Copper(II)-2,2′-isopropylidenebis[(4*R*,5*S*)-4,5-diphenyl-2-oxazoline]ditriflate {[Cu(4*R*,5*S*)-bis-Ph-box](OTf)$_2$} catalyzes intramolecular additions of sulfonamides to terminal alkenes with concomitant addition of the stable oxygen radical (2,2,6,6-tetramethylpiperidine-1-yl)oxyl (TEMPO) (see the figure, panel G) (*17*). The mechanism involves concerted intramolecular addition of R_2N-[Cu^{II}] across the alkene followed by C-[Cu^{II}] homolysis and subsequent carbon radical coupling with TEMPO. A different ring-forming alkene amino-functionalization strategy involves electrophilic addition of a chiral organocopper(III) complex (formed in situ by oxidation of a chiral Cu^{I} complex with [Ph-I-Ph]AsF_6) (where Ph is phenyl) to the electron-rich alkene of an indole (see the figure, panel G) (*18*). Amine addition to the resulting iminium ion forms a new C–N bond, and reductive elimination provides the chiral C–C bond.

Finally, diamination is one of the more sought-after alkene difunctionalization reactions, and it can occur as an intermolecular copper-catalyzed reaction of dienes with diaziridinones with complementary regioselectivity by either a two-electron or single-electron mechanism, depending upon the ligands used (see the figure, panel H) (*19*). Oxidative addition of [Cu^{I}] into the diaziridinone gives a new [Cu^{III}] species that can be in equilibrium with a [Cu^{II}] species by Cu–N homolysis. Electrophilic addition of the copper(III) species to the alkene occurs at the more electron-rich, internal alkene, generating the internal diamine. This preference can be changed by addition of the bulky PCy_3 (tricyclohexylphosphine) ligand, which shifts the equilibrium to the copper(II)-aminyl radical species that favors addition to the terminal alkene carbon.

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GENETICS

The Maturing Brain Methylome

DNA methylation patterns in the developing and adult mammalian brain point to a role in synaptic development and maturation.

Harrison W. Gabel and Michael E. Greenberg

The methylation of DNA in mammalian genomes regulates gene expression, guiding differentiation and maintaining cellular identity within tissues. However, it may have a distinct function in the brain. On page 629 of this issue, Lister *et al*. (*1*) present a comprehensive analysis of DNA methylation and hydroxymethylation at single-base resolution in the mammalian frontal cortex. The authors chart out striking postnatal alterations in neuronal methylation profiles that occur as synapses develop and are refined, from the fetal to adult stage. The patterns suggest that DNA methylation is important in the maturation of neurons in the developing brain.

DNA methylation represses gene expression in all mammalian cells. The methylation of cytosines in the context of cytosine-guanine dinucleotides (mCG) is a stable repressive mark on DNA. However, the Tet family of enzymes converts methylcytosine to hydroxymethylcytosine (hmC), an oxidized form that can be demethylated (*2*, *3*). hmC is enriched in stem cells and neurons, suggesting that they might be particularly susceptible to changes in DNA methylation state (*2*, *4*). Intriguingly, stem cells and brain tissue also contain substantial cytosine methylation outside of the CG context [mCH, where H is adenine (A), thymine (T), or cytosine (C)], which is rare in most somatic cells (*5*, *6*). Although the role of hmC and mCH in stem cells has been extensively investigated at base-pair resolution (*5*, *7*), there has been limited examination of DNA methylation at high resolution in the brain.

Using high-throughput sequencing to profile the mouse and human cortex from the fetal to adult stage, Lister *et al*. revealed a conserved, genome-wide increase in mCH amounts in the brain after birth. Although the period during which mCH accumulates differs between mice and humans (several weeks versus several years, respectively), the increase coincides with the peak of synaptogenesis and synaptic pruning in the brain for each organism (see the figure). Thus, the acquisition of mCH may be linked to neuronal maturation. High amounts of mCH were found in neurons compared to low amounts in nonneuronal (glial) cells. Although the average percentage methylation detected in neurons at CH is quite low (~2 to 6%) and quite high at CG (~80%), because CG is rare in mammalian genomes relative to CH, a similar number of total mCH and mCG events occur in neurons. Indeed, in adult human neurons, the total number of mCH sites surpasses that of mCG sites. Rather than a minor addition to the methylation modifications in the genome ("methylome"), mCH is likely a major substrate for gene regulation in the maturing brain.

hmC builds up in neurons with a timing similar to that of mCH (*8*), raising the possibility that, in addition to occurring at CG, hydroxymethylation also takes place at CH in the brain. Lister *et al*. used another high-throughput sequencing method (*9*) to profile hmC at single-base resolution in mouse fetal and adult frontal cortex. Surprisingly, even though hmC and mCH both accumulate as the brain matures, hydroxymethylation occurs almost exclusively in the hmCG context. If mCH is converted to hmCH at all, it must be extraordinarily short-lived, as it is essentially undetectable at steady-state amounts.

To better understand how methylation contributes to transcriptional regulation, Lister *et al*. performed integrated analysis

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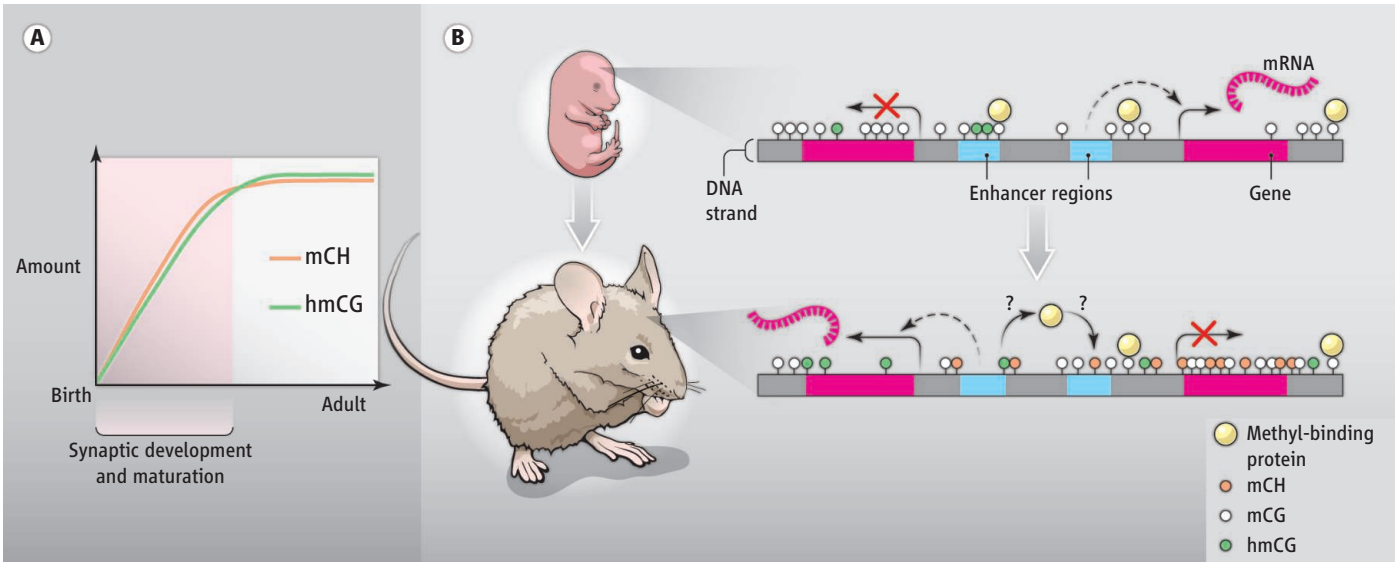

A distinct neuronal methylome. (**A**) High mCH and hmCG accumulate in mammalian neurons postnatally, coinciding with the period of active synapse development and maturation. (**B**) mCH builds up in adult neurons and is enriched at repressed genes and enhancers. hmCG associates with active genes and can premark enhancers in the fetal brain that will become demethylated and active in the adult. Differential affinity to hmCG and mCH may alter the binding profile of methyl-DNA–binding proteins and thereby control distinct neuronal transcription.

of hmC, mCG, and mCH profiles, mRNA expression, and epigenomic data sets from the brain. The authors observed an expected enrichment of mCG and mCH at genes and distal regulatory elements correlating with transcriptional repression, whereas hmC is deposited across active genes (*6*, *8*). Previously undescribed patterns of methylation in the brain also were uncovered, such as enrichment of mCH at genes that escape X-chromosome inactivation in females. Lister *et al*. also noted that, while glial genomes contain low global amounts of mCH, mCH is enriched at genes that are repressed in either neurons or glia, indicating that mCH may enforce cell-type–specific transcription in both of these related cell lineages. Further, the authors detected thousands of developmental-stage– and cell-type–specific methylation sites at putative enhancers. Thus, some inactive enhancers in the fetal brain are marked with hydroxymethylation, which likely leads to subsequent demethylation and activation in the adult.

The findings of Lister *et al*. pave the way for examining how the brain-specific methylome is established. For example, the enzymes that generate high amounts of hmC and mCH in neurons have not yet been described. A strong candidate is Dnmt3a, a methyltransferase that peaks in expression in neurons during the period of mCH deposition and displays methylation activity on CH dinucleotides (*10*). Likewise, it has not been fully determined which Tet enzyme(s) drive the buildup and patterning of neuronal hmC. Because hmC and mCH profiles display a high degree of conservation and reproducibility across individuals, they likely result from regulated processes. However, it is unclear to what extent the patterns of hmC and mCH in neurons result from sequence-directed targeting mechanisms or if they occur secondarily, guided by accessible chromatin structure or transcriptional activity.

Although the profiles point to regulatory roles for mC and hmC in the brain, the mechanisms by which these marks are read out to affect gene expression await elucidation. It will be essential to understand which DNA binding factors are recruited to mCG, mCH, and hmCG to mediate their regulatory functions. Multiple proteins differentially bind to unmethylated, methylated, and hydroxymethylated DNA (*11*, *12*). In neurons, high hmCG amounts at some CG sites could block proteins from binding, whereas mCH may provide an array of new binding sites in the genome. Notably, the methyl-DNA–binding protein MeCP2 accumulates in the brain with a timing similar to that of the increase in mCH and hmC (*13*). It is possible that in neurons, MeCP2 binds to the additional sites afforded by mCH to repress genes. Because disruption of MeCP2 leads to the neurodevelopmental disorder Rett syndrome (*14*), it will be important to determine how mCH affects the function of this protein.

Could the epigenomic rearrangements uncovered by Lister *et al*. participate in the development and maturation of synaptic connections? Because the frontal cortex develops postnatally in concert with input from the environment, it is possible that neuronal-specific DNA methylation contributes to sensory input–dependent changes in gene expression and synaptic development. Alternatively, high amounts of mCH and hmC may facilitate the generation of diverse neuronal subtypes; acquisition of the neuronal-specific methylome coincides with the final stages of neuronal differentiation that give rise to the many neuronal subtypes of the brain. The propensity of mCH to mark cell-type–specific genes and the differential methylation of enhancers suggest that the methylation profile of a neuron may dictate its specific gene expression profile and functions within the context of a neural circuit. Future studies should determine whether epigenomic rearrangements affect neuronal cell-type diversity and/or sensory-dependent synapse maturation and circuit formation in the developing brain.

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