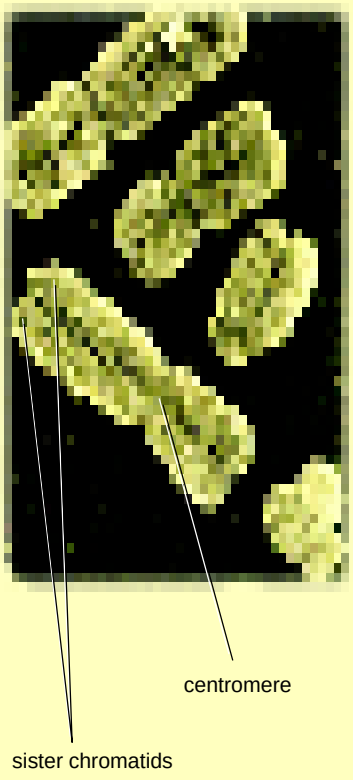
CHAPTER

13

Chromosomes



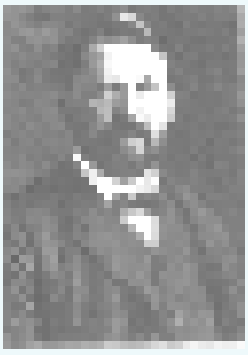
Human chromosomes during mitosis.

The chromosomes are the nuclear components of special organisation, individuality and function. They are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species.

HISTORICAL

Karl Nagli (1842) observed rod-like chromosomes in the nuclei of plant cells. **E. Russow** (1872) made the first serious attempt to describe chromosomes. **A. Schneider** (1873) published a most significant paper dealing with the relation between chromosomes and stages of cell division. **E. Strasburger** (1875) discovered thread-like structures which appeared during cell division. **Walter Flemming** (1878) introduced the term chromatin to describe the thread-like material of the nucleus that became intensely coloured after staining.

W. Roux (1883) suspected the involvement of the chromosomes in the mechanism of inheritance. **Benden** and **Bovery** (1887) reported that number of chromosomes for each species was constant. The present name **chromosome** (Gr., *chrom*= colour, *soma*=body) was coined by **W. Waldeyer** (1888) to darkly stained bodies of nucleus. **W. S. Sutton** and **T. Boveri** in 1902 suggested that chromosomes were the physical structures which acted as messengers of



Theodor Boveri (1862-1915).

heredity. **Sutton** (1902) observed that the chromosome pair in synapsis is made up of one maternal and one paternal member. He believed that chromosomes, acting in this way, may be the physical basis for the Mendelian laws of heredity. He is credited as the originator of the theory of the chromosomal basis for heredity. **Thomas Morgan** and **Hermann Muller**, in the early 1900s, established the cytological basis for the laws of heredity. Working with *Drosophila* chromosomes, they located 2000 genetic factors on the four chromosomes of the fruit fly in 1922. In 1914, **Robert Feulgen** demonstrated a colour test known as **Feulgen reaction** for the DNA. In 1924, he showed that chromosomes contain DNA. In 1942, using cytochemical procedures, **Brachet** demonstrated the presence of another nucleic acid, RNA, and not long there after, **Mirsky** and **Pollister** (1946) showed that there were proteins associated with chromosomal material. **Heitz** (1935), **Kuwanda** (1939), **Geitter** (1940), and **Kaufmann** (1948) have described the morphology of chromosomes. **Dupraw** (1965) suggested 'folded fibre model' of the chromosome to suggest that it was made of a highly folded single molecule of DNA which is wrapped in chromosomal proteins. **R. D. Kornberg** (1974) proposed the 'nucleosome model' of the basic chromatin material. The term 'nucleosome' was coined by **P. Outdet** *et al.*, (1975).

CHROMOSOME NUMBER

The mumber of the chromosomes is constant for a particular species. Therefore, these are of great importance in the determination of the phylogeny and taxonomy of the species. The number or set of the chromosomes of the gametic cells such as sperms and ova is known as the gametic, reduced or **haploid** sets of chromosomes. The haploid set of the chromosomes is also known as the **genome**. The somatic or body cells of most organisms contain two haploid set or genomes and are knows as the **diploid cells**. The diploid cells achieve the diploid set of the chromosomes by the union of the haploid male and female gametes in the sexual reproduction. The suffix "—ploid" refers to chromosome "sets". The prefix indicates the degree of the ploidy.

The number of chromosomes in each somatic cell is the same for all members of a given species. The organism with the lowest number of the chromosomes is the nematode, *Ascaris megalocephalus univalens* which has only two chromosomes in the somatic cells (*i.e.*, 2n =2). In the radiolarian protozoan *Aulacantha* is found a diploid number of approximately 1600 chromosomes. Among plants, chromosome number varies from 2n = 4 in *Haplopappus gracilic* (Compositae) to 2n = >1200 in some pteridophytes. However,the diploid number of tobacco is 48, cattle 60, the garden pea 14, the fruit fly 8, etc. The chromosome number of some animals and plants is tabulated in Table 13-1. The diploid number of a species bears no direct relationship to the species position in the phylogenetic scheme of classification.

Table 13.1. **Chromosome number of some organisms.**

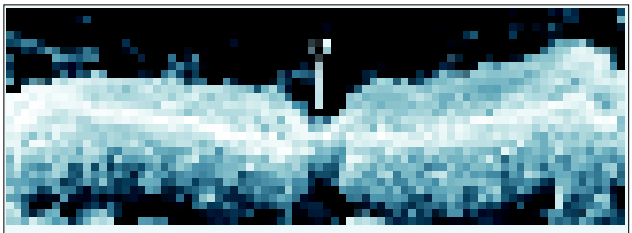
Group	Common name	Scientific name	Chromosome number
Animals :			
Protozoa	Paramecium	*Paramecium aurelia*	30–40
Cnidaria	Hydra	*Hydra vulgaris*	32
Nematoda	Round worm	*Ascaris lumbricoides*	24
Arthropoda	House fly	*Musca domestica*	12
	Mosquito	*Culex pipiens*	6
Chordata	Frog	*Rana esculenta*	26
	Pigeon	*Columba livia*	80
	Rabbit	*Oryctolagus cuniculus*	44
	Gorillia	*Gorilla gorilla*	48
	Man	*Homo sapiens*	46

Group	Common name	Scientific name	Chromosome number
Plants :			
Algae	Chlamydomonas	*Chlamydomonas reinhardii*	10?; 12? 16? (Haploid sets)
Fungi	Bread mold	*Mucor heimalis*	2
Gymnosperm	Yellow pine	*Pinus ponderosa*	24
Angiosperm	Cabbage	*Brassica oleracea*	18
	Coffee	*Coffea arabica*	44
	Potato	*Solanum tuberosum*	48
	Sugar cane	*Saccharum officinarum*	80
	Onion	*Allium cepa*	16

Lastly, while '*n*' normally signifies the gametic or haploid chromosome number, '*2n*' is the somatic or diploid chromosome number in an individual. In polyploid individuals, however, it becomes necessary to establish an ancestral primitive number, which is represented as '*x*' and is called the **base number**. For example, in wheat *Triticum aestivum* 2*n* = 42 ; *n* = 21 and *x* = 7, showing that common wheat is a hexaploid (2n = 6*x*).

Autosomes and Sex chromosomes

In a diploid cell, there are two of each kind of chromosome (these are termed **homologous chromosomes**), except for the **sex chromosomes**. One sex has two of the same kind of sex chromosome and the other has one of each kind. For example, in human, there are 23 pairs of homologous chromosomes (*i.e.*, 2n = 46 ; a chromosome number which was established by **Tijo** and **Levan** in 1956). The human female has 44 non-sex chromosomes, termed **autosomes** and one pair of homomorphic (morphologically similar) sex chromosomes given the designation XX. The human male has 44 autosomes and one pair of heteromorphic or morphologically dissimilar sex chromosomes, *i.e.*, one X chromosome and one Y chromosome.



A mitotic chromosome; c = centromere.

MORPHOLOGY

Size

The size of chromosome is normally measured at mitotic metaphase and may be as short as 0.25 µm in fungi and birds, or as long as 30 µm in some plants such as *Trillium*. However, most metaphase chromosomes fall within a range of 3µm in fruitfly (*Drosophila*), to 5µm in man and 8µm to 12µm in maize. The organisms with less number of chromosome contain comparatively large-sized chromosomes than the chromosomes of the organisms having many chromosomes.

The monocotyledon plants contain large-sized chromosomes than the dicotyledon plants. The plants in general have large-sized chromosomes in comparison to the animals. Further, the chromosomes in a cell are never alike in size, some may be exceptionally large and others may be too small. The largest chromosomes are lampbrush chromosomes of certain vertebrate oocytes and polytene chromosomes of certain dipteran insects.

Shape

The shape of the chromosomes is changeable from phase to phase in the continuous process of the cell growth and cell division. In the resting phase or interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread-like stainable structures, the chromatin

threads. In the metaphase and the anaphase, the chromosomes become thick and filamentous. Each chromosome contains a clear zone,known as **centromere** or **kinetocore**, along their length. The centromere divides the chromosomes into two parts, each part is called **chromosome arm**. The position of centromere varies form chromosome to chromosome and it provides different shapes to the latter which are following (Fig. 13.1) :

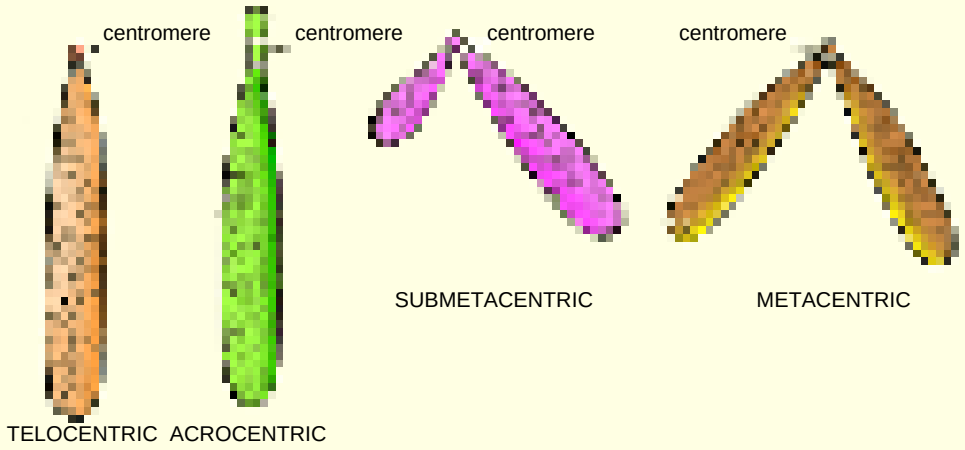


Fig. 13.1. The four morphological types of chromosomes according to the position of the centromere.

1. Telocentric. The rod-like chromosomes which have the centromere on the proximal end are known as the **telocentric chromosomes.**

2. Acrocentric. The acrocentric chromosomes are also rod-like in shape but these have the centromere at one end and thus giving a very short arm and an exceptionally long arm. The locusts (Acrididae) have the acrocentirc chromosomes.

3. Submetacentric. The submetacentric chromosomes are J- or L-shaped. In these, the centromere occurs near the centre or at medium portion of the chromosome and thus forming two unequal arms.

4. Metacentric. The **metacentric chromosomes are V-shaped** and in these chromosomes the centromere occurs in the **centre and forming two equal arms**. The amphibians have metacentric chromosomes.

Structure

While describing the structure of the chromosomes during various phases of cell cycle, cell biologists have introduced many terms for their various components. Let us become familiar with the following terms to understand more clearly the structure of the chromosomes (Fig. 13.2) :

1. Chromatid. At mitotic metaphase each chromosome consists of two symmetrical structures, called **chromatids**. Each chromatid contains a single DNA molecule. Both chromatids are attached to each other only by the centromere and become separated at the beginning of anaphase, when the sister chromatids of a chromosome migrate to the opposite poles.

2. Chromonema (ta). During mitotic prophase the chromosomal material becomes visible as very thin filaments, called **chromonemata** (a term coined by **Vejdovsky** in 1912). A chromonema represents a chromatid in the early stages of condensation. Therefore, 'chromatid' and 'chromonema' are two names for the same structure : a single linear DNA molecule with its associated proteins. The chromonemata form the gene-bearing portions of the chromosomes.

According to old view, a chromosome may have more than one chromonemata which are embedded in the achromatic and amorphous substance, called **matrix**. The matrix is enclosed in a sheath or **pellicle**. Both matrix and pellicle are non-genetic materials and appear only at metaphase

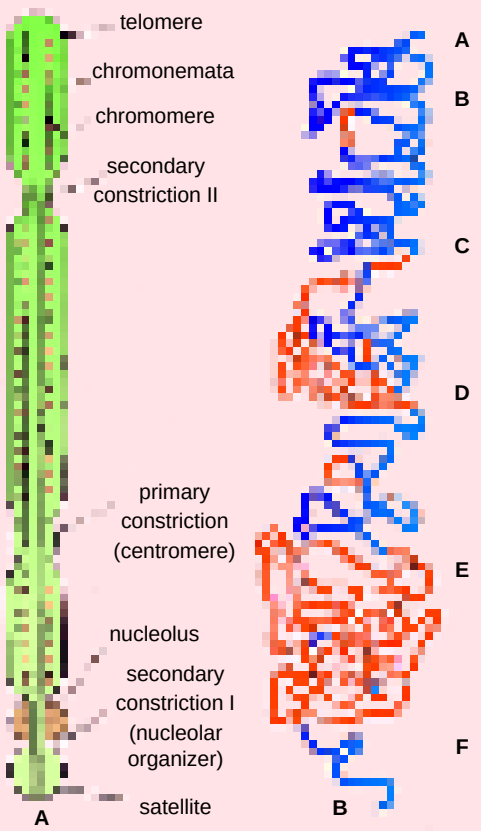


Fig. 13.2. A—Structure of a typical chromosome; B—Model of constitutive heterochromatin in a mammalian metaphase chromosome. A—Constitutive heterochromatin; B—Secondary constriction; I or nucleolar organizer; C—Primary constriction or centromere; D—Euchromatin; E—Secondary constriction. II—possible site of 5S rRNA cistrons; F—Telomere (after De Robertis *et al.*, 1975).

when the nucleolus disappears. It is believed that nucleolar material and matrix are interchangeable, *i.e.*, when chromosomal matrix disappears, the nucleolus appears and vice versa. Electron microscopic observations, however, have questioned the occurrence of pellicle and matrix in them.

3. Chromomeres. The chromomeres are bead-like accumulations of chromatin material that are sometimes visible along interphase chromosomes. The chromomere-bearing chromatin has an appearance of a necklace in which several beads occur on a string. Chromomeres become especially clear in the polytene chromosomes, where they become aligned side by side, constituting the chromosome beads (Fig. 13.2). At metaphase the chromosomes are tightly coiled and the chromomeres are no longer visible.

Chromomeres are regions of tightly folded DNA and have great interest for the cell biologists. They are believed to correspond to the units of genetic function in the chromosomes (see **De Robertis** and **De Robertis, Jr.**, 1987). In fact, for long time most geneticists considered these chromomeres as genes, *i.e.*, the units of heredity.

4. Centromere and kinetochore. Originally it was considered that the centromere consists of small granules or spherules. The centromere of the chromosome of the *Trillium* has the diameter of 3µm and the spherules have the diameter of 0.2 µm. The chromonema remains connected with the spherules of the centromere. Currently it is held that centromere is the region of the chromosome to which are attached the fibres of mitotic spindle. The centromere (a term much preferred by the geneticists) lies within a thinner segment of chromosome, the **primary constriction**. The regions of chromosome flanking the centromere contain highly repetitive DNA and may stain more intensely with the basic dyes. (*i.e.*, it is a constitutive heterochromatin, Fig. 13.2B). Centromeres are found to contain specific DNA sequences with special proteins bound to them, forming a disc-shaped structure, called **kinetochore** (a term that is much preferred by the cytologists). Under the EM, the kinetochore appears as a plate - or cup-like disc, 0.20 to 0.25 nm, in diameter situated upon the primary constriction or centromere. In thin electron microscopic sections, the kinetochore shows a trilaminar structure, *i.e.*, a 10 nm thick dense **outer proteinaceous** layer, a **middle** layer of low density and a dense **inner** layer tightly bound to the centromere (Fig. 13.3). The DNA of centromere does not exist in the form of nucleosome (**Ris** and **Witt**, 1981). Further, emanating from the convex surface of outer layer of kinetochore, in addition to the microtubules, a "corona" or "collar" of fine filaments has been observed. During mitosis, 4 to 40 microtubules of mitotic spindle become attached to the kinetochore and provide the force for chromosomal movement during anaphase. The main function of the kinetochore is to provide a centre

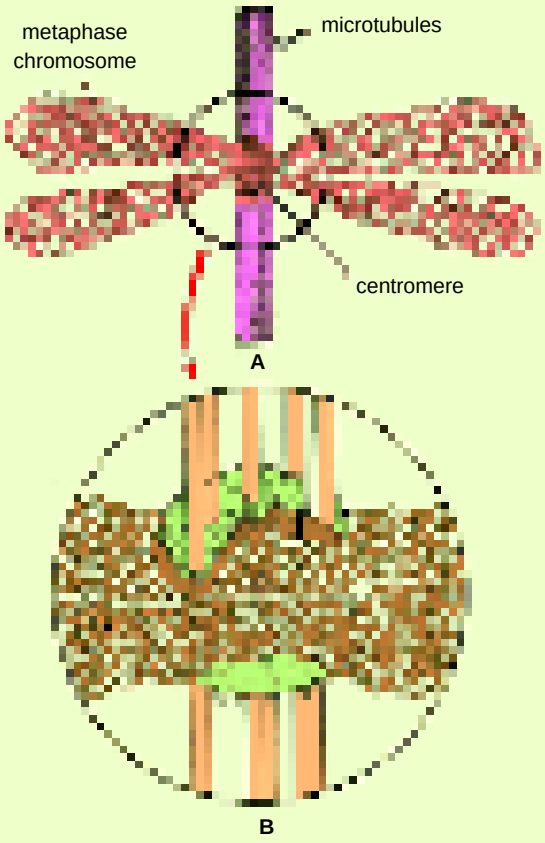


Fig. 13.3. A—A metaphase chromosome showing the folded fibre structure and the centromere with implanted microtubules; B—An inset a higher magnification, showing the convex electrone dense layer and the fibrillar material forming the "corona" of the centromere (after De Robertis *et al.*, 1975).

of assembly for microtubules, *i.e.*, it serves as a nucleation centre for the polymerization of tubulin protein into microtubules (**Telzer** *et al.*, 1975).

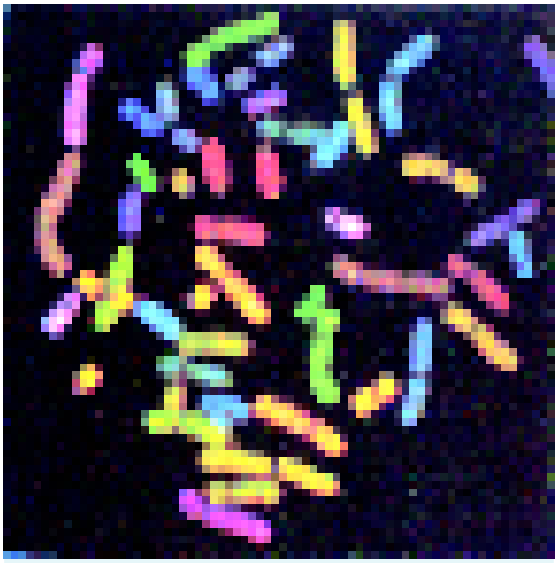
The chromosomes of most organisms contain only one centromere and are known as **monocentric** chromosomes. Some species have diffuse centromeres, with microtubules attached along the length of the chromosome, which are called **holocentric chromosomes**. The chromosomes of the *Ascaris megalocephala* and hemipterans have diffused type of the centromere. In some chromosomal abnormality (induced for example by X-rays), chromosomes may break and fuse with other, producing chromosomes without centromere (**acentric chromosomes**) or with two centromeres (**dicentric chromosomes**). Both types of these chromosomal aberrations are unstable. The acentric chromosomes cannot attach to the mitotic spindle and remain in the cytoplasm. The dicentric chromosomes lead to fragmentation, since, two centromeres tend to migrate to opposite poles.

5. Telomere. (Gr., *telo*=for; *meros*=part). Each extremity of the chromosome has a polarity and therefore, it prevents other chromosomal segments to be fused with it. The chromosomal ends are known as the **telomeres**. If a chromosome breaks, the broken ends can fuse with each other due to lack of telomeres.

6. Secondary constriction. The chromosomes besides having the primary constriction or the centromere possess secondary constriction at any point of the chromosome. Constant in their position and extent, these constrictions are useful in identifying particular chromosomes in a set. Secondary constrictions can be distinguished from primary constriction or centromere, because chromosome bends (or exhibits angular deviation) only at the position of centromere during anaphase.

7. Nucleolar organizers. These areas are certain secondary constrictions that contain the genes coding for 5.8S, 18S and 28S ribosomal RNA and that induce the formation of nucleoli. The secondary constriction may arise because the rRNA genes are transcribed very actively and, thus, interfering with chromosomal condensation. In human beings, the nucleolar organizers are located in the secondary constrictions of chromosomes 13, 14, 15, 21 and 22, all of which are acrocentric and have satellites.

8. Satellite. Sometimes the chromosomes bear round elongated or knob-like appendages known as **satellites**. The satellite remains connected with the rest of the chromosome by a thin chromatin filament. The chromosomes with the satellite are designated as the **sat chromosomes**. The shape and size of the satellite remain constant.



Photograph of a cluster of mitotic chromosomes. Pairs of homologous chromosomes can be identified and karyotypes can be prepared with their help.

Chromosome satellites are a morphological entity and should not be confused with satellite DNAs which are highly repeated DNA sequence.

Karyotype and Idiogram

All the members of a species of a plant or the animal are characterized by a set of chromosomes which have certain constant characteristics. These characteristics include the number of chromosomes, their relative size, position of the centromere, length of the arms, secondary constrictions and satellites. The term **karyotype** has been given to the group of characteristics that identifies a particular set of chromosomes. A diagrammatic representation of a karyotype (or morphological characteristics of the chromosomes) of a species is called **idiogram** (Gr., *idios* = distinctive; *gramma* = something written). Generally, in an idiogram, the chromosomes of a haploid set of an organism are ordered in a series of decreasing size. Sometimes an idiogram is prepared for the diploid set of chromosomes, in which the pairs of homologues are ordered in a series of decreasing size.

A karyotype of human metaphase chromosomes is obtained from their microphotographs. The individual chromosomes are cut out of the microphotographs and lined up by size with their respective partners. The technique can be improved by determining the so-called **centromeric index**, which is the ratio of the lengths of the long and short arms of the chromosome.

Some species may have special characteristics in their karyotypes; for example, the mouse has acrocentric chromosomes, many amphibians have only metacentric chromosomes and plants frequently have heterochromatic regions at the telomeres.

Uses of karyotypes. The karyotypes of different species are sometimes compared and similarities in karyotypes are presumed to represent evolutionary relationship. A karyotype also suggests primitive or advanced features of an organism. It may be symmetric or asymmetric. A karyotype exhibiting large differences in smallest and largest chromosomes of the set and containing fewer metacentric chromosomes, is called an **asymmetric karyotype** (Fig. 13.4). In comparison to a symmetric karyotype (*e.g.*, *Pinus*; Fig. 13.4 A), an asymmetric karyotype (*e.g.*, *Ginkgo biloba*, Fig. 13.4 B) is considered to be a relatively advanced feature. **Levitzky** (1931) suggested that in flowering plants there is a prominent trend towards asymmetric karyotypes. This trend has been well studied in the genus *Crepis* of the family Compositae. In many cases it was shown that increased karyotype asymmetry was associated with specialized zygomorphic flowers.

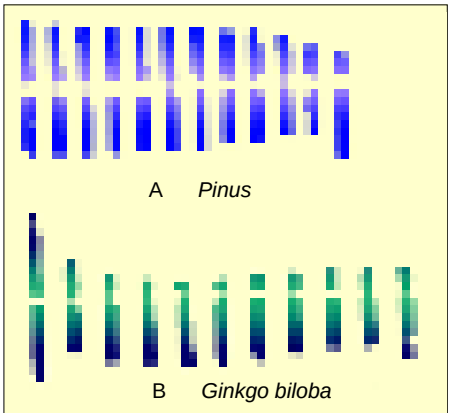


Fig. 13.4. Karyotypes : A–Symmetric; B– Asymmetric.

MATERIAL OF THE CHROMOSOMES

The material of the chromosomes is the chromatin, the structure of which has already been described in previous chapter (Chapter 12). Depending on their staining properties, the following two types of chromatin may be distinguished in the interphase nucleus :

1. Euchromatin. Portions of chromosomes that stain lightly are only partially condensed; this chromatin is termed **euchromatin**. It represents most of the chromatin that disperse after mitosis has completed. Euchromatin contains structural genes which replicate and transcribe during G_1 and S phase of interphase. The euchromatin is considered genetically active chromatin, since it has a role in the phenotype expression of the genes. In euchromatin, DNA is found packed in 3 to 8 nm fibre.

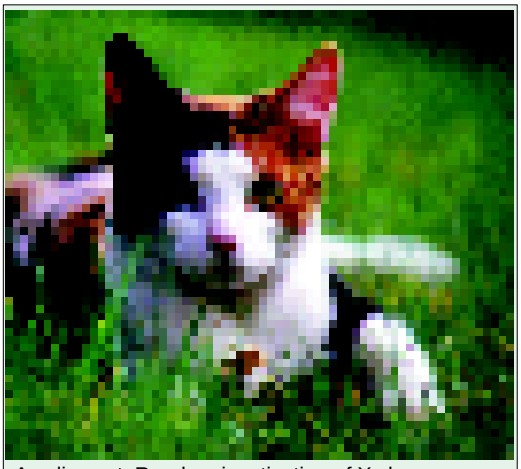
2. Heterochromatin. In the dark-staining regions, the chromatin remains in the condensed state and is called **heterochromatin**. In 1928, **Heitz** defined heterochromatin as those regions of the chromosome that remain condensed during interphase and early prophase and form the so-called **chromocentre**. Heterochromatin is characterized by its especially high content of repititive DNA sequences and contains very few, if any, structural genes (*i.e.*, genes that encode proteins). It is **late replicating** (*i.e.*, it is replicated when the bulk of DNA has already been replicated) and is not transcribed. It is thought that in heterochromatin the DNA is tightly packed in the 30 nm fibre.

Types of heterochromatin. In an interphase nucleaus, usually there is some condensed chromatin around the nucleolus, called **perinucleolar chromatin**, and some inside the nucleolus, called **intranucleolar chromatin**. Both types of this heterochromatin appear to be connected and together, they are referred to as **nucleolar chromatin**.

Dense clumps of deeply staining chromatin often occur in close contact with the inner membrane of the nuclear envelope (*i.e.*, with the nuclear lamina) and is called **condensed peripheral chromatin**. Between the peripheral heterochromatin and the nucleolar heterochromatin are regions of lightly staining chromatin, called **dispersed chromatin**. In the condensed chromosomes, the heterochromatic regions can be visualized as regions that stain more strongly or more weakly than the euchromatic regions, showing the so-called **positive** or **negative heteropyknosis** of the chromosomes (Gr., *hetero* = different + *pyknosis* = staining).

Heterochromatin has been further classified into the following types :

1. Constitutive heterochromatin. In such a heterochromatin the DNA is permanently inactive and remains in the condensed state throughout the cell cycle. This most common type of heterochromatin occurs around the centromere, in the telomeres and in the C-bands of the chromosomes. In *Drosophila virilis*, constitutive heterochromatin exists around the centromeres and such **pericentromeric heterochromatin** occupies 40 per cent of the chromosomes. In many species, entire chromosomes become heterochromatic and are called **B chromosome**, **satellite chromosomes** or **accessory chromosomes** and contain very minor biological roles. Such chromosomes comprising wholly constitutive heterochromatin occur in corn, many phytoparasitic insects and salamanders. In the fly *Sciara*, large metacentric heterochromatic chromosomes are found in the gonadal cells, but are absent in somatic cells. Entire Y chromosome of male *Drosophila* is heterochromatic, even though containing six gene loci which are necessary for male fertility (see **Suzuki**, *et al.*, 1986).



A calico cat. Random inactivation of X chromosome creates a mosaic of tissue patches. An example of facultative heterochromatin.

Constitutive heterochromatin contains short repeated sequences of DNA, called **satellite DNA**. This DNA is called satellite DNA because upon ultracentrifugation, it separates from the main component of DNA. Satellite DNA may have a higher or lower G + C content than the main fraction. For example, the mouse satellite DNA is a 240 base pair sequence that is repeated about 1000,000 (10^6) times in the mouse genome, constituting 10 per cent of the total mouse DNA. The exact significance of constitutive heterochromatin is still unexplained.

2. Facultative heterochromatin. Such type of heterochromatin is not permanently maintained in the condensed state ; instead it undergoes periodic dispersal and during these times is transcriptionally active. Frequently, in facultative heterochromatin one chromosome of the pair becomes either totally or partially heterochromatic. The best known case is that of the X-chromosomes in the mammalian female, one of which is active and remains euchromatic, whereas the other is inactive and forms at interphase, the **sex chromatin** or **Barr body** (Named after its discoverer, Canadian cytologist **Murray L. Barr**). Barr body contains DNA which is not transcribed and is not found in males. Indeed, the number of Barr bodies is always one less than the number of X chromosomes (*i.e.*, in humans, XXX female has two Barr bodies and XXXX female has three Barr bodies; **M.L. Barr**, 1959).

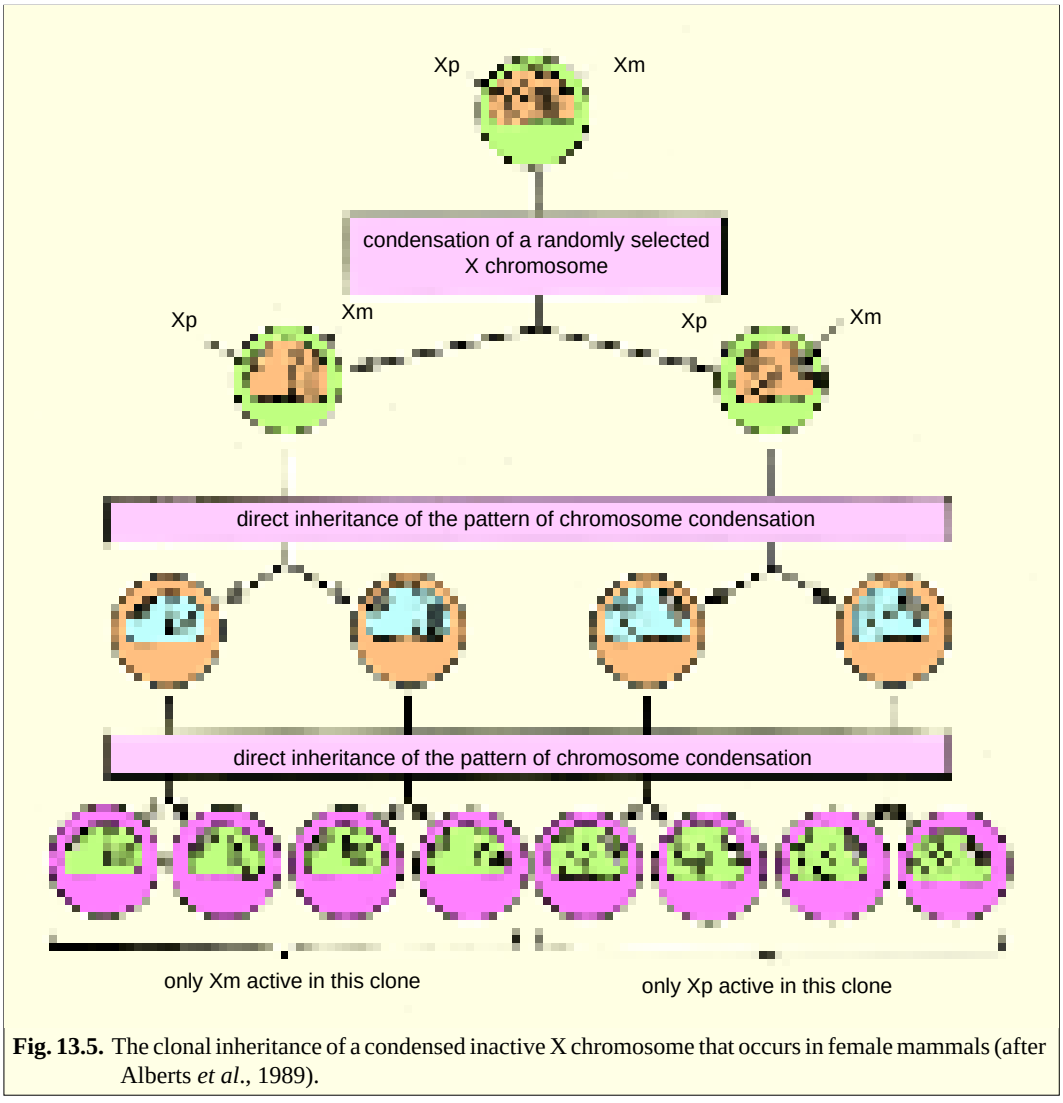


Fig. 13.5. The clonal inheritance of a condensed inactive X chromosome that occurs in female mammals (after Alberts *et al*., 1989).

Dosage compensation and lyonization. In mammals all female cells contain two X chromosomes, while male cells contain one X and one Y chromosome. Presumably because a double dose of X chromosome products would be lethal, the female cells have evolved a mechanism for permanently inactivating one of the two X chromosomes in each cell (this process is called **dosage compensation)**. Process of X chromosome inactivation is often termed **lyonization** after the name of British cytogeneticists **Mary Lyons**. In mice, this occurs between the third and the sixth day of development,

when one or the other of the two X chromosomes in each cell is chosen at random and condensed into heterochromatin (or Barr body). Because the inactive chromosome is faithfully inherited, every female is a **mosaic** composed of clonal groups of cells in which only the paternally inherited X chromosome (Xp) is active and a roughly equal number of groups of cells in which only the maternally inherited X chromosome (Xm) is active (Fig. 13.5).

ISOLATION METHODS

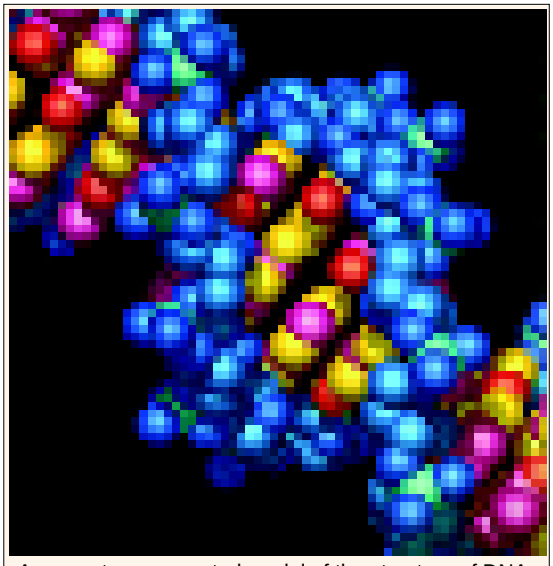
Several methods have been developed over the years to prepare chromatin for microscopical examination. A relatively simple approach is to first prepare purified nuclei from thymus, liver, or any other desired source. Nuclei are then lysed with detergent and the chromatin pelleted at 20,000 rpm (about 50,000 g) in a preparative centrifuge. Chromatin can also be isolated biochemically by purifying nuclei and then lysing them in hypotonic solutions. When prepard in this way, chromatin appears as a viscous, gelatinous substance.

Chromosomes are best observed in **squash** preparations. Fragments of tissues (*e.g.*, root tips of onion) are stained with basic stains (*e.g.*, orcein, Giemsa or acetocarmine) and then squashed between slide and coverslip by gentle pressure. Sometimes hypotonic solutions are used prior to squashing to produce swelling of the nucleus and a better separation of the individual chromosomes. The morphology of the chromosomes is best studied during metaphase and anaphase, which are the periods of maximal contraction.

Salamanders and grasshoppers contain high DNA content and very large chromosomes that produce vary beautiful meiotic preparations; among plants, broad beans (*Vicia fava*) and onion (*Allium cepa*) have particularly large chromosomes that produce fine preparations of mitotic cells from the root tips.

CHEMICAL COMPOSITION

Chromatin which has been isolated from rat liver contains DNA, RNA and protein. The protein of chromatin is of two types : the histones and the non-histones. Rat liver chromatin has been used as a model for chromatin. It possesses a histone to DNA ratio near 1 : 1, a non-histone protein to DNA ratio of 0.6 : 1 and a RNA/DNA ratio of 0.1 : 1.



A computer - generated model of the structrue of DNA

1. DNA

DNA is the most important chemical component of chromatin, since it plays the central role of controlling heredity. The most convenient measurement of DNA is picogram (10^{-12} gm). DNA of chromatin represent the following two phenomena :

The C-value. The DNA in nuclei was stained using the **Feulgen reactions** and the amount of stain in single nuclei was measured using a special microscope (called **cytophotometer**). Both of these techniques demonstrated that nuclei contain a constant amount of DNA. Thus, all the cells in an organism contain the same DNA content (**2C**) provided that they are diploid. Gametes are haploid and, therefore, have half the DNA content (1C). Some tissues such as liver, contain occasional cells that are polyploid and their nuclei have a correspondingly higher DNA content (4C or 8C) (see Table 13-2).

Table 13.2. DNA content and chromosome component (after **De Robertis** and **De Robertis, Jr.**, 1987).

Cells	Mean DNA-Feulgen content	Presumed chromosome set
1. Spermatid	1.68 (1C)	Haploid (n)
2. Liver	3.16 (2C)	Diploid (2n)
3. Liver	6.30 (4C)	Tetraploid (4n)
4. Liver	12.80 (8C)	Octoploid (8n)

Thus, each species has a characteristic content of DNA which is **constant** in all the individuals of that species and has, thus, been called the **C-value**.

The C-value paradox. Eukaryotes vary greatly in DNA content but always contain much more DNA than prokaryotes. Lower eukaryotes in general have less DNA, such as nematode *Caenorhabditis elegans* which has only 20 times more DNA than *E.coli*, or the fruit fly *D. melanogaster* which has 40 times more DNA (*i.e.*., 0.18 pg or picogram per haploid genome). Vertebrates have greater DNA content (about 3 pg), in general about 700 times more then *E. coli*. One of the highest DNA content is that of the salamander *Amphiuma* which has 84 pg of DNA. Man has about 3 pg of DNA per haploid genome, or 3×10^9 base pairs, *i.e.*, the human genome could accommodate about 3 million average sized proteins if all the DNA were coding (or containing structural genes) and if this was true, salamanders would have 30 times more genes than human beings. This is called **C-value paradox** (**Gall**, 1981). It was detected quite early that there was little connection between the morphological complexity of eukaryotic organisms and their DNA content. For example, *E. coli* (containing 3,400,000 base pairs in its DNA) has about 3000 genes. Although it is difficult to estimate how many different genes exist in the human genome, there are probably not more than 20,000 to 30,000 genes (Note : According to a most recent estimate, there are 100,000 genes in human genome, see **Deviah**, 1994). There is no reason to believe that salamanders should have any more. From these facts, it can be easily concluded that most of the DNA in the eukaryotic genomes must be of a non-coding nature.

2. Histones

Histones are very basic proteins, basic because they are enriched in the amino acids arginine and lysine to a level of about 24 mole present. Arginine and lysine at physiological pH are cationic and can interact electrostatically with anionic nucleic acids. Thus, being basic, histones bind tightly to DNA which is an acid. There are five types of histones in the eukaryotic chromosomes, namely H1, H2A, H2B, H3 and H4.

One of the important discoveries that has come from chemical studies is that the primary structures of histones have been highly conserved during evolutionary history. For example, histone H4 of calf and of garden pea contains only two amino acid differences in a protein of 102 residues (**DeLange**, 1969). Likewise, the sequence of histone H3 from rat differs only in two amino acids from that of peas, out of 102 total amino acid residues. These organisms are estimated to have an evolutionary history of at least 600 million years, during which they diverged structurally. This conservation of structure suggests that over the eras, histones have had a very similar and crucial role in maintaining the structural and functional integrity of chromatin. Such an evolutionary conservation suggests that the functions of these two histones involve nearly all of their amino acids so that a change in any position is deleterious to the cell.

Histone H1 is the least rigidly conserved histone protein. It contains 210 to 220 amino acids and may be represented by a variety of forms even within a single tissue. H1 is present only once per 200 base pairs of DNA (in contrast to rest of the four types of histones each of which is present twice) and is rather loosely associated with DNA. H1 histone is absent in yeast, *Saccharomyces cerevisiae*.

Histones besides determining the structure of chromatin, play a regulatory role in the repression activity of genes.

3. Non-histones

In contrast to the modest population of histones in chromatin, non-histone proteins display more diversity. In various organisms, number of non-histones can vary from 12 to 20. Heterogeneity of these proteins is not conserved in evolution as the histones. These non-histones differ even between different tissues of the same organism suggesting that they regulate the activity of specific genes.

About 50 per cent non-histones of chromatin have been found to be structural proteins and include such proteins as **actin**, and **α-**and **β-tubulins** and **myosin**. Although for sometime these contractile proteins were thought to be contaminants, it is now believed that they are vital ingredients of the chromosome, functioning during chromosome condensation and in the movement of chromosomes during mitosis and meiosis (see **Thorpe**, 1984). Many of the remaining 50 per cent of non-histones include all the enzymes and factors that are involved in DNA replication, in transcription and in the regulation of transcription. These proteins are not as highly conserved among organisms, although they must carry out similar enzymatic activities. Apparently they are not as important as the histones in maintaining chromosome integrity.

ULTRASTRUCTURE

The field of ultrastracture of the chromatin is still the area where electron microscope had failed to provide us a clear picture of the organization of DNA in the chromatin. For the study of chromosomes with the help of electron microscope, whole chromosome mounts as well as sections of chromosomes were studied. Such studies had demonstrated that chromosomes have very fine fibrils having a thickness of 2nm—4nm. Since DNA is 2nm wide, there is a possibility that a single fibril corresponds to a single DNA molecule.

Single-stranded and Multi-stranded Hypotheses

When chromosomes are compared in related species which differ widely in DNA content, such differences may be attributed to one of two causes : (1) **lateral multiplication** of chromonemata leading to multiple or multi-strandedness, or (2) **tandem duplication** of DNA or chromonemata where lenghthwise duplication is responsible for chromatin differences. This latter condition will retain the single stranded feature of chromosomes.

Although multiple strandedness has been demonstrated in several cases of plants such as *Vicia faba* and animals such as dipteran salivary gland chromosomes, there are evidences against such hypothesis to become a generalization. In all these cases, however, tandem duplication of chromonemata (or DNA) evidently takes place. Indeed, there are many evidences to support the idea of single-stranded nature of chromatin. This was confirmed by the technique of pulsed gel electrophoresis that in yeast *Saccharomyces cerevisiae*, each chromosome is formed from a single linear DNA molecule (**Kavenoff** *et al.*, 1974).

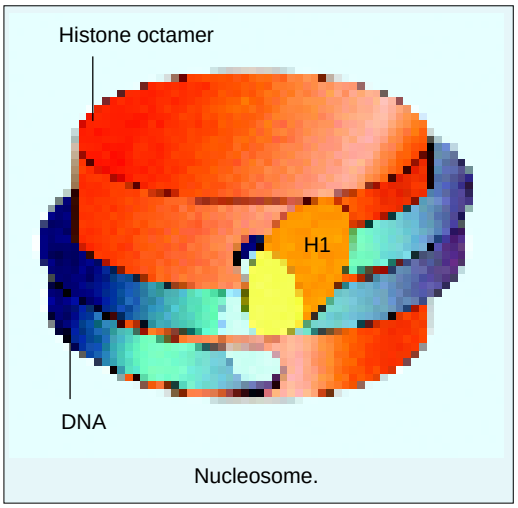
Folded-fibre Model and Nucleosome Concept

If we presume that a single chromatid has a single long DNA molecule, we have no choice but to believe that DNA should be present in a coiled or folded manner. The manner of coiling and folding of DNA was a matter of debate and dozens of models were available for this purpose ; of them only two stand out and are important. A popular model was the **folded-fibre model**, proposed by **E.J. Dupraw** in 1965. According to it, the bulk of the chromosome is visualized to be composed of a tightly folded fibre which has a rather homogeneous diameter of 200 to 300 A°. This folded fibre is supposed to contain the DNA histone helix (of 30A° diameter) in a supercoiled condition (Fig. 13.6). Another model is most significant and universally accepted one and is called **nuclesome model** which was proposed by **R.D. Kornberg** (1974) (Fig. 13.7) and confirmed and christened by **P. Oudet** *et al.*, (1975). Thus, while in the folded-fibre model, it was proposed that the histones were bound on the

outside of the DNA coils (*i.e.*, histone shell around DNA), the nucleosome model has proposed the converse (*i.e.*, histone particle with DNA round it). In other words, the earlier theory that basic chromatin fibre had DNA core surrounded by histones was incorrect (**Berns**, 1983). In fact, from a genetic perspective, a significant feature of packing mechanism through the nucleosomes lies in its topology : *at no point is the DNA buried* ; instead, it is freely exposed along the entire surface of the "spool", available for genetic expression. Nucleosomes seem to be universal device for compacting the long DNA molecules of eukaryotic cells.

Nucleosomes and Solenoid Model of Chromatin

In eukaryotes, DNA is tightly bound to an equal mass of histones, which serve to form a repeating array of DNA-protein particles, called **nucleosomes**. If it was stretched out, the DNA double-helix in each human chromosome would span the cell nucleus thousands of time. Histones play a crucial role in packing this very long DNA molecule in an orderly way (*i.e.*, nucleosome) into nucleus only a few micrometres in diameter. Thus, nucleosomes are the fundamental packing unit particles of the chromatin and give chromatin a "**beads-on-a-string**" appearance in electron micorgraphs taken after treatments that unfold higher- order packing (**Olins** and **Olins**, 1974).



Nucleosome.

The nucleosome 'beads' can be removed from long DNA "string" by digestion with enzymes that degrade DNA, such as bacterial enzyme, **micrococcal nuclease**. After digestion for a short period with micrococcal nuclease, only the DNA between the nucleosome beads is degraded (Fig. 13.7). The rest is protected from digestion and remains as double-stranded DNA fragments 146 nucleotide pairs long bound to a specific complex of 8 nucleosome histones (the **histone octamer**). The nucleosome beads obtained in this way have been crystallized and analyzed by X-ray diffraction.

Each nucleosome is a disc-shaped particle with a diameter of about 11 nm and 5.7 nm in height containing 2 copies of each 4 nucleosome histones–H2A, H2B, H3 and H4. This histone octamer forms a protein core [(*i.e.*, a core of histone tetramer (H3, $H4)_2$ and the apolar regions of 2(H2A and H2B)] around which the double-stranded DNA helix is wound 1¾ time containing 146 base pairs. In undigested chromatin the DNA extends as a continuous thread from nucleosome to nucleosome. Each nucleosome bead is separated from the next by a region of **linker DNA** which is generally 54 base pair long and contains single H1 histone protein molecule. Generally, DNA makes two complete turns around the histone octamers and these two

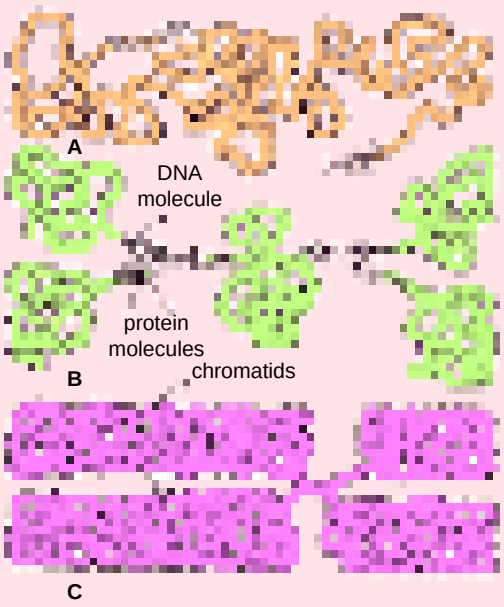
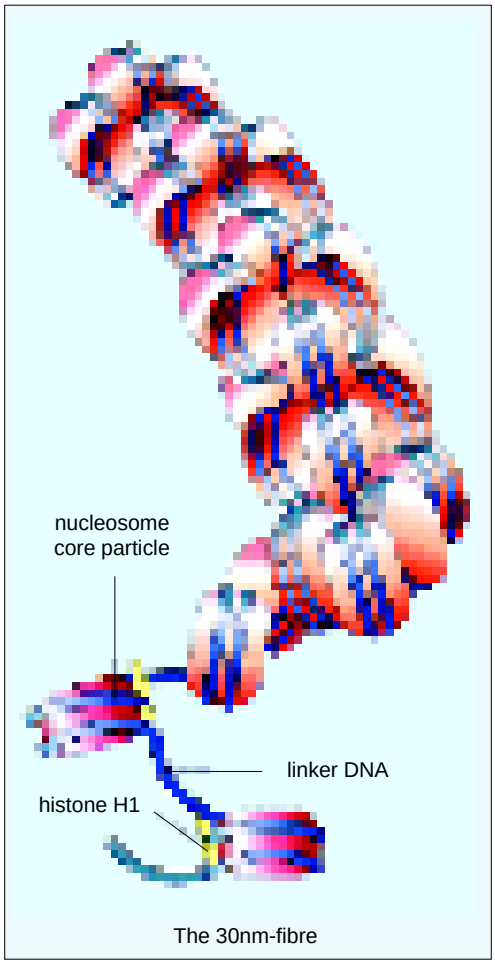


Fig. 13.6. Dupraw's folded fibre model of chromatin in interphase (A and B) and in metaphase (C).

turns (200 bp long) are sealed off by H1 molecules. (**Note** : In some organisms nucleosome DNA may vary from 162 base pairs (*e.g.*, rabbit cortical neurons) to 242 base pairs (*e.g.*, sea urchin sperm); **Reid** and **Leech**, 1980). Thus, on an average, nucleosomes repeat at intervals of about 200 nucleotides or base pairs. For example, an eukaryotic gene of 10,000 nucleotide pairs will be associated with 50 nucleosomes and each human cell with 6×10^9 DNA nucleotide pairs contains 3×10^7 nucleosomes.

Solenoid Models

H1 is reported to be phosphorylated just before mitotic and meiotic cell division to make possible the higher levels of coiling (see **Mays**, 1981). During mitosis or meiosis, the prophase is the stage during which the chromosomes become shorter and thicker due to multiple coiling as proposed by **Dupraw** and others. The hypothesis of a solenoidal structure, with coils of coils had renewal since nucleosomal sub-structure has been discovered. Thus, due to solenoid coiling of nucleosome containing fibre, the following types of chromosomal structures can be observed during the cell cycle (Fig. 13.12) :



The 30nm-fibre

1. The 10-nm fibre. When nucleosomes are in close apposition, they form the **10-nm filaments**, in which packing of DNA is about five-to seven-fold, *i.e.*, five to seven times more compact than free DNA.

2. The 30-nm fibre. When nuclei are very gently lysed onto an electron microscopy grid, most of the chromatin is seen to be in the form of a fibre, with a diameter of about 30 nm. Such **30-nm fibres** can be observed in metaphase chromosomes and in interphase nuclei and it probably represents the natural conformation of transcriptionally inactive chromatin.

The 30-nm fibre consists of closely packed nucleosomes. It probably arises from the folding of the nucleosome chain into a **solenoid structure** having about six nucleosomes per turn (**Klug** and coworkers, 1976, 1979 and 1985). The DNA of 30-nm solenoid has a packing that is about 40-fold.

Histone H1 molecules are found responsible for packing nucleosomes into the 30-nm fibre. The H1 histone molecule has an evolutionarily conserved globular core or central region linked to extended amino-terminal and carboxyl-terminal "arms", whose amino acid sequence has evolved much more rapidly. Each H1 molecule binds through its globular portion to a unique site on a nucleosome and has arms that are thought to extend to contact with other sites on the histone cores of adjacent nucleosomes, so that the nucleosomes are pulled together into a regular repeating array (Fig. 13.10). The binding of H1 molecule to chromatin tends to create a local polarity that the chromatin otherwise lacks.

3. Radial loops of 30-nm fibre and metaphase chromosome. The nucleus is typically about 5µm (5×10^{-4} cm) in diameter. The packaging of DNA into a 30-nm chromatin fibre leaves a human chromosome about 0.1 cm long, so there must be several higher orders of folding. The probable nature of one further level of folding was originally suggested by the appearance of specialized chromosomes—the lampbrush chromosomes and polytene chromosomes. These two types of chromosomes

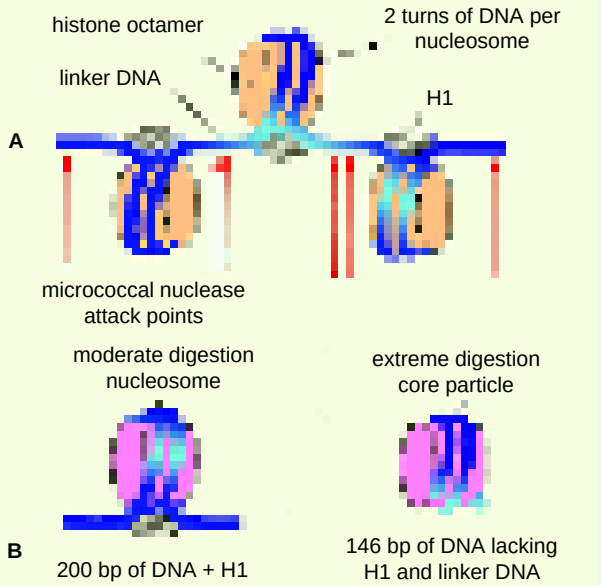


Fig. 13.7. Diagram showing the effect of nuclease enzymes on chromatin. A— Intact chromatin, note that the nucleo some is flat and that each histone octamer has two turns of DNA sealed off by histone H1. In living cells, the nucleosomes would be touching each other to form a 10 nm fibre and not stretched out (to form beads-on-a-string preparation) ; B— A nucleosome released by moderate digestion ; C— Core particle of nucleosome obtained by extensive digestion (after De Robertis and De Robertis, Jr. 1987).

seem to contain a series of **looped domains**— loops of chromatin that extend at an angle from the main chromosome axis. Since such loops do occur in *E. coli* chromosome, so the presence of loops may be a general feature of chromosomes.

In principle, looped domains in chromatin could be esta- blished and maintained by DNA binding proteins that clamp two regions of the 30- nm fibre together by recognizing specific DNA sequence that will form the neck of each loop. Alternatively, they could be formed by binding of DNA at the base of loop to a chromosome axis. Structural non-histone proteins could be involved in organizing the 30-nm fibres into loops. In an experiment, the histones are removed from the metaphase chromosome by adding the polyanion dextran sulphate. Histone-depleted chromosomes are found to have a central core of **scaffold** surrounded by a halo made of hoops of DNA (Fig. 13.11 A). The scaffold is made of non-histone proteins and retains the general shape of the metaphase chromosome. Each chromosome has two scaffolds, one for each chromatid, and they are connected together at the centromere region. When the histones are removed, the DNA which has packed about 40-fold in the 30-nm chromatin fibre, becomes extended and produces loops with an average length of 25µm (75,000 base pairs). In each loop the DNA exits from the scaffold and returns to an adjacent point. On the basis of these observations a model of chromosome structure has been proposed by **Laemmli** and coworkers (1979, 1984). In Laemmli's radial loop model DNA is arranged in loops anchored to the non-histone scaffold. Because the lateral loops have 25µm DNA, after contracting 40-fold in the 30-nm fibre, they would be only about 0.6µm long, a length consistent with the diameter of metaphase chromosome (1µm). Figure 13.11 B shows how the chromatin is arranged in loops which during metaphase, become arranged so that the base of the loops forms a scaffold in the centre of the chromatid. The base of the loop might be arranged on a helical coiled path (*e.g., Trillium* and *Tradescantia*).

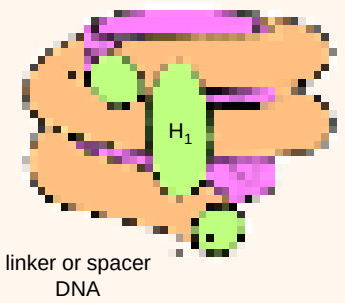


Fig. 13.8. A nucleosome (after Thorpe, 1984)

The chromatin in mitotic chromosomes is transcriptionally inert : all RNA synthesis ceases as the chromosomes condense. Presumably the condensation prevents RNA polymerase from gaining

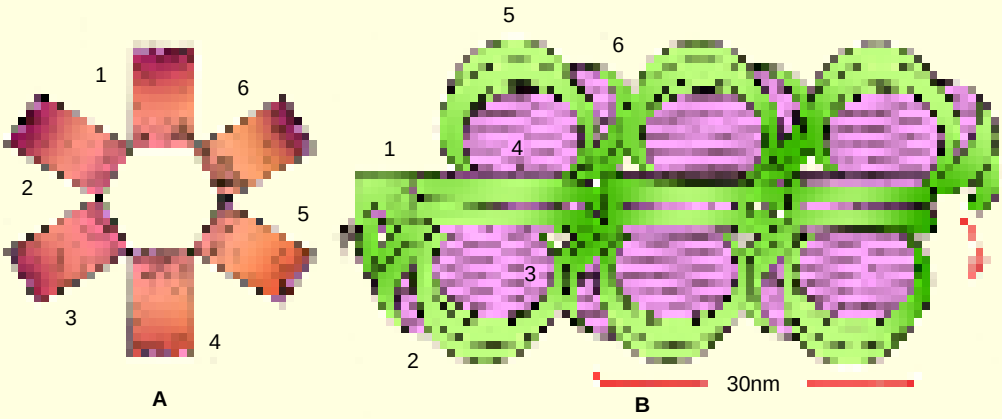


Fig. 13.9. A model suggested to explain how the "Beads-on-a-string" form of nucleosomes is packed to form the 30-nm fibre. A— Top view of 30-nm fibre; B—Side view of 30-nm fibre (after Alberts *et al*, 1989).

access to the DNA, although other controlling factors might also be involved.

FUNCTIONS

The function of chromosomes is to carry the genetic information from one cell generation to another. DNA being the only permanent component of chromosome structure, is the sole genetic material of eukaryotes.

GIANT CHROMOSOMES

Some cells at certain particular stages contain large nuclei with giant or large-sized chromosomes. The giant chromosomes are the **polytene** and **lampbrush** chromosomes.

1. Polytene Chromosome (Salivary Gland Chromosomes)

An Italian cytologist **E.G. Balbiani** (1881) had observed peculiar structures in the nuclei of certain secretory cells (*e.g.*, of salivary glands) of midge, *Chironomus* (Diptera). These structures were long and sausage-shaped and marked by swellings and cross striations (transverse bands). Unfortunately, he did not recognize them as chromosomes, and his report remained buried in the literature. It was not until 1933 that **Theophilus Painter**, **Ernst Heitz**, and **H. Bauer** rediscovered them in *Drosophila* and recognized them as the chromosomes. Since these chromosomes were discovered in the salivary gland cells, they were called **salivary gland chromosomes** (Fig. 13.13). The present name **polytene chromosomes** was suggested by **Kollar** due to the occurrence of many chromonemata (DNA) in them.

Thus, some cells of the larvae of the dipteran insects such as flies (*e.g.*, *Drosophila*), mosquitoes and midges

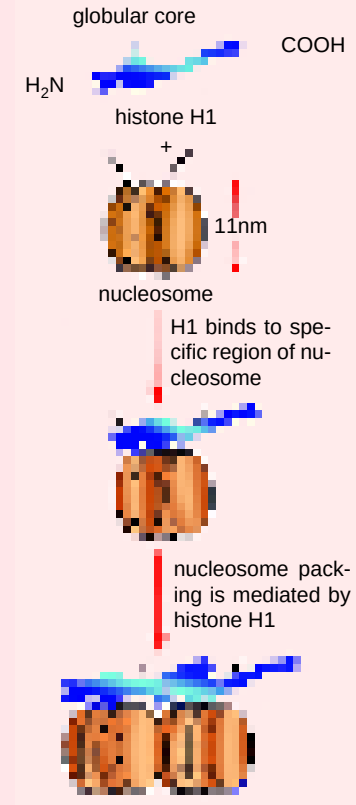


Fig. 13.10. The way histone H1 is thought to help to pack adjacent nucleosomes together (after Alberts *et al.*, 1989).

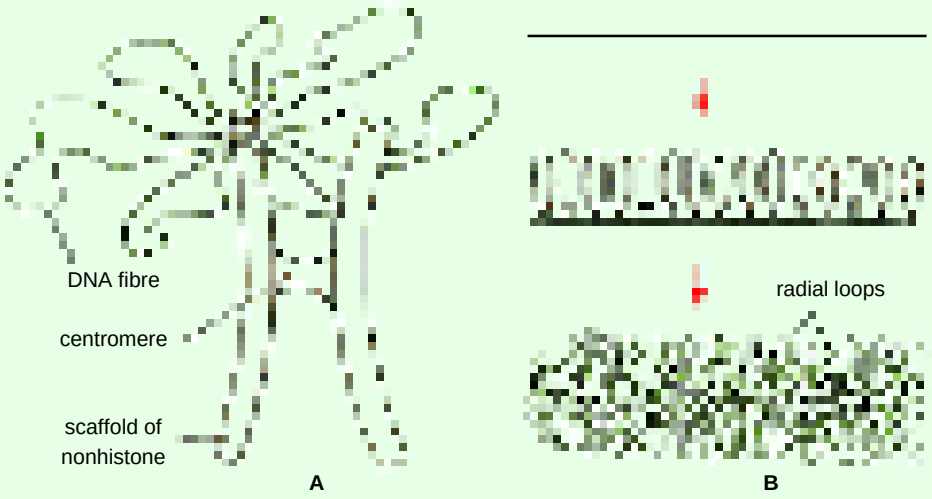


Fig. 13.11. Two methods by which DNA loops may form a metaphase chromosome. A—The non-histone proteins form two scaffolds, one per chromatid, while the naked DNA fibres form a halo around it; B— Laemmli's radial loop model of chromosome structure showing how 10-nm fibre form 30- nm fibre which further folds into radial loops (after De Robertis and De Robertis, Jr., 1987).

(*Chironomus*) become very large having high DNA content. These cells are unable to undergo mitosis and are destined to die during metamorphosis (Those cells of larva which are destined to produce the adult structures after metamorphosis, *i.e.*, imaginal discs remain diploid). Such polytenic cells are located most prominently in the salivary gland, but also occur in Malpighian tubules, rectum, gut, foot pads, fat bodies, ovarian nurse cells, etc. Polyteny of giant chromosomes is achieved by replication of the chromosomal DNA several times without nuclear division (endomitosis); and the resulting daughter chromatids do not separate but remain aligned side by side. In the process of endomitosis the nuclear envelope does not rupture and no spindle formation takes place. In fact, polyteny differs from polyploidy, in which there is also an excess DNA per nucleus, but in which the new chromosomes are separate from each other.

A giant chromosome of a midge (a small fly)

A polytene chromosome of *Drosophila* salivary gland has about 1000 DNA molecules which are arranged side by side and which arise from 10 rounds of DNA replication ($2^{10} = 1024$). Other dipteran species have more DNA, for example,*Chironomus* has 16000 DNA molecules in their each polytene chromosomes. Further, the polytene chromosomes are visible during interphase and prophase of mitosis. In them the chromomere (regions in which the chromatin is more tightly coiled) alternate with regions where the DNA fibres are folded more loosely (Fig. 13.15). The alignment of many chromomeres gives polytene chromosomes their characteristic morphology, in which a series of dark transverse **bands** alternates with clear zones called **interbands**. About 85 per cent of the DNA in polytene chromosomes is in bands and rest 15 per cent is in inter bands. The crossbanding pattern of each polytene chromosome is a constant characteristic within a species and helps in chromosome mapping during cytogenetic studies. For example, in *Drosophila melanogaster* there are about 5000 bands and 5000

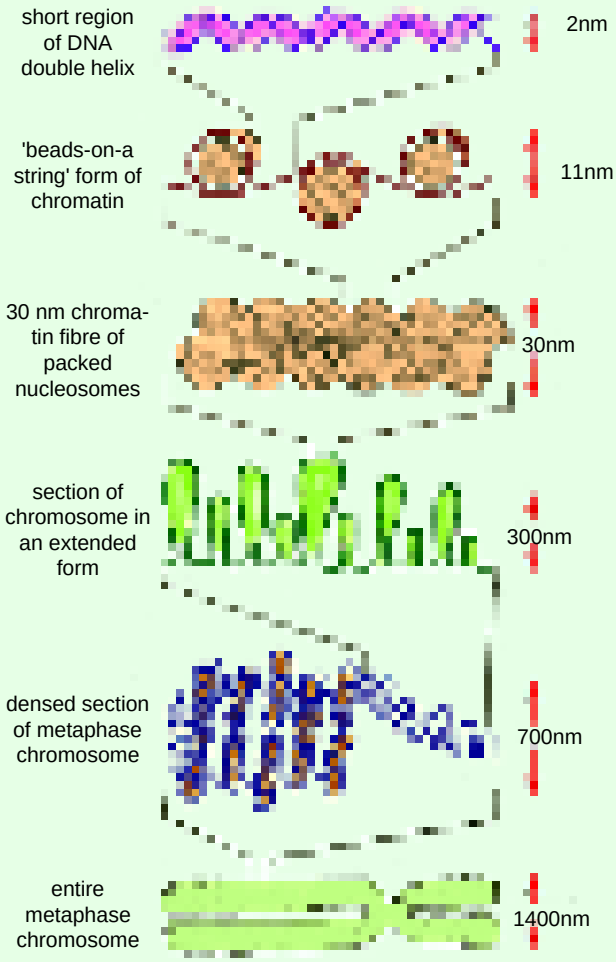


Fig. 13.12. Schematic diagram of some of many orders of chromatin packing which may give the highly condensed metaphase chromosome (after Alberts *et al.*, 1989).

interbands per genome and each band and interband represent a set of 1024 identical DNA sequences arranged in file.

Another peculiar characteristic of the polytene chromosomes is that the maternal and paternal homologous chromosomes remain associated side by side. This phenomenon is called **somatic pairing**. Consequently in the salivary gland cells the chromosome number always appear to be half of the normal somatic cells, *e.g.*, *D. melogaster*, has only 4 polytene chromosomes. In *Drosophila*, pericentromeric heterochromatin of all polytene chromosomes also coalesces in a **chromocentre**.

The preparation of a slide of the polytene chromosomes of dipterans for light microscopy is rather easy. The larvae are taken at the third instar stage and the salivary glands are dissected out and squashed in aceto-carmine. In such preparations, these chromosomes in aggregate reach a length of as much as 2000 µm in *D. melanogaster*. In female *Drosophila*, the polytene chromosomes are found in the form of five long and one short strands radiating from a single more or less amorphous chromocentre (Fig. 13.15). One long strand corresponds

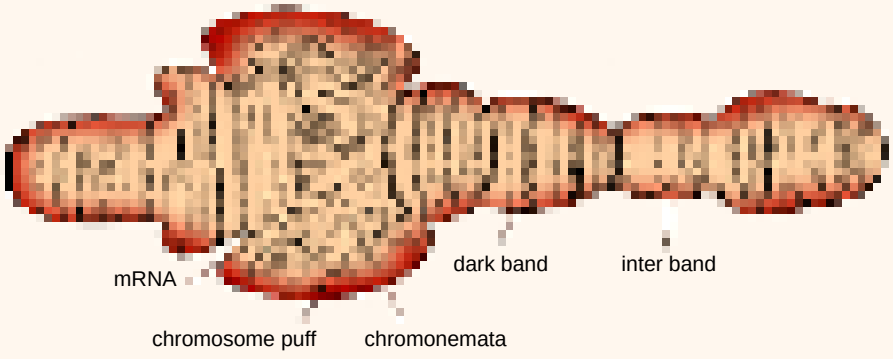


Fig. 13.13. Polytene chromosome of an insect, showing bands and interbands and a puff or Balbiani ring.

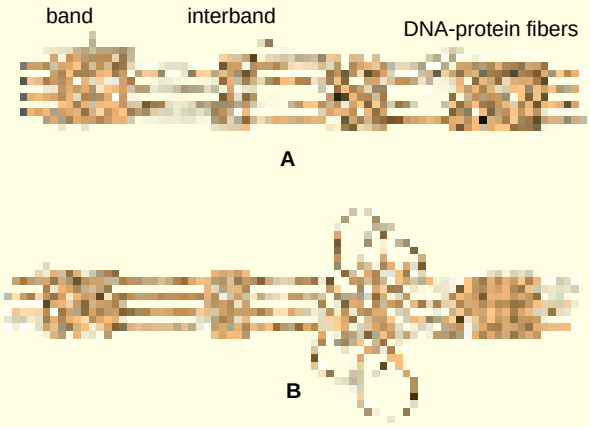
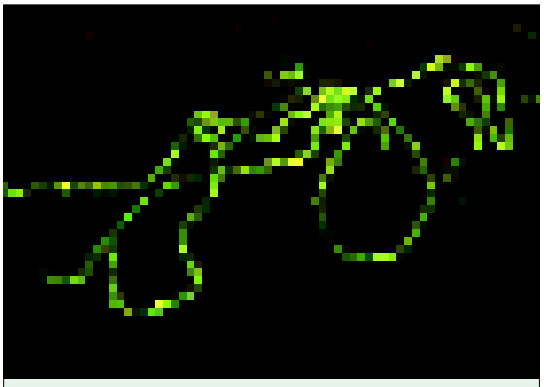


Fig. 13.14. A— Model of Dupraw and Rae showing band and interband structure in giant chromosome. B— The same model showing a possible method of "puffing" in one of the bands.

to the X chromosome and remaining four long strands are the left and right arms of II and III chromosomes. The shortest strand represents the small dot-like IV chromosome. Each of these chromosomes contains maternal and paternal homologues in somatic pairing which lacks in the sex chromosomes of male fruit flies. Thus, in male *Drosophila*, X chromosome remains single and thin and Y chromosome exists indistinctly fused with the chromocentre.

One-gene, one-band hypothesis. The fixed pattern of bands and interbands in a *Drosophila* polytene chromosome suggested the early cytologists such as **Painter** (1933) and **Bridges** (1936) that each band might correspond to a single gene. Accordingly, they concluded that *Drosophila* might contain only 5000 essential genes. It was also believed that bands were the sites of genes (DNA) and interbands were relatively inert linker regions. Recent data, however, have contradicted this simple "one-band, one-gene" hypothesis, now it is held that bands as well as inter-bands contain active genes and a band may even contain more than one genes.



In the giant chromosomes of the *Drosophila* active genes appear as brighter bands.

At this juncture a question arises, why is the single long strand of chromatin in each chromosome subdivided into so many distinct regions ? Exact explanation of this question is still not known. However, **Alberts** *et al.*, (1989) believed that this type of organization (*i.e.*, banding and interbanding of chromosomes in general) may help to : (1) keep the DNA organized; (2) isolate genes from their neighbours and thereby prevent biological "crosstalk", or (3) regulate gene transcription for the cytodifferentiation, for example, constitutively expressed "**housekeeping**" **genes** could be located in interbands, whereas **cell-type-specific genes** could be confined to the bands.

Chromosome puffs or Balbiani rings. Chromosome puffs or **Balbiani rings** are the swellings of bands of the polytene chromosomes (Fig. 13.16) where DNA unfolds into open loops as a consequence of intense gene transcription (*e.g.*, mRNA formation). In 1954, **W. Beerman** compared the polytene chromosomes of different tissues of *Chironomus* larvae and showed that although the pattern of bands and interbands was similar in all tissues, the distribution of puffs differred from one tissue to another. **Beerman** and **Bahr** (1954) have studied the fine structure of these puffs. According to them these puffs represent regions where the tightly coiled chromosomal fibres open out to form many loops. In fact, puffing is a cyclic and reversible phenomenon : at definite times and in different tissues of the larvae, puffs may appear, grow and disappear. In salivary glands the appearance of some puffs has been correlated with the production of specific proteins which are secreted in large amounts in the larval saliva (**Grossbach**, 1977). The process of puffing involves several processes such as the accumulation of acidic proteins, despiralization of DNA, accumulation of **RNA polymerase II** (an

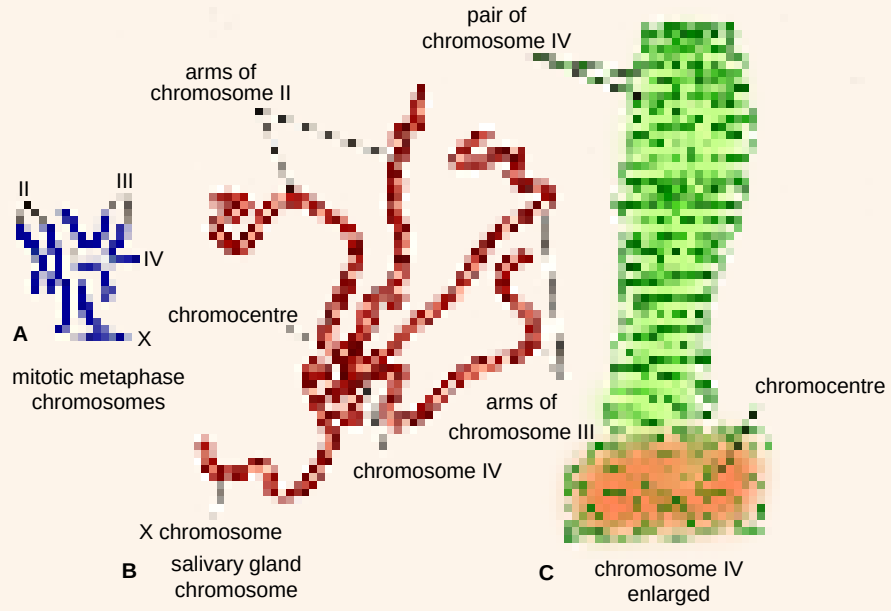


Fig. 13.15. Normal and polytene chromosomes of *Drosophila melanogaster*. A—Normal mitotic chromosomes; B—Polytene chromosomes of female; C—An enlarged IV chromosome.

enzyme involved in the transcription of m RNA molecules), synthesis of mRNA and release of newly synthesized mRNA in the cytoplasm.

2. Lampbrush Chromosomes

The lampbrush chromosomes were first observed in salamander (amphibian) oocytes in 1882. He coined the name because the chromosomes look like the brushes which were used for cleaning the glass chimneys of old-fashioned paraffin or kerosene lamps. They were described in detail in shark oocytes by **R. Ruckert** in 1892. **Thorpe** (1984) and **Burns** and **Bottino** (1989) preferred the term **test tube brush chromosomes** for them. However, due to recent investigations of **Gall** and coworkers (1962, 1983) the structure of these exceptionally large-sized chromosomes has been interpreted in

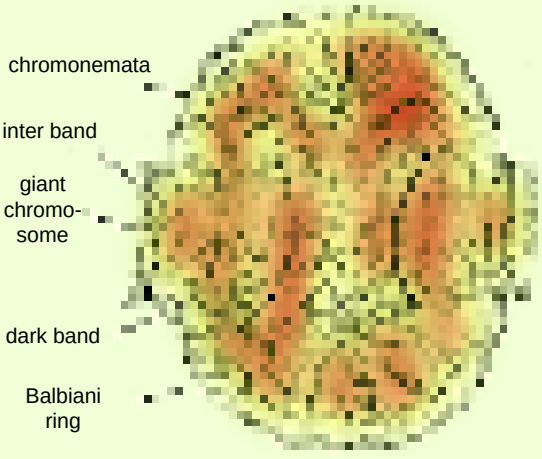


Fig. 13.16. A Balbiani ring of a polytene chromosome.

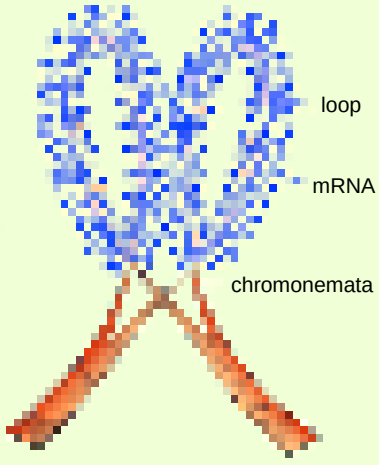


Fig. 13.17. Suggested form of the individual loops in chromosome puffs. Note the tiny fibrils of RNA and proteins attached to the loop.

functional terms, *i.e.*, now they are merely visualized as means of "*turning on and turning off*" of the genes.

The lampbrush chromosomes occur at the diplotene stage of meiotic prophase in the primary oocytes of all animal species, both vertebrates and invertebrates. Thus, they have been described in *Sagitta* (Chaetognatha), *Sepia* (Mollusca), *Echinaster* (Echinodermata) and in seveal species of insects, shark, amphibians, reptiles, birds and mammals (humans). Lampbrush chromosomes are also found in spermatocytes of several species, giant nucleus of *Acetabularia* and even in plants (**Grun**, 1958). Generally, they are smaller and "hairy" in invertebrates than in vertebrates. Lamphrush chromosomes are best visualized in salamander oocytes because they have a high DNA content. For example, the largest chromosome having a length up to 1 mm have been observed in urodele amphibian. Thus, lampbrush chromosomes are much larger (longer) than the polytene chromosomes of insects.

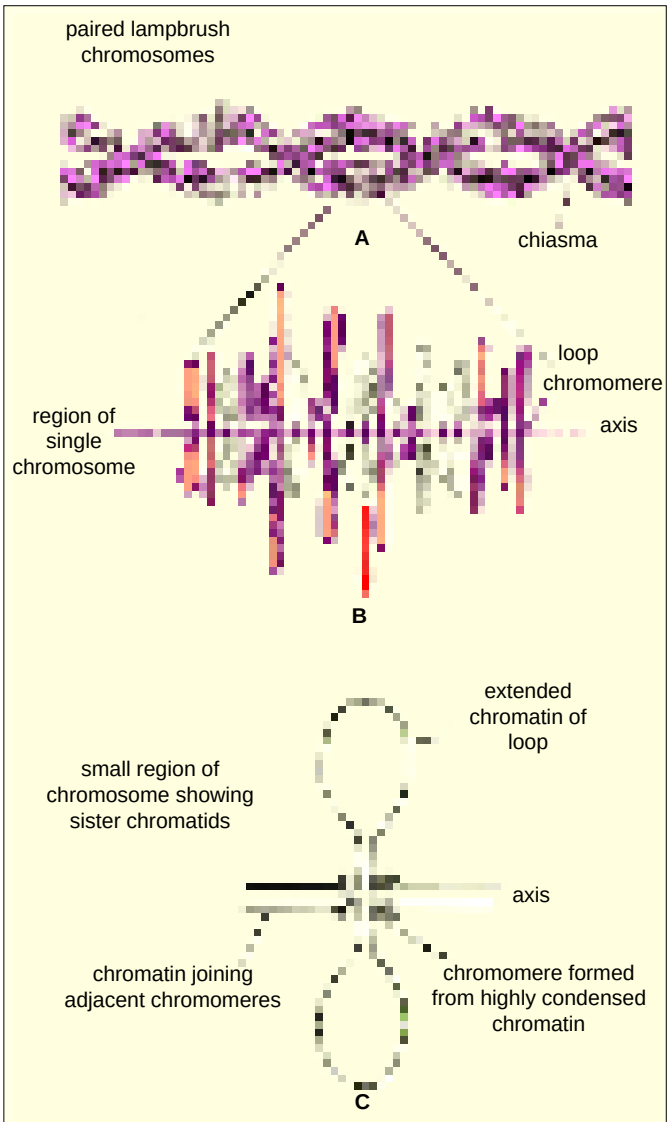


Fig. 13.18. Lampbrush chromosome structure. A—Bivalent or paired homologous chromosomes in pairing showing chiasmata, B—A part of one homologue showing paired loops given out by two chromatids; C—Single pair of loop (after Alberts *et al.*, 1989).

Since the lampbrush chromosomes are found in the prolonged diplotene stage of meiotic prophase I, they are present in the form of **bivalents** in which the maternal and paternal chromosomes are held together by **chiasmata**, at those sites where crossing over has previously occurred (Fig. 13.20). The paired homologues are not condensed as usual chromosomes would be; instead, they are very long and stretched out. Each bivalent has four chromatids, two in each homologue. The axis of each homologue consists of a row of granules or **chromomeres** from which **lateral loops** extend. The loops are always symmetrical, each chromosome having two of them, one for each chromatid. The loops can be categorized by size, thickness and other morphological characteristics. Each loop appears at a constant position in the chromosome ; this fact helps in the chromosome mapping. There are about 10,000 loops per chromosome set or haploid set (*e.g.*, oocytes of slamander *Triturus* ; see **Grant**, 1978). Each loop has an **axis** which is made of single DNA molecule that is unfolded from the chromosome for the intense RNA synthesis. Thus, about 5 to 10 per cent of the DNA exists in the lateral loops, the

rest being tightly condensed in the chromomeres which are transcriptionally inactive. The centromeres of the chromosomes bear no loops.

Each loop of lampbrush chromosomes is found to perform intense transcription of **hn RNA** or **heterogeneous RNA** molecules. (*i.e.*, precursors of mRNA molecules for various ribosomal proteins and for five types of histone proteins). Electron microscopy of the loops has shown that **RNA polymerase** enzyme molecules are attached to the principal axis (DNA) of the loop from which RNA fibrils of increasing length extend. As transcription continues along the DNA strand of loop, the fibrils of RNA (*i.e.*, hnRNA) lengthen. Proteins get associated with these RNA fibrils as they are formed and ultimately ribonucleoprotein product is released.

Thus, each lateral loop is covered by a **matrix** (Fig. 13.19) that consists of RNA transcripts with hn RNA-binding proteins attached to them. Generally this matrix is asymmetrical, being thicker at one end of the loop than at the other. RNA synthesis starts at the thinner end and progresses toward the thicker end. Preparations spread for electron microscopy exhibit the typical '**christmas tree**" images with nascent ribonucleoprotein chains attached perpendicularly to the DNA axis. Many of the loops correspond to a single transcriptional unit (or single gene) and they are transcribed continuously from end to end; they form a continuous thin-thick matrix. However, other loops contain several units of transcription (or many genes); such loops include an extended section of chromatin that is not transcribed at all. For example, **Gall** *et al.*, (1981) found that in the American newt *Notophthalmus viridescens*, clusters of the five histone genes are tandemly repeated in the genome but separated by about 50,000 base pairs of repeats of a 225-base pair satellite DNA.

Further, the number of pairs of loops gradually increases during meiosis till it reaches maximum in diplotene. Such a lampbrush stage may persist for months or years as the primary oocyte builds up a supply of mRNA molecules and other materials required for its ultimate development into a new individual. As meiosis proceeds further, number of loops gradually decreases and the loops ultimately disappear either due to disintegration or by reabsorption back into the chromosome. For example, the addition of histone proteins to the lampbrush chromosomes stops the syntheisis of RNA on the loops and causes the loops to retract into the chromosomes (see **Ambrose** and **Easty**, 1979).

Certain hypotheses regarding nature of loops. The loop of the lampbrush chromosomes can be viewed in the following two ways :

1. It may be static, unchanging in length and constantly exposing the same stretch of chromosome fibre.

2. It may be dynamic, with new loop material spinning out of one side of a chromomere and returning to a condensed state on the other side. This is called **spinning out and retraction hypothesis** (**Gall** and **Callan**, 1962). It means that 100 per cent of the genome can be expressed during the lampbrush stage.

Recent, DNA-RNA hybridization experiments have rejected the spinning out and retraction hypothesis (see **Grant**, 1978).

Master and slave hypothesis. **Callan** and **Llyod** (1960) suggested that each loop pair and thus each chromomere is associated with the activity of one specific gene. Their master and slave hypothesis was postulated to explain the large size of the chromomere and of lampbrush loop; presently this hypothesis has become obsolete, but still holds interest. This hypothesis postulates that each loop consists not of one gene, but of a

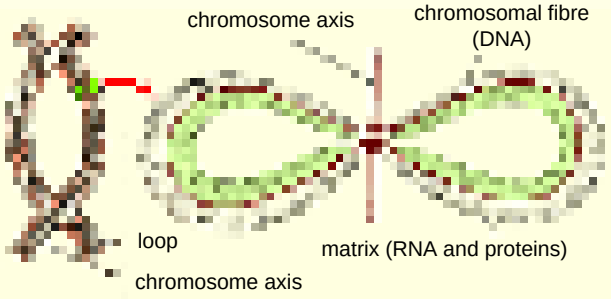


Fig. 13.19. Lampbrush chromosome. A—At low magnification; B—Loop magnified.

number of duplicate copies, linearly arranged, of one gene. There is a "**master**" **copy** at each chromomere and information is transferred from this to each of the "**slave**" **copies** which are matched against it to ensure that they are all identical to the master. The master copy of the gene does not take part in RNA synthesis, but the slave copies of the gene existing in the loop have a role in transcription. The advantage of having a number of duplicate copies of a gene is that a higher rate of RNA synthesis is possible.

Study of both polytene and lampbrush chromosomes provided the evidence that eukaryotic gene activity is regulated at the level of RNA synthesis (or transcription). Lampbrush chromosomes also show the possible way of gene amplification which is required during the growth phase of oocytes.

REVISION QUESTIONS

1. Give an account of the morphology, ultrastructure and chemistry of the chromosomes.
2. Distinguish between the members of each pair :
   (i) diploid-haploid; (ii) chromatid-chromosome; and (iii) euchromatin-heterochromatin.
3. Describe the basic structure of chromatin as we understand it. What is the role of histones in this structure ?
4. Describe the structue of the prokaryotic chromosome.
5. Write short notes on the following :chromosomal proteins ; nucleosome ; polytene chromosome; and lampbrush chromosomes.
6. Why the study of the chromosomes has become very significant in the field of biology ?
7. Write short account on the following : (i) Nucleosome concept and solenoid model ; (ii) Karyo-type ; (iii) Kinetochore ; (iv) Heterochromatin ; (v) C-value and C-value paradox; (vi) Salivary gland