Matti Myllykoski, Maria A. Eichel, Ramona B. Jung, Sørge Kelm, Hauke B. Werner, Petri Kursula

1 Faculty of Biochemistry and Molecular Medicine, University of Oulu, Finland
2 Department of Neurogenetics, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany
3 Georg August University School of Science, University of Göttingen, 37077 Göttingen, Germany
4 Centre for Biomolecular Interactions Bremen (CBIB), University of Bremen, Bremen, Germany
5 Department of Biomedicine, University of Bergen, Bergen, Norway

*Correspondence:
petri.kursula@uib.no (P.K.)
Department of Biomedicine, University of Bergen
Jonas Lies vei 91, N-5009 Bergen, Norway
Tel.: +47-5586438

OR
Hauke@em.mpg.de (H.B.W.)
Max Planck Institute of Experimental Medicine
Hermann-Rein-Str. 3, D-37075 Göttingen, Germany
Tel.: +49 (551) 3899-759
Fax.: +49 (551) 3899-758

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**Abstract**

The close association of myelinated axons and their myelin sheaths involves numerous intercellular molecular interactions. For example, myelin-associated glycoprotein (MAG) mediates myelin-to-axon adhesion and signalling via molecules on the axonal surface. However, knowledge about intracellular binding partners of myelin proteins, including MAG, has remained limited. The two splice isoforms of MAG, S- and L-MAG, display distinct cytoplasmic domains and spatiotemporal expression profiles. We used yeast 2-hybrid screening to identify interaction partners of L-MAG and found the dynein light chain DYNLL1 (also termed DLC8). DYNLL1 homodimers are known to facilitate dimerization of target proteins. L-MAG and DYNLL1 associate with high affinity, as confirmed with recombinant proteins in vitro. Structural analyses of the purified complex indicate that the DYNLL1-binding segment is localized close to the L-MAG C terminus, next to the Fyn kinase Tyr phosphorylation site. The crystal structure of the complex between DYNLL1 and its binding segment on L-MAG shows 2:2 binding in a parallel arrangement, indicating a heterotetrameric complex. The homology between L-MAG and previously characterized DYNLL1-ligands is limited, and some details of binding-site interactions are unique for L-MAG. The structure of the complex between the entire L-MAG cytoplasmic domain and DYNLL1, as well as that of the the extracellular domain of MAG, were modeled based on small-angle X-ray scattering data, allowing structural insights into L-MAG interactions on both membrane surfaces. Our data imply that DYNLL1 dimerizes L-MAG, but not S-MAG, through the formation of a specific 2:2 heterotetramer. This arrangement is likely to affect, in an isoform-specific manner, the functions of MAG in adhesion and myelin-to-axon signalling.

**Introduction**

Myelination of axons facilitates rapid nerve conduction in the vertebrate nervous system. Molecular interactions between the extracellular surface of myelinated axons and the myelin sheath are crucial for the development and lifetime maintenance of axon/myelin-units. Our knowledge about extracellular glia-axon interactions has greatly improved in recent years, regarding *e.g.* the formation of the nodes of Ranvier (Rasband and Peles 2015; Salzer and Zalc 2016) and the glia-dependent support of axonal integrity (Edgar & Nave, 2009; Philips and Rothstein, 2017). On the other hand, little is known about the intracellular interactions of myelin membrane proteins at the molecular level.

While the ultrastructure of myelin as a multilayered membrane appears similar in the CNS and PNS, functional and biochemical differences exist (Nave and Werner 2014). For example, the immunoglobulin-like cell adhesion molecule (Ig-CAM) myelin-associated glycoprotein (MAG)
(Arquint et al. 1987; Lai et al. 1987; Salzer et al. 1987) constitutes, according to quantitative mass spectrometry, 1% of the total myelin protein in CNS myelin (Jahn et al. 2009), but only 0.3% in PNS myelin (Patzig et al. 2011). In the CNS, MAG functions as a repulsive oligodendrocytic surface molecule, representing one of several myelin-associated inhibitors of axonal regrowth after injury (Filbin 2003). In the PNS, expression of MAG by myelinating Schwann cells regulates the developmental survival of motoneurons (Palandri et al. 2015), axonal caliber (Yin et al. 1998), and the long-term maintenance of axonal integrity (Fruttiger et al. 1995; Kinter et al. 2013). MAG is involved in human neurological diseases, and anti-MAG IgM monoclonal gammopathies are relatively common neuropathic conditions (Bosch and Smith 1993; Dalakas 2010; Steck et al. 2006). Recently, missense mutations in the extracellular domain of MAG were identified in patients with a heritable hypomyelinating leukodystrophy (Lossos et al. 2015; Roda et al. 2016). Taken together, MAG is a pathobiologically relevant myelin-specific Ig-CAM, with specific functions in myelin-to-axon signalling.

Crystal structures of the extracellular domain of MAG (MAGex), comprised of five Ig-like domains (Lai et al. 1987; Salzer et al. 1987), were recently reported (Pronker et al. 2016), suggesting conformational and oligomeric state determinants of the interaction between MAG and its axonal binding partners, including gangliosides. Interestingly, the crystal structures implied a homodimeric arrangement, while the suggested homodimerization interface in Ig-like domains 4 and 5 displayed surprisingly low affinity (Pronker et al. 2016). In contrast to membrane-associated MAG, soluble forms of its extracellular domain are unable to bind to neurons (Sadoul et al. 1990), which implies a need for dimerization and/or cytoplasmic binding partners as prerequisites for proper adhesion. How this dimerization could be linked to the cytoplasmic interactions of MAG, and vice versa, has remained unknown.

Two isoforms of MAG emerge via alternative splicing of exon 12 of the Mag gene (Lai et al. 1987; Salzer et al. 1987; Tropak et al. 1988). Exon 12 encodes 10 amino acids at the C terminus of S-MAG, whereas exon 13 encodes the C terminus of L-MAG, with 54 specific amino acids (Lai et al. 1987; Salzer et al. 1987). Thus, the two MAG isoforms have identical extracellular, transmembrane, and juxtamembrane cytoplasmic domains, but are unique in their respective cytoplasmic C termini.

Little is known about the molecular interactions of MAG with cytoplasmic proteins in myelinating cells. Considering the distinct spatiotemporal expression profiles (Erb et al. 2006; Ishiguro et al. 1991; Pedraza et al. 1991) and the phenotypes of mutant mice (Fujita et al. 1998), we hypothesized that differences between the MAG isoforms are determined by specific interactions of their respective cytoplasmic domains, in addition to their individual expression profiles. The S-MAG cytoplasmic domain was suggested to interact with zinc and microtubules (Kursula et al. 1999; Kursula et al. 2001), while the L-MAG cytoplasmic domain (L-MAGct) is a target for Fyn tyrosine phosphorylation near the C terminus (Jaramillo et al. 1994; Umemori et al. 1994). Phosphorylation events in the MAG cytoplasmic domains are likely to be of importance in regulating its interactions and function, probably in an isoform-specific manner.

We set out to identify specific intracellular interaction partners of L-MAG. We identified the dynein light chain LC8-type 1 (DYNLL1, also termed DLC8) as a high-affinity binding partner of the L-MAG-specific domain (L-MAGspec). The heterotetrameric complex was crystallized, and extended complex structures were further studied in solution using small-angle X-ray scattering (SAXS). High-affinity dimerization of the L-MAG cytoplasmic domain by DYNLL1 is likely to facilitate the dimerization of...
the entire L-MAG protein. Balancing the expression of S-MAG and L-MAG could, thus, regulate the oligomeric state of MAG, and thereby its interactions with binding partners on the axonal surface.

Materials and methods

Materials

All chemicals were from Sigma-Aldrich (Research Resource Identifier (RRID):SCR_008988), unless otherwise indicated. The sources for special chemicals and materials are given within the text below.

Ethical statement

Experiments were performed in compliance with the animal policies of the MPI of Experimental Medicine approved by the German Federal State of Niedersachsen and thus with the German law on the Protection of Animals, in compliance with EU Directive 2010/63/EU, ARRIVE guidelines. Only non-burdening procedures were performed on non-burdened animals, i.e. exclusively sacrificing of wild-type mice to obtain sciatic nerve tissue. The study did not involve pre-registration.

Statistics

No statistical methods were employed to predetermine sample size of any of the presented experiments. Randomization or blinding were not employed. Outliers in SAXS data were removed using standard protocols in the field, incorporated into automated data processing pipelines; in essence, multiple consecutive X-ray exposures of the sample were automatically compared, and only the frames presenting no signs of radiation damage were averaged and used further in the analyses. Fits of models to the SAXS raw data ($\chi^2$ values) were calculated by the modelling programs as specified below. Normality of the data for statistical purposes was not assessed in this study, and no specific statistical software or tests were used. Crystallographic data collection and refinement statistics were calculated using standard protocols by the specific programs given below.

Yeast two-hybrid screen

The C-terminal cytoplasmic domain of mouse L-MAG (YITQTRKKNVTESSSFSGGDNPHVLYSPEFRISGAPKYESEKQRLGSERRLLGLRGESPELDSLYSHSDLGKRPTKDSYTLTEELAEYAEIRVK) was fused with the GAL4-binding domain by PCR amplification of the corresponding cDNA using primers 5'-'CGGGATCCTATACATCACCACCCAGACGAGAAG and 5'-GGATCCCTACTGGACTCGATTCTGC and subsequent cloning into the pGBT9 vector (Clontech; RRID:SCR_004423) via the BamHI restriction site. The resulting bait construct was validated by DNA sequencing and designated pGBT9 L-MAG\textsuperscript{C\text{\text{D}}}o. Using the lithium acetate method, the yeast strain CG1945 was transformed sequentially with pGBT9 L-MAG\textsuperscript{C\text{\text{D}}}o and a postnatal mouse brain MATCHMAKER cDNA library in pACT2 (Clontech), as previously described (Stegmüller et al. 2003). Transformants were grown on SD medium plates deficient for the amino acids Leu, Trp, and His (‘triple dropout’). 5 mM 3-amino-1,2,4-triazole was added to the medium to suppress leaky HIS3

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reporter gene expression. Positive clones were tested for β-galactosidase gene activity; yeast colonies were grown on SD-Leu-Trp-His, transferred onto reinforced nitrocellulose membrane, submerged in liquid nitrogen, and placed on a Z-buffer/X-gal-soaked Whatman paper (Z-buffer: 16.1 g/liter Na₂HPO₄·7H₂O, 5.5 g/liter NaH₂PO₄·H₂O, 0.75 g/liter KCl, 0.246 g/liter MgSO₄·7H₂O, pH=7; Z-buffer/X-gal solution: 100 ml of Z-buffer, 0.27 ml of β-mercaptoethanol, 1.67 ml of 20 mg/ml X-gal stock solution). Blue color was allowed to develop for 30-120 min. DNA sequencing revealed 14 independent clones that all comprised the entire open reading frame of DYNLL1.

For yeast two-hybrid control experiments, the C-terminal domain of mouse S-MAG (YITQRKKNVTESSSFAGGDPHVLYSPEFRISGAPKYESREVSTRDC) was fused with the GAL4-binding domain by PCR amplification of a template plasmid supplied by N. Schaeren-Wiemers using PCR primers 5’-CGGGATCTCCTATACATCACCAGACGAGAG-3’ and 5’-CGGGATCCTCAGTGAATACCCCGGTTAGAG and cloning into pGBT9 via the BamHI restriction site. The C-terminal domain of zebrafish L-MAG (GDDSDYQSVGPMAGMERQELNYAALEFLHGRHREGVFRRADGDGSDYTEIKAK) was fused with the GAL4-binding domain by amplification of a template plasmid supplied by P. Roey using PCR primers 5’-GGATCCGGATCCGGGACCGACTTACC-3’ and 5’-GGATCCTCATTAGCTTTAATTCCGAT-3’ and cloning into pGBT9 via the EcoRI and BamHI restriction sites. All plasmids were validated by molecular sequencing. For control experiments, transformants were first grown on SD plates deficient for leucine and tryptophan (‘-Leu-Trp double dropout’) with 25 mM 3-amino-1,2,4-triazole before assessing viability on triple dropout plates. β-galactosidase gene activity was tested for yeast grown on double dropout plates as above.

**Recombinant protein expression and purification**

A full-length codon-optimized DYNLL1 (Uniprot: P63168-1) expression construct in the pJExpress401 plasmid (Catalogue number pJ401) was purchased from DNA2.0 (California, USA). Codon-optimized constructs corresponding to residues 534-626 (L-MAG534-626; corresponds to L-MAGct) and 570-626 (L-MAG570-626, corresponds to L-MAGspec) of mouse L-MAG (Uniprot: P20917-1) and residues 534-582 of mouse S-MAG (Uniprot: P20917-2) were purchased from DNA2.0. The coding sequences, including the N-terminal TEV protease cleavage site, were amplified in two sequential PCR reactions to add the attB recombination sites to both ends of the products. The primers used for the first PCR were 5’-GCTCTGAGATCTgatctttcaacta-3’ (forward), 5’-AGAAAGCTGCTGTCatgaattg-3’ (S-MAG reverse), and 5’-AGAAGCTGGGTTCgattatxacttc-3’ (L-MAG reverse), and for the second 5’-GGGGACAAGTTTACAAGGCTCGAGATACT-3’ (attB forward) and 5’-GGGGACCAAGTTTACTACAGAAGCTGGGT-3’ (attB reverse). The PCR products were then subcloned into the pDONR221 entry vector (Invitrogen) and the pDEST-trx expression vector (Tsunoda et al. 2005), coding for thioredoxin (TRX) fusion proteins, with the Gateway system (Invitrogen).

DYNLL1 was produced in *E. coli* BL21 (DE3) cells (RRID: NCBITaxon:511693), grown in LB medium at 37°C, whereby 3 h overexpression was induced with 0.5 mM IPTG (Cat# I6758, Sigma-Aldrich). TRX-His₅-MAG fusion proteins were overexpressed in *E. coli* BL21 (DE3) grown in ZYM5052 autoinduction medium at 30°C for 24 h (Studier 2005). Proteins were purified from lysed bacteria with Ni-NTA (Cat# 30230, Qiagen, RRID: SCR_008539) chromatography. Initial purification was followed by TEV protease cleavage of the fused tag. Cleaved tags were removed with a second Ni-NTA chromatography step, and target proteins were further purified with size exclusion chromatography (SEC). For pulldown assays, thioredoxin fusion tags were not removed from the
MAG constructs, but the fusion proteins were subjected to size exclusion chromatography after the first Ni-NTA step.

MAG constructs containing Ig domains 1-3 and 1-5 (MAGd1-3 and MAGd1-5) were expressed in Chinese hamster ovary cell lines CHO-Lec3.2.8.1 (RRID:CVCL_3440) and CHO-Lec1 (RRID:CVCL_K173), respectively, essentially as described (Koliwer-Brandl et al. 2011). The MAG domains were fused to the Fc part of human IgG. The proteins were purified from the culture medium using protein A sepharose, and the Fc fusion tag was cleaved with 3C protease. SEC was used for further purification.

**Peptides**

A synthetic peptide corresponding to residues 604-620 (KRPTKDSYTLTEELAEY) from the mouse L-MAGct was purchased from Genscript (NJ, USA; RRID:SCR_002891). The N terminus was acetylated and the C terminus amidated.

**Pulldown assays**

30 µM DYNLL1 was mixed with 20 µM TRX-tagged MAG fragments or TRX alone in buffer containing 10 mM Na phosphate pH 7.5, 0.2 M NaCl, 5% glycerol, and 20 mM imidazole at 4°C for 30 min. Ni-NTA equilibrated with same buffer was added, and the mixtures were applied to small columns, washed with same buffer, and finally eluted with the buffer containing 0.3 M imidazole. Eluted fractions were analyzed with SDS-PAGE on precast 4-20% TGX gradient gels (Bio-Rad, RRID:SCR_008426).

**Detection of the protein complex by SEC**

DYNLL1 and L-MAG570-626 were mixed, and complex formation was analyzed by SEC. SEC was carried out on a Superdex 75 10/300 column (GE Healthcare, RRID:SCR_000004), in a running buffer containing 10 mM HEPES pH 7.5, 200 mM NaCl, 0.1 mM TCEP. DYNLL1 was run both alone and mixed with L-MAG570-626. Samples from the observed peaks were further analyzed with SDS-PAGE.

**Isothermal titration calorimetry**

Thermodynamics of the molecular interactions were studied using isothermal titration calorimetry (ITC). L-MAG570-626 and DYNLL1 were dialyzed into a buffer containing 5 mM HEPES pH 7.5, 100 mM NaCl, and 0.1 mM TCEP. The experiment was done using an iTC200 instrument (Microcal). L-MAG570-626 at 450 µM was injected into 45 µM DYNLL1 at 25 °C. Binding thermodynamics were analysed with Microcal Origin (RRID:SCR_002815), using a one-site binding model.

**Crystallization, data collection, and refinement**

Crystals of the complex between L-MAG570-626 and DYNLL1 were grown in sitting drops using vapour diffusion, with 0.1 M bis-tris (pH 5.5), 0.2 M lithium sulfate, and 25% (w/v) PEG 3350 as well solution. The well solution with added 15% (v/v) PEG 400 was used to cryoprotect the crystals, which were then vitrified in liquid nitrogen. Initial crystals were obtained from a sample of the complex that had been stored on ice. Similar crystals did not grow with a freshly purified sample. Diffraction data were collected at the ESRF (Grenoble, France) beamline ID29, at 100 K.

For crystallizing the peptide complex, DYNLL1 and the L-MAG peptide 604-620 were mixed in a 1:1 ratio. The mixture was crystallized in sitting drops using a well solution containing 0.1 M Bis-Tris pH 6.5, 0.2 M lithium sulfate, and 25% (w/v) PEG 3350. Crystals were briefly soaked in well solution

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supplemented with 15% (v/v) PEG 400 and vitrified in liquid nitrogen. Diffraction data were collected at 100 K at the BESSY II (Berlin, Germany) beamline 14.2.

Data were processed with XDS (Kabsch 2010) (RRID:SCR_015652). As both crystals were quite anisotropic, the data were truncated and scaled with the Diffraction Anisotropy Server (Strong et al. 2006). In both cases, the resulting electron density maps were improved, and the anisotropically truncated data were used in subsequent refinement steps.

The structures were solved with molecular replacement using PHASER (McCoy et al. 2007) (RRID:SCR_014219) and DYNLL1 from PDB (RRID:SCR_012820) entry 3ZKE (Gallego et al. 2013) as the search model. Crystals were in space group P6\(_2\)2\(_2\), and one polypeptide chain of both DYNLL1 and the L-MAG fragment was found in the asymmetric unit. The DYNLL1 dimer was formed via crystallographic symmetry. Models were refined using phenix.refine (Adams et al. 2010; Afonine et al. 2012) (RRID:SCR_014224) and manually rebuilt using COOT (Emsley et al. 2010) (RRID:SCR_014222). The refined structures were validated with MolProbity (Davis et al. 2007) (RRID:SCR_014226). The final structure coordinates and the structure factors were deposited at the PDB with codes 6GZJ and 6GZL for the DYNLL1–L-MAG\(^{570-626}\) and the DYNLL1–peptide complex, respectively.

**Solution structure determination by SAXS**

For the complex between DYNLL1 and L-MAG\(^{570-626}\), the two proteins were mixed and applied to Superdex 75 10/300 GL (Ge Healthcare) column for SEC. Peaks for the individual proteins and the complex were collected and dialysed against buffer with 10 mM Na HEPES pH 7.5, 0.2 M NaCl, and 0.5 mM TCEP. Batch mode SAXS data of these samples (individual proteins and the complex) were collected on the Diamond Light Source beamline B21 (Didcot, UK). The dialysis buffer was used as the blank. Data were processed using Primus (Konarev et al. 2003).

For a SEC-SAXS experiment, DYNLL1 and L-MAG\(^{534-626}\) were mixed at a 1:1 molar ratio, and the mixture was applied to a Biosec 3-300 SEC column (Agilent; RRID:SCR_013575). SAXS data were collected on the SWING beamline at the SOLEIL synchrotron (Saint-Aubin, France). The running buffer contained 10 mM HEPES pH 7.5, 100 mM NaCl, and 0.1 mM TCEP. SAXS data were collected from the single elution peak, from a region preceding and containing the maximum of the peak. The data were initially processed with FOXTROT3 (SOLEIL synchrotron). Highly similar frames were averaged, buffer was subtracted, and the data were further processed and analyzed with the ATSAS 2.8 package (Franke et al. 2017) (RRID:SCR_015648).

MAG extracellular domains were analyzed by SAXS in batch mode on the beamlines P12 (Blanchet et al. 2015) at EMBL/DESY (Hamburg, Germany) (MAGd1-5 with and without sialic acid) and B21 at Diamond (MAGd1-3 with and without deglycosylation). The proteins were in a buffer containing 10 mM HEPES pH 7.5, 150 mM NaCl.

For both the MAG-DYNLL1 complexes and the extracellular domains, ab initio and hybrid models were built with standard protocols. Briefly, chain-like ab initio models were made using GASBOR (Svergun et al. 2001) and hybrid modelling, using known domain structures and building missing linkers and flexible termini, with CORAL (Petoukhov et al. 2012). Both programs are part of the ATSAS 2.8 package.
For batch mode measurements, data were corrected for beam intensity and exposure time, and data were normalized to a protein concentration of 1 mg/ml. It should be noted that for glycosylated proteins, this procedure leads to an overestimation of particle mass. Molecular masses were determined based on the forward scattering, I(0), of the sample compared to that measured from a standard protein measured in the same setting, such as bovine serum albumin or monomeric myelin CNPase.

Immunolabelling of teased fibers

Teased fibers were prepared essentially as described (Patzig et al. 2016). Male P11 C57BL/6N mice (MPI-EM animal facility) were sacrificed by cervical dislocation. 2-5 mice had been husbanded/cage. Sciatic nerves were dissected and transferred into ice-cold PBS buffer on a glass slide. Using two fine forceps, the epineurium was removed and small pieces of the sciatic nerve were transferred onto a dry coverslip. Axons were separated by pulling the fiber bundles apart using fine forceps. Teased fiber samples were stored at -20°C until further processing. Teased fibers were postfixed in 4% PFA (5 min), permeabilized with ice cold methanol (5 min), washed in PBS (3 x 5 min), and blocked in blocking buffer (10% horse serum, 0.25% Triton X-100, 1% BSA in PBS) for 1 h at RT. Primary antibodies were applied in incubation buffer (1.5% horse serum, 0.25% Triton X-100 in PBS) overnight at 4°C. Samples were washed in PBS (3 x 5 min), and secondary antibodies were applied in incubation buffer for 1 h at RT. Samples were washed in PBS (2 x 5 min), and DAPI was applied (1:50000 in PBS) for 10 min at RT. Samples were briefly washed with 2 x ddH2O and mounted using Aqua-Poly/Mount (Polysciences, Eppelheim, Germany).

Antibodies were specific for MAG (Millipore, clone 513; Cat# MAB1567, RRID:AB_2137847; 1:50) and DYNNL1 (Abcam Cat# ab104603, RRID:AB_10716605, 1:50). Secondary antibodies were donkey anti-mouse Alexa 488 (Invitrogen, 1:1000; RRID:AB_141607) and donkey anti-rabbit Alexa 555 (Invitrogen, 1:1000; RRID:AB_162543). Images were obtained by confocal microscopy (Leica SP5; RRID:SCR_008960), using the objective HCX PL APO CS 63.0 x 1.30 GLYC 20°C UV. To excite the Alexa488 fluorophore, an argon laser with excitation of 488 nm was used, with emission set to 500-555 nm. To excite the Alexa 555 fluorophore, the laser DPSS 561 was used, with emission set to 570-695 nm. DAPI was excited at 405 nm, and emission was set to 415-470 nm. Images were exported and processed using the LAS AF lite software (RRID:SCR_013673), Photoshop (RRID:SCR_014199), and Adobe Illustrator (RRID:SCR_010279).

Results

We set out to identify novel binding partners for the cytoplasmic domain of L-MAG and to characterize the corresponding molecular complexes in structural detail. The membrane-proximal segment of the MAG cytoplasmic domain is identical between S- and L-MAG, while the isoform-specific regions consist of 10 and 54 residues, respectively. The MAG cytoplasmic domain constructs used in this work are shown schematically in Figure 1A.

DYNNL1 is a specific binding partner for L-MAG

To identify cytoplasmic interaction partners of L-MAG, its complete cytoplasmic domain (L-MAGcyto) was used as bait in a yeast two-hybrid (Y2H) screen of a mouse brain cDNA library. Yeast colonies viable on triple dropout medium were further investigated. All 14 plasmids independently selected from the library represented the entire open reading frame of DYNNL1, a subunit of the dynein...
complex that mediates dimerization of various target proteins and vesicular trafficking (Barbar 2008; Rapali et al. 2011b). Apart from being a subunit in the dynein complex, DYNLL1 is known to be a mediator of intrinsically disordered protein (IDP) dimerization (Clark et al. 2015). The frequency of DYNLL1-positive clones is suggestive of a high-affinity interaction, although the high hit rate also reflects the fact that the DYNLL1 open reading frame is small. The observed interaction was verified by reintroducing L-MAG<sup>534−626</sup> and DYNLL1 into yeast cells. Co-transformed yeast were viable on double and triple dropout media and additionally tested positive in the β-galactosidase assay (Table 1). These results imply that DYNLL1 is a novel direct molecular interaction partner of L-MAG.

The two splice isoforms of MAG differ by the C-terminal segment of their respective cytoplasmic domain. To test if the interaction with DYNLL1 is specific to L-MAG, the C cytoplasmic domain of S-MAG (S-MAG<sup>534−626</sup> as TRX fusions (for improved solubility) were incubated with DYNLL1, and the copurification of DYNLL1 with the Histagged segments of MAG was determined (Figure 1B). DYNLL1 was clearly copurified with both L-MAG segments, but not with S-MAG. No interaction was observed with a TRX control. Hence, the pulldown experiments indicated a direct molecular interaction of DYNLL1 towards the L-MAG-specific C-terminal segment of the MAG cytoplasmic domain.

**Verification of the interaction between L-MAG and DYNLL1 with recombinant proteins**

To validate the Y2H results, pure recombinant proteins were used to study complex formation between L-MAG and DYNLL1. His-tagged L-MAG<sup>534−626</sup>, L-MAG<sup>570−626</sup>, or S-MAG<sup>534−582</sup> as TRX fusions (for improved solubility) were incubated with DYNLL1, and the copurification of DYNLL1 with the Histagged segments of MAG was determined (Figure 1B). DYNLL1 was clearly copurified with both L-MAG segments, but not with S-MAG. No interaction was observed with a TRX control. Hence, the pulldown experiments indicated a direct molecular interaction of DYNLL1 towards the L-MAG-specific segment of the MAG cytoplasmic domain.

**The complex forms with high affinity**

To determine the affinity and thermodynamics of the interaction between L-MAG<sup>ct</sup> and DYNLL1, we used ITC to measure the generation of heat, when L-MAG<sup>570−626</sup> was injected into a cell containing DYNLL1 (Figure 1C). The affinity was micromolar (K<sub>d</sub> = 7.3 µM), and binding was exothermic (ΔH = -3.9 kcal/mol, ΔTΔS = -3.2 kcal/mol). Considering that 1:1 and 2:2 stoichiometries are indistinguishable in ITC experiments, the observed result is in agreement with the hypothesis that the interaction between DYNLL1 and L-MAG occurs in a 2:2 heterotetrameric stoichiometry, in a similar manner to previously described interactions between DYNLL1 and other target proteins (Mohan and Hosur 2009; Wang et al. 2003). The interaction between the two proteins is of high affinity in vitro, showing favorable enthalpy.

**A stable protein complex can be purified using size exclusion chromatography**

When running both DYNLL1 and the L-MAG-specific domain together on SEC, a peak shift was observed compared to DYNLL1 alone; this observed peak with higher hydrodynamic radius contained both proteins (Figure 1D,E). Addition of excess L-MAG resulted in the disappearance of the DYNLL1 peak from the chromatogram. The result shows that the two proteins form a stable complex in vitro, which does not dissociate even during a lengthy separation in a SEC column.

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The complex between L-MAG and DYNLL1 was crystallized for high-resolution structure determination. Initially, prior to knowing the exact binding site for DYNLL1 on L-MAG, the entire L-MAG-specific region of 54 residues was mixed with DYNLL1 and subjected to crystallization trials. The formed crystals presented a structure with residues 606-615 of L-MAG visible, bound to DYNLL1 in a heterotetrameric 2:2 complex (Figure 2A-C, Table 2). The remaining parts of L-MAG were not visible in electron density, and it is likely they have been partially degraded during crystallization.

In an effort to get better crystals, a synthetic L-MAG peptide (residues 604-620), corresponding to the observed binding site, was co-crystallized with DYNLL1. However, the diffraction data from these crystals did not extend to a higher resolution (Table 2), and the structure was very similar to the one obtained initially. Residues 606-617 were visible in electron density in this case.

The crystal structure shows a homodimer of DYNLL1, binding two molecules of L-MAG on opposite faces of the dimer, similarly to previously described complexes of DYNLL1 with ligand peptides (Benison et al. 2008). Importantly, the N termini of both L-MAG peptides point in the same direction in the protein-protein complex (Figure 2A-C); this side faces the cytoplasmic leaflet of the adaxonal myelin membrane.

**Structural details of the MAG-DYNLL1 complex**

Based on the crystal structure, a detailed structural analysis of the L-MAG-DYNLL1 complex is possible (Figure 2D). The L-MAG sequence can be numbered as a canonical DYNLL1 ligand, with residue Leu613 considered position 0. The backbone of the bound L-MAG segment in the complex forms β-strand interactions with DYNLL1 all the way from Lys608 (position -5) to Thr614 (position +1). The carbonyl oxygen of the first visible N-terminal residue of MAG, Pro606(-7) interacts with the amide of Thr70, following a turn in the DYNLL1 structure. All in all, 8 backbone hydrogen bonds are formed between the L-MAG peptide and DYNLL1, indicating that canonical β strand interactions are a major contributing factor to DYNLL1 ligand specificity. The strong role of backbone interactions possibly also explains the promiscuity of DYNLL1 recognition of selected ligand proteins, such as L-MAG, which have lost the conserved Gln residue.

The L-MAG side chains interact with the DYNLL1 surface. Lys608(-5) forms a hydrogen bond with Ser610(-3) and a salt bridge with Asp12. Asp609(-4) hydrogen bonds to both Tyr65 and Thr67. Ser610(-3) forms another hydrogen bond to His68 on DYNLL1. The Tyr611(-2) side chain is sandwiched between the side chains of Tyr65 and Lys 36 from two different molecules of DYNLL1. Thr612(-1) forms a hydrogen bond with Ser64 and a water molecule; the latter interaction with a conserved water molecule is common to DYNLL1 complexes. The side chain of Leu613(0) leans against the N terminus of the α2 helix. In canonical interactions, this residue is a Gln (of the TQT motif) and hydrogen bonds with the helix N terminus, interacting with the helical dipole. Thr614(+1) interacts with the main-chain amide of Phe62 and forms a C-H...π interaction with the side chain of the same residue. Glu615(+2) forms a salt bridge with Lys9, and the remaining two visible residues, Glu616 and Leu617 do not have tight interactions.

At least 20 DYNLL1 – ligand structures from human, rat, fruit fly and yeast can be found in the PDB with a sequence search. A sequence alignment of the MAG DYNLL1-binding site and other DYNLL1 ligands from the PDB is shown in Supplementary Figure S1, indicating a very limited sequence homology. Only two of the structures are "similar" to the DYNLL1 – L-MAG complex in that the
The ligand lacks the Gln in position 0 [Lightcap et al. 2008, Bodor et al. 2014]. Canonically, Gln(0) would interact through H bonds with the terminus of helix α2 (Figure 3A); it is clear that this interaction cannot take place with a Leu residue, and Leu(0) in L-MAG forms van der Waals interactions instead. A similar lack of canonical interaction at position 0 is apparent in Myo5a (Figure 3B) and Pak1 (Figure 3C).

The Lys608(-5) and Ser610(-3) side chains are hydrogen-bonded to each other. In canonical DYNLL1 ligands, these positions are often occupied by Ser(-5) and Lys(-3), while a similar interaction between the side chains is present. Hence, residues at positions -3 and -5 appear swapped in L-MAG, compared to many other DYNLL1 ligands (Figure 3A, Supplementary Figure S1).

DYNLL1 has a binding groove for short linear motifs, such as that from L-MAGct, and the distances across this groove can be used as a marker for allosteric changes in DYNLL1 (Benison et al. 2008). Distance between the Ca atoms of Lys9 and Gly63 is 13.1 Å, comparatively short, in our complex, indicating little allosteric effects upon binding (Benison et al. 2008).

**Solution structure of disordered L-MAGct constructs bound to DYNLL1**

In order to obtain a better view into the structure of the complex between DYNLL1 and the L-MAGct, SAXS experiments were carried out on complexes of DYNLL1 and either the full L-MAGct or the L-MAG-specific region (Table 3, Figure 4). DYNLL1 alone is a compact, folded dimer, while L-MAGct remains disordered apart from the DYNLL1-binding site in these complexes, and in effect, DYNLL1 dimerizes two disordered cytoplasmic tails of L-MAG. This interaction is similar to many IDP complexes previously observed for DYNLL1 (Benison et al. 2008; Nyarko et al. 2004; Wang et al. 2004). Importantly, both of the bound L-MAG segments have the same parallel orientation, and the distance to the plasma membrane, which both L-MAG molecules would be integral components of, can be estimated to be ~5 nm between the cytoplasmic membrane leaflet surface and the DYNLL1 dimer bound to L-MAG. The Tyr phosphorylation site on L-MAGct would likely be ~8 nm from the membrane surface; the same applies for the binding site of phospholipase Cγ (PLCγ).

**SAXS analysis of MAG extracellular domains**

In order to understand the conformation of MAGex and its linkage to complexes formed by the L-MAG cytoplasmic domain, we expressed and purified MAGex for structural studies. During the course of this work, the crystal structures and SAXS data of similar constructs were published (Pronker et al. 2016). We carried out additional SAXS analyses on MAGex conformation in solution here, using various modelling approaches (Figure 5).

SAXS data indicate that domains 1-3 are monomeric, and that glycosylation increases the mass of the construct; the mass is slightly overestimated due to the presence of an unknown mass fraction of glycans (Table 3, Figure 5A). The Ig1-3 construct has an elongated conformation, in line with previous reports (Pronker et al. 2016), and the monomeric crystal structure fits the SAXS data well; domains 1 and 2 are linked through a disulphide bridge (Pedraza et al. 1990) and hence form a rigid unit. The kink seen in this unit in the crystal structure is reproduced by the *ab initio* model in solution (Figure 5A-B).
Ig domains 4-5 were shown to mediate homodimerization of MAGex, although with low affinity (Pronker et al. 2016). Our SAXS experiments on MAGd1-5 indicated a concentration-dependent mass, and the highest concentration used for modelling showed very good fits to dimeric models of MAGex (Figure 5C-D). Comparing forward scattering at various concentrations, we could see that a plateau was approached at the concentration used for modelling, indicating high prevalence of dimer in these conditions (Supplementary Figure S3). It should be noted that for the MAGex constructs, due to an unknown degree of glycosylation, the concentrations are underestimated, leading to an overestimation of molecular mass (Table 3). The ab initio chain-like model (Figure 5D) built with the number of residues corresponding to a dimer gave a very good fit and a low chi^2 value of 1.5 and resembled the hybrid model conformation. While it is likely that some amount of monomeric MAGd1-5 is present under these conditions, larger particles will dominate the scattering, and the molecular models give a realistic picture of the true conformation and dimensions of the dimer, even though we admit that glycosylation – not taken into account by the modelling procedure – will cause errors in models for MAGd1-5. The presence of sialic acid, a ligand for Ig domain 1 (Tang et al. 1997), did not affect the scattering curves, indicating no conformational changes or alterations of oligomeric status upon ligand binding (data not shown). Hybrid models conserving the dimer interactions observed in the crystal structure but lacking enforced two-fold symmetry indicate better fits to the raw SAXS data than those built using strict P2 symmetry, suggesting the MAG dimer does not follow strict two-fold symmetry. This is as expected for a large elongated molecule with a limited interaction surface between the monomers. One must keep in mind that due to several restrictions in the experimental settings here for MAGex SAXS studies, the obtained models will not accurately represent a single true structure. However, our data give clear indications of both flexibility and the lack of strict symmetry in MAGd1-5 dimers.

The dimeric models of MAGex may illuminate possible interactions at the glia-axon interface. The distance between apposing leaflets of the adaxonal myelin membrane, containing MAG, and the axonal surface was estimated to be ~12-14 nm (Trapp and Quarles 1982). The SAXS models of MAGex in our study are in excellent corroboration with this distance (Figure 5E-F), and they give further insights into extracellular flexibility of MAG in addition to the high-resolution, but rigid, crystal structures (Pronker et al. 2016).

**Localization of MAG and DYNLL1 in teased sciatic nerve fibers**

To determine the localization of MAG and DYNLL1 in myelinating cells, we carried out immunohistochemistry of teased mouse sciatic nerve fiber preparations (Figure 6). By confocal microscopy imaging, MAG immunolabelling was detected in all non-compact myelin subcompartments, i.e. in Schmidt-Lanterman incisures (SLI), as well as in adaxonal, abaxonal, and paranodal myelin, in agreement with earlier reports (Erb et al. 2006; Patzig et al. 2016; Trapp and Quarles 1984). DYNLL1 immunolabelling was most intense in the cell bodies of Schwann cells and the paranodal myelin subcompartment, but comparatively weak in SLI and both adaxonal and abaxonal myelin. On the other hand, MAG and DYNLL1 immunolabelling co-distributed well in paranodal myelin, suggesting that this subcompartment could be a site of interaction in myelinating glia.
Evolutionary aspects

In order to shed light on the conservation of the observed interaction, L-MAG cytoplasmic domain sequences from various selected species were aligned. Groups of aligned sequences are shown in Figure 7. The entire L-MAG cytoplasmic domain is highly conserved among mammalian species (Figure 7A). Interestingly, in several reptiles, the C-terminal 20 residues, including the DYNLL1 binding site, are fully conserved (Figure 7B). This highlights the C terminus as a functionally important site. On the other hand, in birds, fish, and amphibian species, the DYNLL1 binding site is not present, although the phosphorylation site Tyr620 is conserved (Figure 7C). It is likely that L-MAGct from these species does not bind DYNLL1, as indeed seen in the Y2H assay between DYNLL1 and the zebrafish L-MAGct (Table 1).

Discussion

MAG has long been known as a myelin-specific Ig-CAM with functional relevance in myelin-to-axon signalling and adhesion (Fruttiger et al. 1995; Fujita et al. 1998; Jaramillo et al. 1994; Kinter et al. 2013; Montag et al. 1994; Palandri et al. 2015; Pronker et al. 2016; Shimizu-Okabe et al. 2001; Tang et al. 1997; Yin et al. 1998). However, important aspects of its function at the molecular level are only now beginning to be resolved. For example, it was recently reported that dimerization of MAG is a conformational requirement (Pronker et al. 2016) for its binding to gangliosides on the axonal surface (Schnaar and Lopez 2009). The functional relevance of the two MAG isoforms that differ only by the C-terminal ends of their respective cytoplasmic tails has remained largely speculative. To obtain a better understanding of these phenomena, it is crucial to identify direct intracellular interaction partners of the two MAG isoforms in myelinating cells. Previously suggested cytoplasmic ligands for MAG include tubulin and PLCγ (Jaramillo et al. 1994; Kursula et al. 2001). However, no detailed assessment of these interactions at the molecular level has been published, and especially structural information on the MAG cytoplasmic domains and their putative complexes with other proteins has been lacking until now.

We performed a yeast 2-hybrid screen to identify proteins specifically interacting with L-MAG and obtained strong hits for the dynein light chain DYNLL1. The interaction site was localized to the L-MAG-specific C-terminal cytoplasmic domain segment. This domain is intrinsically disordered; this feature is common in DYNLL1 target proteins (Barbar 2008). DYNLL1 is known to dimerize IDP segments in its targets (Barbar 2008), in agreement with our present data for L-MAG. No obvious consensus sequence for DYNLL1 binding is present in the L-MAGct, however.

The interaction between L-MAGct and DYNLL1 was characterized in detail by X-ray crystallography. The sequence conservation between the binding site and previously known and structurally characterized DYNLL1 ligands is very limited, and based on sequence alone, we were initially not able to highlight a specific DYNLL1-binding site in L-MAG after observing the interaction in vitro. However, attempts to crystallize the complex using DYNLL1 and the entire L-MAG-specific segment resulted in a complex that contained ~12 residues of L-MAG well-defined in electron density, while the rest possibly had degraded during crystallization, or were disordered within the crystal. This experiment, thus, accurately mapped the DYNLL1 binding site in L-MAG. Crystallization was repeated with a peptide covering the observed likely binding site, and essentially the same structure was obtained, confirming the correct assessment of the binding site. The affinity of 7 µM is well in line with the high-affinity recognition of a linear sequence element by a small protein; for example SH3
domains, PDZ domains, and profilins bind their short peptide ligands often at low micromolar affinity, with $K_d \sim 1$-100 µM (Dalgarno et al. 1997; Kursula et al. 2008; Wiedemann et al. 2004).

DYNLL1 has been detected in myelin using quantitative proteomics approaches; in the PNS, its developmental expression profile parallels that of MAG (Patzig et al. 2011). Both DYNLL1 and DYNLL2 were downregulated after nerve injury (Saunders et al. 2014). Here, we have shown expression of DYNLL1 in myelinating Schwann cells, whereby colocalization with MAG could be observed in the paranodal compartment. These data are in agreement with the critical role of the dynein complex in neurodevelopment and myelination, as recently shown by analyzing both PNS and CNS of zebrafish carrying a mutation in the gene encoding dynein heavy chain (dync1h1) (Langworthy and Appel 2012; Yang et al. 2015). On the other hand, DYNLL1 is recognized as having independent functions outside the dynein complex, which often relate to interactions with, and dimerization of, IDPs (Barbar 2008; Clark et al. 2015). Hence, the L-MAG-DYNLL1 interaction may also be distinct from any larger dynein complex.

Sequence analyses indicate that L-MAGct is a non-canonical ligand for DYNLL1, especially in that the central Gln residue of the consensus motif is replaced by a Leu. The L-MAG DYNLL1-binding site conforms poorly to the suggested consensus motif presented in the literature (Rapali et al. 2011a; Benison et al. 2008; Lo et al. 2001). The high affinity of DYNLL1 towards L-MAG, on the other hand, shows that this apparent deterioration of the binding motif at the sequence level does not lead to a dramatically lowered binding affinity. Comparing to the recently published sequence matrix of DYNLL1 binding sites, several of the L-MAG residues in the binding site are among those proposed to be unfavorable for the corresponding position, including Leu at position 0, which is arguably the most non-favored residue for the position (Erdős et al. 2017). According to this sequence matrix, other unfavorable-appearing residues in L-MAG are Ser(-3) and Tyr(-2) (Erdős et al. 2017). To conclude, L-MAG is a novel, non-canonical interaction partner of DYNLL1, not harbouring the conserved TQT anchor (Clark et al. 2016).

Comparison of L-MAG cytoplasmic domain sequences between species (Figure 7) reveals strong conservation of the C-terminal segment in mammals, including both the Tyr620 phosphorylation site and the DYNLL1-binding site. It is noteworthy that the C-terminal 20 residues of L-MAGct, including the DYNLL1 binding segment, are also fully conserved in reptiles, despite otherwise poor conversation of the L-MAG cytoplasmic domain. On the other hand, sequence comparison shows the absence of an apparent DYNLL1-binding segment in fishes and amphibians. The latter is also true for the SMP protein, which is the MAG homologue in birds (Dulac et al. 1992), as well as zebrafish L-MAG, which was tested in our Y2H experiments. The lack of binding between DYNLL1 and zebrafish L-MAGct is explained by the absence of the DYNLL1 binding site in its sequence, and probably reflects the emergence of a new functional protein-protein interaction in neurodevelopment during vertebrate evolution, at about the transition from amphibia to reptiles.

The dimerization of the L-MAG cytoplasmic domain by DYNLL1 could have effects on the extracellular interactions of MAG, by increasing its avidity and/or changing its conformation. Importantly, S-MAG is not dimerized by DYNLL1, since no direct interaction can be observed. Recent structural data have provided snapshots of the MAG extracellular domain in a dimeric form (Pronker et al. 2016); our results on the extracellular domain conformation essentially replicate these data. It is possible that MAG has a tendency to dimerize both extra- and intracellularly, and ligand proteins could affect this behavior. Importantly, a general view has been that MAG may be clustered through...
binding to extracellular ligands, which could then trigger cytoplasmic signalling events. Our results imply that cytoplasmic partners may per se regulate MAG dimerization, and thereby affect extracellular binding properties of MAG. The MAG isoforms might have different oligomeric states depending on the expression levels of their respective interaction partners in the myelinating cell cytoplasm. This could explain the differential effects of the MAG isoforms on e.g. neurite outgrowth (Shimizu-Okabe et al. 2001).

DYNLL1 is known for its ability to promote dimerization of IDPs (Clark et al. 2015). The L-MAGct is an IDP, as predicted and shown here, and gets dimerized through DYNLL1. This dimerizing interaction will translate through the single transmembrane domain to the extracellular domain. On the other hand, the isolated MAGex homodimerizes, albeit with a much lower affinity (Pronker et al. 2016) than observed here for the DYNLL1-L-MAGct complex. This suggests that DYNLL1 may be a major contributor in regulating L-MAG oligomeric state on the myelin membrane. On the other hand, should L-MAG already be dimeric prior to DYNLL1 binding, it is likely that the affinity for the L-MAGct – DYNLL1 interaction is much higher than that measured for the isolated cytoplasmic domain. Dimeric binding sites have been shown to increase DYNLL1 affinity from micromolar to the nanomolar range (Radnai et al. 2010). In our experiments, the affinity for the MAGex homodimer seemed higher than reported before (Supplementary Figure S3), but still much lower than the L-MAGct – DYNLL1 interaction. It is likely that DYNLL1 binding is involved in the regulation of the MAG extracellular domain arrangement, which could further translate to altered functional affinities towards extracellular ligands of MAGex. It is also conceivable that S-MAG – by being unable to bind DYNLL1 – would not be prone to homodimerize through the cytoplasmic domain. The DYNLL1 interaction, thus, may be the mechanism mediating and/or regulating localization or functional differences between the MAG isoforms, on both faces of the myelin lipid membrane.

Phosphorylation sites in the L-MAGct have been characterized, including Tyr620 phosphorylated by Fyn kinase, as well as Ser/Thr phosphorylation sites. Thr607 in the L-MAG cytoplasmic domain has been suggested to be a phosphorylation site for protein kinase A (Kursula et al. 2000); such a post-translational modification (PTM) would be expected to inhibit DYNLL1 binding, and thus, dimerization of L-MAGct. The phosphorylated Tyr620 is a target for PLCγ SH2 domain binding (Jaramillo et al. 1994), and the binding sites for DYNLL1 and PLCγ are very close together, if not overlapping. It is unknown, whether Tyr620 phosphorylation might affect DYNLL1 binding and vice versa; we also do not know if DYNLL1 and an SH2 domain can simultaneously bind L-MAG, or if binding is mutually exclusive. Dimerization of L-MAG by DYNLL1 might, thus, affect the interactions of MAG both extra- and intracellularly, as well as be linked to its PTMs.

The affinity between L-MAGct and DYNLL1 is ~7 µM based on our ITC experiments. Several studies have been performed using ITC and SPR to deduce affinity for DYNLL1-ligand interactions, and the highest affinities of known DYNLL1 ligand peptides have been in the range of 1 µM (Clark et al. 2016; Erdős et al. 2017; Rapali et al. 2011a). Thus, among the DYNLL1-interacting proteome, L-MAG can indeed be considered a high-affinity binder. The fact that a total of 14 positive DYNLL1 clones were originally isolated from the Y2H screen further points towards a high-affinity, stable interaction between L-MAG and DYNLL1. As the binding properties of DYNLL1 and the highly homologous DYNLL2 are considered largely similar, it could be possible that L-MAG also interacts with DYNLL2; however, no clones for DYNLL2 were isolated in the original Y2H screen.
In conclusion, the specific interaction between DYNLL1 and the L-MAGct provides a means for isoform-specific L-MAG dimerization induced in the cytoplasm of myelinating cells. This mechanism probably translates into conformational and/or oligomeric state changes of L-MAG towards its axonal interaction partners with likely consequences for adhesion and myelin-to-axon signalling.

Figure legends

**Figure 1. Direct molecular interaction between DYNLL1 and L-MAG.**
The binding first observed in a Y2H screen was validated using different methods and purified proteins. A. Constructs used in the study. B. Pulldown of the DYNLL1-MAG complex. 1, input sample; 2-3, wash fractions; 4, eluted fraction. DYNLL1 (arrowhead) is pulled down with both L-MAG constructs, but not with S-MAG or the TRX control. C. ITC analysis of binding affinity. D. Detection of complex formation in SEC. DYNLL1 was run alone (orange trace) and mixed with L-MAG570-626 (blue). SDS-PAGE analysis from fractions (0.5 ml each) of the complex sample, from between 10-13 ml, covering the two peaks, are shown below. While the first peak contains both proteins together, the second one contains only the excess L-MAG.

**Figure 2. Crystal structure of the DYNLL1-MAG complex.**
A. Overall structure of the 2:2 complex. DYNLL1 is in pink/orange and the two MAG segments in blue/cyan. The termini of the peptides are labelled. B. Stereo view of the complex, with one DYNLL1 monomer shown as a Cα trace, to highlight the parallel orientation of the L-MAG peptides. C. Top view of the complex, from the side of the plasma membrane towards the L-MAG C terminus. D. Stereo view of the binding interactions. Hydrogen bonds are shown with green dashed lines.

**Figure 3. Comparison between L-MAG and selected DYNLL1 complexes.**
A. Comparison to a canonical DYNLL1 ligand peptide, harboring a central TQT sequence motif. Shown is the peptide from the complex between DYNLL1 and the cytoskeletal adaptor protein Swallow (Benison et al. 2008). The sequences of the peptides are shown aligned below the structures. Note the swapping of the Lys and Ser residues at positions -3/-5. B. Comparison to the non-canonical interacting peptide of myosin 5a (Bodor et al. 2014). C. Comparison to the non-canonical peptide from Pak1 (Lightcap et al. 2008).

**Figure 4. Solution structure of the disordered L-MAG cytoplasmic domain bound to DYNLL1.**
A. SAXS data. The data have been displaced along the y axis for clarity. Samples are as follows: blue, L-MAGspec; orange, DYNLL1; red, DYNLL1-L-MAGspec complex; green, DYNLL1-L-MAGct complex. The same colouring is used in the following panels. Guinier plots for all SAXS data are shown in Supplementary Figure S2. B. Dimensionless Kratky plot, indicating rigid folding of DYNLL1 and high flexibility of L-MAGspec. The cross indicates the theoretical peak position for a perfectly folded globular protein. C. Distance distribution. D. Molecular models based on SAXS data. From left to right: DYNLL1, L-MAGspec, DYNLL1-L-MAGspec complex, DYNLL1-L-MAGct complex. E. UV traces from the SEC-SAXS chromatogram of the DYNLL1-L-MAGct complex. Note that the complex between DYNLL1 and L-MAGct is a heterotetramer, in which the segments of L-MAGct outside the DYNLL1 binding site remain extended and flexible.
Figure 5. Implications for full-length MAG structure.

A. Scattering data for MAGd1-3. Red, deglycosylated; black, glycosylated. B. Chain-like model for deglycosylated MAGd1-3 superposed on the crystal structure (Pronker et al. 2016). The arrow indicates the sialic acid-binding site. C. SAXS data for MAGd1-5 at two concentrations. The higher concentration (black) was used for modelling. For a view on concentration-dependence of forward scattering, see Supplementary Figure S3. D. Models of the dimeric MAGd1-5. Top: crystal structure (Pronker et al. 2016). The arrows indicate the sialic acid-binding sites. Middle: hybrid model based on SAXS. Bottom: chain-like model based on SAXS. E. Model of the SAXS-based extra- and intracellular dimers of L-MAG, indicating the approximate dimensions. The black lines represent the locations of the transmembrane domains, and the Ig1-2 unit is coloured in orange and Ig5 in magenta. F. Schematic model for dimerization of L-MAG, but not S-MAG, through DYNNL1.

Figure 6. MAG and DYNNL1 co-distribute in the paranodal myelin subcompartment.

Confocal microscopic analysis of immunolabelled teased fiber preparations of WT sciatic nerves at P11 detects MAG (green) in noncompact myelin (adaxonal, abaxonal and paranodal myelin, Schmidt-Lanterman incisures (SLI)). DYNNL1 immunolabelling (red) was prominent in the cytoplasm of Schwann cell bodies and in paranodal myelin. Scale bar: 5 µm.

Figure 7. Sequence alignments of the L-MAG cytoplasmic domain from different species.

The DYNNL1 binding site is highlighted with a red line, and the Tyr620 phosphorylation with a green arrowhead. A. Various mammalian species show high conservation of the entire L-MAG cytoplasmic domain. B. The last 20 residues of L-MAG, including the DYNNL1 binding site, are fully conserved between several mammalian and reptile species. C. The DYNNL1 binding site is not present in an amphibian (frog), a bird (quail), and two fish species (coelacanth, zebrafish). Conversely, the very C terminus including the Tyr phosphorylation site is conserved across all species examined

Supplementary Figure S1. Conservation of the DYNNL1 binding sequence in various complexes from the PDB.

Supplementary Figure S2. Guinier plots for the SAXS data.

Supplementary Figure S3. Correlation between forward scattering intensity and protein concentration for MAGd1-5 in SAXS experiments.
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Conflict of interest

The authors declare that they have no conflict of interest.

Institutional approval

Institutional approval was not required for this study.

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glycoprotein is a zinc-binding protein. J Neurochem 73, 2110-2118.


Table 1. Binding of the C-terminal domain of mouse L-MAG to DYNLL1. Yeast cells were cotransformed with DYNLL1 and the cytoplasmic domains of mouse L-MAG, mouse S-MAG, or zebrafish L-MAG and tested for growth on double and triple amino acid deficient medium, respectively. Note that only cotransformation of mouse L-MAGct and DYNLL1 allowed growth on triple dropout medium, indicating specificity of the interaction. +, robust growth; -, no growth.

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Table 2. Crystallographic data collection and refinement statistics. Values in parentheses correspond to the highest-resolution shell. For data processing, statistics are shown both for regularly processed data and the anisotropically truncated data used in structure refinement.

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<td>26.8</td>
<td>49.8</td>
<td>44.2</td>
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<tr>
<td>Refinement statistics</td>
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<tr>
<td>(R_{\text{cryst}}) (%)</td>
<td>21.22</td>
<td>19.15</td>
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<tr>
<td>(R_{\text{free}}) (%)</td>
<td>25.11</td>
<td>22.89</td>
<td></td>
<td></td>
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<tr>
<td>RMSD bond length (Å)</td>
<td>0.008</td>
<td>0.013</td>
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<tr>
<td>RMSD bond angle (°)</td>
<td>0.945</td>
<td>1.135</td>
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<tr>
<td>Mean (B) value (Å(^2))</td>
<td>26.4</td>
<td>46.2</td>
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<tr>
<td>Ramachandran plot</td>
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<tr>
<td>Res. in favoured regions (%)</td>
<td>96.94</td>
<td>96.81</td>
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<td></td>
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<tr>
<td>Res. in allowed regions (%)</td>
<td>3.06</td>
<td>3.19</td>
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<tr>
<td>Outlier residues (%)</td>
<td>0</td>
<td>0</td>
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<td></td>
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<tr>
<td>MolProbity score/percentile</td>
<td>1.51/96\textsuperscript{th}</td>
<td>1.74/89\textsuperscript{th}</td>
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<td></td>
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<tr>
<td>PDB entry</td>
<td>6GZL</td>
<td>6GZJ</td>
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</table>
Table 3. SAXS data collection and analysis.

The $R_g$ values are determined from the Guinier plot. *n.d.* = not determined in the SEC-SAXS experiment.

<table>
<thead>
<tr>
<th>Sample</th>
<th>c (mg/ml)</th>
<th>Monomer MW (kDa) from sequence</th>
<th>MW (kDa) from I(0)</th>
<th>$R_g$ (Å)</th>
<th>$D_{max}$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DYNLL1</td>
<td>2.5</td>
<td>10.5</td>
<td>26.2</td>
<td>20.2</td>
<td>83</td>
</tr>
<tr>
<td>L-MAGspec</td>
<td>2.9</td>
<td>6.7</td>
<td>6.6</td>
<td>16.8</td>
<td>72</td>
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<tr>
<td>DYNLL1 - L-MAGspec</td>
<td>2.3</td>
<td>17.2</td>
<td>31.7</td>
<td>22.2</td>
<td>80</td>
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<tr>
<td>DYNLL1 – L-MAGct</td>
<td>n.d. (SEC)</td>
<td>21.0</td>
<td>n.d.</td>
<td>34.4</td>
<td>160</td>
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<td>MAGex (d1-3)</td>
<td>1.2</td>
<td>36.0</td>
<td>60.7</td>
<td>31.9</td>
<td>120</td>
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<tr>
<td>MAGex (d1-3 deglycosylated)</td>
<td>1.1</td>
<td>36.0</td>
<td>51.6</td>
<td>30.1</td>
<td>120</td>
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<tr>
<td>MAGex (d1-5)</td>
<td>1.6</td>
<td>56.7</td>
<td>337</td>
<td>89.9</td>
<td>400</td>
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<tr>
<td>MAGex (d1-5) + sialic acid</td>
<td>1.2</td>
<td>56.7</td>
<td>317</td>
<td>88.0</td>
<td>400</td>
</tr>
</tbody>
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