in alkaline conditions. RNA's larger helical grooves mean it is more easily subject to attack by enzymes.
- RNA strands are continually made, broken down and reused.
- RNA is more resistant to damage from UV light than DNA.
- RNA's mutation rate is relatively higher.
- Unusual bases may be present.
- The number of RNA may differ from cell to cell.
- Rate of renaturation after melting is quick.
- RNA is more versatile than DNA, capable of performing

FUNCTIONS OF RNA

- RNA is a nucleic acid messenger between DNA and ribosomes.
- It serves as the genetic material in some organisms (viruses).
- Some RNA molecules play an active role within cells by catalyzing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals.
- Messenger RNA (mRNA) copies DNA in the nucleus and carries the info to the ribosomes (in cytoplasm).
- Ribosomal RNA (rRNA) makes up a large part of the ribosome; reads and decodes mRNA.
- Transfer RNA (tRNA) carries amino acids to the ribosome where they are joined to form proteins.
- Certain RNAs are able to catalyse chemical reactions

Chemical composition of Eukaryotic chromosomes:

Introduction:

- Prokaryotes are **less complex** than eukaryotes in both genetically and biochemically

- Prokaryotic are monoploid they have **only one set of gene**.
- Higher animals and many higher plants are **diploid** having two complete sets of genes one from each parent
- some higher plants are **polyploidy.**

- Eukaryotes contain **many times the amount of DNA of prokaryotes** but this **DNA is packaged in several chromosomes** and each chromosome is present in two (Diploids) or more (polyploidy) copies.
- The chromosome of **E. coli contain (DNA) 1100µm** or about **1mm** length.

- But the haploid genome of the **human contains about 1000mm of DNA**. This is sub divided among 23 chromosome of variable size and shapes **each**

Questions:

1. How this DNA was arranged in chromosomes?
2. Is there one molecule of DNA per chromosomes as in prokaryotes or many molecules of DNA per chromosome?
3. If many, how they are arranged?
4. How does the 85 mm (85000nm) of DNA of largest human chromosome **get condensed** into mitotic metaphase structure of 0.5 µm in diameter and 10 µm long?

Chemical structure

- Interphase chromosomes are not visible with light microscope.

- However, the chemical analysis, electron microscopy and x-ray diffraction studies on **isolated chromatin** (the complex of DNA, chromosomal protein & other chromosomal constituents isolated from nuclei) have **provided a solid framework of chromosome structure in eukaryotes.**

- Chemical analysis of isolated chromatin shows that it consists of the following.
    1. DNA
    2. Protein
    3. Lesser amount of RNA
- The proteins are two major classes
    - ✓**Histones** - the basic protein (+ positively charged at neutral pH)
    - ✓**Non-Histones** - A heterogeneous acidic group of protein (-ively charged)

Histones:

- Histones play a major structural role in chromatin.
- They are present in the chromatin of all higher eukaryotes in amounts equivalents to the amount of DNA (w/w).
- The histones of higher organisms consists of **5 different major proteins**

1. **H_1**
2. **H_{2a}**
3. **H_{2b}**
4. **H_3**
5. **H_4**

In all cell types exceptionally in sperms, Histones are replaced by another class of small basic protein called **protamines.**

- The five histones are present in the molar ratios of H_1: H_{2a}: H_{2b}: H_3: H_4

1: 2: 2: 2: 2

They are specifically complexed with DNA to produce the **basic structural subunits of chromatin,**
small (110 A^o diameter by 60 A^o high) **"beads" called nucleosome.**

- The histones are basic because they contain 20-30 percent **arginine and lysine**, the two positively charged amino acid.
- The exposed $-NH_3^+$ groups of arginine and lysine allow histones to act as **polycations.**
- This is important in their interaction with DNA, which is **polyanionic** because of the negatively charged phosphate groups.

➢ Histones H_{2a}: H_{2b}: H_3: H_4 in all cell types of an organism and even between widely divergent species **it is constant and consistent.**

Non-Histones:

➢ protein fraction of chromatin consists of a large number of very heterogeneous proteins.

➢ Their composition various widely among different cell types of the same organism.

➢ **Thus, the non-histone chromosomal proteins are likely candidates for roles in the regulation of expression of specific genes or set of genes.**

One Giant DNA molecule per chromosome

There are some questions regarding the structure of DNA molecule per chromosome.

a) How 1-20 cm of DNA arranged in highly condensed mitotic & meiotic structures seen in light microscope?

b) Are there many DNA molecules run parallel throughout the chromosome? (Multineme / Multistand)

c) Is there just one DNA double helix extending from end to end of chromosome? (Unineme/ Single –stand model) ie. Here single strand means a DNA double helix

d) Are there many DNA molecules joined end to end (or) arranged in some other fashion in the chromosome?

e) Does one giant, continues molecule of DNA extend from one end to the other in a highly coiled and folded

- The evidence supporting the Unineme model of chromosome structure is now over whelming.
- In addition, solid evidence presently supports the concepts of

  chromosome –size DNA molecules.

ie. each chromosome appears to contain a single, giant molecule of DNA that extends from one end through the centromere all the way to the other end of the chromosome.

Packaging the Giant DNA molecules into chromosomes

- The largest chromosome in the human genome contains about 85mm (85,000 µm) of DNA.
- This giant DNA molecule somehow gets packaged into a **metaphase structure that is about 0.5µm in diameter and about 10 µm in legth.**
- This represents **a condensation of almost 10^4 fold in length** from the naked DNA molecule to the metaphase chromosome.

With regard to the above concept,

the following questions arises????.

a) How does the condensation occur?

b) What components of the chromosome are involved in the packaging process?

c) Are DNA molecules packaged in different chromosomes in different ways? (or) Is there a universal packaging scheme?

d) Are there different levels of packaging?

Meiotic and mitotic chromosome are more extensively condensed than Interphase chromosome

e) What additional levels of condensation occur in these special structures that are designed to assure the proper segregation of the genetic material during cell divisions?

f) Are DNA sequences of genes that are being expressed packaged differently than those of genes that are not being expressed?

Nucleosome Structure

- When isolated chromatin is examined by electron microscopy, it is found to consist of **a series of ellipsoidal "beads" of about 110Aº in diameter and 60Aº high joined by thin threads.**
- Further evidence for a regular, periodic packaging of DNA has come from studies on the digestion of chromatin with various nucleases.
- These studies indicated that **segments of DNA of 146 nucleotide pairs in length were protected from degradation by nucleases.**
- Moreover, partial digestion of chromatin with these nucleases yielded fragments of DNA of smallest size fragments.
- The above result clearly explained that the chromatin has a repeating structure **the "bead" (seen by electron microscopy) within which the DNA is packed in a nuclease resistant form.**
- This "bead" (or) chromatin subunit is called the nucleosome.
- The "interbead" threads of DNA (or) linkers are susceptible to nuclease attack.

- The nuclease resistant nucleosome consists of

  i) a 146- nucleotide –pair length of DNA

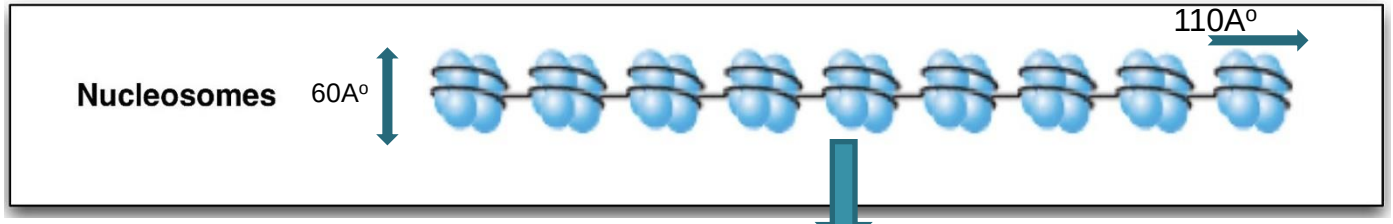
  ii) two molecules each of histones H2a, H2b, H3 and H4 (Octomer)
- Physical studies (x-ray diffraction) of nucleosome core crystal shown that **the DNA is wound as $1^{3/4}$ turns of a superhelix around the outside of the histone octamer**.
- The complete chromatin subunit consists of

  a). The nucleosome core

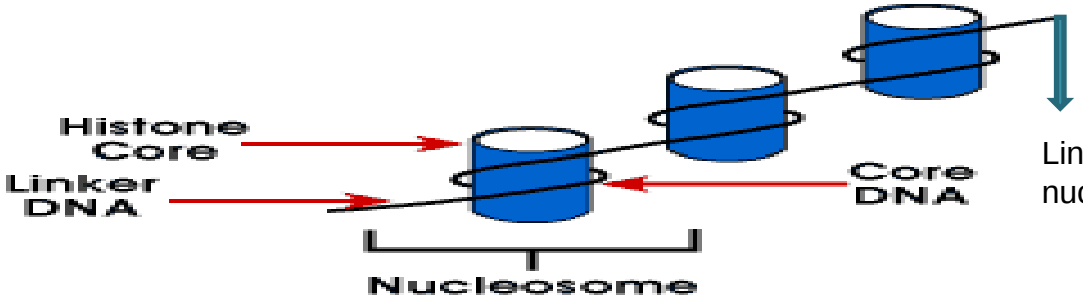
  b). The linker DNA (8-114 nucleotide –pair long varying length)

  c). One molecule of histone H1

  d). Non histone chromosomal proteins
- Some evidence suggests that the complete nucleosome contains one molecule of histone H1, which stabilizes the two full turns of DNA super helix on the surface of the histone octamer.



Chromatin fibers ‘structured’ during preparation for electron microscopy revealing linker DNA between nucleosome core



Linker DNA varing in length from 8 to 114 nucleotide pairs

Nucleosome core
146 nucleotide –pairs of DNA wrapped as 13/4 turns around an ctamer of histones.

Structure of a Nucleosome Core

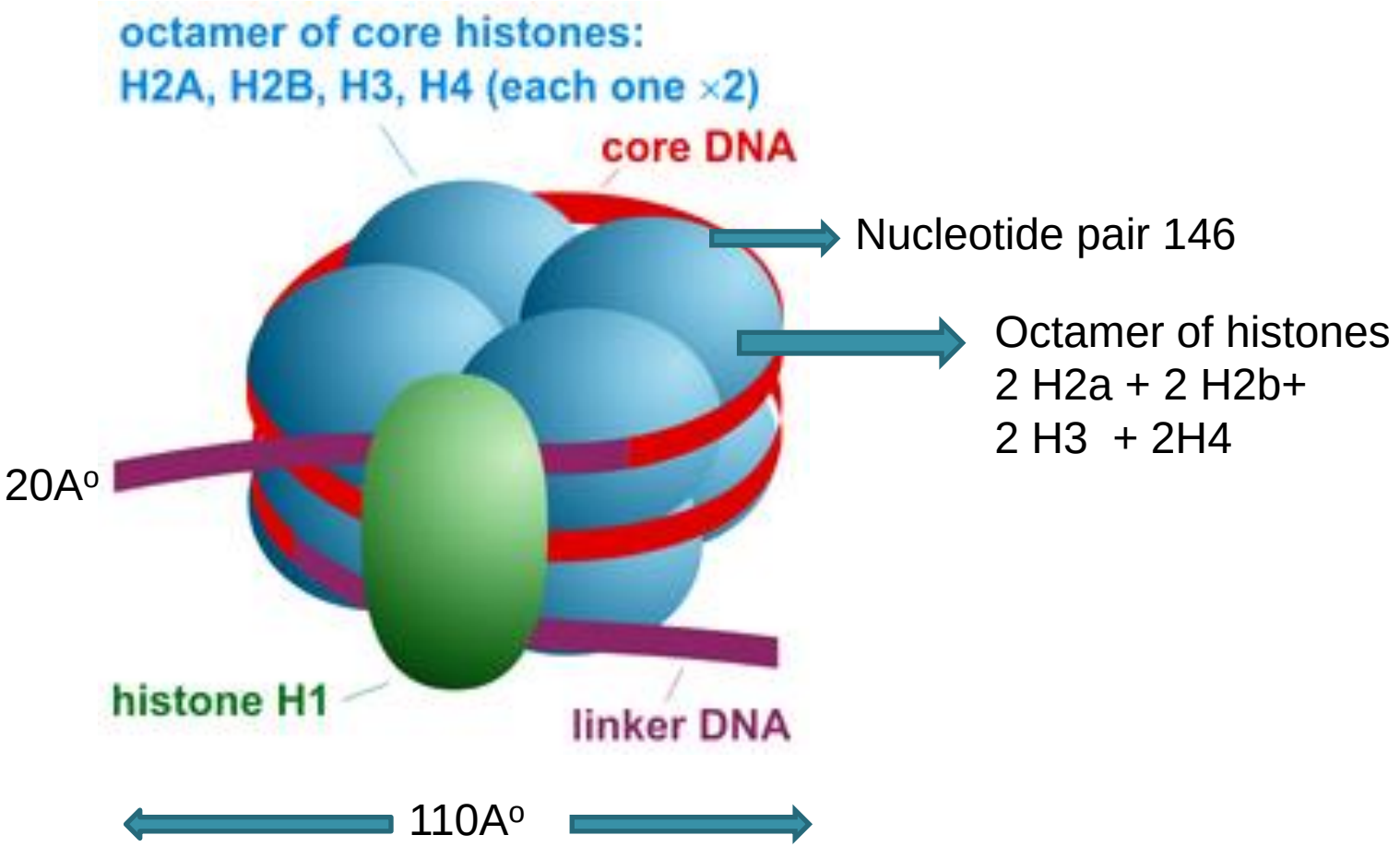
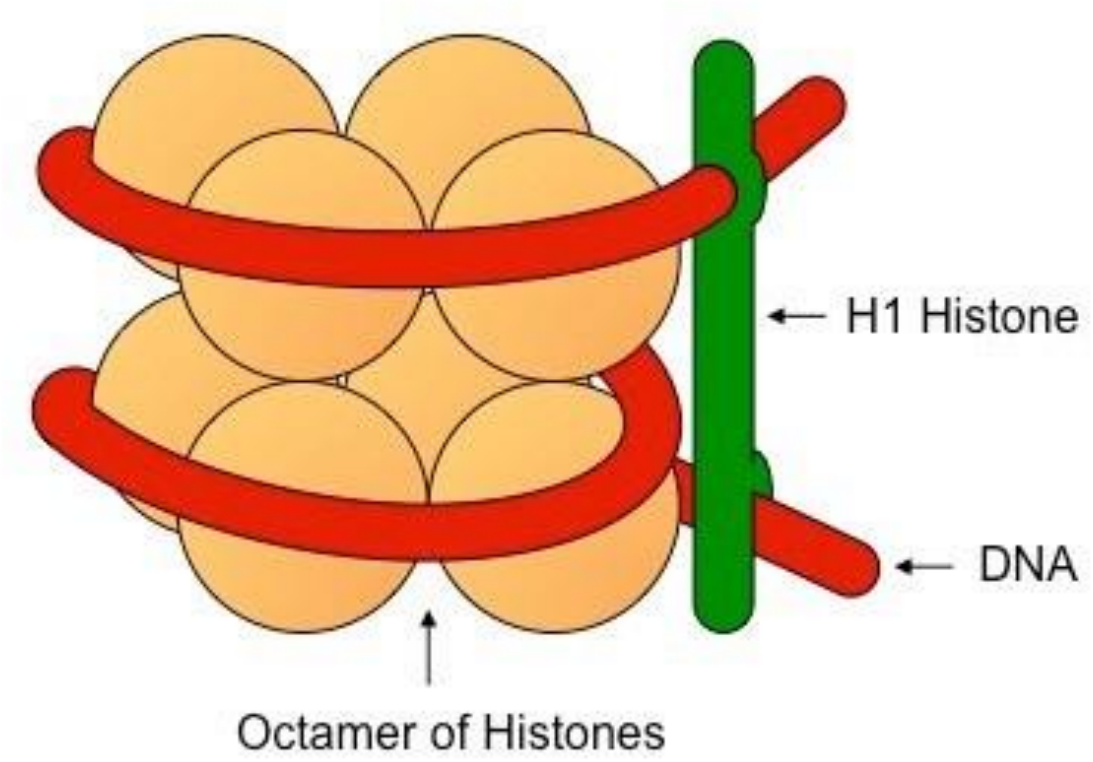


Diagram of a Nucleosome (SIDE VIEW)



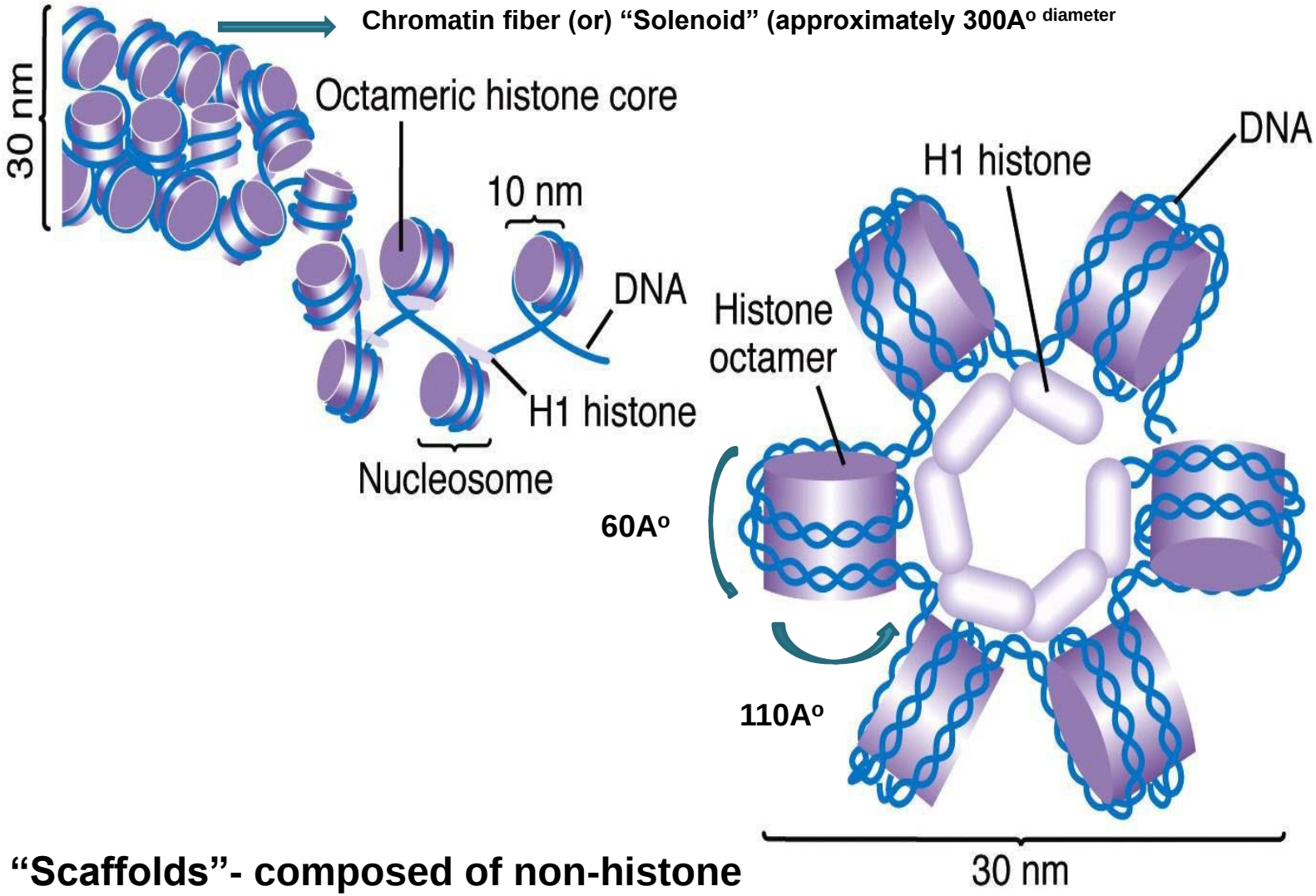
Role of histone H1 in stabilising two complete turns of DNA super helix around the octamer of histones

The 300A^{o} chromatin Fiber

- Electron micrographs of isolated metaphase chromosome show **masses of tightly coiled (or) folded lumpy fibers**. These chromatin fibers have an average diameter of 300A^{o}
- The DNA is wound as a supercoil around a histone octamer to yield the 100A^{o} diameter nucleosome.
- *In vivo*, the nucleosome are in direct juxtaposition with each other without detectable linker regions and they will form a 100A^{o} nucleosome fiber.
- If this 100A^{o} fiber, in turn, is wound in a higher-order supercoil (a “super- super coil” (or) Solenoid) a 300A^{o} fiber can easily generated. It represents some type of **solenoidlike** structure.

- Metaphase chromosomes contain the maximum degree of condensation observed in normal eukaryotic chromosome.
- The role of these highly condensed chromosome is to organize and package the giant DNA molecules of eukaryotic chromosomes into structures that will facilitate their segregation to daughter nuclei.
- The basic structure unit of metaphase chromosome is the 300A^o chromatin fiber.
- How these 300A^o fibers further condensed into the metaphase structure? Unfortunately, there is still no clear answer.
- There is evidence that, the metaphase structure is not dependent on histones.

“Scaffolds”- composed of non-histone chromosomal proteins



“Scaffolds”- composed of non-histone chromosomal proteins

Summary

- At least three levels of condensation are required to package the 10^3 to 10^5 µm of DNA in a eukaryotic chromosome into a metaphase structure a few micron long.

1. First level of condensation involves packaging DNA as a supercoil into nucleosomes. This produces the 100A^o diameter interphase chromatin fiber. This clearly involves an octamer of histone molecules (two each of histones H2a, H2b, H3 and H4).
2. The second level of condensation involves an additional folding and (or) supercoiling of the 100A^o nucleosome fiber to produce the 300A^o chromatin fiber. Characteristics of mitotic and meiotic chromosome. Histone H1 is involved in this super coiling of the 100A^o nucleosome fiber to produce the 300A^o chromatin fiber.
3. Finally, non-histone chromosome protein form a “Scaffold” that is involved in condensation of the 300A^o chromatin fiber into the tightly packed metaphase chromosomes.

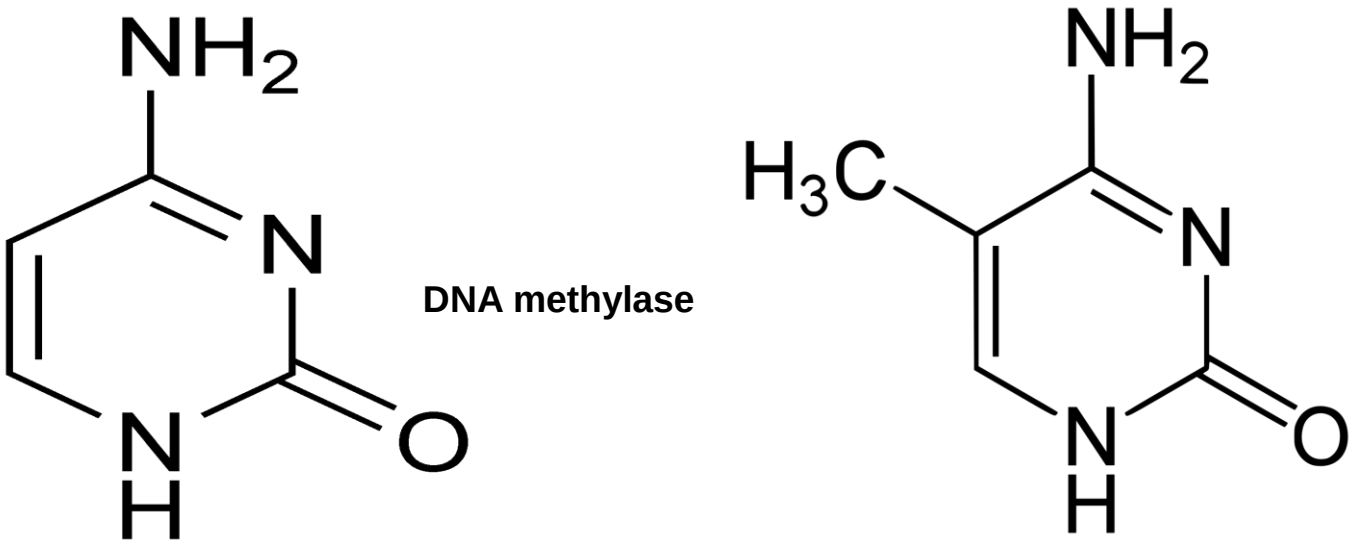
The third level of condensation appears to involve the segregation of segments of the giant DNA molecules present in eukaryotic chromosomes into independently super coiled domains (or) loops. The mechanises by which the third level of condensation occurs is not known.

Euchromatin and Heterochromatin

- When chromosomes are stained by various procedures such as **Feulgen reaction**, which is **specific for DNA** and are examined by light microscope
- some regions of the chromosomes are **stained darkly**, where as **other regions stain only lightly.**
- When examined by electron microscopy, **the intensely staining chromatin** called heterochromatins consists of densely packed chromatin fibers (300A^{o}).
- **The lightly staining chromatins** called Euchromatin, which is composed of less tightly packed 300A^{o} fibers.
- Heterochromatin shown to remain highly condensed throughout the cell cycle, where as euchromatin is not visible with light microscope during interphase.
- Genetic analysis indicate that **heterochromatin is largely genetically inactive**. **Most of the genes of eukaryotes that have been extensively characterized are located in euchromatin regions** of the chromosome.

DNA Methylation

- In most higher plants and animals, the DNA is often modified after synthesis by the enzymatic conversion of many cytosine bases to 5-methylcytosine bases.
- The extent of methylation various from species to species.
- In mammals, 2-7% of the cytosine residues are methylated.



Cytosine **5- methylcytosine**

- Methyl groups on the 5-carbons of pyrimidines occupy exposed positions within the major grooves of DNA molecules, thus they have the potential to play influential roles in the interactions of DNA with specific proteins.

- In *E. coli* Lac operon, the addition or removal of a single methyl group, can sharply change the affinity of the repressor for the operator DNA.

- Thus, the potential role of 5-methyl group on pyrimidine base is well established.

- Since there is no direct proof of the role of methylation in the eukaryotic gene regulation, the following are the arguments for the involvement of methylation in the control of gene expression.

- A correlation between the level of gene expression and the degree of methylation.

  Low methylation - high gene expression

  High methylation - Low gene expression

- Methylation patterns are tissue specific

- The drug (base analog) 5-azacytidine, which cannot be methylated after it is incorporated into DNA, which has been shown to result in the expression of gene in tissues where they normally are not expressed

Methylation pattern

- More than 90% of the methylation in the eukaryotic DNA occurs in CG dinucleotide sequences

- These sequences are symmetrically methylated

- Semi conservative replication of such symmetrically methylated sequence will yield two half- methylated sequences.

(Refer the diagram in handouts)

- The key step in any model for the regulation of gene expression (or) differentiation through DNA methylation involves the formation of the tissue specific methylation pattern.
- The most popular hypothesis is that the patterns are formed during development by tissue specific demethylases, which remove methyl groups from critical sites in genes that are scheduled to be expressed in a particular cell type.
- Recently, the methylation blocking drug 5-azacytidine resulted in the expression of the fetal (haemoglobin) and embryonic (e) β -like haemoglobin genes of anaemic adult baboons and adult humans with severe β -thalassemia (an inherited disease - inability to synthesis the β -hemoglobin chain of adult haemoglobin) and with sickle-cell anaemia.

- These embryonic and fetal genes are normally not expressed in red blood cells of adults.

- However, in this study, the DNA in the region of γ-hemoglobin (fetal) and ε- hemoglobin (embryonic) genes was shown to contain fewer methyl groups in the red blood cells of the individuals after treatment.

- Thus, these results not only support the hypothesis that methylation is important in the regulation of gene expression **but also suggest a possible approach to the treatment of these inherited disease.**

Chromosome Karyotype and Idiogram

- The general morphology of a set of chromosome at the metaphase stage of an individual (or) species is known as karyotype.

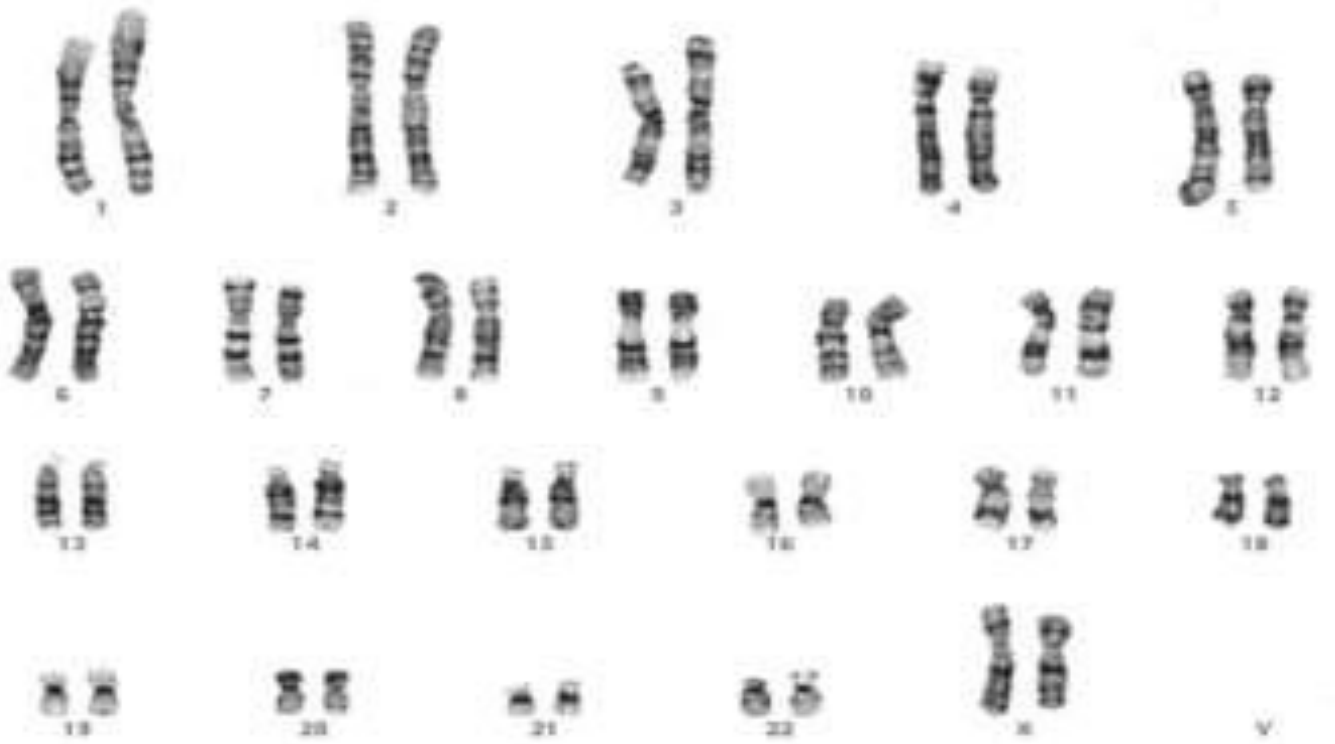
- **Karyotype**: is the general morphology of the somatic chromosome. Generally, karyotypes represent by arranging in the descending order of size keeping their centromeres in a straight line.

- **Idiotype**: the karyotype of a species may be represented diagrammatically, showing all the morphological features of the chromosome; such a diagram is known as **Idiotype.**

It deals mainly with

- The number of chromosome in the cell
- Length of the arms
- Chromosome relative size
- Position of the centromere
- Secondary constrictions and

Human Karyotype



Idiogram of Human Chromosome

Idiogram of human chromosomes

ref: http://www.pathology.washington.edu/Idiogram_select.html

- Karyotype is characteristics of an individual species, genus (or) larger grouping and may be represented by a diagram in which the pairs of homologous are ordered in a series of decreasing size, such an arrangement is called ideogram.

- A karyotype of chromosome is usually obtained from microphotographs.
- The individual chromosome are cut out of the microphotograph and are then lined up by size with their respective patterns.

- The technique can be improved by determining centromeric index.

- Centromeric index is the ratio of the long and short arms of the chromosome.

- Recently, a system has been introduced that involves a computer controlled microscope and several accessories that permit the scanning of slides, locating of the cells in metaphase, counting chromosomes and transmission of digitally expressed images for computation and storage. Thus, karyotype could be done easily and rapidly.

- By karyotype technique, the chromosome of a cell can be grouped according to their size and position of the centromere.
- 23 pairs of human chromosome are grouped into seven groups - A to G

Group	Pairs	Description
A	1-3	Large, almost metacentric chromosome
B	4-5	Large, submetacentric
C	6-12 & x	Medium, Submetacentric
D	13-15	Large, Acrocentric with satellites
E	16-18	No.16 metacentric No. 17&18 small sub-mentacentric
F	19-20	Small metacentric
G	21-22 & y	Short, acrocentric with sateliites Y-has no satellites.

- Karyotype of different groups are sometimes compared.
- Similarities in karyotypes are presumed to represent **evolutionary relationship**.
- A karyotype showing **large differences** between smallest and largest chromosome of the set and having **fewer methacentric** chromosome is called asymmetric karyotype.
- All the members of the chromosome are **nearly equal in size and the difference in size is little** between the larger and smaller chromosome is called symmetric karyotype.
- G.A. Levitzky (1931) suggested that in flowing plants there is a predominant trend towards karyotype asymmetry. The asymmetry is increased among the most advanced zygomorphic flowering plants.
- In some species, the chromosome set may be of same length and their centromeric position cannot be distinguished. In these cases, the karyotype preparation is not successful for the identification of chromosome.
- Development of chromosome banding technique proved very useful for the karyotypes preparation.

Chromosome banding techniques

- When the chromosome set may be of some length and their contromeric position cannot be distinguished, the karyotype preparation cannot be successful for the identification of chromosome.
- In such cases, development of chromosome banding technique proved very useful for the karyotype preparation.
- The distinct banding patterns in these chromosomes of same morphology can be easily distinguished.
- The banding technique allow distinction of GC (or) AT rich regions (or) the regions of repetitive DNA.
- A variety of different kinds of bands namely Q, C, G, R bands have been used in animal materials
- Giemsa C bands and N-bands have been utilized in plant materials.

1) G-banding:

- The bands stained with Giemsa are designated as G-bands
- Both G and Q bands correlate with chromosomes observed in leptotene and pachytene chromosome during meiosis.
- During cell cycle, G-bands replicate in the second half of S-phase.
- G-bands are richer in AT bases.
- G banding has become important tool in analysis of mammalian, avian, reptilian and amphibian chromosomes.
- G-bands have not been found in plant chromosomes because of more DNA content per unit length.

2) Q-banding:

- In this banding technique a fluorescent dye, quinacrine mustard is used.
- The bands stained with quinacrine along the length of the chromosome were named as Q-bands.

3) C-banding:

In this banding technique, a Giemsa staining is used which results in intense staining in kinetochore regions corresponding to the localization of heterochromatin.

4) R-banding:

Reverse of the Q and G-banding of chromosome.

5) T-banding:

Allows the staining of telomeric regions of chromosome

6) N-banding:

Allows the selectively staining of nucleolar organiser region of the chromosome.

- Banding pattern provides us the new features of human chromosome.
- In addition to telomere, kinetochore, arms, special land marks ie well defined bands were selected to subdivide the arms into regions, designated as 1, 2, 3, 4 moving from the kinetochore towards the telomeres.

- By the banding technique, reciprocal translocations can be identified.
- The use of the banding techniques has permitted the detection of more than 30 chromosomal syndromes.
- These technical advances have permitted the identification of chromosome defects.

G-banding: karyogram of human male using Giemsa staining.

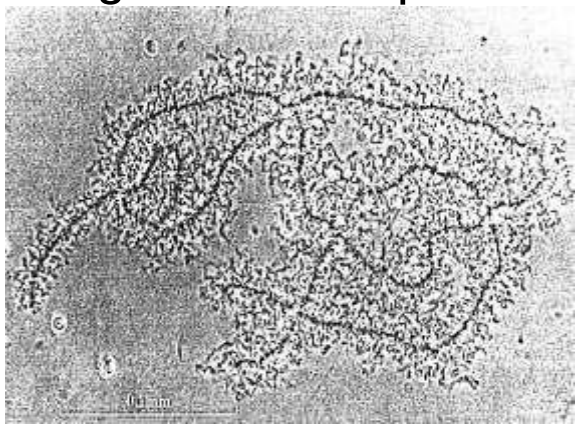
Giant chromosome

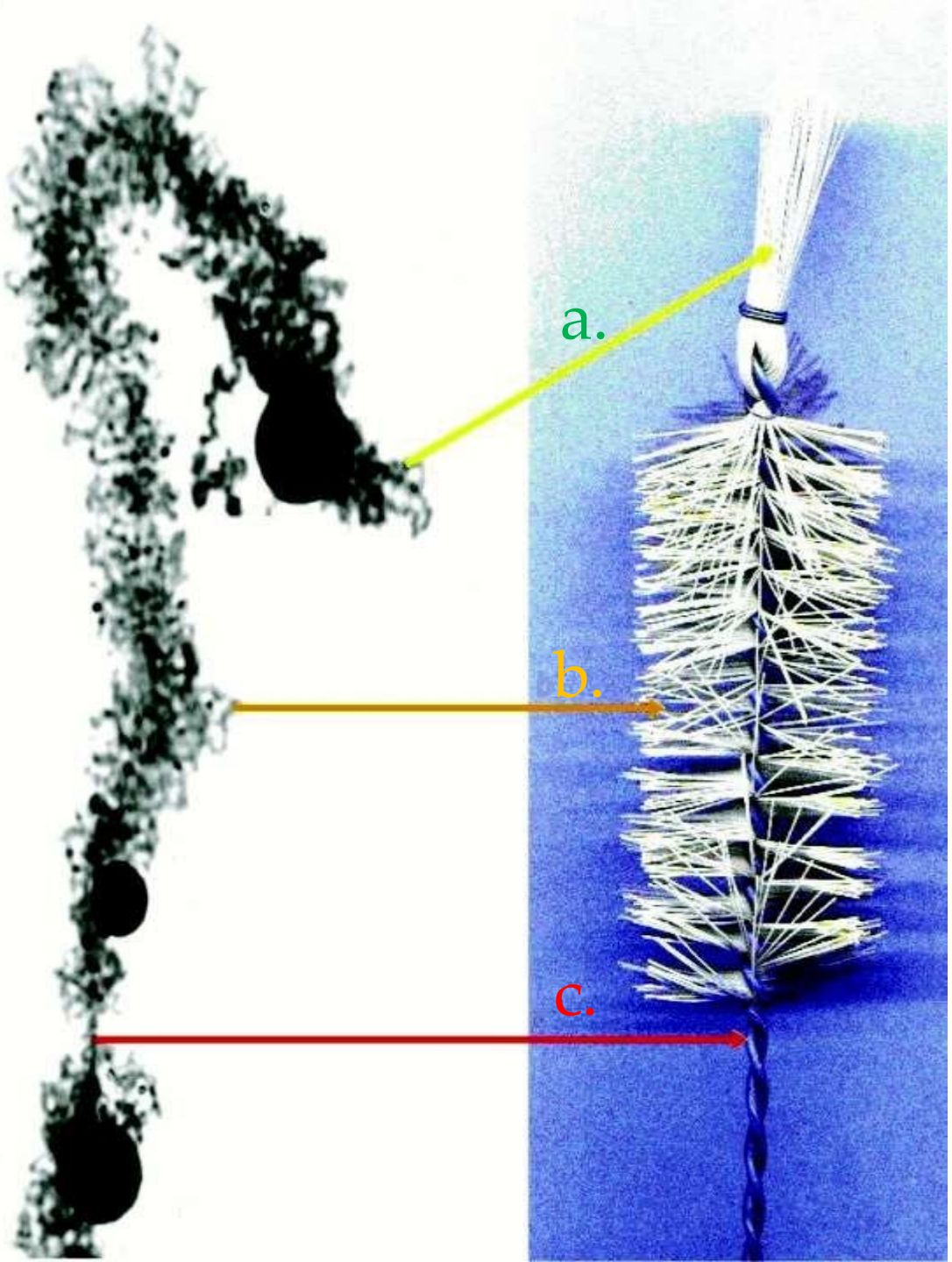
- Chromosomes are decondensed during interphase.
- Some exception are lampbrush chromosomes of vertebrate & polytene chromosome of insect.
- In both these chromosome The region that are actively synthesizing RNA are least condensed.
- Giant chromosome are very long & thick (200 times) during metaphase.
- Hence they are know as “Giant chromosomes”.

Lampbrush chromosome

INTRODUCTION

- First discovered by Ruckert in 1892.
- Occur in oocytes of vertebrates as well as in some invertebrates.
- Found in those cells which produce a lot of RNA and their cytoplasmic and nuclear volume increases.
- Their detailed structure have been studied during the diplotene stage of
  meiotic division.
- During diplotene stage, certain chr. Stretch out large loops of DNA, causing the chr to resemble a lamp brush.
- They are visible under the light microscope.





A lampbrush chromosome & the "original item"

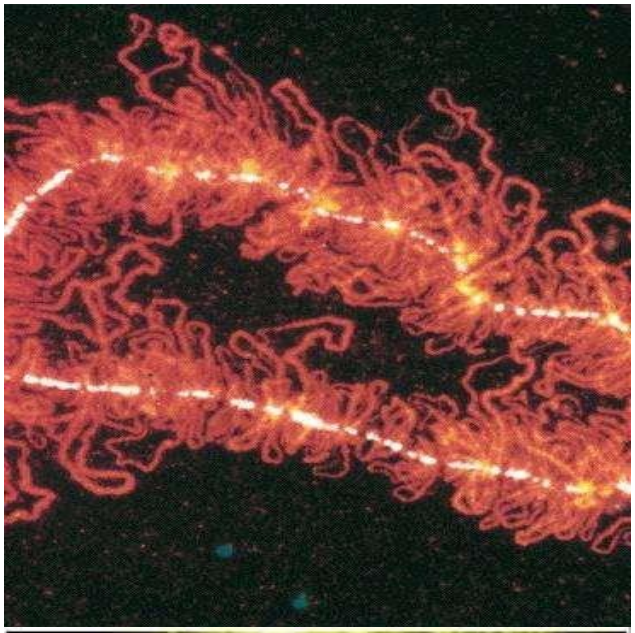
a- telomeric loop,

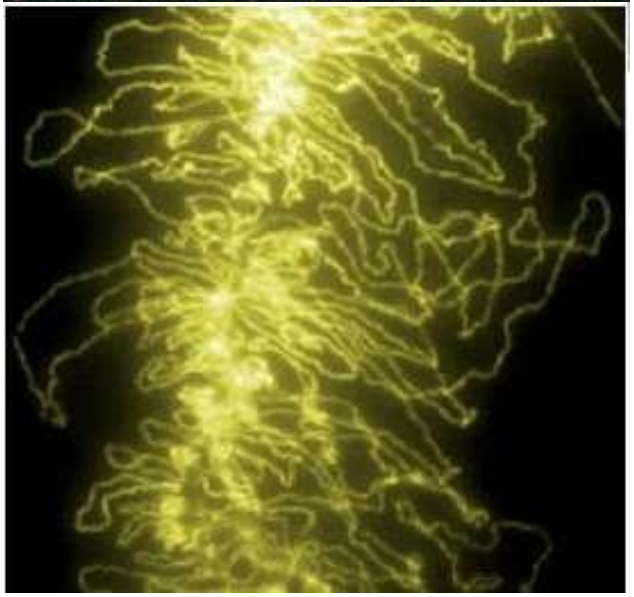
b. side loops,

c.a chromatid without loops.

Morphology

- Each LBC'sconsists of a main axis having two chromatids.
- Main axis has a row of granules known as chromomeres, which are held together by fine axial fibre.
- Lateral loops in pairs project from the chromomeres.
- About 1 to 9 loops may arise from a single chromomere, Their size varies.
- They are held together at points of chiasma formation.
- The loops of a paired chromosome form mirror- image structures.
- This stage can last several months.





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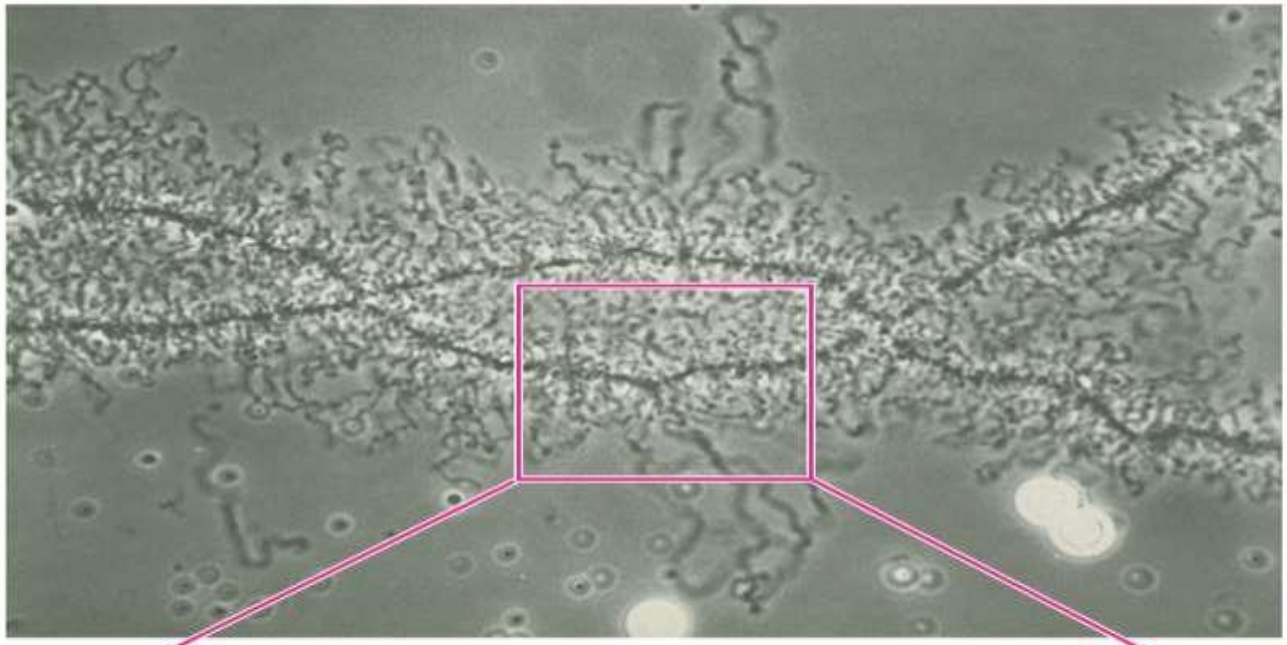

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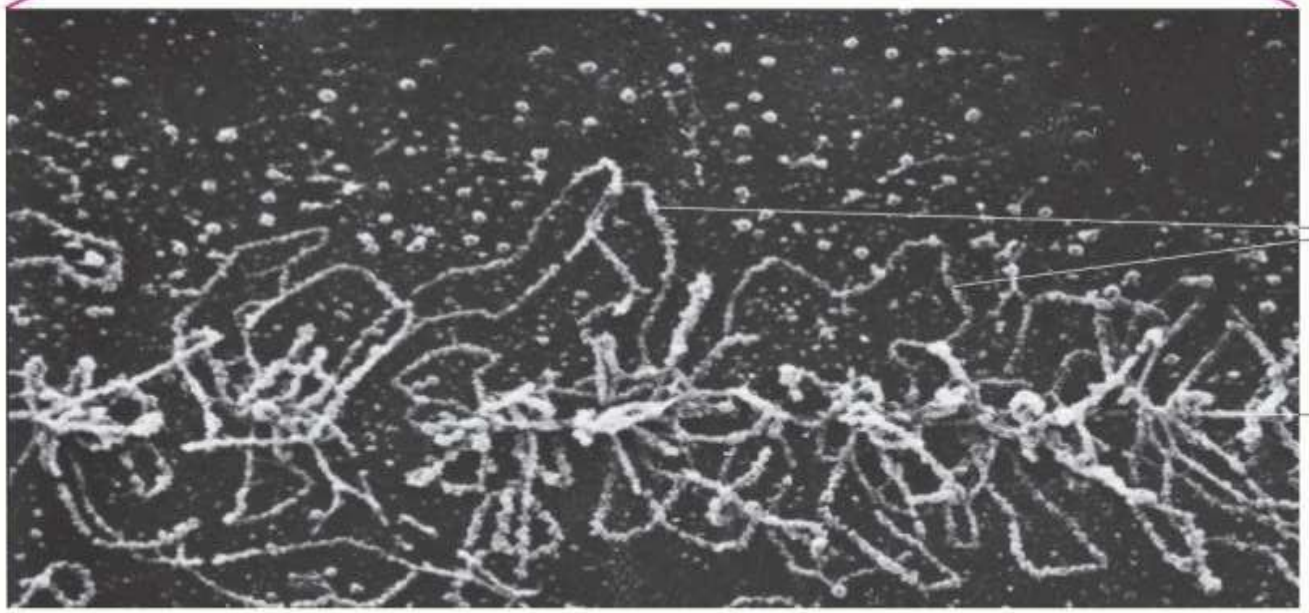
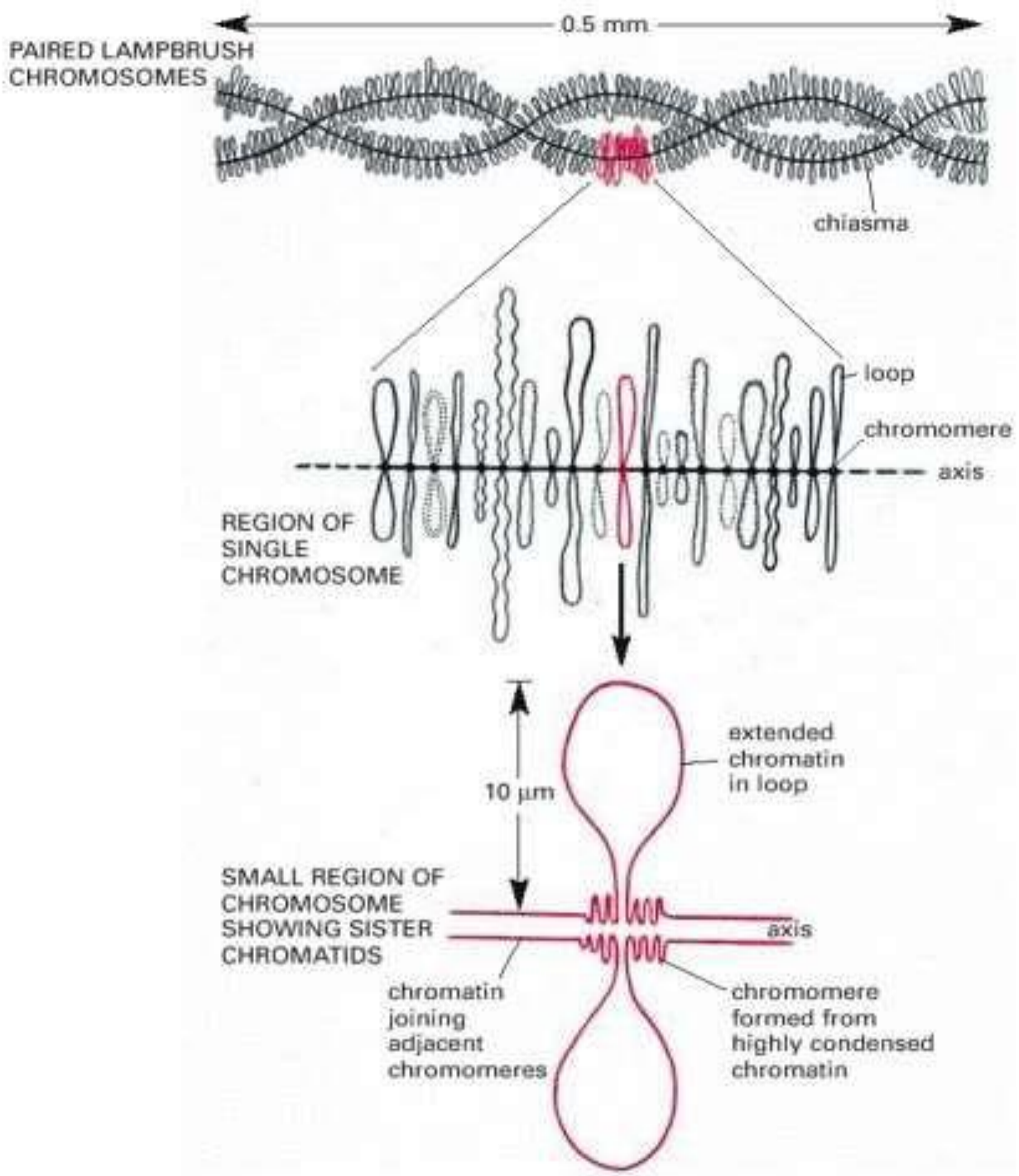
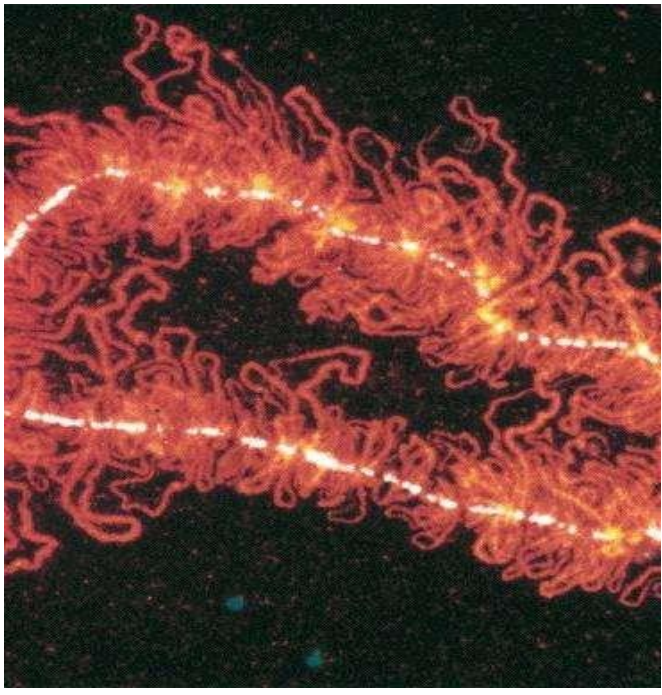


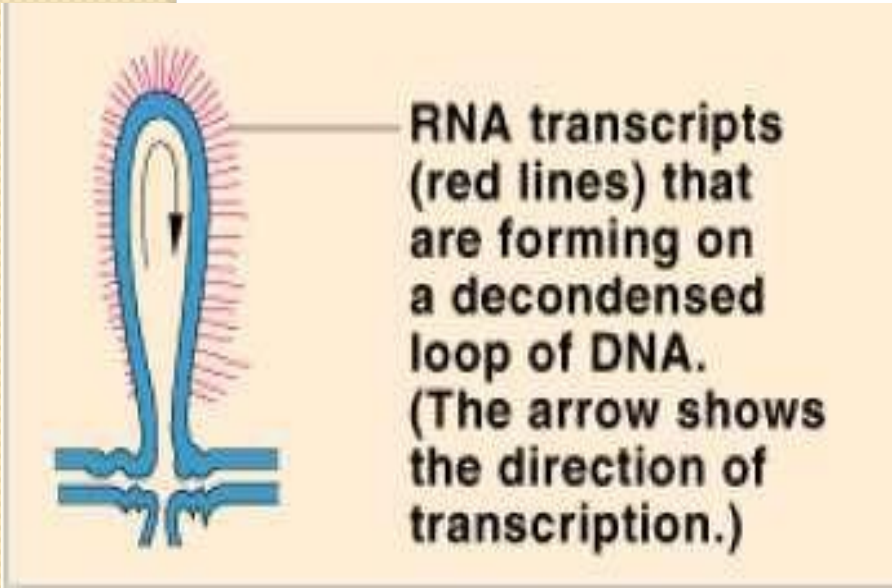


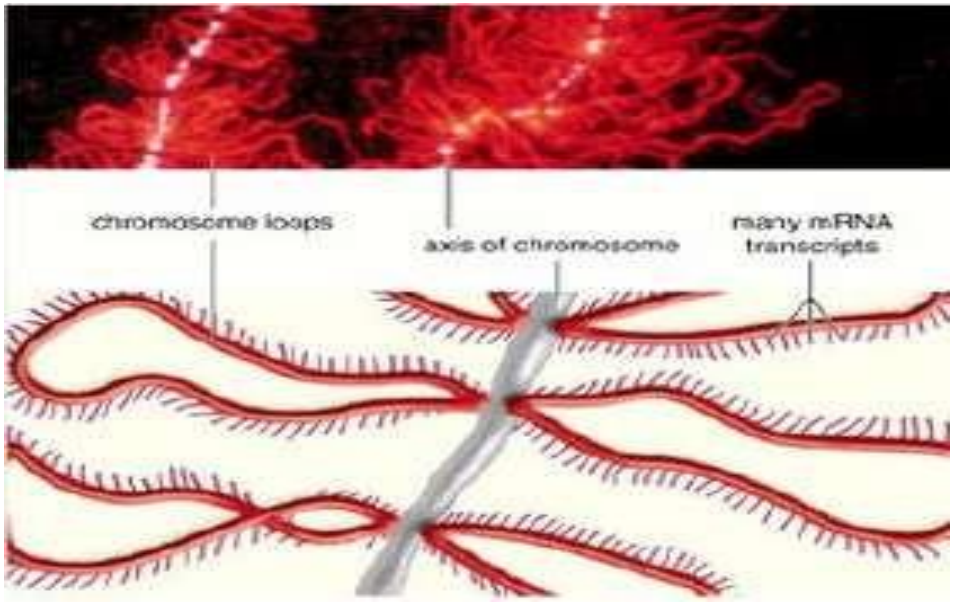
Detail structure of a Lampbrush chromosome.



LBC transcription

- Transcription occurs either along the whole loop or at a parts of a loop.
- At the beginning of meiosis, when DNA replication is complete, the homologous pairs lie immediately next to each other & form characteristic structures composed of 4 chromatids.
- Lampbrush chromosomes are distinguished by an especially high rate of RNA transcription.





Function

- Lamp brush chromosomes are involved in the synthesis of RNA& proteins.
- Each loop is believed to represent one long operon consisting of repetitive cistrons.
- Each locus codes for RNA.
- The loop is supposed to synthesis at a high rate because of repetitive
  gene sequence.
- There are also reports that the LBC help in the formation of yolk material in the egg.

Polytene chromosomes

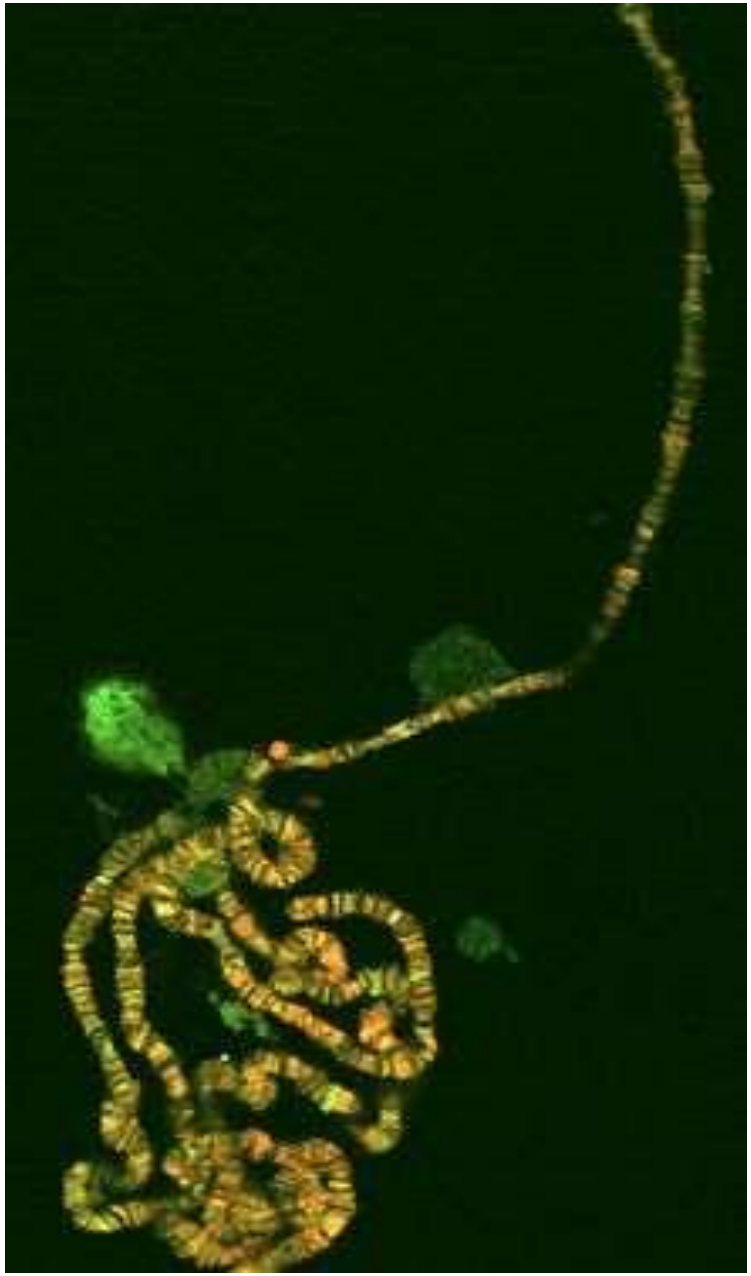
INTRODUCTION

- First discovered by E.G *Balbiani* in 188, in squash of salivary cells of Chironomous.
- They also occur in rectal epithelium & Malphigian tubules.
- They are many times larger than the normal chromosomes reaching a length of 200*μm* and are visible even under a compound microscope.
- The enormous size is due to the duplication of chromonema which do not separate.
- According to an estimate, the polytene chromosomes have 1000 times more DNA than the normal somatic chromosomes.
- Because of these chromosomes actually consist of many strands, they are called as Polytene chromosome.

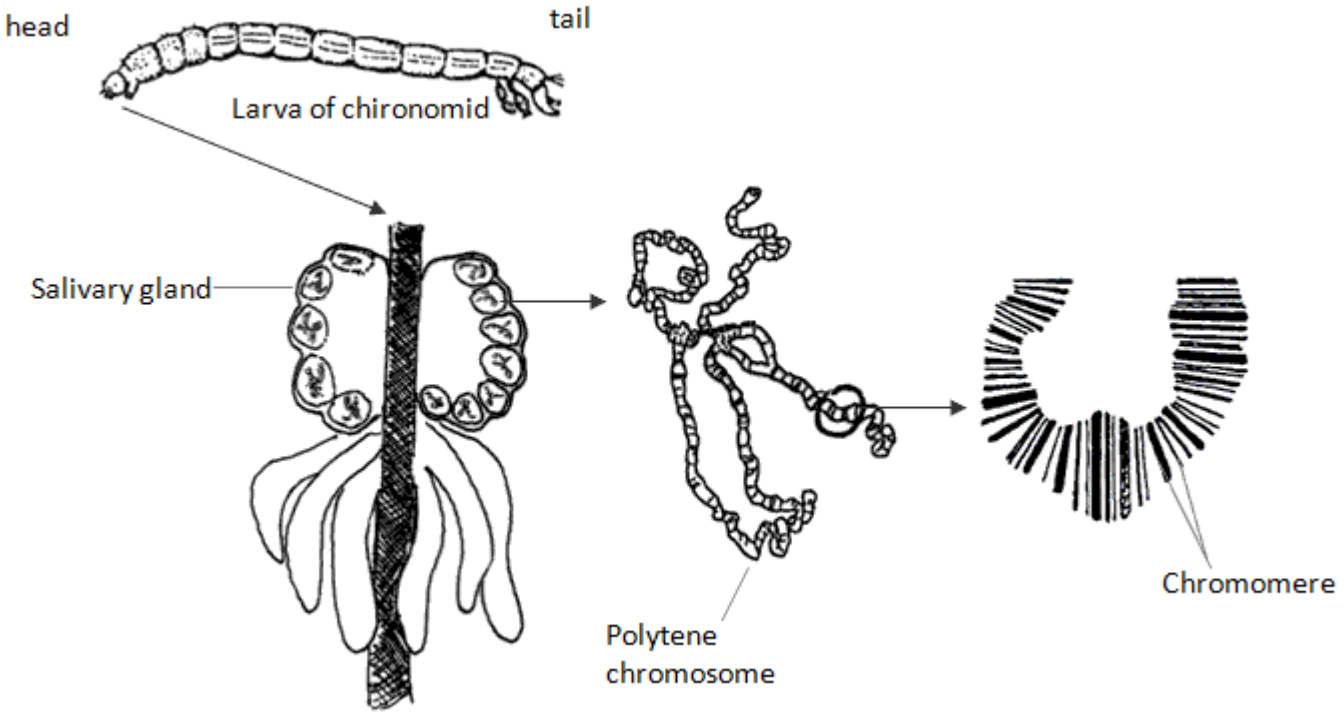
Morphology

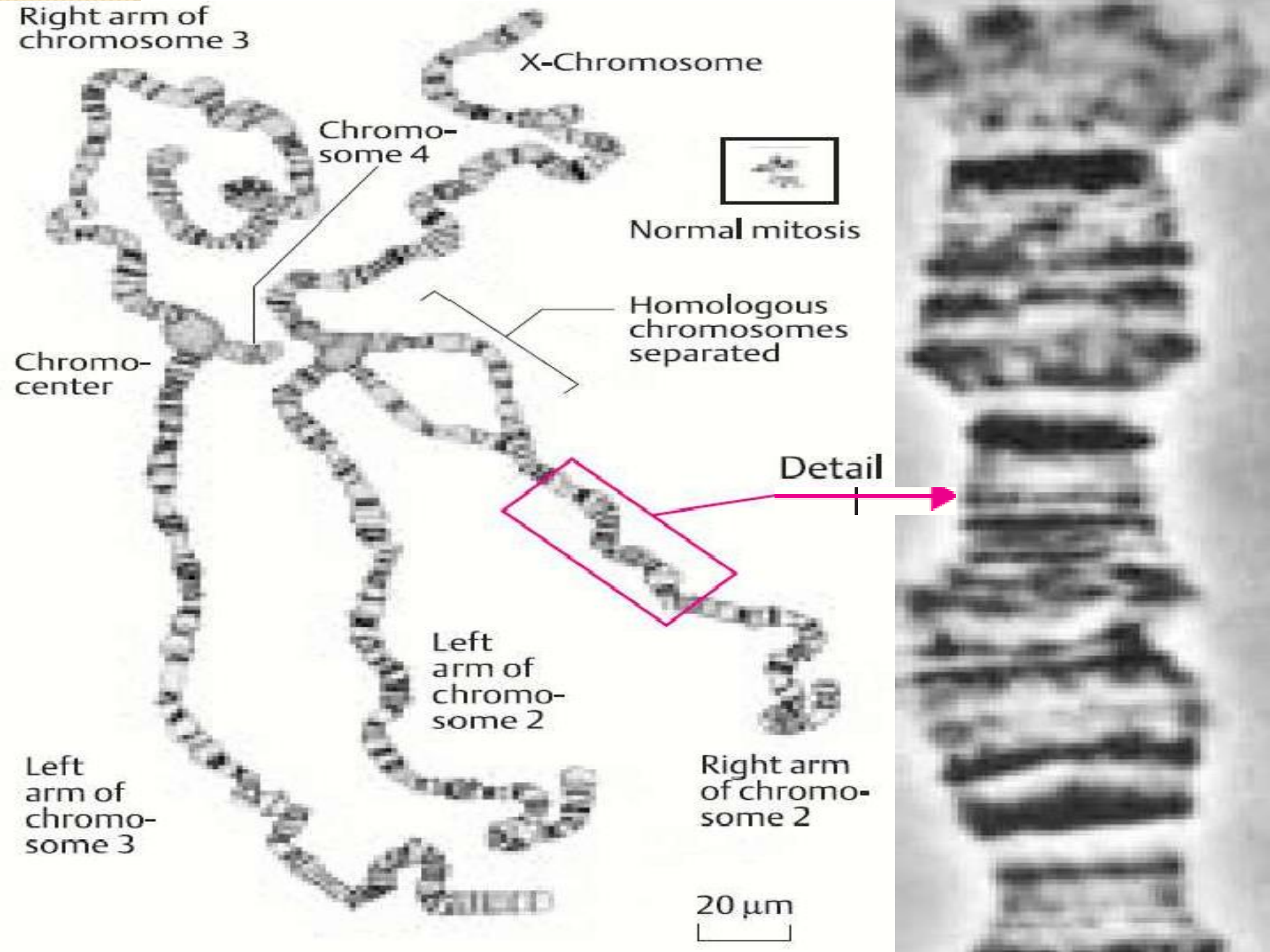
- Contain 5 long & 1 short arm radiating from a central point called chromocentre, formed by the fusion of centromeres all the 8 chromosomes found in the cell.
- Of the 6 arms, the short arm represents the fused IV chromosome & the longest represents the fused sex chr.
- About 80% of the DNA is located in bands, & about 15% in interbands.
- The chromatin in Darkly stained band is more condensed than chromatin in interbands.
- Intensely stained chromosomal segments correspond to high degree of packing & are genetically inactive (heterochromatin).
- Less tightly packed segments stain less distinctly & correspond to
segment with genetic activity (euchromatin).

- In Drosophila, 5000 bands have been found in the 4 chromosomes of salivary gland cells.
- Chromomeres in bands are at right angle to
  the long axis of chromosome.
- Bands have a high DNA content & absorb
  U.V light.
- Painter (1933) & Bridges (1936) showed that in Drosophila the bands are associated with genes.



- Found in salivary glands & other tissues of flies.
- Seen in the nucleus during interphase.
- Show linear series of alternating bands & interbands, distinctive banding for each chromosome in a given species.



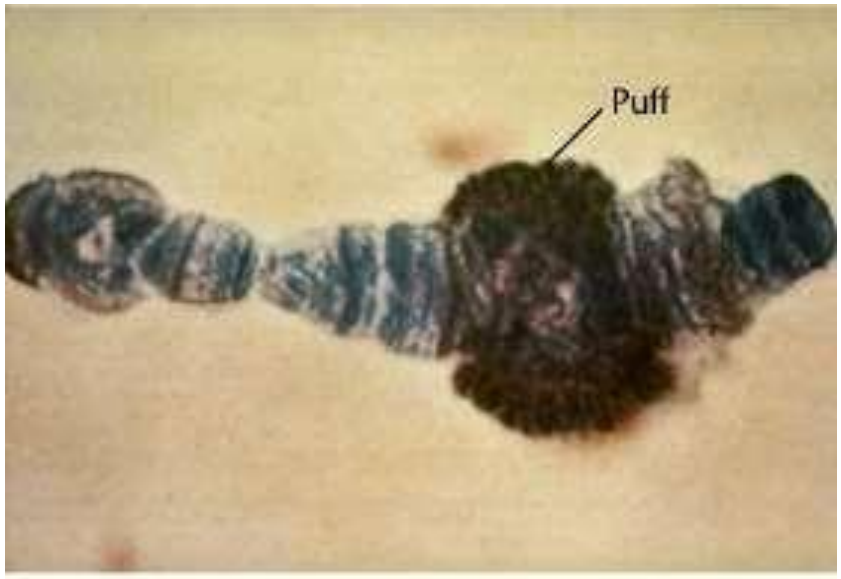

Right arm of chromosome 3
X-Chromosome
Chromo-some 4
Normal mitosis
Homologous chromosomes separated
Chromo-center
Detail
Left arm of chromo-some 2
Left arm of chromo-some 3
Right arm of chromo-some 2
20 µm

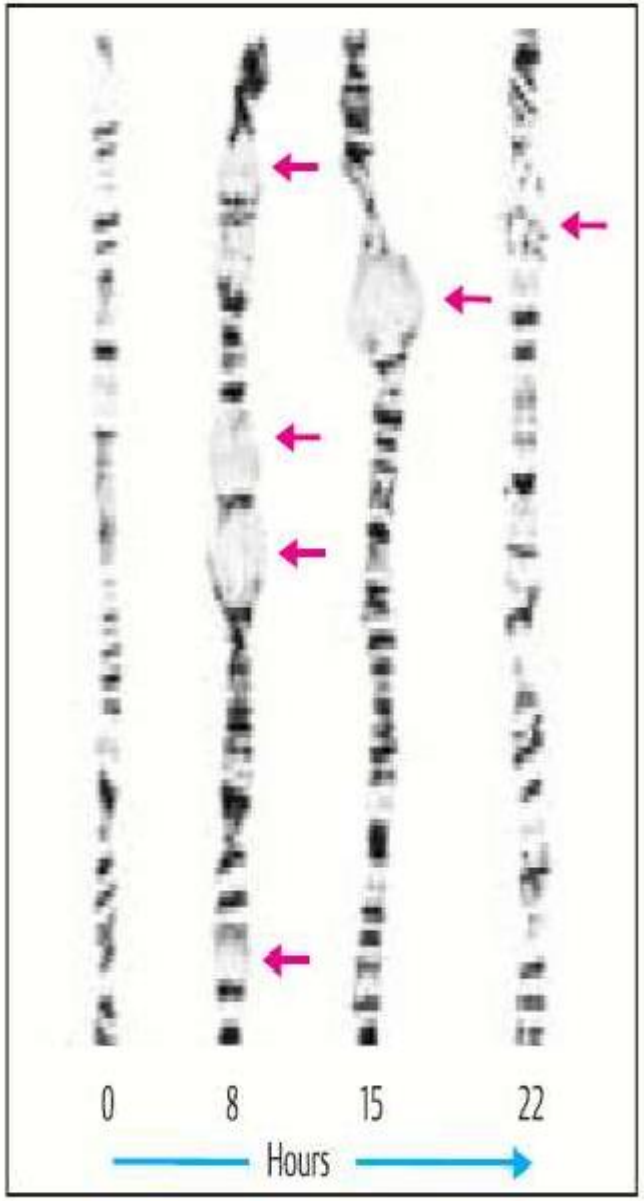
Functional stages in polytene chromosomes

- Polytene chromosomes form structures that correlate with the functional state
- During the larval development of drosophila, a series of expansions
  (puffs) appear in temporal stages in the polytene chromosomes.
- Chromosome puffs are decondensed, expanded segments that represent active chromosomal regions, i.e., regions that are being transcribed.
- The location and duration of the puffs reflect different stages of larval development
- The incorporation of radioactively labeled RNA has been used to demonstrate that RNA synthesis, a sign of gene activity (transcription), occurs in these regions

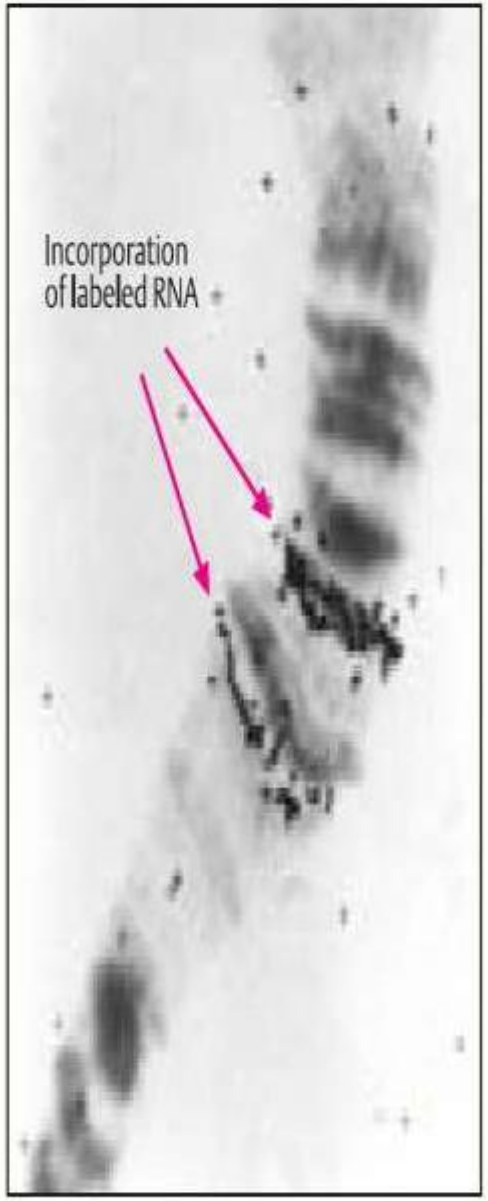
Chromosome puffs

- There are certain intresting structure associated with the bands in the giant chromosomes called as chromosome puffs or Balbiani rings.
- The swellings are called as chromosome puffs.
- These puffs are associated with the metabolic activities & represents areas of active RNA synthesis.

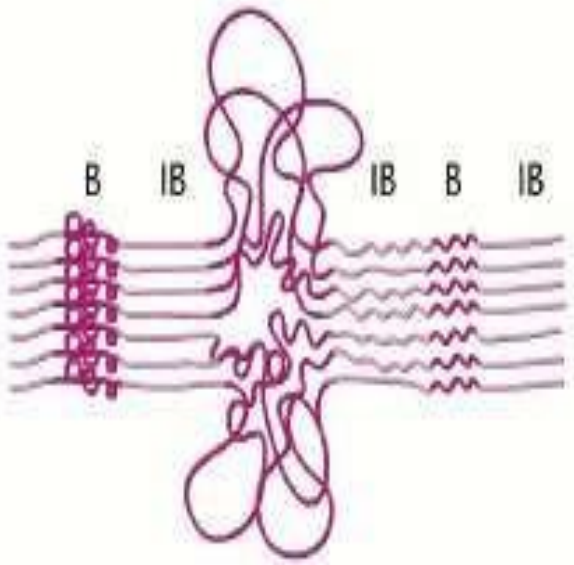




1. Formation of puffs (arrows)



2. Evidence of gene activity



B. Functional stages in polytene chromosomes

Function

- Increasing the volume of the cells' nuclei and causing cell expansion.
- Metabolic advantage as multiple copies of genes permits a high level of gene expression.
- In *Drosophila melanogaster*, the chromosomes undergo many rounds of endoreduplication, to produce large amounts of glue before pupation.
- There is tandem duplication of various polytene bands located near the centromere of the X chromosome which results in the Bar phenotype of kidney-shaped eyes.