Chromatin is a Dynamic Structure*

Rajiva Raman

Eukaryotic cells carry a vast amount of DNA packaged in their nucleus as chromatin. During cell division, it further condenses into individual Chromosomes. The basic unit of chromatin is nucleosome which comprises 200 bp of DNA wrapped around an octamer of positively charged histone proteins (2 molecules each H2A, H2B, H3, and H4), and one molecule of Histone H1. The octamer forms a cylinder around which DNA is wrapped (~146 bp/octamer) and continues to the similar adjacent unit (~60 bp of linker DNA), forming a string of nucleosomes. The H1 histone brings nucleosomes closer by binding with the linker DNA. These 10–11 nm thick threads further condense into 30 nm fibres, achieving a higher level of compaction by binding with nonhistone chromosomal proteins (e.g., topoisomerase II, condensins and cohesins) to form a scaffold through which the 30 nm fibre passes, forming loops. The average size of the loops is 600 nm, each accommodating approximately 60 kb of DNA. Whereas 85% of DNA is distributed in the loops, about 15% is associated with the scaffolds. The chromatin which is condensed even at interphase is 'heterochromatin'. Constitutive heterochromatin comprises tandem repeats of short DNA sequences and is generally devoid of genes, transcription and recombination. A potentially active part of the genome forms the 'euchromatin', which also has a differential distribution of DNA that shows up as mutually exclusive G- and R-bands rich in tissue-specific and housekeeping genes, highlighting a highly organised compaction of DNA in the chromatin. Compared to these bands, >100 kb long 'topologically associating domains' (TADs) have been identified at the genome level as the

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functional unit of chromatin. For its differential functional states, chromatin constantly undergoes condensation and decondensation, mediated by the post-translational modification of histones (e.g. acetylation, methylation of lysine) and the displacement of nucleosomes by an ATP-dependent complex of proteins (e.g. SWI/SNF). DNA methylation is another mode of chromatin modification that affects chromatin function. Thus, a highly dynamic system of chromatin organisation not only encapsulates a large amount of DNA in the nucleus but ensures differential gene function in an orderly fashion.

Chromatin and Chromosome: A History

When we see cells under a microscope, the most common site is that of cells having nuclei homogeneously stained through their entire area. Sometimes, we see cells with no nucleus. Instead, they show thread-like entities either on the equator or projecting towards respective poles. The cells with uniformly stained nuclei are interphase cells having chromatin in the nucleus (*Figure* 1a), while the latter are the dividing cells with chromosomes (*Figure* 1b). This property of the chromatin was discovered by Flemming in 1882, who showed that the stainable thread-like constituents visible during cell division undergo a periodic condensation-decondensation cycle. He named the decondensed diffused mass in the nucleus as chromatin, which later condenses into a discrete entity during the cell division. This enables the ordered distribution of chromatin into daughter cells, following which they unravel again into a thread-like structure, and this cycle continues through the life cycle of a cell. The term chromosome was coined by Waldeyer in 1888. At metaphase during cell division, each chromosome has two threads; each is a copy of the other and is called 'chromatid'. The chromatids tend to join at a place called the 'centromere', through which the chromosome is segregated at anaphase of cell division to the daughter cells as single chromatids. Following the discovery of Mendel's laws of inheritance in 1900, Wilson and Sutton, in 1902, proposed that the chro-

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Figure 1. **(a)** Shows an interphase nucleus with chromatin spread in it. The arrow points to the heterochromatin body seen at the nuclear periphery. **(b)** Chromatin compacting into distinct chromosomes during cell division. (Source: Cytogenetics Laboratory, Zoology, BHU)

mosome is the carrier of the hereditary material. Though it was nearly half a century later that deoxyribonucleic acid (DNA) was established as the hereditary (genetic) material, the presence of an acid (DNA) in the nucleus was already shown by Swiss scientist Friederick Miescher in 1871. This nitrogen and phosphorus-rich acid in the nucleus, called 'nuclein' by him, was isolated as a complex with proteins. The major proteins present in the nucleus bound to the nuclein were extracted by Albrecht Kossel in 1884 and were termed 'histones'. Thus, much before being identified as the carriers of hereditary factors, the chromatin/chromosome was known to comprise a nucleic acid and proteins, the nature and significance of which were unknown.

J H Taylor and his colleagues in 1957 showed that chromosomes comprise a single molecule of DNA throughout its length while demonstrating semi-conservative replication of DNA. However, caught in the then prevailing controversy about whether there was one DNA molecule or more in the chromosome, they did not expressly state so. Later, more sophisticated techniques (visco-elasticity, pulsed-field gel electrophoresis) confirmed that there is only a single DNA molecule in a chromatid. With the whole

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genome sequences available from numerous organisms, it turns out that a eukaryotic cell has a massive amount of DNA; a haploid human cell, for example, has 3300 million base pairs (3300 Mb). In a diploid cell, the cumulative length of DNA will add up to approximately two meters! How is so much DNA accommodated in a 40–50 μm nuclear space? This dilemma intuitively reveals the importance of histone proteins in the nucleus and their association with the DNA to form chromatin. As we will see later, this binding of DNA with the histones enables orderly compaction of the massive volume of DNA into chromatin housed in the nucleus.

Histones

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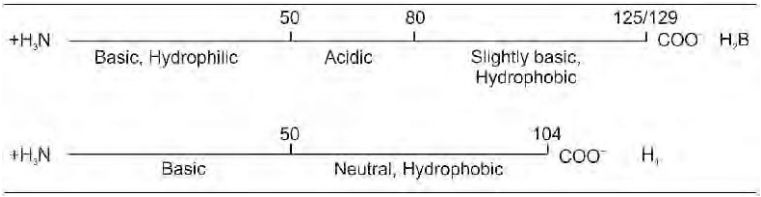

Figure 2. A schematic illustration of the distribution of amino acids in histones. Notice that the first 50 residues are mostly basic and hydrophilic (bind with DNA), and those towards the carboxy-terminal are neutral, hydrophobic, acidic or low on basic amino acids, which enables their binding with histones and other proteins.

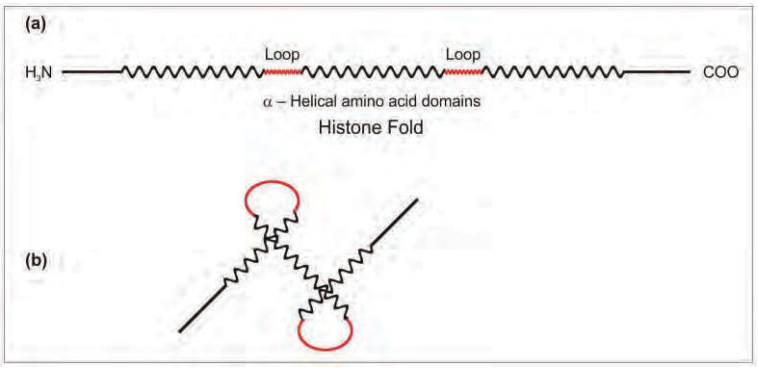

Figure 3. Histone fold. All the histones have a conserved pattern of organisation with **(a)** 3 blocks of amino acids in α-helical configuration joined with two short loops which enable it to fold **(b)** on itself and interact with other histones.

as among themselves and other chromatin-binding proteins (*Figure* 2). Though all the histones can bind with each other, there is a preferential attraction between H3 and H4 and between H2A and H2B. As a result, these histones occur almost always as dimers. However, H1 remains a monomer. The structural conformation of the amino acids is also highly conserved. The 'histone fold', as these formations are called, comprises three α helical stretches (the polypeptide takes helical shape) joined by two small 'loops'. This pattern is at the core of all the histones with slight changes in the sequences beyond this fold (*Figure* 3a). This conserved structure helps these proteins to fold on themselves and bind with other histones, which is an important aspect of chromatin organization (*Figure* 3b).

Organization of DNA-Histone in Chromatin

As earlier mentioned, each nucleus houses a massive amount of DNA whose successful accommodation in a small nuclear space

is facilitated by its binding with histones. How they bind and accommodate in the nucleus has been a question of abiding interest. Calculations have shown that the molecular mass of around 100 bp long DNA was equal to the combined mass of one molecule of each histone. For long, it was believed that histones wrapped around DNA. In 1969, a scientist couple Olins and Olins visualized chromatin under the electron microscope and, unlike previous observations, reported a "beads on string" orientation in which the thin chromatin fibre had a globular knot ("bead")-like structure at even intervals. A much clearer picture of these was published by them in 1974.

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Interestingly, 1974 is a landmark year in the history of chromatin structure, as three different methodologies – electron microscopy (by Olins and Olins), X-ray crystallography (Jacob and Kornberg) and DNA gel electrophoresis of the nuclease-treated chromatin (Noll) – provided critical pieces of evidence that elegantly complemented each other in unravelling the basic unit of chromatin structure. X-ray pictures of the chromatin led to the resolution of a cylindrical structure of around 11 nm width and 6 nm height that occurred repeatedly through the length of the chromatin. The cylinder comprised an octamer of 4 histones (2 molecules each of H2A, H2B, H3 and H4). It was further clarified that whereas H2A and H2B occurred as two dimers, H3 and H4 made a funnel-shaped tetramer into which the two dimers of H2A and H2B fitted from two sides, making it a solid cylinder. In conformity with the earlier calculations, it was clear that around 200 bp of DNA could wrap this octamer with nearly two rounds. Considering that a single polymer of DNA went around successive octamers, the DNA that ran between the two cylinders was the linker DNA. It was shown that a single molecule of H1 bound with the linker DNA and the adjacent cylinders to bring them closer to each other (*Figure* 4a). Noll treated chromatin with a nuclease (the enzyme that cuts DNA). He extracted DNA from it and resolved it by gel electrophoresis. He found DNA fragments of 200 bp or its multiples (400, 600, 800 bp, etc.) rather than of random sizes. This simple experiment explained that in the chromatin, DNA was exposed to

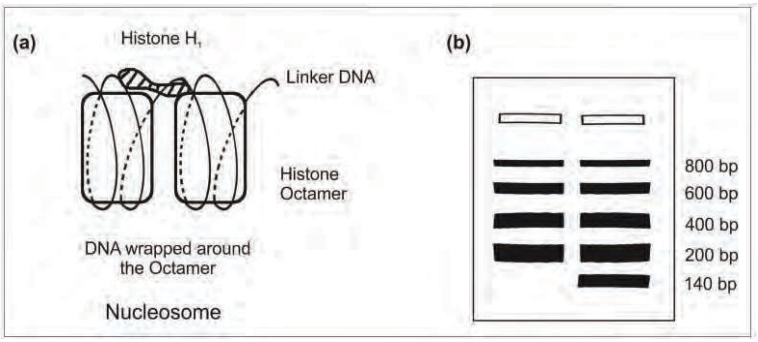

Figure 4. Nucleosome. **(a)** ~200 bp DNA is wrapped around a cylindrical structure made up of an octamer of histones [(H2A–H2B)2 and (H3–H4)2], of which approximately 146 bp remain attached to the octamer and 60 bp form the linker DNA between the two adjacent octamers. H1 joins the two nucleosomes and protects the linker DNA. **(b)** A diagrammatic representation of an electrophoresis gel depicting Noll's experiment on resolution of DNA fragments following chromatin digestion with micrococcal nuclease (MNase). Lane 1 shows DNA bands of 200 bp or its multiples. In lane 2, histone H1 was removed from the chromatin and then digested with MNase, leading to the release of an additional fragment of 146 bp.

the nuclease only at intervals of 200 bp as this length of DNA was bound with histones, leaving only the linker DNA unprotected for the nuclease activity.

Following up, Noll removed H1 histone prior to nuclease digestion of the chromatin. He demonstrated that besides the 200 bp (or its multiples) fragments, there were repeats of around 146–160 bp, confirming that the linker DNA was around 40–60 bp long and was attached with H1 histone, and that around one and a half turn of DNA wrapped around the histone octamer (*Figure* 4b). Convergence of these incisive experiments enabled Kornberg [1] to propose that the basic unit of chromatin comprises two molecules each of histones H3, H4, H2A and H2B, one molecule of H1 and 200 base pairs of DNA which wraps around it, forming a cylinder of 11 nm width, and named it, the 'nucleosome'. What is instantly clear is that length-wise, nearly 60 nm DNA (200 bp) is compressed in an 11 nm entity. Subsequent studies clarified that DNA being a solid helix, does not tightly attach to the histone octamer; rather, it attaches through the minor groove of its B-conformation, thus attaching with histone after every 10 bp interval. Similarly, histones also have an unattached region with DNA that can project out and undergo certain modifications.

Histone Chaperons Mediate DNA-Histone Binding

As expected, during replication, DNA serially dissociates from histones to become available to the replicative machinery, and

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soon after replication, it binds with histones again to form the chromatin. CAF-1 was the first protein identified that channelised the DNA-histone binding during replication. A long list of proteins has been identified that mediate DNA-histone association following replication and independent of it, e.g., for transcription, DNA repair or chromatin remodelling. This group of proteins is collectively termed 'histone chaperons'. Chaperons such as Asf1 and Nap1 mediate cytoplasm to nuclear transfer of histones. While Asf1 transfers (H3–H4)2 into the nucleus, CAF1 deposits them on the DNA. Nap1, with the help of the importin KAP114, brings H2A–H2B into the nucleus. CAF-1 is majorly involved in associating H3–H4 during replication by distinguishing the new and old molecules. However, the replication-independent deposition of H3–H4 dimers is generally a function of various post-translational histone modifications catalysed by other chaperons. Apparently, the process of H2A–H2B transfer onto DNA is similar in replication and other events [2].

30 nm Chromatin Fibre

If one looks at the chromosome at prophase or metaphase, each chromatid is ~500 nm thick, and many ultra-structural studies have elucidated its coiled-coiled structure. Obviously, the nucleosome must compress by several more orders to be seen as 500 nm chromatid at metaphase. The most stable picture of chromatin at interphase is that of a fibre with a width of 30 nm. There has been much discussion on how nucleosomes align to compress into the 30 nm fibre. The first model was that of a solenoid, proposed by Aaron Klug, which envisaged the alignment of 6 nucleosomes in a cylindrical order brought in proximity by the H1, which is on the outside between two nucleosomes (*Figure* 5a). This cylinder will occupy 1200 bp (200 bp $\times$ 6) DNA in 30 nm width. However, different high-resolution electron-microscopy, X-ray crystal, and computer simulation studies have suggested various conformations, such as zig-zag and other orientations (*Figure* 5b). There is also evidence that neighbouring nucleosomes interdigitate and achieve 30 nm width. Recent studies support that a large segment

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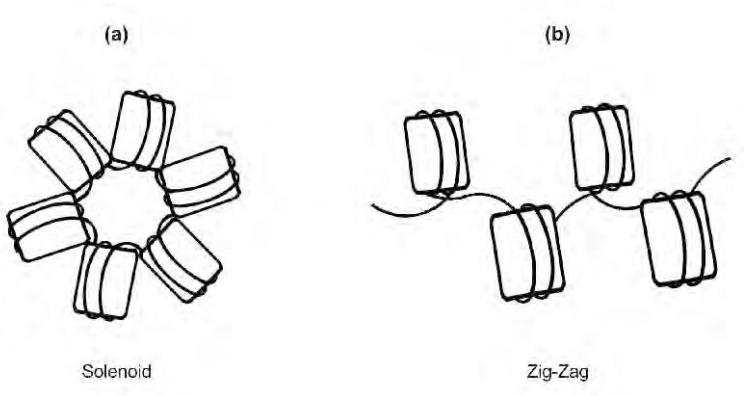

Figure 5. 30 nm chromatin fibre. Neighbouring nucleosomes further compact into a 30 nm fibre by taking different conformations such as **(a)** a solenoid or **(b)** a zig-zag. Other possible conformations are not shown here. Their coming together is helped by the H1 monomers (not shown here) that interact between the nucleosomes.

of chromatin may have alternating patterns of organization for the 30 nm chromatin fibre. Additional studies on chromatin of individual nuclei in situ by combining super-resolution microscopy with computer simulations have demonstrated that instead of a compact 30 nm fibre, nucleosomes are arranged in nanoclusters of a variable number of nucleosomes (nucleosome clusters), leaving also nucleosome-free spaces between the clusters. More recently, using the same technology, Maria Cosma and her collaborators have demonstrated the transcription process on these clusters, showing that the smaller the cluster, the more efficient the transcription process [3]. However, irrespective of the primary alignment of nucleosomes, further compaction is necessary to compress DNA into chromatin as it progresses into becoming a chromosome during cell division.

Chromosomal Scaffold and Nuclear Matrix

In order to test the organization of the histone-depleted chromosomes, Paulson and Laemmli [4] treated an enriched pool of metaphase chromosomes with dextran sulphate or 2M sodium chloride to remove histones. Observing under the electron microscope, they found a clear skeletal network of proteins 'core' surrounded by a dense 'cloud' of DNA (see the figures in [4]). A similar pattern was obtained in the interphase chromatin. They reported that the chromosomal 'core' is made up of non-histone

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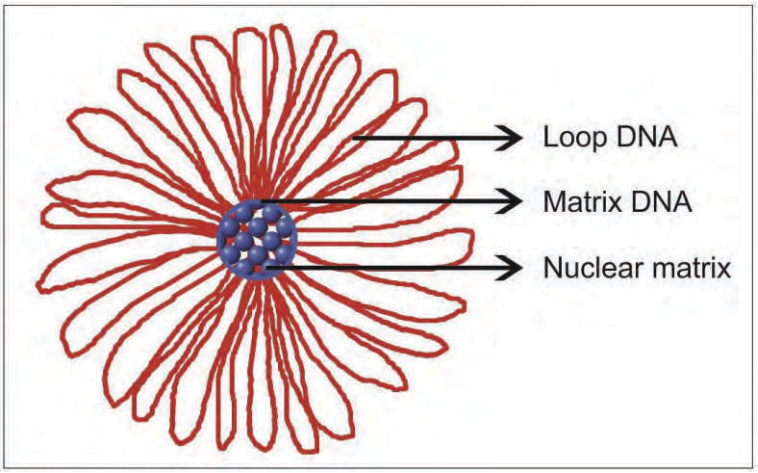

Figure 6. A diagrammatic representation of histone depleted nuclei showing a large volume of DNA in the loop domain passing through the matrix (comparable to the scaffold at metaphase). (Source: Cytogenetics Laboratory, Zoology, BHU)

chromosomal proteins (NHCP) through which DNA passes, forming loops of one continuous double helix. Laemmli called the core the 'scaffold' anchoring 30 nm chromatin fibre. The DNA that projects out of the scaffold forms the looped domain, and that embedded within the core is the 'scaffold attached region' (SAR). Though dynamic and variable in size, an average loop is around 200–300 nm long, comprising ~60 kb DNA/loop. In interphase, the protein complex is named the 'nuclear matrix' (around 70% of the proteins between the matrix and the scaffold are common), and the DNA embedded in the matrix is called MAR (matrix associated region) (*Figure* 6). On average, ~85% DNA is in the looped domain and 15% in the SAR/MAR, which is predominantly 'AT' nucleotide rich. It has been computed that a cylindrical structure formed of the layers of 200–300 nm loops of the 30 nm chromatin fibre would achieve a width of around 500 nm and gather much more DNA in a comparatively smaller space. A rough calculation envisages that the occurrence of 18–20 loops in one layer of the cylinder, 'mini band', would mean 12 million base pairs (12 Mb) of DNA per layer. Though not an accurate estimation of the DNA per layer of loops, it does suggest how a large amount of DNA undergoes structured compaction with the help of proteins to form the chromatin and then highly compacted chromosomes.

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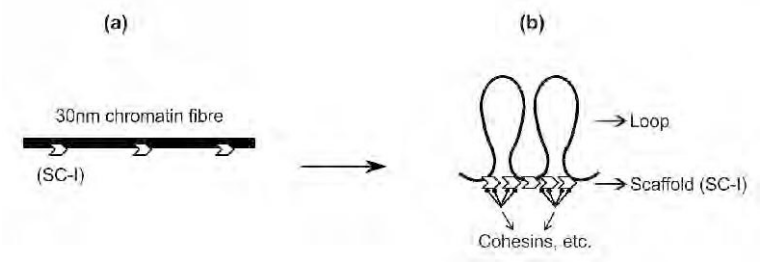

Figure 7. Dynamics of chromatin condensation into chromosome: Binding of the intermittently distributed SCI proteins (Topo-II family protein) on the 30 nm chromatin fibre initiates higher-order compaction through the loop domain. Spring-like condensins and cohesins attach with the chromatin and help it condense further to become chromosomes.

Unlike the histone core, the scaffold is composed of various proteins. The most abundant of them, SC1, is an enzyme from the topoisomerase II family. As the name suggests, Topo-II changes the topology of the DNA by affecting its coiling. In general, DNA occurs as a supercoiled structure in the nucleus. This enzyme acts by cleaving either one strand (Topo-I) or both strands (Topo-II) of a DNA molecule and pulling the other strand through it, adding or releasing DNA of its supercoils. It plays an important role during DNA replication. SC1 is a permanent part of the chromosomal core spread throughout the length of the chromosome. Condensins and cohesins are another group of spring-like proteins that abounds chromatin through interphase and metaphase. The latter plays a crucial role in keeping the two chromatids together by attaching the axes of both the chromatids. Condensins, on the other hand, bind not only with the axis of the chromatid but also with the surface on the outer side of the chromatid to bring the loops closer to each other. These spring-like proteins are a dimer of two alpha-helical polypeptides bound to a globular unit at their amino- and carboxy termini which imparts them necessary flexibility (*Figure* 7).

Condensins and cohesins are group of spring-like proteins that abounds chromatin through interphase and metaphase. Condensins, bind with the axis and surface on the outer side of the chromatids to bring the loops closer to each other, while cohesins keeps the two chromatids together by attaching the axes of both the chromatids.

Organization of Chromatin into Metaphase Chromosome

How these differently folded domains of chromatin get organized into what is seen as a metaphase chromosome remains a matter of much discussion. Certain simple chromosome staining techniques reveal interesting features of the metaphase chromosomes. When treated with a mild trypsin solution or with hot phosphate buffer and stained with the Giemsa stain, chromosomes get dif-

Figure 8. G-Band. A metaphase plate treated with trypsin showing transverse bands along the length of the chromosomes. Compare it with the metaphase plate in *Figure* 1b, where chromosomes show no banding. These chromosomes are of the Indian barking deer, *Muntiacus muntjak*, which has the lowest number of chromosomes for a mammal (2n 7♂/6♀). (Source: Cytogenetics Laboratory, Zoology, BHU)

ferentially stained through their length, revealing chromosome-specific banding patterns called G- (trypsin-Giemsa) (*Figure* 8) and R- (Reverse) bands, respectively. Interestingly, an identical band pattern is revealed when chromosomes are stained with AT- (comparable to G-banding) or GC nucleotide (comparable to R-banding)-specific fluorochromes[1], confirming that these G-/R-bands are no artefacts but an integral part of chromosome organization.

[1] Reagents which shine up only when seen under a fluorescence microscope.

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In recent years, several high-resolution techniques, viz., X-ray crystallography, cryo-electron microscopy and chromatin conformation capture, especially the high-throughput version, i.e., Hi-C, have been developed, enabling the visualization of chromatin in a dynamic state and facilitating an in-depth study of the higher-order organization of chromatin/chromosome. The crystal studies of chromatin subscribe to the nucleosomal organization, and its higher-order alignment could take solenoid and/or zig-zag formation. The chains of nucleosomes may interdigitate within the spaces available between them. Recent cryo-electron microscopic

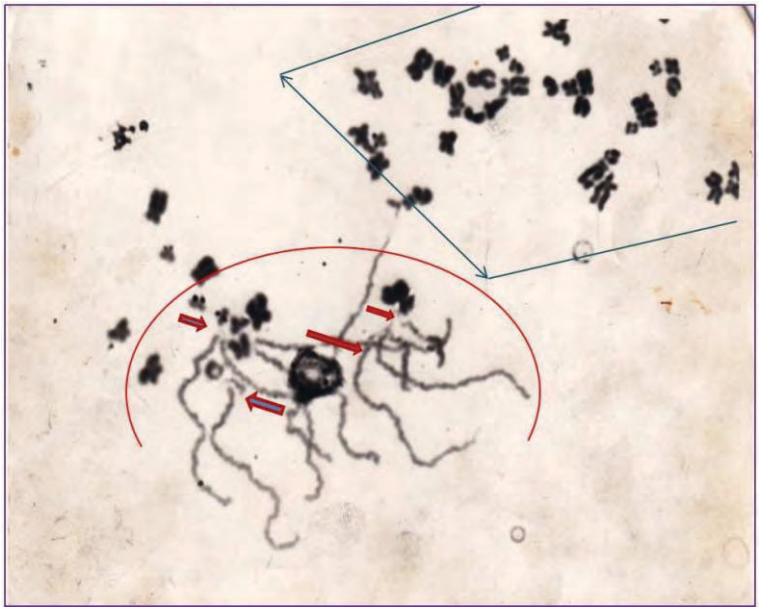

Figure 9. Rabl orientation of chromatin in interphase. A metaphase cell (see small condensed chromosomes; bracketed with straight blue lines), when fused with an interphase cell (bracketed with a curved red line, G1 stage), induces its chromatin into chromosomes. See chromatin of the G1 stage cell condense into thin, long, single chromatids. Arrows point at the centromeres of all the G1 chromosomes oriented poleward in one direction as at the anaphase stage of cell division. (Source: Cytogenetics Laboratory, Zoology, BHU)

studies have invoked an arrangement of the nucleosomes as sheet-like layers in the chromatin. While these structures are still debated, chromatin domains analogous to the chromosome bands have been resolved. The Hi-C technique has succeeded in capturing several kilobase (100 kb to 1 Mb) long stretches of DNA as topologically associating domains (TADs) with distinct boundaries that separate one TAD from another. These TADs generally comprise functionally related genes and, therefore, are likely to replicate and transcribe as a group. The significance of TADs in chromosomal folding can be understood from the fact that disruption of TAD structures can lead to gene misexpression resulting in developmental defects and cancer. It is reasonable to expect that the TADs occupy the looped domain of the chromatin while boundary elements reside in the scaffold core. One of the well-studied boundary elements is the CTCF domain which binds with the cohesins that comprise the nuclear matrix or scaffold in the chromosomes [6–8].

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Heterochromatin and Euchromatin

Presently, the term heterochromatin is also used for the chromatin that is temporarily condensed and transcriptionally inert in tissue- and cell stage-specific manner.

Though most of the chromatin in interphase nuclei is diffusely stained, in many cell types, patches of darkly stained chromatin, either close to the nuclear periphery or as clusters in different regions, are seen. Due to their differential staining, Heitz (1928) called these darkly–stained regions 'heterochromatin' and the rest of the chromatin 'euchromatin' (*Figure* 1). It turns out that the heterochromatin (i) remains condensed throughout a large part of the cell cycle, (ii) is largely devoid of genes and is transcriptionally inert, (iii) is structurally distinct as being composed of blocks of DNA sequences repeated in tandem, and (iv) binds with specific HP proteins (heterochromatin proteins) that lead to higher and prolonged condensation of chromatin. Generally, centromeres of most of the chromosomes are heterochromatin rich. Presently, the term heterochromatin is also used for the chromatin that is temporarily condensed and transcriptionally inert in tissue- and cell stage-specific manner. A loosely used term for this condition-specific heterochromatin is 'facultative heterochromatin' as against 'constitutive heterochromatin'[2] described above. Euchromatin, on the other hand, is characterized by the single-copy, gene-rich DNA sequences that are potentially active and produce gene products. Within euchromatin, there are also stretches of DNA which occur in multiple copies, but unlike the heterochromatic DNA, these repeats are smaller in size and are distributed all over the genome (chromosomes). Called by different names, we collectively call such low copy, interspersed repeat DNA as variable number of tandem repeats (VNTR) that may or may not be part of genes.

[2] Having distinct repeat sequences of DNA on homologous chromosomes.

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Spatial Organization of Chromatin in the Nucleus

Since only a uniformly stained interphase nucleus is seen under the light microscope, the question is, "in 3-dimension in the nucleus, is the chromatin randomly spread, or is there a topological order to its assemblage?" It is, therefore, creditable that Rabl, more than a century ago in 1885, proposed that chromosomes re-

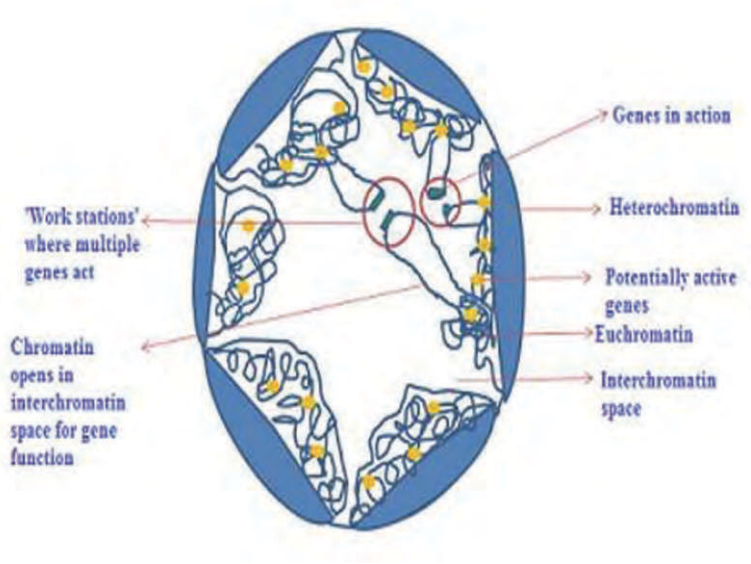

Figure 10. Chromatin distribution in the nucleus. Condensed heterochromatin attaches to the nuclear periphery while euchromatin is diffused and projected inward in the nucleus. There are interchromatin spaces (ICP) in the nucleus where gene (yellow dots) action takes place at 'workstations' while multiple functional strands from different sites act simultaneously.

tain their polar orientation of the anaphase stage of cell division in interphase too (centromeres towards and chromosome arms away from the pole). There is evidence to support the Rabl orientation (*Figure* 9). Now that it is possible to see specific chromosomes and chromatin fragments in the interphase, an acceptable picture of the nuclear architecture envisages each chromosome to have its area fixed in the nucleus with a certain degree of flexibility. The heterochromatin occupies the peripheral part of the nucleus, while the euchromatin projects inwards from the periphery in the nucleus, and there are 'interchromatin' spaces. These spaces are the actual sites of chromatin function (*Figure* 10).

As a general principle, that part of the chromatin which is functional (for transcription, replication, DNA repair or recombination) decondenses, opens up and projects out in the interchromatin space so that the operational machinery (the enzymes, proteins, etc.) gets space to align itself on the chromatin and act. At any given time, many genes, either from neighbouring regions or from different regions and different chromosomes, are active to different degrees. There are 'workstations' in the interchromatin spaces where multiple stretches of DNA from the same or dif-

There are 'workstations' in the interchromatin spaces where multiple stretches of DNA from the same or different chromosomes assemble and perform the function. These workstations are highly dynamic bodies with DNA stretches constantly shuffling back and forth from their condensed state to open conformation and retreating to the condensed state.

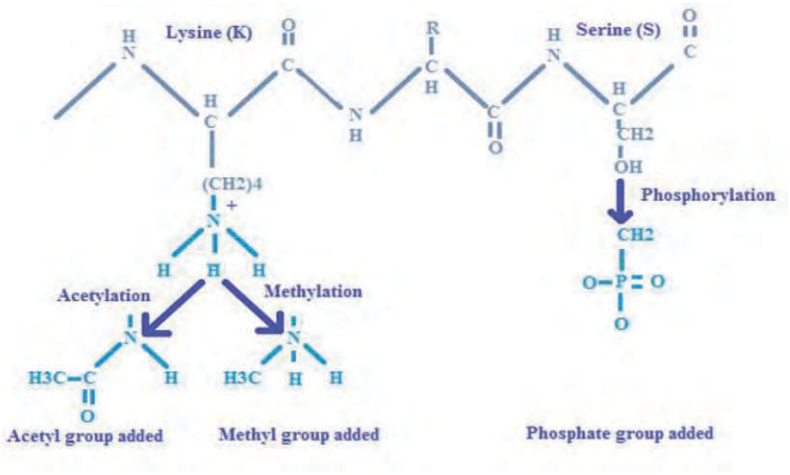

Figure 11. Modification of lysine (K) and serine (S). NH_3^+ group in the side chain of the amino acid lysine loses the +ve charge when replaced by an acetyl group or one or more methyl groups. In serine, an OH group in its side chain is modified by the incorporation of a phosphate group.

ferent chromosomes assemble and perform the function. These workstations are highly dynamic bodies with DNA stretches constantly shuffling back and forth from their condensed state to open conformation and retreating to the condensed state (*Figure* 10). Also, the chromatin with a set of genes that are unlikely to transcribe in a given cell will be closer to the heterochromatic zone (towards the nuclear periphery). Those required to be active will be away from the periphery and more towards the central zone of the nucleus. Even the replication timing of the DNA is grossly dependent on the functional state of the chromatin. Whereas the R-band region (housekeeping genes) is the first to replicate, the heterochromatic DNA replicates the last in the S phase, and the G-band positive regions (tissue-specific genes) replicate between these time zones. Thus, there is a function related organization of chromatin/chromosome in the nucleus, and their topology is highly dynamic subject to its functional status.

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Chromatin Modification and Reorganization

As elucidated, chromatin shuffles between the active and repressed states, passing through cycles of condensation and decondensation. Since DNA-histone interaction is at the root of the chromatin structure, largely because of their opposite charges (–ve

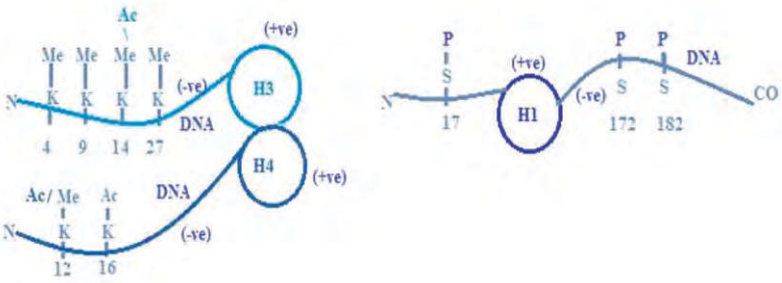

Figure 12. A schematic representation of the specific amino acids in H3, H4, and H1 histones that undergo modification through methylation (Me), acetylation (Ac) and phosphorylation (P).

charge of DNA and +ve charge of histone), its functional dynamics must depend upon the extent of their binding. Various types of post-translational modifications in histones have been reported; the addition of a methyl ($-CH_3$) or acetyl ($-C_2H_5$) groups to the +vely charged amino acid, Lys (designated as K), neutralises its +ve charge. Consequently, the DNA-histone binding becomes loose at that site (e.g., K16 in H4). Unlike acetylation, methylation of Lys causes both loosening and tightening of the chromatin depending upon the position of the amino acid in the histone. For example, methylation of K4 in H3 histone leads to chromatin loosening, but that of K9 in H3 causes compaction. Phosphorylation of the amino acid serine (Ser), on the other hand, leads to tighter binding among histones, especially H1 (*Figures* 11, 12). A combination of these changes in the string of contiguous nucleosomal histones, called 'histone code', in the regulatory regions of a gene determines the state of DNA-histone binding and the gene's potential to act or be suppressed. These histone modifications attract other chromatin-binding proteins, such as transcription factors, which further consolidate the structure as loose or more compact, ensuring the durability of a given state.

Not only histones but DNA also undergoes post-replication modification.

Not only histones but DNA also undergoes post-replication modification. The pyrimidine base, cytosine, tends to modify by adding a $-CH_3$ group at its 5th position (C_{me}). This change is perpetuated when C occurs as a doublet, CpG, implying that the complementary strand at this site is also CpG. A group of enzymes called DNA methyltransferase (DNA MTase) methylates the CpG couplet. Those which methylate an unmethylated CpG on both the strands ($C_{me}pG/C_{me}pG$) are called de novo DNA MTase. In

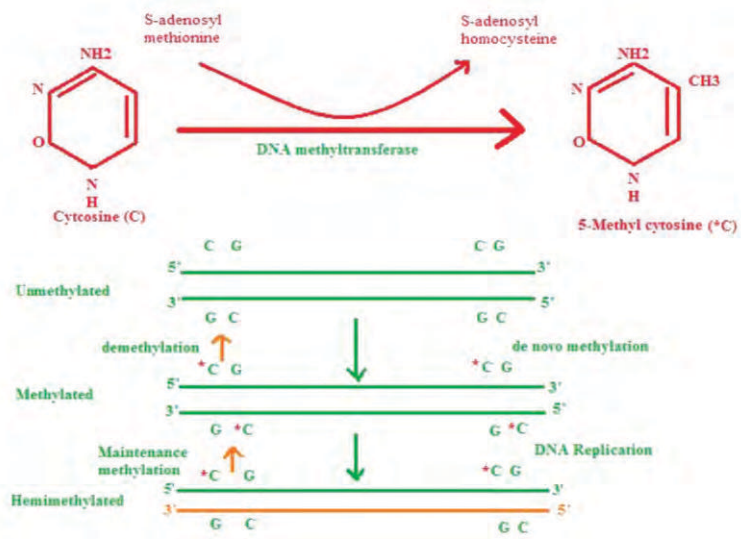

Figure 13. DNA modification. A 5-adenosyl methionine donates a $-CH_3$ group to cytosine to make it 5-methyl cytosine. De novo DNA MTase methylates C in both DNA strands (1st green arrow). Post replication, the DNA is hemimethylated (2nd green arrow; new strand (red) not methylated). It is remethylated by the maintenance Mtase (last red arrow). Thus DNA methylation perpetuates through cell generations.

subsequent replication cycles in which the new DNA strand does not have methyl C (CmepG/CpG), maintenance MTase, methylate the CpG in the new strand, restoring its $C_{me}pG/C_{me}pG$ status after every division cycle (*Figure* 13). Cytosine methylation allows the binding of methylation-specific proteins (MeCPs) to the chromatin, which interacts with histones such that the modified chromatin is repressed for a long period, even across generations.

Chromatin Remodeling and Its Prolonged Activity

The genetic basis of chromatin remodelling was discovered in mutants of yeast which failed to perform important cellular functions, such as switching sex (SWI) and glucose fermentation (SNF).

Modifications, as described above, effect local changes in chromatin to loosen DNA-histone binding. In the cell, however, certain regions of chromatin harbour clusters of genes that act together for a prolonged period. In such cases, the template DNA must remain exposed for an extended period. For example, one can recall rRNA (ribosomal RNA) cistrons that occur in clusters and transcribe large amounts of rRNA, especially during oogenesis, for a long time. In these conditions, chromatin sheds its nucleosomal organization and undergoes a remodelling achieved by energy-driven dislocation of nucleosomes from their original position along a large stretch of DNA. The genetic basis of

chromatin remodelling was discovered in mutants of yeast which failed to perform important cellular functions, such as switching sex (SWI) and glucose fermentation (SNF). The SWI/SNF mutants revealed that for prolonged chromatin function, energy-releasing enzymes, ATPase[3] and other proteins together move nucleosomes from their original site either on the same chromosome or on another chromosome. Thus, histones are removed from a stretch of chromatin for the DNA to be functional. Multiple groups of such protein complexes have now been identified in almost all the cell types and organisms.

[3] The enzyme that cleaves ATP, the energy molecule of the cell, to generate energy.

In contrast, certain cell types develop densely compact chromatin. A substantial part of the sperm chromatin is highly condensed and inactive. In this case, nucleosomes are dismantled, and histones are replaced by even more positively charged proteins—protamines—that bind with DNA in long sheets and interdigitate through disulphide (S-S) bonds. The sperm chromatin opens up after fertilization and reorganizes into nucleosomal conformation. Thus, an active shuffling of the chromatin continues through the life cycle of a cell.

Concluding Remarks

DNA, being the genetic material which holds the blueprint of all bodily functions of an individual's life, needs a robust system for (i) its accommodation in the nucleus, (ii) protection from catabolic enzymes, and (iii) differential activity within a cell and in a group of cells from the single-celled yeast to highly complex multicellular vertebrates. The evolution of DNA-histone interaction and chromatin formation fulfilled that requirement successfully. The constituents and process of chromatin formation are conserved throughout the living kingdom, and so is its dynamic reorganization. The long history of studies on chromatin organization has been exciting and intriguing and remains an active area of exploration. Its three-dimensional organization in the nucleus, the disease conditions associated with impaired chromatin organization, epigenetic modifications in chromatin and cellular

The constituents and process of chromatin formation are conserved throughout the living kingdom, and so is its dynamic reorganization.

differentiation and many such areas are among the most vigorously followed fields of research [9].

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