Mini-Symposium

Epigenetic Etiology of Intellectual Disability

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Intellectual disability (ID) is a prevailing neurodevelopmental condition associated with impaired cognitive and adaptive behaviors. Many chromatin-modifying enzymes and other epigenetic regulators have been genetically associated with ID disorders (IDDs). Here we review how alterations in the function of histone modifiers, chromatin remodelers, and methyl-DNA binding proteins contribute to neurodevelopmental defects and altered brain plasticity. We also discuss how progress in human genetics has led to the generation of mouse models that unveil the molecular etiology of ID, and outline the direction in which this field is moving to identify therapeutic strategies for IDDs. Importantly, because the chromatin regulators linked to IDDs often target common downstream genes and cellular processes, the impact of research in individual syndromes goes well beyond each syndrome and can also contribute to the understanding and therapy of other IDDs. Furthermore, the investigation of these disorders helps us to understand the role of chromatin regulators in brain development, plasticity, and gene expression, thereby answering fundamental questions in neurobiology.

Key words: **α-thalassemia mental retardation syndrome; Claes-Jensen syndrome; DNA methylation; histone posttranslational modification; Kleefstra syndrome; neuroepigenetics; Rett syndrome; Rubinstein-Taybi syndrome; X-linked intellectual disability**

Introduction

Intellectual disability (ID) disorders (IDDs) are characterized by impaired cognitive abilities, commonly defined by an IQ < 70, and severe deficits in the capability to adapt to the environment and social milieu. With a prevalence of 2%–3% worldwide, these neurodevelopmental disorders represent one of the biggest medical and social challenges in our society. The causes of IDDs are heterogeneous and include environmental factors, chromosomal aberrations, and single gene mutations. Human genetics and clinical research in the last decade have led to the identification of hundreds of genes responsible for these disorders. Notably, a large number of such genes encode for epigenetic regulators: by proteins that exert their function through genome-wide post-translational modification of histones, DNA base modifications and other covalent and noncovalent changes of the chromatin (Kramer and van Bokhoven, 2009). Indeed, of the 750 genes currently linked to ID and autism spectrum disorder (ASD) (Kochinke et al., 2016), at least 55 correspond to chromatin regulators (Kleefstra et al., 2014). Several of these ID-linked chromatin factors have been found to directly interact with one another in complexes that regulate chromatin structure at genes important for neurodevelopment and/or neuroplasticity (Kleefstra et al., 2014).

The development of the nervous system is a highly organized process that requires precise spatial and temporal regulation of gene expression programs involved in differentiation, maturation, and survival of neurons, but also the repression of alternative cell fates and restriction of cell type-specific gene expression (Lilja et al., 2013). Such dynamic expression patterns are sustained by extensive changes in the epigenome. It is therefore not surprising that the mutation of genes encoding chromatin modifiers and readers lead to severe neurodevelopmental defects (Kleefstra et al., 2014; Bjornsson, 2015). However, the mechanisms by which these mutations cause IDDs and ASD are still largely unknown. Given the overall prevalence of IDDs and their large medical and social costs, it has become increasingly important to understand their molecular etiology.

Emerging evidence suggests that epigenetic regulation of gene expression plays a crucial role in processing experience-driven synaptic activity required for long-lasting modifications of neural circuits and neuronal properties in the adult brain (Gupta et al., 2010; Baker-Andresen et al., 2013; Sweatt, 2016). Neurons in memory-related networks respond with patterns of activity that

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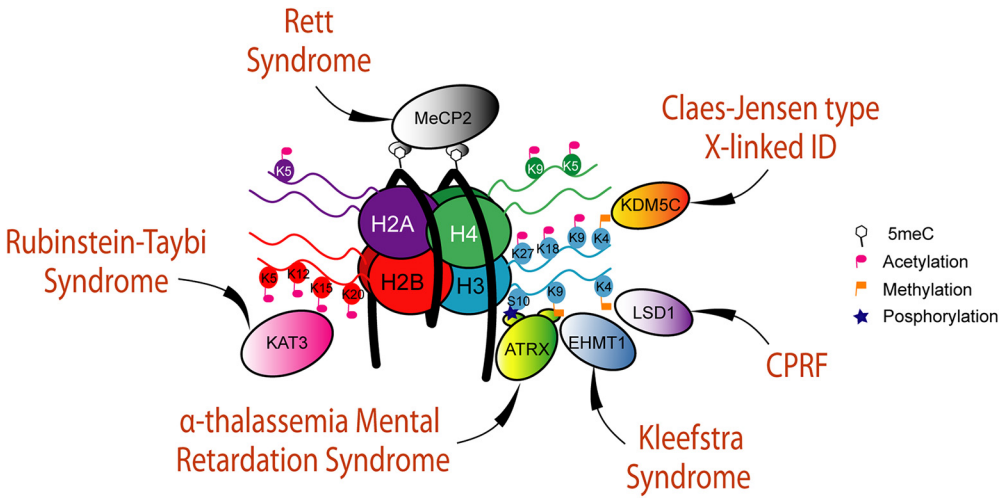

Figure 1. Six important chromatin-related IDDs. The six IDDs discussed in this review are caused by mutations in different chromatin regulators that converge in related molecular processes. In the scheme, these chromatin factors are depicted in the proximity of their respective chromatin targets. All the acetylation marks presented in the scheme are possible substrates of KAT3 proteins. K, lysine; 5meC, 5-methylcytosine.

relay and encode information in the form of alterations in synaptic strength that modify neuronal connectivity and contribute to cognitive processes, such as learning and memory. Recent findings illustrate the dynamic nature of chromatin marks in mature neurons, demonstrating that DNA methylation and post-translational modifications of histone proteins, such as histone phosphorylation, acetylation, and methylation, actively contribute to the activity-dependent modulation of neuronal networks (Heyward and Sweatt, 2015; Bonnaud et al., 2016). Such changes have also been shown to correlate with memory formation and consolidation (Gupta et al., 2010; Lubin et al., 2011; Mews et al., 2017). As a result, during the last decade, there has been great interest in deciphering the role of chromatin modifications in neuronal plasticity processes in the brain (Zocchi and Sassone-Corsi, 2010; Sweatt, 2013; Lopez-Atalaya and Barco, 2014).

In the next sections, we review recent progress in understanding the molecular etiology of six genetic syndromes associated with ID. These IDDs are caused by mutations in chromatin regulators, ranging from chromatin remodelers to histone-modifying enzymes (Fig. 1). Much of this progress stems from the generation and characterization of appropriate mouse models for these conditions. The study of these genetic models allows us to determine the functions of ID-linked chromatin factors throughout life, dissect the cognitive and neurological defects based on their developmental or adult origin, and identify potentially druggable targets for therapy (Fig. 2). The precise understanding of the chromatin-based etiology of these IDDs will eventually help to cure or ameliorate disease phenotypes.

ATRX and α-thalassemia mental retardation syndrome

Mutations in the *ATRX* gene cause α-thalassemia mental retardation syndrome (ATR-X; OMIM #301040). ATR-X syndrome patients present ID, microcephaly, dysmyelination, seizures, autistic-like behavior, microcephaly, α-thalassemia, dysmorphic faces, short stature, skeletal defects, and urogenital abnormalities (Gibbons et al., 2008). The disease is X-linked and confined to males, whereas female carriers display highly skewed X chromosome inactivation toward the mutant allele and are usually phenotypically normal. All inherited *ATRX* mutations identified to date are hypomorphic, suggesting that null mutations are lethal.

ATRX is a chromatin-remodeling enzyme that uses ATP hydrolysis to move nucleosomes on the DNA template or promote nucleosome exchange (Xue et al., 2003). The protein contains a C-terminal Swi2/Snf2-type ATPase/helicase domain and an N-terminal histone reader domain for the histone post-translational modifications H3K9me3/H3K4me0 and H3K9me3/H3S10P (Picketts et al., 1996; Iwase et al., 2011; Noh et al., 2015). ATRX and the histone chaperone DAXX form a complex that deposits the histone variant H3.3 at pericentric heterochromatin and telomeres (Goldberg et al., 2010; Lewis et al., 2010; Wong et al., 2010). ATRX was also demonstrated to regulate gene expression in the mouse CNS. For a subset of genes, ATRX has a positive effect on transcription, by aiding transcriptional elongation and RNA polymerase II passage through G-rich regions and histone H3.3 incorporation in the gene body. One such gene is *Ngln4X*, a known autism-related gene (Levy et al., 2008, 2015). Conversely, ATRX has suppressive effects at imprinted genes in the neonatal brain by promoting long-range chromatin interactions mediated by CCCTC-binding factor (CTCF) and cohesin (Kernohan et al., 2010; Kernohan et al., 2014).

Multiple conditional *Atrx* knock-out (KO) mouse models have been developed, allowing for temporal and spatial control over gene inactivation. Conditional deletion of *Atrx* in the developing forebrain results in extensive neuronal apoptosis, microcephaly, and reduced postnatal survival (Bérubé et al., 2005; Seah et al., 2008). Loss of ATRX in this mouse model causes DNA replication stress in neural progenitor cells, resulting in increased DNA damage and p53 activation (Watson et al., 2013). Overall, the identified defects in the ATRX-null developing brain are intimately linked to cell proliferation and might provide an explanation for the microcephaly phenotype observed in a subset of ATR-X syndrome patients. However, whether loss of ATRX strictly in postmitotic cells of the CNS leads to cognitive deficits had not yet been investigated. To specifically interrogate the importance of ATRX in learning and memory, N.G.B. and colleagues mated *Atrx* floxed mice to mice expressing Cre recombinase in postmitotic forebrain pyramidal neurons under the control of the CaMKII gene promoter (Casanova et al., 2001). These conditional KOs (referred to as *Atrx*-CaMKIICre) survived to adulthood and did not display microcephaly. Normal activity levels were observed in the open field test and in the Y-maze test for working memory. However, hippocampal-dependent spatial

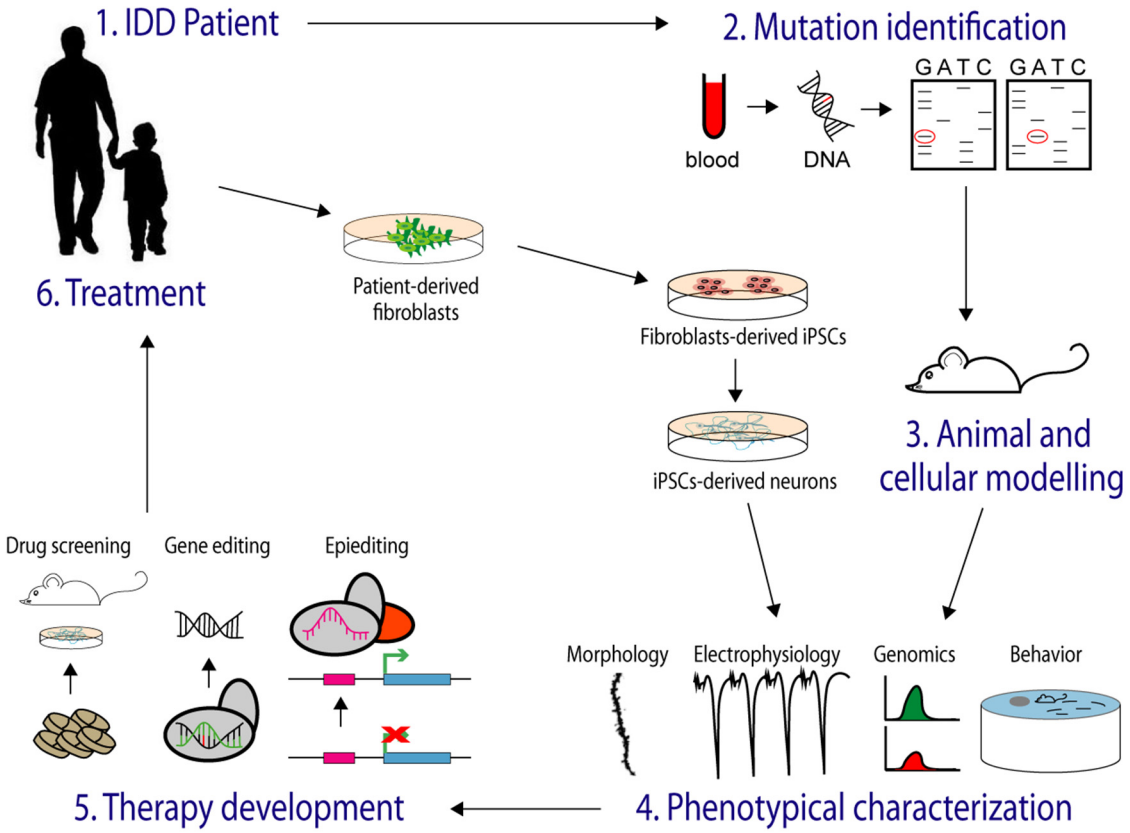

Figure 2. From identification of ID-related genes to therapy. Schematic representation of the long, and still unaccomplished, path that goes from the identification of the IDD-causing mutation to therapy. After identification of the mutation, the generation and characterization of animal model reproducing the same genetic defects enable the description of the molecular mechanisms underlying the disease and the assessment of therapies. Complementing the studies of animal models, iPSCs derived from the patients can be also used to investigate pathoetiology and assess possible therapies. Therapeutic strategies that provide positive results in the cellular and animal models, such as drug treatment and gene editing or epi-editing, will be eventually evaluated in clinical trials.

learning and memory were impaired in *Atrx*-CaMKIICre mice when tested in different memory task (unpublished results). These results would suggest that ATRX in differentiated neurons is required for normal spatial learning and memory. The *Atrx*-CaMKIICre mice, therefore, represent an ideal model to further investigate the molecular and cellular underpinnings of cognitive defects caused by *ATRX* mutations and could be used in preclinical trials.

MeCP2 and Rett syndrome

Rett syndrome (RTT; OMIM #312750) is an X-linked neurological disorder characterized by regressive loss of neurodevelopmental milestones and acquired motor and language skills. It represents one of the most common causes of ID among young girls (Chahrour and Zoghbi, 2007). Approximately 95% of RTT cases are caused by mutations in the X-linked gene encoding methyl-CpG binding protein 2 (*MECP2*) (Amir et al., 1999). MeCP2 is a ubiquitously expressed nuclear protein that binds to methylated DNA and is thought to mediate transcriptional repression or activation, but the identification bona fide transcriptional targets of MeCP2 has been challenging because hundreds of genes have been found to be slightly altered in MeCP2 mutant cells, and the subtlety of these gene expression changes varies among different studies (Lyst and Bird, 2015). In addition, the apparently ubiquitous distribution of MeCP2 across the genome remains perplexing and further complicates the identification of direct targets and, therefore, the understanding of the molecular etiology of RTT (Skene et al., 2010; Guo et al., 2014; Chen et al., 2015; Gabel et al., 2015; Rube et al., 2016).

The complexity in identifying MeCP2 transcriptional targets is further confounded by the heterogeneity of cellular transcriptomes in the mammalian brain. The brain comprises a myriad of intermixed cell types that differ in morphology, function, and electrophysiological properties. Recent studies have reported that cellular identity and diversity are established and maintained by distinctive transcriptional and epigenomic programs, including the cell type-specific genomic distributions of methylated and hydroxymethylated cytosines (Lister et al., 2013; Mo et al., 2015). Thus, MeCP2, as a methyl-cytosine binding protein, may exhibit distinctive binding patterns across different cell types and thus regulate different genes in different types of cells. Overcoming cellular heterogeneity, particularly for neurons in the brain, would be an imperative first step to identify the target genes of MeCP2. Other than cellular heterogeneity, the cells themselves comprise a heterogeneous pool of nuclear and cytoplasmic RNAs at different stages of synthesis, processing, transport, translation, and degradation (Maniatis and Reed, 2002). The bulk of protein-coding mRNAs are enriched in the cytoplasm and subjected to extensive post-transcriptional regulation. In contrast, protein-coding nuclear RNA transcripts lie upstream of most post-transcriptional mechanisms and are thus ideal for studying transcriptional dynamics in the cell (Buxbaum et al., 2015). Therefore, whole-cell mRNA from individual brain regions reflects composite profiles that can obscure cell type-specific expression

changes due to the loss of MeCP2 and impede appropriate assessment of MeCP2 function at the transcriptional level. Finally, RTT is an X-linked dominant disorder in heterozygous females, but the majority of RTT research has focused on hemizygous male mouse models because of the limits imposed by the mosaic expression of MeCP2 in females as a consequence of random X-chromosome inactivation (Lyst and Bird, 2015). Thus, overcoming X-linked cellular heterogeneity and distinguishing cell autonomous from non–cell-autonomous effects upon MeCP2 loss in ~50% of the cells are particularly pertinent for RTT research.

To address the confounding effects of cellular heterogeneity at multiple levels, Z.Z. and colleagues have engineered genetically modified mice whereby MeCP2 is labeled with biotin in a Cre-dependent manner. To understand the molecular impact of RTT-associated mutations on cell type-specific gene expression *in vivo*, they have also developed tagged knock-in mice bearing one of two frequent and molecularly distinct RTT missense mutations, T158M and R106W. When combined with FACS, this strategy effectively circumvents the cellular heterogeneity of the mouse brain and allows for the isolation of neuronal nuclei from cell types of interest (Johnson et al., 2017). First, they examined nuclear RNAs to assess the primary effects of RTT mutations on transcriptional activity. Upon systematic profiling of nuclear transcriptomes from distinct neuronal cell types in MeCP2 wild-type, T158M, and R106W male and female mice, the laboratory of Z.Z. identified transcriptional features that are specific to each cell type and correlate with the severity of MeCP2 mutation. They found that lowly expressed, cell type-enriched genes are preferentially disrupted by MeCP2 mutations. Upregulated genes demarcate functional categories related to synapse morphology and function, whereas downregulated genes are enriched for functions related to transcription and chromatin regulation. Furthermore, these analyses uncovered that genome-wide transcriptional changes in the nucleus are opposed by post-transcriptional compensation of RNAs in the whole cell in a gene length-dependent manner. This approach effectively circumvented cellular heterogeneity associated with X-chromosome inactivation in heterozygous females through the transcriptional profiling of neighboring wild-type and mutant neurons, thereby discerning cell autonomous from non–cell-autonomous transcriptional effects. The comprehensive analysis across different neuronal settings in an allelic series of RTT mouse models has led to the proposal of a contextualized model by which cell-autonomous and non–cell-autonomous transcriptional changes in different cell types contribute to the molecular severity of neuronal deficits in RTT, providing new directions for therapeutic development (Johnson et al., 2017).

EHMT1 and Kleefstra syndrome (KS)

KS (OMIM#610253) is characterized by moderate to severe ID, autism, microcephaly, and dysmorphic features (Kleefstra et al., 2009). Most individuals have severe expressive speech delay with hardly any speech development. About a decade ago, KS was identified as a neurodevelopmental disorder caused either by a submicroscopic 9q34.3 deletion or an intragenic euchromatin histone methyltransferase 1 (*EHMT1*) mutations, leading to haploinsufficiency of this gene (Kleefstra et al., 2006). More recently, mutations in *EHMT1* have also been associated with isolated idiopathic ASD (Bock et al., 2016) and schizophrenia (Talkowski et al., 2012).

EHMT1 cooperates with its mammalian paralog EHMT2/G9a and exhibits enzymatic activity in histone 3 lysine 9 monomethylation and dimethylation (H3K9me1 and H3K9me2), which is known to promote heterochromatization and gene repression (Tachibana et al., 2008). During development, the EHMT1/EHMT2 repressive complex is important for cell differentiation (Fiszbein et al., 2016). This is exemplified by the fact that both *Ehmt1*$^{-/-}$ and *Ehmt2*$^{-/-}$ mice show early embryonic lethality, whereas heterozygous *Ehmt1*$^{-/+}$ and *Ehmt2*$^{-/+}$ mice are viable and fertile (Tachibana et al., 2008). Loss of EHMT1 function in mice and *Drosophila* reduces H3K9 methylation and lead to learning and memory impairments (Schaefer et al., 2009; Maze et al., 2010; Kramer et al., 2011). Initial studies reported that *Ehmt1*$^{-/+}$ mice show cranial abnormalities (Balemans et al., 2014), hypoactivity, reduced exploration, increased anxiety, and aberrant social behavior compared with their wild-type littermates (Balemans et al., 2010). This recapitulates the autistic-like features and hypoactivity seen in KS patients (Vermeulen et al., 2017). In follow-up studies, the same group showed that *Ehmt1*$^{-/+}$ mice were also impaired in fear extinction learning as well as novel and spatial object recognition (Balemans et al., 2013). More recently, they tested whether *Ehmt1*$^{-/+}$ and wild-type mice differ in several cognitive tests in a touchscreen-equipped operant chamber. Surprisingly, *Ehmt1*$^{-/+}$ mice were mostly unaffected except in the location discrimination test of pattern separation in which they outperformed their wild-type littermates. In line with this, they detected increased cell proliferation in the subgranular zone of the dentate gyrus (Benevento et al., 2017). At the cellular level, a reduction in dendritic branching and spine density, and an increase in paired-pulse ratio indicative of a presynaptic deficit were observed. However, there was no change in long-term plasticity, a Hebbian form of synaptic plasticity (Balemans et al., 2013).

Contrary to Hebbian synapse-specific mechanisms, homeostatic synaptic plasticity acts to maintain a fine-tuning of overall neuronal excitability by monitoring and scaling globally all synapses (Turrigiano, 2012). Intriguingly, EHMT1 is required for homeostatic synaptic scaling (Benevento et al., 2016). H3K9me2 levels are bidirectionally altered in response to enduring stimulatory or inhibitory neuronal network activity. Specifically, EHMT1 is critical for the repression of *Bdnf*, which encodes for a neurotrophin critically involved in homeostatic plasticity (Desai et al., 1999), during synaptic scaling up. As a consequence, loss of EHMT1 prevented synaptic scaling up *in vitro* and *in vivo*. EHMT1, through the regulation of H3K9me2 levels, functions as a permissive tag to recruit the machinery that adds more stable repressive marks, such as H3K9me3, H3K27me3, and DNA methylation (Mozzetta et al., 2014; Rothbart and Strahl, 2014; Yearim et al., 2015). According to this view, H3K9me2 levels correlate with the activity state of a neuronal network and could serve as a general mechanism for poising a state in the genome in a "ready to repress" mode. This hypothesis is in line with data showing that neurons lacking EHMT1 (and having reduced H3K9me2) show few and modest changes in gene expression under basal conditions but were unable to respond to changes in activity through the repression of genes involved in synaptic scaling (including *Bdnf*). H3K9me2 can thus be viewed as a dynamic regulatory histone mark in euchromatic regions, and not merely a mark for heterochromatin.

In conclusion, the role of EHMT1 in homeostatic plasticity is in line with recent reports supporting the dynamic nature of histone methylation in activity-dependent gene transcription and neuronal plasticity (Heller et al., 2014; Rusconi et al., 2016; Webb et al., 2017). Impaired chromatin regulation due to mutations in *EHMT1* may thus lead to a pathophysiological change in neuronal excitability, resulting in aberrant network activity and seizures, which are present in KS patients. The laboratory data of

N.N.K. measuring neuronal network development *in vitro* do indeed show that loss of Ehmt1 in rat cortical neurons during development leads to neuronal network miswiring (Martens et al., 2016).

LSD1, a novel chromatin regulator associated with ID

Very recently, a novel and still unnamed form of neurodevelopmental disorder featuring distinctive traits of facial dysmorphisms and associated to ID has been linked to point mutations in the *Lysine Specific Demethylase 1* (*LSD1*) gene (Rauch et al., 2012; Tunovic et al., 2014; Chong et al., 2016) (the disorder is referred as cleft palate, psychomotor retardation and distinctive facial features, or CPRF, in the OMIM database; OMIM #616728). This new autosomal dominant pathology, displaying partial phenotypic overlap with Kabuki syndrome, but representing a distinct condition, still needs to be fully characterized at the clinical point of view. Three missense mutations in the *LSD1* gene have been described so far, all entailing single amino acid substitution in the catalytic domain of this chromatin modifier (Pilotto et al., 2016).

LSD1 is a flavin-dependent enzyme that erases monomethyl and dimethyl groups from histone H3 Lysine 4 (H3K4me1/2) (Shi et al., 2004). H3K4me1/2 represent "permissive" histone marks, which is why their removal can contribute to transcription repression. Indeed, LSD1 is an epigenetic transcriptional corepressor that participates in a macromolecular complex, including CoREST and histone deacetylases HDAC1/2 (Shi et al., 2005). The three *de novo LSD1* point mutations associated with this new form of ID (E403K; D580G; Y785H) partially affect the ability of LSD1 to demethylate H3K4 but do not modify LSD1 binding to other molecular partners, including transcription factors and cofactors, such as CoREST and HDAC1/2 (Pilotto et al., 2016). Relevantly, while LSD1 KOs are embryonic lethal, heterozygous deletion of *LSD1* does not trigger pathologic traits in rodent models (Wang et al., 2007), suggesting a possible dominant negative pathogenic function of the annotated human mutations, competing with wild-type allele-derived LSD1 for complex assembly.

The importance of LSD1 as a regulator of neuronal physiology was first unveiled with the discovery of a neurospecific splicing isoform, referred to as neuroLSD1, involved in neuronal maturation (Zibetti et al., 2010). NeuroLSD1 differs from LSD1 by the inclusion of an additional exon (the microexon E8a) at the mRNA level, encoding 4 amino acids that form a protein loop located in the vicinity of the substrate entry site (Zibetti et al., 2010). The neuroLSD1 isoform does not substitute LSD1 in neurons. Rather, both isoforms take part in a neuronal-restricted mechanism of fine transcriptional modulation (Toffolo et al., 2014; Rusconi et al., 2016). Interestingly, whereas LSD1 can exert a repressive action toward transcription, neuroLSD1, which shares the same gene targets, is not able to accomplish this molecular task *in vivo* (Laurent et al., 2015; Wang et al., 2015). In this frame, it has been proposed that neuroLSD1 represents a dominant negative isoform, competing with LSD1 and modulating LSD1-related repressive strength in accordance to their relative abundance in neurons (Rusconi et al., 2016). Notably, the three *LSD1* mutations associated with ID can modify the function of both LSD1 and neuroLSD1 because they map within exons that are shared by the two isoforms (Pilotto et al., 2016).

The study of the physiological role of LSD1 in controlling neuronal plasticity began a few years ago, with two papers describing LSD1/neuroLSD1-mediated modulation of activity-dependent transcription and behavior in mouse models. Because LSD1 KOs are embryonic lethal, the neuroLSD1^{KO} mouse model represents a very important tool to investigate the relevance of LSD1 and neuroLSD1 in neurons and their specific role in ID. Several independent observations provide a perspective on how *LSD1* mutations can affect cognition. Mice with ablation of the neuroLSD1-specific microexon E8a, but preserved LSD1 expression, show learning-related disabilities and deficits in stress-related plasticity (Wang et al., 2015; Rusconi et al., 2016). In particular, Rosenfeld's group reported that neuroLSD1^{KO} display significant memory impairment (Wang et al., 2015), whereas Battaglioli's group showed that neuroLSD1 haploinsufficiency translates in an aberrant acquisition of stress-related plasticity, leading to decreased anxiety-like behavior in neuroLSD1^{HET} (Rusconi et al., 2016). Remarkably, convergent evidence in both models indicates that activity-dependent transcription of plasticity-related immediate early genes (IEGs), such as *Fos*, *Egr1*, *Npas4*, *Nr4a1*, and *Arc,* is impaired upon neuroLSD1 deletion, which indicates that either neuroLSD1 acts as positive transcriptional regulator for these genes (Wang et al., 2015) or that its ablation results in increased level of LSD1 (Rusconi et al., 2016). Both the formation of new memories and the acquisition of a correct emotional phenotype in terms of anxiety (i.e., the ability to turn stressful experiences into protective warnings) require an efficient experience-driven transactivation of plasticity genes (Rusconi et al., 2016) modulating structural plasticity in the hippocampus, prefrontal cortex, and amygdala in a highly coordinated fashion (Felix-Ortiz et al., 2013; Calhoon and Tye, 2015). Although the origin of IEG transcriptional impairment caused by loss of neuroLSD1 still needs to be fully understood, the dual LSD1/neuroLSD1 system is clearly implicated in the process of activity-dependent transcription in the brain. A formal demonstration that mutations in the *LSD1* gene associated to ID alter IEG transcription in neurons is still lacking, but the implication of LSD1 in cognition-related processes via direct control of neuronal plasticity transcriptional programs further envisages aberrant IEG transcriptional modulation in LSD1-related new form of ID.

KDM5C and Claes-Jensen Type X-Linked ID

Mutations into the *KDM5C* gene account for at least 2% of X-linked IDs, which is more frequent than most of the X-linked ID genes. The KDM5C-associated cases are referred to as mental retardation, X-linked, syndromic, Claes-Jensen type (MRXSCJ; OMIM #300534). Patients with these mutations, in addition to ID, often show epilepsy and aggressive behavior.

KDM5C encodes for the lysine (K)-specific demethylase 5C, an eraser enzyme for dimethylated and trimethylated histone H3 lysine 4 (H3K4me2/3) (Iwase et al., 2007; Tahiliani et al., 2007). ID-associated missense mutations decrease demethylase activity, suggesting that mutations lead to loss of function. Therefore, this disorder, like the one discussed in the previous section, seems to be also caused by altered H3K4 methylation (although the specific forms affected vary since LSD1 removes monomethylations and dimethylations instead of dimethylations and trimethylations). Iwase et al. (2016) have recently shown that *Kdm5c*-KO mice closely recapitulate the behavioral abnormalities of human patients, including impaired learning and augmented aggression. While gross morphological abnormalities were not noted in the *Kdm5c*-KO brains, a decreased density of dendritic spines was found in pyramidal neurons of the amygdala. RNA-seq analysis in these animals revealed brain region-specific expression changes in hundreds of genes. In addition, the levels of H3K4me2 and H3K4me3 were increased at the promoters of genes, some of which are relevant to neuronal maturation, in cultured *Kdm5c*-deficient neurons (Iwase et al., 2016). *Kdm5c*-KO mice represent

the first mouse model of ID caused by defective erasure of histone methylation, thereby providing a link between the dynamic regulation of histone methylation and cognitive development.

More recently, A.B. and colleagues developed and analyzed inducible and forebrain-restricted cKOs for *Kdm5c* (referred to as *Kdm5c*-ifKOs). In these mice, KDM5C was specifically deleted in excitatory forebrain neurons of adult mice. In contrast to the severe phenotype and broad behavioral alterations of KOs, *Kdm5c*-ifKOs displayed highly specific spatial memory defects (Scandaglia et al., 2017). These results suggest that the most severe impairments observed in *Kdm5c*-KOs originate during development. It will be important to test in future experiments whether this is also the case after deleting *Kdm5c* in other cell types, including inhibitory neurons and astrocytes, as KDM5C is also expressed in those cells (Iwase et al., 2016). Parallel genomic screens in *Kdm5c*-KOs and ifKOs enabled a fine dissection of the genomic actions of Kdm5c during neuronal differentiation, maturation, and maintenance. These analyses indicated that Kdm5c functions as an epigenetic repressor of germline genes during early development, as a fine-tuner of activity-regulated enhancers during neuronal maturation, and it prevents illegitimate activation of non-neuronal and cryptic promoters in mature neurons (Scandaglia et al., 2017).

Before their removal by KDM5C, the H3K4me marks are placed by a group of lysine methyltransferases (KMTs) with specificity for this residue (H3K4me writer enzymes). The human genome encodes seven H3K4me writer genes that are ubiquitously expressed; however, the functional relationship between KDM5C and the H3K4me writer enzymes is not known (Vallianatos and Iwase, 2015; Garay et al., 2016). How does the balance between writers and erasers of histone methylation influence cognitive development? Do one or more specific H3K4me writer enzymes mediate the abnormal brain development caused by the loss of KDM5C? Answering these questions will open the possibility that inhibition of those enzymes compensates for the loss of KDM5C. Identifying H3K4me writer enzymes that counteracts with KDM5C may thereby provide a specific drug target for MRXSCJ.

Moreover, it is possible that the same drug could be also used to ameliorate the form of ID associated with LSD1 deficiency. As aforementioned, KDM5C and LSD1 act on the same target (H3K4), and both suppress transcriptional enhancers of actively transcribed genes and can be found in the same protein complex (Whyte et al., 2012; Shen et al., 2016; Agarwal et al., 2017). Furthermore, similar to the neuroLSD1-deficient neurons, *Kdm5c*-KO neurons display aberrant expression of some neuronal activity-dependent genes (Iwase et al., 2016; Scandaglia et al., 2017). Further research is warrant to test whether the cleft palate, psychomotor retardation, and distinctive facial features (*LSD1* deficiency) and MRXSCJ (*KDM5C* deficiency) can be grouped as a clinical condition associated with impaired erasure of H3K4me, and similar amelioration strategies may apply to this group of IDD in the future.

KAT3 proteins and the Rubinstein-Taybi syndrome (RSTS)

RSTS is a sporadic autosomal dominant disorder caused by mutation in the genes *CREBBP* (RSTS1; OMIM #180849) or *EP300* (RSTS2; OMIM #613684), which account for ~70% and 5% of the cases, respectively (Negri et al., 2015; Rusconi et al., 2015; Spena et al., 2015). The syndrome is characterized by mental impairment of variable severity and a wide spectrum of congenital anomalies, being usually diagnosed based on the concurrence of ID and broad thumbs and toes (Rubinstein and Taybi, 1963).

The genes involved in RSTS encode for two very large (>250 kDa) paralog proteins belonging to the lysine acetyltransferase (KAT) 3 family: CBP (*aka* KAT3a) and p300 (*aka* KAT3b). Both proteins are known to act as a molecular bridge between different transcription factors and the RNA polII complex, and as molecular scaffolds that bring a variety of enzymatic activities to the promoter. In addition, their KAT domains catalyze the transfer of acetyl groups to lysine residues in the histones tails (likely affecting chromatin accessibility) and in numerous nonhistone substrates, including transcription factors, components of the RNA pol II complex and other chromatin regulators (Valor et al., 2013; Lopez-Atalaya et al., 2014). Both proteins display widespread occupancy of transcriptional regulatory regions, including many putative enhancers (Wang et al., 2009). Although the function of KAT3 proteins has been extensively studied, downstream events of CBP and p300 deficiencies responsible for neurodevelopmental and cognitive deficits in RSTS patients remain obscure. Outstanding questions still under investigation are the unique and redundant roles of these two proteins regulating neuronal chromatin acetylation and their distinct genomic targets during the development of the nervous system and in neuronal plasticity processes in the adult brain.

The use of animal models has demonstrated that CBP and p300 are expressed in almost identical patterns in the mouse embryo, but rather than compensate for each other, both factors are required for embryonic development and viability (Valor et al., 2013). As a result, both the CBP and p300 KOs display defects in neural tube closure and cell proliferation and are early embryonically lethal (Yao et al., 1998; Tanaka et al., 2000), which makes it difficult to ascribe the developmental abnormalities to deregulation of specific genes. This limitation has been circumvented by the investigation of conditional and inducible KOs, focal gene ablation using viral vectors, and tissue-restricted expression of dominant negative transgenes. Despite significant differences across KAT3-deficient strains and laboratories, these approaches have shown that some cognitive impairments associated with CBP deficiency are not due to developmental defects but result from the continuous requirement of CBP and p300 activities throughout life (Korzus et al., 2004; Chen et al., 2010; Barrett et al., 2011; Valor et al., 2011; compare Lopez-Atalaya et al., 2014).

The A.B. laboratory and others have found that CBP deficiency causes severe deacetylation of specific lysine residues at the histone tails in the brain of mouse models (Alarcón et al., 2004; Chen et al., 2010; Barrett et al., 2011; Valor et al., 2011) and in cell lines derived from patients (Lopez-Atalaya et al., 2012). The use of next-generation sequencing-based techniques (Telese et al., 2013; Maze et al., 2014) now provides the ideal conditions for establishing a detailed and multilayered map of these alterations at a genomic scale. These acetylation deficits can have a major role in the etiology of ID because a number of studies have shown that histone acetylation correlates with memory formation in diverse paradigms, pinpointing a role for this process in cognition (Gräff and Tsai, 2013). Supporting this view, inhibitors of Classes I and II histone deacetylases (HDACi), a heterogeneous group of compounds that increase histone acetylation levels, have been found to ameliorate cognitive deficits in RSTS mouse models (Alarcón et al., 2004; Korzus et al., 2004; Stefanko et al., 2009), although there are also some inconsistencies between studies that still need to be clarified (Chen et al., 2010; Barrett et al., 2011). These studies identified HDACi as promising drugs to treat RSTS, a hypothesis that is currently been tested in clinical trials (https://clinicaltrials.gov/ct2/show/NCT01619644). In addition, to pharmacological approaches, recent experiments using the

Clustered Regularly Interspaced Short Palindromic Repeats system have opened a new avenue for therapy, although its application in clinical settings seems to be still far away. By directing the KAT activity of p300 to specific loci, Hilton et al. (2015) demonstrated that it is possible to manipulate the acetylation status of a given loci with unprecedented precision. Therefore, upon identifying hypoacetylated loci downstream of CBP/p300 loss involved in neuropathology, the novel tools for epi-editing (Konermann et al., 2013) could be used for correcting these lysine acetylation defects.

Conclusion

Eradicating rare congenital IDDs caused by *de novo* mutations will require detailed knowledge of their etiology and pathogenesis. The progress brought by studies in animal models, such as those outlined above, together with the availability and characterization of neurons differentiated from induced pluripotent stem cells (iPSC)-derived from patients and the precise description of abnormal chromatin profiles in the different models, should lead to the identification and evaluation of novel drugs, and the implementation of innovative therapeutic strategies based on genetic or epigenetic editing (Fig. 2). However, before reaching that goal, many challenges toward the treatment of IDDs will need to be overcome. Therapeutic time window is one such challenge. As described earlier, adult-neuron-specific KO resulted in cognitive deficits in the model of ATR-X syndrome, RSTS, and MRXSCJ (although deficits were clearly milder than in conventional KOs). The pioneering study by Guy et al. (2007) revealed that reexpression MeCP2 after onset of the symptoms in Mecp2-null mice alleviated motor and cognitive regression, indicated that ID-related phenotypes can be reversed in adult state. These observations point to importance of determining developmental time windows, in which a given chromatin regulator is required and/or sufficient for normal brain development and function in gene-by-gene basis. As discussed in the MeCP2 section, another forthcoming challenge is the ubiquitous presence of chromatin regulators and heterogeneous impacts of their malfunction in different cell types. The presence of both cell autonomous and non–cell-autonomous impacts of deficient chromatin regulation represents perhaps greater hurdle for rationalized treatments. Harnessing state-of-the-art genetics/genomics and cell biology tools combined with mouse models will likely help us to understand the complexity of IDD and ASD pathophysiology.

As clearly illustrated in the examples described here, the development of treatment strategy for IDDs will greatly benefit from discoveries in the molecular biology/biochemistry of individual syndromes. For example, the activity-dependent transcriptional response appears to be a key target for future drug discovery given that different chromatin regulators, such as CBP/p300 (Visel et al., 2009), KDM5C (Shen et al., 2016; Scandaglia et al., 2017), and LSD1 (Whyte et al., 2012), all regulate transcriptional enhancers positively or negatively. The ATR-X and Rett syndromes may also share phenotypic commonality and mechanistic root because ATRX fails to locate at heterochromatin in MeCP-null cells (Nan et al., 2007), indicating the roles of MeCP2 in ATRX localization within heterochromatin. Therefore, they may be treated with the same strategy. These discoveries may also shed light on the etiology of other neuropsychiatric conditions, such as autism and schizophrenia, because the proteins under study play important roles in the regulation of gene expression programs that govern many aspects of brain development and plasticity. Therefore, in addition to clinical insights, the investigation of these genetic disorders can unveil fundamental mechanisms by which chromatin sculpts the complex neuronal networks underlying cognition.

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