

Mechanisms by which transcription factors gain access to target sequence elements in chromatin

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Transcription factors (TF) bind DNA sequence motifs, but the presence of a consensus DNA element is not sufficient to direct TF binding to chromatin. Recent genomic data have revealed that accessibility, as measured by DNase sensitivity and the presence of active histone marks, is necessary for TF binding. DNA sequence provides the initial specification of the accessibility of DNA elements within chromatin that permits TF binding. In yeast, it is known that poly(dA-dT) tracts directly encode low-nucleosome occupancy at promoters. Recent evidence suggests that CpG islands in mammals are inherently refractory to higher-order chromatin structure and remain accessible, despite favoring nucleosome formation *in vitro*. Taken together, these studies support a model for how accessibility originates and then propagates throughout regulatory cascades and development.

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Introduction

Specific DNA sequence elements are sufficient to direct transcription factor (TF) binding in prokaryotes; however, in higher organisms, chromatin often occludes TF binding [1–4]. In eukaryotes, DNA is wrapped around nucleosomes and forms higher order chromatin structures that restrict TF access. The first high resolution *in vivo* measure of the accessibility of chromatin structure came from the study of candidate heat shock genes [5]. DNase I footprinting revealed that the 5' end of *Hsp70* and *Hsp83* were highly sensitive to digestion before heat shock induction [5]. Post-translational modifications of histones provide an independent measure of chromatin structure.

For instance, histone acetylation is associated with actively transcribed genes [6,7], but can also be a precursor to transcription and permit subsequent activation [8]. DNase I signals overlap with histone acetylation marks [9], suggesting that histone acetylation contributes to the molecular basis for DNase I sensitivity. Multiple types of histone acetylation and H3K4 methylation often co-occur and are associated with transcriptionally active or potentiated chromatin [10^{**},11,12^{**},13]. Recently, André Martins developed a probabilistic model that infers DNase I sensitivity from histone modification data, reinforcing the qualitative link between DNase sensitivity and active histone marks with a quantitative model [14^{*}] (Figure 1).

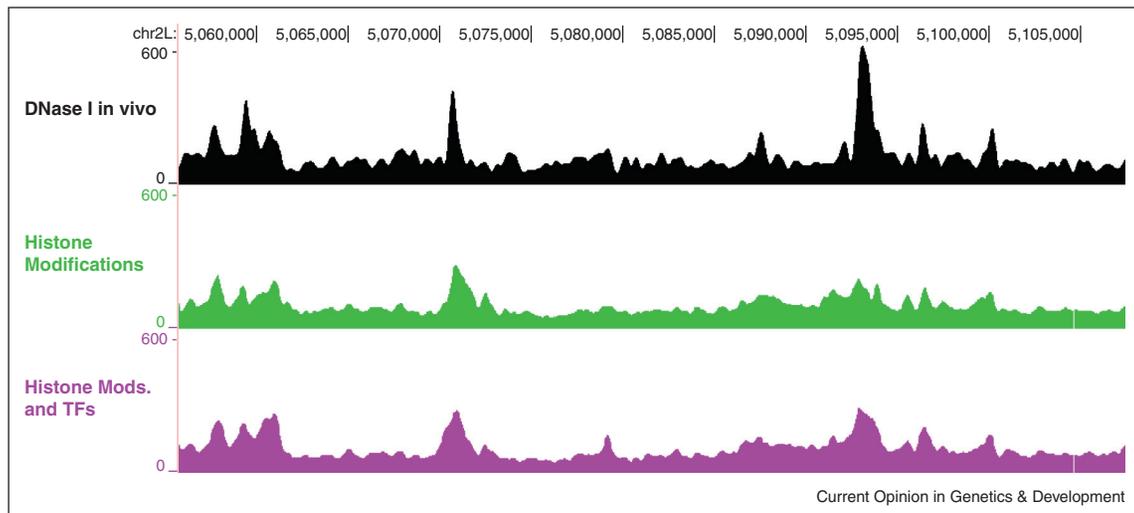
Early studies revealed that TF bound DNA and DNase I sensitivity co-occurred at candidate promoters [5,15]; however, these measurements did not resolve the causality of either event. Did pre-existing DNase I hypersensitivity permit TF binding or did TF binding affect the local chromatin environment? Candidate gene analyses have shown that accessible chromatin correlated with inducibly bound TF binding sites for several factors [16,17,18^{**}]. To test whether accessible chromatin was necessary and sufficient to direct TF binding to consensus elements, genomic assays were needed to examine the comprehensive set of bound target elements and unbound consensus elements. The recent advent of molecular genomics approaches that measure both the accessibility of DNA (DNase-seq, FAIRE-seq, and ChIP-seq) [19–21] and the inducible binding of transcription factors (ChIP-seq) [10^{**},12^{**},22,23] allows this causality to be addressed in a comprehensive manner.

Genomic assays reveal that active chromatin is required for TF binding

The first genome-wide study looking at histone marks and inducible TF binding indicated that active chromatin marks preceded TF binding [24]. The authors found that STAT1 binding sites were marked by H3K4 methylation before interferon-induced STAT1 binding [24]. These data suggested that an active chromatin state is necessary for inducible binding. However, it remained unclear if potential, but unoccupied, STAT1 binding sites also harbored active marks, but remained unbound after stimulation.

To test whether or not potential binding sites in active chromatin remain untargeted, we directly compared the chromatin landscape (histone modification and chromatin

Figure 1



DNase I intensity can be modeled using histone marks and TF binding data. DNase I hypersensitivity landscape is inferred by models that use histone modification profiles and TF profiles. Incorporating non-histone chromatin-bound factors into the model increases accuracy, which is consistent with the role of TFs having an additive effect upon DNase I hypersensitivity [5,12**].

associated factors) at inducibly bound, heat shock factor (HSF) DNA elements (HSEs) to unoccupied HSEs [10**]. We found that inducibly bound HSEs resided in chromatin characterized by histone acetylation and H3K4 methylation and unbound HSEs lacked these histone marks (Figure 2). A similar study showed that the glucocorticoid receptor (GR) specifically binds to target elements that are pre-marked by DNase I hypersensitive signal before ligand treatment [12**]. Taken together, these data indicate that TFs are specifically targeted to consensus elements within a region of active chromatin.

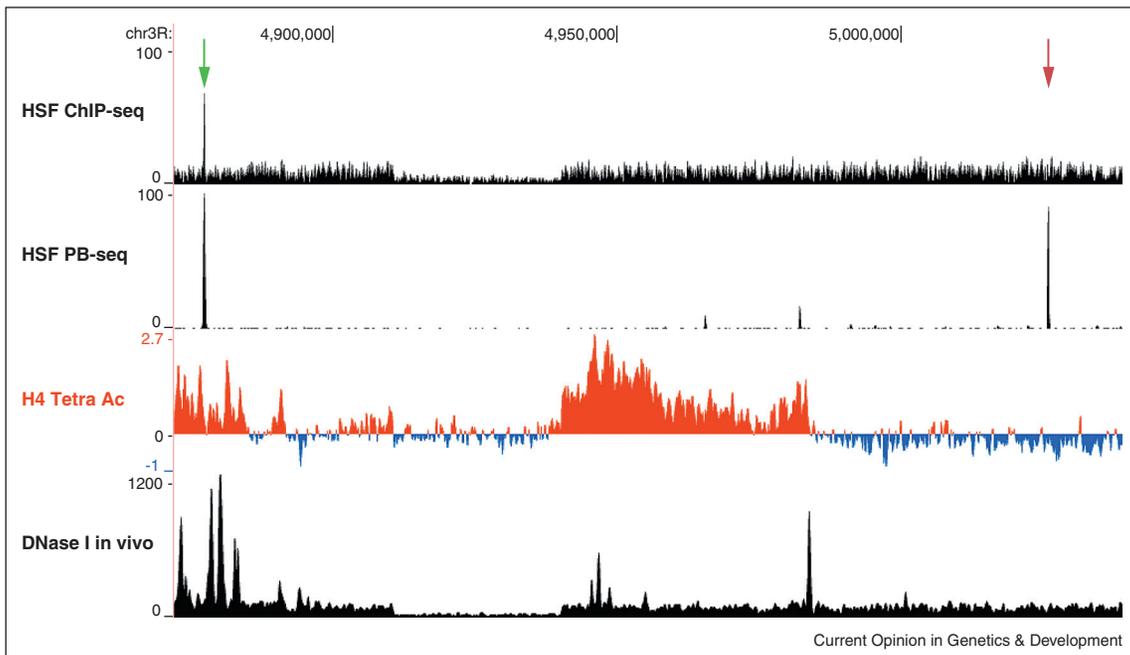
The previously mentioned studies were performed in cell lines with an inducing agent (interferon, hormone, and heat stress), but defining the temporal order of chromatin structural change and TF binding is more difficult within a developing organism. Multiple cell types can confound the quantitative analysis of both TF binding and chromatin structure. Additionally, the analysis requires capturing cells at a stage just before detectable TF binding, which is difficult to accomplish in a manner that does not perturb the developmental process [25,26]. To overcome these limitations and study the context-dependent manner of transcription factor binding in erythroid differentiation, Wu *et al.* generated an inducible GATA1 cell line [27*]. They found that erythroid progenitors lacking GATA1 retain the chromatin state (H3K4 methylation and DNase I sensitivity) that is permissive for binding [27*]. Others have captured the early events in the reprogramming of differentiated cells to induced pluripotent stem cells (iPSC) using an assay that allows for cells to be distinguished by their number of cell divisions

[28]. Reprogramming is initiated by ectopic expression of Oct4, Sox2, Klf4, and c-Myc, and the authors showed that the primary targets of these factors pre-exist in an accessible state [28].

A special class of TFs termed ‘pioneer factors’ are often the first detectable transcription factors binding a region of chromatin *in vivo* and can access nucleosomal DNA *in vitro* [29]. Recent genomic studies have shown that H3K4 methylation and DNase I sensitivity precedes binding of the pioneer factors FoxA1 and GATA1 [18**,27*,30]. FoxA1’s high affinity for nucleosomal DNA [29] likely allows FoxA1 to bind to transiently accessible chromatin that may be inaccessible to other factors (reviewed in [31]), but the *in vivo* evidence suggests that these sites are not heterochromatic. Here we use ‘heterochromatic’ to refer to the annotation of chromatin states defined by the presence and absence of specific chromatin marks that likely result in higher order chromatin structure [11,13]. FoxA1 and other TFs are defined as pioneers, because they precede binding of other TFs. These studies indicate that the mechanism and function of pioneer factor binding is not appreciably different from TFs that are not considered pioneers: both classes bind to relatively decondensed chromatin marked by active histone marks and subsequently reinforce and expand the accessible region [10**,12**,32].

We propose that many factors have the potential to pioneer a region. For instance, AP1 binding precedes GR binding and maintains accessible chromatin at over 70% of GR binding sites, but it seems that GR is acting as

Figure 2



HSF discriminates between potential binding sites based on the pre-existing chromatin state. This region of chromosome 3R contains two strong potential HSF-binding sites (green and red arrows), measured by an *in vitro* protein/DNA-binding assay (PB-seq) [14]. Although the HSF-free motif (red arrow) binds with comparable affinity *in vitro* (PB-seq), chromatin structure restricts HSF occupancy *in vivo* (ChIP-seq). HSEs that are enriched for H4 acetylation and DNase I hypersensitivity during non-HS are preferentially bound by HSF *in vivo* (green arrow).

a classical pioneer at 15% of the binding sites [12^{••},32]. We have found that the presence of GAGA Associated Factor (GAF) at HSEs positively influences HSF binding at a subset of sites [33].

Accessibility increases concomitantly upon transcription factor binding

Just as chromatin landscape influences TF binding, so does TF binding influence chromatin state. The histone marks and DNase I signal that are predictive of TF binding also increase in intensity upon inducible binding [5,10^{••},12^{••},19–23,34,35]. This observation is consistent with the role of TFs in recruiting coactivator complexes that are capable of modifying histone tails and remodeling nucleosomes [24,36–43]. Concomitant increases in locus accessibility may reinforce and expand the boundaries of accessible chromatin to allow access for other TFs and large molecular complexes, a mechanism defined as ‘assisted loading’ by Hager and colleagues [34]. This cascade can lead to accumulation of paused RNA polymerase II near promoters, which can exclude nucleosomes from promoters and help maintain an active and factor-accessible chromatin state [44,45].

Sequence composition encodes accessible chromatin

The mechanism by which accessibility originates to allow TF binding is an unresolved but active subject of

investigation. Ultimately, DNA sequence must direct the origin of accessible DNA. In yeast, the low nucleosome density at promoters is largely specified by DNA sequence, mainly poly(dA-dT) tracts, producing promoters that are generally devoid of nucleosomes permitting TF access [17,46,47].

CpG islands, which are hallmarks of approximately 60% of human and mouse promoters [25,48], favor nucleosome formation *in vitro* [49], but are nucleosome-depleted *in vivo* [50]. It seems counterintuitive that the DNA sequence of CpG island regulatory elements would favor nucleosome formation. We hypothesize that CpG island sequence is inherently refractory to higher order compaction by linker histones and retains the chromatin in a transiently accessible state amenable to TF binding.

It has been known for over 40 years that linker histones, like H1, preferentially bind AT-rich linker DNA (reviewed in [51]). Recently, the molecular interactions that are responsible for this selectivity have been characterized [52]. In short, the nonpolar region of the linker histone’s globular domain preferentially interacts with the thymine methyl groups through hydrophobic interactions [52]. These data support the hypothesis that CpG islands are refractory to higher-order compaction *in vivo*. Nonetheless, this does not completely explain the fact

that CpG island promoters are generally highly accessible *in vivo* [50], despite favoring nucleosome assembly *in vitro* [49]. TFs are highly bound to CpG islands *in vivo*, so it is clear that their elements are initially accessible and that TF binding propagates a more accessible chromatin landscape. Consistent with this idea, CpG islands were found to be sufficient to direct the *de novo* recruitment of the CxxC finger protein 1 (Cfp1) and subsequent H3K4 methylation [53[•]]. Cfp1 is a component of the Set1 H3K4 methylation complex [54], and has been shown to interact with unmethylated CpG-rich DNA *in vitro* [55]. Methylation of H3K4 is inhibitory to DNA methylation, so H3K4 methylation may also reinforce and maintain the unmethylated DNA state of CpG islands [56]. KDM2A, a H3K36 demethylase, also targets linker DNA between nucleosomes in CpG islands by a CxxC zinc finger domain protein [57[•],58^{••}], consistent with the idea that CpG islands do not efficiently incorporate linker histones for compaction.

Non-CxxC zinc finger proteins must also target the linker DNA between nucleosomes at CpG islands, and in fact, mammalian sequence-specific TFs, as a class, have a GC-bias in their cognate binding sites [59] and TFs bind to GC-rich regions *in vivo* [50]. Thus, CpG islands, which constitute a majority of mammalian promoters, have inherent properties amenable for TF binding. Although these GC-rich sequences can interact with nucleosomes, they appear to be dissuaded from higher-order packing and are enriched for TF binding elements. Importantly, CpG island promoters can be found in a repressive state *in vivo*, so repressive TFs and cofactors can also bind to the region and precipitate repressive chromatin and further condensation.

This model of CpG island structure and function is highlighted by the example of embryonic stem cell differentiation. CpG island promoters that are uncommitted to the repressive or active state, so-called bivalent promoters, are highly depleted of DNA elements that are the targets of TFs that are expressed in the embryonic stem cell [60,61]. These same promoters in differentiated cells are highly acetylated and more accessible, presumably because these cells express TFs that are competent to bind the region and tilt the balance from the bivalent to the active state [60,62].

The DNA sequence of *Drosophila*, which is not enriched for CpG-island and poly(dA-dT) tracts in its promoters, can also influence TF binding. A recent study has shown that the high GC content in the 10 kb flanking Male-specific lethal (MSL) binding sites can influence MSL binding [63]. We speculate that the sequence in flanking regions of *Drosophila* also may occlude higher order chromatin compaction.

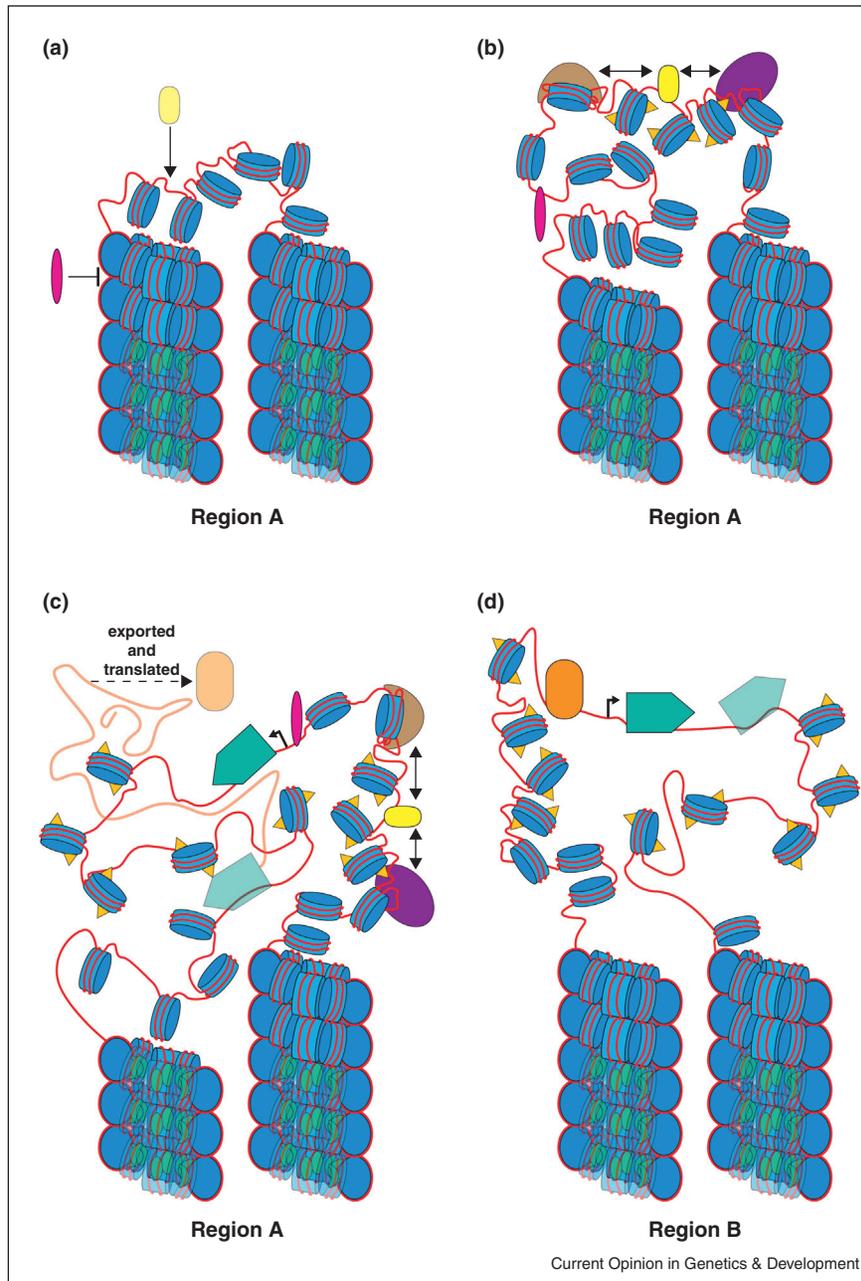
Our model: DNA sequence directs chromatin structure to allow TF binding

We propose that DNA sequences that inherently promote unstable nucleosomes (AT-rich stretches) or disfavor higher order compaction (CpG islands) are sufficient to keep chromatin in a state that is at least transiently accessible to TF binding and uncommitted to the repressive or active transcription state (Figure 3a). Throughout development, or in response to regulatory cascades, previously unexpressed or inactive TFs (often pioneer TFs) and nucleosome modifying enzymes are targeted to their cognate binding sites (Figure 3b) through their interaction with accessible DNA (often CG-rich, unmethylated, linker DNA between nucleosomes). Targeting of the TFs cause subsequent hyper-modification of histones in the region and tilt the balance toward the repressive or active state, as directed by the TFs (Figure 3b). Subsequent recruitment of cofactors or RNA polymerase itself is sufficient to enhance and expand the boundaries of the accessible or inaccessible chromatin (Figure 3c). In some cases, TF binding will directly or indirectly activate or repress the transcription of other sequence-specific TFs or cofactors (Figure 3c). The cognate elements of transcriptionally activated TFs will be targeted if they reside within active chromatin (Figure 3d). The *trans* targets of transcriptionally repressed TFs will return to the default accessibility state, in the absence of factors with redundant functions that reinforce the chromatin state. This cascade can be subject to further perturbation by activating inactive TFs through environmental factors (e.g. heat shock, hormone treatment, stress, etc.). Genomic tracking of chromatin and transcription changes throughout a regulatory cascade will test the validity of the proposed model.

Inference and prediction of transcription factor binding sites

A complete understanding of the DNA and chromatin features that influence TFBS selection would permit accurate prediction of TFBS intensities using only DNA sequence and chromatin landscape. Many TFBS models infer TF binding, rather than predict inducible binding patterns *de novo* [26,64–66]. These models are developed with chromatin state data generated at the same time as TF binding, so the effect of pre-existing chromatin states upon TF binding is confounded by the influence of the TF on the local chromatin environment. These models also use position-specific weight matrices to predict potential binding sites, which can be problematic if the assumption of independence between positions in the binding site is not met. Recently, many protein/DNA binding assays have been developed to directly measure the TF/DNA binding affinity [14[•],67–69]. These assays can be combined with *in vivo* chromatin landscape data to predict *de novo* TFBS intensity from chromatin features. For instance, we used PB-seq [14[•]] to measure the binding energy landscape of all potential

Figure 3



DNA sequence directs chromatin structure and allows transcription factor binding to precipitate regulatory cascades. **(a)** A CpG island is refractory to higher-order chromatin compaction (unraveled chromatin). A regulatory or developmental cascade is precipitated by the activation or expression of a transcription factor (yellow rounded rectangle) targeted to elements within the CpG island. Another TF remains (pink ellipse) unable to access elements with the H1 linker histone (green, interior crescents) condensed chromatin. **(b)** Targeting of the TF to the CpG island directly or indirectly results in the recruitment of nucleosome remodeling factors (brown crescent) and histone acetyltransferases (purple ellipse), which further decondenses the region and allows the other TF (pink ellipse) access to its cognate element. **(c)** Binding of the second TF (pink ellipse) directly or indirectly causes the recruitment and productive elongation of RNA Polymerase II (green pentagon) to a gene encoding a third TF, which is subsequently transcribed (transparent orange line) and translated (orange rounded rectangle). This causes further nucleosome loss, histone modifications (orange triangles), and decondensation of the Region A locus. **(d)** The translated protein (orange rounded rectangle), encoded by region A, is targeted *in trans* to Region B, which is highly acetylated and contains a transcriptionally engaged paused RNA Polymerase II. This binding event leads to the release of the paused RNA Polymerase II and activation of the gene.

binding sites in a genome in a chromatin-free context and chromatin landscape data from the modENCODE consortium [13] to develop a probabilistic TF binding model. Our model revealed that the intensity of each modification could be used to predict more than just whether or not HSF would bind, but also the intensity of binding. As more chromatin data become available, these models will be expanded upon and refined.

Conclusions

Ultimately, the goal is to understand TF binding and function within a developmental or regulatory framework. This understanding of networks would allow one to predict the transcriptional output of binding events and the cascade of regulatory binding and transcription cycles that follows in normal and disease states. Carefully designed kinetic experiments that monitor changes in chromatin structure, TF binding, and transcription throughout stages of development and regulatory networks will be necessary to unravel the complex cascade of molecular events precipitated by differentiation or response to stimuli.

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