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Pioneers Invade the Nucleosomal Landscape

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https://doi.org/10.1016/j.molcel.2018.07.004

Two papers in *Molecular Cell* (Kubik et al., 2018; Yan et al., 2018) explore the mechanisms by which transcription factors bind their sites in chromatin, providing fresh insights into the much-debated question of how transcription factors can be "pioneers."

Sequence-specific transcription factors (TFs) stand at the apex of the gene regulatory hierarchy in all cellular life. TF master regulators of development were identified in classical transformation experiments showing that some TFs can reprogram committed mammalian cells to develop along a particular developmental pathway, and a cocktail of TFs can induce pluripotency. Thus, understanding how master regulator TFs engage their sequence-specific targets has been a central goal of modern developmental biology. However, the mechanisms of action of master regulator TFs remain controversial (Cirillo et al., 2002; Johnson et al., 2018; Soufi et al., 2015). In contrast to their prokaryotic counterparts, binding sites of eukaryotic TFs are occluded by nucleosomes, and we do not fully understand the intricacies of nucleosome dynamics that TFs encounter in the nucleus. To reduce the complexity of the problem, mechanistic studies can be performed in an easily manipulated single-cell model organism, such as the budding yeast *Saccharomyces cerevisiae*. With its small genome size and close packing of genes and regulatory elements, *S. cerevisiae* greatly simplifies the mechanistic study of TF binding in the context of a dynamic nucleosome landscape. The comprehensive catalog of components of yeast regulatory machineries has been enriched by numerous mechanistic and functional studies, revealing a close correspondence between TF binding site specificity *in vitro* and *in vivo*. Taking advantage of these attributes of yeast for elucidating TF binding dynamics, two studies appearing in *Molecular Cell* (Kubik et al., 2018; Yan et al., 2018) probed fundamental principles of TF binding in the chromatin context, with implications for master regulatory factors that govern developmental decisions.

Yan et al. (2018) devised a systematic reporter assay in which a large set of binding site variants for each of 104 TFs with known binding motifs could be scored based on the ability of the corresponding TF to deplete the nucleosome that occludes the site (Figure 1). They chose a position within the middle nucleosome of a well-phased 7-nucleosome array in the cell-cycle-regulated promoter of the *HO* gene, which they permanently inactivated by mutating the binding site for its Swi5 activation factor. For integration into the HO promoter site, they designed 16,667 60-mer oligonucleotides spanning from −45 to +15 around the nucleosomal dyad axis, such that each TF binding site variant would be occluded by the middle nucleosome in the *HO* promoter array. A library of yeast integration mutants was pooled, cells were treated with micrococcal nuclease (MNase) to produce a nucleosome "ladder," and the extracted DNA was gel purified to recover mononucleosomal fragments. Adapters were added to fragment ends, and the integrated region was amplified and sequenced. For each TF binding site in the library, the number of fragments was proportional to the estimated occupancy of the nucleosome occluding the oligonucleotide insertion, and so depletion by a TF binding to the site was seen as fewer fragments than in controls lacking a TF binding site. TFs were then rank ordered based on their inferred nucleosome depleting activity. This assay revealed a wide range of activities. Six factors showed strong nucleosome depletion activity, whereas nearly two-thirds of the factors yielded no significant depletion. Strong nucleosome-depleting factors include the general regulatory factors (GRFs) Abf1, Reb1, and Rap1, which are well known to deplete promoter nucleosomes (Hartley and Madhani, 2009). Nucleosome depletion activity correlated closely with affinity of the factor for its binding site, supporting a simple model in which TF binding affinity determines the degree to which it can deplete a nucleosome (Luo et al., 2014).

TFs are not the only factors with nucleosome-depleting activities at yeast promoters. The essential SWI/SNF family remodeling complex RSC uses the energy of ATP to slide or evict nucleosomes at promoters (Hartley and Madhani, 2009). Kubik et al. (2018) have now disentangled the contributions of GRFs and RSC to nucleosome depletion by showing that RSC action at many promoters does not depend on binding of GRFs or vice versa, suggesting independent action. Furthermore, the strength of RSC action correlates with the spacing and orientation of a pair of short motifs, polyA and G/C (Kubik et al., 2015), consistent with *in vitro* work suggesting that these motifs orient RSC relative to the first nucleosome downstream of the promoter (Krietenstein et al., 2016). Thus, it would appear that the combination of a GRF and RSC sequence specificity clear out promoter nucleosomes. Importantly, the action of RSC is required for the binding of TATA-binding protein (TBP) (Kubik et al., 2018), which in turn assembles the pre-initiation complex to recruit RNA polymerase II. Thus, both a GRF and RSC independently make DNA accessible to facilitate binding of other factors required

for gene activation, each partially fulfilling the original criteria to be classified as a "pioneer factor" (Cirillo et al., 2002).

There are clear parallels between these findings in yeast and the action of pioneer factors in multicellular eukaryotes, in which a special set of nucleosome-depleting TFs facilitate the binding of other TFs to open previously inaccessible chromatin. One popular model for pioneer factors is that they have evolved to bind directly to the nucleosome and destabilize it, and in the case of the FoxA endoderm-specific TF, winged helix domains resembling those found on linker histones can facilitate its nucleosome depletion action *in vitro* (Cirillo et al., 2002). Other TF motifs on pluripotency factors have been hypothesized to act similarly in binding to and destabilizing nucleosomes (Soufi et al., 2015). However, essential nucleosome-remodeling complexes in multicellular eukaryotes that are orthologous to RSC no doubt play central roles in nucleosome depletion (Johnson et al., 2018), and so the actions of animal pioneer factors and remodelers *in vivo* have not been disambiguated. Furthermore, TFs and nucleosomes compete behind the replication fork in *Drosophila*, a process that is associated with the Brahma remodeler, the single fly ortholog of yeast RSC and SWI/SNF (Ramachandran and Henikoff, 2016). Perhaps the situation in multicellular eukaryotes is the same as in yeast, whereby pioneer factors provide the specificity of binding, using the energy of ATP exerted by SWI/SNF remodelers to mobilize nucleosomes. Given the range of TF binding affinities for sites throughout the genome and the multiplicity of factors that govern nucleosome stability, we expect that pioneering will depend not only on features specific to a TF, but also on regional distinctions. Whether or not site-specific differences impact pioneering activity *in vivo* is a question that advancing genome-wide technologies are poised to address.

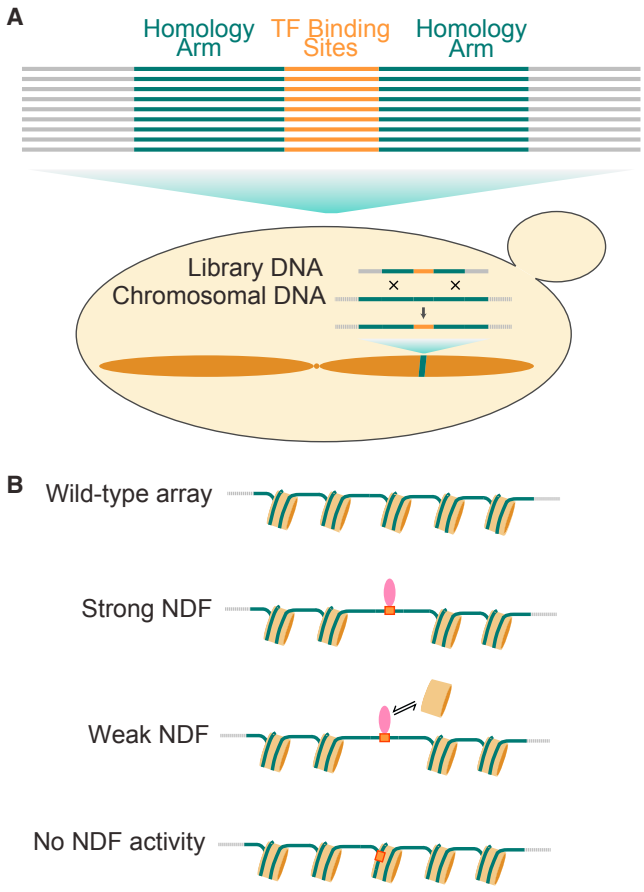

Figure 1. A Systematic *In Vivo* Assay for Nucleosome Depletion at TF Binding Sites

(A) A library of TF binding sites with flanking sequences that are homologous to a single chromosomal location are transformed into budding yeast, in which recombination results in exchange of the wild-type sequence with the designed binding site.

(B) Nucleosome profiling of the transformed yeast pooled together reveals three classes of nucleosome-displacing factors (NDFs) based on the effect of designed binding sites for the factor on occupancy of the nucleosome *in vivo*: strong NDFs in which the nucleosome is strongly displaced, weak NDFs in which the nucleosome is partially displaced, and factors with no nucleosome-displacing activity. The orange-red box depicts the TF binding site inserted by homologous recombination and the pink oval depicts a TF.

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