Review

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Transcribing through the nucleosome

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The packaging of DNA into chromatin limits sequence accessibility, which affects all DNA-based processes including transcription. Indeed, the fundamental unit of chromatin, the nucleosome, presents a strong barrier to transcription *in vitro*. Since the discovery of the nucleosome barrier, the question of how the RNA polymerase II (Pol II) machinery overcomes nucleosomes at high speeds *in vivo* has remained a central question in chromatin biology. In this review, we discuss the nature of the nucleosomal barrier to transcription and highlight recent findings that provide new insights into the mechanism of transcription through nucleosomes.

Transcription in the context of chromatin

Virtually all eukaryotic life forms package their DNA into chromatin composed of repeating units of 147 bp of DNA wrapped around eight histone proteins called the nucleosome (see Glossary; Figure 1A). This fundamental structure organizes DNA within the confined space of the nucleus, protects against DNA damage, and provides a structural scaffold for ensuring equal distribution of genetic material during cell division. However, such packaging limits sequence accessibility, which affects all DNA based processes such as repair, replication, recombination, and transcription. Early biochemical studies have established that nucleosomes pose a strong barrier to Pol II at various stages of transcription (Box 1). At the initiation stage, a single nucleosome positioned at the promoter region blocks Pol II loading and formation of the pre-initiation complex (PIC) [1]. Furthermore, a nucleosome downstream of the elongating Pol II is also sufficient to inhibit Pol II elongation *in vitro* [2,3]. Therefore, transcription of a chromatin template *in vivo*, which occurs at speeds comparable to naked DNA templates despite nucleosomal barriers, is a remarkable feat.

Eukaryotic cells have evolved multiple mechanisms to overcome the nucleosome barrier. For instance, many promoters and transcription start sites contain sequences that resist bending and are thus thermodynamically unfavorable for nucleosome formation [4]. These nucleosome depleted regions allow *cis*-regulatory elements increased accessibility to transcription factors and the Pol II machinery. Furthermore, certain transcription factors have gained the ability to bind cognate sites in nucleosomal DNA and induce nucleosome remodeling, either through their intrinsic properties or through recruitment of chromatin remodeling factors [5]. Once Pol II is loaded and begins to transcribe, it encounters an ordered array of nucleosomes [6,7]. How Pol II transcribes through these nucleosomes at high speeds has been a subject of intense research for the past few decades, but recent advances in single molecule techniques and genomic technologies have generated unprecedented insights into this process (Figure 2). In this review, we examine the detailed mechanical and biochemical properties of the nucleosome barrier as observed *in vitro*, and discuss new technologies that have defined the nature of the barrier *in vivo*. We also provide an overview of the cellular players and highlight recent studies that expand our understanding of how Pol II overcomes the nucleosome barrier.

The nature of the nucleosomal barrier to Pol II transcription

Understanding the nature of the nucleosome barrier first requires knowledge of some basic properties of Pol II. Pol II functions as a linear Brownian ratchet, catalyzing the addition of nucleotides one base at a time in a unidirectional manner. However, Pol II is susceptible to intrinsic stalling and backtracking even on bare DNA templates, events that are partly governed by the underlying sequence [8]. When Pol II backtracks, the 3′ end of the

Glossary

+1 Nucleosome: in yeast, this is defined as the nucleosome located precisely at the transcription start site. In higher eukaryotes, the transcription start site is generally depleted of nucleosomes, and the +1 nucleosome is located ~50–80 bp downstream.
Dyad axis: the approximate two-fold symmetry of the nucleosome has an axis of symmetry located at the interface between two H3–H4 dimers.
Histone post-translational modification: these include acetylation, phosphorylation, methylation, ubiquitylation, and ribosylation of histones, which can either alter the interaction between DNA and histones or recruit other factors that modify nucleosomes.
Nucleosome: the fundamental repeating unit of chromatin composed of two each of H3, H4, H2A, and H2B, in which two dimers of H3–H4 form the central tetramer core flanked by two dimers of H2A–H2B on either side. The 147-bp DNA wraps in a left-handed direction forming a mirror-image symmetrical structure with the center located at the H3–H3 dimerization interface called the dyad.
Pol II backtracking: the reversible sliding of Pol II towards the 5′ end of the gene, resulting in dislodgement of the 3′ end of the nascent RNA from the active site.
Pol II stalling: there are various uses of this term in the literature, but for the specific purpose of this text, it is defined as a significant decrease in rate of nucleotide addition by Pol II.

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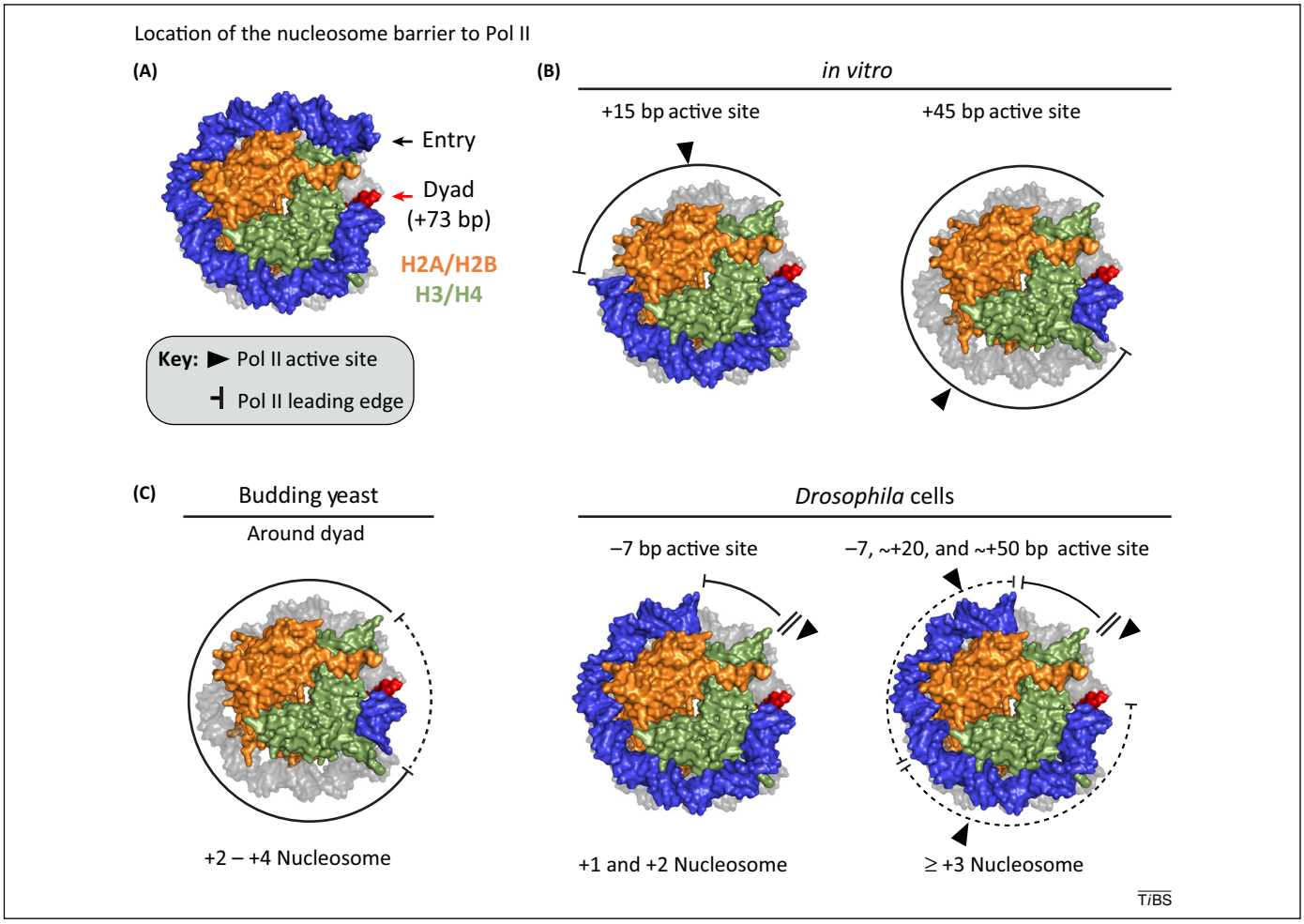

Figure 1. Location of the nucleosome barrier to RNA polymerase II (Pol II). **(A)** The nucleosome. DNA (blue) is wrapped around two dimers each of histones H3–H4 (green) and histones H2A–H2B (orange) in a two-fold symmetry about the dyad axis (red). The bottom half of the symmetrical nucleosome is in gray. The site of Pol II entry into the nucleosome is marked, along with the leading edge and active site of Pol II. **(B)** *In vitro* assays have mapped the locations on the nucleosome that present the strongest barrier to Pol II: shortly after the entry site (+15 bp active site) where the Pol II leading edge reaches the contact between DNA and the H2A–H2B dimer (left), and at +45 bp active site where the leading edge of Pol II reaches the dyad axis (right). **(C)** *In vivo* maps of the nucleosome barrier for budding yeast and *Drosophila*. The nucleosome barrier in budding yeast resembles *in vitro* data. *Drosophila* displays context specificity for the nucleosome barrier where the +1 and +2 nucleosomes (left) presents different sites of Pol II stalling than downstream nucleosomes (right).

nascent transcript is dislodged from the active site, preventing further elongation [8]. Elongation can restart if Pol II randomly diffuses forward to realign the 3′ end or if the backtracked RNA is cleaved [9,10]. This intrinsic stalling and backtracking of Pol II is exacerbated when it encounters any obstacles and can lead to permanent arrest [11]. Hence, transcription through nucleosomes presents a special challenge. Histones form extensive nonuniform interactions with the DNA template and the strength of these interactions define the mechanical barrier that must be overcome. Histone–DNA interactions at the entry and exit sites are particularly weak and are prone to transiently unwrap. By contrast, the contacts at the dyad axis and ±40 bp from the dyad are strongest [12–14]. Thus, *in vitro*, a single nucleosome forms a strong barrier to Pol II that cannot be efficiently overcome and causes the enzyme to stall, backtrack, and permanently arrest [15].

Based on the structural and biophysical studies described above, Pol II would be expected to stall differentially throughout the nucleosome. Indeed, *in vitro* transcription assays that map the single-base location of Pol II on a defined template show that Pol II arrests where the nucleosome contacts are strongest, just in front of the dyad and at the dyad axis [16]. Electrostatic interactions between histones and Pol II itself might also contribute to Pol II arrests [17]. Recently, single-molecule experiments that track real-time trajectories of Pol II demonstrated that the nucleosome causes an increase in both the duration and density of stalls, while significantly decreasing stall-free velocity [18,19]. Furthermore, selective modification of nucleosomes followed by single molecule tracking of Pol II has revealed that Pol II slows down at select regions of the nucleosome corresponding to the entry site, loosely defined as up to –35 bp from the dyad, and the central region that includes the dyad axis [18,19]. Consistent with previous bulk transcription assays, these single molecule studies have also found that the major nucleosome barrier is at the dyad [16,18–20]. Combined, these studies point to a basic mechanism where Pol II, rather than actively separating DNA from histones, must wait for fluctuations in the interaction between DNA and histones to transcribe the nucleosome *in vitro* [19].

Despite the recent advances in defining the nature of the nucleosomal barrier *in vitro*, investigation of this process

Box 1. Pol II stages and the transcription cycle

The transcription cycle begins at the initiation stage when, in response to inter- and intra-cellular signals, sequence-specific transcription factors bind to promoters and enhancers [94]. This binding event triggers the hierarchical recruitment of general transcription factors along with Pol II to form the pre-initiation complex (PIC). PIC maturation leads to the melting of promoter DNA to form the transcription bubble, and to loading of the template strand onto the active site of Pol II. At this stage, the active complex remains somewhat unstable such that transcription of the first 10 base pairs typically results in backtracking, arrest, and re-engagement, which is called abortive transcription. After a certain RNA length, the RNA–DNA–Pol II complex is stabilized, and Pol II proceeds to dissociate from the PIC in a process termed promoter clearance. For many metazoan genes, Pol II pauses at ~30–50 bp downstream of the transcription start site (TSS), awaiting further signals during the promoter proximal pausing stage. Once Pol II escapes the pause site, it enters the elongation stage and elongates efficiently across the gene 3′ end and the poly-adenylation site. During the termination stage, Pol II disengages from the DNA, and the whole process can start anew [94](Figure I).

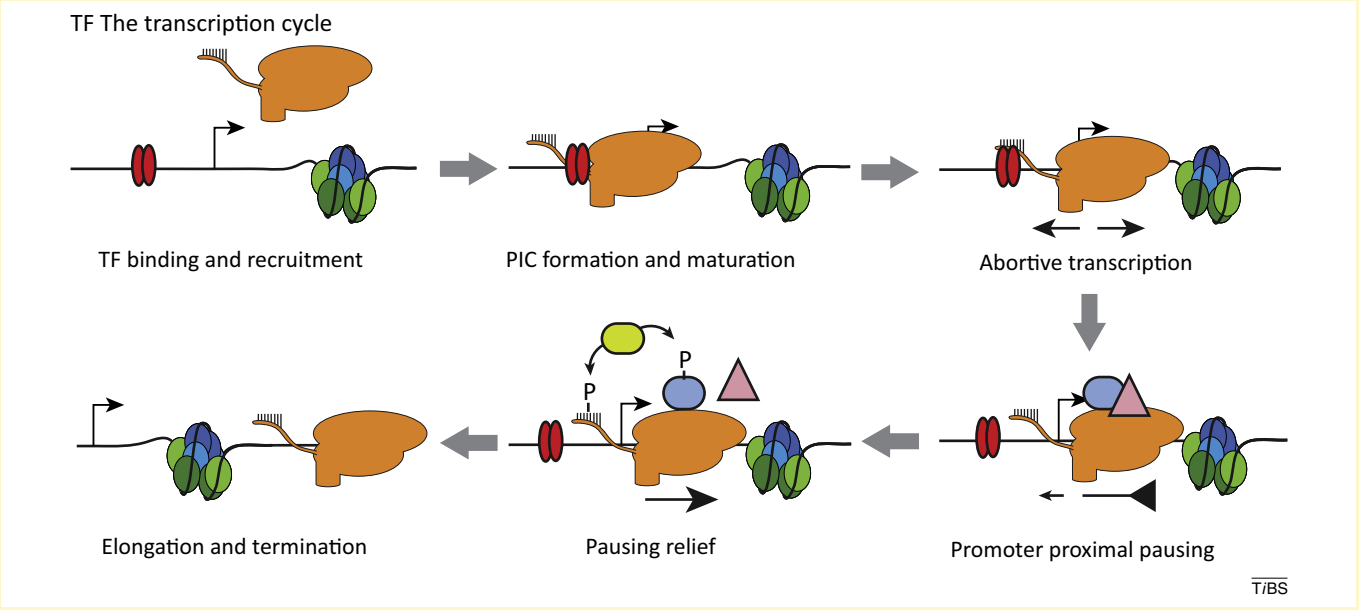

Figure I. The transcription cycle. Pol II cycles through the different stages of transcription as described in Box 1.

in vivo has been hampered by the lack of technology with sufficient resolution to quantify Pol II trajectory within cells. However, recent advances in genomics technology now enable measurements of Pol II positions at single-base resolution *in vivo*. These new approaches, first with native elongating transcript sequencing (NET-seq) [21], and later with precision nuclear run-on sequencing (PRO-seq) and 3′ end of nascent transcripts (3′NT) [22,23], determine the precise position of Pol II genome-wide by mapping either the last base incorporated (NET-seq and 3′NT) or a single base run-on product (PRO-seq). These precise maps of Pol II can then be compared with nucleosome positions to examine the nucleosome barrier *in vivo*. One surprising discovery resulting from these new methods is the context-specificity of the nucleosome barrier *in vivo*; that is, depending on the genomic location of the nucleosome, the position and magnitude of the barrier differs. The application of 3′NT in *Drosophila* cells show that the consensus nucleosome barrier location for all genes was only ~13 bp into the nucleosome, a site not observed *in vitro*, whereas nucleosomes over gene bodies (third nucleosome and beyond) also had barriers in front of and near the dyad, similar to *in vitro* studies. Furthermore, the magnitude of the barrier was found to be approximately three times higher at the +1 nucleosome than at downstream positions, causing extensive Pol II backtracking [23]. Intriguingly, this context-dependency is not observed in budding yeast. NET-seq experiments in highly expressed budding yeast genes revealed that the nucleosome barrier *in vivo* resembled the trend observed *in vitro*, such that Pol II stalls most near the dyad [21]. However, using nucleosome position defined by chemical cleavage for all genes revealed a periodic pausing throughout the nucleosome reflecting the helical turn of DNA throughout the nucleosome [24]. The difference in nucleosomal barrier between budding yeast and *Drosophila* can likely be attributed to differences in chromatin architecture. The +1 nucleosome in budding yeast is typically located at the transcription start site of most genes, and is generally displaced during Pol II loading, which prevents analysis of Pol II transit. In metazoans, the +1 nucleosome is much further downstream, and in *Drosophila*, it is the largest barrier. This hypothesis is supported by the fact that in *Drosophila* barriers of downstream nucleosome positions more closely resemble the trend observed in yeast and *in vitro*.

Future studies are needed to define why the +1 nucleosome is a much larger barrier in metazoans compared with downstream nucleosomes. One possible explanation is that Pol II requires a set of elongation factors in order to efficiently transcribe through any nucleosome. Recruitment of these factors may be tightly regulated, perhaps through phosphorylation of the C-terminal domain of Pol II [25]. Therefore, by being the first barrier, the metazoan +1

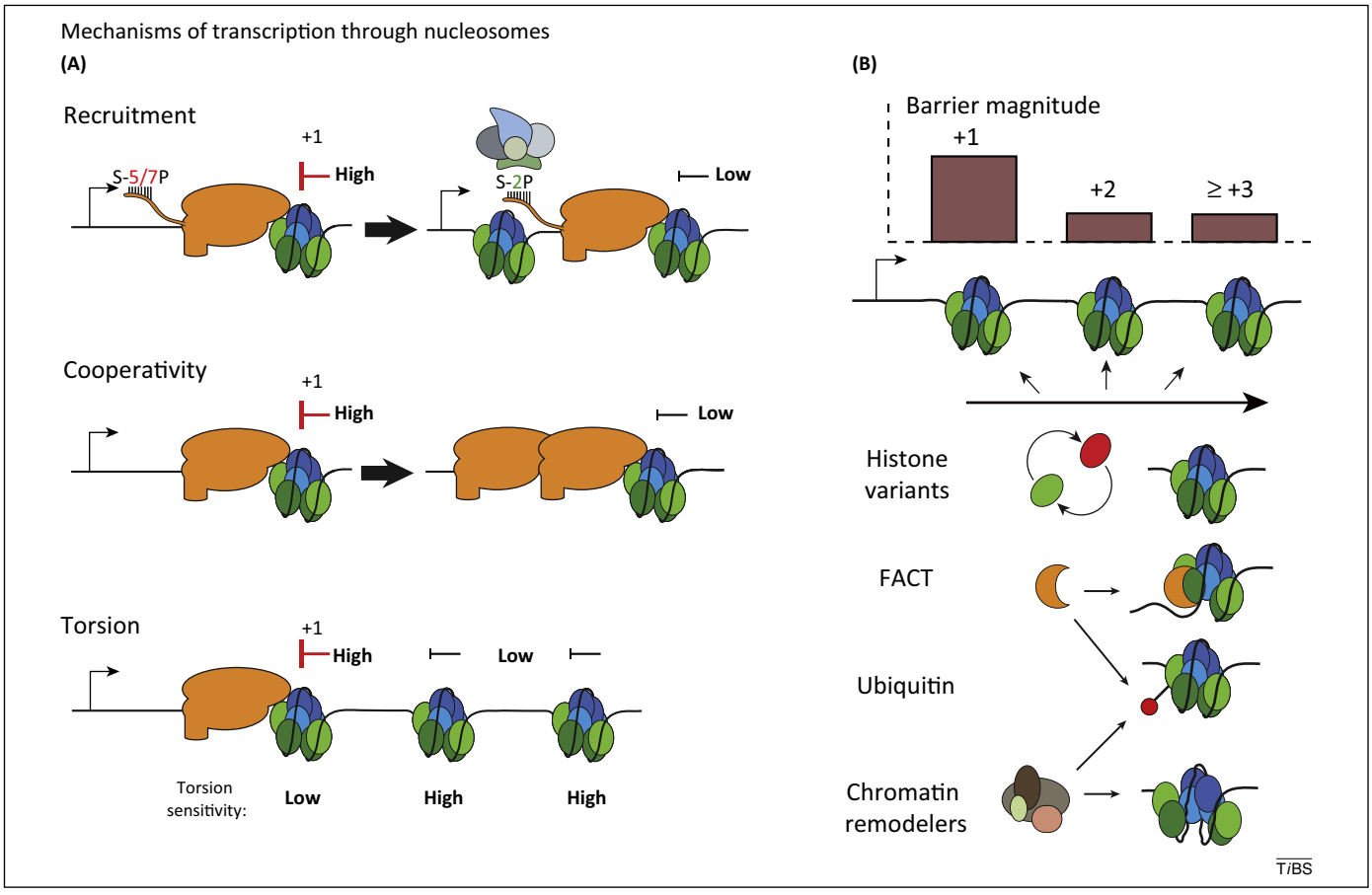

Figure 2. Mechanisms of transcription through nucleosomes. **(A)** Potential models for the context-specificity of the nucleosome barrier for metazoans *in vivo*. **(B)** General mechanisms for modulating the nucleosome barrier. In metazoans, the +1 nucleosome presents the strongest barrier to RNA polymerase II (Pol II). Several mechanisms exist *in vivo* to modulate the barrier and facilitate Pol II transcription, including histone modifications, histone variant replacement, histone chaperones, and nucleosome remodelers. These mechanisms affect nucleosomes in general but may contribute to context specificity depending on other factors that affect targeting of function. Abbreviations: FACT, FAcilitates Chromatin Transcription.

nucleosome might block Pol II until the necessary elongation factors and chromatin remodelers are recruited and allow full maturation of the elongation complex. This might serve as a redundant mechanism to ensure that Pol II is fully competent before transcription proceeds further and to prevent transcriptional arrest downstream, which could result in genome instability [26] (Figure 2A, top). An alternative explanation for the large +1 nucleosome barrier comes from *in vitro* work showing that two Pol II complexes can cooperatively overcome the barrier [27,28] (Figure 2A, middle) In this model, the first transcribing Pol II evicts a dimer from the +1 nucleosome. The passage of a second Pol II then destabilizes the +1 nucleosome further and results in complete eviction. The presence of a second Pol II may also prevent backtracking of the first Pol II and facilitate a more efficient elongation. This mechanism may serve as a regulator of transcription level such that lowly transcribed genes become more susceptible to the nucleosome barrier. A third possibility relates to the topological state of DNA. As Pol II transcribes along the DNA template, it induces positive supercoils in front and negative supercoils behind [29] (Figure 2A, bottom) (discussed later). Positive supercoiling is known to destabilize nucleosomes [30–32] and differences in the topological state may determine the magnitude of the barrier. For example, the +1 nucleosome may be a larger barrier because less positive torsion has accumulated due to a shorter distance travelled by Pol II. These potential roles of the +1 nucleosome as a context-specific barrier are not mutually exclusive and may in fact work synergistically to ensure proper Pol II transcription.

In vitro studies have shown that nucleosomes are barriers to Pol II transcription, and *in vivo* studies have revealed that this barrier is context-specific. How then do cells overcome this barrier to ensure efficient and well-regulated transcription programs?

Modulating the nucleosome barrier

The recent findings described in the previous section suggest that mechanisms must exist *in vivo* for modulating the nucleosomal barrier so that transcription proceeds efficiently. These mechanisms fall into three broad classes: mechanisms that alter nucleosomes (chromatin modifiers), mechanisms that mobilize nucleosomes (chromatin remodelers), and mechanisms that facilitate Pol II activity (elongation factors) (Figure 2B). Recently, the structure of DNA itself has emerged as a mediator of nucleosome dynamics that can also affect the strength of the barrier. In the next sections, we discuss the most recent discoveries in each category and their contributions to modulating the nucleosome barrier.

Histone modifications and variants

H2Bub1. One of the best studied mechanisms for modulating the barrier acts by altering the histone–DNA contacts within the nucleosome through post-translational modification of histones [33,34]. Much of the research has focused on H3 modifications and their strong correlation with transcription, and recent reviews provide extensive coverage of the potential role of these modifications in transcription. However, in recent years, mono-ubiquitylation of H2B (H2Bub1) has emerged as a major yet understated player in modulating the nucleosome barrier.

H2Bub1 levels are dynamically regulated within the cell, with the ubiquitin-ligating complexes and deubiquitylating enzymes conserved from yeast to humans [35,36]. This modification has been implicated in many processes, including tumor suppression, DNA recombination, repair, co-transcriptional mRNA splicing, and transcription [37,38]. Perhaps its most recognized function, H2Bub1 is required for the methylation of histone H3 at lysine 4 and 79 (H3K4 and H3K79), modifications that mark active promoters and gene bodies, respectively [39]. However, H2Bub1 has transcription elongation functions independent of H3 modifications. The addition of ubiquitin on H2B stabilizes the nucleosome both *in vitro* and *in vivo*, which seems contradictory to its elongation function [40]. However, H2Bub1 also stimulates the activity of the histone chaperone FAcilitates Chromatin Transcription (FACT) [41,42], one of the minimally required factors for Pol II elongation through chromatin templates (discussed later), and ubiquitylation levels are dependent on the nucleosome remodeler Chd1 (discussed later) [43]. These characteristics suggest that H2Bub1 aids Pol II elongation by stimulating nucleosome remodeling ahead of Pol II and facilitating nucleosome reassembly behind Pol II through FACT and Chd1. In support of this model, a budding yeast strain harboring the mutant *H2BK123A*, which is unable to be ubiquitylated, shows reduced nucleosome occupancy in highly expressed genes [39]. A similar mutant in fission yeast also results in reduced Pol II levels within the bodies of active genes, which is mediated through a positive feedback interaction between the transcription elongation factor P-TEFb and H2Bub1 [44], thus further implicating H2Bub1 in transcription elongation. Therefore, in addition to mediating histone H3 methylation at various lysine residues, H2Bub1 modulates the nucleosome barrier to facilitate Pol II elongation.

In addition to modulation by histone modifications, the nucleosome barrier can also be altered by incorporation of histone variants. In contrast to canonical histones, histone variants are expressed and deposited into nucleosomes in a replication-independent manner [45]. Several histone variants exist with cell-specific functions. In the next sections, we focus on the involvement of two histone variants, H3.3 and H2A.Z (Box 2), in facilitating transcription through the nucleosome.

H3.3. Because of its expression pattern outside of DNA replication, it has long been assumed that the histone variant H3.3, which differs from canonical H3 by only 4–5 amino acids, is simply a replacement histone when nucleosomes are disrupted during DNA-templated processes such as transcription [46]. Indeed, nucleosome turnover in *Drosophila* correlates strongly with H3.3 levels, and is dependent on active transcription [47,48]. This replacement pathway is proposed to maintain chromatin integrity and protect from DNA damage during nucleosome-disrupting events [49]. However, some evidence suggests a more active role of H3.3 in facilitating transcription. For example, activation of signal-responsive genes, such as those activated by retinoic acid [50], interferon-gamma [51], UV [51], and heat shock, is dependent on H3.3 deposition [52]. Furthermore, transcription recovery at sites of UV damage requires H3.3 deposition by the H3.3-specific chaperone HIRA [53]. These studies suggest that the replacement function of H3.3 may be separate from its transcription-modulating function, and that activation of signal-responsive genes may be more susceptible to H3.3-modulating functions. It still

Box 2. Histone variants H3.3 and H2A.Z

The histone variant H3.3 differs from canonical H3 by only 4–5 amino acids (Figure I). Despite this high degree of similarity, these histones differ in chaperones, interacting proteins, temporal deposition, and distribution throughout the genome [95]. Canonical H3 is expressed only in the S phase and interacts with the chaperone CAF1 for replication-coupled deposition onto newly synthesized DNA. By contrast, the H3.3 variant is expressed throughout the cell cycle, and the H3.3-specific HIRA chaperone complex deposits H3.3–H4 onto DNA in a replication independent manner primarily in transcribed regions. Recently, H3.3 has also been shown to interact with the histone chaperone DAXX/ATRX to deposit H3.3 in telomeres and heterochromatin regions [96,97]. When incorporated into nucleosomes, H3.3 imparts different biochemical properties. For example, H3.3 decreases the interaction between adjacent nucleosomes such that chromatin compaction is impaired [50]. Furthermore, mutations of *H3.3* to the canonical H3.2 sequence alters its genome-wide distribution patterns [98], suggesting that these 4–5 amino acids are sufficient to impart differences in function and specificity.

The histone variant H2A.Z diverged from canonical H2A early in eukaryotic evolution, and is thus highly conserved across eukaryotes [99]. In metazoans, H2A.Z is essential for viability [100]. Similar to H3.3, H2A.Z is expressed throughout the cell cycle, and is deposited into chromatin in a replication-independent manner by H2A.Z-specific chaperones [99]. The Swr1 complex catalyzes the exchange of H2A–H2B dimers with H2A.Z–H2B dimers, whereas the INO80 complex catalyzes the reverse reaction [101,102]. H2A.Z is enriched in nucleosomes surrounding TSSs, and in metazoans, is roughly correlated with gene expression [45]. However, this variant has also been implicated in formation of constitutive heterochromatin, maintenance of heterochromatin boundaries [103,104], and proper centromere function [105].

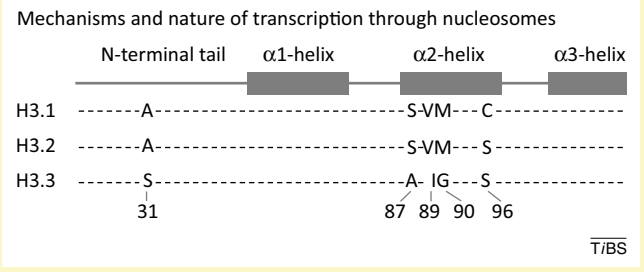

Figure I. H3 variants. The differences in amino acid between H3.1, H3.2, the canonical histones, and H3.3, the replication independent variant, are shown.

remains unclear, however, exactly how a difference of 4–5 amino acids between the two histones can impart such dramatically different functionalities. One mechanism for effecting specificity may be through protein–protein interactions mediated by H3.3-specific chaperones. For example, HIRA interacts with a histone methyltransferase WHSC1, which in turn interacts with transcription elongation factors, providing a direct link between H3.3 deposition and Pol II elongation [51]. The exact significance of H3.3 in facilitating transcription still remains unclear, especially given that *Drosophila* flies survive without H3.3 (although they are sterile) [54,55].

H2A.Z. The histone variant H2A.Z is highly conserved across eukaryotes, yet displays only ~60% similarity in amino acid sequence to canonical H2A. The differences include a unique C-terminal tail, an altered docking domain that is predicted to weaken interaction with the H3–H4 tetramer, and an extended acidic patch located at the surface of the histone octamer. These structural differences appear to contribute only minor differences in the stability between canonical H2A and H2A.Z-containing nucleosomes, however this remains controversial [56]. H2A.Z nucleosomes are enriched around transcription start sites (TSSs). The SWR1 complex that deposits H2A.Z–H2B dimers onto nucleosomes has a preference for nucleosome-free sequences in promoters, suggesting a mechanism for H2A.Z targeting in and around TSSs [57,58]. In *Drosophila*, incorporation of H2A.Z at the +1 nucleosome lowers the barrier to Pol II elongation and decreases Pol II stalling [23]. Exactly how H2A.Z facilitates Pol II progression through the nucleosome remains unclear. A recent study shows that in budding yeast, one outcome of exchanging H2A for H2A.Z is an increased rate of nucleosome turnover, suggesting that facilitated exchange provides an opportunity for Pol II elongation, resulting in nucleosome turnover [58]. This is consistent with previous studies of H2A.Z function in budding yeast, where the loss of H2A.Z from nucleosomes of silent genes is required for their transcriptional activation [59]. Furthermore, H2A.Z deletion results in decreased rate of Pol II elongation [60]. However, in metazoans, H2A.Z appears to have different functions albeit with similar outcomes. In *Drosophila*, H2A.Z-containing nucleosome occupancy correlates with decreased nucleosome turnover [23], and in mouse ES cells, the loss of H2A.Z–H2B dimers is required for activation of retinoic acid inducible genes [50]. The recent characterization of a mammalian chaperone specific to H2A.Z, ANP32E, provided evidence for the removal of H2A.Z from nucleosomes on and near the TSS [61,62]. H2A.Z dynamics facilitate nucleosome depletion and exposure of transcription factor binding sites for efficient gene activation [63,64]. Therefore, despite the ambiguities surrounding the physical properties of H2A.Z-containing nucleosomes, the emerging role of H2A.Z in facilitating Pol II transit is to increase accessibility of nucleosomal DNA through dynamic turnover of H2A.Z–H2B dimers.

Chromatin remodelers and cofactors

In addition to histone modification and variant replacement, several chromatin interacting factors participate in modulating the nucleosome barrier, including histone chaperones, nucleosome remodelers, and Pol II elongation factors.

Biochemical approaches identified FACT as among the minimal factors required for *in vitro* transcription of chromatin templates [65]. *In vivo*, FACT has been shown to affect multiple stages of transcription. At the initiation stage, FACT participates in maintaining a dynamic nucleosomal environment in promoter regions [66]. During elongation, FACT travels with Pol II and functions to reassemble nucleosomes behind the elongation complex and prevent cryptic transcription initiation [67]. Despite the consensus that FACT is important for transcribing nucleosomal DNA, the precise mechanism by which FACT modulates the barrier remains unclear. Biochemical evidence suggests that FACT functions as a histone chaperone with a strong preference for H2A–H2B dimers [68,69] mediated by the N-terminal tail of H2B [42]. Purified human FACT has been shown to displace an H2A–H2B dimer from nucleosomes [68], leading to the proposed model that FACT evicts a dimer ahead of Pol II to destabilize the nucleosome, and deposits the dimer behind the elongation complex to maintain chromatin integrity. Recent studies, however, suggest that FACT may not require dimer eviction to modulate the barrier during transcription. For example, FACT enhances accessibility of nucleosomal DNA to endonucleases without H2A–H2B displacement [70], suggesting that FACT may disrupt histone–DNA contacts within the nucleosome. *In vitro* transcription assays also show that FACT relieves the major stall site of Pol II on the nucleosome dimer–tetramer interface equally well on native or cross-linked histone octamers [71]. However, the same study showed that FACT-mediated transcription requires the presence of H2A–H2B dimers on the transcribed nucleosome, leading the authors to suggest a model whereby FACT destabilizes histone–DNA contacts to allow Pol II progression, and, through its interaction with the H2A–H2B dimer, facilitates the survival of the transcribed nucleosome [71]. Further research is required to fully characterize the mechanisms of FACT action, but the global role of FACT in modulating the nucleosome barrier to facilitate Pol II progression remains consistent (for recent reviews on FACT, see [66,72]).

Histone chaperones facilitate dimer exchange, but certain enzyme complexes act on the whole nucleosome. Indeed, the realization that nucleosome remodeling complexes can use ATP to move, slide and evict nucleosomes, and to exchange nucleosomal subunits, can potentially resolve the paradox of the nucleosome barrier *in vivo* (Box 3). Although we know the various members of each remodeler family, how these remodelers function at a biochemical level, and where they act in the genome, their precise function in facilitating Pol II progression *in vivo* remains unclear because of the built-in redundancy among the various nucleosome remodeling complexes. For example, in budding yeast, the remodeler RSC (Remodels the Structure of Chromatin) of the SWI/SNF (SWItch/Sucrose NonFermentable) family is the only remodeler required for viability [73], and individual deletions of other remodelers showed very little effect on gene expression. Among the four major subfamilies of nucleosome remodelers, perhaps the least understood is the CHD (Chromodomain Helicase

Box 3. Nucleosome remodelers

Nucleosome remodelers use energy from ATP hydrolysis to assemble, evict, or slide nucleosomes. There are four main subfamilies of remodelers as classified by their conserved ATPase domain: SWI/SNF, ISWI, INO80, and CHD. The conserved SWI/SNF subfamily disrupts chromatin structure by evicting nucleosomes. By contrast, most members of the ISWI subfamily of remodelers assemble nucleosomes and slide them into regularly spaced arrays. The INO80 subfamily, including Swr1, also spaces nucleosomes [106], but is better known for its function in H2A.Z deposition and removal. The CHD subfamily has also been shown to regularly space nucleosomes, but the precise function of its members remains unknown [107].

One role of nucleosome remodeling in facilitating gene expression is best exemplified at the yeast *PHO5* promoter chromatin. Under normal growth conditions, the *PHO5* gene is silenced, with well-positioned nucleosomes covering the TSS and promoter regions of the gene. *PHO5* becomes highly activated upon phosphate starvation, but nucleosome remodeling at the promoter is a prerequisite for gene activation. The search for nucleosome remodelers that facilitate the removal of nucleosomes in *PHO5* promoter has revealed an extensively redundant network involving members of all four subfamilies of remodelers. SWI/SNF and INO80 cooperate to remodel promoter chromatin to activate the gene, but the absence of either or both remodelers is insufficient to prevent opening of the promoter region and gene activity. Deletion of Isw1 and Chd1 did not fully inhibit promoter remodeling or gene activation. Recently, RSC, the only essential remodeler in yeast, has been shown to play a crucial role in *PHO5* promoter remodeling. Although these studies provide evidence for remodeler redundancy at the initiation stage of transcription, it is likely that such redundancy is also a factor in remodeling genic nucleosomes to allow efficient Pol II elongation [108].

DNA binding) subfamily. In budding yeast, Chd1 has been shown to act with chromatin remodelers Isw1 and Isw2 to maintain evenly spaced nucleosomes within gene bodies and repress cryptic transcription [74,75] by preventing histone loss [76], suggesting an inhibitory role of Chd1 in transcription. However, Chd1 has also been shown to facilitate nucleosome turnover at the 5′ end of genes in *Drosophila* and yeast, while increasing stability of nucleosomes at 3′ ends [77], providing further clues to its role in transcription. Recently, studies in mouse embryonic fibroblasts show that Chd1 is recruited to promoters of actively transcribed genes, where it facilitates the majority of transcription-mediated nucleosome turnover [78]. The catalytic activity of Chd1 is also required for efficient Pol II escape from promoters [78], suggesting that nucleosome remodeling by Chd1 alleviates a strong barrier to Pol II progression. Despite the uncertainties regarding function and mechanism, Chd1 is emerging as a key player in modulating the nucleosome barrier to transcription.

Although most of the mechanisms described thus far act on nucleosomes to modulate the barrier, other factors also act on Pol II itself. For example, several Pol II-associated factors decrease intrinsic stalling of Pol II on both DNA and nucleosomal templates, and thus decrease the rate of backtracking and arrest. This is primarily facilitated by the transcription factor S II (TFIIS), which facilitates cleavage of the nascent RNA in arrested Pol II to realign the active site with the 3′ end of nascent RNA through synergistic cooperation with transcription factor F II (TFIIF) [15] and the Mediator complex [79] to reduce the probability of Pol II stall entry and decrease overall stall duration [80,81]. By modulating Pol II processivity, TFIIS, TFIIF, and the Mediator complex facilitate transcription through the nucleosome [80]. Another TFIIS interacting complex, the polymerase-associated factor complex (Paf1C), has also been shown to regulate Pol II elongation [82]. Paf1C co-localizes with Pol II at gene promoters and mediates transcription-coupled histone modifications, providing a connection between Pol II- and nucleosome-directed barrier modulation. In particular, Paf1C is required for H2B mono-ubiquitylation [83]. Recently, Paf1C has been shown to facilitate transcription through chromatin in a synergistic cooperation with TFIIS, suggesting that Paf1C may also function to modulate the nucleosome barrier independent of histone modifications [82]. These studies suggest cooperative interactions between Pol II-associated factors and chromatin modifying complexes to alleviate the nucleosome barrier.

DNA topology and nucleosome dynamics

Studying the modulators of the nucleosome barrier has largely focused on histones and chromatin co-factors, but recently, DNA itself is emerging as a potential player in nucleosome structure and stability. During transcription, the melting of promoter DNA and subsequent translocation of the Pol II machinery generates bidirectional torsional forces: positive torsion ahead of and negative torsion behind the elongating Pol II [84] (Box 4). Recent biochemical, single-molecule, and *in vivo* characterizations of torsion during transcription have revealed a dynamic role for DNA structure and topology in facilitating transcription through nucleosomes. First, torsional stress generated during transcription can inhibit Pol II activity by increasing Pol II stall frequency and duration [85]. Second, DNA topology is intricately connected with nucleosome structure and stability. Early nucleosome assembly studies showed that negative supercoiling promotes assembly whereas positive supercoiling inhibits it [86]. Studies that examine the effects of torsion on already assembled nucleosomes, however, had varying results. Electron microscopy showed that nucleosomes possess structural plasticity when subjected to increasing torsional stress, undergoing chiral transitions to mitigate its damaging effects [87,88]. Nucleosomes also lose H2A–H2B dimers when torsion is increased [89], suggesting a possible mechanism for loosening the nucleosome barrier. Recently, methodological advances in detecting torsional states *in vivo* have provided an unprecedented genome-wide view of transcription-generated DNA supercoiling, from the surrounding gene bodies [90,32] to large supercoiling domains [91]. As seen *in vitro* and in early single-gene studies [92,93], accumulation of DNA supercoiling during transcription inhibits Pol II progression [32]. Furthermore, torsional stress induces large scale chromatin re-organization and movement [91], and more pertinently, results in increased nucleosome dynamics within transcribed genes [32]. Combined with the *in vitro* characterization of torsion, these studies suggest that transcription-generated torsional stress destabilizes nucleosomes ahead of Pol II to facilitate elongation and promotes nucleosome reassembly behind to maintain chromatin integrity.

Box 4. Twin supercoil domain model

The double helical structure of DNA, with two strands intertwined, is subject to torsional forces that affect DNA twist, defined as the number of times the two strands wrap around each other, and DNA writhe, defined as the coiling of the double strand about itself [109]. In a relaxed state, DNA has one twist for every ~10 bp, with the writhe equal to zero. When torsional force is applied to DNA, it manifests as a change in DNA twist (local melting), or a change in writhe (DNA supercoiling) [109] (Figure IA). Most DNA binding proteins alter DNA structure at varying levels, applying torsional force on the double helix structure. Indeed, the wrapping of DNA around the histone octamer in a left-handed direction produces constrained supercoiling. However, the Pol II machinery is among the strongest molecular motors that exert torsional force on the DNA that results in unconstrained supercoiling [85,110]. The melting of the promoter DNA to form the transcription bubble and subsequent translocation generates positive and negative torsional force downstream and upstream of the elongating Pol II, respectively, defined as the twin-supercoil domain model [84] (Figure IB). Previously, detecting torsional forces has been confined to *in vitro* studies or those using mini-chromosomes in yeast [111]. However, recent advances in microarray and sequencing technologies have allowed detection of torsion genome-wide in various eukaryotes, including yeast, *Drosophila*, and human [90,32,91]. These technologies shed light on the role of DNA topology in different cellular processes.

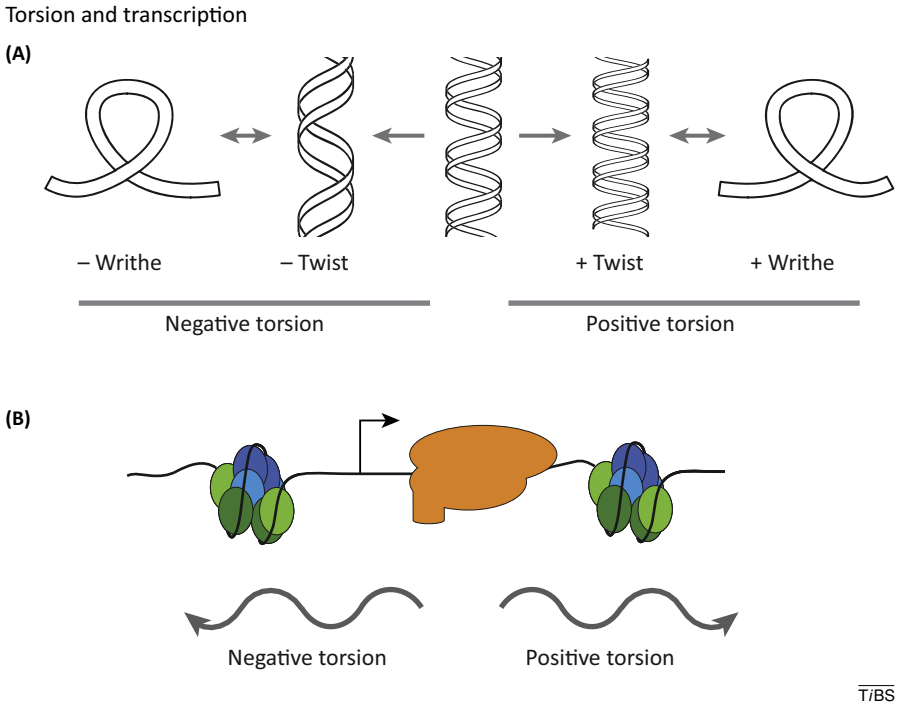

Figure I. Torsion and transcription. **(A)** The changes in DNA structure upon positive and negative torsion are shown. **(B)** Twin supercoil domain model states that, as Pol II elongates, it generates positive and negative torsion ahead and behind, respectively.

Concluding remarks

Since the discovery of the chromatin packaging of eukaryotic DNA, many have speculated that nucleosomes pose a strong barrier to Pol II at various stages of the transcription cycle. Indeed, research from the past few decades, especially recent advances in single-molecule technologies and genome-wide assays, have revealed detailed properties of the nucleosomal barrier both *in vitro* and *in vivo*. Almost in parallel, a plethora of convergent and often redundant mechanisms *in vivo* have been discovered to modulate the nucleosomal barrier and allow efficient Pol II progression. These mechanisms include ways to modify and move nucleosomes, to assist Pol II, and to use the structure of DNA as a mediator of nucleosome dynamics. Despite these great advances in understanding the nature of the nucleosome barrier and the means of overcoming it, many questions still remain to be answered. For instance, the discovery that the nucleosome barrier *in vivo* is context-specific, with the +1 nucleosome posing the strongest barrier, raises several questions. What determines the context-specificity of the +1 nucleosome? Are the mechanisms for overcoming the +1 nucleosomal barrier distinct from those for other nucleosomes? Furthermore, research into modulating the nucleosome barrier *in vivo* is beginning to converge into a more dynamic view of nucleosomes, rather than viewing them as static packaging units, which raises the question of how the various mechanisms for modulating the barrier contribute to overall dynamics of nucleosomes. Perhaps as we begin to address these questions, we may begin to understand the fundamental roles of nucleosomes in regulating transcription and in epigenetically maintaining gene expression patterns.

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