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Chromatin remodeling by RNA polymerases

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Chromatin packages DNA tightly into the eukaryotic nucleus and maintains its proper functioning. Recent studies suggest the existence of two distinct mechanisms of progression of RNA polymerases through chromatin. The first is characteristic of eukaryotic RNA polymerase III, bacteriophage RNA polymerases, and probably ATP-dependent chromatin remodeling complexes. In this mechanism, nucleosomes are translocated without release of the octamer into solution. By contrast, transcription by RNA polymerase II (Pol II) involves displacement of one H2A–H2B dimer. Nucleosomes can present a barrier for transcribing Pol II that can be regulated *in vivo*. Analysis of the mechanisms of transcription through chromatin should provide important information about mechanisms of chromatin remodeling and gene regulation at the level of transcript elongation.

Chromatin consists of repeating subunits called nucleosomes, each comprising 147 bp of DNA wrapped in 1.7 superhelical turns around a histone octamer containing two each of histones H2A, H2B, H3 and H4 [1]. One molecule of linker histone H1 binds where the DNA enters and exits the nucleosome. Nucleosomes are further compacted into a 30-nm diameter fiber, which, in turn, are further compacted into structures not yet fully understood.

Compact nucleoprotein organization causes severe problems for processes such as DNA-replication, -recombination, -repair and -transcription, as well as for binding of regulatory proteins to DNA *in vitro*. Modulation of chromatin structure plays a central role in the regulation of such cellular processes [2]. The changes in chromatin structure that accompany (and often are involved in the regulation of) transcriptional activation or repression are collectively called chromatin remodeling. Chromatin is remodeled before or during transcription initiation (by ATP-dependent remodelers) and during transcript elongation. There are at least two different mechanisms of transcription-dependent chromatin remodeling – nucleosome mobilization (i.e. movement of the octamer on the DNA) and H2A–H2B dimer depletion. Recently, it has become apparent that the mechanisms of nucleosome mobilization by RNA polymerases and ATP-dependent remodelers are very similar. This review focuses on recent progress towards analysis of the mechanism of transcription-dependent chromatin remodeling and possible ways in which it might be regulated.

Transcription through chromatin *in vivo*

Eukaryotic RNA polymerases deal with chromatin in different ways *in vivo* and *in vitro*. Small polymerase III (Pol III)-transcribed genes are thus covered with transcription factors that could prevent the formation of nucleosomes on active genes [3]. However, the yeast Pol-III-dependent RNase P RNA1 gene, *RPR1*, is sufficiently long (369 bp) to contain at least one nucleosome [4]. Nucleosomes are disrupted on active Pol-I-transcribed genes [5] but the mechanism of Pol-I-dependent chromatin remodeling is unknown.

Pol-II-transcribed genes retain nucleosomal structure [6]. At the same time, Pol-II-transcribed nucleosomes are transiently depleted of ~50% H2A and H2B histones [7], and nucleosome positioning is often changed [6]. Extensive transcription-dependent exchange of H2A and H2B histones [6] and possibly H3 and H4 histones [8] has been observed. The SH groups of histone H3 in Pol-II-transcribed nucleosomes become transiently accessible to various probes [6]. It has been suggested that high accessibility of the SH groups reflects the transient partial displacement of H2A and H2B histones during Pol-II transcription [9]. Higher-order chromatin structure is also strongly disrupted during Pol-II transcription [6] and, like the partial disruption of nucleosomal structure, is also transcription-dependent and results in chromosome decondensation of the transcribed domain of chromatin [10].

In summary, Pol II is a powerful chromatin remodeler, modifying both nucleosomal and higher-order chromatin structure. It is possible that the transient Pol-II-induced

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changes of chromatin structure could, under some circumstances, result in longer-term changes in chromosome architecture. For example, it has been suggested that transcription by a pioneering Pol II across a chromatin domain could serve as a regulatory event, making the domain accessible for subsequent activation of transcription [11]. Indeed, large intergenic transcripts are precisely localized within three open (DNase-I-sensitive) subdomains of the human β-globin chromatin domain. Appearance of these transcripts and opening of the domains occur in parallel during development [11]. Moreover, the intergenic transcripts are initiated at the boundaries of the domains, at least one of which is likely to be important for domain opening [12].

Mechanisms of chromatin remodeling

Recently, it has become apparent that nucleosome mobilization (a change of nucleosome positioning) is involved in every known process of chromatin remodeling [13]. Two possible mechanisms have been proposed to account for nucleosome mobilization: the 'bulging model' and the 'twisting model' (Figure 1) [14]. The bulging model has been proposed to explain transcription-dependent nucleosome translocation and suggests that nucleosomes move on DNA through formation and rotation of a bulge on the surface of the octamer [15]. The twisting model suggests that a remodeling activity could apply torsion to DNA and alter its twist on the nucleosomal surface [16]. Directional propagation of the twist along nucleosomal DNA could result in nucleosome mobilization. The bulging mechanism certainly operates during chromatin remodeling by RNA polymerases and is probably involved in ATP-dependent chromatin remodeling as well.

Nucleosome transfer mechanism of chromatin remodeling by RNA polymerases

Several different RNA polymerases (*E. coli*, Pol II, Pol III, and bacteriophage SP6 and T7) can transcribe through

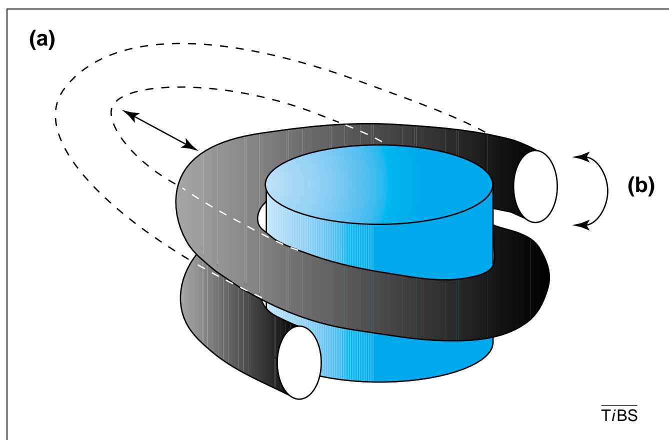

Figure 1. Possible mechanisms mediating nucleosome mobilization. Nucleosomes could be relocated along DNA as a result of one of two processes. **(a)** The 'bulging model'. DNA could be partially displaced from the surface of the octamer and then re-bound forming an intranucleosomal DNA loop. The loop could rotate around the octamer (blue) by breaking DNA–histone interactions on one side of the loop and re-forming them on the other side. **(b)** The 'twisting model'. Rotation of DNA along its long axis initiated at one end of a nucleosome changes the twist and could be propagated along nucleosomal DNA. Rotation of DNA by 36° would result in 1-bp DNA translocation.

nucleosomes *in vitro* (see [17] for review), and, at least in the case of transcription by SP6 bacteriophage polymerase and Pol III, complete nucleosomes can survive transcription [18]. In agreement with these earlier *in vitro* studies, experiments in yeast and *Drosophila* suggest that nucleosomes are not disrupted during transcription by these enzymes [19,20]. The initial clue about the mechanism associated with this process was obtained using a plasmid template carrying a single nucleosome core positioned just downstream of the SP6 promoter [21]. It was found that nucleosomes survived transcription but were translocated (mobilized) to multiple positions over the entire plasmid with a preference for the region immediately upstream of the promoter [21].

To analyze further the mechanism of transcription-dependent nucleosome mobilization, short linear templates carrying a single positioned histone octamer were used [15,22]. After transcription by bacteriophage polymerase, the octamer was transferred backward in a transcription-dependent way [15]. The data suggested that the octamers were either displaced from the DNA and then recaptured by the DNA fragment, or directly transferred out of the path of the advancing polymerase [15,22]. Support for the direct transfer model came from the analysis of the fate of single nucleosomes located at different positions on identical DNA templates [15]. Each positional isomer undergoing transcription generated a distinct set of octamer positions after transcription [15]. It is difficult to understand how such memory of the starting positions could be retained if the octamer was released into solution and then recaptured. Moreover, under regular transcription conditions, DNA-free octamers fall apart (in 1 s or faster [23]) and cannot be re-assembled on DNA *in vitro*. Therefore, the ability of the octamer to survive transcription suggests that it was transferred directly. Further studies indicated that this nucleosome transfer mechanism is used by both yeast Pol III and SP6 polymerase [18].

The transfer mechanism is characterized by a relatively weak barrier to transcription, which, nevertheless, slows down transcription at least three- to five-fold at physiological and lower ionic strength [24,25]. A nucleosomal barrier is first detected when the polymerase progresses ~25 bp into the nucleosome; it disappears when the polymerase approaches the nucleosomal dyad and the octamer is transferred [24,26]. Surprisingly, the barrier is never observed at the nucleosomal border, suggesting that DNA-bound histones might be transparent to the polymerase and that transfer intermediates form the barrier [24]. This possibility was confirmed by studies that analyzed the structures of the intermediates formed during transcription through the nucleosome (Figure 2) [27]. Two kinds of intermediates were observed by cryomicroscopy (Figure 2a). The closed intermediates [Figure 2a(i) and (iii)] were always observed when the polymerase paused in the nucleosome, suggesting that these constitute the barrier. In these intermediates, RNA polymerase is surrounded by DNA–histone contacts; the data suggest that DNA–histone interactions in front of or behind the polymerase [Figure 2a(ii)] are not sufficient to slow it down.

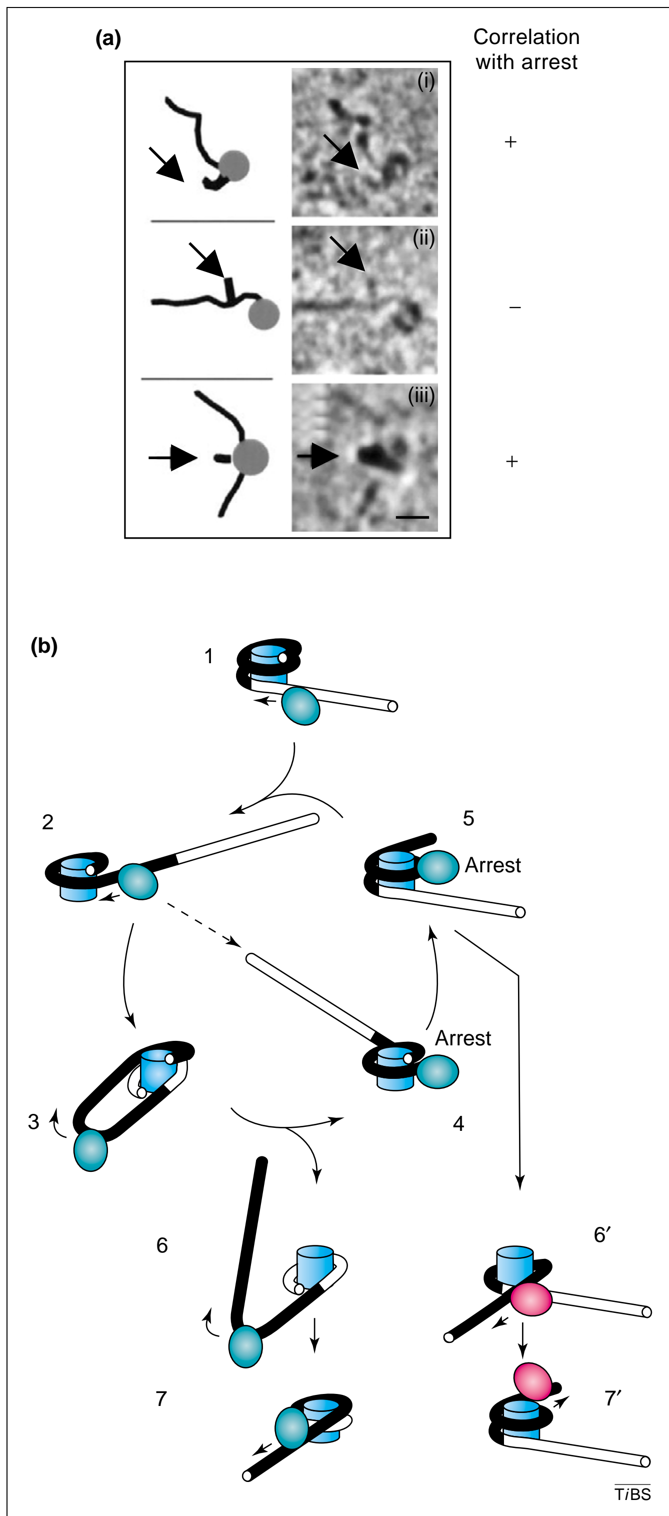

Figure 2. Mechanism of transcription through the nucleosome by RNA polymerase III (Pol III) and SP6 polymerase. **(a)** Structure of the nucleosomal barrier to transcription as analyzed by electron cryomicroscopy [27]. Nucleosomes were formed on the 227-bp templates and transcribed with SP6 RNA polymerase. Three classes of complexes formed during transcription are shown: (i) 'one-tailed' complexes containing polymerase arrested in the nucleosome; (ii) polymerase is approaching the mucleosome; (iii) 'two-tailed' complexes containing polymerase arrested in the nucleosome. The nucleosome is represented by a disc and the transcript by a black cylinder. Arrowheads indicate positions of the transcript. Scale bar, 10 nm. **(b)** Mechanism of transcription through a nucleosome by Pol III and SP6 RNA polymerase [18,27]. (1) RNA polymerase rapidly transcribes the first ~25 bp of nucleosomal DNA causing (2) partial dissociation of DNA from the octamer (blue). (3) The DNA behind the RNA polymerase (green) transiently binds to the exposed surface of the octamer forming a loop. As the enzyme cannot rotate in the loop, advance of the polymerase leads to opening and collapse of the loop and formation of closed intermediates (4,5), where further movement of enzyme is inhibited. Eventually, DNA dissociates from the octamer, and the configuration (2) is restored. This cycle of events can be repeated several times. When the polymerase has penetrated ~60 bp into the core, the downstream portion of DNA dissociates from the octamer (6), completing the transfer of the octamer. Transcription continues freely to the end of the template (7). The orientation of the octamer is fixed in all the drawings. Escape from intermediate 5 could also be possible without nucleosome translocation. In this case, DNA dissociates from the octamer in front of the enzyme (6′) and nucleosomes remain at the original position (7′). It is also possible that the intranucleosomal DNA loop involving the DNA region located upstream of the original nucleosome (3) does not form [the (2) → (4) shortcut]. The (5) → (7′) and/or (2) → (4) pathways are probably used during transcription through the nucleosome by Pol II (pink). Adapted from Ref. [27].

There is strong experimental evidence to suggest a model of transcription-dependent nucleosome mobilization that involves DNA bulging during transcription. First, the octamer is transferred over 20–900 bp in a direction opposite to the course of polymerase movement [15,21]. If transfer were achieved through twisting of DNA by the polymerase, the nucleosome would be expected to move in the opposite direction. No evidence of such forward nucleosome movement was obtained. Second, the octamer remains at the original position on DNA until RNA polymerase transcribes more than 50–60 bp into the nucleosome, and sufficiently long DNA (presumably enabling formation of the loop) is displaced from the surface of the octamer [24,27]. Finally, because the octamer never leaves the DNA during transfer, the only reasonable way for the octamer and RNA polymerase to pass each other is through formation of a bulge [15].

Figure 2b outlines the nucleosome transfer mechanism of transcription through the nucleosome [27]. As RNA polymerase enters the nucleosome, DNA is partially dissociated from the octamer, enabling formation of a looped intermediate. The advance of the polymerase leads to opening and collapse of the loop and formation of the closed intermediates in which further movement of the enzyme is inhibited. Eventually, the enzyme moves up to ~60 bp into the nucleosome, where the majority of nucleosomes are translocated [15,27].

Can DNA supercoiling be accumulated during transcription through the nucleosome? Under certain circumstances (immobilized enzyme and fixed DNA ends), transcription can induce changes in the DNA twist even on histone-free DNA [28]. During transcription through the nucleosome, the polymerase progresses without difficulties up to 40 bp into the nucleosome [24]; it is unlikely that DNA supercoiling is accumulated at this stage. At the same time, formation of a small intranucleosomal DNA loop and closed intermediates [Figure 2b(3–5)] creates suitable topological conditions for accumulation of torsion in DNA. Indeed, DNA ends are immobilized on the surface of the octamer, and the enzyme cannot rotate freely in the small DNA loops. This makes possible the transient accumulation of superhelical stress at this point of the process, although this has yet to be established experimentally. Even if stress is accumulated during transcription through the nucleosome, it is unlikely that the nucleosome is transferred by the twisting mechanism. However, DNA supercoiling could facilitate nucleosome transfer from in-front to behind the polymerase by

destabilizing histone-octamer–DNA interactions in front of the enzyme and stabilizing them behind (see [29] for discussion).

Most notably, in all intermediates, only approximately one superhelical coil of DNA remains associated with the octamer, while the second DNA coil is displaced by the enzyme (Figure 2b). It is probable that formation of these key uncoiled intermediates could explain many of the properties of chromatin remodeling (Figure 3).

Considerable uncoiling of nucleosomal DNA occurs during both transcription and ATP-dependent remodeling (Figure 3, and see later). One end of the nucleosomal DNA is partially displaced from the surface of the octamer. In these uncoiled DNA intermediates, open octamer surface is available for formation of a bulge, and rotation of the bulge on the surface of the octamer might result in nucleosome translocation in *cis*. If an excess of a competitor DNA is present in the reaction, it can also bind to the open octamer surface and result in direct octamer transfer in *trans*. This latter reaction, first demonstrated in transcription through the nucleosome [15], has now been described for various ATP-dependent remodelers [30,31]. More importantly, formation of the uncoiled intermediates during chromatin remodeling enables analysis of the remodeled state – a dimer formed by non-covalent association of two nucleosomes (Figure 3) [30–33].

In summary, the nucleosome transfer mechanism is characterized by a relatively low nucleosomal barrier for

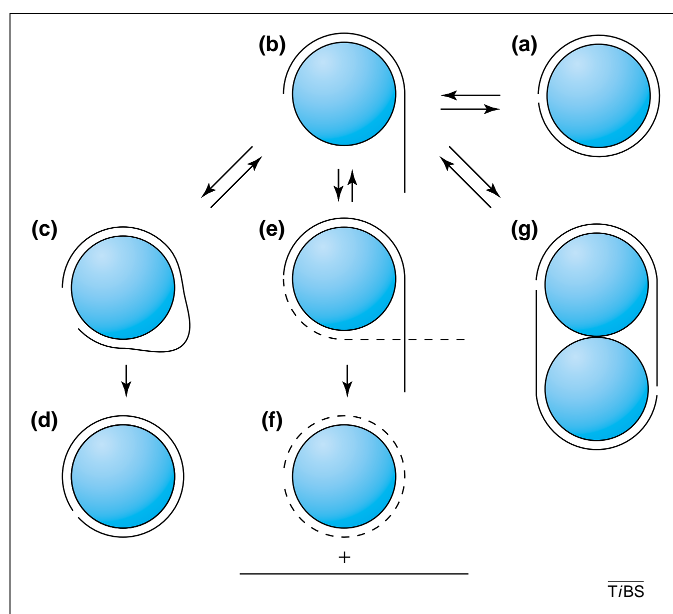

Figure 3. Partial displacement of DNA from the surface of the histone octamer during remodeling. During remodeling of a nucleosome **(a)**, DNA is partially displaced from the surface of the octamer (blue) starting at one end **(b)**. Next, a bulge can be formed **(c)**, and its rotation on the surface of the octamer might result in a different final position of the nucleosome (mobilization in *cis*) **(d)**. If a competitor DNA (broken line) is present in the reaction at high concentration, it can bind to the open octamer surface **(e)** and completely displace the original nucleosomal DNA (transfer in *trans*) **(f)**. Finally, DNA displaced from the surface of one octamer could interact with the partially open octamer surface of another nucleosome to form a dimer **(g)**. The dimers (a hypothetical structure is shown) are formed during ATP-dependent remodeling. However, it is unknown whether they can be formed during transcription-dependent remodeling. For clarity, only one superhelical coil of nucleosomal DNA is shown. Note that other pathways are also possible in the case of ATP-dependent remodeling (see main text).

transcription and by mobilization of the complete octamer (no histones are lost during the octamer transfer) [27] over a range of distances (20–900 bp). Clearly, remodeling occurs by a bulging mechanism; no evidence for a role of the twisting mechanism has been obtained. The ability to move along DNA and uncoil (or stabilize the uncoiling of) DNA from the surface of the octamer are two key activities required for nucleosome mobilization. That bacteriophage SP6 RNA polymerase (which does not encounter nucleosomes *in vivo*) can move nucleosomes with almost no difficulties suggests that there is no requirement for a special mobilizing activity and, possibly, that any enzyme having these properties could mobilize nucleosomes.

Mechanism of DNA uncoiling from the nucleosome

Although certain polymerases can transcribe through a nucleosome by a bulging mechanism (Figure 2b), it was not clear how this process could be initiated. This issue was addressed by incisive studies from the Widom's laboratory. Nucleosome core particles were reconstituted with a 150-bp DNA fragment containing a series of different restriction enzyme sites located progressively toward the dyad axis within the nucleosome [34]. These sites were accessible to the enzymes, with decreasing but non-zero rates of digestion for sites further towards the dyad. Comparison of digestion kinetics between the nucleosome and naked DNA led to the conclusion that the rate-limiting step for cutting is a reaction in which DNA at conformational equilibrium is transiently released from the nucleosome surface starting at one end. The probability of this event decreases with increasing lengths of released DNA.

Determining what the rate-limiting step might be in transcription through the nucleosome is more complicated. It has been proposed that as polymerase approaches or contacts the nucleosome, accumulation of torsion in DNA as writhe could change the superhelical DNA path and push it off the nucleosome surface [14]. It has also been suggested that the rotating enzyme could physically displace the DNA. Arguments against these models have been presented by Protacio *et al.* [26], who propose that the advancing polymerase does not itself contribute significantly to the measured rate of transcription. This, in turn, supports the idea that site exposure by transient liberation of DNA from the nucleosome surface (as described earlier) is probably the rate-limiting step in transcription under these conditions. However, the situation for Pol II *in vivo* is probably different (see later).

Mechanism of nucleosome mobilization by ATP-dependent chromatin remodelers

There are two classes of ATP-dependent chromatin remodeling protein complexes, and the mechanisms of action of both have strong similarities to that described earlier for transcription on chromatin templates. Some ATP-dependent remodeling protein complexes [notably those belonging to the imitation switch (ISWI) family] can induce short-range (10–100 bp) stochastic nucleosome mobilization. This characteristic activity could explain many of the properties of these remodelers (see [13] for review), and it seems probable that a bulge propagation

mechanism is responsible. However, two kinds of experimental results have been interpreted in terms of a mechanism involving twisting, rather than bulging (Figure 1). First, considerable unconstrained negative DNA supercoiling is accumulated during the remodeling [35]. Second, remodeling of nucleosomes assembled on a small circular DNA is strongly inhibited [36]. Neither of these observations unambiguously supports the twisting mechanism. DNA supercoiling could accumulate during transcription-dependent remodeling, where the bulging mechanism is clearly used (see earlier). Furthermore, both bulging and twisting would be strongly inhibited on small DNA circles. In fact, other experiments designed to discriminate between the two models support the bulging mechanism. The key features of the twisting model are requirements for unimpeded rotation of nucleosomal DNA on the octamer surface and absence of single-stranded nicks in the DNA that could relieve the stress accumulating as a result of DNA rotation. However, the introduction of nicks into nucleosomal DNA did not prevent mobilization by ISWI-family remodelers [37]. Moreover, nucleosomes containing branched DNA that forms a steric block to twist-diffusion are successfully mobilized [38]. Taken together, the available evidence does not support the twisting model of chromatin remodeling.

The second class of ATP-dependent remodeling complexes – members of the SWI/SNF family – appears to use a somewhat different mechanism [39]. When SWI (homothalic switching deficient)–SNF (sucrose non-fermenting) operates on a single nucleosome it unwraps DNA from one end and moves the octamer beyond the DNA end by the familiar bulge propagation process [40]. However, when the nucleosome that is remodeled lies between two others in a tri-nucleosome array, the central octamer does not move but is retained in place [39]. The DNA wrapped around this nucleosome has a stable bulge that can be detected by restriction endonucleases. This could be a kinetically trapped intermediate in a process that is similar to that described for the ISWI complex [39] or it might be a novel structure in which the nucleosome and its DNA are significantly rearranged. In either case, a bulged DNA structure is involved. Furthermore, experiments with DNA carrying hairpins indicate that, as for the ISWI family, SWI/SNF-driven bulge propagation does not require twist diffusion [38,41]. We suggest that in all these cases the same path is followed to bulge formation.

Can a bulging mechanism similar to the transcription-dependent nucleosome transfer mechanism operate in the case of ATP-dependent chromatin remodeling? The following evidence indicates a high mechanistic similarity of ATP-dependent and transcription-induced remodeling. First, many relevant activities of ATP-dependent remodelers and RNA polymerases are similar. These include short-range mobilization of nucleosomes, partial displacement of DNA from the octamer [39,40,42–44], and transfer of the octamer in *trans* [30,31]. All these activities are probably related to the DNA uncoiling activity (Figure 3) detected during transcription-dependent and ATP-dependent remodeling [13,15]. Second, the histone octamer remains intact after remodeling [27,42,45]. Third, both types of chromatin remodeling are coupled with hydrolysis of nucleotide triphosphates (NTPs). Finally, at least some ATP-dependent remodelers can move along DNA. All ATP-dependent remodelers have essential helicase-like motifs (but do not have helicase activity; see [14] for review) and are DNA translocases [46,47] that can directionally move along DNA, most probably by rotating around the DNA double helix. Thus, ATP-dependent remodelers have all the properties that seem to be involved in transcription-dependent nucleosome mobilization and could work by a similar mechanism (Figure 4).

In summary, it is probable that similar mechanisms are used during ATP-dependent and transcription-dependent nucleosome transfer remodeling that could also be used in other processes such as DNA replication. Indeed, the histone octamer is transferred during replication of SV40 mini-chromosomes or nucleosomes assembled *in vitro* without displacement into solution [48–50]. Moreover, efficient replication and octamer transfer do not require dissociation of the octamer [51].

Pol-II-type transcription-dependent chromatin remodeling

In the case of Pol II, experimental progress has been severely limited by lack of an appropriate experimental system. Recently, a novel technique was developed for assembling elongation complexes [52,53]. Using this new approach, it was shown that one H2A–H2B dimer is displaced from the histone octamer during transcription of mononucleosomal templates [54]. As a result, nucleosomes are converted to hexasomes. Transfer of a complete histone octamer is a hallmark of the mechanism of transcription through the nucleosome by Pol III and SP6 polymerase [18]. By contrast, the histones do not change their positions along the DNA on transcription by Pol II [54]. Thus, there are at least two distinct types of transcription-dependent chromatin remodeling.

The observation that nucleosomes are not translocated during transcription by Pol II could be explained in either of two ways. One possibility is that Pol II uses a mechanism similar to the transfer mechanism [Figure 2b, pathway $(2) \rightarrow (3) \rightarrow (4) \rightarrow (7')$] when a larger transient DNA loop is formed but the octamer chooses to stay at the original position. Alternatively, the nucleosome could collapse in the initial position without formation of a large transient DNA loop [Figure 2b, pathway $(2) \rightarrow (4) \rightarrow (7')$]. Further experiments are required to discriminate between these models. It is worth noting that nucleosome transfer might not be an obligatory outcome of a single-round transcription by Pol III and SP6 enzymes, and, in principle, these polymerases might use different pathways to overcome the nucleosome barrier. The reported predominant nucleosome transfer is a result of several rounds of transcription [18], and, if all the nucleosomes are not transferred in the first round, there is an opportunity for an eventual transfer in subsequent rounds. By contrast, the assay used for the Pol II studies allows for only a single round of transcription.

It has been shown that the same nucleosomes represent much higher barriers for Pol II than for Pol III or SP6 polymerase [54]. In fact, even a single nucleosome can be an absolute barrier for Pol II [54,55]. Why is the

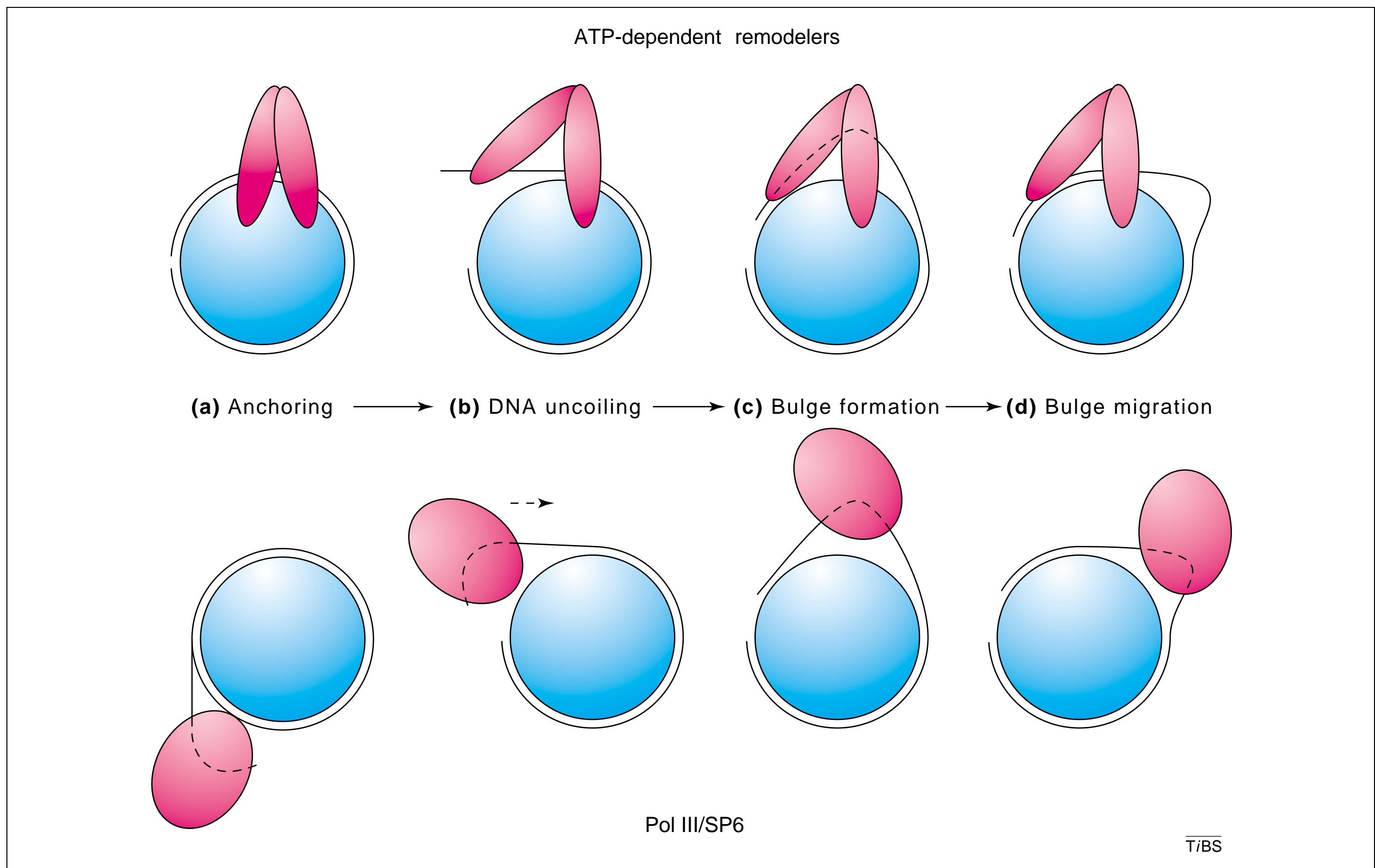

Figure 4. Bulging mechanisms of chromatin remodeling by some ATP-dependent chromatin remodelers (top), and by yeast III and SP6 RNA polymerases (bottom). The mechanism of ATP-dependent remodeling is highly hypothetical [13,27]. Elongating RNA polymerases introduce a ~90° bend in DNA [69,70]. **(a)** Anchoring: the remodeling complex binds to or approaches nucleosomal DNA. **(b)** DNA is uncoiled from the surface of the octamer. **(c)** The remodelers introduce a bend in the DNA that forces formation of an intranucleosomal DNA loop (a bulge). It has been proposed that ATP-dependent remodelers have both DNA-binding and octamer-binding sites, and that an ATP-hydrolysis-dependent change in conformation of the protein complex introduces a bulge in the DNA. **(d)** The bulge is relocated within the nucleosomal DNA either spontaneously (ATP-dependent remodelers) or by traveling together with the transcribing enzyme.

nucleosomal barrier the highest for Pol II? Pol II transcribes long nucleosomal arrays *in vivo* and it might be expected that its design would accommodate transcription through nucleosomes. One possibility is that the nucleosomal barrier is used for regulation of gene expression by changing the rate of transcript elongation. Indeed, transcript elongation blocks located 20–200 bp downstream of the promoters, and which are relieved during gene activation, have been identified in a growing number of eukaryotic genes, including proto-oncogenes c-*myc* and c-*fos* and the gene encoding HIV-1 polyprotein (see [56] for review). Moreover, at least in one case, the nucleosome was established as a key element of regulation of the transcript elongation rate. The first nucleosome positioned in the transcribed region of the human *hsp70* gene presents a strong barrier for elongating Pol II *in vivo* and *in vitro* and the nucleosome is removed during transcription activation [57].

An inability to overcome the nucleosome is not strictly a Pol-II-specific feature. RNA polymerase from *E. coli* is remarkably similar to Pol II in the pattern of transcription through a nucleosome template [58]. Thus, the height of the nucleosomal barrier and the fate of the transcribed nucleosome probably depend on the general properties of the transcription elongation complex. Properties of the elongation complex that affect transcription of the chromatin template remain to be identified experimentally.

The potency of the nucleosomal barrier necessitates the involvement of mechanisms facilitating chromatin transcription (Figure 5). Various factors facilitate progression of RNA polymerase through the nucleosome via diverse mechanisms. These include factors that bind to histone proteins, Pol-II elongation factors, complexes inducing non-covalent ATP-dependent chromatin remodeling, and factors responsible for covalent modifications of histones.

The only known factor that can stimulate transcription through chromatin in a highly purified system is the heterodimeric protein complex FACT (facilitates chromatin transcription) [59]. *In vivo*, FACT displays kinetics of recruitment and chromosome tracking similar to Pol II [20]. There is also significant genetic evidence connecting FACT with transcript elongation *in vivo* [60]. Recent data suggest that FACT facilitates Pol-II-induced displacement of H2A–H2B dimer from the nucleosome, probably via direct interaction with the H2A and/or H2B histones [61]. FACT specifically interacts with all core histones *in vitro* and has intrinsic histone chaperone activity [60,61]. This activity might explain *in vivo* observations suggesting that FACT not only participates in Pol-II-induced nucleosome disruption, but is also required for nucleosome re-assembly behind the transcribing enzyme [62,63].

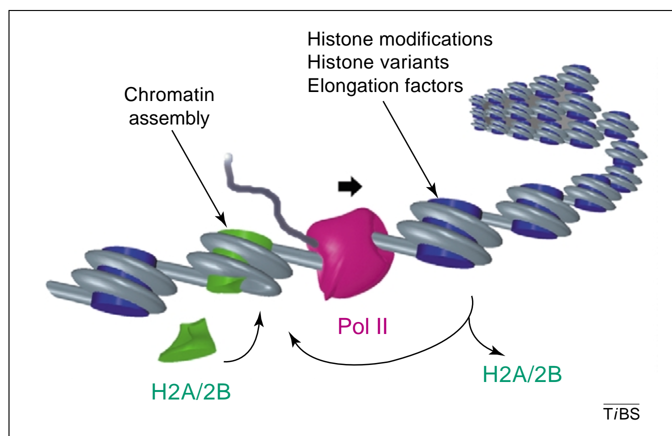

Figure 5. Hypothetical mechanism of transcription through chromatin by polymerase II (Pol II) *in vivo*. As Pol II (pink) travels along a eukaryotic gene, it converts DNA-bound octamers (blue) in its path to hexamers (green; a smaller green shape is the displaced H2A–H2B dimer). Facilitation of this process could be achieved with the aid of elongation factors such as FACT (facilitates chromatin transcription), histone modifications and/or histone variants. Transient loss of the H2A–H2B dimer from the nucleosome could create a window of opportunity for chromatin remodeling factors or DNA-binding proteins. Unless the disrupted state is stabilized, complete nucleosomes are eventually restored. Transcription might also result in nucleosome redistribution and partial nucleosome depletion.

Some ATP-dependent remodelers have the ability to perturb nucleosomes in ways that could assist polymerases both in initiation and elongation. For example, the human SWI–SNF complex is required for both initiation and elongation on the human *hsp70* gene *in vitro* [57]. Further evidence for the role of SWI–SNF during elongation was obtained as a result of analysis of human heat shock factor 1. This factor can stimulate both initiation and elongation using distinct residues of the activation domain for each of these functions [64]. Although it can recruit SWI–SNF to a chromatin template, the biggest negative effect on recruitment is seen when the residues responsible for elongation are mutated, suggesting that chromatin remodeling occurs in this system as part of the process of transcript elongation [64]. Additional evidence for a role of SWI–SNF in elongation *in vivo* comes from studies of mutations in yeast genes encoding SWI–SNF subunits (*SWI1*, *SNF5*, *SWI2 or SNF2*), which are synthetic lethal in combination with disruption of the pyrimidine pathway regulatory 2 gene, *PPR2*, encoding transcription elongation factor IIS (TFIIS) [65]. Yeast and human SWI–SNF complexes are associated with Pol II [66,67], suggesting that they could be recruited together. In summary, some ATP-dependent remodelers can facilitate transcript elongation, but it would be unsurprising if future experiments reveal that transcription could also facilitate ATP-dependent remodeling.

For several elongation factors, only either genetic or biochemical evidence is available to indicate their involvement in transcription through chromatin (see [6] for review). These include high-mobility group 14/17 (HMG14/17) proteins, Pol II protein kinase I, DNA topoisomerase IIα, and the elongation factors TFIIS, Elongator and suppressor of Ty *SPT6*. Modifications of histones by acetylation, methylation, phosphorylation or ubiquitination, as well as histone variants such as H3.3 and H2A.Z, are also associated with modulation of gene expression at the elongation step (see [6] for reviews). Taken together, these data suggest that transcript elongation could be regulated as elaborately and extensively as initiation (Figure 5). Future experiments should clarify both the mechanistic and regulatory aspects of this process.

Concluding remarks and future directions

It has become clear that chromatin is a dynamic structure exploited by the cell for regulation of gene activity. Recent experiments indicate that partial unfolding of DNA from the octamer and nucleosome mobilization are probably the most important components of various kinds of remodeling. Evidence suggests that at least ATP-dependent remodeling and transcription-dependent remodeling involving transfer could share the same bulging mechanism. The mechanism of transcription through the nucleosome by Pol II is distinct, although probably mechanistically related. Most remarkably, the histone octamer has intrinsic acrobatic abilities that assist passage of RNA polymerase through the nucleosome without even transient displacement of the octamer into solution. At the same time, the discovery that RNA Pol II transiently displaces one H2A–H2B dimer from the nucleosome highlights the ability of the nucleosome and its histone octamer to undergo internal structural changes.

Whereas the nucleosome transfer mechanism is relatively well understood, the Pol-II-type mechanism involving conversion of the nucleosome to a hexasome clearly needs further analysis. The studies of Pol II and Pol III transcription discussed here have largely omitted examination of the effects of cofactors known to facilitate and regulate transcription initiation and elongation. To be meaningful, much of this work will have to be carried out with polynucleosomal templates capable of folding into higher-order structures, and under conditions closer to the physiological state.

The importance for medicine of the regulation of transcript elongation was highlighted by recent discoveries showing that numerous elongation factors play roles in various human diseases, including HIV-1 infection and acute myeloid leukemia (see [56] for review). The SWI–SNF ATP-dependent remodeler contains and interacts with tumor suppressors and is clearly linked to human cancers (see [68] for review). Thus, further analysis of the mechanism and regulation of transcription in chromatin is important for a better understanding of both the basic and clinical aspects of the transcriptional process.

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