

# Norbin

## A promising central nervous system regulator

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Norbin, a neurite-outgrowth promoting protein, has been found to interact with and regulate several membrane proteins, including metabotropic glutamate receptor 5 (mGluR5). The disruption of both Norbin alleles leads to early embryonic death between 3.5 and 6.5 day post coitus.<sup>1</sup> Forebrain specific Norbin knockout (KO) mice are defective in synaptic plasticity,<sup>2</sup> an interesting feature considering that Norbin was initially discovered in the context of chemical-induced long term potentiation (LTP),<sup>3</sup> a form of synaptic plasticity extensively studied in the context of learning and memory.<sup>4</sup> The behavioral phenotypes associated with Norbin conditional KO suggest reduced mGluR5 function. Because of its fundamental functions, Norbin is emerging as a key neuronal regulator. The aim of the present review is to summarize current knowledge about Norbin while emphasizing its role in the nervous system.

### Norbin Identification

In a search for novel proteins involved in LTP, rat hippocampal slices treated with a potassium channel blocker (tetraethylammonium) known to induce LTP, were analyzed using a subtractive screening approach and the Norbin gene was found to be upregulated.<sup>3</sup> Norbin [neurite-outgrowth related rat brain protein (also called neurochondrin)] is a 75 kDa protein that is evolutionarily conserved from invertebrates (e.g., arthropods, echinoids) to vertebrates (e.g., mammals, amphibians, birds, fishes) (Fig. 1) presenting 98% amino acid identity among the rat, mouse and human orthologous proteins. Interestingly, the Norbin primary amino acid sequence does not share any significant homology with other proteins and does not present obvious domains or functional motifs. However, based on secondary structure predictions and sensitive fold recognition tools, it is clear that Norbin is enriched in  $\alpha$ -helices (65%) (Fig. 2A) that are likely organized as tandem repeats.

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### Norbin Chromosomal Localization and Gene Expression

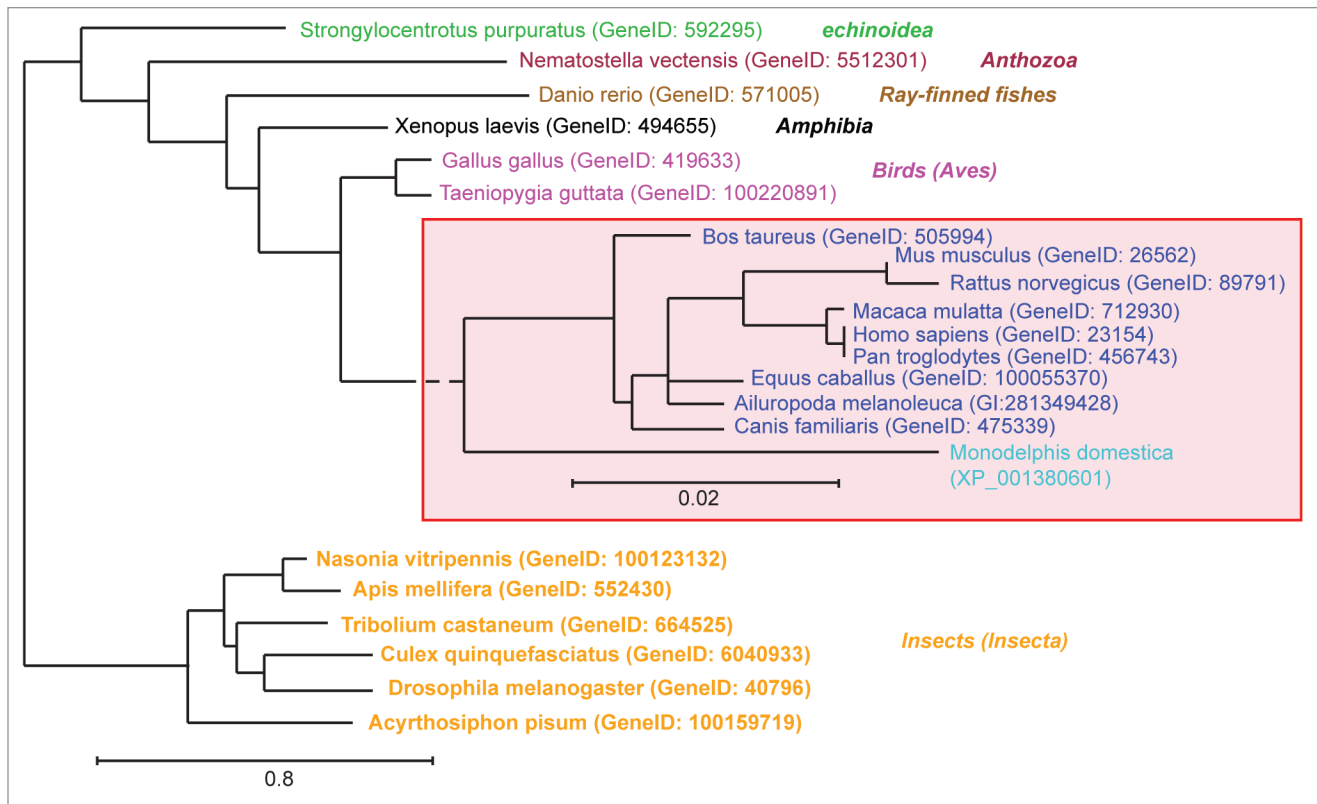
Norbin gene (Ncdn; MGI:1347351) localizes on mouse chromosome 4D2.1, rat chromosome 5q36 and human chromosome 1p34.3.<sup>5,6</sup> It contains 7 exons and codes for two splice variants in mouse and human.<sup>6</sup> Several potential binding sites for transcriptional regulators were identified in exon 1 of the Norbin gene.<sup>7</sup>

Norbin is predominantly expressed in the nervous system.<sup>3</sup> Norbin mRNA was first detected embryonically in the hind-brain and spinal cord at day E10.5 by in situ hybridization.<sup>8</sup> By day E18, the expression was also detected in forebrain and mid-brain. The expression of Norbin is detectable by western blotting at day E18 and increases until day P21, which temporally correspond to the time of dendrite outgrowth.<sup>9</sup> In the adult brain, Norbin is highly expressed in the bed nucleus of the stria terminalis (BNST), amygdala and hippocampus and more moderately in septum, piriform cortex, striatum and cortex.<sup>2,8</sup> BNST and amygdala are the two components of the brain circuitry regulating fear and anxiety, whereas hippocampus is essential for learning and memory. Norbin is specifically expressed in neurons and presents a somato-dendritic distribution.<sup>9</sup> Subcellular fractionation experiments indicated that the majority of Norbin exists in the cytosolic fraction. However, a fraction of Norbin remains insoluble in 1% triton, suggesting a membrane or cytoskeleton association.<sup>9</sup> In agreement, Norbin was found to localize to dendritic spines and post-synaptic densities (PSD).<sup>2,10</sup>

Notably, besides Norbin upregulation in response to LTP induction, Norbin expression was reported to be upregulated in amygdala when a mouse was exposed to the odor of a predator (cat),<sup>11</sup> indicating that Norbin's expression is regulated in response to environmental stimuli. In addition, Norbin expression might be differentially regulated in the brain cortex of male and female mice.<sup>12</sup>

### Norbin Interacts with Membrane Proteins and Binds Phospholipids

Norbin was found to interact with several membrane proteins enriched in the brain, including Sema4C,<sup>13</sup> several G-protein coupled receptors (GPCRs) from the family A such as the melanin-concentrating hormone receptor 1 (MCHR1)<sup>14,15</sup> and from



**Figure 1.** Phylogenetic analysis of Norbin sequences. Full length Norbin amino acid sequences (FASTA format) were aligned using the program MUSCLE. The phylogenetic analysis was performed on this alignment using PhyML and the tree was drawn using TreeDyn. The GeneID or accession numbers corresponding to each of the sequences used are indicated between parentheses. The mammalian species were grouped (red box) and were analyzed independently to increase the resolution. The class to which the species presented belong is indicated on the right in italics.

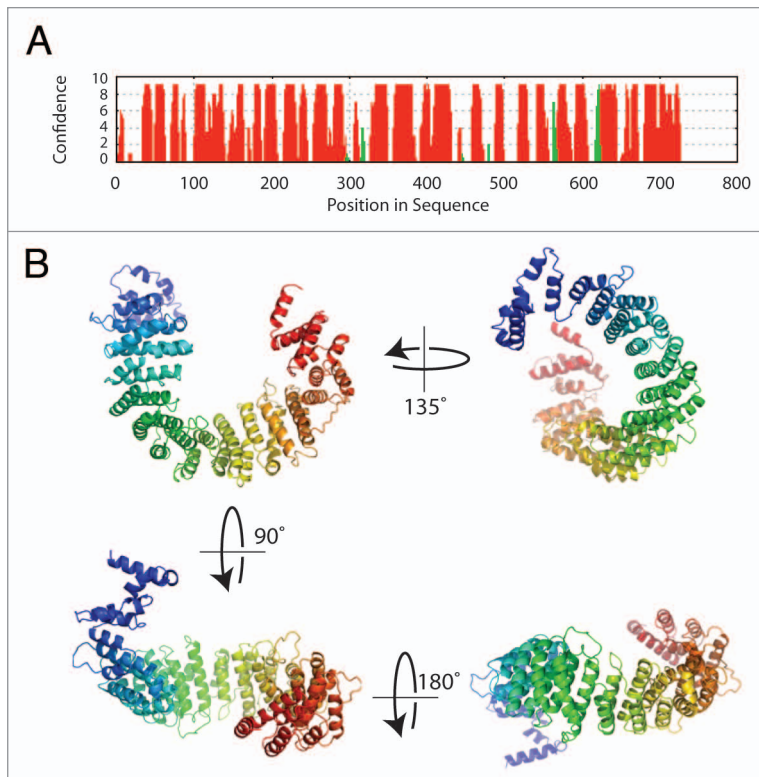
the family C (mGluR1 and mGluR5).<sup>2</sup> Truncation and mutagenesis studies indicate that the interactions between Norbin and membrane proteins involve the C-terminal part of Norbin and the membrane-proximal region of the membrane proteins.<sup>2,13,14</sup> However, the structural basis of these interactions is not understood. Recently, two sites responsible for Norbin binding were mapped on mGluR5.<sup>2</sup> Notably, there is no apparent sequence homology either between those two sites or among other Norbin-binding regions (e.g., Sema4C or MCHR1).

How does Norbin engage different proteins that lack sequence similarity? As mentioned earlier, structural prediction methods suggest that Norbin is almost entirely comprised of  $\alpha$ -helical repeat units which show features mostly resemble HEAT repeats ([www.embl.de/~andrade/papers/rep/search.html](http://www.embl.de/~andrade/papers/rep/search.html)).<sup>16</sup> The crystal structure of HEAT repeats protein importin  $\beta$ 1 illustrated that adjacent  $\alpha$ -helices stack upon each other to form a right-handed elongated super-helix. The superhelical structure creates a shallow groove that is a protein-protein interaction platform.<sup>17</sup> Using comparative modeling methods based on the fold recognition results, we propose a 3D structural model for Norbin (Fig. 2B). The concave surface of the curved Norbin helical fold is the likely area dedicated to protein binding. In this eventuality, Norbin would be capable of capturing proteins that present particular structural features instead of relying on primary sequence identity.

Until recently and despite the studies of the physical protein-protein interactions with Norbin, the functional and physiological consequences of interactions involving Norbin remained obscured. It has been reported that Norbin expression increases the steady-state cell surface level of MCHR1 and mGluR5,<sup>2,14</sup> suggesting that Norbin plays a key role in the trafficking of transmembrane proteins. Interestingly, mGluR5 C-terminal tail, including the Norbin-binding-region, contains several potential phosphorylation sites.<sup>2,18</sup> Phosphorylation of receptor intracellular domains is crucial for their endocytosis and recycling.<sup>19,20</sup> Norbin, by physically interfering with these modifications, might alter receptor phosphorylation and consequently its trafficking. Along those lines, the central region of Norbin was found to bind to phosphatidic acid, a membrane phospholipid.<sup>21</sup> This observation suggests that Norbin may function as an adaptor or scaffold protein linking membrane receptors and lipids, thus facilitating receptor trafficking to the cellular membrane and/or recycling between different cellular membrane compartments.

### Involvement of Norbin in Cell Signaling and Neuronal Function

Norbin was recently shown to modulate the signaling pathway of mGluR5.<sup>2</sup> mGluR5 primarily responds to the excitatory amino acid neurotransmitter glutamate, localizes at perisynaptic



**Figure 2.** Sequence analysis and structure prediction of Norbin. (A) Secondary structure prediction of Norbin. The full length amino acid sequence of Norbin was analyzed using PsiPred (v2.6). (B) Norbin helical fold architecture. The helical HEAT repeats detected in the Norbin sequence by fold recognition methods were used to build a plausible three-dimensional structure of human Norbin using comparative modeling techniques.<sup>16</sup> The seventeen helical repeats shown in (B) (colored in a blue-to-red N-terminal to C-terminal gradient, respectively) form a curved structure with a well defined inner (or concave) surface that forms the likely area of protein interaction.

sites<sup>22,23</sup> and plays important roles in normal brain function as well as in several pathological disorders, including schizophrenia.<sup>24-26</sup> A recent study indicates that Norbin expression enhances mGluR5 signaling in an *in vitro* expression system, while the conditional KO of Norbin in transgenic mice reduces mGluR5 function. In line with reduced mGluR5 function, Norbin KO mice show defects in pre-pulse inhibition and psychostimulant induced locomotor activity, two behavioral phenotypes typically observed in rodent models of schizophrenia.<sup>2</sup>

Norbin has also been shown to modulate the signaling pathway downstream of other G<sub>αq</sub> coupled GPCRs such as inhibiting MCHR-induced G-protein activation and downstream calcium influx. It is hypothesized that Norbin, by binding to MCHR1, sterically competes with the binding of G-proteins to the receptor and thereby inhibits G-protein-coupled signal transduction.<sup>14</sup> MCHR1 is the melanin-concentrating hormone receptor involved in the regulation of feeding behavior and energy balances.<sup>27</sup> Whether Norbin affects metabolic energy through regulating MCHR1 awaits further study.

As mentioned earlier, the expression of Norbin is upregulated during chemical-LTP.<sup>3</sup> LTP is the long-lasting enhancement of synaptic strength between neurons, which is an important

cellular mechanism underlying learning and memory.<sup>4</sup> At least two biochemical phases exist for LTP: an early phase involving modification of existing synaptic proteins and a late phase conveyed by *de novo* transcription and translation.<sup>28</sup> Our recent study showed that fore-brain specific Norbin KO abolished both early and late phase hippocampal LTP.<sup>2</sup> Consistently, behavioral studies indicated that Norbin deletion impaired spatial learning and memory.<sup>29</sup> Activation of mGluR5 is required for hippocampal LTP induction. Considering the positive regulation of mGluR5 by Norbin, it is very likely that Norbin's role in LTP is at least partially dependent upon its interaction with mGluR5. However, the LTP defects seen in Norbin KO mice are slightly more severe than the ones described in mGluR5 KO mice,<sup>2,30</sup> suggesting that Norbin might play roles in addition to regulating mGluR5. One obvious possibility is that Norbin deletion also affects mGluR1 function as Norbin also interacts with mGluR1.<sup>2</sup>

Norbin was found to induce neurite-outgrowth when ectopically overexpressed in cultured neuroblastoma N2a cells. The first 100 amino acids of Norbin contain the activity necessary to induce this phenomenon.<sup>3,31</sup> However, the molecular mechanism underlying this neurite-outgrowth promoting activity is not known. Recently, Norbin was found to interact with Dia1, an actin nucleation factor that stimulates barbed end actin filament elongation.<sup>31</sup> Although Norbin does not affect the actin polymerizing activity of Dia1, recruiting Dia to a specific sub-cellular localization might contribute to the neurite-outgrowth function of Norbin. Additionally, the capacity of Norbin to direct the trafficking of key membrane receptors toward specific cellular compartments, may also contribute indirectly to neurite outgrowth.

## Conclusions

Over the last few years, progress has been made in understanding the function of Norbin in the nervous system. Norbin plays essential roles during development and for synaptic plasticity and interacts physically with several membrane proteins. Structure prediction of Norbin suggests that Norbin may function as a scaffold or adaptor protein that regulates the trafficking and/or signaling of membrane proteins. However, questions are also raised: (1) Is the interaction between Norbin and receptors regulated? Ligand induced GPCR phosphorylation regulates the interaction between GPCRs, GRKs and arrestins.<sup>32</sup> As mentioned earlier, Norbin binding domains on mGluR5 include several phosphorylation sites. It will be interesting to test how receptor phosphorylation affects Norbin binding. (2) Could Norbin catalyze receptor crosstalk? Norbin is capable of binding several receptors. Unpublished data indicate that Norbin exists at least partly as a dimer. Assuming that each of the two subunits of a Norbin dimer binds different receptors, Norbin might mediate the coupling and crosstalk among receptors. (3) What is the physiological significance of Norbin interaction with membrane receptors? Norbin

interacts with several membrane receptors. With conditional Norbin mice, how Norbin affects receptor function can now be examined by *in vivo* studies. (4) Is Norbin involved in anxiety/fear responses? Norbin is highly expressed in BNST and amygdala, two brain regions involved in fear and anxiety responses. Forebrain specific Norbin KO mice provide a useful tool to understand this question. In summary, more systematic behavioral studies of Norbin KO mice and future work using molecular

and cellular methods will improve our understanding of Norbin function in the CNS.

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