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Review
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The Role of Chromatin during Transcription

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Chromatin structure imposes significant obstacles on all aspects of transcription that are mediated by RNA polymerase II. The dynamics of chromatin structure are tightly regulated through multiple mechanisms including histone modification, chromatin remodeling, histone variant incorporation, and histone eviction. In this Review, we highlight advances in our understanding of chromatin regulation and discuss how such regulation affects the binding of transcription factors as well as the initiation and elongation steps of transcription.

Introduction

Ever since chromatin structure was recognized as a repeating unit of histones and approximately 200 bp of DNA (Kornberg and Thomas, 1974), it has been speculated that its function extends beyond simple DNA compaction. Indeed, the discovery that nucleosomes impede transcription in vitro (Knezetic and Luse, 1986; Lorch et al., 1987) and that deletion of histones or their basic tails elicits specific effects on gene expression in vivo (Han and Grunstein, 1988; Kayne et al., 1988) provided a glimpse of chromatin's importance. The biochemical isolation and characterization of the first nuclear histone-modification enzyme (Brownell et al., 1996) and the first chromatin-remodeling complex (Cote et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994) proved the genetic predictions for the functions of these factors (Struhl, 1998). Indeed, these findings led scientists to realize the pivotal roles of nonhistone proteins in regulating chromatin structure. Importantly, these discoveries provided new approaches for manipulating chromatin both in vitro and in vivo. The explosion in chromatin research efforts has made it increasingly apparent that chromatin structure imposes profound and ubiquitous effects on almost all DNA-related metabolic processes including transcription, recombination, DNA repair, replication, kinetochore and centromere formation, and so forth. Given that information, other than DNA sequence (genetic) information, that is contained in chromatin structure can be inherited, chromatin research has also moved to the forefront of modern epigenetics. In this Review, we discuss the role of chromatin in transcription regulation, which is the area that has brought the field into the limelight. However, the transcription-centric perspective of chromatin has revealed principles that apply to other DNA-related processes such as DNA replication and repair (see Review by A. Groth et al., page 721 of this issue).

Transcription on "Naked" DNA

The principles and mechanisms underlying transcription on naked DNA are remarkably similar between eukaryotes and prokaryotes despite the increased complexity of eukaryotic transcription machinery (Hahn, 2004). The typical RNA polymerase II (Pol II) transcription cycle begins with the binding of activators upstream of the core promoter (including the TATA box and transcription start site). This event leads to the recruitment of the adaptor complexes such as SAGA (Green, 2005) or mediator, both of which in turn facilitate binding of general transcription factors (GTFs; Thomas and Chiang, 2006). Pol II is positioned at the core promoter by a combination of TFIID, TFIIA, and TFIIB to form the closed form of the preinitiation complex (PIC). TFIIH then melts 11–15 bp of DNA in order to position the single-strand template in the Pol II cleft (open complex) to initiate RNA synthesis. The carboxy-terminal domain (CTD) of Pol II is phosphorylated by the TFIIH subunit during the first 30 bp of transcription and loses its contacts with GTFs before it proceeds onto the elongation stage. Meanwhile, the phosphorylated CTD begins to recruit the factors that are important for productive elongation and mRNA processing (Buratowski, 2003).

Regulation of Nucleosome Dynamics

The packaging of the template into nucleosomes appears to affect all stages of transcription from activator binding and PIC formation to elongation (reviewed in Workman and Kingston, 1998). We will summarize the prevailing view of how chromatin structure is regulated and then discuss how chromatin exerts effects on transcription initiation and elongation.

The nucleosome core is composed of 147 bp of DNA wrapped 1.65 turns around the histone octamer; there are 14 contact points between histones and DNA (Luger et al., 1997). These multiple interactions make the nucleosome one of the most stable protein-DNA complexes under physiological conditions; because of this, it is well-suited for its packaging function. However, the nucleosome is not a simple static unit. It possesses dynamic properties that are tightly regulated by various protein complexes.

Table 1. Histone Modifications Associated with Transcription

Modifications	Position		Enzymes				Recognition Module(s)[a]	Functions in Transcription
			S. cerevisiae	*S. pombe*	*Drosophila*	Mammals		
Methylation	H3	K4	Set1	Set1	Trx, Ash1	MLL, ALL-1, Set9/7, ALR-1/2, ALR, Set1	PHD, Chromo, WD-40	Activation
		K9	n/a	Clr4	Su(var)3-9, Ash1	Suv39h, G9a, Eu-HMTase I, ESET, SETBD1	Chromo (HP1)	Repression, activation
		K27				E(Z)	Ezh2, G9a	Repression
		K36	Set2			HYPB, Smyd2, NSD1	Chromo(Eaf3), JMJD	Recruiting the Rpd3S to repress internal initiation
		K79	Dot1			Dot1L	Tudor	Activation
	H4	K20		Set9	PR-Set7, Ash1	PR-Set7, SET8	Tudor	Silencing
Arg Methylation	H3	R2				CARM1		Activation
		R17				CARM1		Activation
		R26				CARM1		Activation
	H4	R3				PRMT1	(p300)	Activation
Phosphorylation	H3	S10	Snf1				(Gcn5)	Activation
Ubiquitination	H2B	K120/123	Rad6, Bre1	Rad6		UbcH6, RNF20/40	(COMPASS)	Activation
	H2A	K119				hPRC1L		Repression
Acetylation	H3	K56					(Swi/Snf)	Activation
	H4	K16	Sas2, NuA4		dMOF	hMOF	Bromodomain	Activation
	Htz1	K14	NuA4, SAGA					Activation

[a] The proteins that are indicated within the parentheses are shown to recognize the corresponding modifications but specific domains have yet to be determined.

Histone Modifications and Transcription

Both histone tails and globular domains are subject to a vast array of posttranslational modifications (see Review by T. Kouzarides, page 693 of this issue). These modifications include methylation of arginine (R) residues; methylation, acetylation, ubiquitination, ADP-ribosylation, and sumolation of lysines (K); and phosphorylation of serines and threonines (Table 1). Modifications that are associated with active transcription, such as acetylation of histone 3 and histone 4 (H3 and H4) or di- or trimethylation (me) of H3K4, are commonly referred to as euchromatin modifications. Modifications that are localized to inactive genes or regions, such as H3 K9me and H3 K27me, are often termed heterochromatin modifications. Most modifications are distributed in distinct localized patterns within the upstream region, the core promoter, the 5′ end of the open reading frame (ORF) and the 3′ end of the ORF (Figure 1). Indeed, the location of a modification is tightly regulated and is crucial for its effect on transcription. For instance, as we will discuss later in more detail, Set2-mediated methylation of histone H3K36 normally occurs within the ORF of actively transcribed genes. However, if Set2 is mistargeted to the promoter region through artificial recruitment, it represses transcription (Landry et al., 2003; Strahl et al., 2002).

Typically, histone acetylation occurs at multiple lysine residues and is usually carried out by a variety of histone acetyltransferase complexes (HATs; Brown et al., 2000). Distinct patterns of lysine acetylation on histones have been proposed to specify distinct downstream functions such as the regulation of coexpressed genes (Kurdistani et al., 2004). Another view posits that the biological functions of histone acetylation rely primarily on the number of lysines modified (e.g., a cumulative effect) with the one known exception of H4K16Ac (Dion et al., 2005). In contrast to acetylation, histone methylation, phosphorylation, ubiquitination, etc. are often catalyzed by a specific enzyme at a specific site and result in unique functions

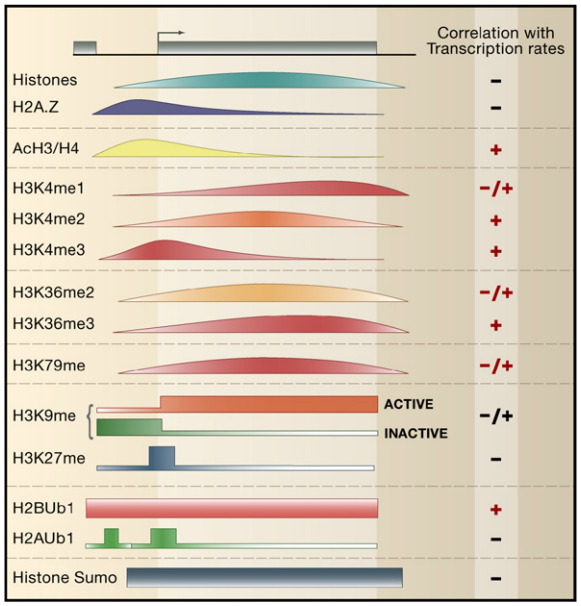

Figure 1. Genome-Wide Distribution Pattern of Histone Modifications from a Transcription Perspective

The distribution of histones and their modifications are mapped on an arbitrary gene relative to its promoter (5′ IGR), ORF, and 3′ IGR (original references were reviewed in Shilatifard, 2006; Workman, 2006). The curves represent the patterns that are determined via genome-wide approaches. The squares indicate that the data are based on only a few case studies. With the exception of the data on K9 and K27 methylation, most of the data are based on yeast genes.

(Table 1). The reason for the distinction between acetylation and other modifications is currently unknown, but biophysical changes caused by histone acetylation (see below) may offer a partial explanation.

Since their identification decades ago, histone modifications have been proposed to have a number of different functions (reviewed in Workman and Kingston, 1998). However, a consensus has begun to emerge in recent years. First, with the exception of methylation, histone modifications result in a change in the net charge of nucleosomes, which could loosen inter- or intranucleosomal DNA-histone interactions. This idea is supported by the observation that acetylated histones are easier to displace from DNA both in vivo (Reinke and Horz, 2003; Zhao et al., 2005) and in vitro (Chandy et al., 2006; Hassan et al., 2006; Ito et al., 2000). Second, it is well accepted that protein modifications can be recognized by other proteins (reviewed in Seet et al., 2006). Given the diversity of covalent modifications, it has been proposed that individual histone modifications or modification patterns might be read by other proteins that influence chromatin dynamics and function (Jenuwein and Allis, 2001; Strahl and Allis, 2000; Turner, 2000). Therefore, the outcome of a particular modification is dependent on the effector proteins that recognize it. Third, some modifications directly influence higher-order chromatin structure. For instance, acetylation of H4 K16 inhibits the formation of compact 30 nm fibers (Shogren-Knaak et al., 2006). Finally, the mechanisms discussed above are not necessarily mutually exclusive. For example, acetylation of H4 K16 also impairs the efficiency of ATP-dependent chromatin assembly and mononucleosome mobilization by the ACF histone chaperone (Shogren-Knaak et al., 2006), thus suggesting that a single modification can elicit multiple effects on chromatin structure.

Chromatin Remodeling and Histone Eviction

The second major class of chromatin regulators are the protein complexes that utilize ATP hydrolysis to alter the histone-DNA contacts; because of this, they are generally referred to as chromatin-remodeling complexes (See reviews Flaus and Owen-Hughes, 2004; Saha et al., 2006; Smith and Peterson, 2005). The consequences of remodeling include transient unwrapping of the end DNA from histone octamers, forming the DNA loop, or moving nucleosomes to different translational positions (sliding), all of which change the accessibility of nucleosomal DNA to transcription factors (TFs).

For a period of time after the discovery of ATP-dependent chromatin-remodeling enzymes, it had been thought that chromatin remodeling did not involve complete displacement of histones from DNA (but see Owen-Hughes et al., 1996). Nevertheless, recent pulse-chase studies suggested a highly dynamic turnover of histones at active genes (see review by Clayton et al., 2006). In addition, a cytological study found that histone H2B rapidly exchanged in and out of a nucleosome relative to H3 and H4 (Kimura and Cook, 2001), which suggests that histone displacement does occur in vivo. More recent genetic and biochemical studies confirmed these early observations at a molecular level (for review, see Workman, 2006). In general, histone dimers of H2A and H2B can be rather easily exchanged in and out of nucleosome, which is consistent with the prediction based on the crystal structure of the nucleosome (Luger et al., 1997). Entire histone octamers, including H3 and H4, can also be displaced (evicted) or exchanged under certain circumstances as discussed later.

The mechanisms of histone eviction involve numerous activities. Detailed biochemical studies suggest that cooperative TF binding (Adams and Workman, 1995; Owen-Hughes and Workman, 1996; Workman and Kingston, 1992); chromatin-remodeling complexes such as Swi/Snf (Bruno et al., 2003; Ito et al., 2000; Owen-Hughes et al., 1996; Phelan et al., 2000) and RSC (Lorch et al., 2001, 2006); and actively transcribing Pol II (Kireeva et al., 2002) can all mediate histone displacement. Because histones that are displaced from DNA can rebind to the same DNA molecule, the addition of proper histone acceptors, such as histone chaperones (Asf1, Nap1, and nucleophosmin), or free DNA into reactions overcomes such a barrier in vitro (Chen et al., 1994; Lorch et al., 2006; Swaminathan et al., 2005; Walter et al., 1995). Importantly, these chaperones are essential for histone eviction in vivo (Adkins et al., 2004; Schwabish and Struhl, 2006).

Table 2. Histone Variants Involved in Transcription Regulation

Histone	Variant Forms	Role(s) in Transcription	Localization	Structural Features	Functions
H3	H3.3	Transcription activation	Transcribing region	Different from canonical H3 in only four amino acids.	Active transcription triggers deposition and removal.
H2A	macroH2A	X chromosome inactivation	Inactive X chromosome	C-term nonhistone-like region is responsible for most of functions; histone-fold prevents sliding; prefers to form hybrid nucleosome.	Repressing initiation but not elongation; interfering histone acetylation by p300; it blocks sliding by ACF and remodeling by Swi/Snf; it inhibits transcription factor binding (NFkB).
	H2AZ	Transcription activation/ repression	Promoter, heterochromatin boundary	Loop1 differs from H2A, disfavors formation of hybrid nucleosome; C-term α helix is essential for recognition.	Facilitates TBP binding; is evicted upon activation; prevents elongation-associated modification and remodeling at promoter
	H2ABbd	Transcription activation	Active X chromosome and autosomes	Lack of C term; it only organizes 118–130 bp pf DNA and leaves each side 10 bp free DNA.	Swi and ACF fail to mobilize the H2ABbd nucleosome but can increase its accessibility. p300- and Gal4-VP16-activated transcription is more robust on H2ABbd nucleosomes; H2A.Bbd histone fold domain is responsible for the unusual properties of the H2A.Bbd nucleosome.
	H2A.X	Repression	Canonical in yeast, generally distributed	A conserved C-term SQ(E/D) motif that becomes phosphorylated upon DNA damage.	

Histone Variant Incorporation

The S phase-synthesized core histones were once considered the universal common component of all nucleosomes (Kornberg and Lorch, 1999). More careful examination, however, revealed that many variant forms of histones exist throughout different organisms (Table 2; Kamakaka and Biggins, 2005). Histone variants are distinguished from canonical core histones mainly by the fact that they are expressed outside of S phase and incorporated into chromatin in a DNA replication-independent manner. Recent studies suggest that the variant histone H2A.Z can be deposited into a nucleosome either through ATP-dependent histone exchange reactions (Mizuguchi et al., 2004) or with the help of a replication-independent chaperone Nap1 (Park et al., 2005). In addition, transcription activation can trigger deposition and removal of H3.3 from the *Drosophila* genome (Schwartz and Ahmad, 2005).

Differences between variants and canonical histones can be found in the histone tails (MacroH2A; Doyen et al., 2006a), in the histone fold domains (H2ABbd; Doyen et al., 2006b), or even in the difference of a few key amino acid residues (H3.3; Henikoff and Ahmad, 2005). Their incorporation impacts chromatin structure in various ways (Table 2). Interestingly, many sites of modification are conserved between variants and canonical histones (McKittrick et al., 2004). Thus, they are likely interchangeable, and the variants may not affect nucleosome recognition by various chromatin-regulatory proteins.

Transcription Factor Recruitment

Eukaryotic and prokaryotic TFs share universal properties in targeting and binding to sequence-specific binding sites in the context of free DNA (Hahn, 2004). However, when recognition sites are buried in chromatin, eukaryotic TFs have to exploit various strategies to achieve proper binding. Early biochemical experiments suggested that TFs can bind to nucleosomal DNA in a cooperative manner (Adams and Workman, 1995; Taylor et al., 1991). This has been confirmed by in vivo studies showing that activator Pho4 can bind to the *PHO5* promoter before nucleosome disassembly (Adkins et al., 2004). Furthermore, the concept that nucleosomal DNA is somewhat accessible to TFs has been reinforced recently. Bucceri et al. found that rapid repair was observed in various nucleosomal regions of the genome including inactive and active genes as well as repressed promoters. Since the dissociation rate of histones is too slow to account for rapid

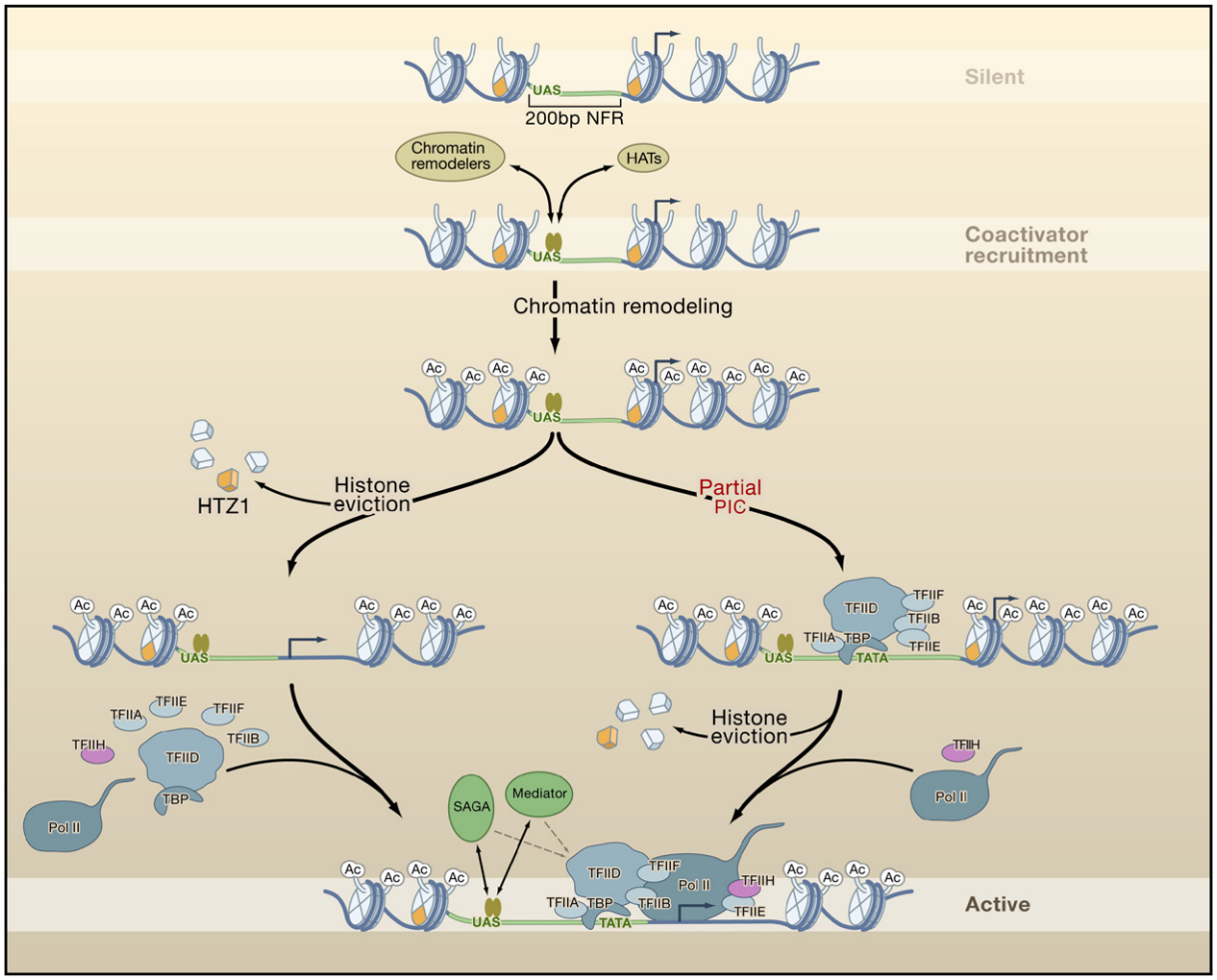

Figure 2. Models of Chromatin Regulation during Transcription Initiation

At the silent promoter, Htz1-containing nucleosomes flank a 200 bp NFR on both sides. Upon targeting to the upstream-activation sequence (UAS), activators recruit various coactivators (such as Swi/Snf or SAGA). This recruitment further increases the binding of activators, particularly for those bound within nucleosomal regions. More importantly, histones are acetylated at promoter-proximal regions, and these nucleosomes become much more mobile. In one model (left), a combination of acetylation and chromatin remodeling directly results in the loss of Htz1-containing nucleosome, thereby exposing the entire core promoter to the GTFs and Pol II. SAGA and mediator then facilitate PIC formation through direct interactions. In the other model (right), which represents the remodeled state, partial PICs could be assembled at the core promoter without loss of Htz1. It is the binding of Pol II and TFIIH that leads to the displacement of Htz1-containing nucleosomes and the full assembly of PIC.

repair, it was concluded that spontaneous unwrapping of nucleosomes, rather than histone dissociation or chromatin remodeling can provide DNA access (Bucceri et al., 2006). However, numerous examples (Workman and Kingston, 1998) have made it apparent that chromatin-remodeling complexes can further stimulate binding of TFs to nucleosomal sites (Utley et al., 1997).

In different studies TF-binding sites have been mapped either to the nucleosome-free region or within a nucleosome. Recent genome-wide studies found that nucleosome density at promoter regions is typically lower than that in the coding region (Bernstein et al., 2004; Lee et al., 2004; Sekinger et al., 2005). Strikingly, Yuan et al. used high-resolution tiling microarrays to discover that in yeast there exists a 200 bp nucleosome-free region positioned approximately over gene promoters (Figure 2). This region is flanked on both sides by positioned nucleosomes (Yuan et al., 2005). The earlier analytical studies and the recent rigorous mathematic modeling led to the hypothesis that organizational information for positioning nucleosomes is embedded within the sequence of the genome (reviewed in Richmond, 2006). Remarkably, the models predict that there is low-level nucleosome occupancy at functional TF-binding sites and that there are more stable nucleosomes at the nonfunctional sites. Therefore, it seems that eukaryotic cells tend to position sequence-specific TF-binding sites within accessible regions. Thus, the first step of gene activation (activator binding) could be more responsive to signaling pathways than it would be if the binding sites were sequestered

within nucleosomes. However, this oversimplified view apparently cannot account for all activator binding in vastly diverse genomes. In a large-scale screen of the human genome, high levels of histone H3K4/79 methylation and H3 acetylation were found to be strict prerequisites for binding of the Myc transcription activator, which implies that chromatin modifications can actually regulate TF binding (Guccione et al., 2006).

Transcription Initiation

Once activators bind to the promoter, they trigger a cascade of recruitment of coactivator complexes (Figure 2). Coactivators (such as chromatin-remodeling complexes, histone-modification enzymes, and mediator) not only facilitate stronger binding of activators to DNA but also make nucleosomal DNA elements more accessible to GTFs. How do cells adjust chromatin structure to accommodate the proper docking of the massive PIC and its ancillary factors?

Historically, increased histone acetylation at the promoter region has been linked to active transcription (Workman and Kingston, 1998). Recently, using high-resolution tiling microarray, Pokholok et al. demonstrated that acetylation of H3 and H4 peaks sharply at active yeast promoters and that, when normalized to nucleosome density, the level of acetylation is proportional to the transcription rate (Pokholok et al., 2005). Consistent with this observation, Robert et al. reported that the HATs Gcn5 and Esa1 are both generally recruited to promoters genome wide (Robert et al., 2004). In addition, elegant biochemical and genetics studies provide further mechanistic support for such a notion (reviewed in Green, 2005). SAGA is recruited to the promoter through direct interaction between its Tra1 subunit and a bound activator (Brown et al., 2001). SAGA recruitment and histone acetylation occur prior to PIC formation at the *GAL1* promoter (Bhaumik and Green, 2001). Moreover, to make DNA more accessible, promoter-bound activators also target chromatin-remodeling complexes such as Swi/Snf (Cosma et al., 1999; Neely et al., 2002). Interestingly, although the sequence of events leading to recruitment of HATs and chromatin remodelers by the same activators is dependent on their promoter context (Hassan et al., 2001a), their recruitment occurs in a coordinated manner (Green, 2005; Hassan et al., 2001b).

Considering the amount of DNA directly contacted by Pol II/GTFs, the structure of the nucleosome seems to pose a significant obstacle to PIC formation (Kornberg and Lorch, 1999). Indeed, it is clear from both ChIP and topological studies that histones are lost at the yeast *PHO5* and *HSP82* promoters upon gene activation and that nucleosomes are reassembled as a gene turns off (Adkins and Tyler, 2006; Boeger et al., 2004; Deckert and Struhl, 2001; Reinke and Horz, 2003; Zhao et al., 2005). In a genome-wide survey, Zanton et al. found that a large number of promoters' partial PICs, including TFIIA, TFIID (and/or SAGA), TFIIB, TFIIE, and TFIIF, were assembled, whereas in these cass RNA Pol II and TFIIH are generally not present (Figure 2, right). Remarkably, in this case, nucleosomes are not displaced (Zanton and Pugh, 2006), thus implying that engaging template DNA into the Pol II active site might create a reasonable point where DNA-histone contacts must be broken. This is reminiscent of a previous observation where Pol II itself was found to be required for chromatin remodeling at the *RNR3* promoter (Sharma et al., 2003).

The histone variant H2A.Z (Htz1) is preferentially enriched at promoters that are poised for transcription activation (Guillemette et al., 2005; Li et al., 2005; Zhang et al., 2005). High-resolution mapping reveals that two well-positioned Htz1-containing nucleosomes flank a 200 bp nucleosome-free region (NFR; Raisner et al., 2005). Htz1-containing nucleosomes are resistant to transcription elongation-related modifications and to chromatin remodeling (Li et al., 2005). In addition, Htz1 is easily dissociated from nucleosomes, presumably as a dimer with H2B (Zhang et al., 2005). Upon transcription activation, however, Htz1 is rapidly evicted from the promoter, and its loss is required for full transcription (Zanton and Pugh, 2006; Zhang et al., 2005). Therefore, Htz1 is specifically positioned at the promoter, where some nucleosomes have to be removed to accommodate PIC formation. However, it should be noted that although there is solid evidence for histone loss, the promoter is not completely nucleosome free. Acetylated histones H3 and H4 continue to accumulate during gene activation (Pokholok et al., 2005), and Htz1 K14 is acetylated at active promoters (Millar et al., 2006). Hence, the reason for Htz1 removal might be to make room for the mobilization of residual nucleosomes. For example, at the IFN-β promoter, sliding of a nucleosome upon TBP binding is indeed beneficial to transcription (Lomvardas and Thanos, 2001). A second reason would be to make the underlying DNA completely accessible (Zhang et al., 2005).

Transcription Elongation

Transcription elongation begins when Pol II releases from GTFs and travels into the coding region. This event signals the recruitment of the elongation machinery, which includes the factors involved in polymerization, mRNA processing, mRNA export, and chromatin function (Hahn, 2004). At this point, one might expect that Pol II would deal with the downstream nucleosomes in a similar manner. However, the opposite is true. Cells exploit a very sophisticated array of factors to control chromatin architecture during elongation, and the events and factors required at the beginning of the gene differ significantly from those required at the end. This is done not only to promote efficient RNA synthesis but also to ensure the integrity of the chromatin structure while Pol II travels through the body of the gene.

The Elongation Machinery

Unlike transcription initiation factors, which are usually recruited to the promoter through activator interactions, Pol II-elongation factors are bound via direct or indirect interaction with the Pol II CTD (Buratowski, 2003). The Pol II CTD undergoes two major phosphorylation changes during elongation: Ser5 is phosphorylated by TFIIH at the 5′ end of the ORF, and Ser2 is phosphorylated by the Ctk

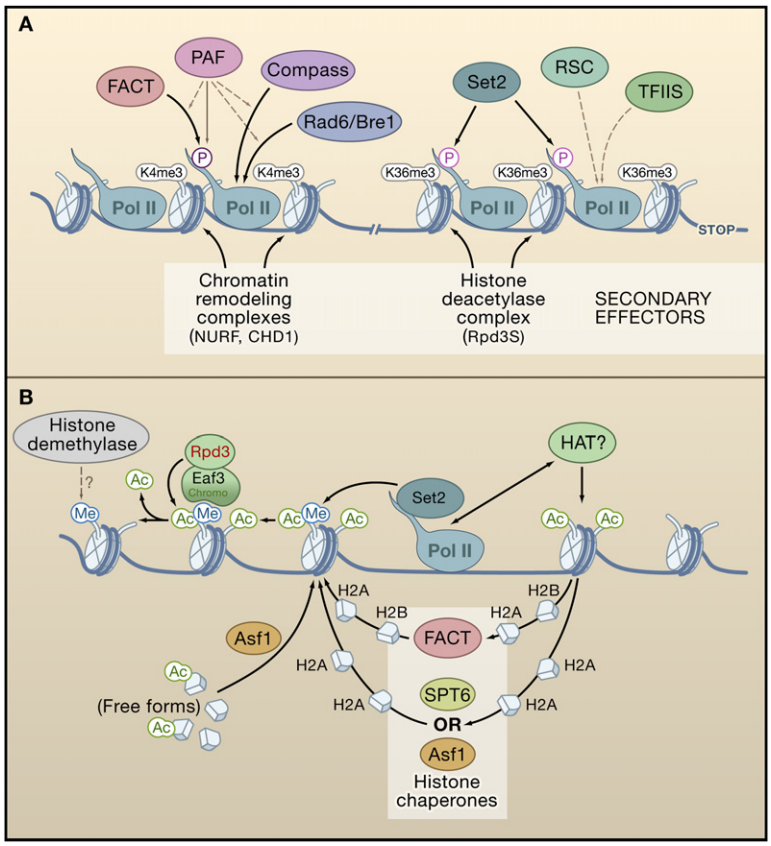

Figure 3. Regulation of Nucleosome Dynamics during Transcription Elongation

(A) The chromatin landscape during elongation is determined by the factors associated with different forms of Pol II. PAF facilitates the binding of FACT, COMPASS, and Rad6/Bre1 to the Ser5-phosphorylated CTD, which results in H2B ubiquitination and accumulation of trimethylation of H3K4 at the 5′ end of ORF. Set2 directly interacts with Ser2-phosphorylated CTD, thus methylating H3K36 at the 3′ end.

(B) Maintenance of nucleosomal stability during transcription. When Pol II migrates into promoter-distal regions, where the influence of activator-dependent HATs is diminishing, Pol II requires other HATs (elongators or those associated with Pol II) to acetylate the nucleosome in front of elongation machinery. The passage of Pol II causes histone displacement. Subsequently, these histones are redeposited onto the DNA behind Pol II via concerted actions of histone chaperones. Alternatively, the free forms of histones in the nucleus are also available for reassembly. These newly deposited nucleosomes are somehow hyperacetylated and are immediately methylated by Set2. Methylation of H3K36 is then recognized by the chromodomain of Eaf3, which in turn recruits the Rpd3S deacetylase complex. Rpd3S removes the acetyl marks and leaves the nucleosome in a stable state. Methylation of H3K36 is eventually eliminated by a histone demethylase when the gene turns off.

kinase as Pol II transits toward the 3′ end. These phosphorylation events appear to control the elongation processes and couple them with alterations in chromatin structure (Figure 3A).

PAF/RTF, a multisubunit complex (Ctr9, Cdc73, Leo1, and Rtf1), is an evolutionarily conserved elongation factor (reviewed in Rosonina and Manley, 2005). With the assistance of Spt4/5 (Qiu et al., 2006), PAF appears to be an early module that is loaded onto the Ser5-phosphorylated CTD. PAF plays a pivotal role in controlling the binding of most Ser5 CTD-associated chromatin regulators (Figure 3A). Although PAF is not required for promoter recruitment of histone ubiquitin ligase Rad6, it is critical for extending Rad6 binding into the ORF and does so presumably by mediating interaction between Pol II and Rad6 (Wood et al., 2003; Xiao et al., 2005). PAF also participates in recruiting the histone H3K4 methyltransferase Set1 complex (COMPASS) for elongating Pol II (Krogan et al., 2003a; Ng et al., 2003b; Wood et al., 2003). Although H2B monoubiquitination by Rad6/Bre1 is required for K4 methylation (Sun and Allis, 2002), particularly di- and trimethylation (Dehe et al., 2005; Shahbazian et al., 2005), PAF appears to directly regulate both H2B ubiquitination and K4 methylation (Krogan et al., 2003a; Ng et al., 2003a, 2003b; Wood et al., 2003). However, PAF is dispensable for monomethylation of K4 that is mediated by Set1 (Dehe et al., 2005), which suggests PAF-independent targeting of Set1. Other chromatin-related factors whose association with Ser5-phosphorylated Pol II are regulated by PAF include the chromatin-remodeling factor Chd1 (Simic et al., 2003) and the histone chaperone-like factors Spt6 and FACT (Adelman et al., 2006; Pavri et al., 2006; Squazzo et al., 2002). In contrast, the histone methyltransferase Set2 targets primarily to Ser2-phosphorylated CTD, while Pol II travels toward the 3′ end of the ORF (Krogan et al., 2003b; Li et al., 2002, 2003; Xiao et al., 2003); thus, it is not dependent on PAF (Figure 3A).

Histone Modifications and Transcription

The well-defined landscape of chromatin modifications observed over the body of a gene (Figure 1) is striking in its detail. However, as we shall discuss below, it is the consequence of an ordered recruitment of various histone-modifying enzymes. This well-choreographed process is likely a consequence of Pol II moving through the ORF while struggling to maintain chromatin structure within the transcribed region.

Histone H3K4 Methylation

The H3K4 residue in yeast is methylated by the Set1 complex across the entire ORF of an active gene (reviewed in Shilatifard, 2006). As shown in Figure 1, monomethylation is enriched toward the 3′ end, and dimethylation peaks in the middle, whereas trimethylation occurs around the transcription start site and the 5′ end of the ORF (Pokholok

et al., 2005). One possible interpretation of this distribution pattern is that K4me1 occurs at a basal level; Set1 associates with elongating Pol II at the beginning of the ORF and converts monomethyl into dimethyl and eventually into trimethyl. Hence, the gradual addition of methyl groups at the 5′ ORF may then lead to the gradient of tri- and dimethyl trailing off at the 3′ end of the ORF. This hypothesis is supported by the observation that in a *paf1*Δ mutant, both H3K4me2 and H3K4me3 are eliminated, but H3K4me1 is not affected. More importantly, H3K4me1 increases at the 5′ ORF (Dehe et al., 2005), presumably due to the failure to convert H3K4me1 into H3K4me2 or into H3K4me3 in the mutant. Therefore, Set1 can catalyze H3K4me1 independent of PAF, but conversion to H3K4me2 or -me3 requires PAF and association with Pol II.

This phenomenon resembles how the MLL/WRD5 complex controls H3K4 methylation in humans, where histones can be mono- and dimethylated at H3K4 without WDR5, a WD-40 domain-containing protein (Wysocka et al., 2005). The MLL/WRD5 complex can specifically recognize the dimethyl marks and convert them into trimethylation (Wysocka et al., 2005). This remarkable similarity accentuates the importance of di- and trimethylation of H3K4 in transcription regulation. Coincidently, H2B ubiquitination is only required for di- and trimethylation (Dehe et al., 2005; Shahbazian et al., 2005), and the extent of both modifications is positively correlated to the frequency of transcription. Therefore, it is conceivable that these two marks enriched at the 5′ ORF may serve as a critical signal for defining the start of the transcribed domain and the frequency with which Pol II travels through it. However, the precise function of H3K4 methylation is still unknown. In a completely defined in vitro transcription system, it has been shown that H3K4 methylation does not affect transcription elongation per se (Pavri et al., 2006), which is consistent with the in vivo observation that Set1 does not affect elongation or processivity of Pol II (Mason and Struhl, 2005). These data imply that the importance of H3K4 methylation might rest primarily in its signaling functions. Recent studies provide some clues in this direction. Chromatin-remodeling factors (NURF) and histone-modification complexes (hTip60, mSIN3/HDAC, yNuA3, etc.) contain PHD domains that can specifically recognize H3K4 methylation (see review by Zhang, 2006), thereby recruiting their respective complexes to activate/repress transcription. In addition, an elongation-related chromatin-remodeling factor Chd1 also recognizes methylated H3K4 (Pray-Grant et al., 2005; Figure 3A). Future studies addressing how these H3K4-binding complexes influence transcription elongation will be of importance for understanding the role of H3K4 methylation.

Histone H2B Monoubiquitination

Histone H2B monoubiquitination (H2Bub1) occurs at both promoters and ORFs (Kao et al., 2004; Xiao et al., 2005) and is dependent on PAF and active transcription (Ng et al., 2003a; Pavri et al., 2006; Wood et al., 2003). One commonly accepted role of H2Bub1 in transcription is to stimulate di- and trimethylation of histone H3K4 (Dehe et al., 2005; Shahbazian et al., 2005; Sun and Allis, 2002). Due to the bulky nature of ubiquitin, it has been speculated that its incorporation into nucleosomes would be disruptive to their structure. However, biochemical studies indicate that ubiquitination of histones has very little effect on nucleosome architecture (Jason et al., 2002). Pavri et al. recently reported that monoubiquitination of H2B enhances the rate of transcription elongation on chromatin templates (Pavri et al., 2006). It is noted that in this system the stimulatory effect occurs while H2Bub1 remains in chromatin. This seems in contrast to the in vivo observation that both ubiquitination and deubiquitination are important for full transcription activation at the *GAL1* promoter (Henry et al., 2003). However, it is possible that in the in vitro assay, deubiquitination may stimulate transcription to a greater extent than does the initial effect of ubiquitination or that deubiquitination is required only at the promoter.

Histone H3K36 Methylation

Histone H3K36 methylation mediated by Set2 is another important landmark on chromatin during elongation. Both di- and trimethylation are enriched at the 3′ ORF, while only trimethylation displays a positive correlation with transcription rates (Figure 1; Pokholok et al., 2005; Rao et al., 2005). Our understanding of the role of K36 methylation in elongation is much more advanced compared to the role of other modifications in this process. H3K36 methylation is recognized by the chromodomain of Eaf3, a subunit of the Rpd3S histone deacetylase complex. Trimethylation leads to the recruitment of Rpd3S and creates a hypoacetylated environment within ORFs (Carrozza et al., 2005; Joshi and Struhl, 2005; Keogh et al., 2005). The biological consequence of the Set2-Rpd3S pathway will be discussed below.

Nucleosomes as Transcription Barriers

The nucleosome forms a strong barrier to Pol II transcription in vitro. Although the phage SP6, T7 RNA polymerases, and yeast Pol III can transcribe through nucleosomal DNA by mobilizing histones along the templates (Clark and Felsenfeld, 1992; Studitsky et al., 1994, 1995, 1997), RNA Pol II can only traverse the nucleosome under conditions in which at least one H2A/H2B dimer is lost (Kireeva et al., 2002, 2005). How does Pol II overcome the nucleosome barrier?

When Pol II transcribes into a nucleosomal template, it pauses at certain sites that are presumably related to the strength or nature of the histone-DNA contacts (Bondarenko et al., 2006; Kireeva et al., 2005). This pausing leads to Pol II backtracking. The prototypic transcription elongation factor TFIIS reactivates the backtracked Pol II complexes and promotes transcription through the nucleosomal templates (Kireeva et al., 2005; Kulish and Struhl, 2001). Consistent with this observation, TFIIS was recently found to be a major component of chromatin transcription-enabling activity (CTEA). CTEA strongly stimulates transcription elongation through nucleosomes

at a post-PIC step and in a manner dependent upon p300 and acetyl-CoA (Guermah et al., 2006).

Biochemical and genetic experiments suggest that the FACT histone chaperone complex can also help Pol II transcribe through nucleosomes (reviewed in Reinberg and Sims, 2006). However, its mechanism is different from that of TFIIS. The requirement for stoichiometric amounts of FACT for nucleosomal transcription initially suggested that FACT might act as a histone chaperone (Orphanides et al., 1999). This notion is consistent with the observation that passage of Pol II through nucleosome at high salt conditions causes a quantitative loss of one H2A/H2B dimer (Kireeva et al., 2002). It was ultimately shown that FACT does act as a chaperone during transcription and that it functions in both disassembly and reassembly of H2A/H2B dimers (Belotserkovskaya et al., 2003).

ATP-dependent chromatin-remodeling complexes have long been suspected of playing a role in helping Pol II pass through nucleosomes (Workman and Kingston, 1998); however, direct evidence did not emerge until recently. Using C-tail DNA templates reconstituted with a histone octamer, Carey et al. demonstrated that RSC can help Pol II transcribe through otherwise paused sites on nucleosome templates. This reaction is further stimulated by SAGA- and NuA4-mediated histone acetylation that presumably utilizes the multiple bromodomains (acetyl-lysine-binding domains) that are contained within the RSC complex (Carey et al., 2006). It will be interesting to see if this effect involves active histone eviction by RSC.

Transcription Memory and Maintenance of Genome Integrity

Accumulating evidence suggests that histones are lost to some extent during elongation, at least partially and/or temporarily (Workman, 2006). However, with the help of histone chaperones, histones evicted in front of elongating Pol II appear to be rapidly deposited onto DNA behind Pol II (Figure 3B). Indeed, it has been shown that H3 redeposition closely correlates with Pol II clearance (Schwabish and Struhl, 2004). In addition, incorporation of the histone variant H3.3 is dependent upon elongation-associated histone turnover in *Drosophila* (Schwartz and Ahmad, 2005). It is thought that failure to redeposit histones back onto transcribed regions would leave free DNA and expose cryptic promoters that would otherwise not be accessible to TFs. Remarkably, mutations in histone chaperones known to affect elongation, such as Spt6, Spt16 (FACT), and Asf1, all lead to the generation of cryptic transcripts initiated from internal start sites within the body of yeast genes (Kaplan et al., 2003; Mason and Struhl, 2003; Schwabish and Struhl, 2004, 2006). Similar phenotypes have also been observed in mutations of factors that comprise the Set2-Rpd3S pathway (Carrozza et al., 2005; Joshi and Struhl, 2005). Apparently, the redeposition and deacetylation of histones are both required to maintain chromatin in a stable conformation within ORFs, and this conformation is repressive to PIC formation and initiation.

Hyperacetylation within an ORF caused by disruption of the Set2-RpdS pathway appears to enable the underlying DNA sequence to become exposed to TFs, thus enabling cryptic promoter-like sequences within the ORF to function as transcription start sites (Carrozza et al., 2005; Joshi and Struhl, 2005). Although this pathway is not essential for viability in yeast, this might not be the case in higher organisms. There are several reasons why internally initiated transcripts could be deleterious to the organism. First, the partial products translated from cryptic transcripts could have dominant-negative effects. Second, if cryptic transcripts are generated from the antisense strand, they might cause severe RNA interference effects. Third, partial transcripts might also bypass critical splicing sites.

Future Directions

With the explosion of novel chromatin-binding domains, it appears that the combination of histone codes is rather limited. How and why do complexes with different functionalities recognize the same mark? For instance, PHD domains seem to exist in both HAT and HDAC complexes (Zhang, 2006); the chromodomain containing Eaf3 is shared by the NuA4 (a HAT) and the Rpd3S (an HDAC). What are the precise roles of HATs that are responsible for initial acetylation prior to Pol II binding or during elongation? Is a ''HAT committee'' required? Is this acetylation directly regulated by Pol II or by more global acetylation events? What are the roles of demethylases (H3K4 and H3K36) during elongation? Do methyl marks need to be removed to release Rpd3S from chromatin after it finishes deacetylation? These and numerous additional intriguing questions promise that the ''role of chromatin during transcription'' is far from being a closed chapter.

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