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Trivalent Chromatin Marks the Way iN

Eriona Hysolli[1] and **In-Hyun Park**[1,*]

[1]Department of Genetics, Yale Stem Cell Center, Yale School of Medicine, 10 Amistad, 201B, New Haven. CT. 06520

Abstract

Recently in Cell, Wapinski et al. investigated the epigenetic mechanisms underlying direct conversion of fibroblasts to induced neurons (iNs). They found that Ascl1 acts as a pioneer factor at neurogenic loci marked by a closed “trivalent” chromatin state in cells permissive to direct conversion but not in restrictive cell types.

Lineage-specific transcription factors define cell fate during development. Intuitively, ectopic overexpression of these transcription factors can re-direct cell fate. One of the most dramatic examples of engineered cell fate change is the derivation of induced pluripotent stem cells (iPSCs) by four transcription factors (classically Oct4, Sox2, Klf4, c-Myc) that reprograms fully differentiated cells to a pluripotent state (Takahashi and Yamanaka, 2006). This successful cellular conversion to pluripotency has also encouraged efforts to directly reprogram one cell type to another. Although examples of direct reprogramming, or transdifferentiation already existed prior to the advent of iPSC technology (Graf, 2011), they have primarily involved switching between related cells in a lineage within the same germ layer, such as the conversion of murine embryonic fibroblasts to myoblasts through overexpression of MyoD (Davis et al., 1987), or mature murine B cells to macrophages through overexpression of C/EBPs (Xie et al., 2004). In 2010, the direct reprogramming field reached an important milestone when Vierbuchen et al. found that three transcription factors (Ascl1, Brn2, Myt1l) are sufficient to convert mesodermal murine fibroblasts to ectodermal neurons (Vierbuchen et al., 2010). Since these reports, several groups have successfully converted somatic cells from various tissue sources into a variety of neuronal subtypes (Yang et al., 2011). In a recent issue of Cell, Wapinski et al. (Wapinski et al., 2013) begin to address the mechanism whereby Ascl1, Brn2, and Myt1l confer neuronal identity to murine fibroblasts. They report that Ascl1 is a pioneer transcription factor occupying closed chromatin regions containing H3K4me1, H3K27ac, and H3K9me3, which subsequently recruits the other factors to activate neural pathways.

Of the three neurogenic factors, Ascl1 was already known to be essential for inducing neuronal fate because its overexpression alone can induce small neuronal features in fibroblasts (Vierbuchen et al., 2010). Wapinski et al (Wapinski et al., 2013) demonstrate that Ascl1 acts as a transcription activator that is responsible for most of the global transcriptional and genome-wide occupancy changes during iN conversion. ChIP-Seq

*Correspondence: inhyun.park@yale.edu.

analyses of Ascl1 binding revealed that Ascl1 occupies its targets in MEFs regardless of whether Ascl1 is expressed alone or with all three factors (Figure 1). In addition, Ascl1 seems to occupy its physiological targets in MEFs, since Ascl1 binding patterns are similar in MEFs and neural progenitor cells (NPCs). Strikingly, Brn2 target binding is misdirected in the absence of Ascl1, but is properly recruited in Ascl1's presence, providing further support for the primary role of Ascl1 in targeting loci for activation during the induced neuron (iN) fate switch.

To determine how Ascl1 can independently target neurogenic loci during reprogramming, Wapinski et al. employed formaldehyde-assisted isolation of regulatory elements followed by massively parallel sequencing (FAIRE-seq) technology that maps genome-wide nucleosome location. Unexpectedly, the authors found that Ascl1 binds more commonly to closed chromatin, while Brn2 and Myt1l occupy regions with active histone marks. These data suggest that Ascl1 acts as a pioneer factor (Zaret and Carroll, 2011), which primes fibroblast chromatin for recruitment of other transcription factors, in addition to activating iN related genes.

The most exciting finding of this study involves the revelation of a trivalent chromatin state in genomic regions of MEFs that are normally occupied by Ascl1 in NPCs. While investigating whether any epigenetic marks were responsible for initially guiding Ascl1 to its intended targets, Wapinki et al. noticed an increased co-occurrence of H3K4me1, H3K27ac, and H3K9me3 histone marks in Ascl1 sites. These trivalent sites are only present in cells permissive to iN reprogramming, such as MEFs, human dermal fibroblasts and human skeletal muscle myoblasts, but are not present in restrictive cells such as human keratinocytes and human osteoblasts. Additionally, some Ascl1 targets not bound in MEFs but occupied in NPCs show less prevalence of the trivalent state. In support of the functional role of the trivalent mark, the authors showed that erasure of the H3K9me3 mark by histone demethylase JmjD2 reduced reprogramming efficiency of MEFs to iNs, further supporting the link between Ascl1 accessibility in the presence of trivalency and iN reprogramming success.

Finally, in order to further explore the functional role of Ascl1 during fibroblast to neuron transdifferentiation, Wapinski et al. tested the Ascl1 downstream target Zfp238 for its ability to functionally replace Ascl1 during iN conversion. Unlike Ascl1, Zfp238 was unable to initiate reprogramming alone, but it was competent in reprogramming in the presence of Myt1l. Even though the interaction between Ascl1 and Zfp238 was not fully examined in detail, taken as a whole, this data supports a central role of the Ascl1 pathway in the neuronal fate switch.

There remain, however, many interesting questions to be addressed in future studies. Ascl1 is sufficient to initiate the reprogramming process. However, when Ascl1 is expressed alone, only occasional Tuj1+ neuronal cells emerge. This observation points to the very strong supportive roles for Brn2 and Myt1l or Zic1, most likely in the later stages of iN reprogramming. In this study, Myt1l marked a minimal number of binding sites despite much optimization effort in ChIP, while its addition to the reprogramming cocktail significantly increased reprogramming efficiency and formation of mature neurons. The low

iN conversion rate (~2–8% depending on cell type) may obscure some interactions among Ascl1 and the supportive transcription factors. Furthermore, it will be interesting to determine how global transcription and epigenetic changes occur in instances when Ascl1 is excluded from the reprogramming cocktail.

Another stimulating question from this study is the existence of trivalent chromatin states or similar unknown chromatin states that enable accessibility to pioneer factors in other transdifferentiation contexts i.e. fibroblasts to hepatocytes and cardiomyocytes, or other known and yet undiscovered conversions (Ladewig et al., 2013). Addressing the binding particulars of how Ascl1 recognizes the trivalent state could be used to predict a more widespread modus operandi of “on target” factors.

Taken as a whole, this study’s accomplishments are twofold. First, by delving into the mechanism of iN reprogramming, this study has provided more support for the soundness of using the direct conversion method, as binding patterns of essential transcription factors resemble those found naturally, i.e. NPCs. Secondly, a trivalent chromatin state is uncovered that further underscores the importance of a more complex combinatorial histone code, just like the discovery of bivalent promoters in embryonic stem cells did previously (Bernstein et al., 2006).

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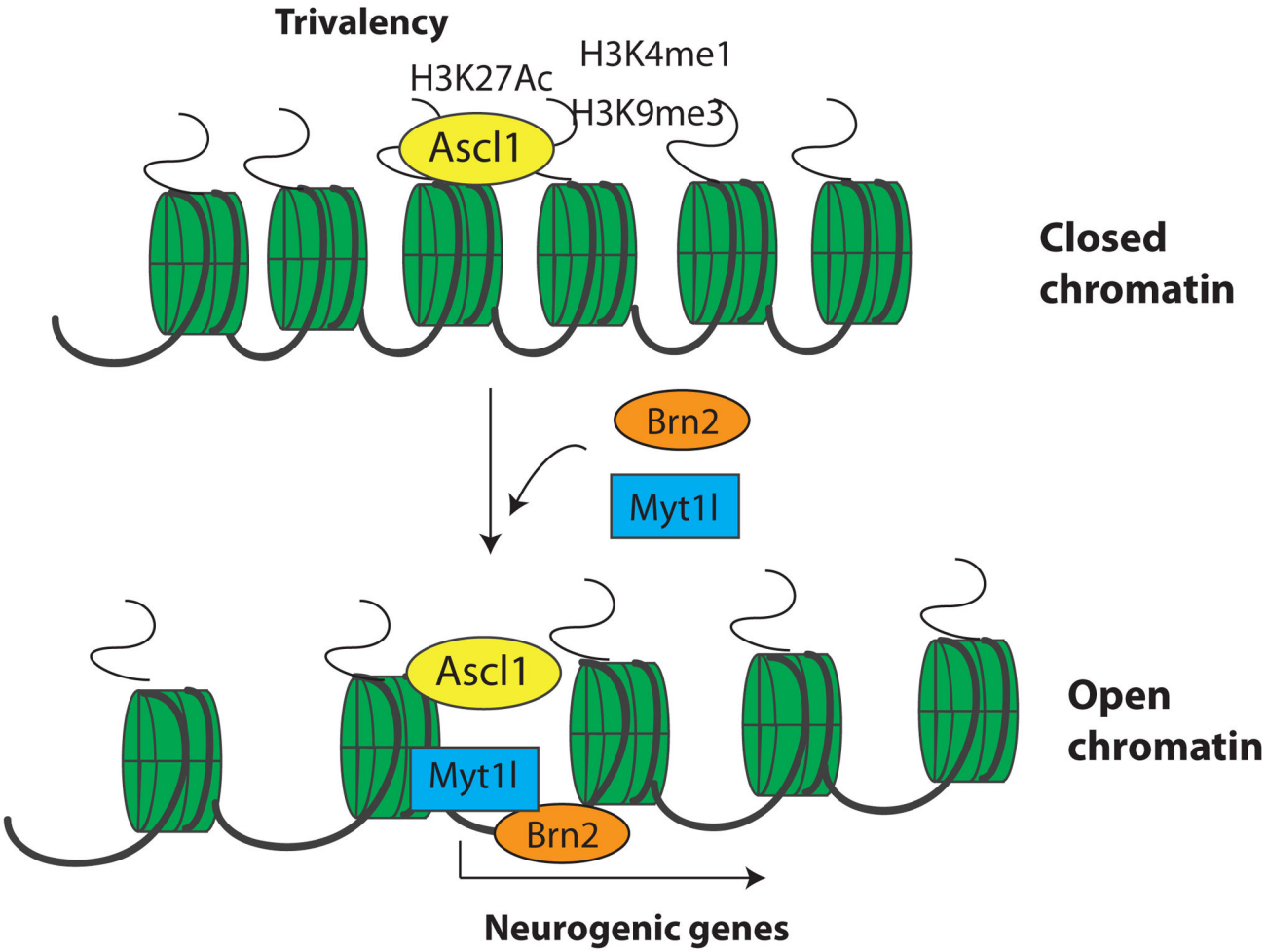

Figure 1.

Pioneer transcription factor Ascl1 is recruited to trivalent chromatin with H3K9me3, H3K27ac, and H3K4me1 histone marks. Brn2 and other transcription factors are recruited to further promote transcriptional activation for neuronal conversion.