**spliterhin der versucb gemacht werden nacbzuweisen, dass sich gewisse patholo­ gische veranderungen arn rlickenrnark nur dann verstehen )assen, wenn man mit der entwickelungsgeschichte des riickenmarkes vollig vertraut ist, und man mag es aus diesem grunde verzeihen, wenn schon in dieser kleinen abhandlung im voraus auf manche pirnkteauf isolationspraparaten, mag man dieselben <lurch zerzupfen frischer rlickenmarksstlickchen oder nach einlegen derselben in verdtinnten losungen von doppelt chromsaurem kali oder ueber­ osmiumsaure hergestellt haben, erkennt man, dass die kerne einen schmalen, homogenen, glanzenden und stark lichtbrechenden rand­ saum besitzen, wahrend ihr lnneres leicht granulirt erscheint, wo­ bei 3-4-5 grobere granula starker hervortreten (vergl.alkohol ferner, mag man denselben in mehr oder weniger diluirtem zustand anwenden, hat stets zur folge, dass sich die zwiscbensubstanz in dei· zuletzt bcschriebenen form dem beobachter prasentirt.es mag gestattet sein, an dieser stelle mit wenigen worten der angaben zu gedenken, welche liber die beziehungen des zellen­ kerns und kernkorperchens zu den ans der entwickelten nerven­ zelle ausstrahlenden fortsatzen gemacht worden sind.um diese behauptung zu erweisen, mag die saure carminliisuug nach schweigger als reagens dienen.die sehr mannichfachen entwickelungs­ formen !assen sich in ihren verschiedenen abstufungen kaum mit wenigen worten schildern, und es mag daher fig.1) in another place (p. 410) rawj.tz also says: »die phy­ siologie mag sich strauben, so vie!serratus mag tricepslow mag nification.form and relationships.— the spinal cord, or medulla spinalis, lies within the vertebral canal and extends the foramen mag num to the level of the inferior border of the body of the first lumbar vertebra.if a mag netizable particle is in the lens, an attempt should be made to draw it into the anterior chamber by means of the giant magnet; if this the foreign body is removed with the small magnet as described above; it if fails, and where the foreign body is non-magnetizable, the lens should be removed by linear extraction in young subjects and by the method employed in senile cataract, in an older person.the endogenous neutral proteinase degrades mag and bp, but not other myelin proteins.studies of wolfgram proteins and mag have not been so precise, but they show that these proteins are not uniformly distributed.this is a matter of some controversy, however, and recent ultrastructural studies show that mag may be uniformly distributed.myelin is isolated in consistently lower yields from these areas, the myelin enzyme cnp is decreased, mag appears to be lost in areas where bp is present, and hydrolytic enzymes, including acid proteinases, are increased.in addition it has been shown that myelin-associated glycoprotein (mag) inhibits axon growth24 25 but, unlike the experiments of schwab and of steeves, mag inhibition has only been demon­ strated so far in vitro26• the injured spinal cords of knockout mice that lack mag still cannot regenerate, but they can do, so in the presence of in-1 (ref.very recently, a neurite growth inhibitory effect of the myelin-associated glycoprotein mag was demonstrated (485,514).the in vivo significance of these findings remains to be shown; mag gene-ablated mice are available (435, 506) .the role of mag as an inhibitor of axonal regeneration in vivo might await the availability of mag monoclonal antibody which can reverse its effect on inhibition.the pn is a favorable environment for cns regrowth for several reasons: (1) it does not contain the inhibitory molecules ni 35/250; (2) al- though it contains some mag it does not seem to inhibit regrowth because the degenerating myelin is rapidly removed and (3) schwann cells are known to divide [123] and form columns of cells within the basal lamina tubes (bungner bands).how- ever, we showed previously that mag null mutants exhibited no gross abnormality in myelination.ultra- structural studies revealed subtle alterations in peri- axonal organisation, indicating a restricted structural role for mag in the formation and maintenance of periaxonal structures (li et al., 1994).here we show that myelination in mag deficient mice is not as finely controlled as it is in wild type mice.these data support the notion of mag being a glial recogni- tion/adhesion molecule.a model is proposed regard- ing the roles mag could play in the formation and maintenance of myelin structure.among myelin specific proteins, mag has been postulated to fulfil such a glia-axon recognition and adhesion role during myelino- genesis.first, mag is expressed at the growing tips of myelin sheath, and in mature fibres it is found in the periaxonal regions of myelinated fibres (bartsch et al., 1989).the periaxonal localisation makes it possible to interact with axonal component(s), presumably a mag receptor (magr).thirdly, in vitro studies have demonstrated that mag can bind specifi- cally to neurones (sadoul et al., 1990) and that up and down regulation of mag result in accelerated and decreased myelination, respectively (owens et al., 1990; owens and bunge, 1989, 1991).hypothesis by generating mag null mutant mice by gene targeting and reported previously that the mag mutants showed no gross impairment of myelination although periaxonal organisation was disrupted in selected fibres (li et al., 1994, montag et al., 1994).this notion of mag being a structural component periaxonally is also consistent with a role for mag as a cell recognition and adhesion molecule in axon-glia interaction.in the present study, we present in vivo evidence which supports a role for mag as a cell recognition molecule.mice deficient for mag at the mrna and protein levels were generated by gene targeting as described (li et al., 1994).genotypes (mag -/- or mag +/+) were determined by tail dna analysis by southern blottings (not shown).**

**nerves from mag deficient mice and age matched wild type control mice were put into montage, scored and compared for their differences in total numbers of axons, the number of myelinated axons and the number of unmyelinated axons.when total numbers of axons were compared, of 32 prints of mag +/+ and 32 prints of mag -/- optic nerves from two litter mates 61⁄2 weeks of age, a consistent reduction in the total number of axons per print in the mag -/- group was observed (fig.comparison of another two pairs of mag -/- and mag-/- and 65.000 ± 2.0615 for mag +/+ (p < 0.01, fig.of the 4 prints for two 12 week old mice, the mean ± sem of axons/print were 30.75 ± 0.781 and 48.970 ± 0.811 for the mag -/- and mag +/+ samples, respec- tively.there seemed to be astrocytosis in mag mutant optic nerve samples, with axons appeared less densely packed consequently (fig.a consistent increase in the proportion of unmyelin- ated axons and a reciprocal decrease in the proportion of myelinated axons in the mag mutant optic nerve samples were also noticed.a pairwise analysis of optic nerves from litter mates of mag -/- and mag +/+ at 14, 12 and 61⁄2 weeks of age gave a proportion of unmyelinated fibers in total fibers of 17.96% (i.e.(258 in 2662) vs. 2.19% (65 in 2968) for the 61⁄2 week old pair of mag -/- and mag +/+ samples, respectively (table i).comparison of a pair of corpus callosum of mag -/- and mag +/+ genotype revealed similarto investigate the impact of the loss of mag on myelinogenesis, electron microscopic prints of opticaxons of mag deficient optic nerves appeared less densely arranged.a and b: representative electron micrographs of mag +/+ and mag -/- optic nerves, respectively, showing a subtle decrease in axon density in the mag -/- sample.a reduction in total number of axons in mag deficient samples for all three pairs were observed.-/- and 65.000 ± 2.0615 of 8 prints for mag +/+ for pair two,14 week olds, and 30.75 ± 0.781 and 48.970 ± 0.811 for the mag -/- and mag +/+, 12 week olds, respectively.consistent with our previous report, we again observed collapsed glial cytoplasmic collars in over 85% of myelinated fibers (table i) at the periaxonal junction of mag deficient optic nerves, thus confirming our previous conclusion that mag was necessary for the normal formation and maintenance of periaxonal structures (li et al., 1994, montag et al., 1994)**.**dysregulated myelination was encountered in mag mutant samplesmost myelinated fibers in mag mutants showed comparable degree of myelination to wild type animals despite the increased ratio of unmyelinated axons.how- ever, dysregulated myelination of selected fibers was noticed occasionally in mag deficient optic nerves.since redundant myelin could occasionally be observed in the wild type optic nerves during myelinogen- esis, three pairs of mag +/+ and mag -/- litter mates were examined and compared for the incidence of redundant myelin.the wild types and the mag null mutants gave a frequency of redundant myelin in the optic nerves at 1.6% vs. 10%, 0.7% vs. 3.1% and 0.3% vs. 4%, respectively.comparison of a pair of spinal cord and corpus callosum of mag -/-and mag +/+ genotype also revealed an increased incidence of redundant myelin in the mag mutants (li et al., unpublished observation).in the pns spinal ventral roots, redundant myelin was encountered in mag deficient samples only, with a frequency of 2.2%, i.e.in contrast, redundant myelin was not observed in mag +/+ wild type samples in a total of 989 myelinated fibers examined (n 2).since redundant myelin was occasionally encountered in the normal mouse cns during early myelinogenesis, the increased frequency in the optic nerves of mag null mutants might represent a delayed in myelination.consistent with this explanation was the increased proportion of unmyelin- ated fibers in mag mutant animals.alternatively, the absence of mag might have compromised glial-axon2. multiple myelination in mag deficient optic nerves.4) in mag deficient spinal ventral root seemed to favor this explanation.3. redundant myelin in the pns of mag mice.compact myelin sheath looping away from the axon, is indicated by arrows in a 12 week old mag deficient l2 spinal ventral root.perturbation of such one to one relationship in the absence of mag therefore, would also support a role for mag as a glia-axon recognition molecule.such abnormalities were not observed in mag +/+ wild type litter mates.consistent with previously published observations (li et al., 1994, montag et al., 1994), myelination was not grossly affected in mag mutant animals, indicating that mag was not essential for myelination.4. a: massive myelin is produced to surround a relatively small axon in mag deficient pns.l2 ventral root from a 14 week old mag null mutant mouse shows a massive myelin indicated by curved arrows.for mag in the normal formation and maintenance of periaxonal organisation.5. myelination of multiple axons in mag deficient spinal cord and optic nerve.61⁄2 weeks old mag deficient spinal cordincrease in the proportion of unmyelinated fibres in the optic nerves from mag mutants.this notion was supported by an increased frequency of redundant myelin in the cns of mag mutant samples.the increased incidence of redundant myelin in mag mutants suggested a delay in myelin maturation, as did the increased proportion of unmyelinated fibres.pns spinal ventral roots of mag mutants whereas it was not encountered in wild type samples scored.in contrast, wild type mice showed some background of redundant myelin in the cns, which exhibited an increased fre- quency in mag mutants.in rare instances, multi-myelination of a group of axons in the cns of mag mutant animals were seen.such failures, however, did not happen in most myelinated fibres in the mag deficient samples.cesses with the axon segment involves the concerted action of mag together with other cell recognition molecules such as n-cam and l1, or a yet to be identified molecule(s).in a mature myelinated fibre, however, mag becomes the molecule that occupies the periaxonal area whereas l1 becomes undetectable and n-cam is dramatically down-regulated.the extracellular part of mag could act as a glue, as well as a spacer, that holds the glial cell membrane and the axolemma in close apposition, with a 12 to 14 nm distance in between.such an interaction may also send signals to the intracellular part of mag so that the cytoplasmic collar is maintained, possibly through its association with a signal transducing molecule, such as fyn (edwards et al., 1988; 1989; umemori et al., 1994; jaramilo et al., 1994), and the cytoskeletal molecules, such as f-actin and spectrin (trapp et al.,1989).in addition to this structural role, mag may very well have other roles in myelinogenesis as well.thus, although mag is not essential for myelin formation, it may facilitate glia-axon recognition and contact in vivo, thereby enabling the proper formation and maintenance of periaxonal structures and optimal myelin production.bartsch u, kirchoff f, schachner m (1989): immunohistological localisation of the adhesion molecules l1, n-cam, and mag in the developing and adult optic nerves of mice.sadoul r, fahrig t, bartsch u, schachner m (1990): binding properties of liposomes containing the myelin-associated glyco- protein mag to neural cell cultures.the mag-deficient mouse was used to test whether mag acts as a significant inhibitor of axonal regenera­ tion in the adult mammalian cns, as suggested by cell culture experiments.these observa­ tions do not support the view that mag is a significantthe additional role of mag as an inhibitor of axonal regeneration is indicated by the observation that in vitro mag exerts a robust inhibitory effect on neurite out­ growth from young cerebellar neurons, adult dorsal root ganglion (drg) neurons (mukhopadhyay et al., 1994), and ng108-15 neuroblastomacells(mckerracheret al., 1994).**

**to test in vitro and in vivo the hypothesis that mag restricts axonal regeneration in the adult cns.behavior of ng108-15, pc12, and 3t3 cells on purified mag and myelin extractsin contrast, 38% ( ± 8%) of the cells plated on proteins extracted from cns myelin of mag+1+ mice (10 µg/well) extended processes longer than the diameter of the cell body, and 50% ( ± 13%) of the cells were inhib­ ited on immunopurified mag from mouse brain (8 µg/well), in agreement with mckerracher et al.**

**myelin preparations from mag 1we therefore tested different cell types for their respon­ siveness to mag as an inhibitory substrate molecule.from 1-day-old (pnd1) animals were about twice as long on mag as those cultured on poly-l-lysine (figure 2).in contrast, neurite elongation from adult dag neurons on mag was similarly poor as on poly-l-lysine and reduced by - 56% when compared with laminin (figure 2).having confirmed the age-dependent responsiveness of these neurons for mag (johnson et al., 1989; schneider­ schaulies et al., 1991; mukhopadhyay et al., 1994), we investigated neurite outgrowth from dag neurons on my­ elin proteins extracted from the cns of mag+'+ and mag+ mice.strong inhibition of neurite elongation by mag has been reported for young cerebellar neurons (mukhopadhyay et al., 1994).to analyze whether mag restricts axonal regeneration incould be seen to grow through ventral and ventrolateral tissue bridges in mag+1+ (n = 10) and mag 1 (n = 12)the maximal distances covered by regenerating fi­ bers varied from animal to animal, and no effect of the absence of mag could be observed.in the present study, we used the mag-deficient mouse to analyze in vitro and in vivo whether mag is a significantthe effect of mag on neurite outgrowth from dag neu­ rons is of particular interest.neurite elongation from young postnatal dag neurons in vitro is significantly enhanced by mag (johnson et al., 1989; schneider-schaulies et al., 1991), whereas neurite outgrowth from adult dag neurons cultured either on transfected chinese hamster ovary (cho) cells expressing the 72 kda isoform of mag or on recombinant mag is significantly reduced when com­ pared with control substrates(mukhopadhyayet al., 1994).age-dependent differences in the extent of neurite elongation from dag neurons on cryosections from peripheral nerves were believed to sup­ port the view that mag is crucial in determining the extent of neurite growth.these results do not support a close and causal relation between presence or absence of mag and the ability of dag neurons to extend neurites on com­ plex substrates.these results demonstrate that mag does not interfere significantly with neurite elongation from adult dag neurons as soon as physiologically more appropriate substrates are used.neurite outgrowth from ng108-15 neuroblastoma cells is reduced on recombinant mag, whereas it is increased on bovine myelin preparations after immunodepletion of mag (mckerracher et al., 1994).in agreement with these observations, we found reduced neurite outgrowth from these cells when purified mag was used as a substrate.neurite outgrowth assays with the immunodepleted myelin extracts after addition of purified mag or of the previously removed material might help to clarify this point.alternatively, a major role of mag as an inhibitor of axonal regeneration might not become apparent in the mag+ mouse, since other neurite growth­ inhibitory molecules that compensate for the lack of mag­ related inhibitory activity could be up-regulated in the mu­ tant.finally, we consider asignificant up-regulation of nl-35 and nl-250 in the mag mutant as unlikely, since in-1 antibodies neutralize the inhibitory ac­ tivity of mag•1 and mag+ cns to a similar extent in all our experiments.on these substrates, an inhibitory activity of mag was no longer apparent, again suggesting that mag plays only a minor role in the control of neurite elongation on complex sub­ strates.that the contribution of other molecules to the inhibitory activity of oligodendrocytes and cns myelin is more relevant than that of mag is also indicated by the observation that n.eurite outgrowth from pc12 cells and spreading of 3t3 fibroblasts is similar on myelin extracts from mag+1+ and mag+ mice but improved after applica­ tion of mab in-1.thus, we found no evidence for a major role of mag in restricting axonal regeneration in vivo.in summary, our observations strongly argue against a major role of mag as an inhibitor of axonal regeneration in the lesioned adult mammalian cns.a comparison of inhibitory activity of mag and neurite growth inhibitors nl-35 and nl-250 in vitro, for instance, demonstrates a strong effect of nl-35 and nl-250 on all substrates tested, while inhibitory effects of mag become apparent only when the purified molecule is offered as a substrate.more importantly, we fo nd no evidence that mag prevents re­ generation in vivo.generation of mice deficient for mag and genotyping of animals have been described (montag et al., 1994).mag was isolated from brain extracts of adult mice by immunoaffinity purification (poltorak et al., 1987), except that the buffer for elution of mag from the immunoaffinity column contained either 0.1% deoxy­ cholate or 0.8% p.octylglycoside.myelln preparations from mag • 1• and mag+ miceneurite outgrowth assay on purified mag or myelin preparations neurite outgrowlh assays were performed in 4-well dishes (greiner) coated overnight with various concentrations of extracts derived from myelin of mag•1 or mag·-1 mice.lmmunoaffinity-purified mag was coated on precoated poly-l-lysine or on plastic tissue culture dishes.or mag • mice and bovine were supplemented with phosphatidyl choline at a protein:lipid ratio of 1:10 and dialysed against pbs for 24-48 hr, fol­ lowed by l15(gfbco) without additives for aminimum of 3hr.optic nerves from mag"' and mag ' mice (at least 2 months old) were quickly removed and frozen in hank's balanced salt solution.we have also observed a loss of mag and plp immun oreactivity in immunologically treated spinal cords.in the present study, we analyzed whether impaired myelination of retinal ganglion cell axons is detectable in adult mag mutants.at both ages, unmyelinated axons in optic nerves of mag mutants were of small caliber.the number of unmyelinated axons decreased significantly in 9-month-old mag mutants when compared to 2-month-old mag mutants, indicative of a slow and long-lasting myelination of axons in the mutant.our observations support the view that mag is involved in the initiation of myelination in the cns.after compact myelin has formed, mag is located in the periax- onal membrane of cns and pns myelin sheaths, and isthe analysis of mice deficient in mag has recently confirmed an involvement of mag in the initiation of myelination, and in the formation and maintenance of morphologically intact myelin sheaths žw2,4,6,9x; reviewed in w3,8x.. in the cns of mag-deficient mice, the oligoden- drocyte periaxonal cytoplasmic collar is reduced in length or completely absent in most myelin sheaths w2,6,9x.more- over, some myelin sheaths of mag mutants contain cyto-here, we demonstrate that optic nerves of 2-month-old mag mutants contain a significantly increased number of unmyelinated axons when compared to age-matched wild- type mice.a difference in the number of unmyelinated axons between wild-type mice and mag mutants is also detectable at 9 months of age, although it is not as prominent as in 2-month-old animals.our observations provide evidence for an involvement of mag in the initiation of myelination, and for a slow and long-lasting myelination of axons in the cns of adult mag mutants.in optic nerves from 2-month-old mag mutants, the oligodendrocyte periaxonal cytoplasmic collar of most1; w2,6,9x.. moreover, some axons of mag-deficient mice were con- centrically surrounded by more than one myelin sheath žsee w2,9x.. these defects were also observed with a similar frequency in optic nerves of 9-month-old mag mutants žnot shown.. another morphological difference between wild-type and mag-deficient mice was the presence of increased numbers of unmyelinated ganglion cell axons in the mutant žfig.is significantly increased when compared to age-matched wild-type mice ža.. note also that the size of unmyelinated axons in mag mutants is similar to that of unmyelinated axons in wild-type mice.1a,b., and was also evident in 9- month-old mice žnot shown.. notably, unmyelinated axons in the optic nerve of 2- and 9-month-old mag mutants were of small caliber and of similar size as unmyelinated axons in optic nerves of age-matched wild-type mice**

**between neurons and oligodendrocytes in vitro w13x, has led to the hypothesis that mag is functionally involved in the initiation of myelination.**

**when expression of mag by schwann cells was experi- mentally reduced w12x.2.. in 9-month-old mag mu-**

**optic nerves of mag mutants w9x, indicating that myelina-in the present study, we demonstrate that the percentage of unmyelinated axons in the optic nerve of 2- and 9-month-old mag mutants is significantly increased when compared to age-matched wild-type mice.deficient mice is statistically significant ž p - 0.005.. re- markably, the percentage of unmyelinated axons in 9- month-old mag mutants decreased by about 50% whenexpression of mag by myelin-forming glial cells al- ready at initial stages of myelination že.g.gether with the observation that mag mediates adhesionmoreover, indirect evidence for a role of mag for the initiation of myelination in the cns has recently been presented in a study by umemori et al.**that mag activates the non-receptor-type tyrosine kinase fyn at initial stages of myelination and žii.moreover, a pronounced dystrophy of distal oligodendro- cyte processes has recently been described in the cns of adult mag mutants, but signs of acute demyelination were only very rarely observed w5x.the small caliber of unmye- linated axons in optic nerves of mag mutants observed in the present study also argues against a significant demyeli- nation of axons in the mutant, but rather suggests that these axons have never been myelinated.and 9-month-old mag mutants when compared to age-matched wild-type animals.w1x u. bartsch, f. kirchhoff, m. schachner, immunohistological local- ization of the adhesion molecules l1, n-cam, and mag in theit is yet to be resolved whether mag or in-i are the major causes of the inhibitory response, but it is clear that embryonic neurons fail to recognize any myelin inhibitory proteins (shewan et al., 1995) and further that in the adult visual pathways retinal axons can overcome this inhibition when given appropriate trophic support (berry et al., 1996).in addition to mag and nogo, there are likely to be many other inhibitors of axon regeneration in myelin.cai d, shen y, de bellard m, tang s, filbin mt: prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.filbin, m. t. (1999) prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.tenascin-r immunoreactivity was again detectable at 6 months after the lesion, correlated with remyelination as indicated by mag immunohistochemistry.monoclonal antibodies 596 and 597 to tenascin-r (pesheva et al., 1989), and 513 to mag (becker et al., 1995) and polyclonal antisera to tenascin-r (bartsch et al., 1993) and tenascin-c (becker et al., 1995) have previously been described.immunohistochemical localization of tenascin-r and mag in the developing and adult retinotectal systemto see whether this is also true for pleurodeles, mag was always assayed in parallel to tenascin-r immunohistochemistry in the same animals as a marker for the presence of myelinating oligodendrocytes (becker et al., 1995).in other parts of the retinotectal system mag immunoreactivity did not correlate with that of tenascin-r. the retina never becomes myelinated (becker et al., 1995), and myelination of the tectum was first observed during metamorpho-

sis, using mag as a marker for myelin (not shown).figure 2. a–g, immunohistochemical localization of tenascin-r (a–c, f, g) and mag (d, e) during development.d, e, mag immunoreactivity parallels that of tenascin-r at metamorphosis in alternating cross-sections of the of the optic nerve of the same animal shown in a and b at the level of the optic foramen (d, compare with a) and closer to the chiasm (e, compare with b).peripheral nerves ( p) in d are also strongly labeled with the mag antibody.postinjury changes in tenascin-r and mag immunoreactivitynote that at 6 months after the lesion, mag immunoreactivity was confined to myelin sheaths (fig.after a crush, mag immunoreactivity was also rapidly lost from the distal optic nerve.at 8 d after the lesion, the lesion-near half of the optic nerve was free of mag immu- noreactivity (fig.7f ), but in the chiasm-near half, mag immu- noreactive myelin debris remained in a small area close to the chiasm for at least 14 d after the lesion (fig.the pattern of mag immunoreactivity was very similar to the distribution of myelin found by electron microscopy during the same period (see below), indicating that mag immunoreactivity is a valid marker for myelin in pleurodeles.at 3 months after the lesion a few individual mag immunoreactive myelin sheaths could again be detected with highest density near the chiasm in two of three animals (fig.thus, clearance of tenascin-r immunoreactivity oc- curring within 8 d after the lesion in the distal optic nerve is even faster than that of mag (fig.however, most mag-immunoreactive debris may be phagocytosed (see below), such that the time course of mag removal from the extracellular environment in the optic nerve may be similar to that of tenascin-r. reappearance of tenascin-r immunoreactivity be- tween 3 and 6 months after the lesion coincides with remyelina- tion of the optic nerve, which was determined by the reappear- ance of mag immunoreactivity.9, inset) were completely removed at 9 d after the lesion between the lesion site and ~800 µm distal to it but remained in the chiasm near-part of the nerve, confirming results from mag immunohistochemistry.during development and regen- eration of the optic nerve, expression of tenascin-r was corre- lated with the appearance of myelinating oligodendrocytes, as demonstrated by mag immunohistochemistry.figure 7. comparison of tenascin-r (a–c, h, i ) and mag (e–g, j–l) immunoreactivities and control without primary antibody ( d) in cross-sections of the crushed distal optic nerve during demyelination ( a–g) and remyelination ( h–l).f, at 8 d after the lesion, mag immunoreactivity is absent from the lesion-near part of the nerve.j, at 3 months after the lesion, mag immunoreactivity indicates that remyelination is scarce in the lesion-near part of the optic nerve.putative inhibitory molecules, such as mag (mckerracher et al., 1994; mukhopadhyay et al., 1994; sch¨afer et al., 1996) (but see bartsch et al., 1995) and tenascin-r, may be phagocytosed along with the myelin (lang and stuermer, 1996) by macrophages and microglial cells or radial glial cells (goodbrand and gaze, 1991; phillips and turner, 1991; wilson et al., 1992; naujoks-manteuffel and niemann, 1994).moreover, both mag and the other myelin-derived growth inhibitory proteins block axon extension by causing growth cone collapse (bandtlow et al.,these studies prompted us to first examine in pc12 cells and cultures of primary neurons the role of rho in growth inhibition by mag and by myelin.my- elin was made from bovine brain corpus callosum, and native mag was purified from myelin after extraction in 1% octylglucoside and separation by ion exchange chromatography (mckerracher et al., 1994).this prep- aration of native mag has some additional proteins, including tenascin (z. c. xiao, p. braun, s. david, and l. mckerracher, unpublished observations).recombinant mag (rmag) was made in baculovirus- infected spodoptera f rugiperda (sf) cells as described previously (shi- bata et al., 1998), except that the sf9 cells were transferred to serum-free conditions before collecting the culture supernatant.we found that all lines were inhibited by both myelin and mag in contrast to a different pc12 line tested under different experimental conditions (rubin et al., 1995).experiments on mag substrates were analyzed by phase-contrast microscopy.figure 1. c3 treatment of pc12 cells plated on inhibitory mag and myelin substrates.a–c, pc12 cells plated on mag remained rounded and did not extend neurites ( a), but cells plated on mag in the presence of c3 ( b) grew neurites.d, quantitative analysis of neurite growth with c3 treatment (open bars) or in scrape-loaded buffer controls ( filled bars) when pc12 cells were plated on poly-l-lysine, laminin, rmag, native mag (nmag), or myelin.we plated three different lines of pc12 cells on both native and recombinant mag sub- strates (fig.on mag substrates, in which neurite formation is inhibited, c3 had a dramatic effect on the ability of cells to extend neurites (fig.moreover, on both mag and myelin substrates, signif- icantly more cells extended neurites, and neurite length was significantly longer after c3 treatment (fig.these results demonstrate that c3 treatment elicits neurite growth from pc12 cells plated on growth-inhibitory myelin or mag substrates.growth of dominant negative rho-transfected cells on mag substratesto test the involvement of rho in the response of primary neurons to mag and to myelin substrates, we purified retinalfigure 4. treatment of retinal neurons with c3 stimulates neurite growth on mag substrates.on native mag substrates, neurite growth is inhibited ( a), but after c3 treatment, retinal neurons plated on native mag substrates extend neurites ( b).d, quantitative analysis of neurite growth of retinal neurons on poly-l-lysine, mag, and myelin substrates, as described in the legend of figure 1. significantly more cells extended longer neurites on mag and myelin substrates with c3 treatment than with buffer-treated controls.neurite outgrowth from these cells was inhibited by mag (fig.treatment of retinal neurons with c3 allowed neurite extension on the growth-inhibitory mag substrates to an extent similar to that observed on control substrates (fig.a quantitative analysis revealed that c3 treatment of retinal neurons plated on mag or myelin substrates had significantly longer neurites, and significantly more cells extended neurites (fig.extend directly on inhibitory substrates of mag or myelin.also, pc12 cells transfected with dominant negative rhoa extended neurites on mag substrates.we report here that c3 inactivation of rho can promote neurite growth of pc12 cells and retinal neurons on mag and myelin.we found that dominant negative rhoa expressed in pc12 cells promoted neu- rite growth on mag but not on myelin, perhaps because rho inhibition by dominant negative constructs can be low (qiu et al., 1995).recently, it was found that priming cells with neurotrophins increases camp levels to block the inhibitory response to mag (cai et al., 1999).growth cone repulsion by mag can be converted into attraction by elevation of intracellular camp levels to activate protein kinase a (pka) (song et al., 1998).several different mag binding partners have been identified (yang et al., 1996; collins et al., 1997), and specific neuronal receptors to myelin inhibitors are likely to exist.cai d, shen y, debellard m, tang s, filbin mt (1999) prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.the authors of this publication demonstrate using mutant transgenic mouse models that blockade of all three myelin inhibitors (nogo, mag and omgp) compared to blockade of any single inhibitor failed to show additive effects (lee et al.cai d, yingjing s, de bellard me, tang s, filbin mt (1999) prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.interestingly, although all three of the prototypical inhibitors nogo, mag, and omgp signal through common receptors, genetic deletion of nogo but not mag or omgp increased sprouting of the intact cst after pyramidotomy (lee et al., 2010).surprisingly, mag deletion was found to decrease axon growth after injury in this model (lee et al., 2010), suggesting that mag and perhaps myelin inhibitors in general have a dual role in modulating axonal sprouting after injury.one of the physiological hurdles for promoting axon regrowth is the presence of growth inhibitors in the cns myelin sheath, including nogo, mag (myelin-associated glycoprotein), and omgp (oligodendrocyte myelin glycoprotein).2 although there are multiple pathways that lead to the inhibition of axon regeneration, neuronal ngr1 has been identified as the converging point for these endogenous myelin-associated inhibitors (mais).3ngr1 is a glycosylphosphatidylinositol-linked leucine rich repeat protein that signals through multiple transducers such as the low-affinity neurotrophin receptor p75, tnf□ receptor superfamily member 19 (troy), and lingo-1,4,5 as well as integrins, egf receptor, b-lymphocyte stimulator (blys), and leucine-rich glioma-inactivated protein 1 (lgi1).6789,10wang kc, kim ja, sivasankaran r, segal r, he z. p75 interacts with the nogo receptor as a co- receptor for nogo, mag and omgp.prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.neurotrophins elevate camp to reach a threshold required to overcome inhibition by mag through extracellular signal-regulated kinase-dependent inhibition of phosphodiesterase.nogo-a, the myelin proteins, mag and omgp, several semaphorins and ephrins as well as chondroitin sulphate proteoglycans have been identified [2].interestingly, these acute interventions gave consistently stronger results than mice ko for nogo-a, double ko for nogo-a and mag, or triple ko for nogo-a, mag and omgp [5,30,31].parallel studies with anti- mag antibodies in human stroke recovery gave indications for improved walking speeds with therapy[55].ldl receptor-related protein-1 is a sialic-acid-independent receptor for myelin-associated glycoprotein that functions in neurite outgrowth inhibition by mag and cns myelin.ngr1 binds to three myelin inhibitors nogo, mag and oligodendrocyte myelin glycoprotein (fournier et al., 2001; fournier et al., 2002; liu et al., 2006; mcgee andstrittmatter, 2003), whereas ngr2 interacts with mag (venkatesh et al., 2005).mag has bidirectional effects on axonal growth; in young neurons, mag promotes axonal growth, whereas in older neurons, it inhibits axonal growth (l., l., ).this bidirectional effect of mag on neurons seems to depend on intra- cellular levels of cyclic amp (camp).further, some studies reported the protective effects of mag on the neurons ( ).deletion of mag reducesthus, mag has both inhibitory and promoting effects on axonal growth in mature neurons.the low-affinity neurotrophin receptor p75ntr was found to be a signal transducer of mag (l., ), and subsequent studies demonstrated that p75ntr associates with ngr to form a receptor complex for mag, nogo, and omgp ( l., ).upon mag stimulation in cultured cerebel- lar neurons, crmp-2 is phosphorylated and inactivated by rock ().previously, deleting mag was reported to have almost on optic nerve regeneration after crush injury ( ).upon mag stimulation, pir- eracts with tropomyosin receptor kinase (trk) neurotrophin ptors, which are known to promote neurite outgrowth.an essential role of mag in mediating axon-myelin attachment in charcot-marie-tooth 1a disease.p75 interacts with the nogo receptor asa co-receptor for nogo, mag and omgp.(66) reported that prior exposure to neurotrophins prevented inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.normally, collapsin-1/semaphorin iiid and mag will, respectively, collapse and repulse growth cones.activation of cgmp inhibits growth cone collapse and converts the repulsive effects of mag to attraction.s. m. mag and omgp synergize with nogo-a to restrict axonal growth and neurological recovery after spinal cord trauma.cai, d.; shen, y.; de bellard, m.; tang, s.; filbin, m. t. prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp- dependent mechanism.prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.further suggesting that crmp-2 is a central target to design strategies to achieve release of myelin inhibition, overexpression of the phospho-resistant mutant t555a-crmp-2 (the rho- kinase phosphorylation site) counteracts the inhibitory ef- fect of mag on postnatal cerebellar neurons [].1999. prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.indeed, mag promotes axon growth of embry- davies, s.j., goucher, d.r., doller, c., and silver, j.the importance of mag in axon–schwann cell communication was indicated by mag-deficient mice in which axonal loss and demyelination occur later in the life (carenini et al., 1997).it is believed that mag is a modulator of axonal properties that regulate a kinase-phosphatase system within the axon, and is also related to the process offirst, it is rarely detect- able at the interface between unmyelinated axons and schwann cells, but as myelination becomes advanced by schwann cells, mag increases and is present at the periaxonal surface (dezawa & nagano, 1996).this result suggests that an intricate temporal and spatial regulation of mag expression exists dur- ing the process of remyelination.myelination detected by the mag signal was colocalized with the gfp signal, recognized, shown as light blue color-coding in fig.(e) mag and neurofilament staining, with ranvier’s nodes indicated by arrowheads.cai d, shen y, de bellard m, tang s, filbin mt (1999) prior exposure to neurotrophins blocks inhibition of axonal re- generation by mag and myelin via a camp-dependent mechanism.filbin, prior exposure to neurotrophins blocks inhibition of axonal regenera- tion by mag and myelin via a camp-dependent mechanism, neuron 22 (1999) 89–101.at least three proteins present in cns myelin, nogo, mag and omgp are capable of causing growth cone collapse and inhibiting neurite outgrowth in vitro.although nogo, mag and omgp lack sequence homologies, they all bind to the nogo receptor (ngr), a gpi-linked cell surface molecule which, in turn, binds p75 to activate rhoa.2. the gene structure of human nogo (a), omgp (b), mag (c) and ngr (d), respectively.curiously, the hnk-1 antigen is also found on mag and is associ- ated with the preferential regeneration of motor ax- ons in peripheral nerves (see below) (low et al., 1994; martini et al., 1994).note that the schematic representation of the mag interaction with ngr is not true to the physical interaction, since experiments using a mutant form of the mag protein, lacking the fourth and fifth immunoglobulin domains, show that it fails to bind to ngr (domeniconi et al., 2002).it should be noted, however, that mag has been found to stimulate, rather than inhibit neurite outgrowth from e17 mouse spinal cord neurons (turnley & bartlett, 1998) and even from neonatal cerebellar neurons in one study (shimizu- okabe et al., 2001).there is evidence that the levels of cyclic amp in neurons mod- ulate the responses to mag and are reduced in neurons when they become sensitive to mag (cai et al., 2001).although mag is gener- ally recognised as a potent inhibitor of axonal growth in vitro, the mag knockout mouse exhibits little or no enhancement of axonal regeneration in the spinal cord (bartsch et al., 1995; but see also li et al., 1996).how- ever, the slow-degenerating mutant mouse provides some evidence that mag can be inhibitory to axonal regeneration in vivo: regeneration is enhanced in the sciatic nerves of these mice if the mag gene is inacti- vated (schafer et al., 1996).as the name siglec suggests, mag is a sialic acid binding protein, which means that it has the potential to bind to a variety of sialylated cell surface molecules.the evidence that the nogo-66 receptor is part of one functional receptor complex for mag on neurons is strong (see below).the mag genethe human gene for mag consists of 12 exons, span- ning nearly 22 kb of genomic dna, and is located on chromosome 19q13.1 (d’eustachio et al., 1988).as well as being the most commonly used alias for myelin asso- ciated glycoprotein, mag is also the official gene sym- bol.briefly, mag is known to possess five regions of inter- nal homology that give rise to five immunoglobulin- like domains within its extracellular amino-terminus (salzer et al., 1987).it has been suggested that the modulation of linked carbohydrate moieties might occur during development as a means of regulating the function of mag in myelinogenesis (pedraza et al., 1991).there is also evidence that the hnk-1 carbohy- drate is linked to mag in a highly differential man- ner in schwann cells supporting motor and sensory nerves of adult mammals (low et al., 1994).a soluble form of mag, capable of inhibiting neurite outgrowth from p6 drg neurons, is released from damaged cns myelin (tang et al., 2001); soluble mag constitutes the great majority of the neurite-outgrowth inhibiting influence releaseda mutant mag protein, mag(d1-3)-fc (which lacks the fourth and fifth immunoglobulin-like domains, as well as the transmembrane domain and c-terminal cytoplasmic tail) does not bind to the nogo receptor or produce growth cone collapse, although retains its ability to bind gangliosides (domeniconi et al., 2002).unlike nogo or omgp, mag is not expressed in neu- rons; it is expressed by glia in the cns and peripheral nerves (martini, 1994).in the cns, l-mag is the ma- jor form synthesized early in development, and it per- sists as a significant proportion of the mag present in the adult (pedraza et al., 1991).furthermore, gain of function studies demonstrated that mag at nanomolar concentrations is capable of inducing growth cone collapse in neurons transfected with ngr.(2002) have recently reported that cerebellar granule cells cultured on polylysine respond to both a nogo-a peptide and mag even after phospholipase treatment.mag also competes with nogo-66 for binding to ngr (domeniconi et al., 2002), but the regions in ngr required for binding mag have not yet been examined by domain deletion analysis.however, this speculation is com- plicated by the fact that the ngr antagonist peptide nep1–40 (which competitively inhibits nogo-66 bind- ing and blocks growth cone collapse), does not compete with mag (liu et al., 2002a).the most likely explanation for the absence of competition between mag and nep1-40 is that mag competes for the active site on ngr whichif nogo, omgp and mag are inhibitory to the regeneration of all axons, other receptors must be present on the neurons that lack ngr.although first identified as a receptor for nogo-66, ngr also appears to be a functional receptor for mag and omgp.nogo-66 lacks sequence homology to omgp or mag, but it has been shown to compete with omgp and mag for binding sites on ngr (wang et al., 2002c; domeniconi et al., 2002; see above).the co-receptors that enable ligand binding to ngr to generate an intracellular signal are still under inves- tigation, but the p75 low affinity neurotrophin receptor was soon shown to be necessary for the inhibition of neurite outgrowth from adult drg neurons and post- natal cerebellar granule cells by mag (yamashita et al.,the interactions of mag with gangliosides have been claimed to mediate the inhibition of neu- rite outgrowth (vinson et al., 2001; vyas et al., 2002; but see also domeniconi et al., 2002), and mag has trophic effects on oligodendrocytes (gard et al., 1996), which have not yet been shown to express ngr.of course, the other mag receptors may mediate func- tions unrelated to growth cone collapse.(2002) claim that phospholi- pase treatment, to remove ngr and other gpi-linked cell surface molecules, does not block all the inhibitory effects of nogo-a or mag on neurite outgrowth from cerebellar granule cells grown on polylysine (but the result for mag contrasts markedly with the findings of domeniconi et al.certainly, if ngr were the only receptor for nogo, mag and omgp, interfering with ngr sig- nalling would be likely to have more marked effects than the removal of individual ligands.the lack of re- generation in the mag knock out mouse (bartsch et al., 1995) supports the view that targeting individual ngr ligands can be disappointing (although full publica- tion of the phenotypes of the various nogo knockout mice may alter this view).niederost et al., 2002), the absence of myelin, mag and nogo from lesion sites which are, none the less, demonstrably inhibitory; and the ability of transplanted and otherwise suitably stim- ulated neurons to grow axons in white matter.the receptors which allow postnatal drg neurons in vitro to show growth cone collapse in re- sponse to cns myelin, mag and nogo are unknown, but in vivo drg neurons do not behave as if they are affected by ngr ligands: their axons do not respond to treatment with the in-1 antibody (oudega et al., 2000), and when drg neurons are transplanted into the dor- sal columns of the spinal cord their axons regenerate rapidly in the white matter (davies et al., 1997, 1999).arginase i and polyamines act downstream from cyclic amp in overcoming inhibition of axonal growth by mag and myelin in vitro.filbin, m. t. (1999) prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.expression of mag isoforms during development.turnley, a. m. & bartlett, p. f. (1998) mag andp75 interacts with the nogo recep- tor as a co-receptor for nogo, mag and omgp.it has been reported that, depending on the age of neurons, mag can either promote or inhibit axonal growth (debellard et al., 1996; cai et al., 2001).for drg neurons, the switch occurs postnatally with a sharp transition from promotion to inhibition by mag by postnatal day 3-4 (p3-4) (debellard et al., 1996; cai et al., 2001).thus, mag is a bifunc­ tional molecule with its role being predominantly inhibitory to nerve regeneration in the adult cns.although mag is normally inserted in the myelin sheath, a soluble form of mag, dmag, also exists in vivo.dmag is a proteolytic fragment of mag and contains the entire extracellular domain, cleaved just before it enters the membrane.this strongly suggests that mag is, indeed, an inhibitory mole­ cule rather than a nonperrnissive substrate for neurite growth (tang et al., 1997).priming neurons with neurotrophins overcomes the inhibition by mag and myelinto test if neurotrophins can indeed overcome inhibition by mag and myelin, a variety of neu­ rotrophins were tested individually with neuronal cultures.however, if cere­ bellar neurons were first cultured overnight (prim­ ing) with bdnf or glial-derived neurotrophic fac­ tor (gdnf), in the absence of mag and myelin,i. model to explain how priming with neurotrophins blocks inhibition by mag and myelin.activation of pka or some as yet unidentified downstream effector molecule then blocks subsequent inhibition by mag or myelin, perhaps by inactivating the small gtpase, rho.if, however, neurotrophin is added to the neuron at the same time as the exposure to mag or myelin, the increase in the camp levels is prevented by mag/myelin activation of a pertussis toxin (ptx)-sensitive g protein (gi \*) and thus inhibition of axonal regeneration is not blocked.activation of g protein by mag or myelin has no direct effect on inhibition of axonal regeneration by mag or myelin.and then transferred onto mag-expressing cells or a myelin substrate, the growth inhibition by mag and myelin was completely blocked.for drg neu­ rons, inhibition by mag and myelin is blocked when the neurons are primed with bdnf, gdnf or nerve growth factor (ngf).a clue to the molecular mechanism whereby neu­ rotrophins block inhibition by mag and myelin came from in vitro studies of growth cone motil­ ity.conversely, a soluble recom­ binant form of mag repels these xenopus growth cones, but the repulsion is switched to attraction by addition of a camp agonist to the culture media (song et al., 1998).if this is the case and if neurotrophins are able to alter the neuronal response to mag and myelin, then the question is whether this switch is dependent on intracellular camp levels.neurotrophins increased neuronal camp levels, and this increase was prevented if mag was also present.further, if neurons were primed with neurotrophins in the presence of a pka inhibitor, the block of mag and myelin inhibition was completely abro­ gated.moreover, artificial elevation of camp levels with an analogue of camp, dibutyryl camp, also blocked the inhibition by mag or myelin.finally, the need to prime was abrogated if neurons were ex­ posed simultaneously to neurotrophins and mag or myelin in the presence of the g protein inhibitor, per­ tussis toxin, inhibition is blocked by neurotrophins without priming.taken together, these results indi­ cate that increased camp levels and activation of the pka pathway can overcome the inhibitory effects of mag and myelin.the elevation of intracellular camp levels in the neuron is prevented by mag or myelin, which, by binding to the neuron, activates an inhibitory g protein that blocks any increase in camp (fig.in addition, the response of a number of different neurons to mag is switched from promotion to inhibition during development.for drg neurons, the switch occurs postnatally with a sharp transition from promotion to inhibition by mag at p3-4 (debellard et al., 1996; cai et al., 2001).thus, young neurons are intrinsically differ­ ent from their older counterparts, and are not inhib­ ited by mag and myelin.the question is, then, what are the intrinsic differences between young and old neurons that determines their response to mag and myelin?since elevation of neuronal camp levels by priming with neurotrophins is able to block the in­ hibitory effect by mag and myelin, we asked if the endogenous camp levels in neurons are regulated during development.pka inhibitors attenuated the growth from young neurons promoted by mag and myelin, whereas an analogue of camp, db-camp, blocked the inhibitory effect of mag and myelin on older neurons (fig.thus, the switch of neuronal response from promotion to inhibition of neurite outgrowth by mag and myelin, which marks the developmental loss of regenerative capacity, is mediated by a developmentally regulated decrease in endogenous neuronal camp levels (cai et al., 2001).furthermore, the growth of drg neurons on mag and myelin was significantly increased, and this improvement of growth could be blocked by pka inhibitors.drg neurons on mag and myelin was even bet-

these results indicate that a conditioning lesion leads to a transient increase in camp levels in the drg, which sets off a cascade of events leading to in- creased growth capacity on mag and myelin.grow on mag or myelin in vitro.in adult neurons, an elevation in camp results in increased growth capacity and overcomes the inhibition by mag and myelin.in addition, intrathecal application of h89 for the entire week after sciatic nerve le­ sion attenuated the improved growth on mag and myelin.importantly, improved growth of drg neu­ rons on mag and myelin was observed 1 day and(1999) prior exposure to neurotrophins blocks inhibition of ax­ onal regeneration by mag and myelin via a camp-dependent mechanism.(1998) mag and mog enhance neurite outgrowth of embryonic mouse spinal cord neurons.fected with dominant negative rhoa (nl 9trhoa) to grow on recombinant mag substrates.transfected nl9trhoa cells and mock-transfected pc12 cells were tested for their ability to extend neurites when plated on inhibitory mag substrates.inactivation of rhoa by dominant negative mutation was sufficient to allow nl9trhoa cells plated on mag substrates to extend neurites, and by contrast, mock transfected cells were unable to grow on the same substrates (lehmann et al., 1999).(1999) prior exposure to neurotrophins blocks inhibition of ax­ onal regeneration by mag and myelin via a camp-dependent mechanism.(1999) showed that putative oligoden­ drocyte inhibitory components other than mag and bni-220 of bovine cns myelin have neurite growth­ inhibitory activity and that one of these repulsive ac­ tivities is via versican v2.we ob- served a slight hypomyelination in optic nerves of mag mutants that was significantly increased in fyn mutants and massive in mag/fyn double mutants.however, data are also compatible with the possibility that mag and fyn act independently to initiate myelination.myelination in the pns, in contrast, was not retarded in the absence of mag (montag et al., 1994).all these results demonstrate that mag performs different functional roles in the cns and pns.

“large” mag isoform (l-mag) is the functionally important isoform in the cns, whereas the “small” mag isoform (s-mag) is sufficient to maintain myelin and axons in the pns (fujita et al., 1998).mag and fyn are coexpressed by oligodendrocytes, coimmunoprecipitation experi- ments revealed an association of fyn with mag, and cross-linking of mag with antibodies stimulated fyn kinase activity in cos cells cotransfected with l-mag and fyn but not in cells cotrans- fected with s-mag and fyn (umemori et al., 1994).during early stages of myelination, l-mag is the predominant mag isoform in the cns (tropak et al., 1988; pedraza et al., 1991), and fyn shows highest kinase activity (umemori et al., 1994).given that several morphological abnormalities of myelin sheaths in mag null mutants are related to the lack of l-mag (fujita et al., 1998), fyn might also be involved in the formation of morphologically intact myelin sheaths.to investigate the proposed functional relationship between mag and fyn in the process of myelination, we performed an ultrastructural analysis of optic nerves of mag-, fyn-, and mag/ fyn-deficient mice.hypomyelination was moderate in mag mu- tants, significant in fyn mutants, and massive in mag/ fyn double knock-out mice.however, results are also compatible with the possibility that mag and fyn act independently to initiate myelination.note that the density of l1-positive axons is significantly increased in mice lacking both mag and fyn ( c) when compared with fyn single mutants (compare c with b).generation of mice deficient in mag or fyn has been described previously (stein et al., 1992; montag et al., 1994).double knock-out mutants deficient in both mag and fyn were obtained by cross-breeding the corresponding single mutants.consecutive cross sections from wild-type nerves were additionally hybridized with the mag antisense probe, and the number of mag-positive cells was determined as described for plp-positive cells.mice deficient in mag and fyn were generated by cross-breeding the respective single mutants (stein et al., 1992; montag et al., 1994).the number of unmyelinated axons was significantly increased in age-matched mag mutants.unmy- elinated axons in mag mutants were all of small caliber, and their number decreased in 4- and 9-month-old mag-deficient mice (data not shown) (bartsch et al., 1997; see below).2a) was significantly increased when compared with age-matched mag mutants.values for 2- and 9-month-old wild-types and mag mutants were taken from bartsch et al.a significantly increased number of unmyelinated axons was present in optic nerves of 2-month-old mag mutants (34.7 ± 5.5%), which decreased with increasing age (20.7 ± 6.6 and 18.2 ± 6.0% for 4- and 9-month-old mag mutants, respectively) (bartsch et al., 1997; present study).compared with 2-month-old mag mutants, a significantly higher percentage of axons was unmyelinated in the optic nerve of age-matched fyn mutants (58.2 ± 5.7%; p < 0.0001 according to the unpaired t test), and this value did not change significantly with increasing age (45.2 ± 9.3% for 4-month-old, and 53.2 ± 3.2% for 9-month-old fyn mutants) (fig.consecutive sections of wild-type nerves were also hybridized with the mag probe and contained almost identical numbers of labeled cells (122.1 ± 11.0).myelin sheaths in the cns of mag knock-out mice show a variety of morphological defects.ultrastructural abnormalities of myelin sheaths in the optic nerve and cervical spinal cord of mag/ fyn double mutants were similar to those found in mag null mutants.note that the formation of well developed periax- onal cytoplasmic collars is dependent on the presence of mag but not on the presence of fyn.in comparison, almost every myelin sheath (i.e., ~95%) lacked a well developed cytoplasmic collar in age-matched mag mutants (fig.moreover, there was no significant difference in the percentage of myelin sheaths with short periax- onal cytoplasmic collars between mag and mag/ fyn mutants (fig.optic nerves of mag mutants, in comparison, contained significantly more morphologi- cally abnormal myelin sheaths (11.9 ± 0.8 and 15.9 ± 1.9% for 2- and 9-month-old mag mutants, respectively).the percentage of morphologically abnormal myelin sheaths in fyn mutants was sim- ilar to that observed in wild-type mice (2.5 ± 0.2% for 2-month-old and 2.2 ± 0.4% for 9-month-old fyn mutants), and the percentage of affected sheaths in mag/ fyn double mutants was similar to that observed in mag mutants (14.7 ± 2.3 and 18.8 ± 0.8% for 2- and 9-month-old double mutants, respectively) (fig.initiation of myelination in the cns of mag-deficient mice is impaired, as indicated by a reduced number of myelin sheaths in optic nerves of young postnatal mag mutants (montag et al., 1994) and a significantly increased number of small-sized unmyeli- nated axons in optic nerves of young adult null mutants (bartsch et al., 1997; this study).alter- natively, mag and fyn might initiate myelination independently from each other, and the severe phenotype of mag/ fyn double mutant mice might result from a combination of phenotypes of the respective single mutants.cantly increased when compared with mag mutants.it is interesting in this respect that high fyn kinase activity has been observed in differentiating oligodendrocyte progenitor cultures be- fore the expression of mag (osterhout et al., 1999).together, the demonstration of mag- independent signaling pathways of fyn in oligodendrocytes is in line with our in vivo observations; hypomyelination in fyn-deficient mice is more pronounced than in mag mutants.interestingly, the degree of hypomyelination in optic nerves of mag/ fyn double mutants was significantly increased when com- pared with mag or fyn single mutants.a possible explanation of this finding is that mag and fyn act independently to initiate myelination; the severe hypomyelination of the double mutant would simply reflect a phenotypic combination of the respective single mutants.in the absence of fyn, other signaling pathways might be activated more efficiently by mag to initiate myelination.simultaneous elimination of both mag and fyn would disrupt these hypothetical compensatory mechanisms, resulting in a phenotype of the double mutant that is more severe than that of each of the single mutants.interestingly, noncompacted regions of myelin, redundant myelin, and multiply myelinated axons were also detected in the cns of mutant mice deficient in the large isoform of mag (fujita et al., 1998).however, morphologically abnormal myelin sheaths typically seen in mag null mutants or l-mag-deficient mice were not observed in fyn null mutants.moreover, myelin sheaths of mag/ fyn double mutants showed similar defects with a similar fre- quency as myelin sheaths of mag null mutants.all these data demonstrate that fyn is not involved in the formation of morpho- logically intact cns myelin and thus indicate that other signaling molecules downstream of mag are involved in the morphological maturation of cns myelin.bartsch u, kirchhoff f, schachner m (1989) immunohistological localiza- tion of the adhesion molecules l1, n-cam, and mag in the developing and adult optic nerve of mice.pedraza l, frey ab, hempstead bl, colman dr, salzer jl (1991) differential expression of mag isoforms during development.schachner m, bartsch u (2000) multiple functions of the myelin-associated glycoprotein mag (siglec-4a) in formation and maintenance of myelin.tropak mb, johnson pw, dunn rj, roder jc (1988) differential splicing of mag transcripts during cns and pns development.since mag is expressed in myelinating schwann cells but not in degenerat- ing ones (martini, 1994; willison et al., 1988), the down-regulation of this inhibitory molecule could contribute to the permissive character of the pns environment (shen et al., 1998).for example, both myelinating schwann cells and oligodendrocytes express proteolipid protein (plp), mag and my- elin basic protein (mbp), although schwann cells express much less.in the rgc re- generation, we found that mag was first barely detectable at the interface region between unmye- linated rgc axons and schwann cells.later on as myelination advanced further, mag increased and continued to be present at the periaxonal surface (not shown).these observations indicate there is an intricate temporal and spatial regu- lation of mag expression during the process of myelination of rgc axons by schwann cells.bartsch, u., kirchhoff, f. and schachner, m. (1989) immunohistological localization of the adhesion mol- ecules l1, n-cam and mag in developing and adult optic nerve of mice.this cascade may be responsible that inhibition by mag or other my- elin components can be overridden, and axons are stimulated to grow.t. (1999) prior exposure to neurotrophins blocks inhi- bition of axonal regeneration by mag and myelin via a camp-dependent mechanism.also present at the lesion site are intact and damaged oligoden- drocytes that express nogo, mag and cspgs.nogo [15–17] tenascin [11] semaphorins [31,32,33,34,35] mag [4] cspg [12] slits [40–42] neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.the authors demonstrate that pre-treatment of drgs with neurotrophins stimulates the camp/pka pathway and permits neurons to grow on normally inhibitory mag and myelin substrates.they also demonstrate that pre-treatment is required because mag and myelin act through het- erotrimeric g proteins to block activation of the camp/pka pathway.growth of pc12 cells and primary retinal neurons was promoted on normally inhibitory myelin and mag substrates by inactivating rho.prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp- dependent mechanism.normally when neurons are plated onto a mag substrate they show poor neurite outgrowth.how- ever, pre-exposure of neurons to certain neurotrophins prior to plating blocks inhibition by mag (cai et al.in the area of over- coming mag inhibition the camp cascade is not the only signalling pathway which has been explored; the other main one is that involving rho gtpase.cai d, shen y, de bellard me, tang s, filbin mt (1999) prior exposure to neurotrophins blocks inhibition of axonal regener- ation by mag and myelin via a camp-dependent mechanism.the inhibi- tory activity of mag was discovered independently by us (mckerracher et al., 1994) and by filbin and collaborators (mukhopadhyay et al., 1994).

the inhibitory activity of mag has been character- ized both in vitro and in vivo (li et al., 1996; mcker- racher et al., 1994; mukhopadhyay et al., 1994; schafer et al., 1996).the extent to which this inhibitor limits outgrowth in vivo has been controversial because of studies performed with mag null mutant mice: two groups were able to show a small improvement in regeneration on mag -/- myelin (li et al., 1996; shen et al., 1998), whereas no change was observed in a third study (bartsch et al., 1995).the inhibitory activ- ity of nogo and mag have been compared, and when tested in vitro these two proteins have equivalent growth inhibitory activity (prinjha, 2000).moreover, pc12 cells transfected with dominant negative rho were able to grow on inhibitory mag substrates (lehmann et al., 1999).treat- ing neurons with neurotrophins to increase intracellu- lar camp before plating on mag substrates, or in- creasing intracellular camp through the use of camp analogues, allows neurons to grow on mag sub- strates and ignore repulsive signaling by mag (cai et al., 1999; song et al., 1998).therefore, increases in camp or direct inactivation of rho allows neurons to grow on inhibitory mag substrates in tissue culture.cai, d., shen, y., debellard, m. e., tang, s., & filbin, m. t. (1999) prior exposure to neurotrophins blocks inhibition of axonal re- generation by mag and myelin via a camp-dependent mecha- nism.although the nerve cell surface ligands for mag remain to be established, evidence supports a functional role for sialylated glycoconjugates.here we review recent studies that reflect on the role of gangliosides, sialylated glycosphingolipids, as functional mag ligands.gangliosides lacking that terminus (e.g., gm1 or gd1b), or having any biochemical modification of the terminal neuac residue fail to support mag binding.notably the mag level in these animals is dysregulated.taken together these observations implicate gangliosides as functional mag ligands.myelin-associated glycoprotein (mag), a quantitatively minor protein in myelin, serves both as a myelin- stabilizing factor and an inhibitor of nerve regenera- tion presumably, mag binds to specific targets on the axon or nerve cell to elicit its physiological effects.the discovery that mag is a sialic acid acid binding lectin led to the hypothesis that it acts via specific binding to nerve cell surface gangliosides.when siaload- hesin was cloned its sequence similarity with cd22, mag and a cd33 led crocker et al.this proposal was advanced when mag and cd33 were also shown to bind sialic acids eventually leading to the designation of the ‘siglec’ family of sialic acid immunoglobulin-family member lectins this family, which has rapidly grown to at least 10 members, appears to have evolved to exploit different sialic acid linkages in nature.certain siglec family members are highly specific for a single type of neuac linkage (e.g., neuac α3 gal, neuac α6 gal, or neuac α6 galnac), whereas others appear less stringent with the exception of mag (siglec 4) siglecs are found in cells of the hemopoietic lineage (mag and the

closely related avian protein ‘schwann cell myelin pro- tein’ (smp) are designated siglec 4a and siglec 4b respectively sialic acid binding specificities for mag and smp are not experimentally distinguishable for the purposes of this review, the two are treated as homologs and designated siglec 4).interestingly, mag was found to bind preferentially to the sequence neuacα3gal �3galnac which is the terminus of the major brain gangliosides gd1a and gt1b (see ).to mag, which is concentrated on the innermost ‘peri- axonal’ myelin membrane, directly apposing the surface of the axon the presence of a mag binding glycan sequence on gangliosides, and their localization on the axon surface, led to the hypothesis that gangliosides, the major sialylated glycoconjugates in the brain, may be endogenous ligands for mag, and may mediate mag’s physiological functions.the mag binding termini may also occur on sialylated glycoproteins in the brain, and there remains controversy whether mag ligands are carried on gangliosides, glycoproteins, or both.in support of glycoproteins as mag ligands, treatment of nerve cells with a high concentration of protease moderately attenu- ated binding to mag-fc, a soluble recombinant form of mag furthermore, mag-fc has been used to precipitate various proteins from extracts of primary nerve cells and neuroblastoma cell lines although the functional roles of these proteins have yet to be esta- blished.whether or not glycoproteins can act as func- tional mag ligands, recent data support such a role for gangliosides, as detailed below.to test whether gangliosides support mag binding, and to probe the fine glycan specificity of mag, a cell adhesion-based assay was developed ganglio- sides of varied structure were adsorbed at concentrations ranging from 1–300 pmol/well onto microwell plastic plates in a background lipid monolayer consisting of phosphatidylcholine and cholesterol.upon expression of mag at the cell surface (confirmed by flow cytometry) the cells bound to gangliosides in a highly specific manner () in particular, major brain gangliosides bearing the ‘neuacα3gal�3galnac’ terminus (gd1a, gt1b) avidly supported mag-mediated cell adhesion, whereas closely related gangliosides with- out that terminus (e.g., gm1, gd1b) failed to support any adhesion.after 48 h of culture to allow mag expression, cells were collected, pretreated with neuraminidase (to enhance adhesion), and placed in microwells previously adsorbed with an artificial membrane monolayer containing phosphatidylcholine, cholesterol, and the indicated gangliosides.blocking or removing the neuac carboxylic acid, or modifying the neuac n-acetyl group each blocks mag binding.along with prior work demonstrating that mag binds relatively poorly to ‘neuacα3gal�4glcnac’ ter- mini and does not bind to α6-linked neuac, the above studies demonstrate that mag has evolved as one of the most specific and stringent of sialic acid binding lectins.the molecular basis of the fine structural recog- nition of mag for the ‘neuacα3gal�3galnac’ terminus has yet to be determined (no crystal structure of mag is available).these data are consistent with side-by-side comparisons of sialoadhesin and mag binding, in which hydroxyls on one face of the sialic acid are required for sialoadhesin binding, whereas those on the other face (the face facing the solvent in the crystal structure) are not the finding that hydroxyls on both faces of the terminal neuac are required for mag binding indicates that additional constraints, eitherknowledge of the specificity of mag-ganglioside binding, along with the availability of a genetically engineered mouse lacking a key enzyme in the synthesis of the major brain gangliosides provided an opportunity to test the hypothesis that gangliosides bearing the ‘neuacα3gal�3galnac’ terminus are functional mag ligands.unusual myelin anomalies, such as doubly myelinated axons with cytoplasm between the two com- pact myelin sheathes are found both in mag(-/-) (reviewed in and galnact(-/-) mice another interesting finding is that galnact(-/-) mice display selective dys- regulation of mag expression mag levels are normal in young animals, but decline in both the central and peripheral nervous systems to about 30% of normal levels (expressed relative to other myelin proteins) as the animals age thus, it appears that complex ganglio- sides are intimately involved in myelin stabilization and in mag expression.in part this is due to well described inhibitory proteins in myelin, including two molecularly well char- acterized proteins, mag and nogo pre- sumably, these proteins bind to specific targets on axons and nerve cells and provide intracellular signals inhibitory for axon outgrowth.treatment of nerve cells in vitro with neuraminidase, which converts mag-binding gan- gliosides gd1a and gt1b to the non-binding ganglioside gm1, reversed mag-mediated inhibition of neurite out- growth similarly, treatment of nerve cells with an inhibitor of glycosphingolipid biosynthesis (dl-threo-1- phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol) rendered them markedly less sensitive to mag together these data are consistent with gangliosides being the functional ligands for mag-mediated inhibition of nerve regeneration.the molecular mechanism by which the binding of mag to apposing gangliosides in the outer leaflet of a nerve cell plasma membrane generates an intracellular inhibitory signal in the nerve cell has yet to be determined.similarly, cross-linking of gangliosides gd1a or gt1b on the nerve cell surface by mag may modulate intracellular signaling molecules in lipid rafts, initiating an inhibitory signal.schachner m., bartsch u., multiple functions of the myelin- associated glycoprotein mag (siglec-4a) in formation and main- tenance of myelin, glia 29 (2000) 154–165.neurite growth inhibitory properties in vitro (table 1): nogo-a [16,78], mag [51,59] and also certain chon-furthermore, mice overexpressing mag in schwann temporarily diminishing chemorepulsive signaling in the cells also display retarded pns regeneration [39].[68,110], e.g., mag and sema3a induce growth cone regenerationneurons may require neurotrophic support for their survival second, the repulsive effects of sema3a and mag can be and/ or to initiate axon regrowth.a brief discussion of both mag and cspgs follows.when cells expressing mag are used as substrate for neurons derived from mature animals, inhibition of neurite outgrowth is observed (mukhopadhyay and oth- ers 1994).furthermore, immunodepletion of mag from inhibitory fractions of solubilized cns myelin pro- teins reduces the inhibitory activity of the fractionsthese studies suggest that mag is a potent inhibitor of neurite outgrowth from mature neurons in vitro.the potency of mag as a neurite outgrowth inhibitor in vivo is less clear.however, it has been suggested that mag is cleared more rapidly after injury to peripheral nerves.indeed, studies of peripheral nerve regeneration in mice with delayed clearing of peripheral myelin (wld6) exhib- it a close correlation of mag levels with the rate of nerve regeneration (schafer and others 1996).in con- trast, investigation of cns axonal regeneration in mag- deficient mice suggests that there is no difference in the regenerative capacity of damaged spinal axons of mag knockout and wild-type mice (bartsch and others 1995).thus, further studies are needed to determine the contri- bution of mag to the nonpermissiveness of the cns environment.interestingly, mag has a similar stage-specific inhibitory effect on drg neurons (mukhopadhyay and others 1994; bartsch and others 1995).1999. prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-dependent mechanism.database links nogo-a | glutamate decarboxylase | gap-43 | mag | brevican | versican | nt-4/5 | bdnf | nt-3 | trkb | trkc | semaphorin 3a | ephb3 encyclopedia of life sciences traumatic centralcai, d., shen, y., de bellard, m., tang, s. & filbin, m. t. prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp- dependent mechanism.denervated schwann cells express a variety of cell adhesion molecules of the im- munoglobulin superfamily, including ncam, l1, mag and n-cadherin [27,83,84].as a consequence of the loss of ax- onal contact after injury, schwann cells downregulate mye- lin constituents like mag and upregulate l1 and ncam [84].(1999) prior exposure to neurotrophins blocks inhibition of axonal regeneration by mag and myelin via a camp-depen­ dent mechanism.other immunological approaches to promoting axonal regeneration following cns lesions have involved the generation of dis-myelinat­ ing lesions with anti-galactocerebroside antibodies combined with complement (dyer et al., 1998), and by a vaccination paradigm involving immunization of mice against mag (huang et al., 1999).cafferty wb, duffy p, huebner e, strittmatter sm (2010) mag and omgp synergize with nogo-a to restrict axonal growth and neuro- logical recovery after spinal cord trauma.the classical myelin-associated inhibitors, nogo (184), omgp (185), and mag (186), bind to transmembrane receptors on the axon (187 - 189), which modulates axon growth.m. v., and filbin, m. t. (2005) mag induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite out- growth.cafferty wb, duffy p, huebner e, strittmatter sm (2010) mag and omgp synergize with nogo-a to restrict axonal growth and neurological recov- ery after spinal cord trauma.one gene that was up-regulated under both conditions is metallothionein (mt)-i. we show here that treat- ment with two closely related isoforms of mt (mt-i/ii) can overcome the inhibitory effects of both myelin and mag for cortical, hippocampal, and drg neurons.adult drgs from mt-i/ii-deficient mice extend significantly shorter processes on mag compared with wild-type drg neurons, and regeneration of dorsal column axons does not occur after a conditioning lesion in mt-i/ii- deficient mice.inhibitory effects of mag and myelin and promote axonal regeneration (15).when camp is elevated in drg neurons by lesioning the sciatic nerve, the ability of these neurons to extend neurites on mag and myelin in culture is significantly improved, a phenomenon known as the conditioning lesion effect (16, 17).the study described here shows a novel role for mt-i/ii in overcoming inhibition by mag and myelin for a variety of neu- ronal populations in vitro and promoting in vivo regeneration in the injured optic nerve.mt-i/ii expression is up-regulated after a conditioning lesion—previously, we and others showed adding bt2camp to primary neurons can overcome both mag and myelin inhibi- tion, and this effect is transcription-dependent (27, 30).we have similar results when we performed a conditioning lesion and subsequently cultured the drgs on mag or myelin; the neurons are not inhibited from putting out long neurites, and this effect is also transcription-dependent (27, 30).mt-i/ii overcomes inhibition of neurite outgrowth by mag and myelin—previously, we showed that the proteins from a variety of camp-regulated genes can overcome mag inhibi- tion of neurite outgrowth (27, 30).2a shows that mt-i/ii blocks the inhibition of neurite outgrowth by mag in a dose-dependent manner.looking at images of the neurons on cho cells expressing mag (fig.furthermore, mt-i/ii is as effective as bt2camp in blocking inhibition, because adding mt-i/ii to the culture media at the time of plating overcomes mag inhibition (fig.drg neurons are also inhibited by mag (fig.2, k and l), as with hippocampal neurons, blocked the inhibition of neurite outgrowth by mag once again as potently as bt2camp (fig.adding mt-i/ii to the culture media at the time of plating the cortical neurons overcomes mag inhibition (fig.these results show that mt-i/ii cannot only overcome the inhibitory effects of mag but in all the inhibitors found in myelin and that a variety of neurons are responsive to mt-i/ii.intrathecal delivery of mt-i/ii blocks inhibition of drg neu- rons by mag in culture—to begin to assess whether mt-i/ii could encourage regeneration in vivo, we first performed an experiment to determine whether mt-i/ii delivered into the cerebrospinal fluid would overcome inhibition of mag by adult drg neurons that are subsequently cultured on mag- expressing or control cho cells (fig.because the mt-i/ii is only applied intrathe- cally suggests that mt-i/ii promoted molecular changes in the drgs neurons in vivo, which enabled them to overcome mag inhibition once in culture.mt-i/ii expression is required for the conditioning lesion effect—because mt-i/ii is up-regulated in drg cell bodies after a conditioning lesion and overcomes inhibition by mag/ myelin, we next wanted to determine whether mt-i/ii-defi- cient mice respond to a conditioning lesion in its ability of drg neurons to overcome mag inhibition.drgs from wt mice with a conditioning lesion are able to overcome mag inhibition at both 1 and 7 days post-lesion, compared with nonlesioned littermate controls.in contrast, the ability of drgs from lesioned mt-i/ii-deficient mice compared with wt mice is strongly reduced in overcoming inhibition by mag for both the 1- and 7-day time points (\*\*, p < 0.01; \*, p < 0.05).mt-i/ii can overcome mag inhibition of p1 hippocampal neurons plated on either control (con) or mag-expressing cho cells for 24 h. for con- trol (nontreated) neurons, mag inhibited neurite outgrowth by greater than 60%; however, mt-i/ii directly added to the culture media overcomes the inhibition in a dose-dependent manner (a).statistics per- formed are anova, \*, p < 0.05, or \*\*, p < 0.01. representative images of hippocampal neurons shown are plated on control-expressing (b) or mag- expressing cho (c) cells and camp-treated on mag (d) and mt-i/ii on mag (e).repre- sentative images of drg neurons plated on mag display how inhibited they are in g–i;1 mm camp (j),5 (k), and 20 (l) µg/ml mt-i/ii put out significantly longer processes.the addition of mt-i/ii promotes adult wt drgs to overcome mag inhibition (fig.vehicle con- trol neurons grew long processes on control cho cells (b) but were inhibited in the presence of mag by an average of 40% (c).rats that received mt-i/ii had significantly longer neurites on mag at 0.125 µg/µl (d) and a dose-de- pendent increase in length with 0.25 (e and f) and 0.5 µg/µl (g).wt and mt-i/ii-deficient mice with cl overcome mag inhibition compared with their non-lesion controls, respectively.however, mt-deficient mice 1 or 7 days post-cl put out significantly shorter processes on mag com- pared with their wt littermates, where mt-i/ii-deficient drgs had ~50% shorter processes 7 days post-cl on mag compared with wt age-matched controls (\*, p < 0.05, and \*\*, p < 0.01).b–e, in the representative images below, wt (b and c) and mt-i/ii-deficient (d and e) drgs 7 days post-cl are plated on mag and stained for (3-iii tubulin.to determine whether mt-i/ii’s ability to promote neurite outgrowth in the presence of mag could simply be due to functioning as a zinc chelator, we applied ca-edta to our neurite outgrowth assay with the mag-expressing cho cells using primary cortical neurons (fig.8e) overcomes this inhibitory effect of mag; however, ca-edta has no effect on overcoming mag inhibition (fig.the addition of mt-i/ii strongly overcomes this inhibitory effect of mag; however, ca-edta has no effect on overcoming mag inhibition suggesting that chela- tion of zinc alone is not sufficient to promote neurite outgrowth on mag.representative images of cortical neurons shown are plated on control (b) or mag-expressing cho (c) cells, ca-edta-treated on mag (d), camp-treated on mag (e), and mt-i/ii-treated on mag (f and g).simply binding up all the free zinc is not efficient to overcome mag inhibition.rather an alternative mechanism for mt-i/ii must be involved to overcome mag inhibition.mt-i/ii inhibits a-secretase (tace) activity and is down- regulated in wt drg after conditioning lesion—to further investigate the mechanism behind mt-i/ii to promote axonal regeneration in the presence of myelin-mediated inhibitors, we looked at the inhibitory cascade initiated by mag binding to its receptor complexes.together, these results indicate that the ability of mt-i/ii to overcome mag inhibition is likely to be interfering with the mag-induced activation of pkc and rho.we found that mt-i/ii is up-regulated in drg neurons after the conditioning lesion and that it can overcome mag and myelin inhibition in three neuronal types.drg neurons that had intrathecal delivery of mt-i/ii in vivo are not inhibited by mag when grown in culture.drg neurons from wt mice with conditioning lesion overcome myelin-associated inhibi- tors, whereas the neurons from mt-i/ii-deficient mice do not overcome mag inhibition.mechanis- tically, mt-i/ii’s ability to overcome mag inhibition is transcription-dependent, as adult drg neurons can no longer overcome mag inhibition with the exogenous application of mt-i/ii in the presence of the transcriptional inhibitor drb.not overcome mag inhibition.we now add to these findings by dem- onstrating that mt-i/ii overcomes mag and myelin-mediated inhibition of neurite outgrowth in vitro for a variety of primary neurons, including hippocampal, cortical, and drg (figs.first, addition of mt-i/ii overcomes mag and myelin-associated inhibitors only when administered to freshly isolated neurons that have all their neurites severed by the isolation process.this is sup- ported by the observation that mt-i/ii’s ability to overcome mag inhibition is transcription-dependent, as adding the tran-the ability of mt-i/ii to overcome mag inhibition does not appear to be dependent on global zinc che- lation, as applying ca-edta in our neurite outgrowth assay did not overcome mag-mediated inhibition of cortical neurons.(2002) p75 interacts with the nogo receptor as a co-receptor for nogo, mag andcai, d., deng, k., mellado, w., lee, j., ratan, r. r., and filbin, m. t. (2002) arginase i and polyamines act downstream from cyclic amp in overcom- ing inhibition of axonal growth mag and myelin in vitro.domeniconi, m., zampieri, n., spencer, t., hilaire, m., mellado, w., chao, m. v., and filbin, m. t. (2005) mag induces regulated intramem- brane proteolysis of the p75 neurotrophin receptor to inhibit neurite out- growth.targeting inhib- itory ligands such as cspg (), nogo ( ; ), mag ( ), and omgp (), or their neuronal receptors and signal transduction factors such as ngr1 ( ; ), pirb (), ptprs ( ), rhoa (), and rock (cafferty wb, duffy p, huebner e, strittmatter sm (2010) mag and omgp synergize with nogo-a to restrict axonal growth and neurological recov- ery after spinal cord trauma.