A Novel Model for an Older Remodeler: The BAF Swap in Neurogenesis

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Changes in chromatin state contribute to the switch in gene expression programs that characterizes the transition of dividing neural stem cells toward a neuronal fate. In this issue of *Neuron*, Lessard et al. show that this process is regulated by specific cofactor exchanges within the SWI/SNF chromatin remodeling complex.

In order to develop specialized cell types and tissues, multicellular organisms depend on epigenetic mechanisms—processes that initiate and maintain specific gene expression patterns that are passed on to progeny without altering the DNA sequence. A wealth of cell-type-specific master transcription factors that orchestrate global gene expression patterns during differentiation is known. However, transcription of the genes they regulate is critically dependent on the accessibility of chromatin at these gene loci by the transcriptional machinery. The transitions from proliferating neural stem cells or progenitors toward committed precursors, and finally, to terminally differentiated cells of the neuronal, astrocytic, or oligodendrocytic lineage, are subject to extensive epigenetic control (reviewed in Hsieh and Gage 2004, 2005), but our understanding of the details of these processes remains sketchy. By carefully examining the multisubunit, ATP-dependent SWI/SNF-like chromatin remodeling complex in the developing nervous system, Lessard et al. (2007), in this issue of *Neuron*, now show that the precise cofactor composition of this complex determines whether neural stem cells continue to divide or differentiate.

The cell has two basic tools at its disposal to dynamically regulate chromatin state: covalent modification of chromatin, which includes histone modifications such as acetylation and methylation at specific positions, and ATP-dependent chromatin remodeling. The latter process is carried out by enzyme complexes that utilize the energy released by ATP hydrolysis to slide or peel away histone octamers around which nucleosomal DNA is wrapped, thus exposing the DNA and facilitating the binding of sequence-specific transcription factors (reviewed in de la Serna et al., 2006). Emphasizing their diverse roles in regulating gene expression, several families of chromatin remodelers are known to date, each with unique compositions and specialized tasks. Among these, the best understood chromatin remodelers belong to the family of Brahma-related gene (Brg)/Brahma (Brm)-associated factor (BAF) complexes, also known as SWI/SNF complexes.

Like most large enzyme machines, SWI/SNF complexes are composed of a set of central subunits that provides the core enzymatic activity of the complex, along with several associated factors that serve specialized roles in substrate targeting, recruitment of additional regulatory machinery, or both (Figure 1). What makes the vertebrate SWI/SNF complex so special, however, is the fact that its subunit composition varies according to cell type, suggesting a functionally relevant combinatorial assembly (Olave et al., 2002; Wang et al., 1996). Since, in neurogenesis, at least, Brg does not appear to be highly regulated (Seo et al., 2005), any specialized function of the complex must therefore be mediated by the accessory subunits. Previous work from the Crabtree group had shown that, indeed, a subset of BAFs is encoded by families of highly homologous genes that are differentially expressed across cell types. For example, while BAF53a was found in all tissues examined (albeit at varying levels), BAF53b was shown to be exclusively expressed in the adult brain (Olave et al., 2002). Likewise, BAF60 comes in three flavors (BAF60a, b, and c), and the expression pattern of these homologs varies according to cell type (Wang et al., 1996). These findings raised the intriguing possibility that the homologs of individual BAFs are assembled into the SWI/SNF complex in a mutually exclusive fashion, allowing for a combinatorial variety of SWI/SNF complexes with distinct functions during development and in the adult.

By demonstrating two functionally relevant cofactor swaps in the SWI/SNF complex during the development of the murine brain, Lessard et al. (2007) now provide evidence that SWI/SNF subunit composition can indeed regulate cell fate. Biochemical purification of the SWI/SNF complex from the brains of newborn mice yielded, in addition to almost all previously known BAFs, four more homologous proteins, termed BAF45a, b, c, and d. Examination of the expression patterns of BAF45 and BAF53 homologs during brain development revealed a rather tight switch: just around embryonic day 12–13 (E12–13), BAF45a and BAF53a give way to BAF45b/c and BAF53b, respectively. This is around the same time that neural stem/progenitor cells in the developing brain cease to proliferate and start to differentiate into neurons. Indeed, immunofluorescence staining for these BAFs in E12.5 spinal cords

showed mutually exclusive expression patterns, with BAF45a and BAF53a confined to proliferative regions, and BAF45b/c and BAF53b mainly expressed in the differentiated zone.

So, these data strongly suggested the existence of two distinct SWI/SNF complexes during development: a neural progenitor-specific complex, termed npBAF, and the postmitotic neuron-specific complex, nBAF (Figure 1). But do these complexes indeed function differently? To investigate this, Lessard et al. (2007) employed a whole battery of in vivo and in vitro experiments. Consistent with a critical role for npBAF complexes in maintaining neural stem/progenitor cells in a proliferative state, neural-specific BAF45a gain of function led to an increase in the mitotic index of cells both in vivo and in cell culture, while BAF45a loss of function, BAF53a loss of function, or both reduced the cell proliferation rate in cultured cells without affecting the survival or extent of neuronal differentiation. Lessard et al. (2007) further substantiated these findings by crossing mice expressing *Cre* recombinase driven by the *Nestin* promoter with mice carrying a single Brg allele flanked by *LoxP* sites. Since the promoter of the *Nestin* gene is activated around E10.5, i.e., just prior to the time when the switch from npBAF complexes to nBAF complexes occurs, the progeny of this cross lose Brg expression before the nBAF stage. This technique thereby provided a tool to study the role of Brg in proliferating neural stem/progenitor cells. In contrast to a constitutive knockout of Brg, which results in peri-implantation lethality (Bultman et al., 2000), Nestin-Cre/Brg-floxed fetuses develop to term but are born without respiration and have drastically smaller brains, as well as other brain defects of varying severity.

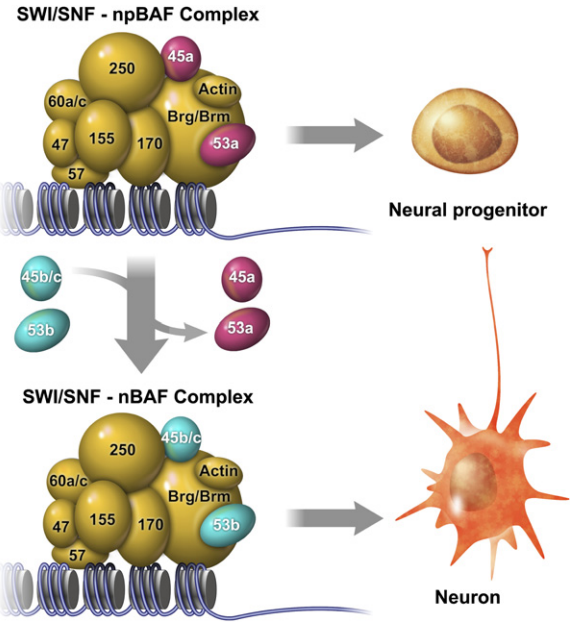

Figure 1. Regulation of Neurogenesis by Differentially Assembled BAF Complexes

BAF chromatin remodeling complexes, also known as SWI/SNF complexes, are comprised of a catalytic subunit (Brg/Brm) and additional core subunits, including BAF250, BAF170, BAF155, BAF60, BAF57, BAF47, and actin. Neural progenitor-specific BAF (npBAF) complexes contain two additional subunits, BAF45a and BAF53a (in red). During neuronal differentiation, these are exchanged for neuron-specific homologous proteins, BAF45b/c, and BAF53b (in green), thus forming postmitotic neuron-specific BAF (nBAF) complexes. While npBAF complexes specify gene expression patterns necessary for neural stem/progenitor cell proliferation, the switch in cofactor composition is critical for neuronal differentiation. Figure credit: Jamie Simon.

These findings highlight the importance of npBAF complexes in keeping neural stem/progenitor cells in a proliferative state. In the absence of Brg, npBAF complexes are catalytically inactive, and may not even assemble into a stable complex. Reduced cell proliferation may then deplete the pool of stem/progenitor cells necessary for proper brain development. In support of this notion, cortical cultures and neurospheres prepared from these Brg-deficient embryos showed reduced mitotic index and self-renewal capacity, respectively (Lessard et al., 2007).

Hints as to what signaling pathways might be regulated by npBAF complexes come from transcript array analyses of telencephalons of npBAF loss-of function mice. Here, several components of signaling pathways and transcription factors previously implicated in neural stem cell maintenance, such as proteins functioning in the Sonic Hedgehog (SHH) and Notch pathways, show aberrant expression levels. Most of these perturbations in gene expression levels probably arose secondarily, via dysregulation of proteins that regulate them. However, Lessard et al. (2007) show by chromatin immunoprecipitation (ChIP) that BAF45a and BAF53a are indeed associated with the promoters of a subset of these genes, thus providing evidence for direct gene regulation by npBAF complexes. Obviously, npBAF and nBAF complexes bind and regulate many more gene targets. With recent advances in high-throughput sequencing technology, it is now feasible to address promoter occupancy on a genome-wide scale (Johnson et al., 2007). Further expression analysis at loci of interest would then reveal downstream candidate genes differentially regulated by npBAF complexes versus nBAF complexes.

These findings clearly show that BAF complexes exert their effect on cell proliferation and differentiation by producing substantial changes in gene expression programs in a chromatin-dependent manner. However, they do not give any clues as to the molecular mechanisms involved. How can a rather subtle change in cofactor composition of the SWI/SNF complex lead to global changes in transcriptional output? While several core components of the SWI/SNF complex have intrinsic chromatin binding activity, the core complex does not bind DNA in a sequence-specific manner. Thus, SWI/SNF complexes are often targeted to promoters by other transcriptional machinery. This dependence on recruiters, therefore, may impart promoter specificity. For example, it was found that BAF57 binds to the CoREST corepressor, and this interaction was proposed to recruit the SWI/SNF

complex to promoters that contain a binding site for the NSRF/REST transcriptional repressor complex (Battaglioli et al., 2002; Ooi et al., 2006). Similarly, BAF45a and BAF53a on the one hand, and BAF45b/c and BAF53b on the other, might differentially mediate recruitment of SWI/SNF to genes via promoter-bound complexes. Support for this notion comes from the finding that despite the large overall homology between BAF45 homologs, it is the most divergent domains of BAF45a that are necessary for its positive effect on cell proliferation (Lessard et al., 2007).

Alternatively, the BAFs might act to block binding of the SWI/SNF complex to promoter-bound transcription factors. Such a mechanism has been proposed for Geminin (Gem), a key regulator of cell cycle progression that has also been shown to directly compete with Brg for binding to proneural transcription factors, including neurogenin and NeuroD, thus preventing SWI/SNF-mediated transcriptional activation of their target genes in progenitor cells (Seo et al., 2005). As cells differentiate toward the neuronal lineage, Gem is downregulated and the Gem-mediated block of transcription factor activation is relieved. Surprisingly, Gem was not recovered in the biochemical approach employed by Lessard et al. (2007). This discrepancy requires further investigation. However, the Gem-Brg interaction was initially identified in a yeast two-hybrid screen, and it is possible that it is not robust enough to survive the high-stringency purification procedure employed by Lessard et al. (2007).

From an epigenetic perspective, the findings in this paper may further our understanding of how different types of epigenetic processes are mechanistically linked. It is well known that SWI/SNF complexes associate with chromatin modifications, such as acetylated histones. Intriguingly, recent work has shown that the PHD finger domain of the nucleosome remodeling factor (NURF), another ATP-dependent chromatin remodeler, binds a specific histone methylation (Wysocka et al., 2006). If the PHD domains found in the novel BAF45 homologs also bind methylated histones, BAF45-containing SWI/SNF complexes will instantly provide biologically well-defined platforms to analyze the coordinated action of different epigenetic mechanisms. Analysis of histone modifications at gene loci identified by ChIP-based studies of BAF components may lead to a better mechanistic understanding of the underlying cohort of epigenetic mechanisms.

In the past few years, much attention has been devoted to the ever-growing body of known histone modifications involved in transcriptional control. This "histone code" (Jenuwein and Allis, 2001) has been suggested to direct diverse chromatin states in a manner dependent on the combination of modifications present at a gene locus. The work by Lessard et al. (2007) now suggests that combinatorially assembled chromatin remodelers could act in an equally complex manner to specify transcriptional activity.

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