Nogo Establishes Spatial Segregation and Extent of Myelination During Development

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Abstract

Myelination of axons is an important developmental process that maximizes the speed and efficacy of action potential propagation throughout the nervous system.

In the developing central nervous system (CNS), myelin is formed by oligodendrocytes, cells with the capacity to form multiple myelin internodes.

What developmental mechanisms control the generation and precise coordination of the appropriate number of myelin internodes?

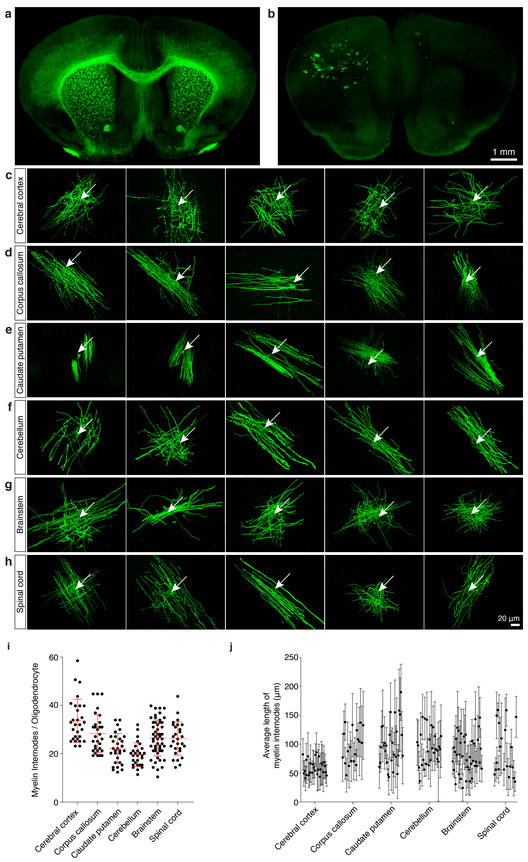
The ability to address this question is hindered by the high density of myelinating oligodendrocytes in vivo. We generated a transgenic mouse with sparsely labelled oligodendrocytes and describe here the remarkable heterogeneity of oligodendrocyte morphology.

We identify the amino-terminal of Nogo-A, expressed by oligodendroglial, as necessary and sufficient to regulate the myelinogenic potential of oligodendrocytes.

In addition, we find that the deletion of Nogo in vivo results in exuberant myelination.

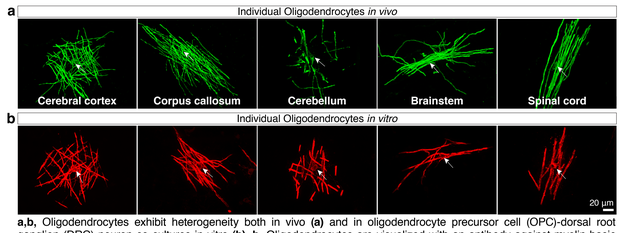
Together these findings support a novel physiological role for Nogo in ensuring the precise myelination of the developing CNS.

1 Individual Oligodendrocytes Exhibit Striking Diversity in the Number and Length of Myelin Internodes Formed In Vivo



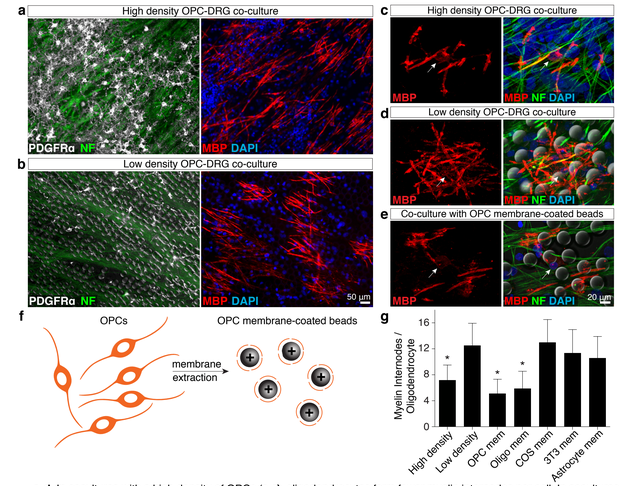
a, Section from the brain of a 2',3'-Cyclic nucleotide 3'-phosphodiesterase (CNP)-GFP transgenic mouse in which all mature oligodendrocytes are fluorescent. **b,** Section from the brain of a transgenic mouse in which the MBP enhancer drives GFP expression in less than 1% of oligodendrocytes. **c-h,** Individual oligodendrocytes from different brain regions of the sparsely labelled transgenic mouse. Arrows point to cell bodies. **i,j** Quantification of the number **(i)** and length **(j)** of myelin internodes in the sparsely labelled mouse. **j,** Error bars show the variable range of internode lengths from individual oligodendrocytes.

2 The Heterogeneous Environment Present In Vivo is not Required to Establish Oligodendrocyte Diversity



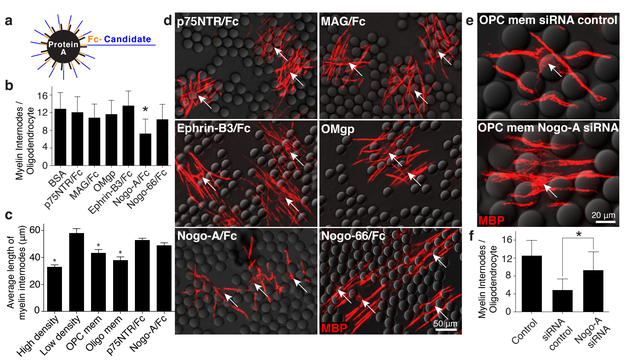
a,b, Oligodendrocytes exhibit heterogeneity both in vivo **(a)** and in oligodendrocyte precursor cell (OPC)-dorsal root ganglion (DRG) neuron co-cultures in vitro **(b)**. **b,** Oligodendrocytes are visualized with an antibody against myelin basic protein (MBP) in red.

3 Membrane-bound Oligodendroglial Cues Reduce the Number of Myelin Internodes Formed by Neighboring Oligodendrocytes



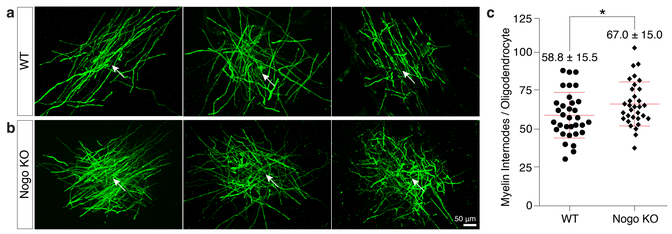
a-d, In cocultures with a high density of OPCs (**a,c**), oligodendrocytes form fewer myelin internodes per cell. In cocultures with a low density of OPCs, where the majority of OPCs are replaced with polystyrene beads (**b, d**), oligodendrocytes form more myelin internodes. **e-g,** To test for the presence of inhibitory cues expressed on oligodendroglial membranes, OPC and oligodendrocyte membranes were extracted and coated onto polystyrene beads. The membrane-coated beads were combined with a low density of OPCs and seeded onto axons. **g,** Quantification of the effect of various cell membranes on myelin internode numbers. **a-f,** OPCs are visualized by immunostaining for platelet-derived growth factor receptor alpha (PDGFRα). Axons are labelled with an antibody against neurofilament (NF).

4 Nogo-A is Sufficient and Necessary to Reduce the Number of Myelin Internodes Formed per Oligodendrocyte



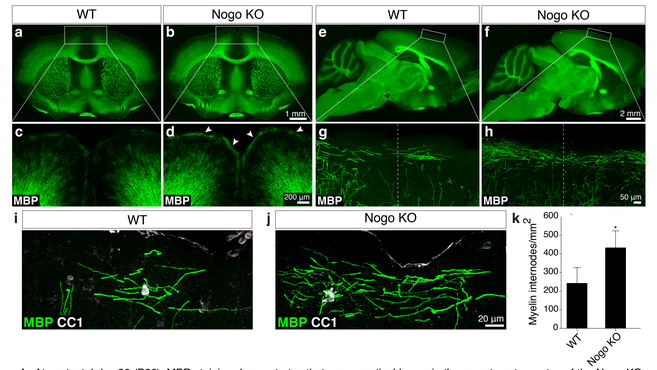
a-d, Fc-fusion constructs conjugated to polystyrene beads and seeded onto OPC-DRG co-cultures allow for localized interactions between oligodendroglia and candidate factors. **b,d** Only the amino terminal of Nogo-A (Nogo-A/Fc) significantly decreases myelin internode numbers. **c,d** Nogo-A is not responsible for the reduction in internode length induced by oligodendroglial membranes. **e, f,** Knockdown of Nogo-A in OPC membranes prior to adsorption onto polystyrene beads leads to an increase in myelin internodes formed per cell. The partial rescue following knockdown of Nogo-A suggests that oligodendroglia express additional membrane-bound cues that regulate myelinogenic potential.

5 The Myelinogenic Potential of Oligodendrocytes Increases in the Absence of Nogo In Vivo



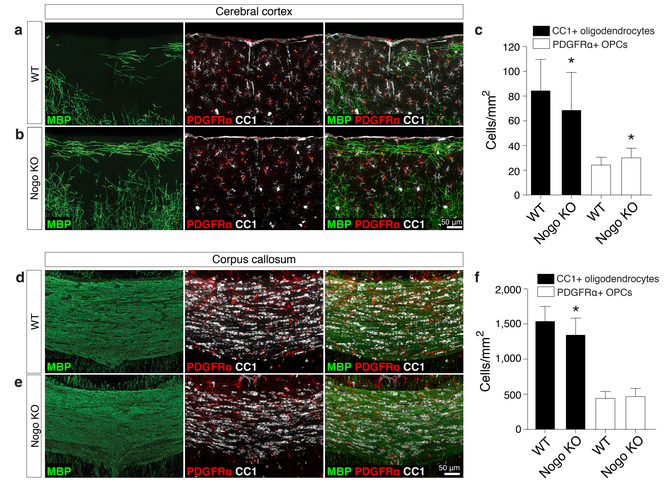
a-c, Sparsely labelled GFP-expressing oligodendrocytes in the cortex of Nogo KO mice **(b)** form significantly more myelin internodes compared to wild-type controls **(a)**.

6 The Deletion of Nogo In Vivo Results in Exuberant Myelin Formation



a-k, At postnatal day 30 (P30), MBP staining demonstrates that upper cortical layers in the somatomotor cortex of the Nogo KO mouse (**b,d,f,h,j,k**) exhibit more myelin internodes as compared to wild-type (WT) controls (**a,c,e,g,i,k**). **a-d,** Coronal view of WT (**a,c**) and Nogo KO (**b,d**) brains. **e-h,** Sagittal views of WT (**e,g**) and Nogo KO (**f,h**) brains. Box (**a,b,e,f**) indicates approximate locations for **c,d,g,h** Dashed lines **(g,h)** indicate approximate cut location in **c,d**. **i-j,** Magnified view of myelin internodes. **k,** Quantification.

7 Exuberant Myelination in the Absence of Nogo is Not Due to an Increase in Oligodendrocyte Numbers



a-b, Magnified coronal views of the cerebral cortex at postnatal day (P) 30 demonstrate exuberant myelin in Nogo KO mice (**b**) as compared to controls (**a**). **c**, Quantification demonstrating that there are significantly fewer oligodendrocytes and more OPCs in the cerebral cortex of Nogo KO mice. **f-h,** Similar to the cerebral cortex, at P30, there are significantly fewer oligodendrocytes in the Nogo KO corpus callosum compared to controls.

Conclusions

Our results suggest that membrane-bound NogoA modulates the myelinogenic potential of individual oligodendrocytes by mediating localized interactions between neighboring oligodendroglia.

We find that NogoA also plays a role in regulating the extent of myelin formation during development.

Targeting the expression of cues such as NogoA may offer new strategies to promote remyelination by maximizing the myelinogenic potential of oligodendrocytes.

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