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Histone modifications controlling native and induced neural stem cell identity

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During development, neural progenitor cells (NPCs) that are capable of self-renewing maintain a proliferative cellular pool while generating all differentiated neural cell components. Although the genetic network of transcription factors (TFs) required for neural specification has been well characterized, the unique set of histone modifications that accompanies this process has only recently started to be investigated. *In vitro* neural differentiation of pluripotent stem cells is emerging as a powerful system to examine epigenetic programs. Deciphering the histone code and how it shapes the chromatin environment will reveal the intimate link between epigenetic changes and mechanisms for neural fate determination in the developing nervous system. Furthermore, it will offer a molecular framework for a stringent comparison between native and induced neural stem cells (iNSCs) generated by direct neural cell conversion.

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Introduction

Cell lineage differentiation of pluripotent stem cells requires the induction of massive gene expression changes that include the down-regulation of stemness-specific genes and the activation of cell-type specific gene transcriptional programs. During this process, extensive regions of the chromatin undergo structural changes to either enhance or reduce the accessibility of specific loci that encode regulatory proteins. Chromatin remodeling is controlled by epigenetic modifiers of histones and DNA that exert crucial roles during the process of cell type specification [1,2]. Although several aspects of the epigenetic control of pluripotent stem cells, including both embryonic stem (ESCs) and induced pluripotent stem cells (iPSCs), has been elucidated, the understanding of the epigenetic control of the identity of the neural precursor cells (NPCs) and, their *in vitro* derivatives the neural stem cells (NSCs), remains incomplete [3]. In development, NPCs emerge as an intermediate step between pluripotent stem cells and more differentiated cell types of the CNS, such as neurons, astrocytes or oligodendrocytes. It is predicted that the epigenetic signature of neural progenitors can be stably transmitted to the progeny to safeguard stem cell renewal, but it is also permissive to modifications for initiating differentiation. A better understanding of these processes is crucial to define how cell fate choices are acquired or erased, and how a somatic cell can be directly reprogrammed into a NSC. Several protocols have been described to convert a differentiated fibroblast into a NSC but the molecular mechanisms underlying this conversion are still poorly understood [4–10]. In this review, we first focus on the dynamics of histone modifications during neural fate specification both *in vivo* and *in vitro*, although a direct comparison of the two systems is still far from being complete. Finally, we survey the epigenetic changes recently associated with direct conversion of fibroblasts into NSCs.

Histone methylation dynamics during neural cell fate specification

During initial neural fate commitment, silencing of pluripotent-specific and alternative lineage-specific genes appears to occur mainly through histone modifications [1,11]. The regulatory regions of lineage specific genes generally present a distinctive histone modification pattern, consisting of large regions carrying the repressive histone 3 tri-methylation of lysine 27 (H3K27me3) modification and smaller regions with the permissive H3K4me3 mark. These 'bivalent' states are thought to maintain low levels of transcription, keeping genes 'poised' for activation or repression during subsequent cell commitment and differentiation [1]. The poised state is resolved by removing H3K27me3 or H3K4me3 marks in order to activate or silence the corresponding genes, respectively [2]. In fact, during neural induction of ESCs the vast majority of the NPC-specific promoters resolve their bivalent signature by loosing the repressive H3K27me3 mark [12]. This is the case for the neural genes Ngn1/2, Sox1 or Ascl1 that retain only the activation mark H3K4me3 [1,13,14]. Similarly, in *vivo* studies

show that the dual histone demethylase KDM7A removes methylation marks on both H3K9 and H3K27, thus promoting neural induction in the epiblast cells of the primitive streak of the early embryo [15,16]. Mechanistically, KDM7A mediates transcriptional activation of FGF4, a signal molecule implicated in neural differentiation, by removing repressive histone marks from the promoter of the gene [17]. Conversely, pluripotent and non-neural genes have been shown to be silenced *in vitro* through H3K9 di-methylation and tri-methylation during neural induction [3,18]. However, these histone modifications *in vitro* accumulate over time as shown recently in a comprehensive transcriptional and epigenomic analysis of progressive stages of NPC differentiation from human ESCs based on cell morphology and Notch activation state [19••], in which total H3K27me3 levels were shown to peak at the transition to late NPCs [19••]. Thus, early neural progenitors retain expression of at least part of the ESC-specific transcriptional program. These findings indicate that the transition from ESC-transcriptional to mature neural precursor-transcriptional program is a slow process that involves discrete cell intermediates characterized by a specific epigenetic state and combination of key developmental TFs [19••].

Polycomb Repressive Complex 2 (PRC2) and neural commitment

Polycomb group (PcG) proteins, initially discovered in *Drosophila* as fundamental regulators of development, are generally responsible for the repressive state of the chromatin [20]. According to the classical model they consist of two complexes: PRC2 that accounts for H3K27me3 mark, subsequently read by PRC1 that catalyzes histone 2A monoubiquitination. The core of PRC2 is formed by three PcG proteins: enhancer of zeste 2 (EZH2) or its homolog EZH1, embryonic ectoderm development (EED), and suppressor of zeste 12 (SUV12). EZH factors, being SET-domain containing proteins, contribute to gene repression by H3K27 methylation [21,22]. In brain development, during the neurogenic phase, the H3K27 methyltransferase Ezh2 controls the balance between NPC maintenance and differentiation. In fact, its ablation in the developing brain induces massive NPC differentiation resulting in a thinner cerebral cortex [23]. In line with this observation, silencing Ezh2 in the embryo by *in utero* electroporation causes a massive exit from cell cycle and differentiation [24]. Conversely, in a recent knockout study of the transcriptional repressor nuclear factor I-b (NfIb), a direct repressor of Ezh2, cortical progenitors were increased in number, suggesting impairment in cell differentiation. As predicted, Nfib-null progenitor cells showed enhanced epigenetic repression on EZH2 downstream target genes, such as the *Ink4A* locus, an inhibitor of cell cycle progression [25]. Collectively, these recent results support a model by which PRC2, as well as the associated repressive histone mark H3K27me3, define the neural progenitor state and prevent neuronal differentiation. In fact, Jmjd3 (Kdm6b), the most abundant H3K27me3-specific demethylase during neural development, promotes neuronal differentiation in both embryos [26–28] and adult NPCs [29•] acting on key elements that favor neurogenic program(s). H3K27me3 is read by the chromobox-domain (CBX) protein subunits of PRC1, which catalyzes the monoubiquitination of H2A on Lys 119 through the E3 ligase of the RING1 family, leading to a closed chromatin state. New players have been recently added to the complex scene of epigenetic control of neural induction. The chromatin component Zuotin-related factor 1 (Zrf1) derepresses developmental genes by displacing the PRC1 complex from chromatin during differentiation. During ESC differentiation into NSCs, Zrf1 acts as downstream effector of the basic helix-loop-helix (bHLH) factor Id1 [30•,31,32]. In the developing cortex, Zrf1-deficient NPCs display defects in self-renewing and, importantly, in their ability to differentiate, which has been linked to impaired beta-catenin signaling [30•]. In fact, Zrf1 is necessary to establish and maintain the expression of Wnt ligands through removal of polycomb-mediated repression from their promoters [30•].

Notably, several distinct PRC1 complexes have been reported based on their protein composition, chromatin affinity, specific interactors and their divergent downstream effects [33•]. Interestingly, in the neural tissue a physical and functional interaction has been demonstrated between PRC1 and AUTS2, a nuclear protein, whose gene mutations in humans are associated with neurodevelopmental syndromes, including autism spectrum disorders (ASD) [34••]. Surprisingly, this interaction promotes gene activation, by contrast with the repressive role of the canonical PRC1 protein complex. In fact, the Auts2-mediated recruitment of casein kinase 2 (CK2) on the PRC1-Auts2 complex and the association with P300 co-activator constitute two independent mechanisms of transcriptional stimulation that ultimately leads to neuronal differentiation [34••]. Taken together these observations underline the complexity of the gene expression regulation controlled by PcG proteins that, in particular within the neural landscape, remains to be fully understood.

Histone acetylation pattern during neural cell fate specification

Beyond methylation, acetylation of histones, has been shown to be crucial for neural fate commitment [35]. Histones H3 and H4 can be acetylated on lysine residues and the presence of this mark in promoter/enhancer correlates with an active transcriptional state [36]. In fact, this leads to a remodeling of the net charge of histone tails and to opening of chromatin. Not surprisingly, this mechanism is conserved across the evolution and is crucial for the sophisticated processes underlying brain development and function. This is supported by evidence that CBP and p300, the major histone acetyl transferases