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Molecules in focus

Molecular regulation of Nogo-A in neural cells: Novel insights into structure and function

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ABSTRACT

Nogo-A is part of the reticulon family of proteins localized to the myelin and oligodendroglial plasma membranes. Nogo-A specifically initiates signal transduction cascades limiting axonal regrowth following injury and disease in the adult mammalian central nervous system (CNS). Recent novel data support the contention that neuronal Nogo-A plays an important role in regulating cytoskeletal re-organization without the requirement of signaling through its cognate receptor (Nogo receptor). These data, along with the recent findings that the N-terminus of Nogo-A can interact with integrins and that NgR1 interacts with the amyloid precursor protein extracellularly, as well as novel findings showing ubiquitin ligases binding with Nogo-A intracellularly add a layer of complexity to its functional role in the CNS.

1. Introduction

Central nervous system (CNS) myelin proteins that inhibit axonal growth were first reported by Caroni and Schwab (1988). Nogo-A, an 1192 amino acid, 256 kDa glycoprotein was later identified to be a prominent inhibitory component of myelin (GrandPre et al., 2000). The role of Nogo-A in regulating neuronal growth during development or importantly following injury has been well characterized (Huber et al., 2002). The chemical structure of Nogo-A and the downstream molecular pathway it can potentiate in neurons have also been well documented (Montani et al., 2009). However, the mechanism of Nogo-A degradation and thus turnover has not received the same attention. Nogo-A is now widely accepted to be an important protein for axonal growth critical during the development of CNS axons (Montani et al., 2009), as well as playing a role in acute injury and neurological disorders (Brosamle et al., 2000; Park et al., 2006; Novak et al., 2002; Papadopoulos et al., 2006). Recent advances have identified a novel interaction between Nogo-A and WWP1, an ubiquitin ligase (Qin et al., 2008), suggesting that ubiquitinylation may be a crucial component in Nogo-A breakdown and hence turnover. With the large array of ubiquitin ligases involved in neurodegeneration in the CNS, identifying the ligases responsible for Nogo-A turnover at the plasma membrane and elucidating the downstream molecular mechanisms may provide insight into novel therapeutic targets for genetic and acquired neurological disorders.

2. Structure

Nogo belongs to the reticulon family of proteins (RTN), which is a large group of membrane-associated proteins present in all eukaryotic organisms and contain a reticulon homology domain (RHD). The RHD is a conserved C-terminal region consisting of two hydrophobic regions flanked by a hydrophilic loop. The diversity of the reticulon family of proteins arises due to alternative splicing resulting in multiple isoforms (GrandPre et al., 2000; Huber et al., 2002). In mammalian cells, there are four reticulon genes encoding for RTN1-4, with RTN4 also known as Nogo (isoforms A, B and C) (GrandPre et al., 2000). The C-terminus of RTNs contains a di-lysine motif resulting in these proteins mainly localizing in the endoplasmic reticulum (ER). However, RTNs are present on the cell surface at lower levels (Dodd et al., 2005). There are three active sites believed to be exposed extracellularly when Nogo is cell surface bound. First, the Nogo-66 domain (located close to the C-terminus) is responsible for inhibiting neurite growth and inducing growth cone collapse (GrandPre et al., 2000). Second, the central inhibitory domain (large 800-amino acid Nogo-A specific domain) also inhibits neurite outgrowth, cell spreading and growth cone collapse (GrandPre et al., 2000). Finally, the N-terminus, which is located in the Nogo-A and B isoforms, inhibits axon growth through the direct or indirect inhi-

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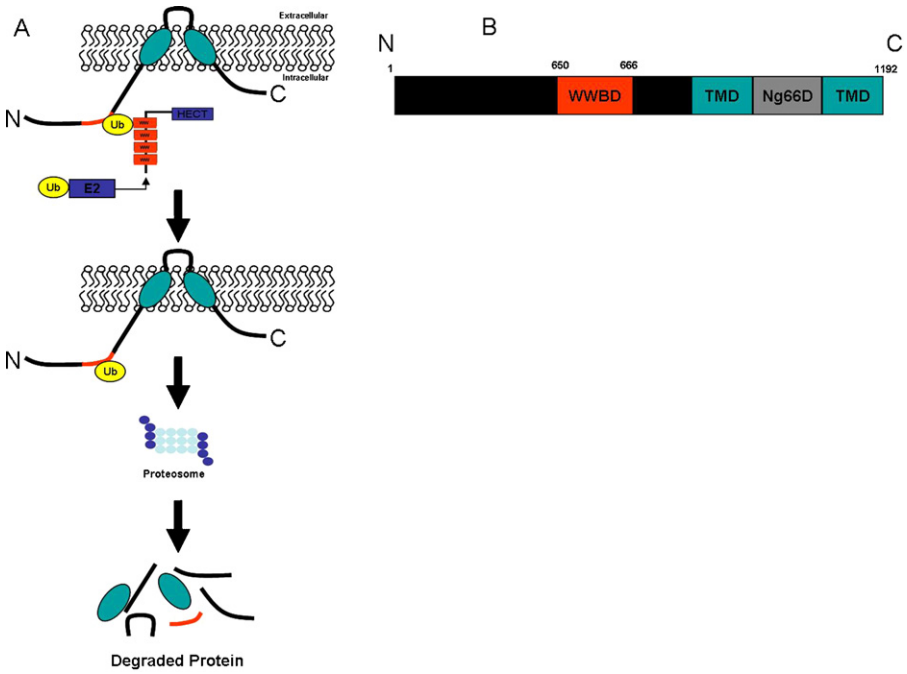

Fig. 1. Central nervous system, full length specific Nogo-A protein (1192 amino acids in length), consisting of two transmembrane domains and a C-terminus flanking the inhibitory Nogo-66 domain, required for interaction with NgR1 (ligand binding domain). Two intracellular tails (one at the N-terminus and one at the C-terminus in the *cis* configuration) (A) in the *cis* configuration, the N-terminus has the capacity to bind the E3 ubiquitin ligase at the PPxY motif and (B) Nogo-A in its *trans* configuration, has its N-terminus in the extracellular compartment which is able to interact with integrin receptors, commonly referred to as amino-nogo and can also be a potent inhibitor of neurite outgrowth. The classical *cis* conformation of Nogo-A in neuronal/oligodendroglial cell membranes. At the N-terminus a WW binding domain (WWBD) has now been identified which can interact with E3 ubiquitin ligases to regulate Nogo-A turnover at the membrane through proteosomal degradation (A and B).

bition of integrins (Hu and Strittmatter, 2008) (see Fig. 1). All three Nogo isoforms share a common 188 amino acid carboxyl-terminus, two hydrophobic domains and a short 66 amino acid loop between these two hydrophobic regions called the Nogo-66 loop (GrandPre et al., 2000). Nogo-A as well as Nogo-C also contains a proline-rich region specific for protein–protein interactions. In particular Nogo-A has SH3-ligand and WW-ligand domains, which are both involved in ligand–ligand interactions (Qin et al., 2008).

Nogo-66 is expressed on the cell surface of oligodendrocytes, in the innermost lamella of the myelin sheath, and is thought to be the important segment of Nogo-A that produces the inhibitory axonal outgrowth properties (Fournier et al., 2001). It can exert these actions by acting on the Nogo-66 receptor (NgR), which is a 473 amino acid glycosylphosphatidylionsitol (GPI)-linked protein. The ligand binding site of NgR is located at the N-terminus, followed by 8 leucine-rich repeats (LRR) and a LRR carboxyl-terminal flanking domain (Fournier et al., 2001). In addition to other ligands such as amyloid precursor protein (APP) and the β-amyloid protein (Aβ) (Park et al., 2006), other CNS myelin proteins such as myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) can also bind NgR in particular the NgR1 (Nogo-66) receptor homologue to mediate their inhibitory effects (Li et al., 2004).

3. Biological function

Nogo-A is present in the processes of oligodendrocytes (Huber et al., 2002) and neuronal Nogo-A is present in axons of neurons (Dodd et al., 2005). Although Nogo-A is mainly ER bound in oligodendrocytes and not highly expressed at the cell surface, it is yet to be determined if neuronal Nogo-A, is predominantly ER bound, or whether when expressed in neurons, it is present on the cell surface potentiating strong repulsion cues common for membrane-bound Nogo-A (Dodd et al., 2005). The localization of both Nogo-A and NgR has been found to be distributed throughout adult mouse brain and spinal cord (Wang et al., 2002). Importantly, Nogo-A mRNA expression has been strongly identified in neurons with an enhanced capacity to regenerate. For example, motor neurons, deep cerebellar nuclei and neurons from the thalamic reticular nucleus show a higher Nogo-A mRNA (Huber et al., 2002).

The mechanism by which Nogo-A may be regulated has yet to be elucidated. It is plausible that Nogo-A requires ubiquitinylation, possibly by the HECT (homology to the E6-associated protein C-terminus) type E3 ligases such as Nedd4 and Nedd4-2 (Neuronally Expressed Developmentally Down-regulated 4 and 4-2). Nedd4 and Nedd4-2 are able to bind to proteins, which consist of a PPxY motif (Ingham et al., 2004), important for protein–protein interactions. Recently, it has been shown that the N-terminus of Nogo-A contains specific protein interaction sites (Qin et al., 2008) that can be recognized by the WW domains (WW1-4) also present in Nedd4 and Nedd4-2. It appears that the most likely mechanism of Nogo-A modulation and degradation is via the ubiquitin-proteosome pathway regulating the expression of Nogo-A (Fig. 1), which may have significant implications in specific neurological diseases.

So far the roles of Nedd4 and its close relative Nedd4-2 have been demonstrated by their capacity to modulate (by ubiquitinylation and degradation) various transporter, receptor and ion channels in the CNS. Nedd4-2 has an integral role in the regulation of insulin growth factor-1 (IGF-1) (Henke et al., 2004) and the surface expression of the excitatory amino acid transporters (EAATs) 1 and 2 (Boehmer et al., 2003, 2006). Furthermore, Nedd4-2 is involved in the cell growth and differentiation by regulating nerve growth factor (NGF) and brain derived neurotrophin factor (BDNF), via its ability to ubiquitinate its high affinity receptor TrkA. Finally, Nedd4 and Nedd4-2 regulate voltage gated K^+ channels (Ekberg et al., 2007). Interestingly, Nogo-A is also able to regulate K^+ channel localization at the paranode during development (Nie et al., 2003). However, the relationship of Nedd4-2 and Nogo-A with regard to K^+ channel regulation has not been established.