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Pioneer Transcription Factors, Chromatin Dynamics, and Cell Fate Control

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Summary

Among the diverse transcription factors that are necessary to elicit changes in cell fate, both in embryonic development and in cellular reprogramming, a subset of factors are capable of binding to their target sequences on nucleosomal DNA and initiating regulatory events in silent chromatin. Such “pioneer transcription factors” initiate cooperative interactions with other regulatory proteins to elicit changes in local chromatin structure. As a consequence of pioneer factor binding, the local chromatin can either become open and competent for activation, closed and repressed, or transcriptionally active. Understanding how pioneer factors initiate chromatin dynamics and how such can be blocked at heterochromatic sites provides insights into controlling cell fate transitions at will.

Keywords

Pioneer factor; FoxA; PHA4; chromatin; development

Pioneer factors: A simple means to initiate regulatory events in silent chromatin

Large eukaryotic genomes fit into the cell's nucleus by distinct levels of chromatin compaction. The first level of compaction is by the DNA is wrapping nearly twice around an octamer of the four core histones, to make nucleosomes (Luger et al., 1997). The second level of compaction arises from the regular spacing of nucleosomes on the DNA, so that the local inter-nucleosomal interactions allow the formation of higher levels of chromatin condensation (Schalch et al., 2005). These levels of compaction of DNA into chromatin limit the amount of free DNA that is available for many regulatory factors to bind. Thus,

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chromatin structure is intrinsically repressive, which helps to stabilize gene expression states and prevents undirected cell fate transitions. How do regulatory proteins gain access to DNA in chromatin, particularly at silent genes that are not yet programmed for expression (or repression), to control cell fate?

One model for transcription factor access to silent DNA in chromatin holds that the DNA would be transiently nucleosome-free during DNA replication. Yet DNA replication is tightly linked to chromatin assembly (Polo and Almouzni, 2006), suggesting little access for stable transcription factor access to DNA. Moreover, early developmental patterning can proceed in the absence of DNA replication (Harris and Hartenstein, 1991) and cell fate can be reprogrammed in the absence of cell division (Chiu and Blau, 1984; Di Tullio and Graf, 2012). Another model holds that the histone themselves may be dynamic on DNA; but measurements of histone exchange in living cell chromatin show that core histones are relatively stable (Kimura and Cook, 2001). Also, it appears that DNA sequences with clusters of transcription factor binding sites, i.e. regulatory DNA, actually stabilize nucleosomes rather than destabilize them (Tillo et al., 2010). Another model holds that ATP-dependent nucleosome remodelers constantly survey the genome, loosening nucleosomal sites to be sampled by transcription factors (de la Serna et al., 2006). But remodelers seem to be concentrated at regulatory sequences, where they are apparently recruited by transcription factors, and remodeling complexes are much larger than the nucleosome and use one ATP per base pair of DNA “remodeled,” and thus are energetically expensive (Clapier and Cairns, 2009). New theories suggest that remodelers may be most important for modulating higher-order chromatin structure (Ho and Crabtree, 2010). Perhaps the simplest model for targeting silent chromatin is by the action of pioneer transcription factors, which are distinguished from other transcription factors by their ability to bind their cognate DNA sites directly on a nucleosome, even in chromatin that is locally compacted by linker histone (Cirillo et al., 1998; Cirillo et al., 2002). Thus, pioneer factors are less impeded by the first level of DNA compaction into chromatin, i.e., by the nucleosome, though as we will see, they can be impeded by higher levels of compaction.

Nucleosome binding as a defining characteristic of pioneer factors

The paradigm pioneer factor, FoxA, was initially discovered in the mouse embryonic endoderm, where it binds to an enhancer sequence at a silent liver gene (Gualdi et al., 1996; Bossard and Zaret, 2000; Lee et al., 2005). FoxA persists at the enhancer in chromatin as the endoderm becomes induced to the liver fate, concomitant with activating the liver gene. Further studies showed that PHA-4, a homolog of FoxA in C. elegans, is similarly the first to bind silent target genes in worm foregut development (Gaudet and Mango, 2002; Fakhouri et al., 2010; Hsu, 2015). Purified FoxA and PHA-4 proteins efficiently bind to their cognate sequences on a nucleosome (Cirillo et al., 1998; Hsu, 2015), explaining how they can target silent chromatin in development. Further studies showed that FoxA can act as a pioneer factor in differentiated cells by opening the local chromatin and allowing hormone-responsive transcription factors to bind (Carroll et al., 2005; Zaret and Carroll, 2011; Pihlajamaa et al., 2014). Indeed, pioneer factors can be discovered by DNA sequence motifs at sites that become DNase hypersensitive in a developmental sequence (Sherwood et al., 2014). Yet the binding of pioneer factors can also lead to repressed chromatin, with binding

adjacent to repressors or corepressors and with reduced local nuclease sensitivity (Sekiya and Zaret, 2007; Watts et al., 2011). The emergent picture is that pioneer factors enable states of competence to be activated or competence to be repressed (Figure 1). This view was underscored by a recent genome-wide study showing that FoxA binding, in endoderm derived from human embryonic stem cells, occurs at sites for target genes that could be either activated or repressed during differentiation to different lineages (Wang et al., 2015).

In mammals, extensive genome-wide chromatin immunoprecipitation studies (ChIP-Seq) have shown that FoxA proteins and other pioneer factors most frequently bind distal regulatory sequences, i.e., enhancers, while also binding to promoters (Motallebipour et al., 2009; He et al., 2010; Serandour et al., 2011). At such distal sequences, during the differentiation of ES cells in vitro, FoxA binding can recruit chromatin remodelers to elicit nucleosome loss (Li et al., 2012). But new studies in C. elegans development, where PHA-4 frequently binds promoters, show that FoxA binding can directly result in recruitment of RNA polymerase II, in either a poised or actively transcribing state (Hsu, 2015). The careful staging of embryos in this system revealed that opening of the local chromatin occurred predominantly after polymerase recruitment, not beforehand, raising the possibility that polymerase could contribute to chromatin opening (Scruggs et al., 2015). Given that enhancers are now known to be transcribed in mammalian cells (Arner et al., 2015), it is of interest to determine whether the initial binding of pioneer factors at distal enhancers in those contexts is also accompanied by polymerase recruitment, prior to local chromatin opening and enhancer activation.

In summary, pioneer factors, as exemplified by FoxA/PHA-4, have been defined by their intrinsic ability to target DNA sites on nucleosomes and where such binding can initiate regulatory events in silent chromatin. Such regulatory events include establishing competence for subsequent induction, e.g. as in development or in response to hormones, establishing a repressed domain, or the direct transcriptional activation of a local promoter.

Pioneer factors in cellular reprogramming

Recent studies on the use of transcription factors to interconvert cells of different types have revealed additional ways that pioneer factors are distinguished from other transcription factors. Consider the collection of transcription factors required to convert fibroblasts to induced pluripotent stem cells (iPS) (Takahashi and Yamanaka, 2006) or fibroblasts to neurons (Vierbuchen et al., 2010). In each case, a group of transcription factors is necessary to elicit the conversion. Yet from a mechanistic point of view, do they all function similarly; i.e., act as pioneers? The answer appears to be no. Direct assessments of the initial binding of the iPS factors Oct4, Sox2, Klf4, and c-Myc showed that Oct4 and Sox2, and to a lesser extent Klf4, but not c-Myc, target predominantly silent, unmarked, and DNase-resistant chromatin when they are first expressed in human fibroblast cells (Soufi et al., 2012). Most of such binding is distal to promoters; i.e., at enhancers, similar to what has been seen for FoxA proteins. By contrast, c-Myc targets “closed” chromatin sites when co-bound with Oct4 and Sox2. A similar assessment showed that the neuronal factor Ascl1, but not Brn2 or Myt1l, targets silent, DNase-resistant chromatin when they are first expressed in fibroblasts; and Ascl1 can recruit Brn2 to closed regions, while Myt1l targets open regions (Wapinski et

al., 2013). Similarly, Pax7 can reprogram a corticotrope cell line into a melanotrope-like cell by targeting closed chromatin sites and recruiting the Tpit factor (Budry et al., 2012). C/EBPα that is ectopically expressed in pre-B cells, in conjunction with endogenous PU.1 (Barozzi et al., 2014), can convert the cells to macrophages by targeting closed chromatin (van Oevelen et al., 2015). In conclusion, not all reprogramming factors are pioneer factors, but reprogramming factor "cocktails" appear to include at least one pioneer factor.

Importantly, these findings were drawn by comparing the genomic sites that are targeted by the factors, when they are first induced in cells, with the chromatin states of the sites in the cells prior to the induction of the factors. That is, it is necessary to know the "pre-bound" state of the chromatin which is being targeted by the factors, in order to assess whether or not the factors are targeting silent chromatin and are thus pioneers. A list of pioneer factors in development and reprogramming has been presented recently, elsewhere (Iwafuchi-Doi and Zaret, 2014)

Mechanism of targeting nucleosomes by pioneer factors and binding cooperativity

What is the extent to which there are substantial parts of the genome that are not "pre-marked" by specific histone modifications, and/or not in open, nuclease-sensitive DNA, and thus might require a pioneer factor for changes in activity? Initial ENCODE studies indicated that about 40% of the genomes studied had "low signal states," i.e. lacked distinguishing chromatin features in a given cell type (Kharchenko et al., 2011; Ho et al., 2014), while more recent assessments suggest that about 67% of a cell's chromatin, including regulatory sequences, is in such unmarked chromatin (Roadmap Epigenomics et al., 2015). More striking, a recent study found a paucity of canonical "active" chromatin marks at genes that are dynamically regulated in development (Perez-Lluch et al., 2015). Thus, there appears to be plenty of "unprogrammed" chromatin for pioneers and other factors to bind during development and reprogramming.

Several parameters govern what allows pioneer factors to target sites on nucleosomes better than other transcription factors. First, in nucleosomes, one face of the DNA helix, along the long axis of DNA, is partially hidden by extensive contacts with the histone octamer (Luger et al., 1997). The "winged helix" DNA binding domain of FoxA proteins interacts with one surface along the DNA long axis (Clark et al., 1993), with extensive specific and nonspecific interactions (Cirillo and Zaret, 2007) that appear compatible with histone binding (Cirillo et al., 1998). In addition, FoxA has a separate, C-terminal domain that interacts with core histones (Cirillo et al., 2002), further stabilizing binding. The Pygo transcription factor contains a domain that binds core histone H3 and its interactions with H3 are governed by H3K4 methylation state and associated factors (Fiedler et al., 2008). Pygo acts like a pioneer factor by initially engaging Wnt target genes and endowing them with the ability to be toggled into active or silent states (Fiedler et al., 2015). Far more work is needed on other pioneer factors to determine the extent to which histone interactions stabilize their ability to target nucleosomes.

A second parameter that allows pioneer factor binding was discovered by curating the nucleosomal targeted sites, separately from the free DNA targeted sites, for enriched sequence motifs. Oct4 and Klf4 were found to target a reduced sequence motif on nucleosomal sites, consistent with the idea that the pioneer factors tolerate reduced protein-DNA interactions during the initial nucleosome targeting (Soufi et al., 2015). The reduced motif could be modeled as being exposed on the nucleosome surface and the pioneers' DNA binding domains, but not that of non-pioneer factors, exhibit structural dynamics that could recognize the reduced motif on the nucleosome surface. Furthermore, while Sox2 normally bends DNA more than could be accommodated on a nucleosome (Remenyi et al., 2003), the Sox2 targeted sites on nucleosomes have a sequence that allows half as much DNA bending, about the amount the DNA is bent on the nucleosome surface (Soufi et al., 2015). Thus the conformation of DNA on the nucleosome may be favorable for certain pioneer factors to bind, and other pioneer factors may have an inherent adaptability of their DNA binding domain that is suitable for nucleosome recognition.

Dynamic monitoring of FoxA factors in living cells showed that they move in the nucleus much more slowly than non-pioneer factors and that both specific and nonspecific DNA contacts are necessary for the slow movement (Sekiya et al., 2009). Taking all of the data together, it appears that pioneer factors have increased residence time on nucleosomal target sites, compared to other factors. Such increased residence time would favor cooperative interactions with other factors, or be associated with chromatin opening that secondarily allows other factors to bind, thus forming a stable regulatory complex that could not be established by factors that cannot target nucleosomes.

Restrictions on pioneer factor binding to chromatin

Despite the ability of pioneer factors to target DNA on nucleosomes, it is clear that they do not occupy all of their cognate sites in a cell; i.e., they exhibit cell-specific DNA binding. It seems likely that as the factors scan sites, they will remain more stably bound to sites where they exhibit cooperative binding with other transcription factors. While such cooperative binding is typical of all transcription factors, the observation remains that such events can be nucleated by the nucleosome targeting capacity of pioneer factors.

Another explanation for the cell-restricted patterns of pioneer factor binding is that they can be actively excluded from certain chromatin domains by repressive features; particularly by the higher order compaction imposed by heterochromatin. This was first demonstrated in a study of where the OSKM factors first bind the human fibroblast genome, during conversion to iPS cells (Soufi et al., 2012). Large, megabase-sized regions of chromatin block OSKM binding to DNA in fibroblasts, but the domains are occupied by the factors in pluripotent cells. Further research showed that these "Differentially Bound Regions" or DBRs are enriched for the chromatin mark H3K9me3, the classic modification associated with heterochromatin (Lachner et al., 2001). The DBRs harbor genes required for late stages of cellular reprogramming to pluripotency (Buganim et al., 2012). Indeed, knockdown of the methyltransferases that impose the H3K9me3 mark (Lachner et al., 2001) allow Oct4 and Sox2 to bind the DBRs and greatly improve the pace of cellular reprogramming. These studies reveal that heterochromatin imposes an impediment to pioneer factor binding and

that understanding the basis for the impediment can inform about ways to enhance the reprogramming process. Further work is needed to understand how other chromatin states may impede pioneer factor binding.

Control of higher order chromatin structure by pioneer factors

In addition to their local effects, pioneer factors alter higher-order chromatin structure. PHA-4/FoxA association with target genes leads to large-scale chromatin decompaction (Fakhouri et al., 2010). Decompaction initiates prior to transcription and may facilitate interactions between cis-regulatory sites. In mammals, FoxA1 and Estrogen Receptor (ER) α associate with enhancers that typically lie tens of kilobases from the target promoter (Carroll et al., 2005; Lupien et al., 2008). FoxA1 promotes ERα binding and looping between enhancers and promoters to activate target genes.

Decompaction can reflect the recruitment of the histone variant H2A.Z by PHA-4/FoxA and consequent histone depletion (Updike and Mango, 2006; Hsu, 2015). In mammals, FoxA2 and CLOCK:BMAL1 recruit H2A.Z to target promoters and promote nucleosome loss (Li et al., 2012; Menet et al., 2014). Nucleosomes containing acetylated H2A.Z, coupled to histone H3.3, form unstable nucleosomes that are easily lost from chromatin (Suto et al., 2000; Bruce et al., 2005; Jin et al., 2009). While H2A.Z and histone loss is observed locally, at the site of pioneer binding, decompaction occurs more broadly, suggesting pioneer factors regulate higher-order chromatin by additional, indirect mechanisms. Curiously though, nucleosome loss occurs less at distal enhancer sequences in differentiated liver cells, where FoxA can be detected bound to nucleosomes in vivo (Chaya et al., 2001; Li et al., 2011).

Future questions for the field

Given that cocktails of transcription factors that elicit cell fate changes can be found to include pioneer transcription factors, or prevail upon pioneer factors pre-existing in cells, it is of great interest to understand how such factors function and their activities could be enhanced, to improve cell reprogramming protocols. As noted above, detailed studies of how core histone interactions can stabilize pioneer factor interactions with nucleosomes will provide a better mechanistic understanding of how the factors function, and possibly how their pioneer activities can be improved. Similarly, a better understanding of the means by which heterochromatic regions can block pioneer factors from binding will shed light on ways to break down barriers to cell trans-differentiation. Recent studies with Hi-C methods have begun to illuminate the higher-order structure of chromatin domains. Given the roles of pioneer factors in local chromatin organization, it will be exciting to determine whether pioneer factors help sculpt the genome in three dimensions. Taken together, further work in these straightforward directions should enhance our ability to control cell fates at will.

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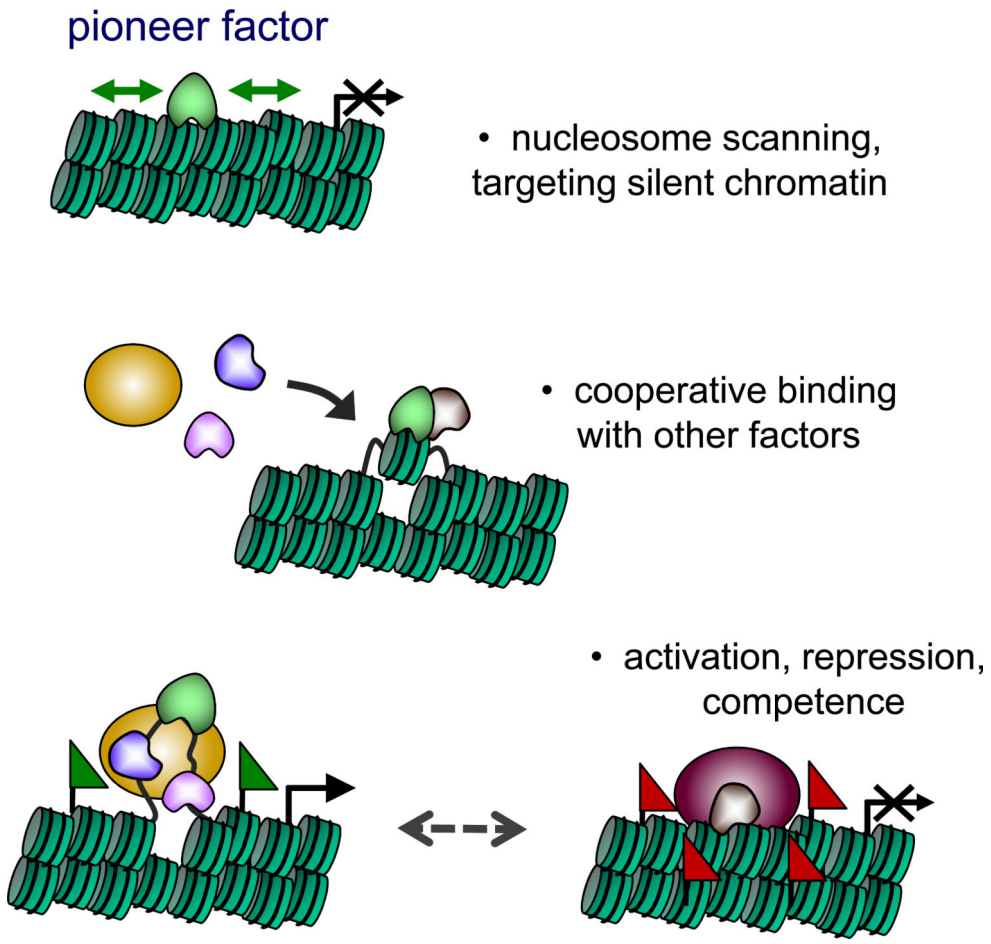

Figure 1. Activities of pioneer factors

(top) Pioneer factors can scan nucleosomal DNA for their target sites in silent chromatin. (middle) Increased residence time at targeted sites allows for cooperative interactions with other factors and more stable binding. (bottom) Binding of pioneer factors can lead to activated sequences (left) with open chromatin features (green flags) and a state of competence to be expressed or direct transcriptional activity, or repressed sequences (right) with closed chromatin features (red flags).