Genetics meets epigenetics: HDACs and Wnt signaling in myelin development and regeneration

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A study shows that the histone deacetylases HDAC1 and HDAC2 stimulate oligodendrocyte differentiation by antagonizing the inhibitory action of Wnt signaling, linking genetic and epigenetic control of oligodendrocyte development.

Oligodendrocytes are the myelinating cells of the vertebrate CNS. The past decade has witnessed considerable success in unraveling the intricate regulatory network that controls successive stages of oligodendrocyte development, from regional patterning of the embryonic neuroepithelium and specification of migratory oligodendrocyte precursors (OLPs) to the mature, myelinating oligodendrocyte phenotype. This network is linked together by extrinsic signaling molecules, intrinsic genetic elements and epigenetic factors[1,2]. In this issue of *Nature Neuroscience*, Ye *et al.*[3] report an interaction between histone deacetylases 1 and 2 (HDAC1/2) and Wnt signaling that is important in the regulation of oligodendrocyte lineage progression and terminal differentiation.

Chromatin conformation determines how accessible the genomic DNA is to transcription factors and thereby controls which genes can or cannot be expressed in a given cell. Acetylation of specific lysine residues in the 'tail' regions of histones by histone acetyltransferases (HATs) reduces their overall positive charge, leading to de-compaction of chromatin structure and increased accessibility of DNA. HDACs reverse the actions of HATs, rendering DNA less accessible. HDACs are essential to many biological processes, including proliferation, differentiation and carcinogenesis[4]. Histone deacetylation also affects oligodendrocyte differentiation and myelin repair[5–7]. To elucidate HDAC function in oligodendrocyte development, Ye *et al.*[3] generated oligodendrocyte lineage-specific knockout lines by mating floxed *Hdac1* and *Hdac2* mice with *Olig1-Cre* transgenic mice. Although neither *Hdac1* nor *Hdac2* single knockouts showed developmental defects, the *Hdac1; Hdac2* double knockouts developed severe tremor and died around postnatal day 14 (P14). In this *Hdac1/2* compound mutant, the OLP-specific markers PDGFRA and OLIG2 disappeared at embryonic day 15.5 (E15.5), and no markers of differentiated oligodendrocytes were detectable after birth at P4. In addition, primary cortical precursors derived from *Hdac1/2* double-null mice were unable to differentiate into oligodendrocytes *in vitro*. These results indicate that HDAC1 and HDAC2 are critical for oligodendrocyte specification and differentiation.

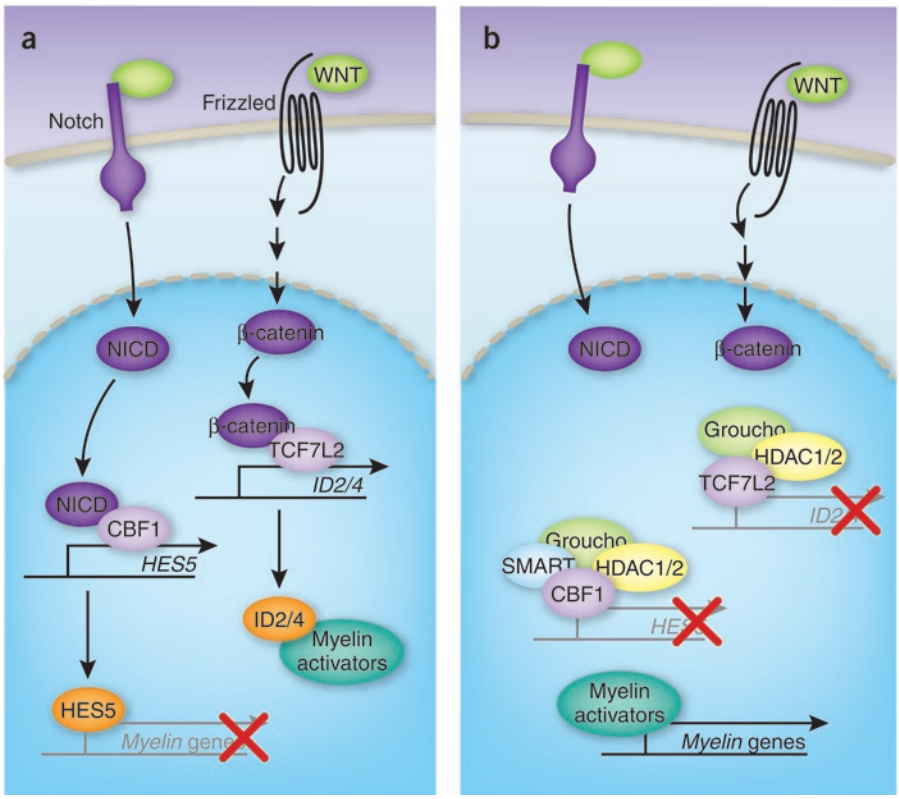

Figure 1 The role of HDACs in oligodendrocyte lineage progression and terminal differentiation. (**a**) Both Notch and Wnt signaling induce transcriptional repressors (HES5, ID2/4) that can inhibit myelin gene expression. (**b**) HDACs, together with co-factors Groucho and SMART, can relieve Notch and Wnt repression by competing with the NICD to bind to CBF1 and competing with β-catenin to bind to TCF7L2. Both scenarios might also increase the state of chromatin compaction around genes such as *HES5*, *ID2* and *ID4* that encode repressors of oligodendrocyte differentiation, thus preventing their transcription and providing permissive conditions for oligodendrocyte lineage progression. This is consistent with the model that cell differentiation is enacted through a sequence of de-repression events, in an overall context of transcriptional repression.

Apart from their epigenetic role in chromatin remodeling, HDACs carry out important functions by deacetylating nonhistone proteins and by physically binding to a number of regulatory partners. Despite their lack of DNA-binding activity, HDACs can interact with transcriptional activators and repressors and become incorporated into large transcriptional complexes. For example, the Notch signaling pathway is regulated by a co-repressor complex consisting of SMART and HDAC1. The SMART/HDAC1 complex can displace the Notch intracellular domain

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(NICD), the active form of Notch, from the CBF1/RBP-Jκ transcriptional complex and, as a result, turns off transcription of Notch target genes[8]. Moreover, HDACs can be recruited by the Groucho-related co-repressors GRO, TLE and GRG, which participate in a wide range of developmental signaling pathways, including the Notch and canonical Wnt pathways[9]. Both Notch and Wnt pathways have been implicated in oligodendrocyte development. For example, data obtained from *in vitro* overexpression of Wnt3a suggests that Wnt signaling inhibits oligodendrocyte differentiation[10]. However, there is still only scant genetic information concerning the role of the Wnt cascade in oligodendrocyte development. Things are now starting to fall into place with the study by Ye *et al.*[3].

At the core of canonical Wnt signaling is activation of the β-catenin pathway, which involves the stabilization, nuclear translocation and accumulation of the β-catenin protein[11]. By breeding the *Hdac*1/2 compound null mutant to BAT-gal reporter mice, which carry a β-galactosidase reporter gene under the control of β-catenin responsive elements, Ye *et al.*[3] found that, in the absence of HDAC1/2, β-catenin is activated at an earlier stage of oligodendrocyte development than usual. Other evidence acquired from primary cortical cultures also pointed to a connection between HDAC1/2 deletion and β-catenin activation.

Ye *et al.*[3] set up mouse models with forced expression of β-catenin in the oligodendrocyte lineage. Forced expression of a constitutively active form of β-catenin caused OLIG2-positive neuroepithelial precursors in the ventral spinal cord to remain in the ventricular zone, apparently unable to generate migratory OLPs. Forced expression of the same active β-catenin construct in OLPs prevented them from differentiating into oligodendrocytes. Expression of the differentiation inhibitors ID2 and ID4 was enhanced, whereas expression of myelin proteins such as myelin basic protein (MBP) and 2′,3′-cyclic nucleotide phosphodiesterase (CNP) was repressed. Therefore, activating the β-catenin arm of the canonical Wnt signaling pathway seems to block normal lineage progression, freezing the cells at their then-current stage of development. Consistent with this, Ye *et al.*[3] also show that conditional knockout of β-catenin in the oligodendrocyte lineage promotes development ahead of schedule. Thus, Wnt signaling negatively regulates oligodendrocyte lineage progression during normal development.

In the nucleus, activated β-catenin does not bind to DNA directly; instead, it forms a transcriptional complex with a member of the TCF family of transcription factors to promote gene expression[11]. Groucho-related co-repressors compete with β-catenin for TCF and, when bound, convert TCF to a transcriptional repressor. Ye *et al.*[3] identified and characterized an oligodendrocyte-specific TCF family member, TCF7L2 (also known as TCF4). In the developing mouse spinal cord, TCF7L2 is first expressed in pre-myelinating oligodendrocytes on E15.5; it is later downregulated in mature oligodendrocytes. Knocking out TCF7L2 in mice blocked oligodendrocyte differentiation, whereas electroporating a dominant repressor form of TCF7L2 into the chick neural tube induced ectopic oligodendrocyte differentiation. These data suggest that a repressor activity of TCF7L2 is required to permit normal oligodendrocyte differentiation.

In a series of co-immunoprecipitation assays, Ye *et al.*[3] found that HDAC1/2 can physically bind to TCF7L2 and that β-catenin can interfere with this interaction. TCF7L2, as with other TCF family members, has a dual role: it can switch from being a transcription activator to being a repressor by swapping its binding partner β-catenin for HDAC1/2. Ye *et al.*[3] propose that HDAC1/2 and β-catenin compete against each other to interact with TCF7L2 and that the outcome of this competition determines the 'stop or go' status of the oligodendrocyte differentiation program. Alternatively, given that Groucho-related co-repressors can recruit HDACs[9], HDAC1/2 might join forces with GRO, TLE and GRG and thereby shut down Wnt signaling to allow oligodendrocytes to differentiate on the appropriate schedule. There is recent evidence that Groucho is expressed in all oligodendrocyte lineage cells[12].

The gene regulatory network that controls oligodendrocyte development involves numerous transcription factors and signaling pathways; the Notch pathway is another area of intense research. Notch signaling inhibits OLP differentiation such that deletion of Notch1 in oligodendrocyte-lineage cells results in increased expression of myelin proteolipid protein and myelin-associated glycoprotein[13]. HES5, a downstream target of Notch signaling, can repress the expression of MBP and SOX10, which is a critical transcription factor controlling oligodendrocyte maturation[14]. HDACs can also switch off Notch signaling by competing with NICD for binding to CBF1 (ref. 8). In general, both Wnt and Notch signaling pathways inhibit differentiation. One established function of Notch signaling in the neural tube is to maintain the pool of neural progenitors in the ventricular zone by preventing them from giving rise to postmitotic neurons or glia until the appropriate time. HDACs, which participate in both Notch and Wnt pathways, might function to release this inhibition by increasing the state of chromatin compaction around genes that encode repressors of differentiation, preventing their transcription and providing permissive conditions for lineage progression (**Fig. 1**). Whether HDAC1/2 can interact directly or indirectly with myelin transcription factors such as Olig1/2 or Sox10 and how the Notch and Wnt pathways themselves impinge on these transcription factors are questions waiting to be answered.

It is generally believed that chronic demyelinated lesions in multiple sclerosis fail to be remyelinated because the local environment in the lesions is nonpermissive for oligodendrocyte differentiation and myelin formation. OLPs are commonly found in nonrepairing multiple sclerosis lesions, but differentiated oligodendrocytes are absent. OLP differentiation could be blocked by inhibitory signaling in the multiple sclerosis tissue (for example, Notch ligands are constitutively expressed in some multiple sclerosis lesions)[15]. Recent work has brought Wnt signaling into focus as another potential inhibitor of remyelination in multiple sclerosis, showing that TCF7L2 protein can be detected in multiple sclerosis lesions and suggesting that Wnt signaling is constitutively active[12]. Together with the study of Ye *et al.*[3], these findings suggest that activation of HDACs might help to overcome the inhibition of oligodendrocyte differentiation in multiple sclerosis lesions. Supporting this idea, the HDAC inhibitor valproic acid reduces remyelination after experimental demyelination in mice[6]. Moreover, activation of Wnt/β-catenin signaling through forced expression of active β-catenin or by using APC^{min} mice (which lack one copy of an endogenous Wnt pathway inhibitor gene) suppresses remyelination[12]. HDACs have been implicated in a range of human pathologies, especially cancer, and enormous effort is already directed toward finding specific HDAC modulators[4].

In conclusion, Ye *et al.*[3] provide important new insight into the mechanisms underlying oligodendrocyte development. HDACs in OLPs appear to serve as a hub integrating several signaling pathways and they might even be responsible for setting the threshold for remyelination in pathological demyelinating conditions. Future studies of the direct and indirect interplay between HDACs and other regulators, as well as further genetic studies of myelination/ remyelination in the conditional *Hdac1/2* null background, can be expected to further illuminate the molecular mechanisms underpinning oligodendrocyte lineage progression and to answer why this is blocked in chronic multiple sclerosis lesions.

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Stop and go GABA

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A recent study shows that GABA switches from stimulating to inhibiting interneuron motility during neocortical development. This change in response is gated by the expression of the chloride transporter KCC2.

Trains, planes and automobiles all rely on a set of extrinsic signals and the ability to signal to each other so as to prevent traffic jams and crashes. Similarly, inhibitory interneurons manage to evenly distribute throughout the developing neocortex. Traffic jams, or disruptions in the distribution of interneurons, are suspected to be involved in a range of neural dysfunctions, including epilepsy and schizophrenia. Previous studies have shown that interneuron invasion and distribution throughout neocortical lamina is regulated by the transcription factors Dlx and Lhx6 (refs. 1,2), the repulsant CRX4 (ref. 3), and by the neurotransmitter GABA. A recent paper by Bortone and Polleux[4], published in *Neuron*, now shows that migrating GABAergic interneurons undergo an intrinsic change that fundamentally alters their response to GABA.

GABA has been implicated in many aspects of neurodevelopment, from the control of cell proliferation in embryonic progenitor cells to the regulation of migration of adult-generated neurons destined for the olfactory bulb[5]. In developing cerebral cortex, GABA in the extrasynaptic space provides a tonic activation of GABA receptors[6,7]. Activation of GABA receptors promotes the migration of both pyramidal neurons[8] and interneurons[4]. GABA also promotes the migration of neuroblasts in the developing hippocampus. In this case, GABA is released by mechanisms distinct from those necessary to release synaptic vesicles[9].

In mature neurons, activation of $GABA_A$ receptors typically results in chloride influx and cell hyperpolarization because the intracellular concentration of chloride in neurons is lower than the extracellular concentration. In neuronal progenitors, however, this gradient is reversed, and GABA therefore typically depolarizes migrating neurons. This is a result of both the expression of the co-transporter NKCC1, which causes intracellular chloride to accumulate, and the relatively low expression of the chloride extruder KCC2. As neurons mature, increasing KCC2 expression results in a net extrusion of intracellular chloride, shifting the chloride reversal potential to a more hyperpolarized potential[10].

In a recent study, Bortone and Polleux[4] used a BAC-transgenic mouse that expresses enhanced green fluorescent protein (eGFP) in a subset of migrating interneurons originating from the medial ganglionic eminence (MGE).

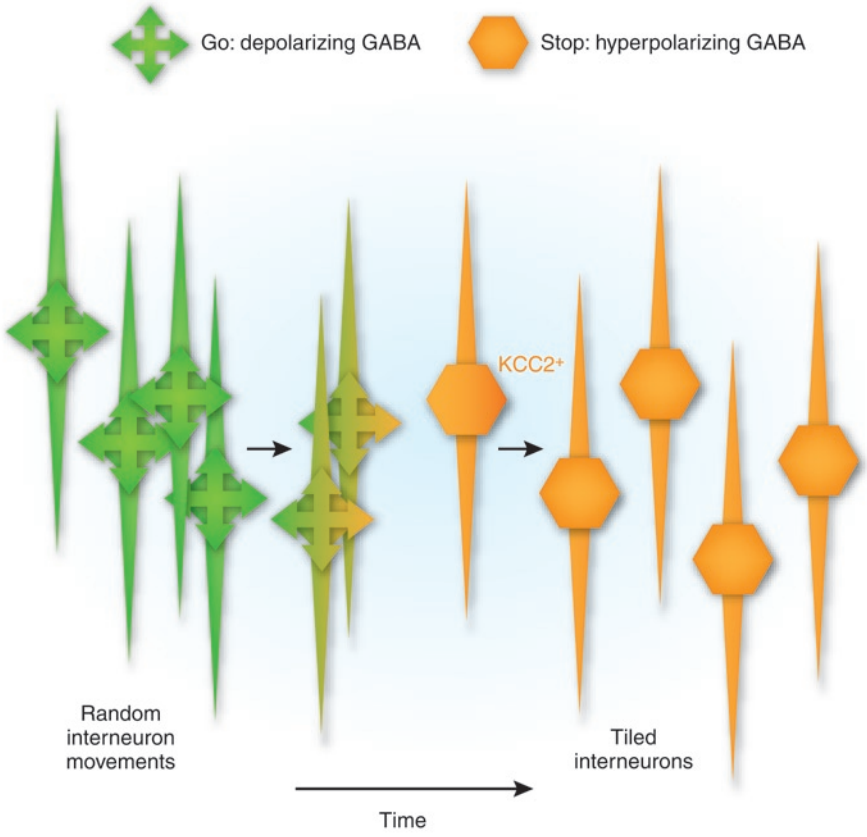

Figure 1 KCC2 expression acts as a molecular switch for interneuron migration. Depolarizing GABA promotes migration of immature interneurons (green cells). Developmentally increasing expression of KCC2 renders GABA hyperpolarizing and reduces interneuron motility (orange cells). This dual function of GABA may promote proper spacing of interneurons within the neocortex.

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