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PirB is a Functional Receptor for Myelin Inhibitors of Axonal Regeneration

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A major barrier to regenerating axons after injury in the mammalian central nervous system is an unfavorable milieu. Three proteins found in myelin—Nogo, MAG, and OMgp— inhibit axon regeneration in vitro and bind to the glycosylphosphatidylinositol-anchored Nogo receptor (NgR). However, genetic deletion of NgR has only a modest disinhibitory effect, suggesting that other binding receptors for these molecules probably exist. With the use of expression cloning, we have found that paired immunoglobulin-like receptor B (PirB), which has been implicated in nervous system plasticity, is a high-affinity receptor for Nogo, MAG, and OMgp. Interfering with PirB activity, either with antibodies or genetically, partially rescues neurite inhibition by Nogo66, MAG, OMgp, and myelin in cultured neurons. Blocking both PirB and NgR activities leads to near-complete release from myelin inhibition. Our results implicate PirB in mediating regeneration block, identify PirB as a potential target for axon regeneration therapies, and provide an explanation for the similar enhancements of visual system plasticity in PirB and NgR knockout mice.

Myelin, an insulating layer surrounding axons, is thought to pose an obstacle to axon regeneration, inhibiting neurite outgrowth in vitro and contributing to regeneration failure in vivo. The NgR, a candidate receptor for the myelin-derived inhibitors Nogo, MAG, and OMgp (1–5), appears to be required for the acute inhibitory activity of these proteins, because genetic removal of NgR blocks acute growth-cone collapse in response to these factors when added in solution (6, 7). However, genetic deletion of NgR does not relieve the chronic inhibition of neurite outgrowth by myelin inhibitors presented as substrates (7, 8). Furthermore, genetic deletion of NgR does not enhance regeneration of corticospinal tract (CST) axons after dorsal hemisection (6, 8), although some regeneration of raphespinal and rubrospinal tracts after spinal cord injury has been reported (6). These data suggest that NgR is important for mediating some of the inhibitory activity of myelin inhibitors but that other binding receptors for these factors remain to be identified. Such putative receptors could work either independently or in concert with NgR.

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Fig. 1. LILRB2 and PirB can bind Nogo66 and MAG. (A) Schematic diagram of LILRB, PirB, and NgR receptors. ITIM, immunoreceptor tyrosine-based inhibitory motif; LRR, leucine-rich repeat. (B) COS cells were transfected with empty vector (control) or PirB or LILRB2 cDNA. After 48 hours, cells were incubated with AP-Nogo66 or MAG-Fc, and bound ligand was detected. NgR and NgR2 were used as positive controls for AP-Nogo66 and MAG-Fc binding, respectively. Scale bars, 200 μm. (C) The affinity of the MAG-Fc-PirB interaction is shown by one representative enzyme-linked immunosorbent assay binding curve. The experiment was repeated twice with similar results.
To identify candidate receptors for myelin inhibitors, we used expression cloning to screen an arrayed library of human cDNA pools (9). Our screen identified only two receptors for Nogo66: (i) NgR and (ii) the human leukocyte immunoglobulin (Ig)–like receptor B2 (LILRB2). LILRB2 is part of the B type subfamily of LILR receptors, which consists of five highly homologous family members in humans (10) (fig. S1). In mice, however, there is a single ortholog, paired immunoglobulin-like receptor B (PirB) (11). PirB shares only ~50% amino acid similarity with LILRB2, and it contains six Ig-like repeats, as opposed to four Ig-like repeats in LILRB proteins (Fig. 1A). Despite this low level of homology, we found that alkaline phosphatase (AP)–Nogo66 can bind PirB (Fig. 1B). The affinity of the AP-Nogo66-PirB interaction was similar to that of the AP-Nogo66 interaction with NgR (fig. S2). Given the unusual promiscuity of NgR binding to Nogo66, MAG, and OMgp, it is possible that LILRB2 and PirB also bind other myelin inhibitors. Indeed, we found that MAG-Fc and AP-OMgp bind PirB (Fig. 1B and fig. S2). The MAG-Fc-PirB interaction is high-affinity (half-maximal saturation of the interaction between purified MAG-Fc and purified PirB ectodomain: 13.8 ± 6 nM) (Fig. 1C).

To address whether PirB is a functional receptor for Nogo66, we focused on juvenile (P7) cerebellar granule neurons (CGNs), whose neurite outgrowth is inhibited when grown on AP-Nogo66 (12). Adult CGNs have been shown to express PirB (13), and we found that is also the case for juvenile CGNs, as assessed by reverse transcription polymerase chain reaction and in situ hybridization (fig. S3). We first tested the ability of a soluble ectodomain of PirB (PirB-His) to interfere with AP-Nogo66 inhibition in vitro. AP-Nogo66 inhibits neurite outgrowth of P7 CGNs to ~66% of untreated control levels (Fig. 2A). Inclusion of PirB-His in this assay reversed AP-Nogo66 inhibition, with neurite outgrowth returning essentially to control levels. These results are similar to those reported using the ectodomain of NgR to block inhibition by Nogo66 (8, 14, 15), and they indicate that PirB can bind the functionally inhibitory domain of Nogo66.
but do not address whether endogenous PirB in CGNs mediates inhibition by AP-Nogo66. Therefore, antibodies to PirB that are capable of interfering with the PirB-Nogo66 interaction were generated. Using a phage display platform (16) directed against the extracellular domain of PirB, we screened multiple clones for their ability to block binding of AP-Nogo66 to PirB (fig. S4). Clone YW259.2 (hereafter referred to as anti-PirB.1), which interfered best with AP-Nogo66-PirB binding, had a dissociation constant \( K_d \) of 5 nM for PirB. Anti-PirB.1 had no effect on the baseline axon growth of CGNs. However, it significantly reduced inhibition by AP-Nogo66 or myelin in cultured CGNs (Fig. 2B), rescuing neurite outgrowth to 59% from 41% on AP-Nogo66 and to 62% from 47% on myelin. Similar results were seen with MAG as an inhibitory substrate or with a different neuronal cell type [dorsal root ganglion (DRG) neurons] (fig. S5). These results suggest that PirB is a functional receptor mediating long-term inhibition of neurite outgrowth.

To confirm this result, we made use of mice carrying a loss-of-function PirB allele, the PirBTM mice, in which four exons encoding the transmembrane domain and part of the PirB intracellular domain have been removed (13). CGNs were cultured from PirBTM mice or wild-type (WT) littermates on a control substrate, AP-Nogo66, or myelin. On the control substrate (PDL/laminin), PirBTM neurons behaved similarly to WT neurons (Fig. 2C). However, neurite outgrowth from PirBTM neurons was markedly less inhibited than that from WT neurons on either AP-Nogo66 or myelin. On AP-Nogo66, outgrowth from WT neurons was inhibited to 50% of control levels, whereas PirBTM neurons were inhibited to 66%. Similarly, on myelin, WT neurons were inhibited to 52% of control levels, whereas PirBTM neurons were inhibited to 70%. Again, we observed similar partial disinhibition of PirBTM DRG neurons on both myelin and AP-Nogo66 (fig. S5). We also saw disinhibition of PirBTM CGNs on MAG and OMgp (fig. S5). These findings indicate that PirB is indeed a functional receptor for AP-Nogo66, MAG, OMgp, and myelin-mediated inhibition of neurite growth. However, loss of PirB activity does not fully rescue outgrowth.

It is possible that PirB and NgR function together to mediate inhibition of neurite outgrowth. To address this concept, both PirB and NgR function were blocked together in CGNs by culturing neurons from NgR-null mice in the presence of anti-PirB.1 (50 \( \mu \)g/ml) (Student’s \( t \) test, \( P < 0.01; n = 6 \) per condition). Scale bars, 50 \( \mu \)m.
inhibitory effect of PirB removal, it is likely that there are additional binding receptors for Nogo66. Although PirB appears to be a more important receptor for substrate inhibition than NgR, inactivation of either PirB or NgR alone is sufficient to block the acute growth-cone collapse caused by the addition of myelin inhibitors. This observation suggests that collapse is a more demanding process, requiring both PirB and NgR activities, acting either in parallel or together. In this context, it is of interest that PirB and NgR receptors have recently been shown to play similar roles in limiting plasticity of synaptic connections in the visual cortex. In mice lacking either receptor, eye closure during a critical development period results in excessive strengthening of connections via the open eye (13, 22). The mechanisms responsible for the effect of both receptors in mediating growth-cone collapse could also underlie the commonality of their role in ocular dominance plasticity.

The mechanism by which PirB signals to inhibit axon growth in response to myelin inhibitors is not clear. However, PirB has been shown to antagonize the function of integrin receptors (23) and to recruit both src homology 2-containing protein tyrosine phosphatase (SHP)-1 and SHP-2 phosphatases (13, 24); either or both of these events could attenuate normal neurite outgrowth. In humans, one or more members of the LILRB gene family might also play a role in regeneration. The blockade of PirB/LILRB activity, either with antibodies or by other means, provides an important target for therapeutic interventions to stimulate axonal regeneration.

References and Notes
5. J. K. Huang et al., Science 310, 1813 (2005); published online 16 November 2005 (10.1126/science.1118313).
9. Materials and methods are available as supporting material on Science Online.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/322/5930/967/DC1
Materials and Methods
Figs. S1 to S5
References
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"Who" Is Saying "What"? Brain-Based Decoding of Human Voice and Speech
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Can we decipher speech content ("what" is being said) and speaker identity ("who" is saying it) from observations of brain activity of a listener? Here, we combine functional magnetic resonance imaging with a data-mining algorithm and retrieve what and whom a person is listening to from the neural fingerprints that speech and voice signals elicit in the listener’s auditory cortex. These cortical fingerprints are spatially distributed and insensitive to acoustic variations of the input so as to permit the brain-based recognition of learned speech from unknown speakers and of learned voices from previously unheard utterances. Our findings unravel the detailed cortical layout and computational properties of the neural populations at the basis of human speech recognition and speaker identification.

In everyday life, we automatically and effortlessly decode speech into language independently of who speaks. Similarly, we recognize a speaker’s voice independently of what she or he says. Cognitive and connectionist models postulate that this efficiency depends on the ability of our speech perception and speaker identification systems to extract relevant features from the sen-