Journal of Neurochemistry

JNC

JOURNAL OF NEUROCHEMISTRY | 2018 | 147 | 712–714

doi: 10.1111/jnc.14597

EDITORIAL HIGHLIGHT

Inside-out or outside-in, a new factor in MAG-mediated signaling in the nervous system

An Editorial for 'High-affinity heterotetramer formation between the large myelin-associated glycoprotein and the dynein light chain DYNLL1' on page 764.

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Abstract

Our nervous system depends on protein-mediated cellular communication and connections for its formation and function. The transmembrane receptor Myelin-Associated Glycoprotein (MAG) plays an important role in the wrapping process of myelin around axons and in life-long maintenance of this important bicellular structure. MAG organizes the adhesion and the signalling between the axon and the myelin. But how does MAG do this? Better understanding of this process is required to treat MAG-function associated neurological disorders. This editorial highlights a study by Myllykoski et al. in the current issue of the Journal of Neurochemistry that describes the identification and characterization of a novel intracellular binding partner of MAG. Using cellular, biophysical and structural techniques, the authors show that the dynein light chain, DYNLL1 recognizes and interacts with only one of two splice forms of MAG, L-MAG. DYNLL1 dimerizes L-MAG at the cytosolic side and this has implications for the signalling and adhesive functions of MAG in our nervous system.

Myelin enables axons to increase the speed of transmitting information, provides life-long axonal integrity and adds an additional layer of complexity to brain functions (Simons and Nave 2015). In the peripheral nervous system, Schwann cells provide the myelin processes whereas in the CNS this is organized by oligodendrocytes. Both cells express myelin-associated glycoprotein (MAG) (also known as sialic acid-binding immunoglobulin-type lectin 4a) on their cell surface to regulate and maintain myelination. At the myelin-axon interface MAG has a threefold role; (i) it forms an adhesion complex with axonal glycolipids, called gangliosides, to maintain a defined myelin-axon spacing (Pan *et al.* 2005), (ii) MAG can function as a ligand to signal from myelin into the axon to regulate neuronal plasticity (Vinson *et al.* 2001) and (iii) MAG can function as a receptor to signal into the myelinating cell to regulate the myelination process (Umemori *et al.* 1994). The Kursula lab has now solved a piece of the puzzle on how MAG is able to organize these three intricate roles (Myllyksoki et al. 2018).

The two isoforms of MAG, a small form (S-MAG) and a large form (L-MAG), have different expression profiles and different roles in the development and function of the nervous system (Erb *et al.* 2006). Nevertheless, only their C-termini are different. MAG is a type-I transmembrane protein that belongs to the immunoglobulin superfamily cell adhesion molecule (IgSF CAM). It consists of five Ig domains on the N-terminal extracellular side, followed by a single-transmembrane helix and an intracellular region that is predicted to be disordered. This intracellular side of MAG can be divided into two regions, a juxtamembrane region of 38 residues and an isoform-specific C-terminal segment that differs in composition and length for L-MAG and S-MAG, consisting of 54 and 10 residues, respectively. The two different C-termini are important for MAG to fulfill its

Received September 14, 2018; accepted September 19, 2018.

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Abbreviations used: CNS, central nervous system; DYNLL1, dynein light chain LC8-Type 1; IgFS CAM, immunoglobulin superfamily cell adhesion molecule; L-MAG, large MAG; MAG, myelin associated glycoprotein; NgR1, nogo receptor 1; PirB, paired immunoglobulin receptor B; PNS, peripheral nervous system; Siglec4a, sialic acid-binding immunoglobulin-type lectin 4a; S-MAG, small MAG.

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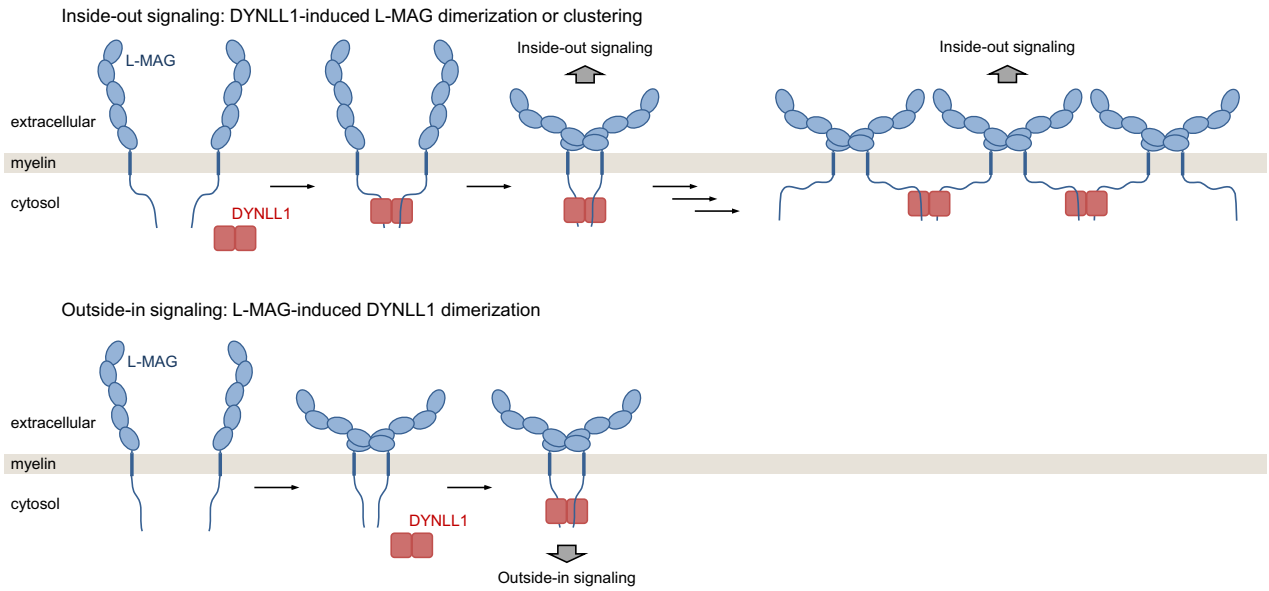

Fig. 1 DYNLL1 may contribute to L-MAG signaling through two possible routes. By binding to the cytosolic segment of L-MAG, DYNLL1 induces L-MAG dimerization or clustering to trigger inside-out L-MAG signaling (top panel). Receptors bound to L-MAG can further transduce this signal, e.g. into the axon (not shown). Extracellular factors (e.g. axonal receptors, not shown) that dimerize or cluster L-MAG may trigger DYNLL1 binding and further cytosolic outside-in signaling in the myelinating cell (bottom panel).

intricate roles, but how this works on a molecular level is not clear.

Myllykoski *et al.* identified the homodimeric protein DYNLL1 (LC8) as a MAG binding partner using the intracellular segment of L-MAG as bait in a yeast two-hybrid screen. The interaction is specific for L-MAG as the S-MAG intracellular region does not recover DYNLL1 in this setup. The affinity for the L-MAG-specific region and DYNLL1 in solution is 7.3 μM and comparable to DYNLL1 interactions with other partners (Radnai *et al.* 2010). Besides its role in dynein function, DYNLL1 on its own is well known to dimerize intrinsically disordered proteins. The crystal structure of the DYNLL1 dimer in complex with the L-MAG-specific region shows a 2 : 2 heterotetramer that dimerizes L-MAG (Myllyksoki et al. 2018).This is an important finding because it was recently shown that the MAG extracellular segment can dimerize via the membrane proximal Ig domains 4 and 5, albeit weakly, and this dimerization is believed to be important for MAG function (Pronker *et al.* 2016). The much higher affinity of the dimeric DYNLL1 for L-MAG is possibly further enhanced by a full-length L-MAG in the context of the cell membrane and suggests that DYNLL1 plays an important role in L-MAG dimerization. The isoform-specific dimerization of MAG at the cytosolic side, either mediated or sensed by DYNLL1, is a new mechanism to control MAG signaling.

How can DYNLL1 contribute to L-MAG signaling? Two mechanisms are conceivable; inside-out or outside-in signaling in which the L-MAG cytosolic region can act as a transmitter or a receiver, respectively. In inside-out signaling, binding of DYNLL1 to two monomeric L-MAG molecules triggers MAG dimerization or larger order clustering (Fig. 1) and this oligomeric change in MAG is sensed at the cell outside. If L-MAG is interacting with an axonal receptor (gangliosides (Pan *et al.* 2005), nogo receptor 1 (Liu *et al.* 2002) and paired immunoglobulin receptor B (Atwal *et al.* 2008)) this change can be transmitted into the axon (Fig. 1). In outside-in signaling the dimerization or clustering of L-MAG by extracellular ligands is transmitted into the intracellular side of the myelin to induce DYNLL1 binding due to much increased affinity arising from avidity (Fig. 1b). The DYNLL1 binding could then trigger further events, such as phosphorylation or co-factor binding, inside the myelinating cell.

The current data suggest cells have four tools available to regulate MAG signaling. (i) By tuning the local concentration of available DYNLL1, cells can control L-MAG dimerization or its response to L-MAG dimerization. (ii) Phosphorylation of the DYNLL1 binding site on L-MAG (residues 606-617) or flanking residues (e.g. Thr607 or Tyr620) may influence DYNLL1 binding and thereby MAG function. (iii) Cells can express L-MAG or S-MAG that have partially overlapping and partially different properties. (iv) Glycosylation at the extracellular side can fine-tune MAG dimerization (Pronker *et al.* 2016) and, possibly, signaling. Which of these mechanisms play a physiological role and in which context, will be a topic for future research.

Further questions can now be raised. Does DYNLL1 influence L-MAG dimerization as a transmitter or does it act as a receiver to signal into the myelinating cell (Fig. 1)? What is the role of posttranslational modifications of MAG in signaling? Which properties determine the promiscuity of

DYNLL1 for binding partners and can a new consensus sequence be defined that allows identification of new interactors by bioinformatics? The L-MAG residues that define the DYNLL1 binding site are conserved, but differ substantially from known DYNLL1 interactors, adding valuable information to defining a consensus sequence. In which cellular substructures does the DYNLL1 – L-MAG combination play a role? Co-localization of DYNLL1 and MAG in the paranodal compartment of myelinating Swann cells suggests a role in myelination (Myllyksoki et al. 2018) but this will need to be verified, e.g. by using L-MAG or DYNLL1 interface mutants that are incapable of complex formation. Last but not least the question arises whether the detailed structure of the DYNLL1 – L-MAG complex presented can be used to design modulators, based on DYNLL1 or on L-MAG, to treat MAG associated pathologies such as nervous system regeneration inhibition and demyelinating disorders? The work by Myllykoski *et al.* (Myllyksoki et al. 2018) adds a new factor and a new mechanism to the isoform-specific function of MAG in our nervous system.

Acknowledgments and conflict of interest disclosure

Bert J.C. Janssen is supported by a European Research Council Starting Grant (677500). B.J.C.J. has no conflict of interest to disclose.

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