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REVIEW ARTICLE

The Form and Function of Chromatin Organization

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ABSTRACT: The structure of chromatin and how it is used for cellular function is fundamental to multicellular organisms. The form and function of chromatin organization affect almost every aspect of gene expression, cell division, and various other cellular processes. This review article aims to provide a comprehensive view of the intricate structural organization of the chromatin and its functional relevance. Beginning with an introduction to DNA and heterochromatin, then focusing on the individual nucleosome and its components, the core histones H2A, H2B, H3, and H4. Subsequently, the article covers chromatin architecture, including its past model of 30 nm hierarchical folding and the current consensus of irregular fibers, as well as how the chromosome is structured around a protein scaffold. Next, the chromatin remodeling complexes, their four categories and distinct functions, and how chromatin is manipulated during mitosis are discussed. Ultimately, I explore how the form of chromatin affects gene expression through physical properties such as liquid-liquid phase separation and nuclear compartmentalization.

KEYWORDS: Molecular and Cellular Biology, Genetics, Chromatin Packaging, Chromatin Compaction, Chromatin Condensation.

Introduction

The diploid cells, with two sets of chromosomes, in a human body contain 6 billion base pairs (bp) of DNA (Deoxyribonucleic acid), which approximately equals 2 meters when stretched. This is packaged in a minuscule nucleus that is 10 micrometers in diameter. Despite this significant compaction, the cell can interpret DNA and the state that it is in to carry out vital processes like cell division and gene expression reliably and accurately. Understanding how chromosomes are organized is an indispensable field of research, as evidenced by the scientific attention and studies conducted about it in the past. It began in the middle of the 19th century when Gregor Mendel postulated that traits were inherited as discrete units without referring to the chemical or structural organization of what is inside the "discrete units". Afterwards, researchers would begin to investigate the basis of heredity, giving rise to the scientific discipline of genetics. Subsequently, Walther Flemming used dyes and basic microscopy to discover the unit of genetic material and named it "chromatin" in 1879.[1] Following that, in 1891, Hermann Henking discovered the X-shaped chromosome while studying spermatocyte divisions of a firebug.[2]

Further insights on DNA arose when the electron micrograph images of the DNA forming "beads on a string" were published in 1974.[3] We now understand that the reason for this appearance is due to the DNA being spooled into the nucleosome. Similarly, there have been massive recent innovations in assays and technologies, such as X-ray scattering, partial decondensation of chromosomes, and ChromEMT (ChromEM tomography, an electron-microscopy staining technique that marks nuclear DNA without changing its structure, permitting better visualization of 3D chromatin conformation). These foundational studies have significantly enhanced our understanding of the structure of chromatin.

Several subsequent studies have allowed scientists to learn how the DNA sequence influences phenotypes and mutations that induce certain diseases. However, it is now known that the proteins that regulate the architecture of DNA are equally key in regulating cellular function. Mutations in genes encoding those proteins can promote conditions like cancer. This new aspect of genetics may be the path that could lead to a multitude of new discoveries and opportunities in treating cancer and chromosomal abnormalities. This could have a widespread impact due to the significant proportion of the population affected by these disorders. Specifically, there were 2,001,140 new cases of cancer in the US in 2024,[4] and an estimated global risk of 25% of getting cancer during a person's life.[5]

In this review, I explore the currently available literature to illustrate the form and function of chromatin structure and how the cell attains it. I discuss how fundamental functions such as cell division and gene expression rely on chromatin architecture and present how misregulation of chromatin can be a catastrophic event for many cells. I specifically focus on how misregulation of key components of chromatin organization leads to damage in cells, which leads to certain disorders such as cohesinopathies and cancer. Understanding the detailed activities of components of chromatin organization and their relevance to the disorders will assist the development of novel treatments by offering potential drug targets.

DNA, Hetero, and Euchromatin:

DNA is the essence of all living organisms on Earth, which encodes all proteins used by cells for every function. It is made of nucleotides that consist of a phosphate group, five carbon sugars, and a nitrogenous base. While all DNA is composed of the same molecules, the unique arrangements of the bases adenine (A), thymine (T), cytosine (C), and guanine (G) store the coding information for every gene and protein.

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The complementary base pairing between adenine and thymine, and between cytosine and guanine, connects two DNA strands that are wrapped around each other in a double helix form, with approximately 10 bp per turn, where it assumes its most stable form.[6] However, as the bond between the sugar and the base is asymmetrical, as the angle formed by the glycosidic bond between the base and sugar is not aligned at exactly 90 degrees,[7] the spaces between backbones form two grooves: the major groove and the minor groove. The major groove is wider than the minor groove. The existence of these grooves is key for proteins to bind and recognize DNA sequences outside the DNA (Figure 1). However, DNA by itself, as an individual entity, cannot contribute to the proper function of the cell.

When DNA is bound to proteins and RNA, chromatin, the building unit of chromosomes, is formed. Chemical interactions with the DNA can only occur if the DNA is physically accessible. Its openness is determined by many factors that will be explained later in this review, such as nucleosome positions, histone modifications, transcription, and chromatin compaction.[8] There are two broad categories for chromatin: hetero or euchromatin. Heterochromatin describes an inaccessible state where the genes in DNA are repressed, while the latter is accessible and has active genes. As such, heterochromatin is much more DNA and nucleosome dense than euchromatin,[9,10] and the diffusion in heterochromatin is also less efficient compared to euchromatin.[11]

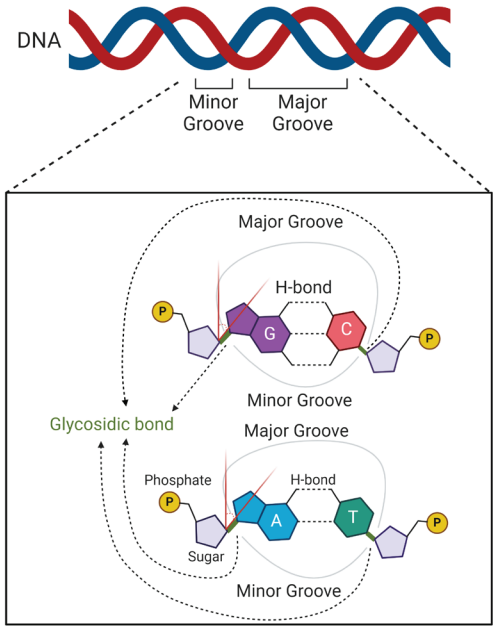

Figure 1: DNA major and minor groove formation. Schematic illustration of groove formation in AT and CG base pairs due to asymmetry in glycosidic torsion angle not equaling 90 degrees (in red line angle). The glycosidic torsion angle is the angle between the phosphate backbone and the glycosidic bond attaching it to the nitrogenous base. This asymmetry is what allows proteins and transcription factors to recognize and bind desired DNA regions.

However, the categorization of chromatin is not a recent development. The nomenclature of heterochromatin and euchromatin originates from Emil Heitz in 1928, when he published the paper 'Das heterochromatin der moose'. He used it to describe the differences detectable by appropriate chromosomal stains. Specifically, euchromatin becomes invisible during late telophase (the final stage of mitosis), while heterochromatin is heteropycnotic, taking up more stain or being more tightly coiled after telophase.[12]

How Does a Cell Package All of its DNA into a Smaller Nucleus? Nucleosomes and Histones:

Since each haploid human cell contains 3 billion bp, each being 0.34nm long,[13] in each diploid cell, there would be 2 meters of DNA. How do cells package all of this DNA into microscopic nuclei with diameters of 5-20μm? A large length compaction of over 100,000-fold. Cells compact the DNA into 'beads on a string' through nucleosomes - the length of DNA wrapped around a histone octamer- wrapping the DNA around the core of a histone octamer (Figure 2). Histones are positively charged proteins that facilitate electrostatic interactions with the negatively charged backbone of DNA to wrap it around themselves, leading to nucleosome formation. There are four core types of histones in humans: H2A, H2B, H3, H4, and a linker histone H1.[14] The four core histones share properties such as their C-terminal domains, which provide histone-histone interactions to form the column-like octamer,[15] which takes up most of their mass.

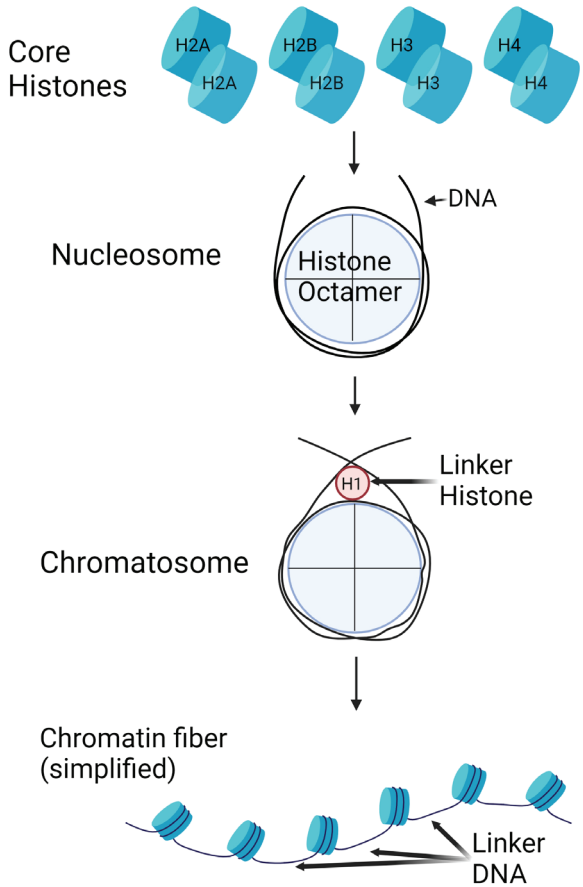

Figure 2: The making of chromatin fiber. Schematic illustration of the process histones go through to become part of a chromatin fiber. With the help of proteins such as assembly remodelers, the core histones form a histone octamer, around which DNA is subsequently coiled, forming a nucleosome. The nucleosome, with the addition of a linker histone, turns into a chromatosome, and the DNA now goes through 2 full turns instead of 1 and ⅔. Many nucleosomes form the chromatin fiber. This makes the DNA very tightly compacted in a regulated manner.

The four histones H2A, H2B, H3, and H4 are called canonical proteins because they are the standard of their respective types, recognized as the norm. However, there are a multitude of variants, such as H3.3, that are non-canonical as they differ from this norm, but I will not discuss them further here as it warrants a focus as a separate topic.[8] Their N-terminal tail provides strong contacts to the DNA that the nucleosomes bind to.[16] The N-terminal tails are readily available for post-translational modifications to be catalyzed by enzymes, playing an important role in epigenetic signaling.[15] They are also in place to conduct inter-nucleosomal interactions in condensed chromatin structures, which help organize higher-order chromatin structures.[17]

Table 1: Glossary of terms.

Term	Definition
Chromosome	A cellular entity containing DNA and protein molecule that is packed into a more complex structure.
Chromatin	The complex of proteins, RNA, and DNA that forms the state of the genome.
Nucleosome	The repeating subunit of chromatin inside the nucleus.
Linker DNA	The length of DNA that connects adjacent nucleosomes (Figure 2).
Chromatosome	The unit of chromatin includes the linker DNA, the nucleosome and the linker histone H1.
Chromatid	Two individual chromosomes that are joined at the centromere that are newly replicated.
Solenoid	A coil in the form of a long cylinder (Figure 3).
Scaffold	A protein structure that provides support for the chromosome. (Figure 3)
Octamer	A complex made of 8 subunits of histones (Figure 2).
ATP (Adenosine Triphosphate)	The universal chemical source of energy in the cell.
ATPases	Enzymes that Proteins that convert ATP to ADP (Adenosine Diphosphate)
Transcription	Synthesis of messenger RNA (mRNA) from DNA sequence as template
Translation	Decoding of mRNA sequence. into amino acids

The product of the 4 abovementioned histones, the nucleosome is the unit of chromatin made up by the spool-like histone octamer wrapping DNA in a left-handed superhelical manner.[15] Its main role is to condense the DNA, but it also has other roles such as repressing processes involving DNA, like transcription, replication, and repair. A nucleosome is created by first gathering two copies of each protein H2A, H2B, H3, and H4 proteins to come together to form a histone octamer, which is what constitutes the nucleosome (Figure 2).[18] The octamer forms through the following steps: firstly, two of the H3:H4 dimers and H2A:H2B dimers are formed, then the two H3:H4 dimers merge through H3:H3 interactions to form a tetramer, and finally, the two H2A:H2B dimers combine with the tetramer via H4:H2B interactions.[15] Nucleosomes are known to condense DNA by around 6~7 times.[8] Specifically, they bend approximately 146 bp of DNA around the histone octamer for approximately 1 2/3 turns.[16,19,20] The core histone octamer domains fold 120 bp, while the remaining 13 bp are bound by H3 N-terminal alpha helices. These interactions help maintain the stability of the nucleosome. The folding is caused by roll-based bending of bp into the minor and major grooves that face the nucleosome.[21] The nucleosome also twists the DNA further, such that the number of bp per turn goes from 10.5 to 10.2.[16]

Although the histone proteins predominantly fold and shape the DNA organization, the sequence of the DNA affects its binding affinity to the histone octamer, creating the phenomenon of sequence-based DNA binding.[15] The affinity varies by 3 orders of magnitude depending on the DNA sequence.[22] It is most optimal for nucleosome binding when bendable bp, such as AT & TA, are at the repeated elements every 10 bp that directly interact with the nucleosome.[23] However, having many repeats of AT/TA is inhibitory to nucleosome binding, thus promoting promoter accessibility, nucleosome depletion, and transcriptional activity.[24,25]

Similarly, the DNA bound by the histone octamer frequently undergoes a process known as "DNA Breathing" that exposes the protein binding sites on the nucleosomal DNA. DNA breathing does this by transiently binding and unbinding to the histone octamer, which can facilitate sequence-specific protein binding if the binding affinity to naked DNA or the local concentration of the protein is high enough to compete with core histone binding.[18,26] Without DNA breathing, the nucleosome would protect the DNA from nuclease digestion, as well as greatly restrict the binding of regulatory factors. Even when breathing, the binding affinity of most DNA-binding factors to nucleosomal DNA is reduced by $10^{3\sim5}$ compared to naked DNA.[15] The length of DNA that connects two nucleosomes is the linker DNA. While the linker DNA length varies from 20-80 bp, the H1 family of proteins, the linker histones, wraps linker DNA near the center and the entry/exit points of the nucleosome, promoting further compaction and stabilization of DNA. This coils an additional 20 bp of DNA, ending up in ~2 oriented full turns around the whole chromatin and linker histone, forming the chromatosome (Figure 2).[27] The functions of linker histones include: promoting folding and assembly of higher-order chromatin structures; changing nucleosome spacing on DNA, regulating specific gene expression, and protecting linker DNA.[28–30]

Not only can histones compact the DNA, but their N and C-terminal tails can be post-translationally modified in various ways, such as acetylation, phosphorylation, methylation, SUMOylation, and ubiquitination. These alter the charge and structure of the tail and thus affect their binding to DNA, ultimately changing the condition of the chromatin and how much the genes are expressed, leading to changes in cellular events such as mitosis. However, in the interest of focus, post-translational modification of histones is not covered at length in this paper. An extensive review on this topic is published elsewhere.[31]

This knowledge about nucleosomes and their post-translational modifications can be useful as they are known to play a role in many cellular processes involving DNA, such as DNA repair and transcription. Moreover, their alteration can affect broader processes, such as development and aging, as well as result in diseases such as cancer and intellectual disability, making their understanding more important.[32]

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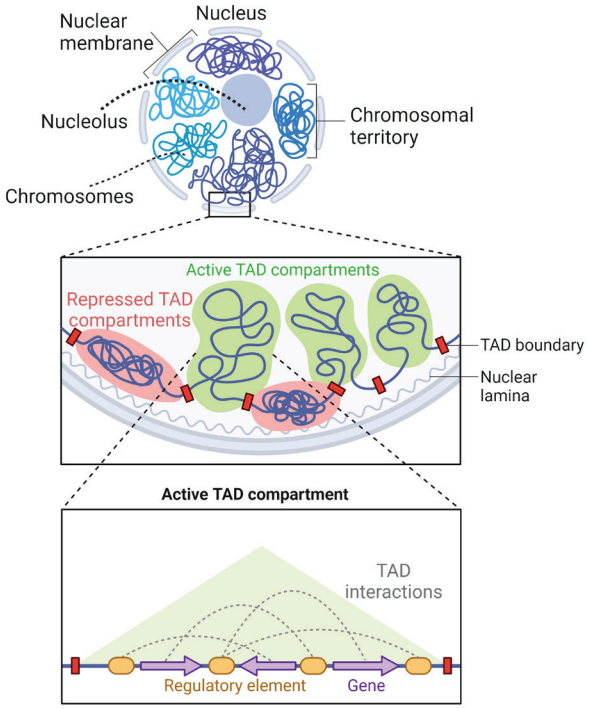

Figure 3: Organization of TADs, the constituents of chromosomes. Schematic diagram showing the nuclear structure and organization of the TADs of the chromosomes that lie close to the nuclear membrane. The TADs are composed of multiple genes and elements that physically interact with each other. The implications of 3D structuring add further complexity to the regulation of gene expression and chromatin organization in the cell.

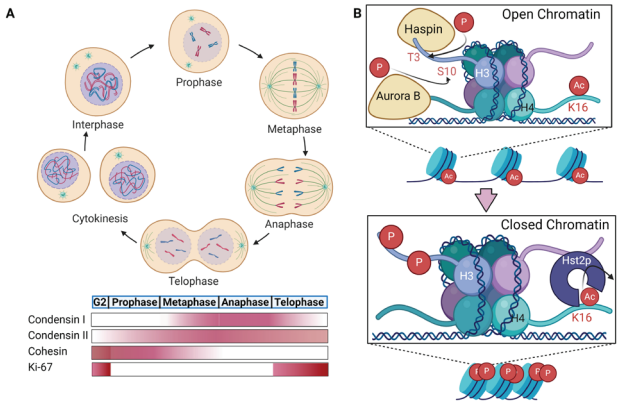

Figure 4: Mitosis and chromatin organization. **(A)** Shows the mitotic cell cycle from interphase to cytokinesis. Shows the nucleolus dissolving, and the genetic material condensing into chromosomes with sister chromatids, which are then separated into separate cells during cytokinesis. The bottom shows the mitotic phases and shows the concentrations of the proteins condensin I, condensin II, cohesin, and Ki-67 at each phase. The lighter the color, the lower the concentration. Adapted from,[37] **(B)** Schematic diagram showing the steps in which histone modifications work to condense chromatin into chromosomes in early mitosis. Haspin modifies the histone H3 by adding a phosphate group to the third threonine (T3). This triggers the phosphorylation of the 10th Serine on the histone H3 (S10) by Aurora B, which brings about the Hst2p that removes the acetyl group from the 16th lysine on the histone H4. This brings about H4 tail and acidic patch interactions with the adjacent nucleosome, thus inducing heterochromatin and chromosome formation. This mechanism demonstrates how important histones are not only in processes such as gene expression but also in mitosis.

Chromatin in Cell Division:

Mitosis is the process that cells undertake, which, in short, replicates and doubles the genetic material, then organizes and arranges it into groups so that the cell can split in half with both daughter cells having equal amounts of DNA. More specifically, the process involves five main phases: interphase, prophase, metaphase, anaphase, telophase, and cytokinesis, in that order. Interphase can be further split into the Growth/Gap (G)1, Synthesis (S), and G2 phases, in that order.

This progression through phases in mitosis is regulated by proteins that add a phosphate group (phosphorylate) to other substances from ATP, called cyclin-dependent kinases, abbreviated CDKs. For example, they can target and phosphorylate the tumor suppressor protein retinoblastoma.[33] CDKs require proteins called cyclins to activate them by binding to them and phosphorylating a certain part of them. Each class of cyclins corresponds to the phase at which they activate CDKs, like the G1-phase cyclins, G1/S-phase cyclins, S-phase cyclins, and M-phase cyclins. The last one is the cyclin, which drives the start of mitosis. There are 20 CDKs and 29 cyclins in humans.[34,35] Early in mitosis, the series of phosphorylations, H3T3 by Haspin, then H3S10 by Aurora B, causes recruitment of a lysine deacetylase Hst2p. It removes an acetyl group from H4K16, a modification that was previously interrupting internucleosomal condensation, to promote chromatin condensation (Figure 4).[36] When chromosomes are most compacted and segregated (anaphase), they are massively condensed longitudinally.

DNA can also be compacted in other ways: while the DNA is simply replicated by DNA polymerases on the irregular fiber of nucleosomes during interphase, during early prophase, the fibers begin to condense into loops around a central chromosome scaffold, as will be covered later in this paper. The condensed chromosomes are segregated by mitotic spindles. In late anaphase and telophase, the chromosome is then decondensed back into nucleosome fibers into its interphase state. However, the mechanism of this decompaction is different from just reversing compaction.

Although entry into mitosis is largely regulated by kinases, to decondense, not only are the relevant kinases deactivated, but the mitotic phosphorylation is also reverted. The phosphatases, removers of phosphates using water, PP1 and PP2A, are the main proteins in this process. PP1γ dephosphorylates the histone H3 at several points after being brought to anaphase chromosomes by the recruiting subunit Repo-Man or by another protein, Ki-67. Meanwhile, PP2A is implied to ensure the timely exit from mitosis through dephosphorylation.[38] In addition to dephosphorylation, Aurora B kinase must be evicted as well. The group of proteins AAA+-ATPase p97 and the cofactors UFD1 and NPL4 guarantee Aurora B's removal. It is implied that they use the ubiquitin proteasome pathway. It is a way that the cell warrants degradation of proteins by attaching a recognizable marker, ubiquitin, to them such that it can be transported to a proteasome for its destruction, as AAA+-ATPase p97 is a system that typically recognizes ubiquitin on tagged proteins.[37] This then implies that Aurora B is ubiquitinated during the later stages of mitosis. It is also speculated that the removal of phosphorylations by Aurora B is

responsible for the halting of the protein condensin's activity for decondensation.[39] Since this decondensation is required for mitotic exit to occur, the absence of factors such as PP2A and its cofactors can delay mitotic exit.[40] This delay could potentially lead to hazardous consequences, such as DNA damage and aneuploidy, a potential cause for tumorigenesis and cancer, making mitotic decondensation a vital topic for further research.

Chromatin Organization in Interphase:

In the last couple of decades, there have been many hypotheses on how the chromatin is compacted. However, there have been more prominent models, such that recently, chromatin has been thought to be mostly compacted by either one of two models, the hierarchical folding model or the radial loop model. (Figure 5) The hierarchical folding model proposes that the chromatin is compacted into a large-scale 30-nm diameter fiber.[41] This is thought to occur by folding into a solenoid or zig-zag shape.[42] These fibers are then progressively used to form higher-order chromatin structures of larger diameters, involving ~100 then ~200 nm, to finally become large interphase chromatin fibers. They may later be anchored to protein scaffolds to form condensed mitotic chromosomes.[8] But of the two models, the current consensus is that the majority of cells use the radial loop model. This is because most of the evidence for the hierarchical model comes from *experiments in vitro*. This is where the artificial chromatin has an array of very regularly spaced nucleosome binding sites with uniform linker DNA length. Unfortunately, there are very rarely regions with these conditions in vivo (meaning inside a living organism), because typically cellular chromatin is found to have variable linker DNA length and histone post-translational modifications, which is a significant critique of this model. There are some instances, such as in some genomic regions and mostly inactive chromatin, like in avian erythrocytes, as well as in regions of very high levels of linker histones. There have also been more data against this model in recent years, and as such will not be discussed further in this paper. [8,9,19,43–45]

The other model, the irregular model, is the current consensus that proposes that the mitotic chromosomes are condensed in irregularly arranged forms, leading to a fractal nature. The decreased physical constraint permits a more dynamic and flexible organization than its static counterpart, the 30 nm diameter fiber.[46,47] Specifically, chromatin in this model is formed into irregular, yet dynamic 10 nm-diameter fibers. The accessibility of the fibers according to computer simulations could come about as a result of nucleosomal fluctuations exposing the genomic DNA and facilitating the mobility of diffusing proteins.[46] The 10nm nucleosomal irregular fibers of chromatin are thought to remain irregular during interphase, but there have been models proposing higher orders of irregular fibers, specifically forming many condensed domains out of these fibers, so that they look like "chromatin liquid drops".[48,49] These drops of chromatin are thought to be formed by the macromolecular crowding effect and specific proteins like cohesin and CTCF (CCCTC binding factor),[50–53] and/or condensin II.[39,54]

Additionally, the physical packaging units of DNA are known as TADs (Topologically Associated Domains). Interestingly, the genes within the same TADs actively interact with each other often, but not with those in other TADs. These TADs correspond to LADs (Lamina Associated chromatin Domains),[55] which are domains that are associated with nuclear lamina - a structure near the inner nuclear membrane, composed of fibrous proteins known as lamins.[56] These chromatin domains serve to help change timing during cell differentiation and transcriptional regulation, as well as for other purposes. Enhancer-promoter interactions are likely limited to regions within the same TAD, as seen in Figure 3.[30]

Nucleosome fibers are arranged into mitotic chromosomes by the activity of certain proteins. This is done so that the chromosomes can be individualized and so that sister chromatids (Table 1) can be separated.[57,58] However, recent research has shown that condensin - a key protein in chromatin condensation into chromosomes – is not required for chromatin to organize into lumps in yeast.[59] This has induced proposals that there is a condensin-independent condensation which occurs in parallel with condensin-dependent condensation, where neighboring nucleosomes are attracted to each other, due to interactions between H4 tails and nucleosomal acidic patches.[36] This has been postulated to be a small-scale form of chromatin compaction which occurs inside the loops of DNA, while the condensins compact at a larger scale, looping long stretches of DNA.[38]

There are some other hypotheses, such as hyperactive condensin DNA supercoiling - the number of DNA bp per turn of the helix decreasing, determining the amount of strain on the DNA, being a contributor to mitotic compaction.[60] This is unsurprising due to how important mitotic compaction is. Therefore, it stands to reason that there would be many types of compaction, including, albeit redundant ones, as any that enhanced compaction would be positively chosen by evolution.[61]

Chromatin Condensation into Mitotic Chromosomes:

The difference in compaction of chromatin between mitotic chromosomes and interphase chromosomes is relatively subtle, where the compaction of one chromosome's worth of DNA is ~10^4 and ~$8.5*10^3$ fold, respectively.[62] This is even though estimations have predicted the difference to be 2-3 fold, which in retrospect seems like an overestimate.[63] The chromatin chain also appears to be more flexible in mitotic chromosomes, allowing for the nucleosomes to be more tightly packed.[9] Although their compaction is relatively similar, their form and arrangement are significantly different. How does the cell cause this? The cells can induce this by using 6 essential proteins that are required for mitotic condensation to occur. The only required proteins to form the chromosome *in vitro* are topoisomerase IIα, cohesin, condensin I and II, and the chromokinesin KIF4A. They also appear to be the major, if not only, essential components of the chromosome scaffold.[64,65] First found by electron microscopy, a central 'scaffold' in the shape of metaphase chromosomes organizes the looping of the DNA along

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the chromatid and is responsible for the chromosome's basic shape as seen in Figure 5.[57,61]

Specifically, condensins and topoisomerase IIα form central axes in the chromatid, while the rest are concentrated along it, around which the nucleosome fibers are wrapped.[46] Condensins use adenosine triphosphate (ATP-energy)-dependent processes that produce loops of DNA, a process known as loop extrusion (Figure 5). Condensin II makes larger loops, which end up forming a spiral staircase arrangement with a protruding loop at each step. Then, condensin I makes nested smaller loops within the condensin II loops.[66] To soundly sort out the topology of this, special proteins called topoisomerase IIα are recruited.[57,67]

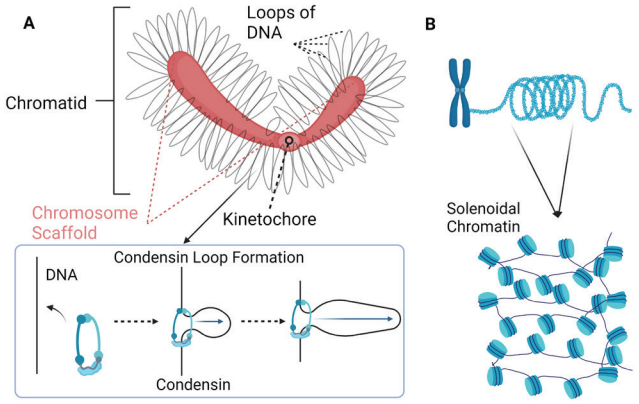

Figure 5: The Chromosome Scaffold and Condensin Loop Formation. **(A)** Schematic diagram of a chromatid with its loops of DNA visible, as well as the steps for condensin loop formation. **(B)** Shows the hierarchical folding model, particularly the solenoidal model of chromatin packaging. The radial loop model's dynamic nature can be seen in (A), while the solenoidal model in (B) is more restricted.

DNA topoisomerase IIα, also known as scaffolding protein 1 (Sc1), is localized at the axes of chromatids,[68] and although the exact role is unknown, it is assumed that Sc1 is essential in preventing the formation of knots and twists as the DNA gets shaped into the rod like arrays of loops representing mitotic chromosomes.[57,67] Sc1 does this by first cutting both strands of the target DNA, then passing an intact double helix through the break, and finally reconnecting the cut DNA. As the DNA is restored, the only difference between its initial and final state is the spatial orientation of the DNA.[69] Their depletion leads to long and thin chromosomes, similarly to condensin II depletion.[57] It is usually active not at prophase, but during late prophase/ the transition phase from prophase to metaphase, known as prometaphase.[70]

The other proteins at the central axes, condensins I and II, are ring-shaped proteins that have distinct yet overlapping functions, but condensin II is inside the nucleus during interphase, while condensin I is in the cytoplasm.[54,71] However, both do end up localizing at the chromatid axes during mitosis, since they are necessary for proper chromosome localization and topoisomerase IIα function.[57,72] They are ATPases as they bind to and hydrolyze ATP in their chemical reactions. The binding and hydrolysis of it regulate the opening and closing of the ring.[73] They shape the mitotic chromosomes by forming chromatin loops via loop extrusion.[74] They do this by binding onto DNA and then, while one part of the ring anchors, the opposite end slides back across the DNA using the motor activity, pulling the DNA along with it, and extruding it as a loop through the ring.[75]

While they both share the same SMC2/4 (Structural Maintenance of Chromosomes) dimer and appear similar, Condensins I and II have notable differences. For example, they have markedly different subunits. They are also different in that condensin I, but not II, requires chromokinesin KIF4A to localize to the mitotic chromosome axis.[76] Condensin II, but not I, requires the enzyme phosphatase 2A to associate with mitotic chromosomes.[77] Condensing activity also requires phosphorylation by kinases like Aurora B to relocate to the condensing chromatin and function there.[71,78] Additionally, condensin II is one of the first to contribute to looping and compacting the DNA from interphase during prophase, although there is a possibility of cohesin being active from prophase as well.[71] At the late stages of prophase, condensin I is brought in from the cytoplasm to the chromosome.[79]

Another essential protein for mitotic condensation, Cohesin is a protein made out of the SMC1/3 dimer that primarily connects sister chromatids during DNA replication,[80] but can interact with chromosomes and form loops during the cell cycle to compact them.[81] Recent studies have also shown that it influences the process of chromosome formation. Cohesin also plays a role in organizing interphase nuclei and regulates patterns of gene expression.[57] The cohesin complex also works with CTCF to organize interphase chromosomes into TADs as well as to help control gene expression.[52] Cohesins are released the most during prophase to act on the chromosome until the end of metaphase.[82] Their malfunction can cause genetic conditions, collectively known as cohesinopathies, including Robert's syndrome and Cornelia De Lange syndrome.

Although equally important, the SMC5/6 complex is elaborate, and thus its function is elusive. However, it is currently thought to play a role in associating with unusual DNA structures such as catenanes - molecules composed of at least 2 cyclic chemicals that are not chemically linked but need chemical link breakage to separate – as well as compacting the surrounding DNA and resolving topological tangles.[83] It can also be recruited by SAGA histone acetyltransferase to certain genes.[84] It seems to function during the S phase of the cell cycle to help replicate DNA and separate repetitive DNA, as well as potentially having roles in DNA repair and recombination. Depletion of it prior to entering S phase causes dramatic chromosome segregation defects.[57,85]

Chromokinesins, on the contrary, are a group of proteins that can move, and KIF4A is one of them. It usually plays a key role in forming the mitotic spindle and intercellular bridges,[86] but it is also connected to cancer if depleted, as it is required for regulating DNA damage responses.[87,88] It can bind to DNA and condensin I. The latter requires KIF4A to localize to the chromosomal axis;[89,90] however, it can only bind to condensin I after it has been phosphorylated.[91] Phosphorylation is also required for lateral chromosome compaction for KIF4A.[91] It has been suggested that KIF4A has a role in forming/stabilizing DNA loops, but has not been verified at the time of writing.[57]

It also works together with condensin and topoisomerase IIα to shape mitotic chromosomes.[90] Depletion causes a decrease in condensin I levels, thus causing short and fat chromosomes to form with defective structures.[90]

All six of these proteins are essential for mitotic condensation to occur, and evidence shows that when they are depleted, chromosomes have defects that can be significantly detrimental to humans, making understanding them illustrative of how errors in cell division can cause diseases. For example, short and fat chromosomes due to condensin I depletion can be found in certain cancers and can cause disrupted brain development.[92]

Chromatin Remodeling:

DNA bound by nucleosomes is very hard to bind to other factors, because it has to compete with the histones at the core of the nucleosomes. However, this closed structure can be opened. This opening, as well as many other processes, is collectively known as chromatin remodeling. There is a special class of proteins that use ATP called chromatin-remodeling complexes that carry these processes out (referred to as remodelers). They make sure of the proper density of nucleosomes and cooperate with site-specific transcription factors (TF) and histone-modifying proteins to move and eject histones from the nucleosome (sometimes entire nucleosomes), to allow the binding of TFs to DNA. Additionally, they help create special regions in the chromosome where canonical histones are replaced by variants. Remodelers are so crucial for regulating almost all chromosomal processes that the lack of them usually leads to many diseases, like cancer.[93,94]

The remodelers can be generally categorized into 4 distinct subfamilies: enzymes: imitation switch (ISWI), chromodomain helicase DNA-binding (CHD), switch/sucrose non-fermentable (SWI/SNF), and INO80.[94,95] While they might seem very different, recent studies have pointed out an important unifying aspect: all of them have an ATP-dependent movement of DNA along the histone surface to break histone-DNA contacts so that DNA can be driven along the histone. This is simply customized by the differing subfamilies to result in their specific actions. There are some other shared properties, such as preferring to bind to nucleosomes rather than naked DNA and having a singular ATPase subunit with a domain that allows interaction with other chromatin proteins.[94]

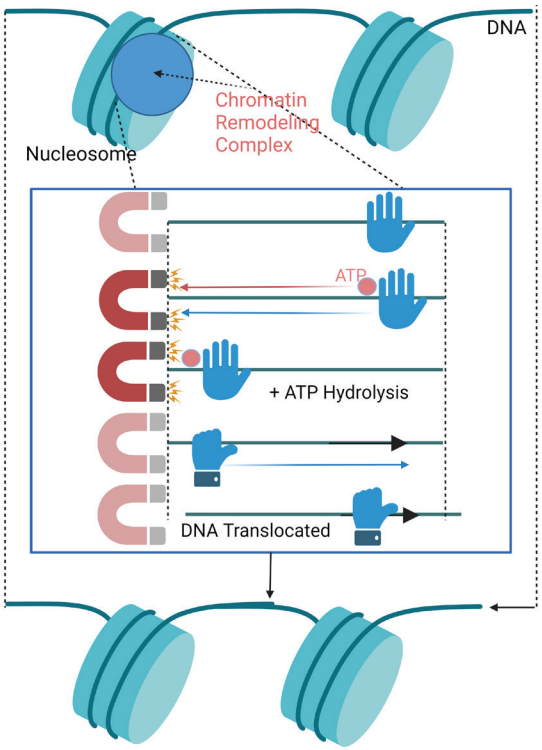

Figure 6: The mechanism of chromatin remodeling complex DNA translocation. Remodelers contain two lobes, where one is depicted as a U-magnet and the other as a hand in the diagram. Both lobes are first bound to the same strand of DNA, with one of them, implied as the magnet, ahead of the other. The hand is either closed or open, depending on how tightly the sequence of DNA that it is associated with binds to it. The lagging hand is moved towards the leading magnet when ATP is bound, but after its hydrolysis, the lagging hand moves backwards, pulling the bound and clamped DNA with it, moving 1-2 bp of DNA.[94] This mechanism is shared among the 4 categories of remodelers, although they all have distinct functions.

When at the nucleosome, and more specifically at the position SHL (Superhelical location) 2,[96] the remodeler binds near the entry site of DNA of the nucleosome and carries out its movement of DNA from the entry to the exit site (Figure 6).[97] Since the DNA is now more concentrated at the exit than the entry, the linker DNA is extended by 1-2 bp to resolve it. This results in overall movement of the histone octamer 1-2 bp along the DNA, which, through repetition, can be moved greater distances.[98] By modifying this process such that DNA is peeled off of adjacent nucleosomes, collision between adjacent ones, or causing major tension, a nucleosome may be ejected from its position. Furthermore, the strong tension can also break histone-DNA contacts to let nucleosome components be evicted and variants be added to restabilize.[94]

The different categories have their own purposes, such as when histone chaperones are carrying the histone polymers to the new DNA formed by DNA replication,[99] assembly remodelers like the ISWI and CHD are the ones to assist the histones and the histone octamer in forming a mature nucleosome. They then also regulate the distance between two nucleosomes so that it is fixed to form an array of nucleosomes. This also occurs at places where the nucleosomes have been ejected, like at sites of transcription. Since TFs need to bind to specific DNA regions called promoters that are blocked by

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the nucleosomes, the remodelers can move the necessary DNA region out of the blockage so it can be transcribed. While assembly remodelers indirectly silence genes, access remodelers like SWI/SNF are the ones mainly responsible for sliding histones, evicting nucleosome components like the H2A/H2B dimer, and evicting full nucleosomes for the purpose of making the chromatin more accessible to proteins and RNA, thus promoting gene expression.[100] Of note, they can also be used for gene repression. Additionally, they are also believed to be a central tumor suppressor.

INO80 remodelers, unlike the previous ones, are independent of replication and can remove a particular histone within a nucleosome and replace it with either a canonical or a variant histone. It is enriched at replication origins and DNA damage sites. Commonly, the histones H2A and H3 are replaced with the assistance of editing remodelers like SRCAP and p400, affecting factor recruitment, exclusion, and activity. INO80 can also relocate nucleosomes by as much as ~15 bp at a time by allowing the DNA loop to exit.[101]

All four of these remodelers' enzymatic activity can be regulated by either subunits that are next to the ATPase domain or by adjacent proteins via one of 'gating', ATP turnover, or 'coupling'. A substrate nucleosome can 'gate' the remodeler from working by altering DNA so that a change in shape is required for the remodeler to modify it. ATP turnover simply refers to the rate at which a remodeler uses ATP or goes through the cycle of binding and then hydrolyzing one ATP to the next. Coupling refers to either the probability that a usage of ATP results in movement of DNA, or the amount of DNA that is moved per one cycle of ATP hydrolysis.[94] Remodelers of all 4 subfamilies can also be affected and regulated by histone modifications and variations, as well as proteins like DNA-binding proteins.[102] They contain domains, such as bromodomains, bromo adjacent homology (BAH) domains, chromodomains, plant homeodomain (PHD) domains, Pro-Trp-Trp-Pro (PWWP) domains, and tryptophan-aspartic acid (WD40) domains. They either bind more or less to certain nucleosomes, depending on the variants, modifications, and nearby activators and repressors.

When their regulation meets errors, such as when SWI/SNF is altered to disrupt chromatin accessibility, oncogene activation and tumor suppressor silencing can occur, leading to cancer.[103] However, there are now clinical trials for inhibitors targeting remodelers to act as therapy against it.

Chromatin Organization and Gene Expression:

Chromatin is mainly separated into two compartments (3D regions of the nucleus), A and B. The A compartment is filled with transcriptionally active components like proteins and histone modifications; it is also associated with early replication. The B compartment is transcriptionally repressed and associated with late replication and includes inactivated genes and silencing modifications.[104,105] Additionally, there are 4 models proposed on how the larger-scale chromatin structure can affect transcription. This is because TF binding is a critical step in gene expression, and thus, the DNA being accessible is a significant part of gene regulation.

Firstly, steric occlusion is just the nucleosome or a series of nucleosome contacts blocking access to essential binding sites of TFs. This exclusion makes the concentration of TFs needed to significantly bind larger. In the extreme case that all binding sites are occupied, the concentration in that area is very low. If chromatin is bound to a gel, then regardless of the chemical properties, proteins are either small enough to enter and access the chromatin through the pores of the gel or too large. If the chromatin also forms a gel, it usually has a very well-defined pore size, leading to the blocking of entry of molecules depending on size. For example, it may shut out RNA Polymerase type II (RNAPII) due to its large size. Similarly, impenetrable crowding agents, such as chromatin, at a particular region can occupy so much solvent volume that it can decrease the concentration of soluble proteins by reducing the volume available for them.[8]

Next, Liquid-Liquid Phase Separation (LLPS) is a phenomenon where groups of molecules with weak interactions with each other separate into two liquid 'phases' with varying concentrations of major molecules to be more energy efficient for the cell than being in one mixture. It has been found that LLPS can occur for chromatin-associated proteins, including HP1α, linker histone, RNA-protein complexes, a diversity of other factors, and for chromatin itself. Therefore, condensed chromatin can exist in both a solid and a liquid state.[105] LLPS is also useful for partitioning as well as regulating transcription and accessibility, as it can prevent proteins and TFs from entering compartments, depending on its chemical properties, like charge. Additionally, the LLPS of chromatin compared to a bulk solution of it is compacted by 10,000-fold, showing that it can act to compact long-range chromatin, so much so that it is suggested that this state of chromatin is the natural 'ground state', although the specific structure at this state lacks scientific consensus.[8]

Examining further, we can see that there are many TFs with reduced residency times on the chromosome. For example, it has been seen that TFs like Sox2 can still access condensed mitotic chromosomes, albeit with decreased binding times due to the loss of stabilization by universal transcriptional inactivation.[106] The looping of chromatin by condensing, forming a spiral staircase, surprisingly does not significantly affect gene expression, and the local changes are most likely due to local factors like the availability of TFs and polymerases to bind DNA. There are some proposals on how condensin could mediate global silencing involving restricting promoter-enhancer interactions, but they will not be discussed further.[107] Pioneer TFs like FOXA1 can bind preferentially to nucleosomal DNA to initiate transcription and/or promote accessibility, while repressive TFs like CTCF do not.[108] All things considered, while the chromatin being condensed into chromosomes globally inhibits transcription, since TFs can still access and bind to chromatin, the genomic structure is more reflective of the local factors and regulatory elements rather than something universal.[8] Additionally, TFs can recruit remodelers and modifiers of histones to the nucleosome that they are near.[109]

Moreover, transcription and gene expression are processes that require initiation, elongation, and termination, and

so it has been observed to localize at less compact regions of chromatin. When genes on the massively silenced and chromatin/nucleosome-dense chromosome X avoid silencing, their transcription tends to localize away from the nearby inactive region of the chromosome. This has been shown to be true for the whole genome as RNAPII - the essential component of transcription that transcribes the DNA into mRNA - prefers to localize at compartments that are less densely packed with chromatin. Furthermore, histone depletion can also preferentially silence regions closer to large repressive domains like telomeres.[8] Interestingly, it has been shown through transcription inactivation studies that it is active transcription that regulates accessibility and short-range chromatin compaction instead of architectural scaffolding.[110,111] Also, transcription can increase accessibility of chromatin by destabilizing nucleosomes through the torsion created by the RNAPII moving along the DNA and processing it into mRNA, although this could be either reserved for a certain subset of genes or accumulated over long-term transcriptional activity.[8]

TADs also play a role in regulating gene expression. For example, the HoxD gene cluster - a cluster that is involved in many stages of vertebrate limb development - has two sequential bursts of gene activation, one for the preceding and central genes, then the next for the succeeding genes. This regulation between one to the next involves a switch in contacts between promoters and regulators, which define two distinct TADs: telomere-proximal TAD and centromere-proximal TAD (referred to as T-TAD and C-TAD). These TADs face each other with the HoxD gene cluster in between. Additionally, the chromatin structure defined by TADs was found likely to influence the phenotypes of certain diseases, such as the formation of limb anomalies.[112,113] Additionally, the chromatin organization at the stages of TADs can also regulate genes such as the sonic hedgehog gene (Shh), as the deletion of CTCF activity causes a 50% decrease in transcription of Shh. This is due to CTCF playing an important role in maintaining TADs. Thus, inhibiting it eliminates strong interactions between the promoter and enhancer.[114]

The ways that chromatin affects gene expression have significant implications for diseases. For instance, mutations in LLPS components can disrupt TAD boundaries, DNA repair, increase genomic instability, and promote oncogenic gene expression, thereby promoting cancer.[115]

■ Conclusion

Through discoveries from several decades of research in cell and molecular biology, scientists have been able to uncover a significant portion of information regarding chromatin and its form. As cell biological technologies improve, we hope that more can be uncovered in the spatial organization of chromatin in the nucleus and in how it affects biological processes.

This review has discussed such findings made by researchers on the topic of chromatin in the context of its organization. Specifically, it has covered the chemical composition of DNA and how the bond angles connecting the nucleic acid to the sugar phosphate backbone form minor and major grooves that proteins use to bind to precise locations. It has also reviewed the categories of chromatin from hetero to euchromatin and how it originates from Emil Heitz. Combining this with the histone octamer forms the nucleosome, the unit of chromatin. Additionally, I have outlined the process of octamer formation and how the chromatosome is formed. Although in the past, chromatin was thought to be hierarchically folded 30 nm fibers of DNA, the current consensus is that nucleosomes are arranged into irregular 10nm fibers. The dynamic irregular fibers are then looped around a chromosome scaffold that gives it its unique "X" shape during metaphase, composed of 6 essential proteins. The fibers of chromatin can be modified to allow for regularity or accessibility by inducing nucleosome maturation, translocation, eviction, or replacement. On the other hand, the decondensation of chromosomes during mitosis requires a combination of histone modifications. Finally, chromatin accessibility is regulated through steric occlusion, nuclear compartments, gel formation, and phase separation, as well as through the spatial organization of TADs and LADs.

In retrospect, ever since Emil Heitz's cytological staining to discover heterochromatin, innovations have allowed biologists to identify how *in vitro* artificial chromatin is organized into 30 nm fibers. Recent advances in assays and experimental techniques like cryo-electron microscopy, tomography, and partial decondensation have allowed us to identify the *in vivo* form of chromatin and how its "irregular" structure enables greater dynamism to control various genomic processes. Thus, in the following years, it is the hope that the remaining unanswered questions may be answered, such as how the key chromatin compaction proteins cooperate to produce the scaffolds to shape the chromosomes, the individual roles played by the different variants of proteins like chromokinesins, as well as how the precise mechanisms of chromatin remodeling complexes work to evict nucleosomes or their components.

To conclude, the study of DNA and how it is packaged remains essential to know due to how it affects gene expression and thus the phenotypes expressed. Yet, since the unit of genetic material, chromatin and its constituents, has been mainly uncovered, I believe there should be increased research attention on how these units contribute towards the larger processes in mitosis during interphase, as well as how they may be different in meiotic and polytene chromosomes. Polytene chromosomes, large chromosomes that occur from repeated rounds of DNA replication without daughter chromatid separation, are present in certain disorders, such as muscular dystrophy,[116] spontaneous abortions,[117] and, notably so in various tumor types in humans,[118,119] making their currently relatively unknown regulation and chromatin organization exceedingly important to research. Additionally, we have observed that the way chromatin impacts gene expression is interestingly usually dependent on its local conditions and, in certain cases, the permeability of the nuclear compartment. Thus, in the future, more on how chromatin organization through chromatin domains like TADs impacts specific gene loci and their expression can be potentially uncovered. However, there are still regions that we know little about, such as how we have only recently discovered the precise dynamics of the linker histone. Thus, there is

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information yet to be uncovered, such as how H1 recognizes the nucleosome and the details regarding the specific sites of interactions between the N and C-terminal domains of H1 within the chromatosome. Additionally, assays for identifying chromatin architecture at the short-range scale of a few nucleosomes have large potential for further development. Thus, as these tools are upgraded, our knowledge of the packaging of chromatin and how it relates to protein binding and gene expression can be elevated further.

By better understanding the form of DNA not only in mitotic but also meiotic cells and learning the interactions between the various proteins like condensin and cohesin, the probability of finding new measures to prevent certain diseases in the near future can be increased. Namely, diseases, such as cohesinopathies, including Roberts and Cornelia de Lange Syndrome, cancer, and developmental disorders like Down syndrome. Breast cancer has found chromatin accessibility among others as a new potential target for treatment.[120] Moreover, epigenetic drugs have recently demonstrated clinical success and have been approved for use against certain cancers.[121]

Fundamentally, according to the currently available data, chromatin is made up of grooved DNA spooled around a histone octamer and decorated by associated proteins and RNAs, which are packaged as irregular 10 nm fibers that are then looped around a protein scaffold to form the X-shaped chromosome. The nucleosome is then made of a histone octamer made of two of each of the core histones H2A, H2B, H3, and H4, that work together to bend the DNA around itself such that DNA can be compacted further. Additionally, from what has been demonstrated about chromatin and how it can affect a multitude of biological processes in the nucleus, there is very high potential in researching the local effects of chromatin on surrounding genes in narrower ranges. This is due to how essential transcription and gene expression are to the cell. However, as there is not as much research in the field done regarding it, I would strongly encourage studies in the area. This field of research has a very exciting future with more discoveries to come.

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