



Review

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An evolving view of epigenetic complexity in the brain

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Recent scientific advances have revolutionized our understanding of classical epigenetic mechanisms and the broader landscape of molecular interactions and cellular functions that are inextricably linked to these processes. Our current view of epigenetics includes an increasing appreciation for the dynamic nature of DNA methylation, active mechanisms for DNA demethylation, differential functions of 5-methylcytosine and its oxidized derivatives, the intricate regulatory logic of histone post-translational modifications, the incorporation of histone variants into chromatin, nucleosome occupancy and dynamics, and direct links between cellular signalling pathways and the actions of chromatin 'reader', 'writer' and 'eraser' molecules. We also have an increasing awareness of the seemingly ubiquitous roles played by diverse classes of selectively expressed non-coding RNAs in transcriptional, post-transcriptional, post-translational and local and higher order chromatin modulatory processes. These perspectives are still evolving with novel insights continuing to emerge rapidly (e.g. those related to epigenetic regulation of mobile genetic elements, epigenetic mechanisms in mitochondria, roles in nuclear architecture and 'RNA epigenetics'). The precise functions of these epigenetic factors/phenomena are largely unknown. However, it is unequivocal that they serve as key mediators of brain complexity and flexibility, including neural development and aging, cellular differentiation, homeostasis, stress responses, and synaptic and neural network connectivity and plasticity.

1. Introduction

Major scientific programmes, including the European Union's Human Brain Project (HBP) and President Obama's Brain Research through Advancing Innovative Neurotechnologies (BRAIN) initiative, have galvanized efforts to unravel the complexity of the human brain, a biological system unmatched in its structural and functional sophistication. Indeed, recent estimates suggest that the adult brain comprises approximately 86 billion neuronal and an equivalent number of glial cells [1]. These highly specialized cell types are organized into anatomical regions reflecting evolutionary advancements, interconnected via trillions of synapses, and assembled into local microcircuits and distributed large-scale networks. In addition to a diverse range of differentiated cellular subtypes, the adult brain also contains populations of neural stem and progenitor cells within specific generative zones, such as the sub-granular zone of the dentate gyrus, which produces 700 new neurons per day, on average [2]. These remarkable properties imbue the human brain with an exquisite degree of environmental responsiveness and underpin higher order human cognitive and behavioural functioning throughout the lifespan, such as learning and memory, language, emotional responses and motor performance.

Recent conceptual and technological advances have provided a strong foundation for the HBP, BRAIN initiative and related programmes by offering insights into the mechanisms responsible for promoting brain evolution, mediating brain development, giving rise to neural cellular diversity, establishing and

maintaining synaptic and neural network connectivity patterns, and coordinating communication across individual synapses and neurons and at the level of neural networks. A growing appreciation for the relevance of epigenetic processes in brain (and other organ systems) and the associated development of tools and techniques for epigenomics are among the most important of these scientific innovations [3].

In parallel, our view of epigenetics has also been evolving. The classical definition refers to changes in gene expression and activity that are fixed and heritable between cells but not associated with changes in DNA sequence, and it embraces molecular events, such as DNA cytosine methylation, histone protein post-translational modifications (PTMs) and chromatin remodelling, as being the principal epigenetic processes. By contrast, our contemporary view of epigenetic mechanisms is more inclusive and accounts for the broader landscape of regulatory and functional interactions between diverse DNA, RNA and protein molecules that are inextricably linked and dynamically occurring throughout the lifecycle of a cell. This perspective has emerged as a consequence of scientific advances made in the post-genomic era, including the ongoing characterization of many novel classes of functional genomic elements, the discovery of pervasive transcription and an appreciation for the significance of biological processes transacted at the level of RNA—particularly, the central roles played by non-protein-coding RNAs [4,5]. Here, we discuss this evolving modern view of epigenetics, which is exceptionally relevant for explaining human brain complexity, focusing on recent insights and emerging areas of interest for future study.

2. DNA methylation

Among the diverse range of epigenetic mechanisms that are now being recognized, DNA methylation is still the best characterized [6,7]. It refers to the covalent modification of cytosine residues catalysed by DNA methyltransferase (DNMT) enzymes, which leads to the formation of 5-methylcytosine (5mC). Various proteins have the ability to recognize and bind to 5mC, including methyl-CpG-binding domain (MBD) proteins, Kaiso and Kaiso-like proteins and SRA domain proteins. These factors are, therefore, described as ‘readers’ of methylation ‘marks’. In turn, these readers recruit various combinations of effector proteins to methylated loci, where they fulfil their specific functions, which can include roles in transcriptional regulation and chromatin modification (see §3). DNA methylation has now been implicated in the execution of myriad biological programmes, including transcriptional regulation, long-term gene silencing, transposable element suppression, genomic imprinting, X-chromosome inactivation and the maintenance of genomic stability.

Nevertheless, our understanding of this process remains protean with recent findings having challenged and significantly advanced our previously held views. One important example is the paradigm-shifting discovery of active DNA demethylation, which clearly established that DNA methylation is a dynamic process [8]. DNA methylation was formerly believed to be a relatively stable, or irreversible, covalent modification that is primarily elaborated during developmental programming. The observation that the ten-eleven translocation family of enzymes can catalyse the oxidation of 5mC into 5-hydroxymethylcytosine (5hmC), and into further oxidized derivatives, represented a watershed moment

in uncovering active demethylation processes. It is now believed that multiple distinct mechanisms might be involved in active DNA demethylation. These include thymine DNA glycosylase-mediated base excision DNA repair pathways acting on 5hmC (or on its further oxidized derivatives, 5-formylcytosine and 5-carboxylcytosine)—which seems to be the most likely scenario *in vivo*—as well as other DNA-modifying processes potentially involving the AID/APOBEC family of DNA/RNA-editing enzymes, Gadd45 protein-mediated nucleotide excision DNA repair pathways, and even demethylation reactions mediated by MBD proteins and DNMT enzymes. Our growing appreciation for active demethylation implies that DNA methylation–demethylation cycles are dynamic and intricately regulated. Also, importantly, the oxidized derivatives of 5mC do not seem to act simply as inert transitional states. Emerging studies have begun to reveal that 5hmC is, like 5mC, distributed throughout the genome in specific gene regulatory regions, inter- and intra-genic sequences and repetitive elements, and that it seems to have functions that counterbalance those of 5mC. Specifically, the presence of 5hmC may disrupt interactions between 5mC and its associated reader and effector proteins, or alternatively, 5hmC may interact with differential profiles of readers and effectors with distinct activities. Together, these observations imply that, in stark contradistinction to the notion of DNA methylation marks being stable, the DNA methylation landscape is dynamic in terms of its genomic distribution, ongoing cycles of DNA methylation maintenance and active and passive DNA methylation–demethylation, patterns of distribution of oxidized derivatives of 5mC, and corresponding interactions with different profiles of readers and their interacting partners.

There is no doubt that this incredible degree of epigenetic flexibility is particularly relevant in the brain. Indeed, one of the seminal studies which identified the presence of 5hmC reported that, in cerebellar Purkinje cell neurons, 5hmC is 40% as abundant as 5mC [9]. Further, high-resolution genome-wide analyses of DNA methylation including dynamic profiling of 5mC and 5hmC (and related factors) in human neuropathological tissues and in model systems have demonstrated that these patterns are connected to evolutionary innovations in brain size and function [10,11], sexually dimorphic [12,13], neuronal and glial subtype specific [14], differentially arrayed across both CpG dinucleotides and non-CpG (CpH) sites [14,15], dynamically regulated throughout the lifespan [16], linked with activity-dependent plasticity underlying cognitive and behavioural processes, such as learning and memory [17–19], and associated with transgenerational inheritance [20]. Furthermore, neural cell-type-specific patterns of methylation including, particularly, 5hmC and CpH methylation being much more prevalent in neurons than in other cells suggests potentially novel and more sophisticated roles for DNA methylation in nervous system compared with other organs [9,14,15].

3. Histone modifications and higher order chromatin remodelling

Chromatin refers to the packaging of genomic DNA, along with histone proteins and associated factors, into a highly condensed form within the nucleus [3,7]. A nucleosome, the smallest unit of chromatin, comprises DNA wrapped around

a histone octamer, containing two of each of the core histones (i.e. H2A, H2B, H3, H4). Nucleosomes are connected via DNA folded around linker histones (i.e. H1) and arrayed into progressively higher order structures. These chromatin states can vary from configurations that are relatively open and accessible (i.e. euchromatin) to those that are closed (i.e. heterochromatin). Chromatin structure is very dynamic and subject to alterations at the level of individual histone proteins and nucleosomes, and also over larger genomic regions. Thus, chromatin serves as a molecular platform for integrating environmental and interoceptive signals at the level of the genome.

Histone PTMs and higher order chromatin remodelling are classical epigenetic mechanisms that are responsible for modulating these chromatin states and, in turn, the accessibility of specific DNA sequences to other nuclear factors, such as the molecular machinery involved in transcription [3,7]. These mechanisms are, like DNA methylation, involved in mediating genomic programmes, including transcriptional regulation, transposable element silencing, genomic imprinting, X-chromosome inactivation and the maintenance of genomic stability. PTMs at specific sites on histone proteins are catalysed by different subclasses of histone-modifying enzymes. Families of histone acetyltransferases/deacetylases and methyltransferases/demethylases are the best characterized, though many more exist. Specific histone PTMs have been associated with particular genomic sites (e.g. enhancers, actively transcribed promoters and gene bodies, and repressed genes) and nucleosomal regions, and we are just beginning to uncover their precise functions within these contexts [21]. It has been hypothesized that histone PTMs present in the relatively free N-termini, or tails, which protrude from the nucleosome, form a combinatorial 'histone code' assimilating complex cross-modulatory and hierarchical relationships between PTMs. A spectrum of chromatin-binding proteins with specialized recognition domains (e.g. bromodomains and chromodomains) have the ability to read these codes and are often part of multimeric complexes containing additional epigenetic effector proteins, including those that can 'write' and 'erase' these epigenetic marks (i.e. remodel chromatin). By contrast, particular PTMs found in histone tails (i.e. H4 lysine [K] 16 acetylation [ac] and H4K20 trimethylation) are implicated in mediating inter-nucleosomal interactions occurring over relatively large genomic regions, thereby influencing the establishment of higher order chromatin conformations directly. More recently described PTMs in histone globular domains, or cores (i.e. H3K122ac), can modulate sites of histone–histone and DNA–histone interactions [22]. It is likely that these types of histone PTMs act in concert with ATP-dependent chromatin-remodelling enzymes to modulate the dynamics of nucleosomes, which undergo extensive rearrangement to expose DNA to regulatory and other factors and to allow RNA polymerases to advance through chromatin when genes are activated for transcription.

Chromatin landscapes—including profiles of nucleosome occupancy and the kinetics of transcription and nucleosome sliding [23]—associated with many different contexts (e.g. development and aging, cell fate determination, and homeostatic and stress responses) are now being studied intensively. Specifically, it is of tremendous interest to characterize the spectrum of different epigenetic proteins that have the capacity to read, erase and write specific chromatin states. In the nervous system, the expression of these factors and

their associated profiles of epigenetic marks (at genomic loci encoding important classes of neural genes) are intricately regulated throughout the lifespan in a highly region- and cell-type-specific and activity-dependent manner. Indeed, an ever increasing number of studies is uncovering how these mechanisms underpin brain patterning [24], neural stem cell maintenance and differentiation, neuronal and glial subtype specification and maturation [25,26], neuronal migration [27,28], the establishment of neural network connectivity patterns [28], the development of sexually dimorphic neuronal circuitry [29], homeostasis [30], plasticity responses (e.g. learning and memory) [31–33] and brain aging [34].

A closely related subject, which has recently become prominent in brain, is the utilization of structurally distinct histone protein variants, which can alter chromatin organization and modulate genomic programmes, such as transcription and DNA repair [35]. Each canonical histone is associated with non-allelic variants that are expressed in cell- and tissue-selective patterns. Variant histones can be incorporated into nucleosomes by replacement of canonical histones in concert with DNA replication, or in a DNA replication-independent manner mediated by histone chaperones and ATP-dependent exchange factors. The latter mechanism is particularly relevant for post-mitotic neurons. The overall functional consequences of variant histone incorporation, the regulation of their genomic distributions, profiles of PTMs and differential rates of turnover [36] are not well understood generally or in the brain specifically. However, recent evidence suggests that certain variant histones are enriched in neuronal cells and play roles in important neural processes, such as activity-dependent gene regulation. For example, in response to neuronal depolarization, the histone chaperone DAXX promotes the incorporation of the H3.3 variant into chromatin associated with activity-dependent genes, leading to the transcriptional activation of these genes [37]. Intriguingly, somatic mutations in the H3.3 variant have recently been implicated in driving the pathogenesis of primary brain tumours, including a high proportion of paediatric gliomas [38–41]. These mutations occur at sites in the tail region that are typically subject to PTMs and lead to alterations in the recruitment of chromatin-remodelling complexes to these sites, aberrant reprogramming of the epigenetic landscape and deregulation of gene expression profiles. It has been suggested that the explicit pathogenic mechanism is the disruption of an RNA-mediated modulatory event [42].

This hypothesis is notable because regulatory RNAs, which are most abundant in brain, serve as one of the principal mechanisms responsible for orchestrating histone PTMs and chromatin remodelling (see §4*a,b*). However, we are still in the early stages of understanding these and other processes involved in establishing, maintaining, transforming and modulating chromatin states and their specific and potentially unique roles in nervous system.

4. Non-coding RNAs

One key insight that has emerged in the post-genomic era is the surprising discovery of pervasive transcription [5,43–46]. Nearly the entire mammalian genome seems to be transcribed in complex overlapping patterns, in both sense and antisense orientations, with each nucleotide serving as a multifunctional transcriptional unit, which can be used in

many different transcripts. This transcriptional activity encompasses regions that are non-protein-coding and can thus lead to the formation of non-coding RNA (ncRNA) transcripts. The identification and characterization of numerous classes of ncRNAs embedded within the genome having diverse and critically important biological roles has upended the central dogma of molecular biology, which states that DNA makes RNA and RNA makes protein [47]. Further, the concept of a 'gene' has been redefined to include not only DNA elements encoding transcripts that are translated into proteins but also those encoding ncRNAs (ncRNA genes) [48]. It is very interesting to note that there are thousands more ncRNA genes in the human genome than protein-coding genes and also that ncRNAs are more abundant by mass than protein-coding mRNAs in human cells, particularly neural cells [49,50]. Moreover, comparative genomic and transcriptomic analyses reveal that non-coding DNA sequences and ncRNA genes exhibit positive selection, accelerated evolution, lineage-related expansion and specificity, preferential expression in brain and selective association with neural genes [47,51]. Together, these observations suggest that the emergence of these factors has played a seminal role in human brain evolution.

ncRNAs have been studied intensively, and our understanding of their biological roles has advanced significantly, though many unanswered questions yet remain. ncRNAs are generally categorized as being short (i.e. small) or long based on their length, with those greater than 200 nucleotides defined as long ncRNAs (lncRNAs). These factors are associated with distinct class- and subclass-specific genomic contexts, structural features, biogenesis pathways, profiles of interacting partners (e.g. Argonaute protein family members), subcellular localizations, intercellular trafficking (i.e. via microvesicles and exosomes), molecular mechanisms of action and biological functions [47].

(a) Short non-coding RNAs

The most prominent classes of short ncRNAs include microRNAs (miRNAs), small nucleolar RNAs (snoRNAs) and piwi-interacting RNAs (piRNAs) [47]. Of these, miRNAs are the most studied. They are typically described as post-transcriptional regulators of mRNA networks that bind to particular sites (i.e. imperfectly complementary sequences) in the 3'-untranslated regions (UTRs) of their target mRNAs and promote the repression or degradation of these transcripts through RNA interference (RNAi) pathways. miRNA expression is developmentally regulated and cell type specific, and is, in turn, responsible for the dynamic and environmentally responsive deployment of gene networks. Given that one of the hallmarks of brain is its exquisite degree of spatial, temporal and activity-dependent control of gene expression, it is not surprising that miRNAs are now implicated in essentially every aspect of neural development and aging, adult homeostasis and plasticity, and disease pathogenesis [47]. However, our view of miRNA biology is still incomplete. Recent studies suggest that miRNA biogenesis can occur through non-canonical pathways [52]; distinct miRNA variants (i.e. isomiRs) can be expressed in developmental stage-, cell-type- and subcellular compartment-specific profiles [53–55]; miRNA precursors can be modified post-transcriptionally via RNA editing [56]; miRNA-binding sites can be present not only in 3'-UTRs but also in 5'-UTRs, coding regions and introns [57];

miRNAs can target other ncRNAs [58]; miRNAs can promote target gene activation [59] and even mediate genomic site-specific chromatin-remodelling events [60]; and miRNAs can be sequestered and their activities modulated by the expression of genomically encoded miRNA sponge transcripts (i.e. competing endogenous RNAs) that harbour large numbers of miRNA-binding sites [61]. Subsequent studies related to these observations are likely to impact very significantly our understanding of the roles played by miRNAs in brain.

snoRNAs are generally thought to serve as guides for post-transcriptional modification of other RNAs, such as ribosomal RNAs [47]. The H/ACA and C/D box families of snoRNAs mediate pseudouridylation and methylation, respectively, although other functions that are particularly relevant in brain have also been identified. For example, brain-specific snoRNAs derived from the snoRNA C/D box 115 cluster modulate alternative splicing of the serotonin receptor 2C [62].

piRNAs were initially believed to participate in the silencing of mobile genetic elements in the germ line via RNAi pathways [47]. However, piRNAs and their interactors, piwi proteins (members of the Argonaute family), were later found in somatic cells, including neurons. Recent data from *Aplysia* further suggests that serotonin-sensitive piRNA activity orchestrates DNA methylation of the cAMP response element-binding protein 2 gene promoter, thereby leading to enhanced long-term synaptic facilitation underlying memory [63]. Intriguingly, it has also been suggested that piRNAs participate in regulating mobile genetic elements in neural cells, including L1 retrotransposons, which are implicated in generating neural cellular diversity and plasticity and in nervous system disease pathogenesis [64].

(b) Long non-coding RNAs

The universal importance of lncRNAs was recognized relatively recently, compared with that of short ncRNAs. These factors are more abundant but also more complex and, current efforts notwithstanding, less well characterized. lncRNAs exhibit great heterogeneity with respect to their genomic contexts, sizes, post-transcriptional processing and transport, molecular interactors and mechanisms of action [47,65]. lncRNAs are often classified in terms of the organization of the genomic loci from which they are transcribed. These contexts can include directionality (i.e. sense or antisense) as well as proximity to protein-coding genes, gene regulatory regions (e.g. promoters and enhancers), mobile genetic elements and other genomic landmarks. The sizes of lncRNAs can be quite large, up to hundreds of kilobases. lncRNAs can be subject to post-transcriptional processing (e.g. 5' capping, polyadenylation, alternative splicing, RNA editing and RNA cytosine methylation) and intracellular and intercellular transport. Because of their large size and ability to form secondary and tertiary structures, lncRNAs can simultaneously engage in a spectrum of sequence-specific and conformational interactions with a diverse range of partners, including DNA, RNA and protein molecules [66]. For example, lncRNAs can recruit non-specific transcriptional and epigenetic regulatory factors (i.e. histone-modifying enzymes and chromatin-remodelling complexes) to particular genomic loci. lncRNAs can also be hosts for short classes of ncRNAs released by cleavage of the lncRNA. Moreover, lncRNAs can form circular transcripts, which act as

miRNA sponges and have other functions [61,67,68]. Furthermore, the process of lncRNA transcription (rather than the transcript) can, itself, be pertinent via direct interactions with other nuclear factors (e.g. the transcriptional machinery). lncRNAs can act via these and additional mechanisms to mediate local and higher order epigenetic states, transcription and post-transcriptional processes, ncRNA regulatory network dynamics, protein PTMs and nuclear architecture. Thus, lncRNAs represent an incredibly versatile and powerful mechanism for coordinating the execution of complex cellular programmes.

Conspicuously, the majority of lncRNAs that have been identified are expressed preferentially in the nervous system, in very selective spatial, temporal and activity-dependent profiles [47,65]. These factors are increasingly being associated, like short ncRNAs, with mediating myriad aspects of brain development and aging, neural cell differentiation, homeostasis and stress responses, synaptic and neural network connectivity and plasticity, and disease pathogenesis [47,69–74]. However, the precise mechanisms of action and biological roles for most lncRNAs have yet to be studied and elucidated, and it is likely that many more lncRNAs remain to be discovered—including those expressed at a low level or in a highly context-specific manner [75].

5. Concluding remarks

Our view of epigenetic mechanisms, and of their specific roles within brain, is evolving rapidly. We have uncovered extremely profound layers of complexity encompassing canonical epigenetic processes, such as DNA methylation and histone modifications and higher order chromatin remodelling. Moreover, our perspective on epigenetic phenomena has been revolutionized by the discovery of pervasive transcription and the characterization of diverse classes of regulatory and functional ncRNAs. These transcripts can act via a broad spectrum of molecular mechanisms through sequence-specific and conformational interactions with DNA elements, RNAs and proteins, and they are thus intimately involved in mediating transcriptional, post-transcriptional, post-translational, and local and higher order chromatin modulatory processes. Although our understanding of when, why, where and how these mechanisms are deployed remains incomplete, it is clear that this exquisite degree of sophistication and refinement is vital for producing the developmental complexity, cellular diversity, activity-dependent plasticity, information processing ability and other emergent network properties that are the hallmarks of brain.

In addition, further layers of epigenetic complexity inter-related with the above processes are rapidly emerging and seem to be similarly important in brain. One recent study demonstrated that depleting MBD3, a component of the Mi-2/nucleosome remodelling and histone deacetylase (NuRD) complex, which couples DNA methylation with histone modification and chromatin remodelling, increases the efficiency of cellular reprogramming to nearly 100% [76]. This finding may be especially relevant for neural cellular differentiation as MBD3 is expressed in the embryonic and adult brain in specific spatial and temporal profiles [77]. Another recent study showed that active transcription of certain ncRNAs, which interact directly with DNMT1, leads to local DNA demethylation [78]. This observation suggests a

novel and genomic site-selective mechanism for modulating DNA methylation profiles associated with thousands of genes, including those with central roles in neural development and plasticity, such as CCAAT/enhancer-binding protein alpha [79]. In contrast to such *cis*-regulatory roles, lncRNAs are also being implicated in the execution of genome-wide programmes through *trans*-regulatory processes. For example, Paupar is a lncRNA transcribed from a locus proximal to the paired box 6 (*PAX6*) gene, this lncRNA binds to and regulates genes on multiple chromosomes in concert with the *PAX6* protein, and Paupar is preferentially expressed in the nervous system [80].

Furthermore, the deployment of lncRNAs and interconnected epigenetic processes is also being linked to the dynamic three-dimensional organization of the genome within the nucleus. These processes encompass, for instance, the compartmentalization of DNA sequences, RNAs and other molecular machinery into functional nuclear domains; enhancer–promoter interactions and DNA looping; inter-chromosomal clustering of co-regulated genes; interactions between genomic elements and nuclear pore complexes; the establishment of chromatin boundary elements and transcriptional ‘poising’ of recently activated genes for rapid reactivation (i.e. cellular memory functions) [81–85]. One example of functional genomic elements involved in this nuclear choreography is developmental enhancers, which exhibit tissue-specific regulatory activity. Intriguingly, many of these developmental enhancers represent ‘human accelerated regions’ that have served as substrates for evolutionary innovations in the human lineage, including those underpinning brain structure and activity [86,87]. Mobile genetic elements (i.e. transposable elements) are another family of genomic elements that are connected to epigenetic processes. These factors have contributed to the evolution of the primate lineage, constitute a large proportion of the human genome, give rise to ncRNAs and significantly impact the transcriptional landscape [88–91]. In the brain, these factors are now being implicated in promoting neuronal genomic mosaicism and in neural development, neural cellular differentiation and plasticity [92]. Conversely, the activity of mobile genetic elements is subject to regulation by epigenetic mechanisms, including potentially short ncRNAs, such as piRNAs [93].

Moreover, it appears that RNA molecules, including protein-coding and ncRNA transcripts, exhibit cell- and tissue-specific and activity-dependent profiles of RNA editing and other more recently recognized post-transcriptional RNA modifications, such as cytosine and adenosine methylation, which are mediated by RNA editing and other distinct RNA modification enzymes. These marks, now being referred to as ‘RNA epigenetics’, can impact RNA secondary and tertiary structure formation, additional post-transcriptional processing, transport, metabolism and functional interactions. Conspicuously, RNA methyltransferase, demethylase and topoisomerase enzymes seem to have emerging roles in stem cell fate determination, neural developmental, synapse formation, neural network activity and cognitive and behavioural functions [94–100].

Intriguingly, this continuum of epigenetic mechanisms seems to be relevant not only in the nucleus but also in mitochondria. For example, the mitochondrial genome exhibits differential patterns of DNA methylation (i.e. 5hmC) in cells of the frontal cortex [101], generates short and long classes of mitochondrial ncRNAs [102,103] and is affected by drugs

that target epigenetic pathways, such as valproic acid, which is widely used in the treatment of neurological and psychiatric disorders [104]. These observations suggest important roles for epigenetic mechanisms in brain-specific mitochondrial functions and offer a mechanism for nuclear–mitochondrial intergenomic communications.

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