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From neural development to cognition: unexpected roles for chromatin

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Abstract

Recent genome-sequencing studies in human neurodevelopmental and psychiatric disorders have uncovered mutations in many chromatin regulators. These human genetic studies, along with studies in model organisms, are providing insight into chromatin regulatory mechanisms in neural development and how alterations to these mechanisms can cause cognitive deficits, such as intellectual disability. We discuss several implicated chromatin regulators, including BAF (also known as SWI/SNF) and CHD8 chromatin remodellers, HDAC4 and the Polycomb component EZH2. Interestingly, mutations in *EZH2* and certain BAF complex components have roles in both neurodevelopmental disorders and cancer, and overlapping point mutations are suggesting functionally important residues and domains. We speculate on the contribution of these similar mutations to disparate disorders.

The specialization that cells must acquire to form an organism from a single zygote is achieved by stepwise changes in gene expression throughout the course of development. These changes occur in response to both extracellular signals and cell-intrinsic genetic circuitries. Chromatin regulators contribute to dynamic changes in gene expression but also maintain cell fates by providing stable, heritable states of gene expression[1–3]. Many chromatin regulators are essential for developmental processes, including the development of the brain, on which this Review focuses (FIG. 1).

At least three mechanisms regulate the assembly and biological states of chromatin. ATP-dependent chromatin remodellers alter the physical state of chromatin probably by moving nucleosomes in relation to the DNA or exchanging nucleosomes into and out of DNA[4,5]. Chromatin modifiers — enzymes that alter the tails of histones projecting from nucleosomes — control the accessibility of DNA to regulatory mechanisms[6,7]. Additionally, modified

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SUPPLEMENTARY INFORMATION
See online article: S1 (table) | S2 (table)

histones may recruit chromatin remodellers and other proteins to chromatin. Therefore, chromatin remodeller 'readers' and histone modification 'writers' and 'erasers' work in concert to regulate chromatin structure and gene expression. We refer to them collectively as chromatin regulators. DNA methylation also contributes to gene regulation; its involvement in neurodevelopmental disorders has previously been reviewed and is not discussed here[8].

Several major events and processes must be precisely orchestrated during normal brain development (FIG. 1); misregulation due to a genetic or environmental insult can result in cognitive deficits and other features of neurodevelopmental disorders. Insights into the role of chromatin in neural development are rapidly emerging from human disease studies using new sequencing and analytical technologies and from more traditional studies of model organisms. A consensus is emerging that chromatin regulatory mechanisms have a key role in many of the major events during neural development[9–13] (FIG. 1).

So far, dozens of mutated chromatin regulators have been causally implicated in human neurodevelopmental and psychiatric disorders, such as autism spectrum disorder (ASD) and schizophrenia (TABLE 1 and Supplementary information S1 (table)). Interestingly, mutations of several chromatin regulators discussed here in a neurodevelopmental context are also involved in human cancer; the implication of this coincidence will be speculated on, but it awaits future investigation (BOX 1).

In this Review, we highlight several chromatin regulators that were recently implicated in human disorders of brain function and that have not been extensively reviewed elsewhere. Additionally, genetic studies in model organisms have provided insight into their roles in normal development, which allows speculation on associated disease mechanisms. We also selected chromatin regulators for which mechanistic links through regulation of common pathways have been indicated. We first discuss selected chromatin remodellers, including SWI/SNF-like or *BRG1*- and *BRM*-associated factor (BAF) complexes and chromodomain helicase DNA-binding protein 8 (CHD8); then we discuss selected chromatin modifiers, including enhancer of zeste 2 (EZH2) and histone deacetylase 4 (HDAC4), speculating on underlying disease-causing mechanisms for these regulators on the basis of their known functions in neural development. Importantly, these four chromatin regulators are united by their genetic dominance in neurodevelopment, meaning that mutation in one allele can confer disease. We conclude our discussion with themes and interesting questions that have arisen from the study of chromatin regulators in neural development and disorders along with ideas for future research directions in this field.

Chromatin remodellers

The canonical role of chromatin remodellers is to alter the placement or position of nucleosomes through a catalytic process powered by ATP hydrolysis, thereby regulating transcription[4]. *In vivo*, however, there may be non-canonical roles for chromatin remodellers, such as regulating higher-order chromatin structure[14]. Recently, exome-sequencing studies have identified causative mutations in subunits of mammalian BAF complexes in patients with various neurodevelopmental and psychiatric disorders, as well as

de novo mutations in the chromodomain helicase DNA-binding protein (CHD) remodeller family in patients with non-familial ASD.

BAF complexes in neural development

Mammalian SWI/SNF-like BAF complexes are ATP-dependent chromatin-remodelling complexes that are made up of 15 subunits. The core ATPase subunit can be either of the two homologues BRG1 (also known as SMARCA4) or BRM (also known as SMARCA2), and the rest of the subunits are known as BAFs. Because 9 of the 15 mammalian subunits are not present in the yeast SWI/SNF complex and because some subunits are homologous to those that are exclusively found in other yeast chromatin-remodelling complexes, we refer to them as BAF complexes rather than SWI/SNF complexes to avoid bias and extrapolation. Mammalian BAF complexes have several subunit positions (such as the ATPase position) that can be filled by one member from an expanded gene family. Through incorporation of 29 different human gene family members by combinatorial assembly, hundreds of distinct complexes are predicted to form, and studies have shown unique assemblies and biological specificities in different tissues[15,16]. Functional specificity is thought to be an emergent feature of the complex, reflecting composite surfaces of adjacent subunits, which may mediate differential BAF genome targeting and/or interaction with distinct partners and signalling pathways.

BAF complexes have important developmental roles in several tissue types outside the nervous system[5]. This widespread role of BAF complexes could explain the syndromic features of neurodevelopmental disorders associated with BAF subunit mutations. Distinct BAF complexes have been shown to bind to and to coordinate with tissue-specific transcription factors to regulate gene expression in resident cells[15,17]. For example, in neural progenitors, neural progenitor BAF (npBAF) complexes interact with the repressive transcription factor REST and its co-repressor to facilitate the inhibition of neuronal genes, thereby maintaining neural progenitor identity[17].

BAF complexes have essential roles in the development of the mammalian nervous system. Mice that lack *Brg*[18], *Baf47* (also known as *Smarcb1*)[19] and *Baf155* (also known as *Smarcc1*)[20] die in pre- or peri-implantation stages. Targeted deletion of *Brg* in the developing nervous system produces mice with smaller brains that lack a cerebellum[16]. Heterozygous mice with null alleles of *Brg* or *Baf155* show defects in neural tube closure, indicating a dosage-sensitive role for BAF complexes in neural development[18,20]. Studies suggest that these defects might be due to failure of neural progenitor self-renewal and differentiation[16,21] (FIG. 2a). The underlying mechanisms could be similar to those in *Caenorhabditis elegans*, in which BAF subunits — SWSN-1, SWSN-4, LET-526 and PBRM-1, which are homologues of BRG, BRM, BAF155 and BAF250, respectively — contribute to asymmetric division of precursor-like T blast cells to generate neural cells[22,23].

During the development of the mammalian nervous system, an essential switch of BAF complex subunits occurs as neural progenitors exit mitosis and initiate differentiation[16] (FIG. 2a). The mammalian npBAF subunits BAF45A and BAF53A are necessary for neural progenitor proliferation, whereas the neuronal subunits BAF53B and calcium-responsive transactivator (CREST; also known as SS18L1) are required for activity-dependent dendritic

outgrowth and axonal development[16,21] (FIG. 2b). The function of BAF53B in dendritic morphogenesis cannot be replaced by BAF53A, demonstrating the functional specialization of different BAF complex subunits[21].

The switch of npBAF to neuronal BAF (nBAF) subunits is initiated when miR-9, miR-9* and miR-124 repress expression of BAF53A of the npBAF complex[24] (FIG. 2b). REST — a transcriptional repressor that is selectively repressed in postmitotic neurons — negatively regulates these microRNAs (FIG. 2a). This triple-negative circuitry, which leads to the npBAF–nBAF switch, appears to occur in all neurons, suggesting that it is a fundamental process of neural development[24]. Excitingly, recent studies showed that synergistic effects of miR-9, miR-9* and miR-124 can convert human fibroblasts to neurons[25] (FIG. 2b). This process recapitulates the BAF subunit switch to nBAF composition, suggesting that subunit switching has an instructive role in neuronal cell fate determination.

Recently, a demanding genetic screen for genes that produce perfect retargeting of olfactory dendritic trees to an incorrect glomerulus uncovered *Bap55*, the *Drosophila melanogaster* homologue of mammalian *BAF53A* and *BAF53B*. No other genes display this phenotype, and human *BAF53B* provides near-complete rescue of the phenotype, demonstrating its conserved function in dendritic targeting[26] (FIG. 2c). Additionally, subunits of BAF complexes were discovered in an RNA interference (RNAi) screen for genes necessary to form and maintain specific dendritic morphologies in *D. melanogaster*[27]. These studies strongly support an evolutionarily conserved role for BAF complexes in dendritic development and also suggest that some of the human diseases produced by mutation of BAF subunits (see below) are related to incorrect dendritic targeting.

BAF mutations in disorders of brain function

The importance of BAF complexes in neural development is further underlined by recent exome-sequencing studies (FIG. 2A; TABLE 1), which identified ~100 mutated BAF subunit alleles affecting human brain development and function. For example, recent studies revealed multiple protein-truncating *de novo* mutations of *BAF250B* (also known as *ARID1B*) as the genetic cause of Coffin–Siris syndrome (CSS), which is a rare autosomal dominant disease that is characterized by intellectual disability with marked language impairment, microcephaly, coarse facial features and hypoplasia of the nail on the fifth finger and/or toe[28]. Another study identified additional *de novo* mutations of *BRG*, *BRM*, *BAF47*, *BAF57* and *BAF250A* (also known as *ARID1A*) as the cause of CSS in 20 out of 23 patients (87%)[29]. Both *BAF250B* and *BAF250A* alleles probably cause haploinsufficiency as only nonsense and frameshift indel mutations were found in these patients. Conversely, *BRG*-, *BRM*-, *BAF47*- and *BAF57*-mutant alleles might be consistent with a gain-of-function or dominant-negative effect because all mutations are either missense or in-frame deletions.

The finding that mutations in multiple BAF subunits result in the same congenital syndrome underscores the fact that different subunits of BAF complexes coordinately regulate chromatin and gene expression as a functional unit. Because these mutations occur in common subunits of both npBAF and nBAF complexes, it could be speculated that some CSS phenotypes, such as microcephaly, may be attributable to the roles of npBAF complexes in neural progenitor proliferation and brain size observed in mouse models. In

addition, the role of nBAF in dendritic morphogenesis and neural circuit wiring may underlie intellectual disabilities in these patients (see above). The systemic symptoms are probably attributable to some of the above-mentioned BAF functions outside the nervous system.

In addition to CSS, multiple *de novo* missense mutations and in-frame deletions of *BRM* were found in 36 of 44 individuals with Nicolaides–Baraitser syndrome (NBS), which includes features of intellectual disability, again with marked language impairment, microcephaly, epilepsy and morphological defects[30,31]. As mutations of *BRM* in both CSS and NBS occur in conserved domains that are thought to be responsible for nucleotide binding or ATP hydrolysis, it is possible that these mutations generate structurally unchanged but functionally defective BAF complexes, which might functionally compete with wild-type complexes. Furthermore, as BRM is a core ATPase of the npBAF and nBAF complexes, its mutation may contribute to microcephaly and intellectual disability as described for CSS above.

Mutations of *BAF250B* can also cause intellectual disability with a lower burden of syndromic features in other tissues[32,33,34]. Two balanced chromosomal translocations that cause truncating mutations of *BAF250B* occur in patients with agenesis of the corpus callosum, intellectual disability and language defects[32,33]. In addition, various *de novo* nonsense mutations, gene deletion and exon duplication alleles of *BAF250B* that are predicted to cause loss of function were discovered as frequent causes of sporadic intellectual disability[34]. Patients in this study showed moderate to severe intellectual disability and speech impairment but no agenesis of the corpus callosum. These studies suggest that haploinsufficiency of *BAF250B* underlies the aetiology of intellectual disability. The differing disease outcome of *BAF250B* haploinsufficiency in these patients compared to the syndromic intellectual disabilities discussed above could be due to genetic background and environmental factors.

De novo mutations of *BAF155*, *BAF170* (also known as *SMARCC2*), *BAF180* (also known as *PBRM1*) and *BAF250B* were noted in non-familial ASD[35,36]. ASD is characterized by impaired social interaction or communication and repetitive behaviours, sometimes accompanied by intellectual disability, with symptoms appearing in early childhood. Although these mutations each appeared only once in the ASD cohorts[35,36], because the subunits are a part of one functional unit, BAF complex mutation is important. Mutations of *REST*, which is a part of the triple-negative circuitry leading to the switch of npBAF to nBAF subunits, were also identified in the same study, suggesting that the switching of BAF complexes in neural development contributes to autism.

Some cases of ASD are associated with symptoms that are apparent from birth, indicating that prenatal neurodevelopmental abnormalities have occurred, possibly through defective functions of npBAF complexes in neural progenitors. Another contributor to ASD characteristics might be postnatal synaptic dysfunction[37,38], possibly caused by dysfunctional nBAF complexes, which regulate dendritic targeting and neural circuit formation[21,26]. Additionally, a potential connection between BAF complexes and CHD

family proteins in modulating the WNT/β-catenin signalling pathway might be implicated in ASD (see 'The role of CHD8 in ASD' section below).

Two *BRM* risk alleles were identified in a genome-wide association study of schizophrenia single-nucleotide polymorphisms (SNPs) in a Japanese cohort[39]. Schizophrenia is a thought disorder that usually appears post-adolescence and is characterized by hallucinations, paranoia, delusions and disorganized speech. Reducing BRM levels in mouse cortical neurons leads to abnormal dendritic spine morphology[40], a function that is controlled by nBAF complexes in postmitotic neurons. *Brm*-knockout mice showed impaired social interaction and prepulse inhibition[39]. These are both features of schizophrenia in humans that are thought to result from abnormalities in synaptic maturation, connectivity and plasticity[40], which are processes that are influenced by nBAF function. Functional interactome studies using bioinformatics showed that *Brm* forms a network with the eight other genome-wide-supported schizophrenia-associated genes, further suggesting the potential involvement of *Brm* in schizophrenia[40]. These studies suggest that large-scale screening of schizophrenic populations for mutations in BAF subunits would be informative.

The discovery of human genetically dominant alleles of BAF subunits aligns with the heterozygous defects in mouse neural development discussed above and demonstrates a dosage-sensitive mechanism for BAF complex function in uncharacterized but crucial aspects of chromatin regulation during neural development. Perhaps the most interesting route to genetic dominance would be that these mutations are haploinsufficient by virtue of being involved in a rate-limiting process in neural chromatin regulation. The observation that directing the assembly of neuron-specific nBAF complexes can convert fibroblasts to functional neurons is consistent with their possible rate-limiting role in neuronal fate determination[25], because rate-limiting roles often define decision points and hubs of regulation in biochemical cascades. Because several of the subunits implicated in neurodevelopmental disorders are not required for *in vitro* nucleosome remodelling, other functions, such as genomic targeting or opposition of Polycomb-mediated repression (see 'The role of EZH2 in development' section below), might contribute to these diseases.

The role of CHD8 during development

Another ATP-dependent chromatin remodeller for which genetic and biochemical studies have led to recent insights is CHD8. *Chd8*-knockout mice die around embryonic day 5.5 owing to widespread p53-induced apoptosis[41], whereas in a WNT-responsive human colorectal carcinoma cell line, *Chd8* knockdown leads to derepression of WNT/β-catenin pathway target genes[42]. Mammalian CHD8 is thought to repress β-catenin and p53 target genes by recruiting linker histone H1 (REFS 41,43,44).

In *Xenopus laevis* embryos, the *CHD8* homologue *Duplin* negatively regulates WNT/β-catenin signalling, especially in the context of head and brain development[43,45]. The WNT/β-catenin signalling pathway also has important roles in mammalian neural development: increased dosage and knockout of the β-catenin protein in the brain lead to central nervous system defects[46–48]. There are not yet any genetic models to test the role of CHD8 in WNT/β-catenin signalling directly in mammalian brain development, although the above studies indicate that it may play an important part. Peptides from CHD8 were

discovered in a proteomic analysis of mouse embryonic stem cell (ESC) BAF complexes, so the two may functionally interact[15]. Interestingly, BAF complexes interact with β-catenin and are important for activating β-catenin target genes[49]. Thus, BAF and CHD8 appear to regulate WNT/β-catenin signalling reciprocally, perhaps providing important modulation of this essential neural development signalling pathway[48,50].

The role of CHD8 in ASD

Recent large-scale sequencing studies have uncovered a handful of genetic risk factors for autism[35,36,51–53], many of which were present just once among the sequenced populations, making it difficult to determine whether they have any link to autism. Mutations in *CHD8* and a few other factors, however, appeared in multiple cases from the patient cohorts. In addition to 13 mutated alleles of *CHD8*, a single mutant allele of *CHD1*, *CHD2*, *CHD3*, *CHD5* and *CHD7* each has been found in the same studies[35,36,51] (TABLE 1 and Supplementary information S1 (table)). This number of *de novo CHD8* mutations is highly unlikely by chance and means that mutation in one copy of *CHD8* and maybe other members of the CHD family contribute to the risk for autism. So far, most of the mutations found in *CHD8* are nonsense or frameshift indels that are thought to cause loss of function, suggesting haploinsufficiency to be the pathogenic mechanism. Macrocephaly was a common phenotype among the 12 CHD8 patients with ASD who had reported head circumference measurements[36,51,52]. Macrocephaly is estimated to occur in 15–35% of autistic children[54], so this commonality could not be coincidental. If *CHD8* function is impaired in these autism patients, one might expect an upregulation of β-catenin-regulated genes. When occurring in certain areas of the brain and/or in neural precursors, this might lead to a brain overgrowth phenotype that is similar to expanded cortex caused by expression of stabilized β-catenin in mouse brain[46]. Indeed, WNT/β-catenin-signalling pathways were previously implicated in the aetiology of autism owing to the prevalence of macrocephaly and genetic linkage of some common variants in the signalling pathway to autism[50]. As mentioned above, BAF complexes are also required for WNT/β-catenin signalling[49], suggesting that CHD8 and BAF might be mechanistically linked in their contribution to ASD development.

The level of contribution of CHD8 to these particular cases of autism is unclear, as *CHD8* mutations may be dominant and causal or a part of a polygenic contribution to this complex disorder. More genome-sequencing studies, in combination with bioinformatics and mathematical analyses, will allow the identification of epistatic interactions and genetic networks underlying ASD. However, these mutations suggest that chromatin remodellers are important for normal neural development owing to their ability to regulate the downstream targets of developmental signalling pathways. It is also possible that after biochemical characterization of CHD8-containing complexes, associated proteins and subunits will emerge as contributors to autism.

Chromatin modifiers

Chromatin modifiers are the ‘writer’ and ‘eraser’ enzymes that post-translationally alter histone proteins, mainly at their unstructured tails, by adding or removing acetylation, methylation, ubiquitylation and phosphorylation[6,7,55]. Certain modifications are associated

with repressive chromatin structure (for example, histone H3 at lysine 27 (H3K27) and H3K9 methylation), whereas others are associated with greater chromatin accessibility (for example, acetylated histones and H3K4 methylation). The discovery of histone deacetylases[56] and demethylases[57] began a transition in chromatin biology from a view that chromatin modifications were epigenetic by virtue of largely being irreversible to the present concept that epigenetic states are actively maintained. This view is supported by the discovery that histone acetylation and methylation are rapidly reversible in living cells and require active maintenance[58,59]. It is important to note that histone modification enzymes have also been reported to modify non-histone proteins, so the complete identification of substrates will be important.

Many chromatin modifiers have been implicated in human neurodevelopmental and psychiatric disorders (TABLE 1 and Supplementary information S1 (table)), including the histone acetyltransferases CBP and p300, and the methyl-CpG-binding repressor MeCP2 in Rubinstein–Taybi syndrome and Rett's syndrome, respectively; their mechanistic roles have been well-reviewed[60–62]. For many modifiers, however, studies are lagging in terms of understanding their functional involvement in neurodevelopmental processes. Here, we will focus on two chromatin modifiers associated with gene repression: EZH2 and HDAC4. We chose these modifiers because emerging evidence of their functional roles is allowing speculation on their mechanistic roles in human mental disorders. Additionally, EZH2 and BAF complexes have opposing roles in chromatin regulation and are both linked to WNT/β-catenin signalling, like CHD8, so comparing their involvement in neurodevelopmental mechanisms will be informative. In addition, studies have shown that blocking HDAC activity with inhibitors facilitated improved learning and memory in mice, and as mentioned before, HDACs can contribute to dynamic chromatin changes, so speculating on the role of an HDAC in cognitive aspects of neurodevelopmental disease is timely and will help to illuminate the role of dynamic chromatin in brain function.

The role of EZH2 in development

The EZH2 enzyme is a Polycomb group protein subunit of the Polycomb repressive complex 2 (PRC2), which methylates H3K27. This methylation mediates recruitment of additional PRC2 and in some cases PRC1 (which ubiquitylates histone H2A), resulting in propagation of repressive chromatin both spatially along the chromosome and through cellular generations[63,64]. Thus, these two complexes can function together to provide stable, heritable gene repression, and the PRC1 complex may also create a more compact chromatin structure[65].

The gene repression mediated by Polycomb complexes is important in many developmental processes, including embryonic mouse development and maintenance of stem cell identity and execution of differentiation programmes[66–68]. In one conditional knockout model that provides almost complete *EZH2* deletion by the onset of neurogenesis around day 12.5, there was an acceleration of neurogenesis in the cerebral cortex: more rapidly cycling neural precursors, an early increase in neuron numbers and precocious production of astrocytes[69]. However, this led to a decreased number of neurons at birth, possibly owing to early exhaustion of neural precursor cells. By contrast, when *EZH2* is deleted at a later time in

neural precursors, close to the shift from neurogenesis to astrogenesis, precursors continued to produce neurons rather than shift to astrocyte production. This was posited to result from lack of repression of the WNT/β-catenin target neurogenin 1, which is normally repressed by EZH2 in neural precursors[70]. Thus, EZH2 has a timing-dependent role in neurogenesis. It is not known whether EZH2 plays an important part in postmitotic neurons. Another link between Polycomb repression and WNT/β-catenin targets was found in the self-renewal of *D. melanogaster* ovary follicle stem cells (FSCs). In the FSCs, *D. melanogaster* Polycomb complex components antagonize the WNT/β-catenin signalling pathway to prevent tumour-like growths of self-renewing FSCs at ectopic sites[71].

Whereas Polycomb complexes have been shown to repress WNT/β-catenin targets[70,71], BAF complexes are co-activators of this pathway and directly interact with β-catenin[49]. Polycomb and BAF complexes have antagonistic roles in relation to WNT/β-catenin signalling in *D. melanogaster*, paralleling their direct antagonistic relationship described in ESCs at leukaemia inhibitory factor (LIF) signal-activated genes[72]. Similar antagonistic relationships between these complexes probably exist in the developing nervous system, where WNT/β-catenin activity drives neurogenesis[46].

The role of EZH2 in Weaver's syndrome

Weaver's syndrome is an autosomal dominant disease characterized by learning disabilities, dysmorphic facial features and general overgrowth, which can include tall stature, obesity and macrocephaly[73]. Recent exome-sequencing studies identified numerous *de novo* and familial missense mutations and in-frame deletions of one copy of *EZH2* in all seven sequenced patients with Weaver's syndrome[74,75]. In a larger cohort of 300 patients, some diagnosed with Weaver's syndrome and others with a nonspecific overgrowth syndrome, targeted EZH2 sequencing revealed another 15 likely pathogenic mutations[75]. As most mutations occur at conserved residues in the catalytic SET domain, it is likely that these mutations are dominant-negative, although the mutations could also result in loss of function and haploinsufficiency if there is a crucial dosage requirement for EZH2. As *EZH2* mutations were not uncovered in all overgrowth patients from the larger cohort, other Polycomb components or other genes may eventually be implicated in causing this syndrome.

The macrocephaly phenotype in Weaver's syndrome patients with *EZH2* mutations is especially interesting in light of the microcephaly in NBS and CSS patients with different BAF subunit mutations owing to the antagonistic relationship between the BAF and PRC2 complexes. Reciprocal regulation of WNT/β-catenin targets, especially those that are crucial for the proliferation of neural progenitors, is one likely mechanism for these opposing phenotypes (FIG. 3B). Indeed, as macrocephaly was also noted in 12 ASD patients with *CHD8* mutations, and as CHD8 is known to repress WNT/β-catenin targets, it seems likely that imbalance of WNT/β-catenin signalling is a key mechanism leading to the opposing micro- versus macrocephaly phenotypes in NBS, CSS and Weaver's syndrome[29,30,48]. This speculation will require much validation but would be a fertile area for future work.

The role of HDAC4 in learning and memory

HDAC4, which is highly expressed in early postnatal mouse brain, is a class IIa HDAC that shuttles between the cytoplasm and nucleus in response to calcium-regulated physiological signals[76,77]. The measured histone deacetylase activity of vertebrate class IIa HDACs is very weak compared to invertebrate forms and to class I HDACs owing to a tyrosine to histidine substitution in the enzyme active site[78]. HDAC4 and other class IIa HDACs may have a non-classical enzymatic activity, but specific targets remain unknown. Despite low histone deacetylase activity, HDAC4 associates with chromatin and developmental transcription factors and is able to mediate gene repression possibly by recruiting co-repressors such as class I HDACs and heterochromatin protein 1 (HP1)[79,80].

Conditional deletion of HDAC4 in mouse forebrain neurons leads to impaired learning, memory and long-term synaptic plasticity[81], in contrast to global HDAC inhibition or HDAC2 deficiency during embryonic development, which led to improved memory and synaptic plasticity[82,83]. Transgenic mice that express a truncated *HDAC4* allele lacking the deacetylase domain and nuclear export signal exhibited defects similar to conditional deletion[84].

Myocyte enhancer factor 2 (MEF2), which is a transcription factor that promotes neuronal survival and synaptogenesis in response to activity-dependent and neurotrophin stimulation[85], may be involved in the phenotypes produced by *HDAC4* deletion in the central nervous system (FIG. 3). MEF2 directly interacts with HDAC4 and is also highly expressed in the mammalian brain. In response to presynaptic glutamatergic inputs, calcium-regulated kinases phosphorylate HDAC4, leading to its nuclear export. MEF2 target genes are repressed by HDAC4 binding, so the calcium-stimulated HDAC4 nuclear export enables MEF2 to activate transcription[77]. HDAC4 and MEF2 antagonistically regulate a common set of genes in cortical neurons that are crucial for synaptogenesis and plasticity of synapses[84,86]. The HDAC4 catalytic domain is dispensable for this repression, which may be mediated instead by its recruitment of co-repressors such as class I HDACs and HP1 (REFS 79,80). *In vitro* electrophysiological tests of cortical neurons indicate that constitutively nuclear HDAC4, with or without the HDAC domain, causes decreased amplitudes of excitatory postsynaptic currents owing to decreased synaptic strength rather than synapse number[84]. Additionally, HDAC4 was shown to have a neuroprotective function in cortical cultures that was not dependent on its deacetylase domain or nuclear gene repression but rather on its presence in the cytoplasm[84].

The role of HDAC4 mutations in BDMR syndrome

Recently, mutations and deletions in one allele of *HDAC4* were found to be responsible for the brachydactyly mental retardation (BDMR) syndrome, which is characterized by intellectual disability, ASD, developmental delay, behavioural abnormalities, sleep disturbance, skeletal abnormalities, dysmorphic features, cardiac defects and obesity[87]. Overlapping *de novo* 2q37.3 chromosomal deletions in multiple patients causing ~50% HDAC4 expression refined the crucial region for BDMR syndrome to the *HDAC4* gene. Targeted sequencing of the gene in two further patients revealed that smaller truncating mutations also gave rise to BDMR syndrome[87,88].

Another report investigated an instance of inherited BDMR syndrome in which a mother with HDAC4 levels at 67% of normal controls and mild BDMR syndrome symptoms gave birth to a son with 23% HDAC4 levels and a more severe phenotype[88]. This lends support to the idea that HDAC4 has dosage-sensitive roles and that haploinsufficiency can be a cause of BDMR syndrome. At least one of the truncating mutations identified by targeted *HDAC4* sequencing, however, did not cause reduced HDAC4 expression. Instead, this allele produced a constitutively nuclear HDAC4 that lacked the deacetylase domain[87]. In mice, expression of a similar truncated allele caused learning, memory and long-term potentiation (LTP) defects[84]. It seems that reduced HDAC4 dosage or constitutive nuclear activity can result in similar phenotypes — a theme noted previously for other genes involved in intellectual disability[89]. Altered HDAC4 activity or dosage may result in BDMR syndrome through misregulation of MEF2 proteins in the developing and adult brain, giving rise to specific defects in neuronal survival, synaptogenesis and synapse strength, as discussed above (FIG. 3a). Interestingly, MEF2 and HDAC4 are also co-expressed outside the brain, in skeletal and cardiac tissues, where major non-neurological features of BDMR syndrome originate.

Conclusion

Human genetic studies have discovered mutations in chromatin regulators in a wide variety of human mental disorders, raising the question of why mutation of these often ubiquitous proteins can lead to selective defects in the nervous system. Although the answer to this question is not clear, it may be that this selective requirement arises from the need to generate hundreds of different cell types in the nervous system. The myriad functions of this array of neuronal cell types are tightly linked to the stability of their morphologies. Hard-wired neuronal circuitry derived from enormous numbers of cells, distinct morphologies and complex connections of neurons almost certainly require stable neuron-specific gene expression. Thus, the frequency of chromatin regulatory mutations that underlie mental disorders may point to an unappreciated role in producing stable levels of gene expression and reducing transcriptional noise that is necessary to maintain stable morphologies over many years. However, the functional tasks of learning and memory require flexible fine-tuning of neuronal circuitries and synaptic plasticity, which might rely on dynamic changes in chromatin structure[90–94]. Thus, creating a balance between flexibility and stability might be a feature of chromatin regulation that becomes particularly important in the development of the nervous system.

Many of the recently uncovered mutations in chromatin regulators appear to be genetically dominant. Genetic dominance can have many different mechanistic underpinnings, including: the production of dominant-negative proteins that can block wild-type protein function; abnormal polymerization processes in which one mutated protein blocks polymer extension; and haploinsufficiency due to mutation of a gene encoding a protein that is involved in a rate-limiting process. ATP-dependent chromatin remodelling is well suited to a rate-limiting role in biological processes, as the rate of chromatin alteration may be limited by the intrinsic rate of energy use by these complexes rather than by ATP levels, which are generally not rate-limiting. The ability of nBAF complex reconstitution to reprogram

fibroblasts into human neurons is consistent with a rate-limiting role for BAF complexes, but how this role is involved in development and human disease needs further investigation.

The fact that *EZH2* mutations also give rise to genetically dominant diseases suggests that the crucial mechanism might be balancing the antagonism between BAF and Polycomb complexes. The opposition was first noted in flies at the homeobox (Hox) genes[95], and recent studies in mice have confirmed the conservation of this relationship[72]. Although the opposing roles of Polycomb and BAF are emerging from studies of both neurodevelopment and cancer[96], we have very little understanding of the underlying biochemical mechanisms. Understanding the balance between these complexes will be crucial to understand fully the growing number of human diseases that arise owing to the genetic disruption of these complexes.

In BOX 1, the known human mutations in *BRG*, *BRM*, *BAF250A*, *BAF250B* and *EZH2* are shown. These mutations demonstrate that BAF complexes based on BRM and BAF250B have crucial roles in human neural development, whereas BAF complexes based on BRG and BAF250A may have more important roles in tumour suppression. As mutations in *EZH2*, *BRG*, *BRM*, *BAF250A*, and *BAF250B* can result in both neurodevelopmental disorders and cancer, it is likely that dosage sensitivity of these proteins is resulting in disparate effects in the context of different developmental stages and cellular environments. The ability of similar mutations in the same protein to produce different diseases probably arises from different genetic backgrounds. For example, we speculate that *BAF250A* mutations in the context of additional mutations in cell cycle regulatory or checkpoint genes might lead to a loss of tumour suppression, whereas the same or similar *BAF250A* mutations in the presence of weak alleles of proteins that encode synaptic or dendritic components might lead to intellectual disability. Thus, searching the exome-sequencing databases for these second site mutations may prove useful.

If it is assumed that chromatin remodellers provide balance to systems that are characterized by processes such as acetylation–deacetylation or methylation–demethylation, then further genetic definition of crucial chromatin regulators in human disease could reveal potential therapeutic targets as their activity can be modulated by small molecules (reviewed in REF. 11; see especially Table 2 in this reference). It could be envisioned that these small molecules be used to reinstate the proper balance between chromatin regulatory activities. Furthermore, a full mechanistic understanding of chromatin regulators in neurodevelopment is necessary to provide effective ways of making human neurons from other cell types. The creation of patient-specific neural cells as therapeutic models and the development of effective neural replacement strategies are two possible goals[97–101].

As the feasibility of large genomic sequencing studies rapidly increases, along with our abilities to analyse, process and store the corresponding data, we should expect that more mutated chromatin regulators, and the genetic background in which they exist, will be defined. This approaching era should provide insight into actual human disease mechanisms, an area in which animal models, although immensely helpful, are not sufficient; genome sequencing will uncover the proteins and domains therein that are most relevant to human disease. As human genomic efforts reveal important functions for new genes in neural

development, basic genetic and biochemical research will need to keep pace to elucidate the underlying mechanisms. We are hopeful that these two lines of research will lead to new insights into the development and function of the human brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Glossary

Exome sequencing	Targeted sequencing of protein-coding regions of the genome (that is, exons). It is a cheaper yet still effective alternative to whole-genome sequencing to identify clinically relevant gene variations that are responsible for both Mendelian and common diseases but does not detect mutations in non-coding regions of the genome
Glomerulus	The glomerulus is a spherical structure located in the olfactory bulb. Each glomerulus is thought to receive input from olfactory receptor neurons (expressing only one type of olfactory receptor). They relay this information into higher brain structure through projection neurons
Autosomal dominant disease	Refers to a disease arising from mutations on non-sex chromosomes that are genetically dominant
Microcephaly	Microcephaly is a significantly smaller head measurement. It can be caused by an abnormal brain size due to loss of any number of cell types or brain features and can even be caused by abnormal ventricular spaces or cerebral fluid
Haploinsufficiency	When the product of one normal allele of a gene is not sufficient to allow the normal function of a gene to be executed. This is another possible cause of genetic dominant diseases
Frameshift indel	An insertion and/or deletion mutation that changes the reading frame of a protein and creates an altered gene product
Dominant-negative	Interference with the function of the normal allele of a gene by a mutated allele. This usually occurs when the mutant product can still interact with the same elements as the wild-type product, but block some aspect of its function. This is another possible cause of genetic dominant diseases

Corpus callosum	A wide flat bundle of neural fibres that connects the left and right cerebral hemispheres and facilitates interhemispheric communication
***De novo* mutations**	Alterations in genes that are present for the first time in one family member as a result of a mutation in a germ cell (that is, an egg or sperm) of one of the parents or in the fertilized egg itself
WNT/β-catenin signaling	The binding of WNT ligand to receptor Frizzled leads to a cascade of events allowing for β-catenin (an integral cell–cell adhesion adaptor protein as well as transcriptional co-regulator) stabilization, nuclear translocation and transcriptional activation. The WNT/β-catenin pathway integrates signals from many other pathways, including retinoic acid, FGF, TGFβ and BMP in many different cell types and tissues
Single-nucleotide polymorphisms	(SNPs). DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome or other shared sequence differs between members of a biological species or paired chromosomes in an individual
Linker histone	The linker histone H1 binds the nucleosome and the entry or exit sites of the DNA, allowing for the formation of higher-order chromatin structures that are thought to lead to chromatin compaction and gene repression
Macrocephaly	Macrocephaly is a significantly larger than average head circumference measurement. It can be caused by an abnormal brain size due to gain of any number of cell types or brain features and can even be caused by abnormal ventricular spaces or cerebral fluid
Polycomb group proteins	These conserved proteins form multimeric complexes that exert their functions by modifying chromatin structure and by regulating the deposition and recognition of multiple post-translational histone modifications. Their epigenetic role appears to arise from their ability to propagate a repressive chromatin modification over several kilobases of DNA

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Box 1

Coincidence of mutations in cancer and neurodevelopmental disorders

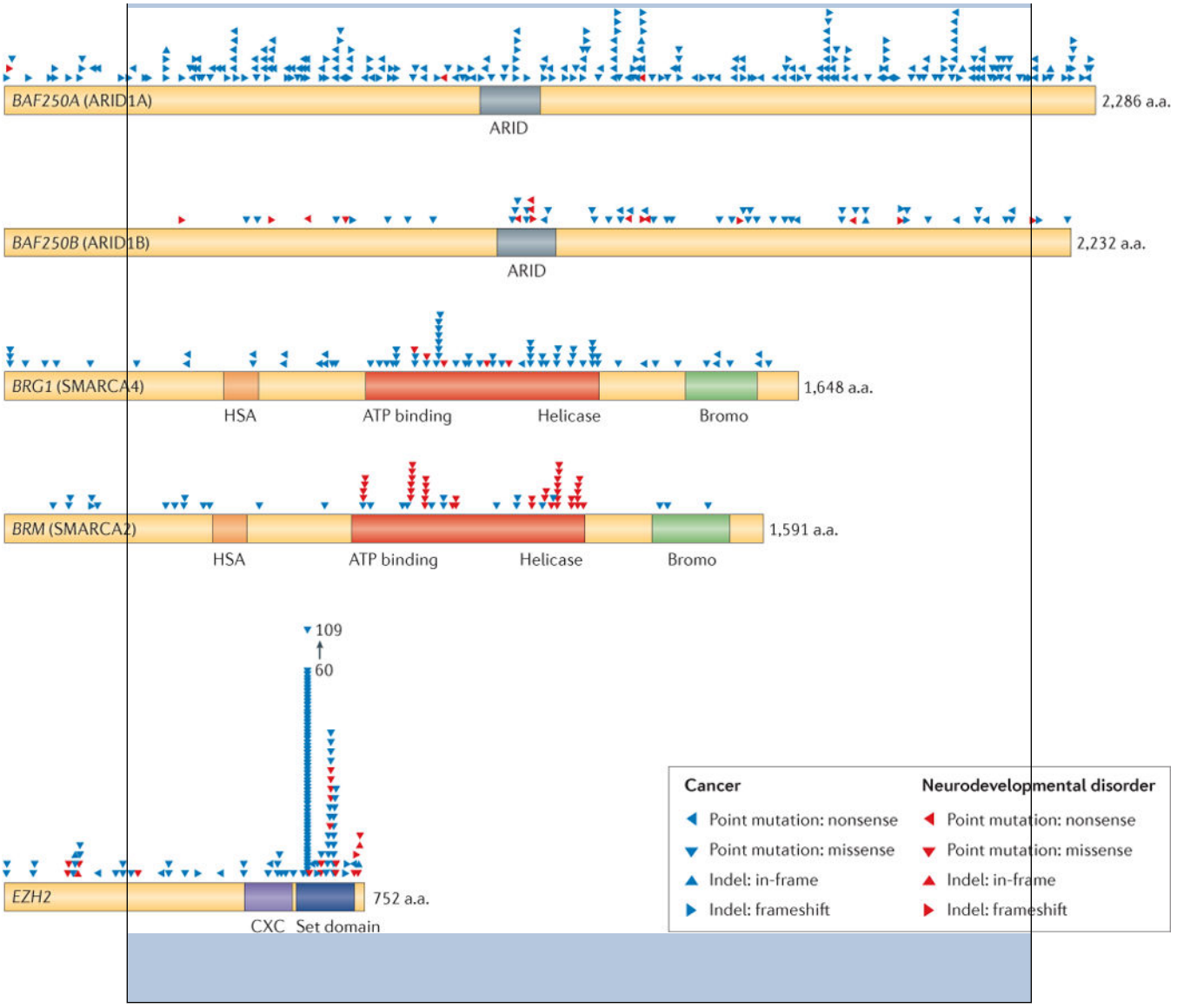
Recently, 44 human genome-sequencing studies have implicated mutations in BAF subunits as potential drivers in human cancer, including some neurological cancers. So far, more than 20% of human cancers are estimated to have mutations in BAF subunits, making BAF complexes the most commonly mutated chromatin regulator in human cancer[102,103]. Strikingly, many of the mutations are similar to those in neurodevelopmental disorders.

BAF250A is the most commonly mutated BAF subunit in human cancer[103–105]. Frameshift and nonsense mutations of *BAF250A* and *BAF250B*, which occupy the same subunit position, are widespread, indicating that there is probably a loss of function for many alleles (see the figure). However, the reported missense mutations may highlight regions of BAF250A and BAF250B with unknown function and should guide further studies of shared and disparate roles of the two homologues. BAF250 subunits are not necessary for *in vitro* chromatin remodelling, so novel mechanistic studies may be required to understand the roles of these subunit homologues in neurodevelopment and cancer.

BRG and *BRM* missense mutations in cancers, Coffin–Siris syndrome (CSS) and Nicolaides–Baraitser syndrome (NBS) highlight the ATPase and helicase domain as a hotspot for genetically dominant, probably dominant-negative, mutations (see the figure). The concentration of mutations in this domain reinforces its fundamental cell biologic role. *BRG* and *BRM* are highly homologous and are co-expressed in many tissues, including the brain, yet there are no reported *BRG* mutations in NBS and no reported *BRM* mutations in CSS. *BRG* is more frequently mutated in cancer, including medulloblastoma, which is a brain cancer. It seems likely that BRG- or BRM-containing complexes may mediate different functions in neural development, as very similar mutations in each protein lead to different diseases.

Enhancer of zeste 2 (*EZH2*) mutations have been implicated as drivers of myeloid malignancies[106]. Some of the germline *EZH2* mutations identified in patients with Weaver’s syndrome are identical to those that are found in somatic cancer mutations. It is not clear whether tumorigenesis is common in Weaver’s syndrome, as it is a rare syndrome, and most reports include only young patients. Analysis of *EZH2* missense mutations in cancer and Weaver’s syndrome reveal that the carboxy-terminal SET domain, the methyltransferase, is most highly mutated (see the figure), reinforcing its important biological role.

The group of mutations in the figure highlights the mystery of how mutational context can lead to very different outcomes. The context of mutations, including timing, genetic background and cell type, may contribute to the differing disease phenotypes, despite similar protein defects. More information about the displayed mutations can be found in Supplementary information S2 (table).



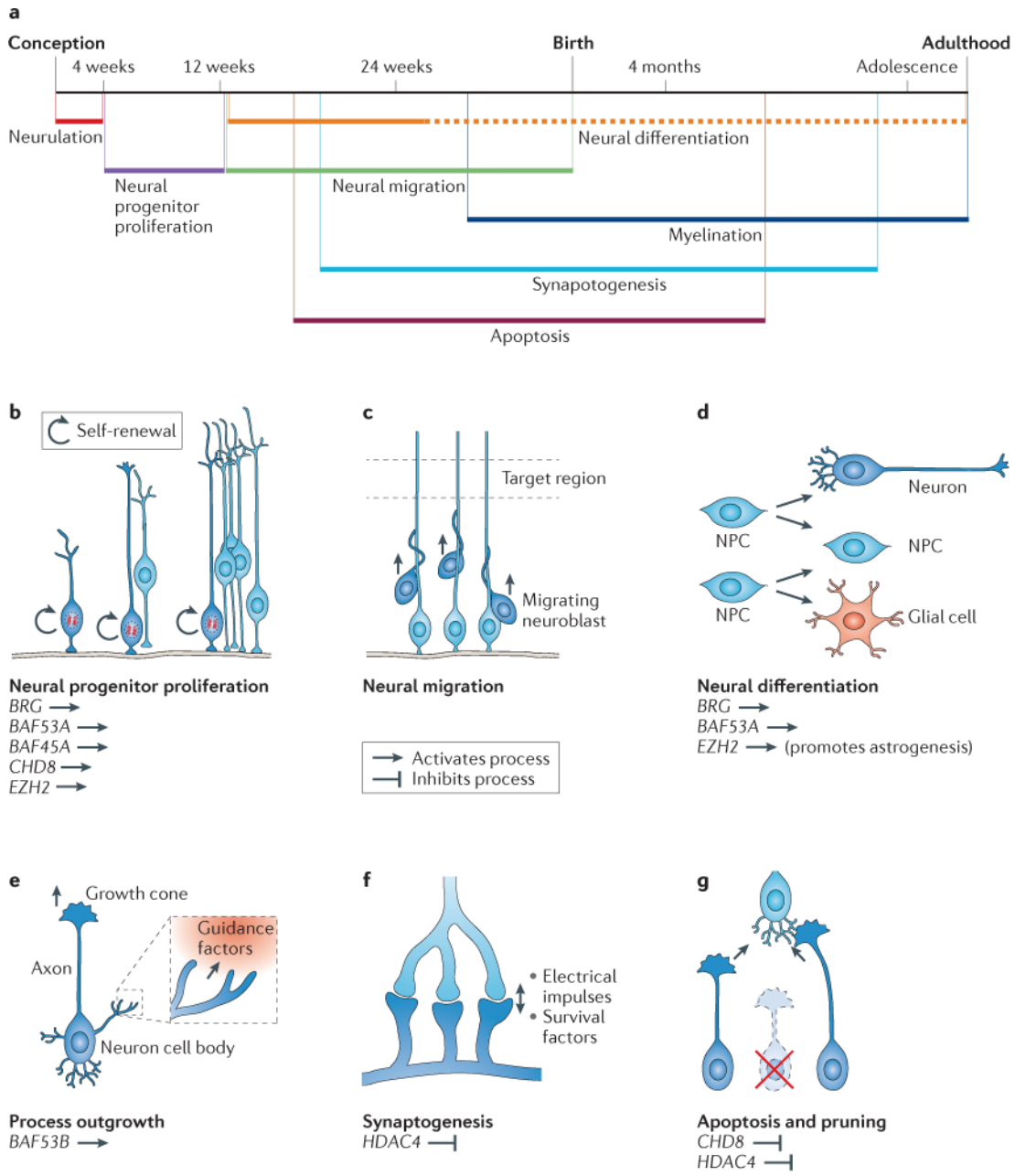


Figure 1. Chromatin regulators have essential roles throughout neural development

The fundamental processes of neural development are illustrated. Chromatin regulators discussed in this Review are noted under the processes in which they have important roles. The key indicates whether a particular regulator promotes or inhibits each neurodevelopmental process. **a** | A timeline of human neural development. **b** | The development of the vertebrate nervous system begins during gastrulation. In the early embryo, neural progenitor cells undergo symmetrical proliferative division. **c** | With the expansion of the number of cell types and the size of the nervous system, the cell bodies of both neural progenitors and resulting postmitotic neurons migrate away from their birthplace to appropriate regions in response to environmental cues. **d** | Neural progenitors asymmetrically divide to give rise to neurons, glial cells or intermediate progenitors. Neural differentiation generates enormous numbers of diverse cell types in the nervous system. **e** | After migrating neurons have reached their destinations, they extend axonal and dendritic processes, which are guided by intricate cellular interactions and guidance molecules to appropriate

target regions, where they further elaborate processes to cover receptive fields and innervate targets. **f** | Mature synapses are formed between neurons that are connected to each other. Synaptogenesis begins during embryonic development, but subsequent synaptic stabilization and plasticity occur throughout life and are adaptive to learning experiences and other activity-dependent environmental inputs. **g** | Active apoptosis and local degenerative pruning events maintain and refine established neuronal morphologies and neural circuit assembly. NPC, neural progenitor cell. Part **a** is modified, with permission, from REF. 107 © American Psychiatric Association.

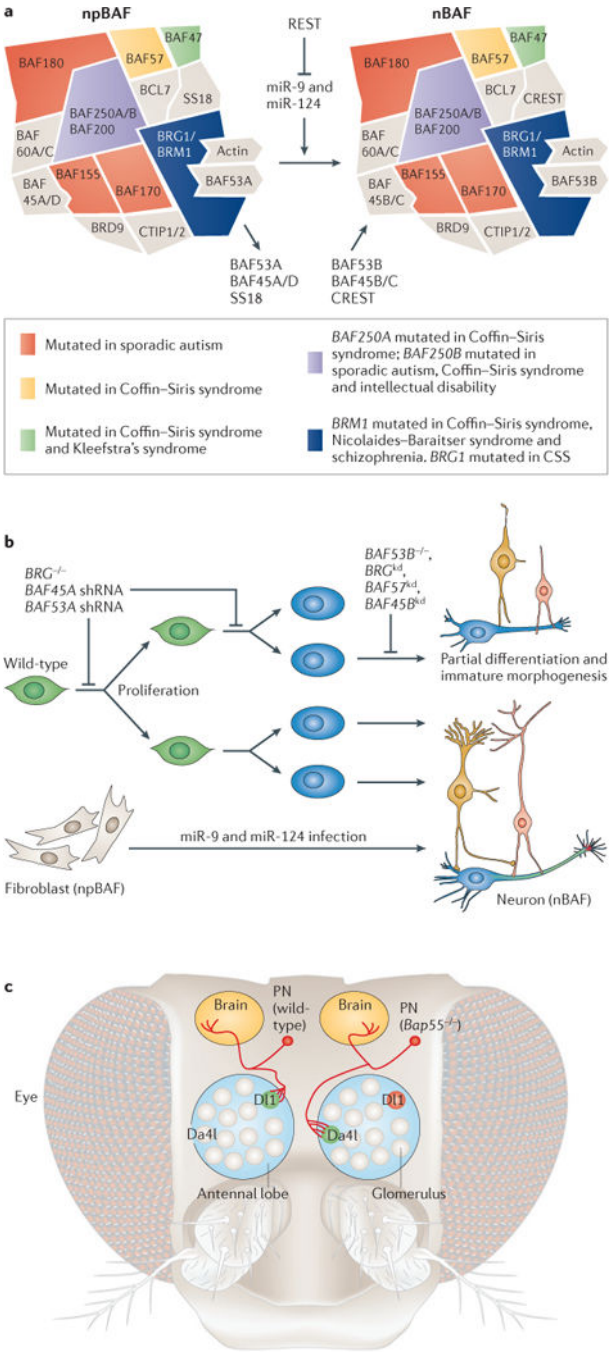


Figure 2. BAF complex roles in neurodevelopment and disorders of brain function

a | The composition of neural progenitor BAF (npBAF) and neuronal BAF (nBAF) complexes is indicated, along with the triple-negative genetic circuit that leads to npBAF to nBAF switching and their involvement in various disorders (right). **b** | Knockout (−/−) of *BRG* and knockdown (kd) of npBAF subunits *BAF45A* and *BAF53A* in neural progenitors (green cells) impede neural progenitor self-renewal and differentiation into postmitotic neurons (blue cells). Loss-of-function of *BRG* and *BAF57* in the developing nervous system and nBAF subunits *BAF45B* and *BAF53B* affects activity-dependent process outgrowth of postmitotic neurons[21]. MicroRNA (miRNA)-mediated direct conversion of human fibroblasts to neurons recapitulates the switching of npBAF to nBAF complexes during normal neural development. miR-9, miR-9* and miR-124 act together by

binding to independent sites in the *BAF53A* 3′ untranslated regions, functioning as a molecular AND gate for this developmental transition. **c** | *Bap55*, the homologue of mammalian *BAF53A* and *BAF52B* in *Drosophila melanogaster*, controls dendritic targeting of olfactory projection neurons (PNs; red). In comparison to wild-type PNs, which target glomerulus Dl1 (green, left), PNs lacking Bap55 are precisely mistargeted to an alternative glomerulus, Da4l (green, right), in the antennal lobe. This dendritic targeting occurs before the axonal patterning of the glomerulus and is thus thought to be mediated through genetic, cell-intrinsic mechanisms and not in response to particular guidance molecules. shRNA, short hairpin RNA.

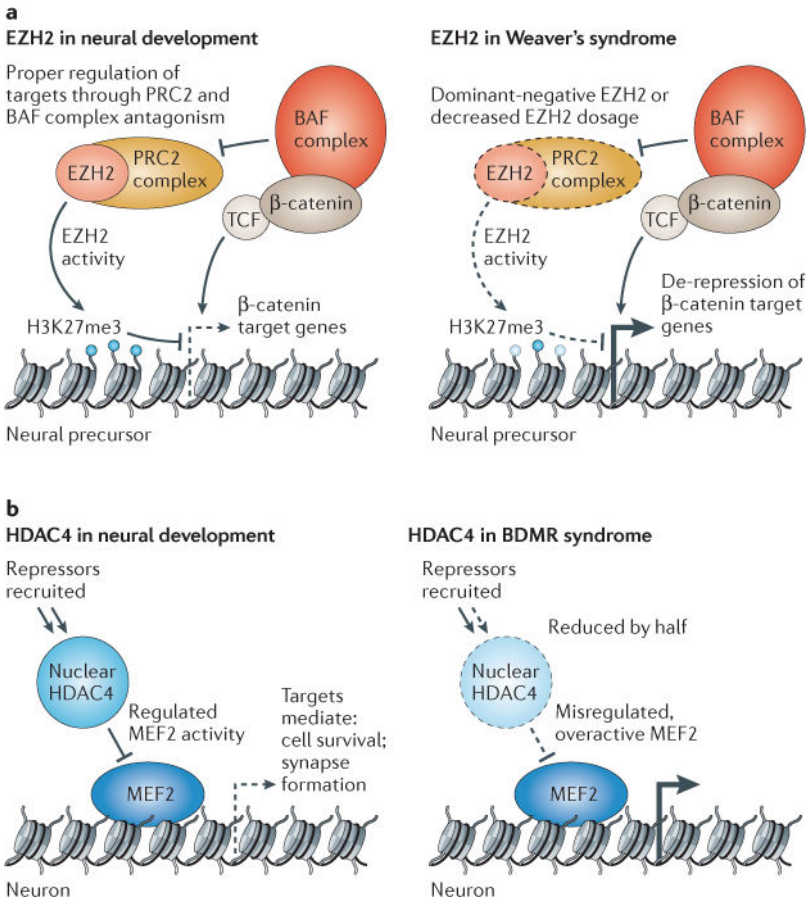


Figure 3. Repressive chromatin modifiers involved in disorders of brain function

a | During later stages of neurogenesis, enhancer of zeste 2 (EZH2) has been shown to repress particular β-catenin target genes in neural progenitors in order to mediate proper cell fate transitions. It is also likely that EZH2 and BAF complexes have antagonistic roles in these cells as they do in embryonic stem cells and *Drosophila melanogaster*. Decreased functional EZH2 dosage (owing to haploinsufficiency or altered function of mutant proteins) will lead to de-repression or over-expression of its targets, leading to altered developmental pathways. Patients with Weaver's syndrome have macrocephaly and learning disabilities of varying severity. **b** | In normal development, histone deacetylase 4 (HDAC4) is dynamically regulated in the cell, moving into and out of the nucleus in response to physiological signals. When localized in the nucleus, HDAC4 binds myocyte-specific enhancer factor 2 (MEF2) transcription factors and recruits repressors such as class I HDACs and heterochromatin protein 1 (HP1) to MEF2 targets. HDAC4 dosage or nuclear residence is critically affected in patients with brachydactyly mental retardation (BDMR) syndrome, probably leading to misregulated MEF2 target gene expression in particular temporal and cellular contexts. H3K27me3, histone H3 trimethylated at lysine 27; PRC2, Polycomb repressive complex 2.

Table 1

Chromatin regulators mutated in human mental disorders

Chromatin regulator*	Disease (or diseases)	Mutations	Chromatin regulator type	Refs
BAF250A (ARID1A; BAF complex)	Coffin–Siris syndrome	Nonsense, frameshift indel	Chromatin-remodelling complex subunit	29
BAF250B (ARID1B; BAF complex)	Intellectual disability, Coffin–Siris syndrome, autism, schizophrenia	Translocation, frameshift indel, nonsense, missense, microdeletion	Chromatin-remodelling complex member	32,33,34,28,36
BRM (SMARCA2; BAF complex)	Coffin–Siris syndrome, Nicolaides–Baraitser syndrome, schizophrenia	Partial deletion, missense, intronic alteration	Chromatin-remodelling complex ATPase	31,30,39,40
BRG1 (SMARCA4; BAF complex)	Coffin–Siris syndrome	Missense	Chromatin-remodelling complex ATPase	29
BAF47 (SMARCB1; BAF complex)	Coffin–Siris syndrome, Kleefstra's syndrome phenotypic spectrum	In-frame deletion, missense	Chromatin-remodelling complex subunit	29,108
BAF155 (SMARCC1; BAF complex)	Autism	Missense	Chromatin-remodelling complex subunit	35
BAF170 (SMARCC2; BAF complex)	Autism	Splice site mutation	Chromatin-remodelling complex subunit	35
BAF180 (PBRM; BAF complex)	Autism	Missense	Chromatin-remodelling complex subunit	36
CHD7	CHARGE syndrome, autism	Missense	Chromatin remodeller	36,109
CHD8	Autism	Nonsense, frameshift indel missense	Chromatin remodeller	35,36,51,52
ATRX	X-linked α-thalassaemia/mental retardation syndrome	Missense	Chromatin remodeller	110
p300	Rubinstein–Taybi syndrome	Large deletions and duplications, misssense	Histone acetyltransferase	111
CBP	Rubinstein–Taybi syndrome	Microdeletions, nonsense	Histone acetyltransferase	112
KAT6B (MYST4 or MORF)	Say–Barber–Biesecker–Young–Simpson syndrome (SBBYSS or Ohdo's syndrome)	Frameshift indel, missense	Histone acetyltransferase	113
HDAC4	Brachydactyly mental retardation syndrome	Balanced chromosomal translocation; deletion	Histone deacetylase	87,88
EZH2	Weaver's syndrome (learning disability)	Missense, frameshift indel	Histone methyltransferase	74,75
EHMT1	Kleefstra's syndrome phenotypic spectrum; autism	Microdeletions, nonsense, frameshift, missense	Histone methyltransferase	36,108,114
MLL	Wiedemann–Steiner syndrome	Nonsense	Histone methyltransferase	115
MLL2	Kabuki's syndrome	Nonsense, frameshift	Histone methyltransferase	116,117
MLL3	Autism, Kleefstra's syndrome	Missense, nonsense	Histone methyltransferase	35,36,108
KDM5C (JARID1C)	Non syndromic X-linked mental retardation	Missense, frameshift, nonsense, intronic alteration	Histone lysine demethylase	118,119
PHF8	X-linked mental retardation	Missense mutation, nonsense	Histone lysine demethylase	120,121
HUWE1	XLMR Turner type	Duplications, missense, copy number gains	Histone ubiquitylation	122
MECP2	Rett's syndrome, Angelman's syndrome, nonsyndromic X-linked mental retardation, autism	Missense, nonsense, frameshift indel, duplication	DNA methylation binding protein	123,124
MBD5	Autism, Kleefstra's syndrome	Frameshift indel, nonsense	DNA methylation binding protein	35,36,108

Chromatin regulator*	Disease (or diseases)	Mutations	Chromatin regulator type	Refs
MED12	Lujan–Fryns syndrome, FG syndrome (also known as Opitz–Kaveggia syndrome)	Missense	REST mechanism for disease, Mediator complex subunit	125,126
MED23	Non-syndromic intellectual disability	Missense	Mediator complex subunit	127
PHF21A (BHC80)	Intellectual disability, craniofacial anomalies	Translocation and deletion	Chromatin reader, histone deacetylase complex member	128

* Protein aliases are given in brackets; if the protein is a member of a complex, the complex name is also given in brackets. EZH2, enhancer of zeste 2; HDAC4, histone deacetylase 4.