Transcription Factor Access to Promoter Elements

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Abstract   In eukaryotes, transcription factors, including both gene-specific activators and general transcription factors (GTFs), operate in a chromatin milieu. Here, we review evidence from gene-specific and genome-wide studies indicating that chromatin presents an environment that is typically permissive for activator binding, conditional for pre-initiation complex (PIC) formation, and inhibitory for productive PIC assembly within coding sequences. We also discuss the role of nucleosome dynamics in facilitating access to transcription factors (TFs) in vivo and indicate some of the principal questions raised by recent findings. J. Cell. Biochem. 102: 560–570, 2007.   © 2007 Wiley-Liss, Inc.

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The discovery in the 1970s that eukaryotic DNA was packaged into nucleosomes in which the nucleic acid component was closely apposed to the histone proteins, and in the 1980s that these nucleosomes could adopt specified positions with respect to DNA sequence, led to a general view that chromatin was likely to be inhibitory to the binding of proteins—transcription factors (TFs)—that was needed to enable production of mRNA in the cell. Nucleosomes were indeed found to inhibit transcription in vitro, and it became clear that cellular mechanisms must have evolved to overcome this potential inhibition. Findings that chromatin structure at promoters sometimes rearranged during transcriptional activation [Almer et al., 1986] fed the notion that nucleosomes might regulate transcription by governing TF access, and the mere presence of positioned nucleosomes at gene promoter regions was frequently taken as prima facie evidence for such regulation. This idea gained further credence with the discovery

of multiprotein complexes, such as the Swi/Snf complex, that could alter nucleosome structure and that function as global transcriptional regulators.

Research showing that histone tails function as transcriptional regulators, and that enzymes that modify those tails act as transcriptional coactivators or corepressors, has provided incontrovertible evidence that transcription is regulated by features of chromatin structure [Wolffe and Hayes, 1999]. The means by which this regulation occurs, however, remain incompletely understood. Here, I will review the current state of our knowledge of the characteristics of chromatin that govern TF access in eukaryotes, and will attempt to point toward some of the major problems in this area to be addressed.

ACTIVATOR BINDING TO CHROMATIN IN VIVO: AN OPEN DOOR POLICY?

Transcriptional activation in eukaryotes may be viewed in most cases as a three-step process: binding of activator(s), recruitment of the pre-initiation complex (PIC), and elongation by RNA polymerase. For many genes transcribed by RNA polymerase II (pol II), the initial and rate-limiting event is activator binding or activation of pre-bound activator (for example, by post-translational modification or binding of a small molecule). The activator then recruits the PIC along with protein complexes that modify or remodel chromatin, which leads to

transcriptional initiation and elongation by pol II. We consider each of these events, in the context of chromatin, in turn.

Activators may contend with chromatin structure in various ways (see reference Morse [2003] for a more extensive discussion of this issue). In rare cases, an activator-binding site can occur on the surface of a nucleosome such that activator binding does not result in nucleosome disruption, but rather results in the formation of a ternary complex. Some activators can outcompete the histones for binding when a binding site is artificially placed into a positioned nucleosome; however, examples for which this occurs in a natural context remain to be described. Finally, the activator-binding site may be situated in a DNA segment that is not associated with histones; that is, in a linker region or a nucleosome-depleted region (NDR).

This last mechanism may be the one most commonly used to allow activator access to eukaryotic promoters in vivo. Specific examples of genes having constitutively accessible cis-acting elements have been known for quite some time, from yeast, humans, and other organisms (discussed in reference Morse [2003]). More recently, new approaches have allowed genome-scale studies of chromatin structure, and these studies have revealed a preponderance of such accessible promoters. A strong indication of widespread nucleosome depletion in promoters was reported in two nearly concurrent publications in 2004 [Bernstein et al., 2004; Lee et al., 2004]. These studies used microarrays to examine the relative abundance of histone-associated DNA sequences throughout the yeast genome, using a protocol in which sonicated chromatin was immunoprecipitated using epitope-tagged histones (myc-tagged H4 in one study and FLAG-tagged H2B in the other) and appropriate antibodies, or antibodies against the carboxy terminus of histone H3. The data obtained were relatively low in resolution, due to both the nature of the arrays and the sonication protocol, which yielded fragments much larger than single nucleosomes; nevertheless, both studies reported depletion of nucleosomes in promoters relative to gene coding sequences. This supported earlier work indicating that the same sequences were more accessible when situated in a promoter than in an open reading frame [Mai et al., 2000], as well as another study demonstrating that promoter and coding sequences could be physically separated on the basis of association with strongly bound proteins (presumably mostly histones) [Nagy et al., 2003]. Further support derived from two follow-up studies, one of which also reported that the general depletion of nucleosomes found in yeast promoters was primarily determined by intrinsically low histone–DNA association relative to coding sequences [Pokholok et al., 2005; Sekinger et al., 2005].

Close on the heels of these studies, Rando and colleagues performed a similar analysis at higher resolution, covering half a megabase of yeast DNA using "tiling" arrays in which 50-mers overlapping every 20 bp were used to monitor enrichment of mononucleosomal sequences compared to total genomic sequences for yeast chromosome III together with selected gene and promoter regions from across the yeast genome [Yuan et al., 2005]. One of the most striking findings from this work was the discovery of an NDR extending about 200 bp from close to the transcription start site into the promoter region of a large fraction of yeast ORFs. Recent work extends this analysis to encompass the entire yeast genome at higher (4 bp) resolution and corroborates the finding of NDRs over the majority of promoters [Lee, Tillo, Bray, RHM, Davis, Hughes, and Nislow, 2007].

How do these NDRs relate to activator binding? One would imagine that for the majority of activators that are bound under the experimental conditions used for chromatin analysis (growth in rich medium, or YPD), occupied binding sites would be found either in linker regions or in NDRs. Both studies found evidence in favor of this scenario. Rando and colleagues used data from a large-scale ChIP-on-chip study, in which binding of 203 yeast TFs (including both activators and repressors) were examined genome-wide, 84 under multiple growth conditions [Harbison et al., 2004], and found that, within the approximately 500 kb of the yeast genome that their analysis covered, 87% of occupied binding sites occurred in linker regions or NDRs, compared to 47% of total intergenic sequences [Yuan et al., 2005]. Lee et al. addressed this issue over the entire yeast genome by examining all yeast promoters for the presence of known or predicted transcription factor-binding sites (TFBSs), and scoring these TFBSs for nucleosome occupancy. Using nuclear localization as a proxy for TF activity, 46 of the factors were considered to be active and

therefore likely to bind their cognate sites. A strong trend was observed for these nuclear-localized TFs having binding sites in regions of low nucleosome occupancy, and a large fraction of these sites were found in the NDRs in the first 100 bp upstream of transcription start sites. However, it was also found that many promoters lack discernible NDRs; it will be interesting to examine more closely in these genes, which are enriched for genes of unknown function, the disposition of activator-binding sites with respect to local chromatin structure. One possibility is that at such promoters, chromatin dynamics or non-targeted activity of chromatin remodeling complexes provide transient access to activators, which then recruit Swi/Snf or other chromatin remodeling activities, thereby generating a local region of open chromatin and stabilizing their own binding by generating a local region of open chromatin through recruitment of Swi/Snf or other chromatin remodeling activities [Gutierrez et al., 2007].

For TFBSs that are not occupied in YPD but are under other conditions, one could envision two alternative scenarios: such sites might be constitutively histone-free, or they might be incorporated into nucleosomes that are remodeled prior to or concurrent with TF binding. Rando and colleagues, again using data from the large ChIP-on-chip study, found that TFBSs not occupied under the growth conditions (YPD) used for chromatin analysis, but bound by activators under other conditions (e.g., by heat shock factor 1 during heat shock) occurred in linker regions or NDRs at the same high frequency found for occupied sites (about 86%). Similarly, a study focusing on the yeast TF Leu3 found that in vivo binding correlated with low nucleosome occupancy, and that this low nucleosome occupancy occurred regardless of the presence of Leu3 [Liu et al., 2006]. Thus, for many TFBSs, an open door policy prevails; the site is constitutively accessible, and no chromatin remodeling is required for factor binding. Put differently, in many cases it appears that chromatin is instructive for TF binding, rather than the binding of TFs determining chromatin structure.

What determines accessibility for such sites? Nucleosome positioning in vivo appears to be governed to some degree by DNA sequence, particularly by phased dinucleotide repeats [Satchwell et al., 1986; Segal et al., 2006].

Improved predictive power was attained by including information on sequence conservation among related yeast species [Ioshikhes et al., 2006], and Lee et al. [2007] found that local structural features of DNA, such as DNA bend and propeller twist, make notable contributions to observed nucleosome occupancy patterns. Thus, sequence-directed accessibility in chromatin appears to have evolved as a prominent mechanism to allow factor access in the context of chromatin.

Another mechanism for establishing accessible TFBSs is through the action of TFs that can outcompete histones for their binding sites and establish local regions of open chromatin. For example, Rap1 is an essential yeast protein that binds to numerous promoters in yeast and functions both in transcriptional activation and silencing [Lieb et al., 2001; Yarragudi et al., 2007]. At the *HIS4* promoter, a Rap1-binding site is needed to help Gcn4 to bind and activate transcription, and placement of a Rap1 site in a nucleosome positioning sequence results in the loss of nucleosome positioning [Yu and Morse, 1999]. Consistent with the notion that Rap1 functions in part to create regions of open chromatin, Rap1-binding sites were found to be enriched in promoters having lowest nucleosome occupancy [Bernstein et al., 2004]. Two other proteins, Abf1 and Reb1, which share functional attributes with Rap1, were found along with Rap1 to associate with promoters having particularly low nucleosome occupancy by Lee et al. [2007] Abf1 can functionally substitute for Rap1, and like Rap1, can outcompete histones in direct competition for its binding site [Yarragudi et al., 2004]. Thus, these proteins can serve as architectural factors that create regions of open chromatin to allow access by TFs that have nearby binding sites.

An intriguing connection has been observed among two of these factors (Abf1 and Reb1), the histone variant H2A.Z, and NDRs at gene promoters in yeast. Genome-wide analysis of the location of H2A.Z showed enrichment at many gene promoters in yeast [Guillemette et al., 2005; Zhang et al., 2005; Li et al., 2005a], and a higher resolution determination found H2A.Z to be enriched in the nucleosomes flanking NDRs at a large number of promoters [Raisner et al., 2005]. In searching for the determinants for H2A.Z localization, one study found a high correlation with binding sites for Abf1 [Zhang et al., 2005], while another

showed that a 22 bp sequence that includes a Reb1-binding site could direct formation of an NDR flanked by H2A.Z-containing nucleosomes [Raisner et al., 2005]. These findings suggest interplay between establishment of NDRs by architectural TFs and the deposition of H2A.Z, but the mechanism underlying this phenomenon remains to be elucidated.

Given the relative compactness of the yeast genome, in which promoter regions are typically only a few hundred base pairs or less, compared to those of higher eukaryotes, it was not clear that NDRs would also be observed in metazoan genomes. However, a recent large-scale study using several human cell lines reports that expressed genes as well as non-expressed genes having PICs present at their promoters in human cells typically exhibit NDRs at their transcription start sites [Ozsolak et al., 2007]; promoters of non-expressed genes lacking PICs did not contain NDRs. Given recent findings that partial PICs assemble at many non-expressed yeast genes [Zanton and Pugh, 2006], the issue of whether PIC assembly is responsible for most NDRs bears closer examination in yeast as well.

ACCESS BY THE GENERAL TRANSCRIPTION MACHINERY

Chromatin remodeling at the proximal promoter region is a common feature of transcriptional activation in eukaryotes. This remodeling typically depends on activator function [Morse, 2003], but in some cases removal of a repressor complex, such as the Tup1/Ssn6 complex in yeast, is sufficient to allow chromatin remodeling and gene activation [Li and Reese, 2001]. Several observations suggest that chromatin remodeling at the proximal promoter is a crucial and regulated event in transcriptional activation, rather than a passive consequence of other critical steps in activation such as recruitment of the general transcriptional machinery. First, at some promoters this remodeling and transcription require the Swi/Snf remodeling complex [Hirschhorn et al., 1992; Fryer and Archer, 1998; Gregory et al., 1999; Sertil et al., 2007]. Second, histone depletion or mutation can sometimes lead to partial gene activation [Han and Grunstein, 1988; Wechser et al., 1997; Wyrick et al., 1999] [He and RHM, unpublished work]. Third, recruitment of TBP via fusions with the LexA or Gal4 DNA-binding domains

can activate some gene promoters, but fails to activate the *CHA1* or *GAL10* promoters, which have positioned nucleosomes occluding their proximal promoter regions [Ryan et al., 2000]. Fourth, some constitutively active genes, such as *ADK1*, *GCY1*, and *PFY1* genes in yeast, appear not to depend on the presence of traditional activators for their transcription, but on architectural factors (Reb1 for *PFY1* and *GCY1*) or DNA-sequence features that keep the proximal promoter region free of nucleosomes [Angermayr and Bandlow, 1997, 2003; Angermayr et al., 2003].

Finally, recent work on *PHO5* regulation in yeast indicates that at least for some promoters, activators may be dispensable for reinitiation of transcription if reassembly of nucleosomes at the proximal promoter region is prevented [Adkins and Tyler, 2006]. This remarkable study used chromatin IP to show that for the *PHO5*, *PHO8*, *ADH2*, *SUC2*, and *ADY2* promoters, which undergo nucleosome loss upon activation, nucleosomes are reassembled upon repression and this reassembly requires Spt6. Furthermore, transcription of these genes in *spt6* mutant yeast continues even after shifting to repressive conditions; for *PHO5*, this occurs in spite of the loss of activating Pho2 and Pho4 from the promoter. Spt6 has been shown to function as a histone chaperone in vitro [Bortvin and Winston, 1996], and its absence can lead to alterations in chromatin structure that cause cryptic initiation to occur within ORFs [Kaplan et al., 2003]. Thus, a strong implication of this work is that a principal function of activators is to clear nucleosomes from the proximal promoter region and to prevent their reassembly during ongoing transcription. This function may sometimes be provided instead by architectural factors or DNA structural elements, as suggested above.

The preceding discussion suggests the existence of at least three distinct types of promoters (Fig. 1). One class comprises constitutively active genes (although they might show increased activation under some circumstances [Angermayr and Bandlow, 2003]) that have "open" proximal promoter regions maintained by architectural factors or DNA sequence elements. A second class consists of genes activated by traditional activators; activation of these genes leads to chromatin remodeling at their proximal promoter regions, allowing access by general transcription factors (GTFs).
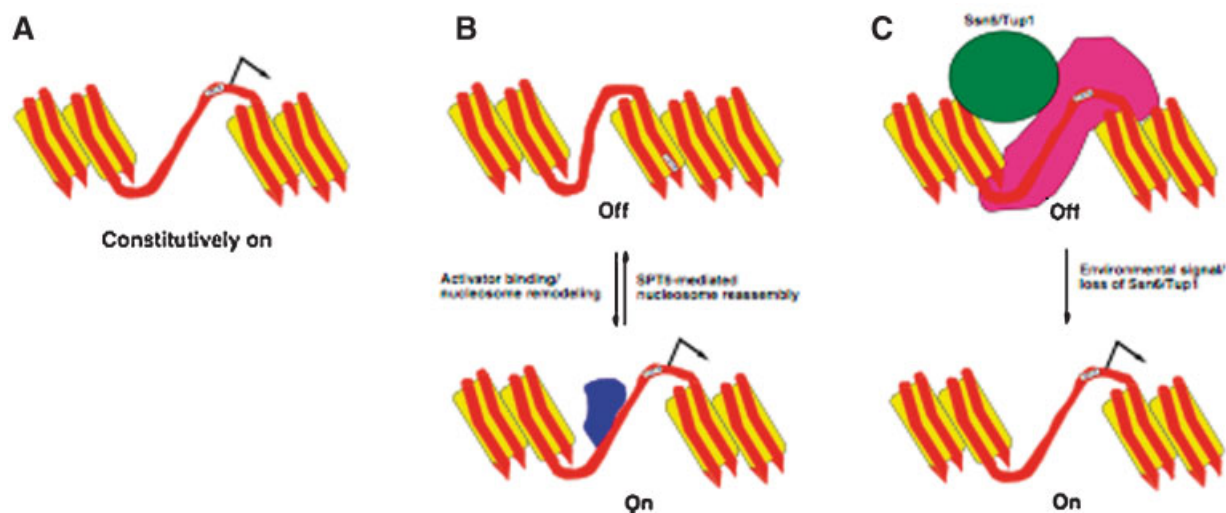
Fig. 1. A simplified view of three categories of promoters and their associated chromatin structures. **A:** Constitutively expressed genes often have open promoter regions, created either by architectural proteins or structural features of DNA. **B:** Conditionally expressed genes often have start sites/TATA elements (small white rectangle) incorporated in nucleosomes when off; activator binding (or activation) leads to chromatin remodeling and transcription. Nucleosome reassembly occurs following repression and requires Spt6 in yeast; in the absence of Spt6, transcription continues independently of activator function. **C:** Some genes are controlled by global repressors such as Ssn6/Tup1 (or heterochromatin) that create a specialized, repressive chromatin environment; environmental signals (or developmental progression) can remove this repression, leading to activation even in the absence of gene-specific activators. See text for more details.

In the absence of active nucleosome reassembly (e.g., in *spt6* mutants), at least some of these genes behave like the first class, having constitutively open proximal promoters that allow continued transcription regardless of environmental conditions. The third class consists of genes that would behave like the constitutively active first class except for the presence of repressors (such as Ssn6/Tup1) that maintain a closed chromatin structure at the promoter.

Although this model provides a useful basis for consideration of how chromatin structure can impact gene regulation, it is oversimplified. For example, although Ssn6/Tup1 represses transcription and is needed to create a "closed" chromatin configuration at some genes, under some conditions the chromatin structure may be altered to the normally active configuration without accompanying gene activation. One instance of this is at the *RNR3* promoter, where Tup1/Ssn6 and Isw2 collaborate to position nucleosomes over the promoter. Although the chromatin structure is altered to the active configuration in *isw2* yeast, *RNR3* remains repressed [Zhang and Reese, 2004]. Similarly, nucleosome positioning at the hypoxic *ANB1* gene is lost in yeast lacking the amino terminus of histone H4, but *ANB1* repression, mediated by Ssn6/Tup1, is not alleviated [Kastaniotis

et al., 2000]. Since Ssn6/Tup1 appears to be able to repress transcription both by chromatin-dependent and -independent mechanisms, interpretation of these results, particularly with respect to the extent to which nucleosomes positioned over the proximal promoter play a role in Ssn6/Tup1-mediated repression, remains murky [Mennella et al., 2003]. Another example in which chromatin remodeling and local nucleosome positioning appear unlinked to transcriptional regulation occurs in the *MET16* and *MET25* promoters [Kent et al., 1994]. These promoters have binding sites for centromere and promoter factor 1 (CPF1), and chromatin structure of the promoters is altered in *cpf1△* yeast, indicating that CPF1 dictates the local chromatin structure. Surprisingly, though, neither basal nor activated expression of *MET16* or *MET25* are affected much by the loss of CPF1, and no chromatin remodeling is observed upon activation of these genes in either CPF1+ or *cpf1△* yeast, even though the chromatin structure differs in these two conditions. Thus, in some cases gene regulation does not correlate strongly with local chromatin structure, indicating that a specific chromatin structure is not a strict requirement for gene regulation.

Remodeling of proximal promoter regions upon activation occurs by more than one

mechanism. As mentioned above, some genes are remodeled by Swi/Snf, just as the textbooks say. Remodeling at the *GAL1* promoter does not depend on Swi/Snf, but has been reported to require Spt3 and the ATPase Mot1, while conflicting reports have been made about *PHO5* requiring Swi/Snf or the chromatin assembly factor Asf1 for remodeling [Steger et al., 2003; Adkins et al., 2004; Topalidou et al., 2004; Korber et al., 2006]. Activation of some well-studied promoters, including *PHO5*, *ADH2*, and *CHA1*, is accompanied by chromatin remodeling that can occur in the absence of most or all known chromatin remodeling complexes [Moreira and Holmberg, 1998; Di Mauro et al., 2000; Boeger et al., 2004; Korber et al., 2006] [He and RHM, unpublished work]. This has led to the suggestion that intrinsic nucleosome stability may play a role in remodeling at some promoters [Hertel et al., 2005]. Proof of this concept may require demonstration of activator-mediated chromatin remodeling in vitro using purified components in the absence of chromatin remodeling enzymes [Guermah et al., 2006].

TRANSCRIPTIONAL ELONGATION: KEEPING THE CHROMATIN TIGHT

Like transcriptional initiation, elongation takes place on a chromatin template and thus must contend with potentially inhibitory nucleosomes. A bewildering array of transactions, involving histone modifications, the carboxy-terminal domain of the large subunit of pol II, and exchange among associated proteins, occurs during this process and has been the subject of intensive research [Hampsey and Reinberg, 2003]. One aspect of this process appears to have evolved specifically to restrict TF access to coding regions. Nucleosomes rapidly reassemble after transcription [Pederson and Morse, 1990; Schwabish and Struhl, 2004]; in yeast, this reassembly is facilitated by Spt6 and Asf1, loss of which results in initiation from cryptic promoters residing in ORFs [Kaplan et al., 2003; Schwabish and Struhl, 2006]. Efficient reassembly of nucleosomes is not enough by itself to prevent such aberrant initiation, though; the reassembled nucleosomal histones must also be deacetylated. This feat is accomplished by recruitment of the Rpd3-S complex to transcribed chromatin via recognition of methylated lysine 36 of histone H3 by

the chromodomain of the Rpd3-S complex constituent Eaf3 [Carrozza et al., 2005; Keogh et al., 2005]. Lysine 36 of H3 is methylated by Set2, which is recruited to elongating RNA pol II; thus, in the absence of Set2, Eaf3, or the chromodomain of Eaf3, or in the presence of H3 carrying a K36A mutation, cryptic initiation is observed from within transcribed coding sequences [Carrozza et al., 2005]. Presumably, this is prevented because its occurrence would be deleterious, but this remains to be demonstrated.

Also yet to be determined is the mechanism by which reassembled, deacetylated nucleosomes inhibit initiation at cryptic sites, or even whether this is indeed sufficient or whether the Rpd3-S complex may also inhibit through additional means. Binding of the activators Gal4 and Gcn4 to coding sequences has been reported in yeast; Gcn4 binding at a site between two positioned nucleosomes in the *PHO8* coding sequence resulted in recruitment of SAGA and Swi/Snf, with concomitant chromatin remodeling, but mediator recruitment was not observed [Li and Johnston, 2001; Topalidou and Thireos, 2003]. Thus, in some cases inhibition may occur at a step subsequent to activator binding. However, preferential binding to promoters compared to coding sequences has been reported for Rap1 [Lieb et al., 2001], suggesting that activator binding may also be inhibited. This latter finding contrasts with work showing that Rap1 can outcompete histones for binding to a site in a nucleosome positioning sequence [Yu and Morse, 1999], but it is possible that histone deacetylation or the presence of the Rpd3-S complex creates a chromatin environment more inhibitory to binding. Indeed, Rap1 access to intermediate affinity sites was found to be regulated by Ssn6/Tup1, which also recruits histone deacetylases [Buck and Lieb, 2006; Malave and Dent, 2006]. It will be interesting to determine whether inhibition of Rpd3-S action on transcribed chromatin affects binding of Rap1 and other TFs to sites residing in transcribed chromatin.

NUCLEOSOME DYNAMICS AND TRANSCRIPTION FACTOR ACCESS

Nucleosomes are not completely static structures; histone–DNA contacts change over time, and these alterations in contacts are likely to

play a role in the access of TFs to DNA in chromatin (reviewed in references Mellor [2005]; van Holde and Zlatanova [2006]). Two distinct types of nucleosome dynamics can be considered. The first concerns the transient release and reformation of individual electro-static contacts between positively charged amino acid residues in the histones and the DNA phosphate backbone, most thoroughly explored by Widom and colleagues [Li et al., 2005b]. These transient changes expose nucle-osomal DNA and occur on a millisecond time scale; they therefore could and likely do affect TF access to nucleosomal sites, as also suggested by a recent study showing that DNA damage at nucleosomal sites can be repaired in vivo on a time scale of seconds [Ahmad and Henikoff, 2002; Morse, 2003; Mellor, 2005; Bucceri et al., 2006]. It will be interesting to determine how histone modifications and/or the presence of repressive complexes such as Ssn6/Tup1 affect these dynamics [Ahmad and Henik-off, 2002; Mellor, 2005].

The extent to which activators find their sites by binding and releasing (i.e., a three-dimensional search), as compared to sliding along DNA (a one-dimensional search) [von Hippel and Berg, 1989], is not generally known. A recent single molecule study reported that lacR in *E. coli* spends most of its time non-specifically bound to and diffusing along DNA; the number of base pairs visited per sliding event was not determined, but an approximate upper bound of 85 bp was reported [Elf et al., 2007]. Clearly, the presence of nucleosomes and the kinetics of histone–DNA transient dissoci-ation could have important ramifications on the use of facilitated diffusion during searches by TFs for their binding sites in eukaryotes.

The second type of nucleosome dynamics concern histone replacement. Three recent reports have examined histone replacement on a genome-wide scale, two in yeast and one in *Drosophila* [Dion et al., 2007; Jamai et al., 2007; Mito et al., 2007]. All three report exchange of histones that occurs independently of replica-tion, with exchange rates being highest at active gene promoters and/or regulatory sites. This exchange, which is almost certainly facilitated by nucleosome assembly/disassembly factors, occurs on a time scale of minutes in yeast [Dion et al., 2007], and so is vastly slower than the transient histone dissociation discussed above. This process may play a role in providing

access of TFs to their binding sites in chromatin, but it would more likely be involved in slow events, such as changes in transcriptional regulation during development, than in rapid responses to environmental cues such as heat shock.

STATIC AND DYNAMIC REGULATION OF TRANSCRIPTION FACTOR ACCESS BY NUCLEOSOMES

In prokaryotes, transcription typically re-quires only that RNA polymerase, via the associated σ factor, binds to specific DNA sequences at -10 and -35 with respect to the site of transcription initiation. These sequence elements are sufficiently complex that their occurrence within coding sequences by chance is negligible; thus, no special mechanism is required to prevent aberrant initiation. In eukaryotes, a different logic applies [Struhl, 1999]. Activators such as Gcn4 and Gal4 can sometimes bind to their recognition sites within coding regions [Li and Johnston, 2001; Topali-dou and Thireos, 2003], and eukaryotic initia-tion sites are of sufficiently low complexity to potentially allow recruitment of the general transcription machinery and aberrant initia-tion. As discussed above, this is prevented by a chromatin-mediated mechanism that requires nucleosome assembly factors and the histone deacetylase complex, Rpd3-S. Because cryptic initiation is observed when this mechanism is corrupted, it is inferred that nucleosome reas-sembly and rapid histone deacetylation pre-vents factor access. Whether this reflects inhibition of activator binding or binding of GTFs is currently unknown; indeed, even the issue of whether transcriptional inhibition in heterochromatin occurs by occlusion of activa-tor or GTF binding is not clear [Sekinger and Gross, 2001; Chen and Widom, 2005].

As discussed earlier, nucleosomes at the proximal promoter prevent transcription from occurring in the absence of activators. Further-more, two examples have been reported in which small changes in nucleosome positioning can change requirements for coactivators, including chromatin remodeling complexes, in transcriptional activation. The IFN-β gene promoter in human cells is induced by viral infection; this induction involves sequential recruitment of coactivators, including the Snf2 homolog Brg1, which catalyzes removal of a

nucleosome from a position that occludes the TATA box to a new site 25 bp downstream [Lomvardas and Thanos, 2001]. Lomvardas and Thanos reported that, by using artificial nucleosome positioning sequences both in vitro and in vivo to alter the position of this nucleosome away from the TATA element, the kinetics of activation of IFN-β were altered and the requirement for Brg1 was alleviated [Lomvardas and Thanos, 2002]. In the second example, movement of a nucleosome that occludes the TATA element of the *PHO5* promoter in yeast by only 2–3 bp induces a requirement for the N-terminal region of histone H4 and the bromodomain factor Bdf1 [Martinez-Campa et al., 2004]. Thus, not only do nucleosomes govern the requirement for activators to facilitate remodeling and PIC assembly during transcriptional initiation, but also their precise position may dictate coactivator requirements and/or activation kinetics.

Evidence also points to a role for chromatin structure in controlling binding of activators in vivo; however, in contrast to the situation at proximal promoter regions and coding sequences, access is allowed rather than prevented [Bernstein et al., 2004; Lee et al., 2004; Pokholok et al., 2005; Sekinger et al., 2005; Yuan et al., 2005; Liu et al., 2006; Ozsolak et al., 2007] [Lee et al., 2007]. However, this is not universally the case; for example, Cpf1 determines local chromatin structure surrounding its binding site at some genes, and binding of the Pho4 activator at the *PHO5* promoter directly perturbs a nucleosome containing a Pho4-binding site [Almer et al., 1986; Kent et al., 1994]. Furthermore, some activators, such as Gal4, Rap1, and Abf1, are able to outcompete histones for their binding sites within nucleosome positioning sequences [Morse, 2003]. It will be interesting to examine the relationship between binding sites for various activators and nucleosome positioning at higher resolution to determine the extent to which correlations observed thus far hold true.

The regulation of factor access discussed above is static; nucleosome positioning, and mechanisms for nucleosome reassembly and modification following transcriptional elongation through chromatin, have evolved to allow or prevent access of TFs as needed (Fig. 2). Predictions of nucleosome positioning at promoters are improved by considering sequence conservation among related yeast species,
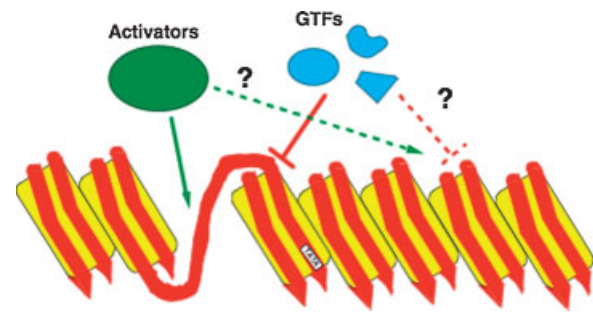

Fig. 2. Chromatin structure helps to govern transcription factor access in vivo. Activator access is generally permitted, often by the presence of NDRs. Access by GTFs and consequent PIC formation is typically, albeit not always, inhibited by chromatin structure at repressed promoters, but allowed at active promoters by an open proximal promoter region (see Fig. 1). Transcriptional initiation from sites within coding regions is typically not observed, but such cryptic initiation is seen under certain circumstances when nucleosome reassembly or histone modifications are prevented. Whether inhibition of cryptic initiation occurs by prevention of activator binding, which has sometimes been observed within coding sequences (green dashed arrow) or prevention of GTF binding is currently unknown.

indicating that governing factor access via chromatin structure is evolutionarily important [Ioshikhes et al., 2006]. In some cases, factor access to sites in chromatin is also governed dynamically, such that nucleosome positioning or chromatin structure is the direct target of a developmental or environmental signal that then affects TF access to allow or prevent transcription. One clear example of this is in regulation by heterochromatin, perhaps most clearly reflected in position effect variegation in *Drosophila* [Ahmad and Henikoff, 2002]. Another is in gene regulation by Ssn6/Tup1, as discussed earlier; environmental signals, such as glucose depletion, that alter Ssn6/Tup1 action at specific promoters may allow increased factor access and consequent transcriptional activation [Buck and Lieb, 2006; Malave and Dent, 2006]. However, given that Ssn6/Tup1 can repress transcription even when nucleosome positioning is altered or lost [Kastaniotis et al., 2000; Zhang and Reese, 2004], and that heterochromatin is not impermissive to factor (or large molecule) binding [Chen and Widom, 2005; van Holde and Zlatanova, 2006], it remains somewhat unclear exactly how such regulated changes in chromatin structure affect factor access. One interesting proposal is that heterochromatin restricts nucleosome dynamics, and thereby inhibits factor access [Ahmad and Henikoff, 2002].

PROSPECTS

As ever, new knowledge raises new questions. Chief among these regarding the relationship between chromatin structure and TF access are the following: How are NDRs created? Why do some promoters lack NDRs and how do TFs gain access at such promoters? What determines which coactivators/remodeling complexes are required at specific promoters, and how can small changes in local chromatin structure alter requirements for transcriptional activation? How do heterochromatin and global repressors such as Ssn6/Tup1 inhibit transcription? How does nucleosome reassembly and Rpd3-S activity inhibit cryptic initiation from within coding sequences? Do activities of Rpd3-S, Ssn6/Tup1, and Spt6 carry over from yeast to higher eukaryotes, or have new and distinct mechanisms evolved to govern access of TFs to chromatin in metazoans? How conserved is nucleosome positioning across related species? Clearly, much more work must be done to achieve a solid understanding of regulation of factor access by chromatin in living cells.

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