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Review

Always on the Move: Overview on Chromatin Dynamics within Nuclear Processes

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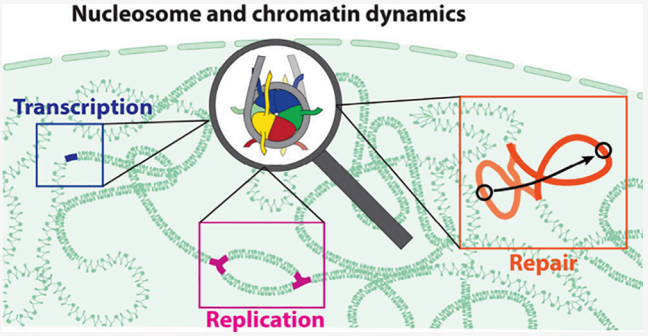
ABSTRACT: Our genome is organized into chromatin, a dynamic and modular structure made of nucleosomes. Chromatin organization controls access to the DNA sequence, playing a fundamental role in cell identity and function. How nucleosomes enable these processes is an active area of study. In this review, we provide an overview of chromatin dynamics, its properties, mechanisms, and functions. We highlight the diverse ways by which chromatin dynamics is controlled during transcription, DNA replication, and repair. Recent technological developments have promoted discoveries in this area, to which we provide an outlook on future research directions.



KEYWORDS: *chromatin dynamics, nucleosome, DNA sequence, chromatin organization*

INTRODUCTION

Each cell in our body contains the same genome, yet each cell performs a specific function. This function is regulated in space (i.e., depends on where the cell is located) and time (i.e., depends on developmental stages). How the genome can integrate this information and simultaneously encode the incredible diversity of functions is a key open question in biology. The answer must lie in how the genome is organized. Chromatin is the packaging system for our genome, which regulates when and where DNA is accessible.[1] Chromatin organization uses repeating structural units named nucleosomes, made of a histone protein core that wraps ±150 base pair (bp) stretches of the genome, locally restraining DNA accessibility.[2−5] These nucleosomes further fold into higher-order structures that functionally organize the genome within the cell nucleus, regulating interactions with myriads of nuclear proteins.[6−9] These diverse layers of chromatin organization regulate the local access to the DNA sequence and the compartmentalization of the genome into functionally distinct domains (e.g., transcribed vs nontranscribed), hereby safeguarding cell identity.[1]

The machineries involved in transcribing, replicating, and repairing DNA have evolved to access its sequence in this chromatin environment, and decades of research have shown that these machineries overcome nucleosomes using a plethora of distinct mechanisms. These mechanisms exploit the dynamic nature of nucleosomes and chromatin.[10−18] Therefore, understanding nucleosome and chromatin dynamics is fundamental to uncovering how cellular functions are sustained. Moreover, this information can also teach us how we may someday tune chromatin dynamics to achieve desired changes in cell function that may be required to treat various diseases and combat aging.

THE DYNAMIC AND COMPLEX NATURE OF NUCLEOSOMES

Nucleosomes are the minimal organizing units of chromatin. Nucleosomes are composed of an octameric core of histone proteins, organized as an $(H3−H4)_2$ tetramer flanked by two H2A−H2B dimers, wrapped by 147 bp of genomic DNA (Figure 1A). Histones are short, positively charged proteins containing a structured histone fold domain that is responsible for their histone−histone interactions within the nucleosome core and unstructured N- and C-terminal tails that protrude outward from the nucleosome core. The DNA wraps around the histone core approximately 1.65 times in a left-handed super helical fashion, entering in proximity of one of the H3 N-terminal and H2A C-terminal tails and exiting at the same site on the opposite nucleosome face (Figure 1A). Through its nucleosomal path, the DNA interacts with the histone core via 14 separate contact points, which organize the Super Helical Locations (SHLs) around the nucleosome dyad (Figure 1A).

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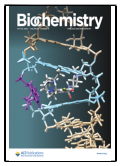

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Figure 1

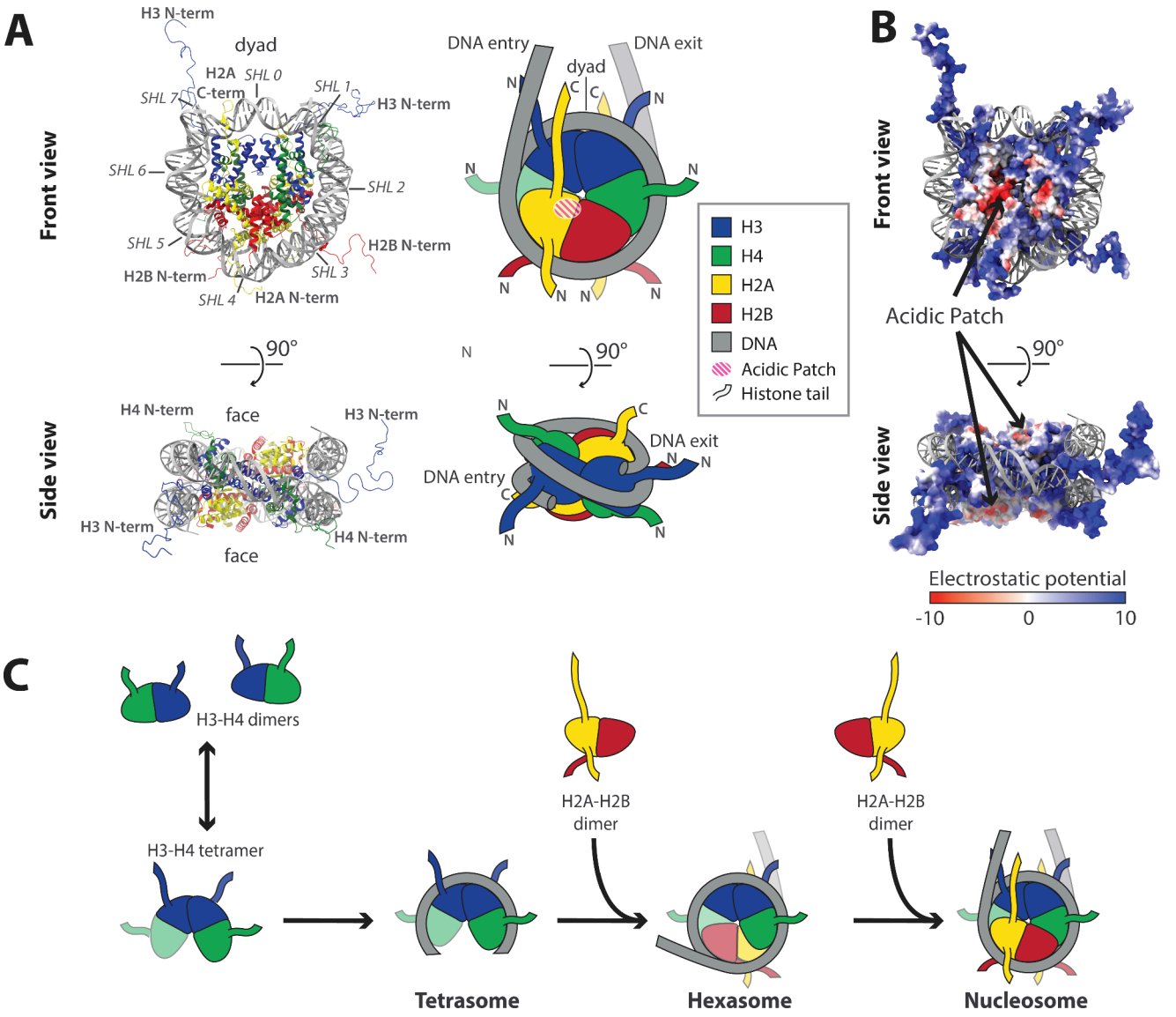

Figure 1. The nucleosome. **A.** Crystal structure (left, PDB 1KX5) and cartoon representation (right) of the nucleosome. Front and side views are shown. The nucleosome dyad (Super Helical Location - SHL 0) is highlighted along with the other SHLs. The DNA entry and exit sites, as well as the histone N- and C-terminal tails, are also shown. A legend of the cartoon is included. **B.** Histone octamer surface electrostatic potential seen from the front and side view of the nucleosome structure (PDB 1KX5, ChimeraX-1.6.1 Coulombic electrostatic potential) shown with its electrostatic potential scale (red, white, and blue for negative, neutral, and positive potential respectively). Arrows point out the nucleosomal acidic patch on the nucleosome faces. **C.** Nucleosomes are assembled in a stepwise manner. First, a $(H3–H4)_2$ tetramer is deposited on DNA to form a tetrasome. Then, the two H2A and H2B dimers are incorporated to form a nucleosome. A hexasome is a nucleosome that misses one H2A–H2B dimer.

The nucleosome faces are characterized by an acidic patch formed by the H2A–H2B dimers on either side (Figure 1B). These features determine the diverse interactions of nucleosomes with nuclear machineries and other nucleosomes, affecting higher-order chromatin organization and its local functions.[3,5,19]

Nucleosome assembly occurs in a stepwise manner. Prior to deposition, histones exist as heterodimers, with H3–H4 being able to tetramerize via the H3–H3′ four-helical bundle, resembling its nucleosomal conformation (Figure 1C).[20,21] This $(H3–H4)_2$ tetramer is deposited onto about 60 bp of DNA, forming a tetrasome, the founding unit of the nucleosome. This is followed by the sequential incorporation of two H2A-H2B dimers on either side of the tetrasome to stabilize the full nucleosome unit (Figure 1C). Nucleosome disassembly occurs in the reverse order, with the sequential removal of H2A–H2B dimers and subsequent removal of the $(H3–H4)_2$ tetramer.[22,23] The kinetics of these assembly and disassembly steps have been well studied in isolated systems where only histone and DNA are present.[22,24–27] However, their physiological kinetics are currently unknown (see Histone Chaperone section below).

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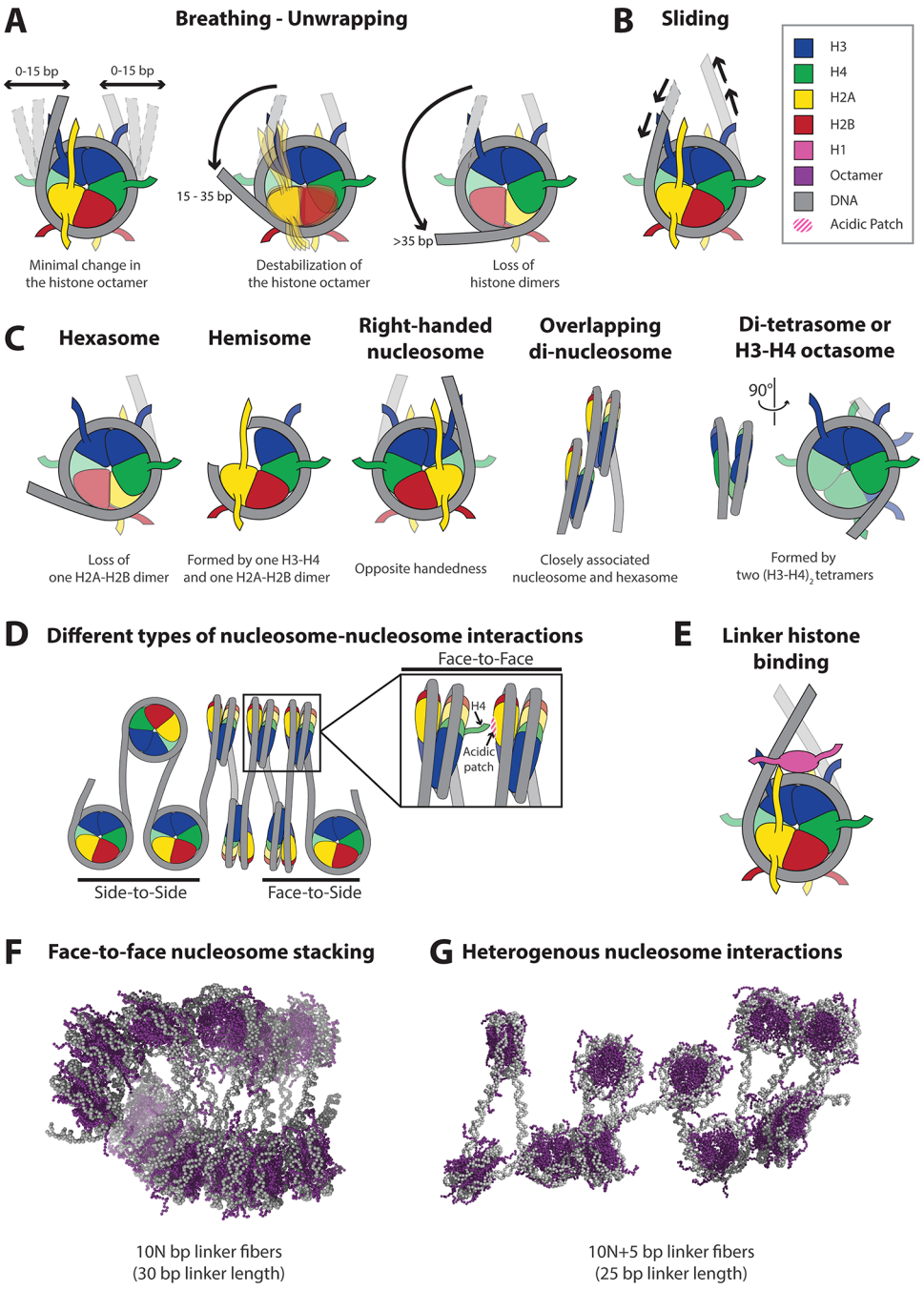

Figure 2. Nucleosome and chromatin dynamics. **A.** Schematic representation of different nucleosome dynamics during breathing - unwrapping. Upon unwrapping of the DNA ends up to 15 bp, the histone octamer remains mostly unchanged, whereas unwrapping of up to 35 bp may cause destabilization of H2A−H2B dimers, inducing their loss when more than 35 bp of DNA is unwrap. **B.** Sliding indicates the movement of DNA along the octamer, to reposition the nucleosome on a different genomic position. **C.** Depiction of different subnucleosomal and noncanonical nucleosome structures: hexasome, hemisome, right-handed nucleosome, overlapping dinucleosome, and ditetrasome. Their key feature is mentioned. **D.** Nucleosome−nucleosome interactions control chromatin fiber organization. Nucleosomes can interact with each other in several ways, namely, side-to-side, face-to-side, and face-to-face. Zoom-in box: the face-to-face arrangement is mediated by the interaction between the H4 tail of one nucleosome and the acidic patch of the adjacent nucleosome. **E.** Linker histones can bind the nucleosome on the dyad position and interact with the linker DNA to control chromatin fiber organization. **F-G.** Simulated structures of chromatin fibers with 10N bp (30 bp, 11 nucleosomes) (F) or 10N+5 bp (25 bp, 12 nucleosomes) DNA linker length (G). The linker length affects the twist of the DNA linker between nucleosomes, resulting in distinct chromatin fiber structures. While the 10N bp array forms an organized fiber characterized by face-to-face interactions between nucleosomes (F), the 10N+5 bp displays a diverse organization with limited stable interactions between nucleosomes (G). The PDB used to generate the figures in panels F and G was a kind gift of Rosana Collepardo-Guevara and Jan Huertas Martin (Chen et al., 2024).

Nucleosomes are intrinsically dynamic.[4] After their formation, nucleosomes can transiently "breathe" or unwrap, and they can slide along the DNA (Figure 2A,B).[23,28−30] Canonical nucleosomes "breathe" about 10 times per second.[31,32] These transient dynamics do not per se jeopardize nucleosome stability, but they do provide opportunities for nucleosome-

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destabilizing events.[4,26,31−34] Unwrapping of the DNA ends enables the binding of protein machineries in proximity to the nucleosome. This is a common mechanism for the initial binding of pioneer transcription factors (TF) to nucleosomal DNA, promoting nucleosome destabilization.[4,35−40] Interestingly, while nucleosomes are symmetric in their composition, DNA unwrapping is asymmetric. Upon unwrapping on one side, the other side is stabilized. The direction of this asymmetric movement is dependent on the DNA sequence, where DNA with lower flexibility is more prone to unwrapping.[41] Recent work demonstrated that such DNA-dependent asymmetry in +1 nucleosomes can either facilitate or hinder transcription, which is in line with other work showing that local DNA mechanics can affect transcription through nucleosomes in proximity of transcriptional start sites.[33,42−44] Nucleosome unwrapping further than 15 bp may lead to the destabilization of H2A−H2B dimers, while the unwrapping of more than 35 bp ultimately leads to the loss of histones within the nucleosome, resulting in noncanonical (sub)nucleosomes (Figure 2A).[29,30] These complexes, such as the hexasome, hemisome, right-handed nucleosome, overlapping di-nucleosome, and di-tetrasome, also called H3−H4 octasome (Figure 2C), have been observed *in vitro*, and evidence for functional roles of such nucleosomes *in vivo* is raising, with interesting implications for their cellular functions (see Transcription section below).[43,45−54] More drastic destabilization of DNA approaching the dyad may lead to full nucleosome disassembly.[27,55] Such drastic dynamics are vital for many nuclear processes that require the passage of molecular machineries through nucleosomes (see DNA Replication and DNA Repair sections below).

As such, nucleosome dynamics sustain nuclear processes, by enhancing, weakening, or changing histone-DNA interactions and/or the interactions with nuclear factors. These ultimately enable DNA replication, transcription, and repair to occur within our genome to maintain cellular life. Therefore, the nucleosome is a versatile, modular, and dynamic structure that structurally and functionally organizes the genome.

THE REGULATION OF NUCLEOSOME STRUCTURE AND DYNAMICS

Dynamics at the nucleosome level are greatly determined by the wrapping of DNA around the histone octamer. As such, DNA and histone sequences and their post-translational modifications (PTMs) have a great influence on nucleosome structure and dynamics.

Our cells express a variety of histone variants, in addition to the canonical histones.[56−61] These variants replace the canonical histones within nucleosomes at specific locations or timing to confer distinct functions. These effects arise from changes in amino acid sequence of these histone variants compared to the canonical histones, directly impacting nucleosome dynamics and/or its interaction with chromatin binding proteins. Some histone variants and their PTMs promote nucleosome dynamics, by increasing the "breathing" of DNA around the histone octamer.[62] For example, the divergent C-terminal tail of the histone variant H2A.Z, which replaces canonical H2A in nucleosomes at transcriptionally active regions, destabilizes the DNA−histone interaction at the DNA-entry and -exit sites.[63] This effect reduces the energy barrier required for nucleosome eviction, which facilitates transcription through the nucleosome.[63] Similarly, the centromere-specific H3 variant, CENP-A, also destabilizes the interaction with the DNA entry and exit sites, via its shorter N-terminal helix.[64] This prevents interaction with linker histones, and likely contributes to centromeric chromatin organization.[65,66]

In addition to variations to the histone sequence, histone tails and their PTMs may also cause effects on nucleosome dynamics.[4,62] The positive charge of histone tails themselves promote the wrapping of DNA around the octamer, facilitated by transient interactions between the histone tails and the nucleosomal and linker DNA.[27,62,67,68] This indicates that while unstructured, the histone tails play a critical role in nucleosome core dynamics. PTMs on these tails are known to tune this potential.[4,62] For example, acetylation of the H3 N-terminus or H2A C-terminus neutralizes the positively charged tails, leading to increased unwrapping of DNA at the entry-exit site, therefore promoting nucleosome dynamics.[69,70] In line with these observations, these modifications are often associated with active and accessible chromatin regions. The direct effect of other histone PTMs on the nucleosome dynamics remains to be explored. However, it is clear that most histone PTMs influence chromatin structure indirectly via action of the effector proteins that they may specifically rectruit.[71] For instance, H3K9 methylation is specifically recognized by the chromodomain of heterochromatin protein 1 (HP1), which acts on the nucleosomal DNA dynamics around the octamer and at the DNA entry and exit sites.[72,73] How these properties promote compaction of chromatin fibers remains to be fully studied, but interestingly phase separation may play a role.[73]

Another factor controlling the nucleosome dynamics is the sequence of DNA around the nucleosome. DNA bending around the octamer distorts the DNA B-form and is dispersed unevenly over the nucleosome. The energetics involved in the distortion of the DNA B-form are sequence-dependent, due to the intrinsic bending propensities and curvature of certain DNA sequences.[74,75] Based on this knowledge, the Widom group established optimized positioning sequences, such as the Widom 601.[76] These sequences have been invaluable for the study of nucleosome structure, and they have provided an important tool for the mechanistic understanding of nucleosome dynamics. However, to understand how nucleosomes behave in the cell nucleus, the use of native DNA sequences is essential.[77] Indeed, recent efforts are elucidating how native sequences shape chromatin dynamics and, in turn, nuclear processes.[78−82]

Finally, DNA topology also contributes to nucleosome dynamics.[83,84] Negatively supercoiled DNA favors nucleosome stability, due to the left-handedness of DNA wrapping around the histone octamer.[83] Changes in DNA topology, that arise when DNA-related machineries (i.e., helicases) approach a nucleosome, may promote nucleosome destabilization by inducing positive supercoiling.[85] This is likely to contribute to overcoming nucleosome barriers during DNA transcription and replication, and they may be used to locally store energy and structural information in the cell nucleus.[84,86−88] Interestingly, the organization of chromatin fibers may contribute to tolerate positive torsion, raising interesting questions to how supercoiling may impact chromatin at different scales.[89] The general lack of tools to measure DNA topology and nucleosome dynamics *in vivo* leaves this area of interest largely unexplored.

Together, the dynamics of the nucleosome are greatly influenced by its components: the histones and DNA. These

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effects within individual nucleosomes ultimately tune chromatin dynamics at a larger scale.

HIGHER-ORDER CHROMATIN ORGANIZATION AND ITS DYNAMICS

Nucleosomes on our chromosomes arrange into a variety of higher order chromatin structures, controlled by nucleosome-nucleosome interactions and nucleosome-binding proteins.[90−95]

The nucleosome-nucleosome interactions driving higher-order chromatin organization depend on the binding of the H4 N-terminal tail of one nucleosome with the acidic patch of the other nucleosome, that allows stacking of nucleosomes in a face-to-face manner (Figure 2D).[96−98] Modifications within the H4 N-terminal tail such as SUMOylation affect internucleosome interactions and thus the formation of chromatin fibers.[96] Similarly, changes on the acidic patch will alter nucleosome−nucleosome interactions.[97] Strikingly, many H2A histone variants have altered acidic patch residues. H2A.Z has an extended acidic region, while H2A.B has a much weaker acidic patch sequence.[99−101] Thus, H2A variants likely control higher order chromatin organization.

In addition to nucleosome−nucleosome interactions, higher-order chromatin organization is controlled by linker histones. Linker histones, such as H1, are proteins containing a winged-helix domain flanked by long positively charged tails.[102−104] Linker histones are positioned on the DNA at the dyad, where they bind the entry and exit DNA stretch, hereby controlling nucleosome dynamics (Figure 2E).[105−110] This binding mode allows linker histones to control chromatin organization, by binding outside of the nucleosome core. Importantly, the different isoforms of linker histone H1 can also bind nucleosomes asymmetrically around the dyad, hereby imparting isoform-specific properties to the orientation and dynamics of the DNA at entry and exit sites.[109] Studying linker histones, their different isoforms, and PTMs is challenging, partially due to redundancy, lack of isoform-specific tools, and cell-type and cell-phase specific effects that are highly local and dynamic. Therefore, information on these key components of chromatin dynamics is only emerging now, and further research will require technical advances.

Recent Highlights in Chromatin Dynamics. Cryo-electron microscopy (Cryo-EM) has recently revealed that H1 binding mode closely crosstalks with the twist and the length of the linker DNA between nucleosomes, to determine the 3D organization of chromatin fibers.[92,93,108] In particular, H1 binding is not compatible with a range of trajectories of the DNA linker between nucleosomes.[95,111,112] So on the one side, H1 promotes the folding of nucleosome arrays by stabilizing the DNA entry/exit site, but there appear to be rules to this effect, that depend on the distance between nucleosomes, also named the nucleosome repeating length (NRL).[95,111] These observations highlight a role for NRL in controlling the stacking of nucleosomes in chromatin fibers. This was recently directly shown by coarse-grained molecular dynamics simulations.[113,114] These studies have now shown that the NRL controls the balance between inter- and intranucleosome interactions in a periodic manner, due to twist deformation energy of the linker DNA between nucleosomes (Figure 2F,G). Specifically, 10N (integer N) bp-long NRLs promote nucleosome−nucleosome stacking within fibers, stabilizing the tight organization chromatin, whereas 10N+5 bp-long NRLs destabilize nucleosome stacking and lead to more interactions between chromatin fibers (Figure 2F,G). This has been functionally linked to the phase-separation properties of chromatin, which contributes to genome compartmentalization.[113,115,116] These studies show that differences in few base-pairs in the NRL have significant consequences for the behavior of chromatin fibers, and these observations may be at the basis of the H1 binding within these fibers. Understanding the principles that organize nucleosomes on the genome have a critical function in also understanding 3D genome organization.[9,117]

PROTEIN FAMILIES THAT CONTROL CHROMATIN DYNAMICS

Chromatin dynamics in cells is mainly controlled by two families of proteins: histone chaperones and chromatin remodelers. Histone chaperones bind histones and control their interactions with DNA to form nucleosomes. Chromatin remodelers use energy from ATP hydrolysis to influence the position of nucleosomes along the genome and in some cases can alter their composition by exchanging H2A−H2B dimers. As these proteins are critical for the assembly and organization of chromatin, it is imperative that we understand how they function.

Histone Chaperones Mediate Nucleosome Dynamics. Nucleosomes cannot spontaneously form inside of our cells. They require histone chaperones for their assembly in physiological conditions.[118−120] Histone chaperones bind histones and shield their positive charges, using short acidic stretches, often intercalated with aromatic residues.[119,121−123] This mode of binding prevents nonspecific interactions of histones with DNA and other proteins, facilitating regulated histone transfer and eventually histone deposition onto DNA.[118,119] Histone chaperone function is purely driven by thermodynamics, and it does not require ATP hydrolysis or any enzymatic reaction.[118] These properties, combined with the hierarchical and modular composition of nucleosomes, underscore the challenge of understanding chromatin assembly at the quantitative level.

The biochemical and physical properties of histone-chaperone interactions have been mechanistically investigated, and these have provided information about the general principles of histone chaperone function. For example, histone chaperones generally interact with histones with tight binding affinities, often within the low nanomolar range.[118] Structural studies have shed light on the interfaces and molecular architecture of some of these complexes.[119] However, how these interactions allow transfer of histones between different histone chaperones and ultimately onto DNA remains to be fully understood. This is challenging to study because of the difficulties in measuring these transitions accurately with currently available methods due to the complexity of the molecules involved and the lack of temporal resolution in the technologies that are available, where histone transfer is often much faster than the readout capacity of existing methods.

Histone chaperones are also highly diverse proteins, where each member of the family displays a unique structural organization and a distinct molecular mechanism of action. This is highlighted, for example, by the diversity in specificity of histone binding. For example, the histone chaperone ASF-1 binds H3−H4 dimers only,[21,124] Spt2 and Mrc1 bind $(H3-H4)_2$ tetramers,[125−128] and CAF-1 and MCM2 can bind a H3−H4 dimer without preventing its tetramerization.[49,123,129,130] Moreover, the histone chaperone NAP1 can

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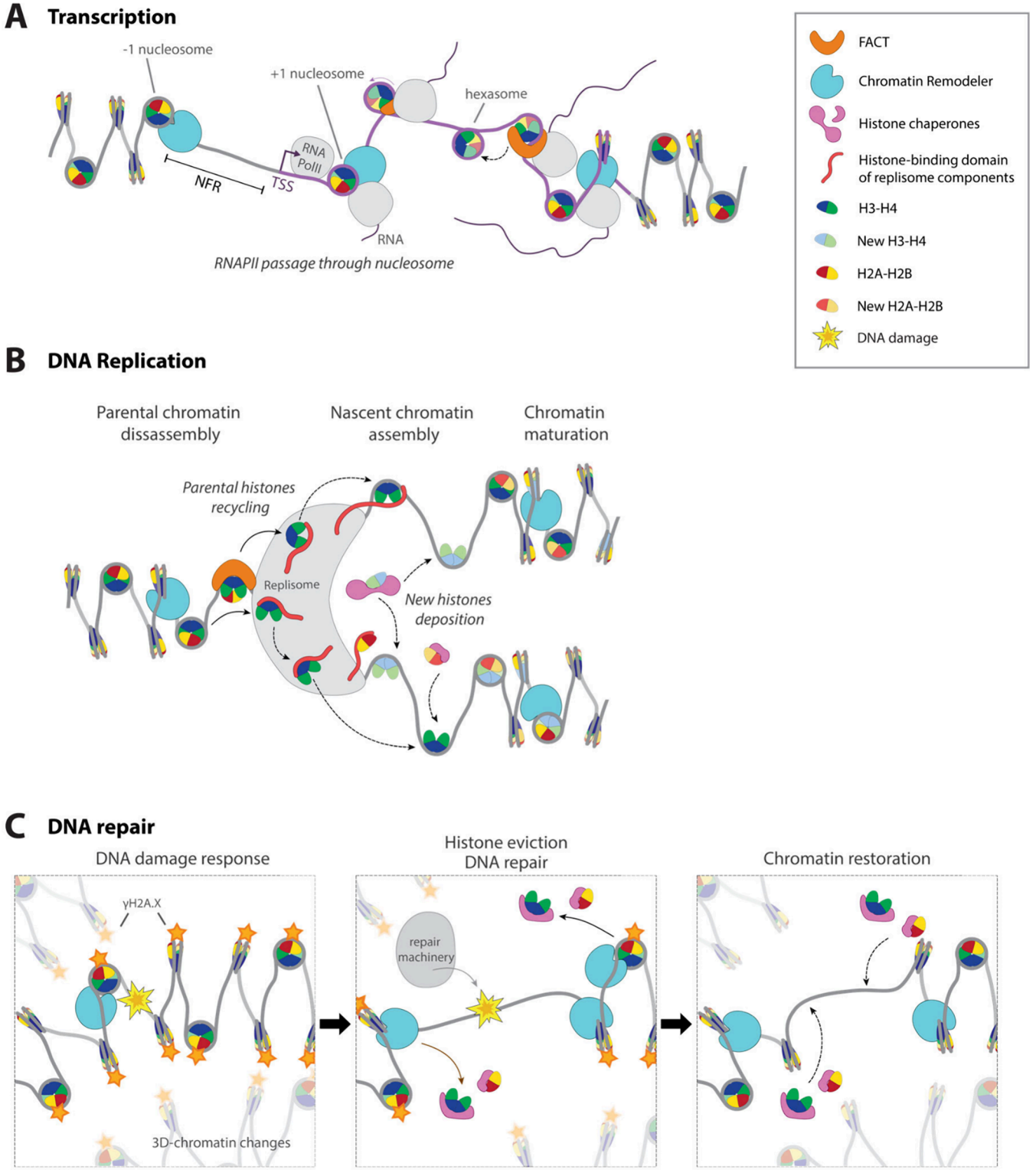

Figure 3. Chromatin dynamics in different contexts. **A.** Chromatin dynamics during transcription. Chromatin remodelers control the positioning of nucleosomes at the Transcription Start Site (TSS), creating a Nucleosome Free Region (NFR) and positioning the +1 and −1 nucleosomes. The RNA polymerase complex (RNAPolII) is transcribed through the nucleosomes, with the support of the histone chaperones FACT and SPT6 (not depicted), and chromatin remodelers. The histones are rapidly transferred from the incoming DNA to the transcribed DNA, with minimal destabilization of the histones from DNA. They may result in H2A-H2B dimer loss, forming hexasomes behind RNAPolII, and they are only slightly shifted from their original position. High frequency of RNAPolII transcription limits the restoration of nucleosomes from hexasomes, resulting in a locally more accessible chromatin landscape. **B.** Chromatin dynamics during DNA replication. Parental chromatin is disassembled ahead of the replisome, and the parental histones are chaperoned by several replisome components to be recycled behind the replisome on newly replicated DNA strands. Separate pathways control the recycling of parental histones on the leading or lagging strand of replication forks (see the text). Concomitantly, newly synthesized (i.e., new) histones are deposited on the replicated strands to maintain nucleosome density. Following the assembly of nascent chromatin, the epigenome is restored by chromatin remodelers and epigenetic complexes (not depicted). **C.** Chromatin

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Figure 3. continued

dynamics during DNA repair. When DNA damage occurs, the surrounding chromatin is marked as part of the DNA damage response pathway. For example, histone H2A.X phosphorylation occurred on Ser139 (gH2A.X). These marks recruit the DNA repair machineries to the site of DNA damage. This process alters the 3D organization of the surrounding chromatin. Generally, nucleosomes are temporarily destabilized leading to histone eviction, to allow DNA repair to occur. Next, nucleosomes are restored on the repaired DNA.

bind either H3−H4 or H2A−H2B in dimeric or tetrameric conformations,[131−133] APLF can bind the histone octamer in its nucleosomal conformation,[122] and the FACT interacts simultaneously with H2A−H2B and a DNA-bound $(H3−H4)_2$ tetramer using independent domains.[134−137] This creates a wide diversity in specificity for histone chaperone mechanisms.

In addition to the histone binding region, histone chaperones contain regulatory domains that regulate the timing and localization of histone chaperone function. These regulatory steps are important to ensure the precise order of histone deposition onto DNA (i.e., H2A−H2B deposition must follow tetrasome formation) and the appropriate location and dynamics in relation to other nuclear processes. These regulatory features lead to much diversity in the histone chaperone family, where virtually every histone chaperone may use a distinct molecular mechanism to perform its function. Therefore, beyond some general principles, there is a variety of histone-chaperone specific mechanisms that need to be unravelled to understand chromatin dynamics in each specific context.[119] It is remarkable that such fundamental processes in life, such as the making of nucleosomes, still remains obscure.

ATP-Dependent Chromatin Remodelers Control Chromatin Organization. Besides the formation of nucleosomes, the chromatin function depends on the position of nucleosomes along the genome. At specific genomic locations such as transcriptional start sites, or at origins of DNA replication, nucleosomes frequently adopt well-defined positions along the DNA.[2,138] This organization can have either stimulatory or inhibitory effects on nuclear processes, such as gene expression, DNA replication, or damage repair, and interestingly, it is often tightly maintained through cellular life, indicating that nucleosome positioning has crucial functions. The positioning of nucleosomes is controlled by chromatin remodelers, which use ATP to slide nucleosomes along the DNA.[139,140] Chromatin remodelers share a conserved ATPase domain that belongs to the DNA helicase superfamily, but they are categorized into four families, based on differences in this domain: SWI/SNF, INO80, ISWI and CHD. Each family regulates chromatin structure through distinct mechanisms fulfilling specific functions.[139−144] Within each family, several remodeler complexes may exist, where a common ATPase subunit form multisubunit complexes with distinct interacting proteins. How the other subunits regulate specificity in timing and localization and what is the degree of redundancy between the different complexes remain to be largely studied.

Recent advances have been made to reconstitute the activities of these complexes and determine their core structures and specificities. For example, the INO80 complex has been recently found to prefer hexasomes over nucleosomes as substrate, changing our understanding of its roles on chromatin.[47,145,146] Moreover, structures of core SWI/SNF complexes have shed light on how these essential factors bind nucleosomes.[147−153]

Beyond these families, noncanonical ATP-dependent chromatin remodelers have been described.[140] One interesting example is SMARCAD1, an ATPase-domain containing protein that can act as a remodeler by sliding nucleosomes in a ATP-dependent manner, as well as a histone chaperone by depositing histones onto DNA in an ATP-independent manner.[154] With this dual function, SMARCAD1 may control chromatin dynamics in a multifaceted way.

An interesting feature of ATP-dependent chromatin remodelers is that they use "barriers" as a reference for the organization of nucleosomes along the genome. Such barriers are often transcription factors, such as CTCF or other tight DNA-binding proteins, or regulatory DNA interacting proteins, such as the origin recognition complex (ORC) that defines DNA replication origins on the genome.[144,155,156] These observations support the idea that nucleosome positioning occurs relative to the large variety of functional events that occur on our genome (i.e., transcription and/or replication) and that is therefore unlikely to be solely determined by the intrinsic properties of histones and DNA *in vivo*. Thus, chromatin dynamics is closely linked to chromatin function, with remodelers playing a central role in this process.

THE CONTEXTS IN WHICH CHROMATIN DYNAMICS MATTERS

In cells, chromatin dynamics is essential to provide access to the DNA for the different molecular machineries involved in DNA transcription, replication, and repair. Because these processes have different functions and use distinct mechanisms, chromatin dynamics have also coevolved with the respective molecular machineries involved.

Transcription. In transcription, chromatin dynamics contributes to determine what genes are transcribed, when, and how much. The mechanisms of transcription through chromatin have been extensively studied and recently reviewed in depth.[14,16] Actively transcribed regions are characterized by an open and organized nucleosome organization, where the promoter is depleted of nucleosomes (i.e., nucleosome-free region, NFR) to allow the recruitment of the RNA polymerase II complex (RNAPolII) (Figure 3A). This local organization is instated by ATP-dependent chromatin remodelers, guided by transcription factors bound at these regions. Once transcription starts, the large RNAPolII must navigate through chromatin along the gene body. Rather than causing full nucleosome disassembly, RNAPolII allows transcription through the nucleosomal DNA by gradual disruption of DNA-histone contacts, handling one nucleosome at the time. This process is assisted by histone chaperones such as FACT, LEDGF and SPT6, as well as chromatin remodelers such as CHD.[157−159] These factors facilitate nucleosome traversal by exploiting the transient unwrapping of the nucleosomal DNA and the destabilization of the H2A-H2B dimers. Incorporation of histone variants at specific regions may aid this process by further reducing nucleosome stability.[57−59,61] Strikingly, RNAPolII is capable of transcription through a nucleosome without completely disassembling the nucleosome. A set of cryo-EM structures captured a variety of active RNAPolII-nucleosome intermediates, revealing the structural basis of

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these mechanisms (Figure 3A).[14,16,160−162] Once RNAPolII reaches the nucleosome dyad, two different outcomes for the nucleosome have been proposed. In one model, the upstream DNA (already transcribed) rewraps around the exposed histone surface to allow direct transfer of the histone octamer from the downstream DNA (not yet transcribed) ahead of RNAPolII, to its wake.[160] In the other model, FACT contributes to this transfer, by dissociating the histone from the downstream DNA.[159] These mechanisms highlight that histones are kept very close to their original location during transcription, with minimal and very transient removal from DNA. In this way, the histones are unlikely to diffuse away from the transcription site and the nucleosome is readily reformed on transcribed DNA.

As transcription in highly active genes involves multiple RNAPolII complexes in very close proximity one after each other, it is plausible that this rapid nucleosome dynamics with minimal histone transfer may prevent nucleosome loss during transcription. It is also interesting to consider that nucleosomes may contribute to the pacing of RNAPolII complexes along the gene.[53] After the transcription machinery has moved through a nucleosome, this can lead to the reassembly of hexasomes instead of nucleosomes.[51,53,159,163,164] It is interesting to consider that the resulting hexasomes may serve two purposes. They may be quicker to remodel than nucleosomes, making it easier to further transcribe.[51] Hexasomes however are also asymmetric and they may create directionality within the system, with distinct effects depending on which H2A-H2B dimer is lost.[53] In this light, chromatin dynamics that occur before and after transcription may contribute to the regulation of gene expression.

DNA Replication. DNA replication ensures the doubling of the genetic material, and during this process, chromatin also needs to be replicated. Chromatin dynamics during DNA replication is a disruptive process (Figure 3B). Nucleosomes ahead of the replication machinery (named the replisome) are disassembled, and the histones are transferred in a highly regulated manner behind the replisome for their reassembly on nascent DNA.[10,17,165−169] $(H3−H4)_2$ tetramers are handled separately from H2A-H2B dimers, indicating that differently than transcription, the histone octamer dissociates during DNA replication (Figure 3B).[170−172] A snapshot of chromatin disassembly ahead of the replisome was visualized by the recent cryo-EM structure of the hexasome-bound FACT complex with the core replisome proteins,[173] confirming that FACT facilitates the progression of DNA replication through chromatin.[174] In addition, it is clear that a handful of replisome proteins contain histone binding domains that control the recycling of the parental histones to the replicated DNA.[17] The discoveries of these dual functions of core replisome components, such as the MCM2, DNA polymerase alpha, DNA polymerase epsilon, CLASPIN/Mrc1, RPA, Ctf4 and DNA polymerase delta, demonstrate that DNA and chromatin replication mechanisms are tightly coupled.[125,127,128,175−183] The histone binding domains of these proteins creates a platform on the replisome for parental histone transfer, where separate mechanisms control leading and lagging strand recycling.[17,169] While some of these histone binding domains have been crystallized with their histone cargos, how these many histone chaperones work together with the replisome remains unclear. Further mechanistic and structural studies of this fascinating machinery will provide insights into these mechanisms.

In parallel to parental histone recycling, replicated DNA is also assembled into nucleosomes that contain newly synthesized histones. These nucleosomes are important to maintain chromatin density, following genome doubling.[10,17,165−168,184] The histone chaperones ASF1 and CAF-1 control this process for H3−H4 histones. ASF1 binds H3−H4 dimers and hands them over to CAF-1, which deposits them onto DNA.[185−188] CAF-1 is recruited to replication forks to specifically act during this process.[189−192] While CAF-1 is not a core component of the replisome, its depletion does have an immediate effect on DNA replication speed, again stressing the close crosstalk between DNA and chromatin during replication.[193−195] The dynamics of H2A and H2B dimers are less understood. While it has been recently shown that parental H2A-H2B are recycled, there is little knowledge on which histone chaperones are involved.[171] This is also the case for newly synthesized H2A and H2B, for which a specific chaperone has not been identified.

Following chromatin assembly, nucleosomes containing newly synthesized histones need to be remodelled and marked by local histone PTMs, to ensure the re-establishment of the local epigenetic landscape along the genome. This chromatin maturation process is controlled by region-specific mechanisms, and requires the action of chromatin remodelers and epigenetic modifiers.[10,17] These complex chromatin dynamics are disruptive for the epigenome, which is first diluted on replicated DNA by the presence of newly synthesized histones and requires careful re-establishment.[17,18] The recent discovery that parental H2A-H2B dimers are also recycled, but independently of H3−H4, has put forward a mix-and-match model that renders epigenome inheritance mechanisms more robust to chromatin dynamics effects.[18,171] Interestingly, these replication-coupled chromatin dynamics may also provide opportunities to tune the epigenome during the cell cycle when necessary, such as during cell fate decisions in development.[196−198] In this light, DNA replication represents the context in which highly disruptive, but tightly regulated, chromatin dynamics are used to ensure accurate copying and inheritance of its epigenetic information.

DNA Repair. DNA can be damaged in many ways, and several mechanisms exist to repair each of these damages specifically. In line with this, distinct mechanisms of chromatin dynamics are expected to take place in each context.[13,199] Generally, chromatin is thought to act in two ways during DNA repair. On the one side, histone modifications are critical signals to trigger the DNA damage response orchestrating the engagement of the appropriate effectors for repair (Figure 3C). This is for example the case for the phosphorylation of the histone variant H2A.X at Ser139 (γ H2A.X),[200,201] or the ubiquitination of H2AK15[202,203] and the methylation of H4K20, which signal and decide repair trajectories.[204−206] On the other hand, chromatin needs to be destabilized to allow access for the DNA repair machineries to the damage site, then reassembled after repair, after which the chromatin landscape needs to be re-established at these sites.[11,13] Therefore, chromatin dynamics in response to DNA damage must strike a balance between allowing signaling and providing accessibility for repair while storing histone-based information temporarily prior to chromatin re-establishment. The access-repair-restore model describes these general principles of chromatin dynamics during DNA repair, yet recent studies have highlighted additional layers of complexity.[11,13,207,208]

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Recent studies that have monitored histone PTMs and effector proteins associated with DNA double strand breaks (DSBs) have shown that these PTMs and effectors are detected well beyond the location of DNA damage. In fact, these are observed hundreds of kilobases up- and downstream of the damage (Figure 3C), and remarkably, they are also seen within the entire topologically associated domain (TAD) where the damage is localized.[11,207,209−212] These large-scale effects also lead to a rewiring of the 3D chromatin organization, with the formation of DSB-specific compartments (D compartment), which has also roles in regulating the transcription of DNA repair genes, providing an important function to these chromatin changes.[211] In fact, DNA damage sites are also known to be relocalized closer to the nuclear periphery, to add to the complexity of the related chromatin dynamics.[13,213] These studies show the penetrant effect that DNA damage has on chromatin modifications and organization, yet the effect on chromatin dynamics specifically, i.e., nucleosomes disassembly and/or destabilization, remains unclear.

Several ATP-dependent chromatin remodelers have been implicated in chromatin rearrangements upon DNA damage.[13,214] These factors are required for providing access to the damaged site, as well as to support a variety of chromatin transactions.[13] Simultaneously to chromatin movements, destabilization of nucleosomal histones has been reported in response to DSBs, which suggests that histones are removed from DNA and stored temporarily during DNA repair.[215,216] This destabilization and storage of histones away from DNA may take longer than in the transcription and replication contexts, as repair kinetics may vary. What factors control this histone dynamics, their storage and redeposition remain to be elucidated. However, it has been proposed that ubiquitin-dependent mechanisms and the INO80 complex are involved.[215,216] It is also expected that histone chaperones play a critical role in this mechanism as they are likely involved in handling the mobilized histones. Once the site has been repaired, nucleosomes need to be reassembled from the stored histones or from new histones. The reassembly of the nucleosomes is mainly controlled by Asf1, CAF-1, and HIRA, and how these factors coordinate their action remains also largely unclear.[11,217,218]

Cells have evolved mechanisms that rewire the 3D genome and control mobilized histone dynamics as DNA is repaired.[13] These are crucial protection mechanisms to avoid severe epigenetic consequences upon the loss of genome stability. There is a lot to discover on how these many events are controlled at the mechanistic level.

NEW TECHNOLOGIES AND OUTLOOK

The last few decades have seen a boom in the development of structural, biochemical, biophysical, and (epi)genomic technologies. These have changed the way we think about chromatin dynamics and provide a wealth of new knowledge and insights. These methodologies are also critical to understand how we can exploit chromatin dynamics to alter cellular function, which would be a game changer in fighting diseases, such as cancer, and aging.

We expect that the advances in cryoEM will continue to bring much needed high-resolution structural data that visualize key interactions and molecular states of the machineries involved in controlling chromatin dynamics. Similarly, single-molecule biophysical measurements, such as optical tweezers or high-speed AFM (atomic force microscopy), are much needed to measure kinetic parameters of these processes. Molecular dynamics simulations have shed light on the forces that drive chromatin dynamics, and progress in these computational approaches is likely to further improve our understanding. These methodologies are further boosted by the increased complexity of biochemical reconstitutions, which can now comprehensively recapitulate chromatin-based mechanisms in test tubes. Prominent examples are the reconstitution of close-to-complete human RNAPolII[159,219] and of yeast and human replisome complexes,[155,174,220,221] which allow unprecedented understanding of the interconnected molecular mechanisms that govern these fundamental processes.

These *in vitro* approaches are complemented by the blooming of microscopy methods, such as cryo-electron tomography (cryo-ET), which provides structural information within cells, at close-to-atomic resolution. Cryo-ET has been used to visualize native chromatin fibers and nucleosome conformations in human cells,[92,222] identify the presence of mono-, di- and trinucleosomes in human heterochromatin,[223] and it has provided insights into the prevalence of non-canonical nucleosomes in yeast.[224] While, currently, the nucleus remains a challenging dense environment for cryo-ET studies, it is expected that upcoming developments in this technology will resolve these challenges, enabling a revolution in our knowledge of chromatin and its organization. Light microscopy approaches, such as expansion microscopy technologies, are also greatly contributing to understanding genome organization. These have enabled the in-depth characterization of chromatin domains and nuclear organization.[90,93,225−230] Technologies that combine light and electron microscopy (cryo-CLEM) could uncover how chromatin compaction, 3D structures, and nucleosome conformations are related, and potentially allow spatial correlation with DNA-dependent processes.[231,232] These technologies have incredible power to change our understanding of the chromatin structure and dynamics *in vivo*.

Finally, epigenomics methods can now provide mapping of chromatin and single nucleosome organization in a single cell, or at a single molecule level. Long-read sequencing approaches are changing our knowledge of chromatin fibers, where we can now monitor subnucleosome dynamics in cells.[43,233−235] Epigenomics has also seen a boost of multimodal methods, where multiple parameters can be analyzed within the same sample, bringing us closer to deriving causal dependencies.[236] Finally, developments toward measuring the temporal aspects of chromatin dynamics in cells, as opposed to taking snapshots of chromatin states, will greatly advance our understanding of these mechanisms and their functions.

These state-of-the-art technologies are bringing a wealth of advances to the field of chromatin dynamics and organization, unlocking questions that could not be pursued until recently. Chromatin dynamics controls all of the genetic and epigenetic processes in our cells. We ought to understand chromatin dynamics and its related mechanisms, as these may provide new ways to control cellular function, treat diseases, and improve lives.[237,238] Integration of expertise and technologies in the chromatin field will shine new light on this process.

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Notes

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