Yin–yang actions of histone methylation regulatory complexes in the brain

Dysregulation of histone methylation has emerged as a major driver of neurodevelopmental disorders including intellectual disabilities and autism spectrum disorders. Histone methyl writer and eraser enzymes generally act within multisubunit complexes rather than in isolation. However, it remains largely elusive how such complexes cooperate to achieve the precise spatiotemporal gene expression in the developing brain. Histone H3K4 methylation (H3K4me) is a chromatin signature associated with active gene-regulatory elements. We review a body of literature that supports a model in which the RAI1-containing H3K4me writer complex counterbalances the LSD1-containing H3K4me eraser complex to ensure normal brain development. This model predicts H3K4me as the nexus of previously unrelated neurodevelopmental disorders.

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The brain is the central organ that allows higher organisms to adapt to an ever-changing environment. At the cellular level, adaptation to a new environment requires chromatin, which integrates environmental information and generates transcriptional responses. The basic unit of chromatin is the nucleosome in which DNA wraps around histone octamers consisting of histones H2A, H2B, H3 and H4. Environmental inputs culminate in chromatin modifications, such as DNA base modifications and post-translational modifications on histones, which in turn influence the transcriptional activity of genes [1,2].

A variety of chromatin modifications act in concert to control gene expression. While cytosine methylation at CpG dinucleotides is the classic DNA modification, which decorates silent gene promoters, recent discoveries of novel DNA modifications, such as cyto-

sine hydroxymethylation, non-CpG cytosine methylation and adenine methylation, have expanded the horizon of chromatin regulation (reviewed in [3–5]). Compared with DNA base modifications, post-translational modifications of histones represent a much larger repertoire. More than ten chemical groups, including methylation, acetylation and phosphorylation, can be placed on over a dozen amino acid residues on a single histone molecule, thereby generating an enormous diversity of nucleosome species [6,7]. Histone variants, such as histone H3.3 and H2AZ, wrap a substantial fraction of the genome to exert specific roles in transcription, providing an additional layer of complexity [8]. We now know that multiple histone and DNA modifications act in concert and they often influence one another [9,10]. Such intricate crosstalk of complex chromatin modifications may provide an ideal platform for cells

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to integrate diverse environmental cues and generate outputs in highly controlled and context-dependent manner.

Dysregulation of histone methylation has emerged as a major genetic driver of neurodevelopmental disorders (NDDs), including intellectual disabilities and autism spectrum disorders [11,12]. Numerous mutations have been described in proteins that place, remove or bind to specific histone methylations (histone methyl-writers, histone methyl-erasers and histone methyl-reader proteins, respectively) [13,14]. However, how histone methylation contributes to brain development and cognitive functions remains largely unknown. Investigating the roles of histone methylations likely offers not only pathophysiological explanations for NDD, but also fundamental insights into neural network assembly, synaptic plasticity and the dynamics of memory.

Histone methylations can be placed on lysine and arginine residues in multiple forms [15]. A lysine residue can be post-translationally modified to be mono-, di- or tri-methylated (me1, me2, me3). Methylations at specific histone lysine residues correlate differentially to the transcriptional status. H3K9 and H3K27 methylations primarily decorate transcriptionally inactive regions [15]. H3K4 methylation (H3K4me) decorates gene-regulatory elements, in other words, promoters and enhancers, at transcriptionally active loci [16]. “Of note, studies often find exceptional cases that do not follow these general patterns” (e.g., H3K9me3 at certain active promoters and 3' ends of genes [17,18]). Functional studies have established that H3K4 writer and eraser enzymes and reader proteins play important roles in generating and maintaining cellular transcriptomes [19–21]. Mutations associated with NDD have been found in most of the H3K4 enzymes and in multiple reader proteins [21], suggesting normal cognitive function is achieved by intricate transcriptional control via precise balance of H3K4me status and its interpretation by reader proteins.

Chromatin regulatory proteins, including H3K4me regulators, generally operate within stable multisubunit complexes [22]. Such a complex typically contains histone modifying enzymes, reader proteins and protein scaffolds. These components are believed to cooperatively control higher order structure of chromatin, suggesting that a given NDD could potentially result from mutations in multiple components of one complex. One such example is Kabuki syndrome (Mendelian Inheritance in Man number (MIM): 147920), a rare NDD characterized by unique facial characteristics [23]. Earlier biochemical studies found that KMT2D, a writer of an active H3K4me mark, cooperates with KDM6A, an eraser of a repressive

H3K27me mark within a large transcriptional coactivator complex [24]. Subsequent human genetics studies revealed that mutations in the *KMT2D* and *KDM6A* collectively explain the majority of Kabuki syndrome cases [23]. These studies highlight the value of considering multisubunit complexes when deciphering the molecular mechanisms underlying NDDs.

Here we review evidence from human genetics, developmental biology and proteomics studies that suggests that the LSD1–CoREST complex and the RAI1-containing complex functionally interact to regulate H3K4me during brain development. The LSD1–CoREST complex and the RAI1 complex contain components genetically associated with multiple NDDs that have not been previously related one another. This review therefore highlights H3K4me dysregulation as an unexpected nexus and the core mechanism underlying a number of neurodevelopmental conditions. We begin by describing the known functions of the LSD1–CoREST complex and their relation to NDDs, and transition to the RAI1 complex by analyzing a key proteomics study, which reveals the link between the two complexes. Additionally, we review possible functions of these two complexes in circadian transcription and activity-dependent gene expression, which may underlie common symptoms of NDDs.

The LSD1–CoREST complex: identification & the mechanism of action

The LSD1–CoREST complex, also known as the BRAF35–HDAC1/2 complex (BHC), was originally isolated as a corepressor complex that suppresses neuron-specific genes (e.g., *Synapsin I*) in non-neuronal cells [25]. Among the components of the BHC was BHC110, the first discovered histone demethylase [26]. BHC110, now commonly called LSD1 or KDM1A, specifically demethylates H3K4me1 and H3K4me2 but not H3K4me3 [26]. In addition to LSD1, the canonical LSD1–CoREST complex is composed of five proteins: an adaptor protein CoREST; histone deacetylases HDAC1 and HDAC2; histone methyl-reader protein PHF21A (BHC80) and a DNA-binding protein BRAF35 (HMG20B) (Figure 1A) [27].

Transcriptional repression of neuron-specific genes by the LSD1–CoREST complex is primarily executed through removal of active transcription-associated histone marks. The LSD1–CoREST complex is recruited to neuron-specific gene promoters in part by REST (NR5F), which is predominantly present in non-neuronal cell types [27]. At the promoter, LSD1 and HDAC1/2 catalyze the removal of methyl and acetyl groups, respectively. Though LSD1 is a potent demethylase when histone H3 N-terminal peptides are used as a substrate, LSD1 alone is not capable of demethylating

Figure 1. Structural features of LSD1–CoREST complex and RAI1 complex components. LSD1–CoREST complex (A) and RAI1 complex (B) components and associated neurodevelopmental disorders.

[†]Known missense mutations associated with neurodevelopmental disorders.

aa: Amino acids; AOD-N: Amine oxidase domain (N-terminal); CC: Coiled-coil domain; ELM2: Egl27 and MtA1 homology domain; ePHD: Extended plant homeodomain; HMG: High mobility group domain; NBD: Nucleosome binding domain; PHD: Plant homeodomain; SANT: Swi3-Ada2-N-cor-Transcription factor domain; TOW: TOWER domain.

Table 1. LSD1–CoREST complex and RAI1 complex components and their published roles in neurodevelopment and brain function.			
Protein	Associated neurodevelopmental disorder(s)	Molecular roles in neurodevelopment	Roles in activity-dependent gene expression Roles in circadian rhythms
LSD1–CoREST complex component			
LSD1 (KDM1A) Demethylates H3K4me1/2	Kabuki syndrome-like neurodevelopmental disorder [32,33]	Represses neuronal genes in non-neuronal cells via demethylation of H3K4me1/2 in LSD1–CoREST complex [25,26] Required for proper migration and morphology of cortical pyramidal neurons [38]	LSD1/neuroLSD1 modulate induction of activity-dependent gene expression [34–36] Demethylase activity-independent coordination of CLOCK–BMAL [37]
HDAC1 Histone deacetylase	–	Represses neuronal genes in non-neuronal cells via deacetylation of H3 in LSD1–CoREST complex [30]	–
HDAC2 Histone deacetylase	–	Represses neuronal genes in non-neuronal cells via deacetylation of H3 in LSD1–CoREST complex [30]	Suppresses memory formation through histone deacetylation in association with CoREST [39] –
CoREST1/2/3 Bridges LSD1 to nucleosomes to facilitate LSD1-mediated demethylation [28]	–	Affects migration and morphology of cortical pyramidal neurons [38] Modulates neuronal precursor proliferation, cortical thickness and brain size [40]	–
PHF21A Binds to unmethylated H3K4 [31]	PSS [41]	–	–
BRAF35 DNA binding protein	–	Mediates the LSD1–CoREST complex's recruitment to DNA or stabilization at DNA [25]	–
BDMR: Brachydactyly with mental retardation; PHD: Plant homeodomain; PSS: Potocki–Shaffer syndrome; PTL: Potocki–Lupski syndrome; SMS: Smith–Magenis syndrome.			

Table 1. LSD1–CoREST complex and RAI1 complex components and their published roles in neurodevelopment and brain function (cont.).

Protein	Associated neurodevelopmental disorder(s)	Molecular roles in neurodevelopment	Roles in activity-dependent gene expression	Roles in circadian rhythms
RAI1 complex component				
RAI1	SMS [42] PTLS [46] Autism [53] Schizophrenia [57] BDMR [58] 2q23.1 deletion syndrome [60]	Craniofacial development [43,44] Learning, memory, motor activity, feeding and social interaction in mouse models of SMS and PTLs [47–51] Binds to an intron of <i>BDNF</i> and promotes its expression [54–56]	–	Associated with sleep deficits in SMS [45] Binds an intron of <i>CLOCK</i> and activates <i>CLOCK</i> expression [52] Influences circadian gene oscillation in cultured cells [52] SMS patient fibroblasts display reduced levels of circadian genes [52] Haploinsufficiency of <i>Rai1</i> causes shortening of free-running period length in mice [59]
TCF20	Autism [61] Intellectual disability [62]	–	–	–
PHF14	Dandy–Walker malformations [63]	–	–	–
iBRAF	–	Mediates neuronal differentiation in P19 cells [64] Diminishes SUMOylation of BRAF35 via heterodimerization [65] Interferes with LSD1-mediated repression of neuronal genes [64,65]	–	–
MLL1 (KMT2A) H3K4me writer enzyme	Wiedemann–Steiner syndrome [66]	Moderates proper neuro- and glio-genesis [67]	Conditional knockout impairs learning-induced <i>Arc</i> expression in mature mouse prefrontal cortex [68]	Controls circadian H3K4me status [69,70]
BDMR: Brachydactyly with mental retardation; PHD: Plant homeodomain; PSS: Potocki–Shaffer syndrome; PTLs: Potocki–Lupski syndrome; SMS: Smith–Magenis syndrome.				

nucleosomes [28,29]. LSD1-mediated H3K4 demethylation at nucleosomes requires HDAC1 and HDAC2 to deacetylate histones [30] and CoREST to bridge LSD1 to nucleosomes [28,29]. PHF21A and BRAF35 appear to contribute to the recruitment of the LSD1–CoREST complex on target genes. PHF21A carries a plant homeodomain (PHD), which recognizes unmethylated H3K4 but not H3K4me [31]. It is proposed that the unmethylated H3K4-binding of PHF21A allows the LSD1 complex to stably bind to target genomic sites after demethylating H3K4me [31]. BRAF35 contributes to transcriptional repression through a yet undetermined mechanism, perhaps by mediating the recruitment of the LSD1–CoREST complex to DNA or stabilizing the complex at DNA [25]. Although the roles of LSD1–CoREST complex components were initially described in non-neuronal tissues, accumulating evidence has pointed to its important roles in brain development and synaptic plasticity as discussed below and summarized in Table 1.

LSD1–CoREST complex & its related NDDs

LSD1(KDM1A) and PHF21 were recently reported independently to have genetic associations with NDDs. Missense mutations in *LSD1* have been identified in three individuals with developmental delay in their ability to speak, sit and walk [32,33]. The developmental symptoms in these individuals are similar to those of Kabuki syndrome (MIM: 147920), characterized by distinct craniofacial features including widely spaced teeth and palatal abnormalities [32,33]. A thorough biochemical study has demonstrated that these mutant proteins exhibit reduced stability and demethylase activity, indicating a loss-of-function mechanism [71].

PHF21A is associated with Potocki–Shaffer syndrome, (PSS, MIM: 601224) an NDD with characteristic craniofacial symptoms and bone malformations including exostoses and enlarged parietal foramina [41]. Multigene microdeletions in chromosomal region 11p11–12 lead to the full spectrum of PSS symptoms [72]. Within this region, *EXT2* and *ALX4* appear to account for the bone malformation [41]. Two individuals, with neurodevelopmental and craniofacial symptoms similar to typical PSS, had balanced translocations in which the *PHF21A* gene was disrupted but all other genes were unperturbed [41]. In the two patient-derived lymphoblastoid cell lines, PHF21A protein expression was reduced, suggesting PSS involves *PHF21A* haploinsufficiency [41]. Disrupted craniofacial and neurodevelopmental effects were similarly seen in zebrafish model with PHF21A knockdown, supporting the idea that reduced PHF21A levels are responsible for these specific symptoms of PSS [72].

In summary, the Kabuki-like *LSD1* (*KDM1A*) deficiency and neurodevelopmental defects in PSS may be attributable to impaired function of the LSD1–CoREST complex. Therefore, future therapeutic efforts for the two conditions can focus on common pathways in which the LSD1–CoREST complex participates. Other components of the LSD1–CoREST complex (HDAC1, HDAC2, CoREST and BRAF35) have not been associated with specific NDD. However, because these components regulate LSD1's enzymatic activity, disruption of these components will likely have a negative impact on the function of the complex. Thus, components of LSD1–CoREST complex are reasonable candidate genes for NDDs for which responsible genes have not been identified.

Roles of LSD1–CoREST complex components in brain development

In addition to human genetics studies, mouse models have provided compelling evidence for the roles of the components of the LSD1–CoREST complex in brain development (Table 1). Here, we review literature describing the roles of individual LSD1–CoREST complex components in brain development.

Homozygous deletion of *Lsd1* (*Kdm1a*) in mice resulted in embryonic lethality by day 7.5 (E7.5) [73], implying an essential role for LSD1 in early development. Pituitary-specific *Lsd1*-knockout (KO) mice displayed impaired cell differentiation in the late stages of anterior pituitary development. Interestingly, LSD1 appears to act as an activator of pituitary genes as well as a repressor of cell cycle-related genes [73]. Although the anterior pituitary is not an organ consisting of neurons [74], this work demonstrated the dual-facet roles of LSD1 in cell specification that may be conserved in brain cell types. To understand the pathophysiology of the Kabuki-like cognitive deficits associated with the heterozygous *LSD1* mutations [32,33], it will be informative to investigate neurodevelopment of *Lsd1*^{+/-} mice, which are reportedly viable and fertile [73].

As discussed earlier, CoREST enables LSD1 (KDM1A) to demethylate H3K4me1/2 on nucleosomes [28]. In mammals, CoREST proteins (CoREST1, CoREST2 and CoREST3) are encoded by the three paralogous genes, *RCOR1*, *RCOR2* and *RCOR3*, with apparently nonredundant function. All paralogs can form a complex with LSD1 and show a distinct expression pattern during neuronal maturation [75]. *Rcor1* was required for proper timing of migration and differentiation of pyramidal cortical neurons in mice [38]. *Rcor2* was required for cortical development and loss of *Rcor2* could not be compensated for by upregulation of *Rcor1* [40]. At the molecular level, recombinant LSD1–CoREST1 showed higher demethylase activity

than LSD1–CoREST3, although the affinity between LSD1 and CoREST1 does not differ from that of CoREST3 [76]. The nonredundant roles of CoREST paralogs may therefore be due to their differential impact on LSD1-mediated H3K4me removal.

BRAF35, the DNA-binding protein in the complex, appears to negatively regulate neuronal differentiation. BRAF35 is expressed highly in immature neurons at the ventricular region and not visibly expressed in mature neurons at the outer layers of cortex of E16.5 mouse embryos [64]. Consistent with this expression pattern, overexpression of BRAF35 inhibited neuronal differentiation in both a P19 embryonal carcinoma cell line and chicken embryonic neural tube [65]. Inhibitory impact of BRAF35 to neuronal differentiation is consistent with the classic roles of the LSD1–CoREST complex in repressing neuron-specific genes.

The neurodevelopmental roles of PHF21A (BHC80), a reader protein for unmethylated H3K4, are less defined compared with other LSD1–CoREST complex components. *Phf21a* loss in mice results in neonatal death and impaired milk suckling behaviors, without overt histological abnormality in the brain [77]. Biochemical studies indicate the role of PHF21A as a negative regulator of the LSD1–CoREST complex. PHF21A was shown to inhibit LSD1-mediated demethylation and transcriptional repression [25,28]. Consistently, PHF21A antagonized the REST-mediated repression of neurosecretion genes in rat PC-12 cell lines [78]. How the unmethylated H3K4-reading capability of PHF21A relates to this negative regulatory function is not well understood.

The roles of HDACs in the brain have been well-documented in the context of neurodegenerative diseases such as Friedreich ataxia and age-related cognitive declines. Excellent reviews summarize the roles of HDACs in neurodegeneration and the clinical implications of HDAC inhibition [79–81]. More recent studies have revealed important roles of HDAC1 and HDAC2 in neurodevelopment. Neuroprogenitor-specific deletion of both *Hdac1* and *Hdac2* in mice caused postnatal lethality with impaired survival of neuroprogenitor cells and impaired differentiation of neurons, but not of astrocytes. This suggests neuronal lineage-specific requirement of HDAC1 and HDAC2 during differentiation [82].

In summary, these observations in rodent and cell culture models agree with human genetics studies, which implicate the pivotal roles of the LSD1–CoREST complex components in brain development. It is important to note that some of the components participate in multiple chromatin-regulatory complexes. For example, HDAC1 and HDAC2 are known to participate in NuRD and Sin3 corepressor complexes [83].

In these cases, it remains unclear which complexes mediate the observed phenotypes. Identification of point mutants that impair physical interactions to one complex but not the other will be a valuable strategy to address this issue. Uncoupling the roles of a given protein in multiple complexes will provide important knowledge to develop therapeutics with minimal side effects in the future.

Reciprocal roles of LSD1–CoREST Complex & iBRAF–MLL1 in neurodevelopment

In addition to the aforementioned canonical components, iBRAF (HMG20A), an HMG-box protein structurally related to BRAF35 (HMG20B), regulates LSD1 (KDM1A)-mediated H3K4me removal. Wydner *et al.* demonstrated that iBRAF and the LSD1–CoREST complex reciprocally regulate neurodevelopment [64]. In contrast to the expression of BRAF35 in immature neurons at ventricular zones, iBRAF is more highly expressed in the mature neurons located in the outer cortices of E16.5 mice. In undifferentiated P19 cells, BRAF35 occupied the promoter of the neuron-specific gene *Synapsin I* to repress its transcription. In response to differentiation induction of P19 cells by serum withdrawal, BRAF35 dislodged from the *Synapsin I* promoter, which coincided with increased *Synapsin I* mRNA levels. Concomitantly, MLL1 (KMT1A), an H3K4me writer enzyme and iBRAF were recruited to the *Synapsin I* promoter, which led to increased H3K4me3 levels. Furthermore, P19 cells depleted of iBRAF were unable to initiate differentiation induction by serum withdrawal, and instead underwent apoptosis. MLL1 occupancy and H3K4me3 levels at the *Synapsin I* promoter decreased upon iBRAF knockdown. Conversely, overexpression of iBRAF induced differentiation and increased expression of neuron-specific genes without serum withdrawal. This work revealed a pro-neuronal differentiation mechanism, whereby iBRAF competes with the BRAF35-containing LSD1–CoREST complex to occupy neuronal gene promoters and to execute MLL1-mediated H3K4me3 installation.

iBRAF may promote H3K4me not only through recruitment of MLL1, but also through interaction with BRAF35. iBRAF can heterodimerize with BRAF35 and so inhibit sumoylation of BRAF35, which is necessary for the antineurodifferentiation function of BRAF35 [65]. Overexpression of iBRAF in P19 cells increased the iBRAF/BRAF35 heterodimerization and promoted neuronal differentiation. Furthermore, iBRAF was capable of substituting for BRAF35 in the LSD1–CoREST complex, thereby attenuating repression of neuronal genes [65]. In summary, iBRAF counteracts the role of the LSD1–CoREST complex via multiple mechanisms. Higher protein levels of

iBRAF compared with BRAF35 appears key to progressing neurodevelopment. This is an exciting discovery that raises important questions. After neuronal differentiation, does iBRAF completely substitute for BRAF35 in the LSD1–CoREST complex? Alternatively, does iBRAF form a distinct complex to execute unique functions in mature neurons? Of interest, then, is identification of the complexes with which iBRAF participates and their roles at different stages of neurodevelopment.

iBRAF forms a novel H3K4me-regulatory complex containing RAI1

Using a unique proteomics approach, Eberl *et al.* discovered that iBRAF is an integral component of a putative chromatin regulatory complex, which is distinct from the LSD1–CoREST complex. The authors used a label-free proteomics approach to identify reader proteins that specifically recognize well-characterized histone methylations including histone H3K4me3 [84]. Brain, kidney and liver tissue lysates taken from adult mice were filtered through columns containing unmethylated H3K4 or H3K4me3 peptides, and eluates were analyzed by MS/MS. Consistent with a previous report [31], PHF21A was identified as a protein that binds to unmodified H3K4 and repelled by H3K4me. In the H3K4me-repelled fraction, they identified a novel protein complex, which consists of iBRAF, RAI1, PHF14 and TCF20/SPBP.

Importantly, mutations in *RAI1* are associated with two NDDs, Smith–Magenis Syndrome and Potocki–Lupski syndrome [45]. Based on the strong genetic association between RAI1 and the two neurodevelopmental conditions, herein, we refer to the new unmethylated H3K4-binding complex as the RAI1 complex (Figure 1B). This proteomics study, therefore, identified iBRAF as the nexus of two H3K4me-regulatory complexes, the LSD1–CoREST complex and the RAI1-complex, in turn revealing an unexpected connection between previously unrelated NDDs. Below, we outline the recent progress regarding the roles of each RAI1-complex component in brain development and chromatin regulation.

RAI1, Smith–Magenis syndrome & Potocki–Lupski syndrome

RAI1 is the primary gene implicated in Smith–Magenis syndrome (SMS, MIM: 182290) and Potocki–Lupski Syndrome (PTLS, MIM: 610883; Table 1). SMS is characterized by low intellectual quotient, delayed motor and speech abilities, altered sleep cycles, obesity, hypotonia, specific craniofacial characteristics and self-injurious behaviors such as face-slapping and polyembolokilomania [42,85]. SMS is most commonly associ-

ated with heterozygous microdeletions of chromosome 17p11, which span the minimal 1.5 Mb region that contains 13 genes including *RAI1* [86]. Truncation and missense mutations in *RAI1* have been reported in individuals with prototypical SMS, implicating *RAI1* as the major gene contributing to the neurodevelopmental and behavioral symptoms (reviewed in [45,54]).

Interestingly, duplication of the same 17p11.2 interval containing *RAI1* is associated with PTLS [87]. PTLS and SMS share similar symptoms of low intellectual quotient and hypotonia. However, in contrast to the obesity and self-injurious behavior in SMS, individuals with PTLS are characterized with reduced body weight, hyperactivity and autistic behaviors. While individuals with SMS experience daytime sleepiness and nighttime awakenings, individuals with PTLS display sleep apnea [45,46]. The 125 kb duplication region common to all PTLS cases overlaps only with *RAI1*, strongly supporting a causative role of *RAI1* duplication in PTLS [88]. Mouse models with syntenic microdeletions or microduplications, *Rai1* KO or *Rai1* overexpression, recapitulate learning disabilities, metabolic disorders, craniofacial features and/or sleep abnormalities observed in SMS or PTLS individuals [47–49,55,56,59]. These observations strongly suggest that RAI1 exerts an evolutionarily conserved, dosage-sensitive role in neurodevelopment.

RAI1 has also been genetically associated with other autism-related conditions [53] and schizophrenia [89]. RAI1 protein levels were altered in post-mortem prefrontal cortex from patients with schizophrenia, bipolar disorder and major depression [90]. In addition, *RAI1* expression levels were shown to be commonly decreased in multiple intellectual disability syndromes that are not directly associated with *RAI1* mutations. These include brachydactyly with mental retardation (BDMR, MIM: 600430), caused by deletion of *HDAC4*-containing chromosome 2q37 region [58], and 2q23.1 deletion syndrome (MIM: 156200) [60]. This suggests *RAI1* might be a downstream effector in other neuropsychiatric conditions.

Roles of other RAI1-complex components in brain development

Two other RAI1-complex components, TCF20 (SPBP) and PHF14, have been genetically linked to neurodevelopmental conditions (Table 1). *TCF20* appears to be a structural paralog of *RAI1* with a high sequence similarity. Two missense variations and an inversion event spanning *TCF20* have been identified in an autism patient cohort with incomplete penetrance [61]. The missense variations hit evolutionarily-conserved amino acids in two PEST sequences within TCF20. Since PEST sequences (sequences that are enriched

in proline, glutamate, serine and threonine) play an important role for rapid protein degradation [91], authors predicted an increased stability of TCF20 variant proteins [61]. Functional validations of identified variations and identification of fully-penetrant missense mutations will be valuable to assess causal roles of *TCF20* alterations in autism. More recently, meta-analysis of exomes derived from patient-parent trios identified *TCF20* as one of the new genes associated with intellectual disability, providing a stronger support for importance of *TCF20* in normal brain development [62].

PHF14 has been associated with Dandy–Walker malformations (DWM, MIM: 220200) [63]. DWMs are a group of heterogeneous abnormalities that commonly affect the cerebellum, fourth ventricle or cisterna magna. A number of chromosomal abnormalities including trisomy of chromosome 18 or chromosome 13, and deletion of chromosome 13q, were previously associated with DWM [63]. In the three fetuses with variable cerebellar vermis hypoplasia and enlarged cisterna magna, imbalanced translocations resulted in deletions or duplication of a genomic region that spans *PHF14* and *NDUFA4*, latter of which encodes a mitochondrial oxidative phosphorylation enzyme. *Phf14*-KO mice showed neonatal lethality likely due to respiratory failure, while heterozygous mice were healthy [92,93]. It remains undetermined if the *Phf14*-KO mice display cerebellar abnormalities. Additional clinical genetics study and further characterization of *Phf14*-KO mice will be necessary to validate the contribution of *PHF14* to DWMs and other NDDs.

Though MLL1 (KMT2A), an H3K4me writer enzyme, was not identified as a component of the RAI1 complex, MLL1 may participate in RAI1-complex through its association with iBRAF. Interestingly, haploinsufficiency of *MLL1* (*KMT2A*) is associated with Wiedemann–Steiner syndrome (WSS, MIM: 605130). WSS is an intellectual disability syndrome coincident with short stature, distinct facial features and distinguished by excessive hair growth around the elbows [66]. In mice, heterozygous neuroprogenitor-specific *Mll1* ablation led to decreased neurogenesis and increased gliogenesis, and lethality within one month of birth [67]. The loss of *Mll1* led to downregulation of an MLL1-direct target gene *Dlx2*, a transcription factor essential for proper neurogenesis. Further investigation is required to determine whether this process is mediated in part or in full by the RAI1 complex.

Molecular biology of the RAI1 complex

Unlike typical chromatin regulatory machineries, none of the RAI1 complex components appear to be histone-modifying enzymes. Notably, PHF14, RAI1

and its paralog TCF20 (SPBP) carry putative methyl-histone recognition modules: PHDs and extended PHDs (ePHDs) (Figure 1B) [94]. Given the role of iBRAF in MLL1 recruitment, the RAI1 complex may act as a reader of combinatorial histone modifications to stabilize MLL1 on target chromatin areas. The RAI1 complex binds to unmethylated H3K4, whereas H3K4me inhibits the interaction [84]. The unmethylated H3K4-binding of the RAI1 complex may serve as a searching mechanism to find yet-unmethylated, and/or recently demethylated H3K4 residues on gene promoters, where the RAI1 complex could then recruit MLL1 to methylate H3K4, thereby promoting gene transcription.

How does the RAI1 complex recognize unmethylated H3K4? The ePHDs of RAI1 and TCF20 (SPBP) and the two PHF14-PHDs have been demonstrated to bind to nucleosomes and histone proteins [92,95,96]. However, it remains unknown if these domains recognize particular histone modifications. The capability of the RAI1 complex to read unmethylated H3K4 and its negative regulation by H3K4me3 might be achieved by one of these PHDs. Of note, RAI1 and TCF20 harbor another nucleosome-binding domain (NBD) upstream of ePHD (Figure 1B) [95]. The NBD-nucleosome interaction appears to be independent of the N-terminal tail of histone H3, which carries H3K4, implying that the NBD is not involved in the unmethylated H3K4 binding. To determine the molecular function of the RAI1 complex, it is necessary to verify the influence of H3K4me status on the histone binding capacity of these domains and its role in MLL1 recruitment.

Some of the histone-binding domains in the RAI1-complex may play a role in development of associated NDDs. Missense mutations in individuals with SMS have been found both in the ePHD and NBD of RAI1, and nonsense or indel SMS mutations truncate these domains [54]. Similarly, one autism-associated missense mutation in *TCF20* (p.Pro1557Leu) falls into PEST sequence embedded within NBD, and another substitution with predicted deleterious effects, p.Pro1937Leu, is localized to the ePHD. Thus, important next step is to determine the exact roles of these histone-binding modules and impact of identified NDD mutation.

Interplay between the LSD1–CoREST & the RAI1 complexes in neurodevelopment

The iBRAF-mediated recruitment of MLL1, an H3K4me writer enzyme and LSD1-mediated H3K4me removal indicate the RAI1- and LSD1–CoREST complexes counterbalance each other at H3K4 (Figure 2). If RAI1- and LSD1-containing complexes were in exact opposition in their function, then PTLS, the *RAI1*-duplication syndrome, should more closely resemble

Figure 2. A model of opposing roles of LSD1–CoREST complex and RAI1 complex on histone H3K4 methylation. The LSD1–CoREST complex facilitates demethylation of H3K4me1/2, repressing expression of neuron-specific genes in neuroprogenitors. Through iBRAF, the RAI1 complex recruits MLL1 to methylate H3K4, which in turn leads to activation of neuronal genes. Note that iBRAF can substitute for BRAF35 in the LSD1–CoREST complex, suggesting multiple modes of regulation between the RAI1 and LSD1–CoREST complex.

individuals with *LSD1* deficiency than SMS, the *RAI1* haploinsufficiency. Indeed, both PTLIS and *LSD1*-associated developmental delay appear to lack the SMS-related features of obesity, altered sleep cycles, and self-injurious behaviors. However, PTLIS characteristics such as autistic behaviors and hyperactivity have not been reported in *LSD1*-deficiency, although both conditions result in intellectual disability. These observations in human conditions imply that the two complexes may act both in common and distinct molecular pathways.

In order to obtain insights into how the LSD1–CoREST and the RAI1 complexes functionally interact, we compiled spatiotemporal expression patterns from the Allen Developing Mouse Brain Atlas [97] for each component (Figure 3). Of note, most of these factors are expressed not only in neurons, but also in astrocytes, oligodendrocytes and epithelial cells, based on a gene expression database of brain cell types [98]. The expression in other cell types suggests the two complexes may exert their roles in neurodevelopment through noncell autonomous manners. Indeed, astrocytes and oligodendrocytes have been implicated as contributing factors to the NDDs [99–101]. However, due to the

scarcity of knowledge on roles of the two complexes in non-neuronal cell types in the brain, we will focus our discussion to neuronal lineage.

As shown in Figure 3, the reciprocal expression of BRAF35 and iBRAF, reported by Wynder *et al.* [64], was recapitulated across the brain regions. Expression of *Rai1* is clearly increased during the perinatal period, while the LSD1–CoREST complex components, except for *Braf35*, display constantly high expression throughout development. A more detailed expression study found a higher LSD1 protein level in mice from E13.5 to P15, which declines in adult brains [38]. Thus, dynamic increase of *Rai1*, and the *iBraf1Braf35* ratio, may be a driving force to replace the LSD1–CoREST complex with the RAI1 complex at neuron-specific genes such as *Synapsin I*.

Conversion of LSD1-mediated H3K4me removal to MLL1-mediated H3K4me placement may stabilize the active transcriptional status of the neuron-specific genes when neurons mature. Once H3K4me3 is installed by MLL1 [108], the LSD1–CoREST complex cannot act on the target genes as LSD1 can only demethylate H3K4me1/2 [26]. Although their roles are distinct,

both complexes show affinity towards unmethylated H3K4 and repulsion by H3K4me. While the RAI1 complex may search for unmethylated H3K4 to recruit MLL1 in differentiating and/or mature neurons, the LSD1–CoREST complex may leverage PHF21A's recognition of unmethylated H3K4 to stably transmit the unmethylated status to daughter cells of dividing neuroprogenitors.

Notably, the expression of *Braf35* is re-induced in juvenile ages of mouse development (Figure 3), suggesting that the fully stoichiometric LSD1–CoREST

complex can form in mature neurons. Thus, in contrast to the differentiation stage, the two complexes may co-exist in mature neural network and modulate H3K4me. Below, we discuss the possible dynamics between the LSD1–CoREST and RAI1 complexes in activity-dependent gene expression and circadian rhythms.

Activity-dependent gene expression

Activity-dependent gene expression refers to the transcriptional changes that occur in response to neuronal

Figure 3. Spatiotemporal expression of LSD1–CoREST and RAI1 complex components in developing mouse brain. (A) Expression of three of the six LSD1–CoREST complex components: *Rcor2* (CoREST2), *Hdac2* and *Braf35* (*Hmg20b*). (B) Expression of three of the four RAI1 complex components *Rai1*, *Tcf20* and *iBraf* (*Hmg20a*). *Braf35* and *iBraf* are reciprocally expressed during development, whereas the components of the RAI1 complex are generally co-expressed at later developmental stages. Time points extend from embryonic day 11.5 (E11.5) to postnatal day 28 (P28). Different subregions of the developing brain are: RSP: Rostral secondary prosencephalon; Tel: Telencephalon; PHy: Peduncular hypothalamus; p3: Prosomere 3; p2: Prosomere 2; p1: Prosomere 1; M: Midbrain. PPH: Prepontine hindbrain; PH: Pontine hindbrain; PMH: Pontomedullary hindbrain and MH: Medullary hindbrain. Corresponding mature brain areas are Hippo: Hippocampus, and Mid: Midbrain. Images are adapted from Expression Summaries obtained from the Allen Developing Mouse Brain Atlas, which compiles *in situ* hybridization data.

The original Allen Developing Mouse Brain Atlas Expression Summaries were found at the following web links: *Rcor2*: [102]; *Hdac2*: [103]; *Braf35* (*Hmg20b*): [104]; *Rai1*: [105]; *Tcf20*: [106]; *iBraf* (*Hmg20a*): [107].

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activity. Proper activity-dependent transcription is essential for learning and memory [109], and multiple forms of neuronal plasticity, such as synaptic scaling, long-term potentiation and long-term depression [110]. While synaptic scaling maintains homeostasis of neuronal excitability, long-term potentiation and long-term depression achieves long-term changes in the strength of individual synapses [111]. The general framework of activity-dependent gene expression is as follows. First, neuronal firing leads to an influx of calcium and a series of calcium-response signaling, which reaches the nucleus within a neuron. Constitutively expressed transcription factors such as cyclic AMP-responsive element-binding protein and serum response factor are then activated via post-translational mechanisms, primarily phosphorylation, which subsequently initiate the 'first wave' of transcription of a set of genes [112]. The first wave genes encode effector proteins such as ARC and BDNF and a number of transcription factors: e.g., FOS, NPAS4, EGR1 and C/EBP β [112]. These transcription factors, in turn, activate a 'second wave' of activity-dependent gene expression [112]. The second wave genes encode many synaptic and dendritic proteins that can modulate neuronal connectivity [113,114].

Given the important roles of histone methylation in transcription, activity-dependent transcription can be a point of convergence of neuronal plasticity and NDDs that are genetically associated with dysregulation of histone methylation. Indeed, MLL1 and LSD1, both of which are implicated in NDDs, appear to be required for optimal activity-dependent transcription (Table 1) [34,68,115]. In addition to these H3K4me regulators, an H3K9 methyltransferase G9a/GLP [116] and an H3K27 demethylase KDM6B [117] engage in activity-dependent transcription, suggesting that the coordinated regulation of both active and repressive histone methylation underlies the exquisite gene response to neuronal activity.

LSD1-CoREST & RAI1 complexes in activity-dependent gene expression

The initial implication for the involvement of the LSD1-CoREST complex in activity-driven gene regulation was documented in the context of synaptic scaling. Pozzi *et al.* demonstrated that REST/NRSF, which is usually expressed in non-neuronal tissues, can be transcriptionally induced by neuronal activity in turn suppresses transcription of Na_v1.2 sodium channel gene *SCN2A* and neuronal excitability [118]. Although the LSD1-CoREST complex was not analyzed in this study, its established role in REST/NRSF-mediated gene silencing [22] implicates the LSD1-CoREST complex in synaptic scaling, though this requires experimental validation.

Recent work revealed essential roles of LSD1 (KDM1A) in activity-dependent transcription, which involves a neuron-specific splicing event of LSD1 [34]. LSD1 appears to have neuron-specific isoforms, neuroLSD1, in which an extra four amino acids are inserted within the catalytic domain [119]. Multiple groups have examined how the demethylase activity of neuroLSD1 differs from canonical LSD1. While Zibetti *et al.* first reported that neuroLSD1 demethylates H3K4me with a reduced efficiency, other groups have since reported that the neuron-specific splicing converts LSD1's substrate specificity from H3K4 to H3K9 [120] or H4K20 [34]. Although the mechanisms of its function require clarification, neuroLSD1 appears essential for proper neuronal function. Expression levels of neuroLSD1 were increased in postnatal stages in mice [119], whereas neuronal firing due to seizure induction or social defeat stress reduced neuroLSD1 mRNA expression, suggesting dynamic roles of neuroLSD1 in synaptogenesis and plasticity [35,36]. Deletion of neuroLSD1, leaving canonical LSD1 intact, led to reduced induction of activity-dependent genes accompanied with impaired spatial learning and stress response [34,35].

Mechanistically, three models are proposed for neuroLSD1-mediated induction of activity-dependent genes (Figure 4A). Firstly, neuroLSD1 promotes transcriptional elongation by RNA polymerase II by removing H4K20me from gene bodies [34]. Secondly, neuroLSD1, with reduced H3K4 demethylation activity, is recruited by serum response factor to target loci where neuroLSD1 acts as a dominant negative form to counteract canonical LSD1 [35]. Thirdly, neuroLSD1 can be phosphorylated at T369 within the neuron-specific exon. The phosphorylation leads to dissociation of HDAC1/2, thereby relieving transcriptional repression [121]. These studies delineated important roles of the LSD1-CoREST complex in activity-dependent transcription and revealed intriguing complexity of chromatin regulation generated by neuron-specific splicing events.

HDAC1 and HDAC2 have distinct roles in mature brain plasticity. HDAC2 appears to suppress the expression of activity-dependent genes [39]. Neuron-specific overexpression or KO of HDAC2, but not HDAC1, leads to alteration in synaptic morphology and learning and memory in mice [39]. As mentioned earlier, HDAC1/2 can associate with the NuRD or mSin3 complexes in addition to the LSD1-CoREST complex [83]. In mature brains of mice, HDAC2 associated with CoREST, but not with members of the NuRD or mSin3 complex [39]. This indicates that HDAC2 inhibits neuronal plasticity through the LSD1-CoREST complex. In summary, the LSD1-CoREST com-

Figure 4. LSD1–CoREST and RAI1 complex components in activity-dependent gene expression and circadian gene expression. Evidence suggests both the LSD1 complex and the RAI1 complex are involved in activity-dependent transcription (A & B) and circadian transcription (C & D). P and Ac regulate the actions of the two complexes. Experimental validations are needed to determine if the RAI1 complex and/or the iBRAF-containing LSD1 complex regulate a variety of activity-dependent genes. Ac: Acetylation; P: Phosphorylation.

plex may employ a variety of mechanisms for neuronal activity-related gene regulation.

The roles of the RAI1 complex components in activity-dependent gene expression remain largely unexplored. However, studies have begun to uncover the roles of RAI1 and MLL1 in this process and their impacts on learning and memory. Deletion of *Rai1* in mice resulted in deficits in contextual memory [47]. Furthermore, RAI1 depletion resulted in diminished expression of brain-derived neurotrophic factor (*Bdnf*) in mouse hypothalamus and frog embryonic brains [55,56]. *BDNF* is a key activity-dependent gene essential for neuronal development and synaptic plasticity [110]. RAI1 occupies an intronic region approximately 1 kilobase upstream of the activity-dependent *BDNF* promoter IV in HEK293 cells, and increases transcription of a luciferase reporter DNA containing this intronic region, suggesting direct involvement of RAI1 in activity-dependent *BDNF* transcription (Figure 4B) [55,122].

Similar to RAI1, mouse models demonstrated the roles of MLL1/KMT2A in learning and memory and activity-dependent gene regulation. Heterozygous loss of *Mll1/Kmt2a* in mice leads to impaired fear memory [115]. Adenovirus mediated ablation of *Mll1* in adult prefrontal cortex was sufficient to cause working memory deficits as well as impaired short-term plasticity, demonstrating the roles of *Mll1* in plasticity of mature circuitry [68]. In this mouse model, learning-induced *Arc* expression was impaired, suggesting a role of MLL1 in activity-dependent gene expression [68]. Important future directions are to determine whether iBRAF, in complex with RAI1 or independently, mediates the recruitment of MLL1 to activity-dependent genes (Figure 4B); and how the LSD1–CoREST and RAI1 complexes interact to achieve timely control of activity-dependent genes.

Combining functional genomics and proteomics approaches will facilitate a deeper understanding of how chromatin regulatory machineries contribute to activity-induced transcriptional response genome-wide. Beyond the protein-coding genes, the inclusion of microRNAs and enhancer RNAs in such analyses will reveal more holistic views of activity-dependent processes [123,124]. An understanding of the histone methylation dynamics underlying the genomic response to neuronal activity will provide insights into molecular etiology of many neurodevelopmental conditions.

H3K4me dynamics in circadian rhythms

NDDs are commonly characterized by sleep abnormalities [125], which often manifest as atypical circadian rhythms. Similar to activity-dependent gene expression, circadian rhythms are the result of an exquisitely

controlled transcriptional process; therefore, dysregulation of histone methylation likely contributes to not only cognitive deficits but also sleep-related symptoms in NDDs. Indeed, mild-to-moderate sleep disturbances have been reported in several histone methylation-related conditions including Kleefstra syndrome (MIM: 610253, caused by haploinsufficiency of *EHMT1*, an H3K9 methyltransferase gene); Kabuki syndrome (MIM: 147920, associated with *KMT2D*, an H3K4 methyltransferase gene or *KDM6A*, an H3K27 demethylase gene); and RAI1-associated SMS and PTLs [60,125,126]. Some of these sleep disorders (e.g., in PTLs) involve obstructive sleep apnea or disordered breathing, which are possibly explained by craniofacial and respiratory abnormalities. However, sleep abnormalities in some NDD are attributable to altered circadian rhythmicity [125]. In this section, we summarize the roles of H3K4me regulatory factors in circadian rhythmicity and their clinical implications with a focus on the LSD1–CoREST and the RAI1-complexes.

Circadian rhythms represent an internal clock for animals to adapt their physiology to the day and night cycle. Recent transcriptome studies using all major organs in mice revealed that approximately 45% of protein-coding genes show circadian expression in at least one organ, and that 10% of genes oscillate in a given cell type [127,128], suggesting pervasive yet cell-type and tissue-specific variations in circadian gene regulations. The core of the oscillatory gene expression is a transcriptional negative feedback mechanism, whereby a heterodimer of the transcription factors CLOCK and BMAL activates transcription of their negative regulators *PER1/2* and *CRY1/2* in a 24 hour cycle [129].

Circadian gene regulation is associated with periodic relaxing and compacting of chromatin structure at gene promoters [130]. Several chromatin regulators, including CLOCK itself as a histone acetyltransferase, have been shown to contribute to the circadian transcriptional program [131]. In reminiscence of activity-dependent gene expression, H3K4me regulators that are associated with NDDs also appear to be key for circadian gene expression (Table 1). These include LSD1, MLL1 and RAI1, the central molecules in this review, as well as KDM5A, an H3K4 demethylase mutated in a recessive intellectual disability syndrome [11,37,69,129,130]. Therefore, sleep disturbance observed in these conditions may be a consequence of impaired circadian transcription.

Both LSD1 (KDM1A) and KDM5A appear to be directly involved in the core CLOCK–BMAL machinery of circadian transcription. LSD1 can be phosphorylated by PKC α at a specific serine residue (S112) in a circadian manner (Figure 4C) [37]. The phosphorylation of LSD1-S112 directly recruits

and/or stabilizes CLOCK–BMAL on chromatin, which in turn activates CLOCK-target genes. Replacement of wild-type LSD1 with the phosphorylation-deficient p.Ser112Ala mutant in mice resulted in impaired circadian rhythmicity at molecular and behavioral levels [37]. H3K4 demethylase KDM5A was also found to form a complex with CLOCK–BMAL and to activate their target genes [132]. Loss of KDM5A led to significantly decreased amplitudes of circadian gene oscillation in mouse fibroblasts and weakened periodicity of locomotor activity in a fly model [132]. Surprisingly, these circadian roles of both LSD1 and KDM5A are independent of their demethylase activities [37,132]. It remains to be determined whether LSD1 and KDM5A exert their roles by interacting with other transcriptional regulators in circadian transcription.

In contrast to the catalysis-independent roles of the demethylases, MLL1 appears to govern circadian oscillation by trimethylating H3K4 [69]. MLL1 was shown to be recruited to promoters via the CLOCK–BMAL complex, in turn activating circadian gene expression in mouse embryonic fibroblasts. In principle, rhythmic expression of genes requires gene activation followed by transcriptional repression. A more recent study showed that MLL1 can be acetylated at several lysine residues, and that the acetylation can be removed by the deacetylase SIRT1 in a circadian manner (Figure 4D) [70]. SIRT1-mediated deacetylation of MLL1 from residues K1130 and K1133 appears to diminish the H3K4 methyltransferase activity of MLL1. MLL1 regulates thousands of genes with broad functions, thus this work raised an exciting possibility that MLL1 generates circadian oscillation of the H3K4me landscape genome-wide, thereby controlling broader physiology in a circadian manner.

In the case of RAI1, clinical analyses first implicated its role in circadian rhythms. Every reported individual with SMS experienced subjective sleep disturbances such as daytime sleepiness and frequent night-time awakenings [133]. The production of melatonin appears to be a primary factor in the observed sleep abnormalities of SMS. Melatonin is a key hormone secreted by the pineal gland during the night, thereby synchronizing the circadian clock throughout the body [134]. While most individuals with SMS display an inverted secretion pattern of melatonin, in rare cases, a normal melatonin secretion pattern accompanies sleep disturbance [126,135]. This raises the possibility that melatonin-independent mechanisms underlie the sleep abnormalities in SMS. Indeed, heterozygous *Rai1* ablation in C57/BL6 mice, which are known to lack melatonin secretion, is sufficient to impair circadian behaviors [59].

Mechanistic studies further support the role of RAI1 in intrinsic circadian regulation, independent of melatonin-mediated endocrine system. *Rai1* knockdown in cultured cells led to reduced and shortened oscillation of CLOCK target genes [52]. The reduced expression of circadian genes was also observed in fibroblasts derived from individuals with SMS [52]. Furthermore, RAI1 was found to directly occupy an intron of the *CLOCK* gene and promote its transcription [52]. Given the aforementioned role of MLL1 in promoting the expression of circadian genes, determining the roles of the RAI1 complex in the MLL1-mediated circadian control (Figure 4D) will provide valuable insights into the mechanisms of sleep disturbance observed in SMS. More broadly, future studies are warranted to elucidate how the LSD1–CoREST and the RAI1 complexes interact to generate oscillation of circadian genes and how this process intersects with activity-dependent transcription.

Conclusion & future perspective

Dysregulation of histone methylation has emerged as a primary determinant of NDDs. However, investigations on the pathophysiology of these conditions are currently limited to focused approaches utilizing single-gene alterations. Many useful insights about individual proteins could be further leveraged by examining the complexes in which they function. Multiple NDDs associated with two chromatin modifying machineries, the LSD1–CoREST complex and the RAI1 complex, unexpectedly converge on a key chromatin modification, H3K4me. Specifically, LSD1-associated Kabuki-like disorder, Potocki–Shaffer, Smith–Magenis, Potocki–Lupski, Wiedemann–Steiner syndromes and Brachydactyly Mental Retardation might be mechanistically linked via H3K4me dynamics. Evidence supports a model in which the LSD1–CoREST complex removes H3K4me and thus opposes the RAI1 complex-mediated deposition of H3K4me during neurodevelopment. Experimentally testing this model will begin to unravel intricate transcriptional processes such as activity-dependent gene expression and circadian rhythms in the central nervous system, paving the path toward therapeutic intervention for a number of intertwined NDDs.

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Executive summary

- Genetic mutations in histone methyl regulators have emerged as a major causative agent of neurodevelopmental disorders (NDDs), including intellectual disabilities and autism spectrum disorders.
- Histone methyl regulators act within multisubunit complexes rather than in isolation.
- Histone H3K4 methylation (H3K4me) is an epigenetic signature associated with open chromatin.
- A number of NDDs might be mechanistically linked by the actions of the LSD1–CoREST complex and the RAI1-containing complexes at histone H3K4.
- The LSD1–CoREST complex demethylates H3K4me1/2, an active histone mark, in turn repressing neuronal genes in non-neuronal cells.
- All LSD1–CoREST complex components have been either genetically implicated in specific NDDs, including Kabuki-like syndrome (LSD1) and Potocki–Shaffer syndrome (PHF21A), or experimentally shown to be involved in neurodevelopment.
- iBRAF (HMG20A) is a negative regulator of the LSD1–CoREST complex during neuronal differentiation.
- iBRAF mediates recruitment of MLL1 (KMT2A), an H3K4me writer enzyme, to gene promoters.
- A proteomics study by Eberl *et al.* discovered that iBRAF is a component of the RAI1 complex containing RAI1, TCF20 and PHF14.
- The RAI1 complex components have been genetically associated with NDDs including Smith–Magenis syndrome and Potocki–Lupski syndrome.
- Evidence suggests a model in which the RAI1 complex promotes neuronal gene activation through iBRAF-mediated recruitment of MLL1, which in turn counteracts LSD1-mediated demethylation of H3K4me.
- LSD1, RAI1 and MLL1 play roles in activity-dependent gene expression and circadian gene expression, which may underlie the altered cognition and sleep behavior in individuals with NDDs.
- Mechanistic examinations of the chromatin regulatory complexes will likely illuminate underappreciated links between NDDs and provide hints for future therapeutic interventions.

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