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Alternate title option:

### **Putting chromatin in its place: the pioneer factor NeuroD1 modulates chromatin state to drive cell fate decisions**

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## **News & Views for:**

NeuroD1 reprograms chromatin and transcription factor landscapes to induce the neuronal program

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### **Title**

**Pioneers of differentiation: targeted chromatin modulation by NeuroD1 drives cell fate decisions.**

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Putting chromatin in its place: the pioneer factor NeuroD1 modulates chromatin state to drive cell fate decisions

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## Abstract

**Cell fate decisions require the deployment of distinct transcriptional programs – how this is controlled and orchestrated is a key question from basic developmental biology to regenerative medicine. In this issue of *The EMBO Journal*, Pataskar and Jung et al. (Pataskar et al, 2015) demonstrate how the transcription factor NeuroD1 acts genome-wide to elicit a specific neurogenic program, including differentiation and migration. Much of that activity is due to NeuroD1 acting as a pioneer factor. NeuroD1 is able to bind its targets within repressive chromatin and can induce a more open chromatin state amenable to cell-type specific regulation.**

## Main

How the genome is regulated to give rise to the many distinct cell types of complex organisms is a primary focus of developmental biology today. With the advent of whole genome assays, it has become almost routine to probe cellular differentiation and identity at the genomic scale. This includes the profiling of transcription factors (TFs) to understand which genes they may regulate, but also the profiling of histone and DNA modifications to gain a more general understanding of the regulatory state of the genome.

*How, where, and when* TFs act is as much a function of their own regulation, as of the specific cellular environment, including cofactor availability and the chromatin they must act within. Examples of the significance of this ‘cellular environment’ include that binding motif presence is often an extremely poor predictor of actual *in vivo* binding (e.g. Wilczynski & Furlong, 2010; Yang et al, 2006), and that individual cell types often demonstrate stark differences in the genome-wide distributions for the same sequence specific TFs (e.g. Cao et al, 2010; Odom et al, 2004). Several TFs have the capacity to fundamentally shape cellular identity and direct cell fate decisions (e.g. Iwafuchi-Doi & Zaret, 2014; Lee et al, 1995) – understanding the mechanism by which these ‘pioneer’ factors are able to accomplish this feat is a matter of intense investigation. Pataskar and Jung *et al.* now show how a proneural TF called NeuroD1 navigates and modifies the local chromatin environment of progenitor cells to trigger differentiation and migration programs.

The bHLH TF NeuroD1 had previously been shown to constitute a potent neuronal differentiation factor (e.g. Guo et al, 2014; Lee et al, 1995). To investigate how

NeuroD1 imposes cell fate, the authors establish an embryonic stem cell (ESC) model, where the selective induction of NeuroD1 *alone* triggers neuronal differentiation, as shown by the loss of pluripotency markers, concomitant gain of neuronal markers, and changes in cellular morphology. Up-regulated genes (URGs) not only include neuronal differentiation factors, but also a significant number of genes implicated in epithelial-mesenchymal-transition (EMT) and migration, which is especially striking considering that NeuroD1 is primarily expressed in the subventricular zone (SVZ) of the developing brain, through which differentiating neurons migrate on their path from the progenitor population towards the cortical plate.

Between the ~3900 genes that change expression in response to NeuroD1 and the ~2400 binding events detected by ChIP, the authors concentrate on how NeuroD1 interacts with the ~200 genes NeuroD1 seems to activate directly via binding to *cis*-regulatory modules (CRMs), such as promoters and enhancers. Since many of these targets are TFs and chromatin regulators, a large part of the remainder of gene expression changes may be attributable to indirect regulation.

Using Bayesian inference, Pataskar and Jung *et al.* searched for classifiers among ESC histone modification and TF binding data that distinguish NeuroD1-bound from non-bound CRMs near URGs. Compared to unbound URG promoters, bound URG promoters showed significantly higher levels of the chromatin condensation mark H3K27me3 and exhibited lower levels of activity hallmarks, such as H3K27ac and general chromatin accessibility in ESCs prior to NeuroD1 induction. Upon NeuroD1 induction the chromatin at target promoters ‘opens’, marked by a decrease in H3K27me3 and increases in H3K27ac, general chromatin accessibility and gene expression. Similarly, target enhancers also gain chromatin hallmarks of activity. Time-course data at several CRMs reveal the sequence of events: NeuroD1 is bound quickly followed by H3K27 demethylation and acetylation, followed by RNA polymerase II engagement and transcription shortly thereafter. These observations strongly indicate that NeuroD1 is able to find and bind its targets in repressed chromatin, which then allows for chromatin remodelling towards a state more conducive to transcription – the very definition of a pioneer TF (Zaret & Carroll, 2011).

Interesting is the temporal requirement of pioneer factors: Is transient activity sufficient to switch chromatin state (and differentiation programs) long-term, or are

they required to maintain the differentiation state? NeuroD1 expression is limited largely to the entry into and migration through the SVZ and is absent from differentiated neurons. The authors argue that a pulse of NeuroD1 induction likely suffices to reorganize chromatin longer-term: Target sites retain marks of chromatin activity state days after *ectopic* NeuroD1 expression is no longer detectable. However, to what degree this may be attributable to autoregulation of endogenous NeuroD1 remains to be resolved.

NeuroD1's effects on gene activity and chromatin state hold up markedly well *in vivo*. Not only are individual URGs identified *in vitro* found to be co-expressed with NeuroD1 in the SVZ, but the actual binding of NeuroD1 to target CRMs was confirmed in the embryonic cortex. In an elegant assay of NeuroD1 overexpression in the developing brain, Pataskar and Jung *et al.* confirm that NeuroD1 increases H3K27 acetylation at most target CRMs, coupled to an increase in target gene expression. Moreover, even the EMT stimulation identified in cultured cells is appreciable in the brain: within 48h after NeuroD1 is induced, cells have left the ventricular zone and lost expression of the neural progenitor marker Pax6.

So here is a pioneer TF that once activated in pluripotent cells triggers wholesale changes in terms of cellular identity and migration behaviour. It does so in part by recognizing its binding motifs and activating differentiation genes even if they are located in repressive chromatin. This is followed by local remodelling of the chromatin towards a more open state, thus allowing for transcription. How does NeuroD1 achieve this? Part of the underlying mechanism will be interaction with other local TFs. The authors show that NeuroD1 binding correlates with displacement of TFs that were associated with the URG promoters (Tbx3) and enhancers (Mbd3, Tbx3). While the mechanisms of this displacement are not clear, the biological implications are: Mbd3 is a component of the nucleosome remodelling deacetylase complex (NuRD) that would help silence enhancers. Similarly, the sequence-specific T-box TF Tbx3 has been described as a potent repressor. Local displacement of factors such as Mbd3 and Tbx3 upon NeuroD1 binding should then allow relief of direct and chromatin-mediated repression at CRMs and allow for regulation by additional non-pioneer (aka. 'settler') TFs. Clearly, the hierarchies and mechanisms underlying chromatin remodelling in response to NeuroD1 and other pioneers remain to be elucidated. Since Utf1, Mbd3 and Tbx3 association were found to be predictive for later NeuroD1 binding, one might speculate whether some of these factors may

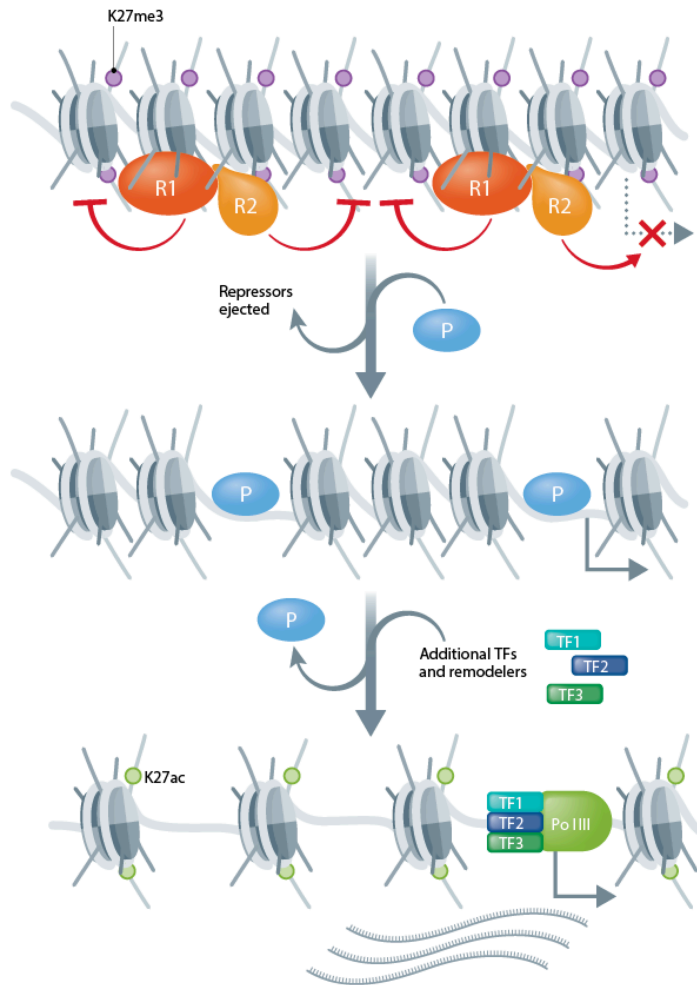
play a role in recruiting NeuroD1 to its targets. Soufi et al. recently proposed that some bHLH pioneers (like Myc) bind their targets primarily through cooperative interaction with local factors, while other bHLHs rely on their own binding activity (Soufi et al, 2015). Where NeuroD1 fits on that spectrum is not yet clear.

Among pioneers, the bHLH TFs may be an especially interesting class. Most are able to form homo- and heterodimers, which affects their precise binding specificity and their molecular activity. Hence, for bHLH pioneers, the specific dimers formed may well dictate *where* they modify chromatin state, as well as *how* they modify it. It is feasible, for example, that the NeuroD1 binding detected in CRMs of up-regulated genes could reflect dimers distinct from NeuroD1 complexes near repressed genes. Furthermore, NeuroD1 may mediate distinct pioneer activities in other progenitor cell types. NeuroD1 (aka. Beta2) is also an essential component in the differentiation and maintenance of the mature pancreatic  $\beta$ -cell state (Jia et al, 2015), where it is likely forming complexes distinct from those in neuronal progenitors.

We are only just beginning to understand the complexities of pioneer activity in terms of how such TFs interact with their chromatinized targets, as well as in terms of the molecular activities they deliver to those targets. Once bound, pioneers like NeuroD1 can be thought of as (re-)partitioners of the regulatable genome: By modulating chromatin state locally in a tissue- or condition-specific manner, they change accessibility for further regulators. A detailed understanding of the hierarchical interactions of ‘pioneers’ and ‘settlers’ with their chromatin environment is an essential prerequisite for predictive modelling of how gene expression programmes are deployed to guide cellular differentiation.

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**Local chromatin remodelling by pioneers.** A genomic region is condensed and genes within are repressed. Repressor proteins (R1-3) such as Mbd3, Utf1 and Tbx3 maintain the repressive chromatin state and reinforce transcriptional silence. Once available, pioneer factor complexes (P) like NeuroD1+bHLH partners find and bind their target sites, including sites within repressive chromatin. This then recruits enzymatic activities to modulate repression locally. For example, repressor proteins like Tbx3 and Mbd3 are ejected and histone trimethylation of H3K27 can be exchanged for acetylation. Chromatin locally decondenses in the process and ‘opens’ for targeting by settler transcription factors (TF1-3) to regulate gene expression either in conjunction with, or independent of the pioneer complex.

Complex context-dependent regulation is achieved by, for example, (i) availability of pioneer binding partners / cofactors, (ii) aspects of the chromatin environment, including recruiting factors already present, (iii) the mode of pioneer interaction with the chromatin (e.g. displacement of proteins, delivery of HAT activities, etc.), and (iv) the specific availability of TFs capable of translating the regulatory information encoded in open chromatin into local gene activity.