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The role of ISWI chromatin remodeling complexes in brain development and neurodevelopmental disorders

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ABSTRACT

The mammalian *ISWI* (*Imitation Switch*) genes *SMARCA1* and *SMARCA5* encode the ATP-dependent chromatin remodeling proteins SNF2L and SNF2H. The ISWI proteins interact with BAZ (bromodomain adjacent to PHD zinc finger) domain containing proteins to generate eight distinct remodeling complexes. ISWI complex-mediated nucleosome positioning within genes and gene regulatory elements is proving important for the transition from a committed progenitor state to a differentiated cell state. Genetic studies have implicated the involvement of many ATP-dependent chromatin remodeling proteins in neurodevelopmental disorders (NDDs), including *SMARCA1*. Here we review the characterization of mice inactivated for *ISWI* and their interacting proteins, as it pertains to brain development and disease. A better understanding of chromatin dynamics during neural development is a prerequisite to understanding disease pathologies and the development of therapeutics for these complex disorders.

1. Introduction

Neurodevelopmental disorders (NDD) have an incidence of 2–3% of the population and present with a wide clinical spectrum and a high degree of genetic heterogeneity. They can be broadly subdivided into disorders with intellectual disability/developmental delay (ID/DD), autism spectrum disorders (ASD), and neuropsychiatric disorders; although cohort sequencing combined with phenomics is suggesting that these classifications may constitute arms within a larger disease spectrum with many clinical comorbidities and a significant convergence of genetic pathways (Kochinke et al., 2016). Indeed, the use of exome and whole genome sequencing on large patient cohorts has resulted in the identification of many new causative genes in the past decade for ID/DD and ASD, further facilitating the identification of genetic networks critical for cognition (de la Torre-Ubieta et al., 2016; Kleefstra et al., 2014; Kochinke et al., 2016; Ropers, 2006; van Bokhoven, 2011). A recent compilation has indicated that there are approximately 750 genes identified that contribute to intellectual disability upon mutation (Kochinke et al., 2016). While categorization of these genes has demonstrated an enrichment (~3-fold) in synaptic function, a similar increase was found for genes important for transcription and chromatin regulation (Fahrner and Bjornsson, 2014; Kleefstra et al., 2014). Other publications indicate that known ASD genes are similarly over-represented with functions in chromatin and transcriptional regulation, particularly ones expressed during the mid-fetal period when neocortical organization and neural circuitry is established (Ben-David and Shifman, 2013; Lasalle, 2013; van Bokhoven, 2011).

Transcriptional regulation is a complex process whose roots lie at DNA and the proteins that compact and organize it into what we refer to as chromatin. The basic unit of chromatin is a nucleosome composed of 146 base pairs of DNA wrapped around a histone octamer containing histones H2A, H2B, H3 and H4 (Luger et al., 1997). The dynamic modulation of the chromatin state throughout development regulates growth, differentiation and cell fate decisions, providing the cell-type specificity and diversity found within the brain. This process is accompanied by a transition from the open and accessible chromatin structure of progenitor cells to a more restricted and cell-type specific pattern of gene expression. This regulation is mediated by multiple mechanisms including DNA methylation (Smith and Meissner, 2013), post-translational histone modifications (Kouzarides, 2007), histone variant incorporation (Volle and Dalal, 2014), non-coding RNA mediated pathways (Fatica and Bozzoni, 2014), and ATP-dependent

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chromatin remodeling (Clapier et al., 2017). Moreover, these modifications are regulated by a large repertoire of enzymes, collectively called epigenetic regulators, that incorporate, remove, or recognize and interact with these specific chromatin modifications in order to regulate all nuclear processes (Xu et al., 2017). Their importance to brain development is recognized by a growing list of mutations associated with many different NDDs that are present in genes encoding these enzymes (reviewed in Fahrner and Bjornsson, 2014; Kleefstra et al., 2014). In this review, we focus on the role of the ISWI class of ATP-dependent chromatin remodeling proteins in brain development and recent evidence implicating their dysfunction in NDD pathologies.

2. ATP-dependent chromatin remodeling in forebrain development and disease

The prototypical ATP-dependent chromatin remodeling protein is SWI2/SNF2, which was identified in two independent yeast screens for mutants affecting mating type switching (SWI) and growth on sucrose (SNF), respectively (Neigeborn and Carlson, 1984; Peterson and Herskowitz, 1992). Molecular characterization determined that SWI2 and SNF2 are encoded by the same gene and that it interacted within a multiprotein complex (now called the SWI/SNF complex) to regulate transcription by nucleosome repositioning using the energy from ATP hydrolysis (Hargreaves and Crabtree, 2011). The *SWI2/SNF2* gene encodes a member of the DEAD/H domain ATPase superfamily and it has two mammalian homologs, *BRG1* and *BRM*. The mammalian proteins also interact with multiple proteins, called BRG1-associated factors (BAFs) that provide additional specificity through targeting and cell-specific expression patterns (Wang et al., 1996). Moreover, this superfamily can be further subdivided into 4 broad classes based on extended homology within the ATPase domain and the inclusion of additional chromatin interacting domains (Clapier and Cairns, 2009; Clapier et al., 2017; Hota and Bruneau, 2016; Narlikar et al., 2013). These include the SWI/SNF, ISWI, CHD, and INO80 families, as well as additional orphan members such as ATRX (Clapier et al., 2017). Similar to other classes of chromatin interacting proteins, members of the DNA-dependent chromatin remodeling family are critical for various aspects of brain development and are mutated in numerous NDDs (see Table 1), as indicated in the following examples.

The cerebral cortex is a six-layered laminar structure that develops from a single layer of neuroepithelial cells and a temporally regulated balance between progenitor proliferation and differentiation to produce neurons of each layer, followed by astrocytes and oligodendrocytes (as reviewed in Costa and Muller, 2014). Mouse inactivation studies have indicated that many chromatin remodeling complexes mediate progenitor proliferation and the transition to differentiation (MuhChyi et al., 2013). This was first described for *Atrx*, where deletion resulted in extensive loss of neural progenitors, small brains and early postnatal death (Berube et al., 2005). Further work showed that Atrx protein was critical for replication through heterochromatin and that loss of Atrx induced stalled and collapsed replication forks, DNA damage and reduction of the progenitor pool leading to poor production of upper layer cortical neurons (Huh et al., 2016). The human *ATRX* gene is mutated as the cause of the α-thalassemia mental retardation X-linked syndrome (ATR-X syndrome), a disorder encompassing severe ID, α-thalassemia and facial as well as urogenital abnormalities (Gibbons, 2006; Gibbons et al., 1995; Gibbons et al., 2008). Similarly, Brg1 was found to be critical for progenitor growth, and this was associated with the inclusion of specific subunits within the complex. The switch from proliferation to terminal differentiation was associated with a change from Brg/Brm interactions with Baf45a/53a subunits in progenitor cells to use of the Baf45b/Baf45c/Baf53b subunits in post-mitotic neurons (Lessard et al., 2007). In another study, competition between the Baf155 and Baf170 subunits was shown to mediate intermediate progenitor cell production and late born cortical neuron output (Tuoc et al., 2013). Indeed, mutations in the genes encoding BRG1 (*SMARCA4*[1]), BRM (*SMARCA2*), BAF47 (*SMARCB1*) and BAF57 (*SMARCE1*) subunits have all been implicated in overlapping ID disorders (Table 1). The CHD proteins Chd3, Chd4, and Chd5 also display differential expression that is critical for the function of NuRD chromatin remodeling complexes during cortical development. The deletion of *Chd4* alone resulted in microcephaly and defects in cortical lamination which were not seen in *Chd3* or *Chd5* mutants which are expressed at a later developmental stage than *Chd4* and were required for migration of newborn neurons (Nitarska et al., 2016). While *CHD3*, *4*, or *5* have not been implicated in ID yet, mutations in *CHD2*, *7*, and *8* are associated with NDDs (Fahrner and Bjornsson, 2014; Kleefstra et al., 2014). The Ino80 family is also implicated in NDDs by the identification of *SRCAP* mutations as the cause of Floating Harbor syndrome (Hood et al., 2012). Collectively, there is a strong link between chromatin remodeling and intellectual disorders and, as discussed below, the ISWI family has recently been added to this growing list.

3. ISWI protein family, interacting proteins, and biochemical properties

3.1. Structure of the ISWI protein family

The ISWI protein was originally purified from *Drosophila* as the ATPase motor of the nucleosome remodeling factor (NURF) complex (Tsukiyama et al., 1995; Tsukiyama and Wu, 1995). Mammals have two homologs of ISWI[2] SNF2L (encoded by the *SMARCA1* gene) and SNF2H (encoded by the *SMARCA5* gene), which have a high identity to each other (approximately 86%) and to their *D. melanogaster* and yeast homologs (78% and 65%, respectively) (Aihara et al., 1998; Lazzaro and Picketts, 2001). In humans, the two genes span ~80 kb comprising 24 exons, with *SMARCA5* on chromosome 4, and *SMARCA1* located on the X chromosome but also containing an extra small exon (exon 13) that functions to disrupt remodeling activity when incorporated (Aihara et al., 1998; Barak et al., 2004; Lazzaro and Picketts, 2001; Lazzaro et al., 2008). The ISWI remodelers contain two key domains (Fig. 1), an N-terminal catalytic ATPase domain which also mediates DNA interactions, and a C-terminal HAND-SANT-SLIDE (HSS) domain, responsible for extranucleosomal DNA binding as well as H4 tail interactions (Alkhatib and Landry, 2011; Clapier et al., 2001; Grune et al., 2003; Toto et al., 2014). Further specificity for ISWI remodeling is mediated by two additional autoregulatory motifs AutoN and NegC that lie adjacent to the ATPase domain (Clapier and Cairns, 2012). In mammals, SNF2H protein levels are abundant and widespread whereas SNF2L protein expression is more tissue restricted and often less abundant (Barak et al., 2004; Eckey et al., 2012; Lazzaro and Picketts, 2001; Ye et al., 2009).

3.2. ISWI-containing complexes

ISWI-containing chromatin remodeling complexes were identified in three different complexes from *Drosophila*: NURF, ACF (ATP-utilizing chromatin assembly and remodeling factor), and CHRAC (chromatin assembly complex) (Ito et al., 1997; Tsukiyama and Wu, 1995; Varga-Weisz et al., 1997). These ISWI complexes are conserved in other species (Barak et al., 2003; Bochar et al., 2000; Guschin et al., 2000; LeRoy et al., 2000; Poot et al., 2000; Tsukiyama et al., 1999), and five additional novel complexes have been identified in mammals, RSF (remodeling and spacing factor), NoRC (nucleolar remodeling complex), WICH (WSTF-ISWI chromatin remodeling complex), CERF (CECR2-

[1] SMARCA4 is an acronym for SWI/SNF-related, Matrix-associated, Actin-dependent Regulator of Chromatin, subfamily A, member 4. Additional subfamilies B-E also exist.

[2] Note that throughout the text, SMARCA1/SNF2L and SMARCA5/SNF2H are used to refer to the human or mammalian gene/protein. *Smarca1*/Snf2l and *Smarca5*/Snf2h is used specifically for mouse. *ISWI*/ISWI is used as a collective term when referring to both mammalian genes/proteins.