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Overcoming the Nucleosome Barrier During Transcript Elongation

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Abstract

RNA Polymerase II (Pol II) must break the nucleosomal barrier to gain access to DNA and efficiently transcribe genes. New single molecule techniques have elucidated many molecular details of nucleosome disassembly and what happens once Pol II encounters a nucleosome. . Our review highlights mechanisms that Pol II utilizes to transcribe through nucleosomes, including the roles of chromatin remodelers, histone chaperones, post-translational modifications of histones, incorporation of histone variants into nucleosomes, and activation of the Poly(ADP-Ribose) Polymerase enzyme. Future studies need to assess the molecular details and the contribution of each of these mechanisms, individually and in combination, to transcription across the genome to understand how cells are able to regulate transcription in response to developmental, environmental, and nutritional cues.

Keywords

Chromatin; Transcription; Chromatin Remodelers; Histone Variants; Histone Post-Translational Modification; Poly(ADP-Ribose) Polymerase

The Nucleosome is a Natural Barrier to Transcript Elongation of Pol II

Eukaryotic organisms efficiently package their genetic information into the nucleus by compacting DNA into chromatin (Glossary). The fundamental repeating unit of chromatin is the nucleosome which is comprised of 147 base pairs (bp) of DNA bound to a central octamer of histone proteins, consisting of two H2A-H2B dimers and one H3-H4 tetramer (Figure 1a). The primary effect of nucleosome formation is a six fold compaction of DNA, which subsequently, can form yet higher order chromatin structures [1].

Although DNA compaction into the nucleosome serves an important structural necessity for packaging genetic information into the nucleus, it also presents an inherent obstacle to any cellular process that requires DNA access, such as gene expression [2]. Transcription of mRNA encoding genes by Pol II can be regulated at many distinct steps [3]. Although nucleosomes can inhibit the stage of transcription initiation by occupying key regulatory DNA sequences near the promoter and transcriptional start sites of genes, these DNA regions are often depleted of nucleosomes *in vivo* [4], and the rules that dictate nucleosome positioning and occupancy near these regions is an area of active research [5, 6].

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Nucleosomes, however, occupy the coding region of nearly all genes and have been shown to severely inhibit the rate of transcription and processivity of purified Pol II complexes *in vitro* [7-9]. More recent *in vitro* studies have identified that the magnitude of the nucleosome barrier to Pol II transcription is dictated by the strength of local histone-DNA interactions and by the positions of these interactions within the nucleosome [10]. Surprisingly, Pol II transcript elongation rates *in vitro* on naked DNA are comparable to the rates that have been measured *in vivo* on genes (~1-4 kb/min) where nucleosomes should, in principle, impede its path [11]. Additionally, nucleosomes that contain DNA sequences that have strong local interactions and impose a polar barrier to transcription *in vitro* do not greatly affect transcription when inserted into the genome *in vivo* [12]. These results indicate that the cell utilizes mechanisms to abrogate the inherent barrier that the nucleosome poses to transcribing Pol II.

Single-Molecule Analysis of Nucleosome Disassembly and Transcription through Nucleosomes

The nucleosome assembles through the addition of an H3-H4 tetramer to DNA followed by sequential addition of each H2A-H2B dimer, and disassembles through the reverse reaction. However, less understood are the intermediate structures formed during this reversible process and the mechanisms by which Pol II and its associated factors facilitate the formation of such intermediates. Recent single-molecule fluorescence resonance energy transfer (FRET) studies using donor or acceptor tags on histones or DNA have uncovered two important findings regarding nucleosome disassembly. First, the frequency of DNA breathing (i.e. spontaneous, localized release of DNA contact with histones) of the first ~20 bp occurs once every 250 ms [13], but the frequency of DNA breathing ~40 bp into the nucleosome progressively and rapidly decreases to once every 10 minutes and even longer closer to the nucleosome dyad [14] (Figure 1a). Second, nucleosome disassembly occurs initially through an opening of the H3-H4 tetramer/H2A-H2B dimer interface followed by the release of H2A-H2B dimers from the DNA [15, 16] (Figure 1b). These results indicate that Pol II is likely able to gain access to the first segment of nucleosomal DNA through its breathing, but it is unlikely to be able to spontaneously access the DNA that is in direct contact with an H2A-H2B dimer, although these experiments have yet to be reported with transcriptionally engaged Pol II. Additionally, one rate-limiting step that might be facilitated by Pol II's traversal of the nucleosome is the loosening of the H3-H4 tetramer/H2A-H2B dimer interface. This is supported by the fact that a single round of Pol II transcription through a nucleosome can remove an H2A-H2B dimer, and the resulting hexamer of histones is then more conducive to Pol II transcription [17, 18].

Single-molecule optical trap experiments have also provided additional high-resolution insight into the DNA-histone interactions and the outcomes of transcriptionally engaged Pol II encountering a nucleosome [19]. Molecular unzipping experiments in which DNA is peeled away from the histone octamer reveal interactions with the DNA every 5 bp with the strongest interactions occurring at the nucleosome dyad, nearest the H3-H4 tetramer, and to a lesser extent 40 bp on either side of the dyad, where DNA contacts the H2A-H2B dimers [20]. These techniques have also been used to measure the effects a nucleosome has on Pol II transcription and, as expected, the nucleosome greatly impeded Pol II's transcription, especially at physiological salt conditions, sometimes resulting in complete arrest [21]. The nucleosome increases Pol II's pause duration as it enters the first ~30 bp of the nucleosomal DNA and decreases Pol II's pause-free transcript elongation rates. These results also support additional biochemical data whereby a transient loop is formed from the upstream, transcribed DNA rewinding around the histone, which consequently, returns histones back to the DNA *in cis* and maintains nucleosome positioning [17, 21, 22]. Both FRET and optical trap measurements demonstrate that under physiological conditions, the nucleosome

provides a formidable barrier to Pol II transcription and that a significant barrier is first encountered after transcription of approximately the first 30 bp of nucleosomal DNA where contacts with the H2A-H2B dimer are strongest.

Chromatin Remodelers are ATP Driven Motors that Slide Nucleosomes and Evict Histones

Chromatin remodelers are enzymes, often a part of large macromolecular complexes, which hydrolyze ATP to translocate nucleosomes along a DNA template, evict or exchange histones, or in some cases completely evict the histone octamer (Figure 1c) [23, 24]. Currently, there are four known main families of chromatin remodelers that carry out these functions: *switch/sucrose nonfermentable* (SWI/SNF), *imitation switch* (ISWI), *chromodomain-helicase-DNA-binding protein* (CHD), and *inositol-requiring 80* (INO80). The combined result of these family members' actions can provide access to underlying DNA elements or change the position, occupancy, or composition of an underlying nucleosome.

Although the SWI/SNF complex has been better studied for its role in nucleosome depletion near promoters [25, 26], it has also been implicated in remodeling nucleosomes in those genes that respond to rapid transcription activation. In yeast, SWI/SNF is present at both promoters and gene bodies and travels with elongating Pol II at many genes [27, 28]. These studies also show that SWI/SNF is needed for the loss of histones and full transcript elongation following gene induction by addition of methionine or heat shock [27, 28]. Just as with yeast, SWI/SNF can stimulate the loss of nucleosomes and transcription activation in humans following heat shock *in vitro* [29] as well as with Tat-activated transcription of the HIV-1 gene [30]. It is likely that the ability of SWI/SNF to eject histones or full octamers [31-33] facilitates the robust transcriptional activation of many genes that respond to environmental stimuli.

Both the ISWI and CHD family of chromatin remodelers have been implicated in their ability to affect transcript elongation. The ISWI family of chromatin remodelers has been shown *in vitro* to possess histone exchange capabilities [33] and to help overcome the nucleosomal barriers to transcription [12]. Although the ISWI complex containing *bromodomain PHD finger transcription factor* (BPTF or NURF-301) is able to bind to H3K4Me₃ and H4K16Ac [34], both marks of active transcription, ISWI is found only on the coding regions of highly transcribed genes [35]. CHD1 is also able to bind H3K4Me₃; but in contrast to ISWI, CHD family members all colocalize with Pol II and can participate in early steps in transcript elongation [36]. CHD1 is also likely to be important during transcript elongation, as it interacts with both the histone chaperone *facilitates chromatin transcription* (FACT) and the Pol II elongation factor *DRB sensitivity inducing factor* (DSIF) [37] and can facilitate replication-independent deposition of H3.3 [38]. However, both ISWI and CHD's major reported function *in vivo* is to slide nucleosomes into ordered arrays throughout gene bodies and promote assembly of chromatin [39]. In yeast, ISWI helps position nucleosomes on the edge of the 5' and 3' nucleosome-free regions [40] and, even more strikingly, in combination with Chd1, ISWI directs the majority of nucleosome positions throughout the gene body into an ordered, repeating periodicity [35]. How or if the establishment of an ordered, periodic repeat of nucleosomes on gene bodies might be able to facilitate Pol II elongation has yet to be discovered.

The split family of ATPases which include the INO80 and SWI/SNF related protein 1 (SWR1) chromatin remodeling complexes facilitate transcript elongation by their ability to exchange histones. The SWR1 remodeling complex is recruited to the 5' ends of many genes and is best characterized for its ability to deposit the variant H2A.Z-H2B dimer into

nucleosomes containing canonical H2A-H2B dimers [41]. Although INO80, like ISWI, slides nucleosomes into evenly spaced arrays [42] it has also been recently shown that INO80 is able to carry out the reverse reaction of SWR1, the removal of H2A.Z-H2B variant dimers and reinsertion of canonical H2A-H2B dimers back into nucleosomes [43]. It is not yet known though if the opposing actions of SWR1's deposition and INO80's removal of H2A.Z is an actively used mechanism to facilitate or hinder transcript elongation of Pol II.

Histone Chaperones Facilitate the Disassembly and Reassembly of the Nucleosome

The cell has provided the nucleus with specific safeguarding proteins that guide the proper incorporation of histones onto DNA and regulate the proper disassembly and reassembly of nucleosomes during passage of Pol II through the nucleosome. Unlike chromatin remodelers, histone chaperones do not use the energy of ATP hydrolysis, but instead have strong affinities for specific surfaces on either the H2A-H2B dimer or the H3-H4 tetramer to facilitate disruption of nucleosomes or deposition of histones [44, 45]. Many histone chaperones have been found to associate with specific histones, histone post-translational modifications, and elongation factors, which are listed in Table 1. We will focus on those chaperones that have been implicated in their ability to facilitate transcription.

As Pol II approaches a nucleosome, the contacts of the nucleosome with the upstream H2A-H2B dimer are the first to be disrupted, and it is not surprising that the actions of the H2A-H2B chaperones, nucleosome assembly protein 1 (Nap1) and FACT, contribute to this process. Both Nap1 and FACT have been used extensively *in vitro* for the assembly and disassembly of H2A-H2B within nucleosomes. Nap1 is able to cooperate with many other factors associated with transcriptionally active regions, such as the remodel the structure of chromatin (RSC) [46] or Chd1 [47] chromatin remodelers, to facilitate the disassembly of H2A-H2B dimers. Nap1 can also act directly on H3K14Ac modified nucleosomes [48] to facilitate this disassembly. Nap1 could affect transcript elongation by managing the relative H2A-H2B density on genes by regulating the deposition of free H2A-H2B dimers onto DNA [49]. As opposed to Nap1, FACT has been shown to directly affect transcription coupled nucleosome disassembly. FACT was first identified by its ability to facilitate Pol II transcription through a nucleosome template *in vitro* [50]. *In vivo*, FACT tracks with elongating Pol II [51, 52] and also physically associates with many known factors that aid transcript elongation (Table 1). Ubiquitination of H2BK123, which is also associated with transcriptionally active regions (Figure 2a), greatly stimulates FACT's ability to aid Pol II's transcription through chromatin assembled templates *in vitro* [53]. Recently, H2BK123 ubiquitination was shown to facilitate transcription elongation *in vivo* through its ability to promote nucleosome reassembly following Pol II transcription [54], perhaps through its association with FACT, or by directly altering chromatin structure [55]. It is not yet clear if Nap1 and FACT have separate or redundant functions, or if their major *in vivo* contribution to transcript elongation comes from aiding in disassembly of the H2A-H2B dimer or, as was clearly shown in yeast [56, 57], in the reassembly of the nucleosome in the wake of Pol II's passage or both.

Of the many histone chaperones that facilitate the disassembly and reassembly of the H3-H4 tetramer (Table 1), anti silencing factor 1 (Asf1) and suppressor of ty homolog 6 (Spt6) facilitate transcript elongation. Asf1 interacts with promoters and gene bodies and mediates H3's eviction and deposition during Pol II transcription [58]. Asf1 also associates with the histone acetylase responsible for H3K56Ac and together can facilitate the replication-independent exchange of H3-H4 within coding regions [59]. Spt6 also possesses H3-H4 chaperone activity and closely associates with elongating Pol II [60] and can positively affect the transcript elongation rate of Pol II *in vivo* [61]. Loss of Spt6 function results in a

genome-wide reduction in H3 density across many transcriptionally active genes, implicating Spt6 in the reassembly of H3 back into nucleosomes after Pol II elongation [62]. It is unknown if the function of Spt6 in facilitating transcript elongation is a result of this reassembly deficient phenotype [63] or the reverse reaction, or if Spt6 affects Pol II elongation through a completely separate mechanism.

Just as canonical histones have specific chaperones, so too do histone variants. Whereas an H2A.Z specific histone chaperone, Chz1, has been identified in yeast [64] as a part of the Swr1 remodeling complex, other organisms may rely on Nap1 for this function or contain a yet to be determined H2A.Z chaperone. H3.3 variants have completely separate chaperones. Both histone regulator A protein (HIRA) [65] and death domain-associated protein (Daxx) [66] are able to aid in the assembly of the histone variant H3.3 into chromatin where it is deposited onto transcriptionally active gene bodies in a replication-independent manner [67]. The Chd1 chromatin remodeler interacts with HIRA and aids in the deposition of H3.3 [38]. Single-molecule experiments, with their ability to track individual transcribing complexes in real time, could provide valuable insights to the mechanisms by which histone chaperones actively destabilize or disassemble nucleosomes in the presence of elongating Pol II.

Histone Post-Translational Modifications Alter Nucleosome Composition and Chromatin Structure

Post-translational modifications (PTMs) of histones can regulate the specific composition and structure of nucleosomes at individual genes [68, 69]. These modifications alter the localized chromatin structure or composition by either directly changing the chromatin structure or by providing a specific binding surface that increases the affinity of particular effector proteins that are able to change chromatin (Text Box 1). New modifications and related functions continue to be discovered [70]. Here, we summarize those modifications that are associated with active transcription, along with the enzymes responsible for the modification, and the protein effectors that can bind to those modifications and further affect chromatin structure and function (Table 2). In this section, we will highlight modifications that specifically alter the structure of chromatin in a way that facilitates Pol II transcription.

The best studied example of how histone modifications can directly affect chromatin structure is through the acetylation of lysine residues within the histone tails. Generally, acetylation neutralizes the positive charge of lysine and thus weakens the ionic interaction with the negatively charged DNA backbone. *In vitro*, the acetylation of the H2A-H2B tails weakens the interactions that are present 40 bp on either side of the dyad, and acetylation of H3-H4 greatly reduces the formation of higher order structures and reduces the amount of DNA bound in the nucleosome [71]. This effect caused by acetylation of H3-H4 agrees with predictions based on the nucleosome crystal structure that show the H4 tail mediating internucleosomal packing by contacting H2A-H2B dimers in an adjacent nucleosome [72]. Acetylation of H4K16 was found to significantly hinder the ability of chromatin to form higher order structures by impeding internucleosomal interactions [73]. A functional result of this modification is seen from dosage compensation in *Drosophila* in which H4K16Ac is enriched on the male X chromosome and results in the 2 fold upregulation of X-linked genes by facilitating transcript elongation [74].

Histone modifications can also influence transcription through their ability to bind to and recruit specific effector proteins. For example, SWI/SNF and RSC chromatin remodelers both contain subunits that have a bromodomain and can bind to acetylated H3 tails. *In vitro*, acetylation of H3 by the SAGA or NuA4 histone acetyltransferase complexes greatly stimulate RSC and SWI/SNF chromatin remodeling activities to facilitate Pol II transcript elongation [75, 76]. Chd1, a chromodomain containing protein, is specifically targeted to the

5' ends of active genes through its ability to bind trimethylated H3K4 (H3K4Me₃) [77] (Figure 2a). Another example is the PHD containing BPTF subunit of an ISWI remodeling complex that, like Chd1, can bind H3K4Me₃ nucleosomes, but it binds more tightly to those nucleosomes containing both H3K4Me₃ and H4K16Ac, thus increasing the specificity of genomic locations where it will remodel nucleosomes [34]. Another PTM associated with transcript elongation, H3K36 trimethylation (Figure 2a), is important for the recruitment of a chromodomain-containing histone deacetylase that aids in nucleosome reassembly following transcription and is necessary for repression of cryptic initiation in gene bodies [78]. The discovery of additional effectors of histone modifications will provide new insight as to how particular histone modifications are able to facilitate Pol II elongation by affecting chromatin structure and composition.

Histone Variants Affect Nucleosome Structure and Stability

In addition to histone PTMs, histone eviction, and chromatin remodeling, *de novo* deposition of specific histone variants can facilitate transcription through the nucleosome [79]. There are numerous examples of different histone variants, whose families have expanded evolutionarily in higher eukaryotes [80]. Almost all eukaryotic organisms have variants of both H2A and H3, some contain H2B variants, but no variants of H4 appear to exist. As with PTMs, some of these variants are positively correlated with transcriptional activation including H2A.Z and H3.3, which we highlight below.

The deposition of H2A.Z into chromatin is an essential process for many organisms and is important for the proper transcription of many genes [80]. The Swr1 remodeling complex exchanges H2A-H2B dimers to create nucleosomes containing H2A.Z-H2B dimers near the 5' ends of genes [41] (Figure 2a). Interestingly, homotypic H2A.Z-containing nucleosomes have been shown to be more stable than canonical H2A containing nucleosomes, creating a dichotomy as to why such a histone variant would facilitate transcript elongation [81].

How H2A.Z facilitates transcript elongation might lie in the deposition of another histone variant H3.3. Deposition of histone H3.3 occurs mainly outside of the period of DNA replication and in a transcription-dependent manner [65]. H3.3 is deposited into promoters, gene bodies, and other gene regulatory elements by the HIRA and Daxx chaperones [66]. Amazingly, H3.3 only differs from canonical H3.1 by 4 amino acids [80]. However, these small changes are enough to provide an *in vivo* mechanism whereby nucleosomes containing both H2A.Z and H3.3 are less stable than those containing canonical H3 or H3.3 histones [82]. Interestingly, the overlap in which nucleosomes contain both H2A.Z and H3.3 are those regions surrounding the promoter and also the 5' ends of transcribed genes (Figure 2a). Taken together, these results imply that less stable nucleosomes contribute to 5' nucleosome-depleted regions *in vivo* and allow Pol II and its regulators access to the underlying DNA to facilitate transcription. In support, both H2A.Z [83] and H3.3 [67] are more rapidly incorporated into gene bodies and promoters (Figure 2a) than their canonical counterparts. Of interest is how chaperones, modifications, or remodelers might synergize with the added instability and turnover of H2A.Z-H3.3 containing nucleosomes.

Activation of Poly(ADP-Ribose) Polymerase Promotes Chromatin Decondensation

Poly(ADP-Ribose) Polymerase (PARP) provides yet another way to alleviate the repressive nature of the chromatin fiber to facilitate transcription. PARP is a chromatin associated factor without any known remodeling or chaperoning activity that can facilitate transcription by altering chromatin structure at many genes. When activated, it uses NAD⁺ as a substrate to synthesize Poly(ADP-Ribose) (PAR) onto target proteins as a PTM (Text Box 2). PAR's

structure is very similar to a nucleic acid and thus can possibly compete or interfere with the functions of factors that bind nucleic acids. PARP and its enzymatic activity have been linked to functional roles in DNA repair, apoptosis, chromatin structure, and transcription [84].

The activation of PARP's enzymatic activity has been long known to bring about relaxation of chromatin structure [84]. Recently, PARP was shown to contribute to the formation of chromosomal puffs that occur at the loci of active transcript elongation in *Drosophila* polytene chromosomes [85]. After heat shock, the loosening of chromatin structure is a result of the activation of PARP's enzymatic activity. The formation of PAR results in the depletion of histone density on the body of heat shock genes that in turn facilitates Pol II transcription [86]. Interestingly, PARP activation and depletion of nucleosomes on the gene body is independent of actively transcribing Pol II [86]. Instead, *heat shock factor* (HSF), the transcriptional activator of heat shock genes, recruits the *Drosophila* Tip60 complex to acetylate H2AK5 which ignites PARP's enzymatic activity, the rapid spread of PAR throughout the region, and correspondingly rapid nucleosome loss [87] (Figure 2b). The activation of PARP *in vivo* is also dependent on the C-terminal phosphorylation of H2A [88], which is a known prerequisite for H2AK5Ac in *Drosophila* [89]. The *Drosophila* Tip60 complex also contains a known homolog of Swr1, whose ability to exchange out H2A is stimulated by H2AK5 acetylation [89]. It will be of interest to know how or if the disruption of nucleosomes through Swr1's exchange can activate enzymatic activity of PARP *in vivo*, how the activation and localized spread of PAR can lead to such dramatic histone depletion and relaxation of chromatin structure, and to what extent this mechanism of chromatin disruption is used at other genes..

Concluding Remarks

The exploration of mechanisms by which cells overcome the nucleosome barrier to allow for efficient transcript elongation has resulted in the discovery of an entire subfield of eukaryotic gene expression. The discovery of a new mechanism of nucleosome displacement by PARP activation has generated numerous intriguing questions that can be addressed with existing and new technologies [86, 87]. The advent of new technologies such as single molecule experiments [19] and protein chemical ligations [55, 90-92] are just beginning to provide mechanistic details as to how chromatin remodelers, chaperones, modifications, and variants are able to destabilize, disassemble, or reassemble nucleosomes and should prove useful for dissecting PARP-based mechanisms. In addition, these technologies are being used to answer fundamental questions about what happens when Pol II engages a nucleosome and how specifications including Pol II density on genes [93], transcription rates [17], and processivity affect the process of transcript elongation through nucleosomes. These views should be augmented with real time and high-resolution imaging approaches [94, 95]. Finally, genome-wide studies of Pol II, the composition of histones, and the presence of particular chromatin associated factors will be useful in evaluating the generality of particular mechanisms in regulating transcript elongation and alleviating nucleosomal barriers.

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Glossary Box

Chromatin	The combination of DNA, histone proteins, and other non histone proteins that together are condensed into a structure that fits within the nucleus of a eukaryotic cell.
Nucleosome	The fundamental repeating unit of chromatin comprised of 147 base pairs of DNA wrapped around a core octamer of histone proteins, 2 H2A-H2B dimers and 1 H3-H4 tetramer.
Chromatin Remodeler	An enzyme that is stimulated by the presence of DNA to hydrolyze ATP which can then be used as energy to ‘pump’ DNA, locally breaking DNA contacts with histones in order to translocate the nucleosome along the DNA template, evict histones, or completely eject the histone octamer from the DNA.
Histone Chaperone	A family of proteins that bind to either an H2A-H2B dimer or an H3-H4 tetramer to prevent inappropriate aggregation of histones onto DNA as well as remove and deposit histones during transcription or replication.
Histone Variant	A protein that is almost identical to one of the four core canonical histone genes with specific amino acid changes and in some cases may contain an additional non-histone fold domain near a terminus of the protein.
RNA Polymerase II	The macromolecular protein complex that transcribes all protein coding genes within eukaryotic cells into mRNA.
Transcript elongation	The specific stage of transcription after RNA Polymerase II has initiated transcription and has progressed through all early stages of transcriptional regulation such as promoter escape.
Poly(ADP-Ribose) Polymerase	An enzyme that binds to aberrant DNA structures as well as nucleosomes and when activated catalyzes the polymerization of linear and/or branched Poly(ADP-Ribose) chains as a post-translational modification.
Poly(ADP-Ribose)	An anionic, nucleic acid-like polymer that is made from precursor NAD ⁺ which can range in size from two joined ADP-Ribose units to upwards of 200 units in either a linear or branched structure.

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Text Box 1: Post-Translational Modifications of Histones

There exists a plethora of data showing that nearly every imaginable post-translational modification (PTM) within the cell can be found on histones. These include classical modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation, but also less well studied modifications including ADP-ribosylation, citrullination, glycosylation, hydroxylation, formylation, and crotonylation. They occur at many residues, particularly on the N-terminal tails of histones and can occur in specific combinations that can increase or decrease the affinity of other effector proteins important for transcript elongation. Although many of these modifications are known to positively correlate with the transcriptional activity of a gene, less causative information exists regarding their ability to facilitate Pol II's traversal of the nucleosome structure by either directly affecting the nucleosome structure or indirectly through facilitating the binding of a chromatin remodeler, histone chaperone, or transcriptional activator. Generally, the most commonly studied PTMs of histones associated with elongating Pol II are the acetylation of the N-terminal tails of H3 and H4, the methylation of H3K4, H3K36, and H3K79, as well as the ubiquitination of H2B's C-terminus. Table 2 summarizes the factors that deposit and bind to the histone PTMs that are associated with active transcription. Additional modifications have recently been discovered that also affect the intrinsic properties of chromatin structure to aid gene expression. These studies take advantage of new technologies in which site specific histone modifications can be made through chemical ligation [90]. The ubiquitination of the C-terminal tail of H2B interferes with chromatin's ability to form higher order structures by creating an open, accessible fiber through a mechanism independent of and synergizes with the acetylation of the H4 tail [55]. Acetylation of H3K56, increases DNA breathing of the nucleosome ~40 bp away from the dyad by 7 fold, allowing DNA that is less tightly wound to gain easier access to proteins such as Pol II [96]. Lastly, phosphorylation of H3T118 was shown to be able to weaken histone-DNA interactions near the dyad thereby increasing accessibility at the dyad 6 fold and increasing nucleosome mobility 28 fold, making it easier to be remodeled by SWI/SNF [92]. This power to create homogeneous samples with site-specific histone modifications will undoubtedly soon reveal how many of the PTMs influence chromatin structure.

Text Box 2: PARP is an Enzyme with Multiple Cellular Functions

Poly(ADP-Ribose) Polymerase (PARP) has been long studied for its role in DNA repair, but it has recently been investigated for its ability to affect chromatin structure and aid transcriptional activation. PARP is an enzyme that uses NAD^+ as a substrate to catalyze the polymerization of *Poly(ADP-Ribose) (PAR)* as a post-translational modification of acceptor proteins. PAR is chemically akin to a nucleic acid with the following key exceptions: PAR contains two negatively charged phosphates per nucleic acid, and PAR can form either linear or branched structures of up to 200 units [84]. The enzymatic activity of PARP is stimulated by many things including broken DNA, aberrant DNA structures, individual histones, and polynucleosomes. PARP binds to both DNA and nucleosomes *in vivo* in a manner similar to that of the linker histone H1 [97]. Both *in vitro* and *in vivo*, the primary target of PARP's enzymatic activity is itself, however other proteins have been identified as targets of PARylation including DNA repair factors, transcription factors, and histones. The ability of PARP to affect a wide-variety of cellular processes is likely a result of the generality of PAR to interact, compete, and interfere with many factors whose function is reliant on their affinity for nucleic acids.

Figure 1. The nucleosome contains specific interactions that provide a barrier to transcript elongation and can be disassembled through chromatin remodelers

(a) The crystal structure of the nucleosome as visualized from PDBid 1AOI [72]. The nucleosome consists of 147 bp of DNA (strands colored black) wrapped 1.7 times around a core octamer of histone proteins with 2 H2A (yellow) H2B (red) dimers associated with an H3 (blue) H4 (green) tetramer. All the subsequent depictions of nucleosomes will use the color coordination depicted here unless otherwise noted. The strongest DNA contacts that exist between the DNA and histones are marked by asterisks with the first being 30 bp into the nucleosome where DNA contacts the H2A-H2B dimer interface and 70 bp into the nucleosome at the nucleosome dyad. This perspective of the nucleosome also shows the direction of DNA movement when breathing, a process whereby DNA spontaneously breaks contact with the histones, which occurs once every 250 ms 20 bp into the nucleosome but once every 10 minutes 40 bp into the nucleosome. The figure on the right is rotated to show the same nucleosome looking at the nucleosome dyad, which is the perspective Pol II takes when it engages the nucleosome. This is the perspective sketched in (b) and subsequent figures.

(b) Diagram depicting the reversible process of nucleosome disassembly/assembly as determined by FRET assays. The first step depicts the H2A-H2B dimers breaking contact with the H3-H4 tetramer in the intact nucleosome state (i) and opening up to form the intermediate shown in (ii). The second step shows the dissociation of the H2A-H2B dimers from the DNA template leaving just the H3-H4 template as shown in (iii). The last step

includes the dissociation of the H3-H4 tetramer from the DNA template resulting in naked DNA (iv).

(c) Chromatin remodelers contain multiple functions which result in either changes in the nucleosome position or composition. These four functions are depicted as reversible processes that can be achieved independently of one another. Chromatin remodelers are capable of sliding nucleosomes along a DNA template to change nucleosome positions (i), ejecting individual histones (ii), exchanging new histones into the nucleosome (H2A.Z is shown to be exchanged as an example in pink) (iii), or completely ejecting a full histone octamer (iv).

Figure 2. Transcriptionally Active Genes are Associated with Specific Histone Post-Translational Modifications that can Trigger the Enzymatic Activity of Poly(ADP-Ribose) Polymerase to Facilitate Transcript elongation

(a) A transcriptionally active gene is shown with the transcription start site of the gene indicated by the arrow, and a representative profile of Pol II density along the gene is shown above in a red line trace. The promoter region of most genes contains a nucleosome depleted region with flanking nucleosomes on either side that are enriched in the histone variants H2A.Z (pink) and H3.3 (purple) which have a higher rate of exchange than their canonical histones found further into the open reading frame. Nucleosomes are periodically spaced flanking the nucleosome-depleted region and may be the result of chromatin remodelers that evenly space the nucleosomes such as Chd1 and ISWI. Additionally, PTMs of histones that correlate positively with gene transcription are depicted above the gene. These include histone acetylation of the N-terminal tails of both H3 and H4, such as H3K9, H4K5, H4K8, H4K12, and H4K16, which are found near the promoters of genes (blue dome) and trimethylated at H3K4 (orange triangles), which are present on nucleosomes flanking the promoter in a monotonically decreasing fashion. Finally, the open reading frames of active genes are progressively modified by H3K36Me₃, H3K79Me₂, and H2BK123Ub (gold triangle).

(b) Model of PARP activation at heat shock loci summarizing [86, 87].

(i) Before heat shock, the *Drosophila Hsp70* gene contains a transcriptionally engaged, paused Pol II (red) and inactive PARP (gray) present near the first well-positioned nucleosome after the transcription start site, indicated by the arrow. *Hsp70* is known to

contain the *Drosophila* H2A.Z variant, H2Av (pink), at the 5' end of the gene before heat shock. It is not known if the C-terminal S137 of H2Av is already in a phosphorylated state.

(ii) After heat shock, the master transcriptional activator, *heat shock factor* (HSF, orange), is recruited to the promoter of *Hsp70* as a trimer. HSF in turn is responsible for recruiting the dTip60 complex (purple cloud), which is part of a chromatin remodeling complex containing a protein homologous to Swr1. dTip60 is responsible for acetylating H2AK5 near the 5' end of the gene.

(iii) Following acetylation of H2AK5, the dTip60 complex is likely to exchange out the H2Av variant as it is known to do *in vitro* with H2Av that is acetylated at K5 and phosphorylated at S137. The combination of the acetylation of H2AK5 and the exchange of H2A triggers activation of PARP's enzymatic activity (now shown in blue).

(iv) The activation of PARP (blue) results in the formation of PAR (blue lines) and the redistribution of PARP throughout the coding region and beyond, but halts at nearby natural chromatin insulating elements (not shown). This rapid, domain-wide process leads to a buildup of PAR throughout the region that locally disrupts and strips histones from the gene allowing for efficient transcript elongation and robust gene activation.

Table 1

Histone chaperones interact with specific histones, histone post-translational modifications, and additional factors that facilitate transcript elongation.

Histone Chaperone	Associated Histones ^a	Associated PTMs ^b	Associated Transcript elongation Factors ^c
Nap1	H2A-H2B (preferred) H2A.Z-H2B H3-H4		FACT, Chd1
FACT	H2A-H2B H2A.X-H2B	H2BK123Ub	Pol II, Chd1, PARP-1, CK2, PAF, Nap1, DSIF, Spt6, TopoI, TopoII, RPA
Chz1	H2A-H2B H2A.Z-H2B	H2BK123Ub	Swr1
Asf1	H3-H4	H3K56Ac, H3K9Ac, H4K5Ac, H4K12Ac, H3K36Me ₃	HIRA, CAF-1, CK2, Set2, Bdf1, Spt15, TFIID, SWI/SNF
Spt6	H3-H4	H3K36Me _{2/3}	Pol II, CK2, Spn1, FACT, DSIF
HIRA	H3.3-H4		Asf1, TFIID, SWI/SNF, Rtt106, DSIF
Daxx	H3.3-H4		Atrx
CAF-1	H3-H4	H3K56Ac, H4K5Ac, H4K12Ac	Rtt106, Asf1,
Rtt106	H3-H4	H3K56Ac	HIRA, SWI/SNF, CAF-1
Vps75	H3-H4	H3K9Ac, H3K23/27Ac	R1109, Pol II, NuA4

^a as reviewed in [44]

^b as reviewed in [98]

^c as reviewed in [44] and mined from physical interaction data from <http://www.thebiogrid.org> and <http://www.yeastgenome.org>

Table 2

Elongating Pol II associates with specific post-translational modifications (PTMs) of histones which a variety of effector proteins bind to and facilitate transcription.

Histone	PTM ^a	Modifying Enzyme ^a	Known Effector Domains and Proteins ^b
H2A	K5 Ac	Tip60/Esa1	
H2B	K5 Ac	P300, ATF2	
	K12 Ac	P300/CBP, ATF2	
	K15 Ac	P300/CBP, ATF2	
	K20 Ac	P300	
	S33/S36 PO ₄	TAF1, AMPK	
	K120/123 Ub	UbcH6/Rad6	COMPASS (Cps35)
H3	R2 Me	CARM1	
	K4 Me _{2/3}	Set1, Set7/9, MLL, ALL-1, Ash1, ALR, ALR-1/2	Chromo (Chd1), PHD (BPTF, TAF3, RAG2, ING2,4, PHF2,8, YNG1) Tudor (JMJD2A,C, Sgf29), MBT, Zf-CW
	K4 Ac	Esa1	
	K9 Ac	Gcn5	
	S10 PO ₄	Msk1, Msk2, IKK-, Snf1, Jil-1	14-3-3, Gcn5, Bmh1, Bmh2
	K14 Ac	Gcn5/PCAF, P300, Tip60/Esa1, TAF1, Sas3	Tandem PHD (DPF3b), Tandem Bromo (Rsc4), Bromo 2 (Polybromo)
	R17 Me	CARM1	Tudor (TDRD3)
	K18 Ac	Gcn5, P300/CBP	
	K23 Ac	Gcn5, P300/CBP, Sas3	
	R26 Me	CARM1	
	S28 PO ₄	Msk1, Msk2	
	K36 Me ₃	Set2, HYPB, Smyd2, NSD1	Chromo (Eaf3, MSL3, MRG15), PWWP (Dnmt3A, Msh-6, BRPF1, NSD1,2,3, N-PAC)
	K56 Ac	Rtt109, P300	SWI/SNF (Snf5), Rtt106
	K79 Me ₂	Dot1	Tudor (53BP1)
H4	R3 Me	PRMT1, PRMT5	P300, Tudor (TDRD3), Dnmt3a
	K5 Ac	P300, ATF2, Tip60/Esa1, HAT1	Bromo (Brdt)
	K8 Ac	P300, Gcn5/PCAF, ATF2, Tip60/Esa1, Elp3	Bromo (Brdt)
	K12 Ac	P300, HAT1, Tip60/Esa1	
	K16 Ac	Gcn5, MOF, Tip60/Esa1, ATF2, Sas2, NuA4	Bromodomain (Gcn5, NoRC)

^aAs reviewed in [99]

^bAs reviewed in [100]