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REVIEW

Manipulating oligodendrocyte intrinsic regeneration mechanism to promote remyelination

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Abstract

In demyelinated lesions, astrocytes, activated microglia and infiltrating macrophages secrete several factors regulating oligodendrocyte precursor cells' behaviour. What appears to be the initiation of an intrinsic mechanism of myelin repair is only leading to partial recovery and inefficient remyelination, a process worsening over the course of the disease. This failure is largely due to the concomitant accumulation of inhibitory cues in and around the lesion sites opposing to growth promoting factors. Here starts a complex game of interactions between the signalling pathways controlling oligodendrocytes migration or differentiation. Receptors of positive or negative cues are modulating Ras, PI3K or RhoGTPases pathways acting on oligodendrocyte cytoskeleton remodelling. From the description of this intricate signalling network, this review addresses the extent to which the modulation of the global response to inhibitory cues may pave the route towards novel therapeutic approaches for myelin repair.

Keywords Myelin repair · Signalling pathways · Oligodendrocytes · Multiple sclerosis · Therapy · Migration

Abbreviations

Akt	Protein kinase B
AMIGO-3	Amphoterin-induced protein 3
ARP2	Actin-related protein-2
Bcl2	B-cell lymphoma 2
BDNF	Brain derived neurotrophic factor
bFGF	Basic fibroblast growth factor
Cdc45	Cell division control protein 45
Cdk5	Cyclin-dependent kinase 5
CNP	2′,3′-Cyclic nucleotide 3′ phosphodiesterase
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
FARP2	FERM, ARH/RhoGEF and Pleckstrin domain protein 2
CSPG	Chondroitin sulfate proteoglycan
CXCL2	C-X-C motif chemokine ligand 2
GAB	GRB2-Associated binding protein
GAP	GTPase, activating protein
GDP	Guanosine diphosphate
GEF	Guanine–nucleotide exchange factor
GFAP	Glial fibrillary acid protein
GPCR	G-protein coupled receptor
GRB2	Growth receptor factor-bound protein 2
GSK3 β	Glycogen synthase kinase-3
GTP	Guanosine triphosphate
GTPase	Guanosine triphosphatase
IGF-1	Insulin-like growth factor 1
IL-6	Interleukin 6
LIF	Leukemia inhibitory factor
LINGO-1	Leucine-rich repeat and Ig domain-containing 1
LKB1	Liver kinase B1
LPC	Lysophosphatidylcholine
NgR1	Nogo receptor 1
Nogo-A	Neurite outgrowth inhibitor
NT-3	Neurotrophin-3
MAG	Myelin-associated glycoprotein
MAIs	Myelin-associated inhibitors
MAPK	Mitogen-activated protein
MBP	Myelin basic protein

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MDM2	Murine double-minute 2 homolog
MS	Multiple sclerosis
MTP-PlexA1	Membrane domain-targeting peptide of Plexin-A1
mTOR	Mammalian target of rapamycin
NF1	Neurofibromin 1
NG2	Neuron glial antigen 2
NRP1	Neuropilin 1
OL	Oligodendrocyte
Omgp	Oligodendrocyte myelin glycoprotein
OPC	Oligodendrocyte precursor cell
P75NTR	Neurotrophin receptor p75
PDGF	Platelet-derived growth factor
PDK1	Protein kinase phosphoinositide-dependent kinase1
PDX	Patient-derived xenograft
PH	Pleckstrin homology
PI3K	Phosphatidylinositol 3-kinase
PIP3	Phosphatidylinositol triphosphate
PKC α	Protein kinase C α
PLA	Proximity ligation assay
PLC	Phospholipase C
PlexA1	Plexin A1
PPI	Protein–protein interaction
PTEN	Phosphatase and tensin homolog
RRMS	Relapsing/remitting multiple sclerosis
RTK	Receptor tyrosine kinase
Sema3A	Semaphorin 3A
SEMA4D	Semaphorin 4D
SOS	Son of sevenless homolog
srGAPs	Slit-Robo-GTPase activating proteins
SREBP	Sterol regulatory element-binding protein
TNFSFR	Tumour necrosis factor super family receptors
TSC2	Tuberous sclerosis complex 2
WASP	Wiskott-Aldrich syndrome protein
WNK1	WNK lysine-deficient protein kinase

Repairing myelin in multiple sclerosis

Multiple sclerosis (MS) is the most common auto-immune disease of the central nervous system and affects 2.5 million people worldwide. This pathology is characterized by the destruction of the myelin sheaths by the immune system. This drives to axonal weakness which disturbs severely neuronal communication and leads to motor and sensory disorders. Main feature of the disease starts with relapse/remitting phases (RRMS) followed after several years (15–20 years) by a progressive phase in the majority of patients. The remaining MS patients (10–15%) have a primary progressive form of the disease, without RRMS, characterized by a slow and progressive neurodegeneration in which the symptoms worsen and disability accumulates from the onset. In early stage of the lesion, disturbance of brain–blood barrier is accompanied by moderate T-cell (CD8 + and CD4 +) and B-cell infiltration which will drive to oligodendrocyte destruction only after a profound invasion of inflammatory cells [1, 2]. Like other neuroinflammatory diseases, infiltration at the site of tissue damage in multiple sclerosis is regulated by cytokines [3, 4]. This deleterious inflammation is characterized by increase of T-cell and B-cell, macrophage infiltration and activation of resident microglia and macrophages. In later stages, granzyme B as well as immunoglobulin and activated complement deposition can be observed in lesions [5, 6]. In classical active lesion architecture, normal-appearing white matter surrounding the lesion contains scattered perivascular inflammatory infiltrates and microglia nodules whose density increases at the border of the lesion. Loss of oligodendrocytes occurs in lesion area where activated macrophages phagocyte myelin debris [7]. Tissue composition of the lesion is ultimately altered by reactive astrocytes and microglia which drives to glial scar formation, supposed to prevent tissue destruction spreading by retaining immune cells and toxic metabolites. Moreover, angiogenesis occurs inside and around the demyelinating lesion, [8]. This profound tissue remodelling is the source of a molecular and cellular barrier impeding myelin repair thereby installing over time severe motor and sensitive deficits (Fig. 1).

Strikingly, remyelination is observed in lesions but is generally incomplete, giving rise to "shadow" plaques where newly generated myelin sheaths are thinner than pre-existing ones [9, 10]. This regenerative process decreases and disappears with chronicity and entrance in progressive phase of the disease. In progressive form of MS, chronic inflammation is maintained in slowly expanding lesions despite a closed brain blood barrier with involvement of activated microglia, T-cells and B-cells. High production of reactive oxygen species contributes to mitochondrial and axonal damages [11].

While the current therapies aim at controlling the inflammatory component of MS, better comprehension of the reasons of remyelination failure in MS would help to develop new therapeutic approaches. In chronic lesions, oligodendrocyte precursor cells (OPCs) derived from neural precursor cells of subventricular zone or coming from perilesional environment are recruited to regenerate the myelin sheet [12, 13] and accumulate at the border rather in the core of the lesion [14, 15]. Whereas many factors promoting OPC recruitment are secreted in demyelinating lesions [16], several repulsive cues contribute to remyelination failure. The massive changes occurring in the lesion regarding matrix and cell composition as well as their activation state produce this particular environment where negative cues outperform remyelinating cues. The counteraction of negative cues or

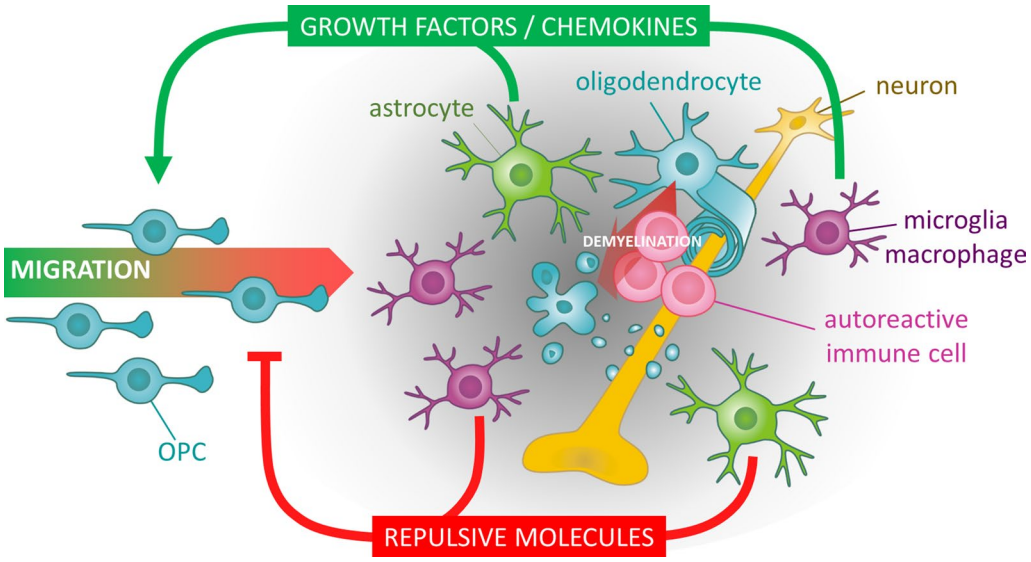

Fig. 1 Opposing molecular factors in demyelinated lesions. In the context of multiple sclerosis, the myelin sheaths are attacked by autoimmune reactive cells. The concomitant pro-inflammatory situation is leading to a glial activation of astrocytes and microglial cells contributing to the secretion of various molecular signals. On the one side, growth factors and chemokines are positively acting on the recruitment of oligodendrocyte precursor cells (OPCs) attracted towards the lesion in order to repair myelin. On the other side, repulsive growth inhibitory molecules accumulating in this perturbed microenvironment create a molecular barrier preventing OPCs to reach the centre of the lesion hence precluding myelin repair

the promotion of positive cues is offering the possibility to develop a therapeutic strategy which could stem on natural capacity myelin repair. To this end, it is important to understand the machinery of this repulsive barrier. In this review, we will highlight signalling pathways involved in remyelination and analyse how repulsive cues alter them.

Intracellular pathways controlling remyelination

Spontaneous remyelination occurring in the early stages of the disease involves proliferation and migration of OPC, followed by their differentiation in mature oligodendrocytes for wrapping up of axons by new myelin sheaths [17, 18]. These steps involve three canonical signalling pathways controlling numerous basic cellular pathways. Ras GTPase, PI3K-Akt pathway and MAPK pathway are central regulators of myelination with already a well-depicted role in developmental myelination (Fig. 2).

The members of the **Ras GTPase** family (HRas, NRas, KRas) cycle between an inactive form bound to GDP and an active form bound to GTP [19]. Activation of receptor tyrosine kinase RTKs by growth factors and chemokines leads to their tyrosine *trans*-autophosphorylation, serving as docking sites for Grb2-Sos complex which activates Ras. G-protein coupled receptors (GPCR) also activate Ras via Ras-GEF activation [20]. Among well-characterized downstream pathways, Ras is known to stimulate PI3K-Akt pathway, Raf-MEK-ERK pathway (also known as MAP Kinases) and Ral/Exocyste complex. R-Ras1$^{-/-}$ and R-Ras2$^{-/-}$ null mice exhibit a diminished oligodendrocyte population with higher proportion of immature oligodendrocytes [21]. In these mice PI3K-Akt and Erk1/2-MAPK pathways are less activated.

The **PI3K** family is composed of regulatory subunits (p85, p87, p110) and p110 catalytic subunit catalysing the phosphorylation of phosphatidylinositol PtdIns(4,5)P2 into PtdIns(3,4,5)P3. It can be directly activated by RTK or via either Ras or Grb2/GAB [22]. PIP3 recruits proteins with PH (pleckstrin homology) domains like PDK1 and Akt allowing Akt activation by PDK1 and mTORC2 phosphorylation. Akt has multiple functions. First it favours survival by inhibiting proapoptotic Bcl-2 proteins, phosphorylating Mdm2 or inhibiting transcription factor NF-kappaB. It regulates also metabolism and differentiation by activating mTORC1 and inhibiting GSK3β [23, 24]. Knock-out of the PI3K-Akt pathway inhibitor PTEN in oligodendrocyte lineage resulted in significant hypermyelination throughout the CNS of transgenic mice associated with increased PIP3 levels and Akt phosphorylation [25, 26]. Similarly, expression of constitutively active Akt in oligodendrocytes resulted in hypermyelination increasing as mice aged up to reach a pathological level [27]. Treatment with the mTOR inhibitor rapamycin inhibited hypermyelination suggesting its involvement downstream Akt [28].

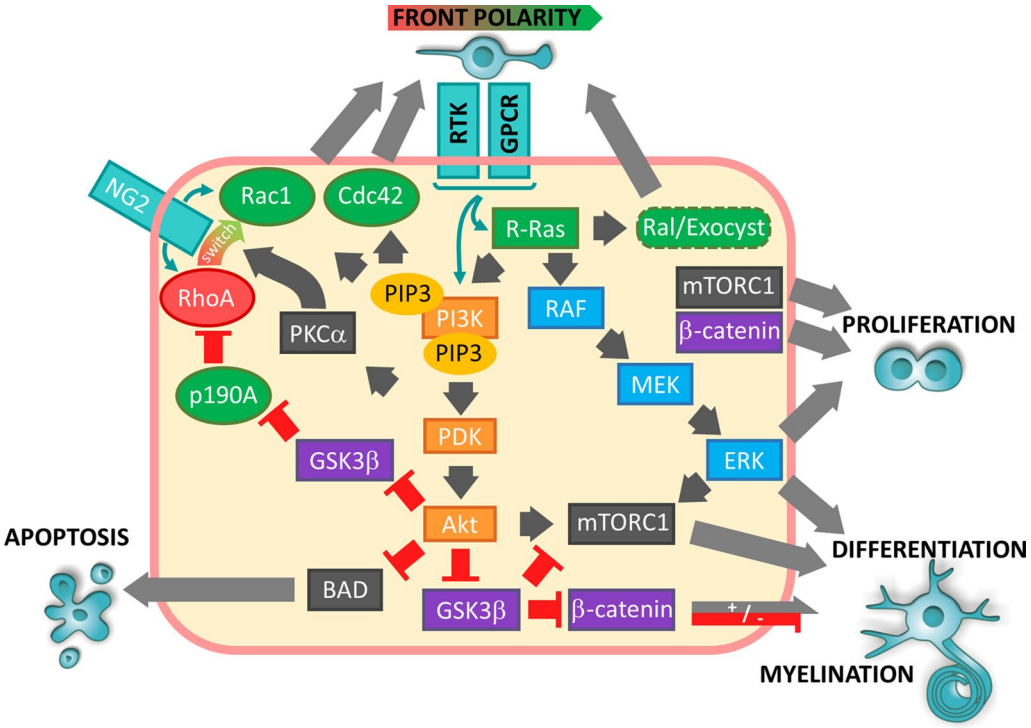

Fig. 2 Involvement of Ras/PI3K/MAPK pathway in remyelination. Migration: the proteoglycan NG2 contributes to front-rear polarity establishment in OPCs in response to growth factors and chemokines. NG2 maintains high RhoA activity at the rear of the cell whereas it stimulates Rac at the migration front via its switch of activity induced by RTK/GPCR dependent PKCα activation. Ras/Raf/MEK/ERK and PI3K/Akt pathways regulate this Rac activation. Conversely, removal of GSK3β mediated inhibition of p190A by Akt probably favours local RhoA inhibition. Proliferation/differentiation: Akt dependent regulation of mTORC1 and β-catenin plays a parallel role to ERK1/2 in proliferation and differentiation of oligodendrocytes. However, β-catenin can have opposite effects on this differentiation depending of its nuclear level. Arrows show activation and truncated red arrows show inhibition. PI3K-Akt pathway is shown in orange boxes, MAPK pathway in blue boxes and GSK3β/β-catenin in purple boxes. Factors shown in green favour establishment of the migration front

Canonical **MAPK pathway** activation starts with Raf phosphorylation by Ras. Then Raf phosphorylates MEK which activates ERK [20]. Phosphorylated ERK moves into the nucleus to regulate expression of target genes. MAPK pathway can be activated either by RTK or GPCR via Ras, adenylate cyclase or Rac-dependent mechanism. As well as Akt, ERK activates mTORC1 [29]. Knock-out of b-raf in neural progenitor cells impaired oligodendrocyte differentiation in developing mice [30]. Erk2 deletion from GFAP-expressing radial glial cells impaired their differentiation in oligodendrocyte in vitro [31]. However, this effect was not found in vivo where Erk1 and Erk2 deletion in oligodendrocyte lineage did not affect OPC differentiation but rather reduced myelin sheath thickness [32]. Therefore, MAPK pathway seems to play a more prevalent role in myelin growth compared to cell differentiation.

The interplay between these pathways is adding another complexity in the signalling machinery controlling OPC differentiation. Fine tuning of these pathways also depends on the integrin-dependent activation of Src family kinases like Fyn. Fyn activation by α6β1 integrin can, therefore, amplify PDGF signalling and switch neuregulin signalling from PI3K to MAPK pathway [33]. Among targets of Fyn identified in OPC, Cdk5 has been shown to transmit PDGF-A induced migration [34] and Fyn also interacts with p130cas to activate Rac [35, 36]. Hence, the concomitant activation of RTK, GPCR and integrins in response to environmental cues is creating intracellular competitions of signalling pathways whose biological consequence on OPC relates on the relative strength of each components [37]. Consequently, the dominance of one or the other pathways impacts the different stages towards remyelination.

Pathways involved in migration

To reach the site of lesions, OPCs must migrate directionally and thus acquire antero-posterior polarity regulated by proteins acting specifically at one cell pole. Phosphatidylinositol 3-kinase (PI3K) and its products, phosphatidylinositol triphosphate (PIP3), are preferably located forward in protrusions [38]. PIP3 recruits at the plasma membrane the Arp2/3/WASP complex which regulates the connection of the actin cytoskeleton and the guanine-nucleotide exchange factors (GEF) which then activate the

Rho GTPases [39]. Rho GTPases Rac and Cdc42 stimulate the polymerization of actin at the migration front, and at the back RhoA stimulates the contraction of actin to advance the cellular body. The receptor tyrosine kinase (RTK) and GPCR of growth factors and chemokines contribute to raise the PIP3 levels locally and define the migration front by activating the PI3K directly or via the GTPase Ras [40]. Downstream Ras, Ral/exocyste pathway coordinates membrane trafficking and actin polymerization contributing also to the front-rear polarity [41]. All these proteins involved in actin-driven protrusions have been found in oligodendrocytes from OPC stage to myelin sheath preparations [42]. Some specificities exist with predominance of actin nucleator WAVE1 in oligodendrocytes, whereas WAVE2 is more expressed in Schwann cells [42]. Loss of WAVE1 leads to a decreased number of OPC processes and hypomyelination in corpus callosum and optic nerve [43].

The contribution of Ras, PI3K-Akt and MAPK pathways to the growth of OPC pool and their involvement in the final steps of differentiation and myelination have overshadowed their involvement in migration, explaining sparse studies about their intrinsic contribution during the remyelination sequence. However, the transmission of promigratory signal obviously involves these pathways. Taking the example of CXCL12 dependent migration of OPCs, its binding to its receptor CXCR4 activates both PI3K-Akt pathway and MAPK pathway whose inhibition reduces OPC migration in vitro [44]. Activation of PKC, known to interact with these pathways, stimulated also OPC protruding activity [45]. Work in zebrafish demonstrated that knockdown of NF1 improved OPC migration presumably via the loss of its GAP inhibitory activity on Ras [46]. One frequent outcome of these pathways ends up on regulation of Rho GTPases like Rac. Whereas growth-factor induced Ras signalling activates Rac via PI3K, Ras-Raf-MAPK pathways can also downregulate expression of the Rac GEF activator Tiam1 [47]. Reciprocally, Rac contributes to the recruitment of PI3K at the leading edge of migrating cells [48] and Rac regulates formation of MEK-ERK1 complexes via PAK [49]. A bFGF dose-dependent activation of Rac determines the switch between directed and random migration of OPCs [50, 51]. The specificity of NG2 proteoglycan expression in OPCs has to be considered to fully understand their migration properties. NG2 intrinsically maintains high RhoA activity at the periphery of the cell via the RhoA GEF Syx [51], resulting in contact inhibition of locomotion and explaining the self-repulsion of OPCs observed in vivo in adult brain [52]. However, the phosphorylation of NG2 by PKCα switches downstream signalling from RhoA to Rac stimulation via recruitment of PAR and CRB complex proteins and activation of the Rac GEF Tiam1 [51, 53]. The activation of PKCα, notably by PLC activity downstream RTK and GPCR, favors a polarized shape where NG2 itself contributes to the high Rac activity at the migrating front and maintain RhoA at the rear of the cell (for extensive review see [50]).

Pathways involved in proliferation/survival

In experimentally induced demyelination, a dramatic increase of OPCs is observed [54]. Several mitogens have been involved in lesion-associated remyelination such as PDGF-A, bFGF, NT-3, IGF-1, CNTF, IL-6, LIF [16]. The consecutive Ras, PI3K-Akt and MAPK cascade controls cell proliferation [55]. It was shown that bFGF and IGF1 synergistically promotes cell cycle progression via enhanced cdk2 and cdk1 activity [56, 57]. This effect was impeded by rapamycin, involving mTOR in oligodendrocyte cell progression. Connexin 47 upregulation in OPC induced by coculture with astrocytes, possibly via heterotypic gap junctions, stimulates OPC proliferation via ERK1/2 [25]. Growth factors promote also oligodendrocyte survival like IGF1 which protects OPCs against TNFα-induced damage via PI3K/Akt dependent phosphorylation of BAD [58]. Knockout in oligodendrocyte lineage of PIKE, an upstream activator of PI3K-Akt, impaired remyelination of corpus callosum treated with lysolecithin. In this model, neural precursor from the subventricular zone usually recruited to repair corpus callosum demyelination had lower proliferation because of PIKE knock-out [59].

Pathways involved in differentiation and myelin sheath formation

Transcription factors, like Olig1, Ascl1, Nkx2.2, Sox10, YY1 and Tcf4, are required for the generation of mature oligodendrocytes [60]. Oligodendrocyte differentiation control by the Wnt/β-catenin pathway is ambivalent since it is mainly described as inhibitor for oligodendrocyte differentiation, whereas its transient activation seems crucial for initiating terminal differentiation [61, 62, 63]. This positive effect has been proposed to be dependent of β-catenin level in the nucleus and its association with the transcription factor Tcf7l2 [64]. GSK3β is a key actor of this balance since it regulates β-catenin availability by controlling its phosphorylation-dependent targeting to proteasome. Indeed pharmacological inhibition or knockdown of GSK3β can either promote or supress OPC differentiation [63, 65].

PI3K-Akt and MAPK pathways play an important role in oligodendrocyte differentiation [66–68]. Their parallel role has been demonstrated by the myelination rescue of Erk1/2 deficient mice by PI3K/Akt constitutive activation [67]. One common target of these pathways is TSC2 whose phosphorylation by Akt or Erk1/2 results in mTORC1 complex activation [69]. Even parallel, these pathways could

have sequential roles since Erk1/2 regulates differentiation in early stages and mTOR in later stages [70]. The function of mTORC1 is interesting since it coordinates myelin protein expression with lipid synthesis and via transcription factors SREBP [69]. Regarding studies focusing on remyelination of lesions, a focal LPC-induced demyelination with CNP- dependent deletion of Cdk5 impaired OPC differentiation and decreased levels of activated AKT [71]. Erk2 conditional knockout in oligodendrocytes resulted in delayed remyelination of LPC-induced lesion in the corpus callosum accompanied by impaired MBP translation [72]. Similarly, sustained activation of ERK1/2 by expression of constitutively active MEK in oligodendrocyte lineage accelerated remyelination of LPC-induced lesions with improved myelin thickness compared to wild-type littermates [73].

Acting downstream of Ras, Ral GTPase and exocyst impact several aspects of cell polarity through vesicular trafficking, The massive membrane remodelling necessary to myelination is, therefore, impaired when these pathways are ablated [74, 75].

The process of myelin membrane wrapping requires a coordination of the cytoskeleton dynamics. Indeed, in the inner tongue, it has been shown that the polarized growth of myelin is determined by elevated levels of PIP3 at the growing edge [68]. Downstream Rho GTPase effectors use this signal to structure cytoskeleton organisation. Indeed, loss of Cdc42 and Rac1 leads to accumulation of cytoplasm of myelin sheaths and myelin out folding, revealing their role in coordinating myelin sheath wrapping [76, 77]. Ultimately, F-actin presents in the leading edge and outermost myelin layer is disassembled in the rest of the myelin sheath with MBP coming [78, 79].

Remyelinating factors secreted in the lesions, a rheostat controlling oligodendrocyte behaviour

Despite the neurotoxic effect consecutive to the proinflammatory role of astrocytes and macrophages/microglia, they can be beneficial for remyelination. Density of O4-positive OPCs is corelated with increased debris-laden macrophages in human MS lesions [80]. Elevated density of HLA-DR macrophages and microglia at the lesion border in MS also correlated with more extensive remyelination [81]. Indeed, depletion of pro-inflammatory macrophages/microglia at early time points post-demyelination impairs OPC proliferation whereas depletion of M2 polarized cells at later points impairs OPC differentiation [82]. This could be explained by the release of several growth factors important for OPC recruitment, proliferation and differentiation: IGF-1, activin-A, endothelin-2, HGF, PDGF-A, bFGF, galectin 3, TNF, IL-1β, IL4, CXCL1, 8, 10, 12 [83–85].

Analysis of MS lesions revealed selective expression of bFGF within active lesion and in the peri plaque of chronic lesions [86]. The stimulation level of factors such as PDGF-A or bFGF affects the oligodendrocytic cell response [50, 51]. Thus PDGF activates the migration of OPCs at low concentrations via PI3K and proliferation at high concentrations via PLCγ [87]. Therefore, the gradient of growth factors and chemokines around MS lesions [88] suggests a concentration-dependent mechanism of oligodendrocyte response.

Neurotrophic factors also modulate remyelination. In demyelinating lesions of the spinal cord, NRG1 promotes remyelination through generation of oligodendrocytes [89] and presumably through stimulation of migration by activation of Rac1 and Cdc42 via the GEF Dock7 [90]. Among neurotrophins, NT3 enhances Schwann cell migration via Rac1 and Cdc42 activation [91], whereas BDNF binding to p75NTR inhibits Schwann cell migration by activation of the GEF Vav2 and RhoA [92]. Conversely, NT3 inhibits peripheral neural system myelination [93, 94] whereas BDNF promotes it [95, 96].

Hence, a gradient of signalling molecules emanating from the lesion probably controls the transition of oligodendrocyte behaviours between migration, proliferation and differentiation. Thus, depending on OPCs or Schwann cells, these lesion-associated factors can specifically favour one of these behaviours or induce different effects depending on their level of activity. Besides positive factors, inhibitory molecules arise from the lesion and block remyelination.

Inhibitors of remyelination, a reminiscence of developmental guidance mechanisms

As usual with biological processes, positive/activating pathways are counterbalanced by negative/inhibitory signals aiming at providing to the cell the right equilibrium adapted to the function. Indeed, facing the numerous factors favouring myelination, several types of guidance molecules known for their critical roles during brain development are impacting OL and OPC functions by triggering complex signalling pathways controlling cytoskeleton remodelling (Fig. 3).

Sema3A/PlexinA1/Nrp1

Downstream signalling deciphering of Sema3A was mainly carried out in neurons [97–102]. Sema3A stimulates the Ras GAP activity of PlexinA1 which directly inhibits Ras [97, 98]. Binding of Sema3A to PlexinA1/Nrp1 complex receptor leads to the release of Rac-GEF FARP2 which activates Rac. GTP-bound Rac is then sequestrated by PlexinA1 and allows the recruitment of Rnd1 which activates the GAP domain of PlexinA1 [99, 100]. Collapsus induced by localized addition of Sema3A

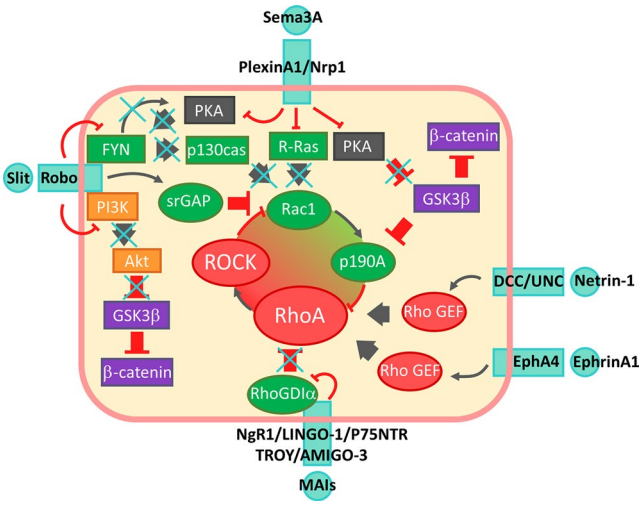

Fig. 3 Signalling pathways affected by inhibitors of remyelination. Rac1/RhoA balance is commonly found altered by all inhibitors of remyelination to the benefit of RhoA. MAIs induce sequestration of Rho-GDI by p75 or TROY, releasing activable RhoA-GDP. Ephrin/Eph association as well as DCC/UNC activation by netrin-1 activates RhoA through RhoGEF. Reading of Sema3 effect on RhoGTPases is complexified by the kinetic of its receptor activation relying on an initial stimulation of Rac1 to subsequently activate its RasGAP domain. However downstream signalling unambiguously favours RhoA signalling. Indeed, PlexinA1 inhibits Ras activity and simultaneously removes RhoA inhibiting activity of p190A through removal of PKA dependent inhibition of GSK3β. Arrows show activation and truncated red arrows show inhibition. PI3K-Akt pathway is shown in orange boxes, MAPK pathway in blue boxes and GSK3β/β-catenin in purple boxes. Elements in favour of Rac activity are shown in green and elements in favour of RhoA activity in red. Crosses show inhibitory effect of inhibitors of remyelination on signalling. Signalling pathways presented have been deciphered in neurons and/or oligodendrocytes

involves RhoA activation and retrograde waves of active Cdc42 [101] showing disorganized cell polarity which could be potentially linked to Ras/PI3K pathway inhibition. Sema3A is also responsible of decreased activity of PKA and reduced phosphorylation of its targets GSK3β and LKB1 [102].

The roles of Semaphorins on oligodendrocyte lineage and its relevance in the context of multiple sclerosis has been well-documented [103, 104].

Sema3A expression has been found in MS lesions as well as EAE and cuprizone experimental demyelination models [105–107]. Sema 3A has been originally identified as a repulsive cue for OPCs during optic nerve development [108, 109]. Sema3A exerts its inhibitory action on oligodendrocytes in multiple ways. Sema3A inhibits process extension [110], migration [105] and also oligodendrocyte differentiation in vitro resulting in remyelination impairing when sema3A is infused in demyelinating lesions [111]. Loss of function of Sema3A receptor Nrp1 rescues OPC recruitments into the demyelinating lesion [112]

NOGO and myelin associated inhibitors

Neurite outgrowth inhibitor (Nogo-A), myelin-associated glycoprotein (MAG) and oligodendrocytes myelin glycoprotein (Omgp) are myelin-associated inhibitors (MAIs) largely described for their inhibitory function on neurite extension in neurons. MAIs are expressed in oligodendrocytes and accumulate in demyelinated plaques exhibiting oligodendrocytes debris. MAIs inhibit neurite outgrowth by interaction with Nogo receptor 1 (NgR1), a GPI-anchored receptor activating RhoA pathway. Since NgR1 has no catalytic domain, it associates with LINGO1 [113], AMIGO3 [114] or some of the tumor necrosis factor super family receptors (TNFSFR): p75NTR or TROY to trigger signalling [113, 115, 116]. These receptors assemble into a signalling platform in which LINGO-1 in association with WNK1 [117] facilitates the binding of Rho-GDIα to p75 or TROY, leading to the release of RhoA-GDP [118, 119]. RhoA-GDP will then be converted into RhoA-GTP by Rho-GEFs (Guanine nucleotide Exchange Factors) to activate RhoA [118]. Interestingly, LINGO-1 homodimerization similarly leads to RhoA activation [120, 121]. LINGO-1 also reduces EGFR phosphorylation by a direct association downregulating MAPK and PI3K pathways [121, 122]. Another study reports that the direct binding of Lingo-1 to ErbB2 blocks its translocation in lipid rafts thereby inhibiting OPC differentiation [123]. TROY has been reported to activate cJunk and PKCα phosphorylation in OPC [124] but the precise mechanism remains unclear.

Netrin/DCC/UNC5

Earliest description of unc-6 (ortholog of netrin-1) in *C. elegans* development suggested its duality, since it performed repulsive effect on axons extending dorsally and attraction on axons extending ventrally [125]. This relies on receptor complexes transmitting netrin-1 signal: homodimers of DCC induce an attraction response, whereas heterodimers DCC/UNC5 induce repulsion [126]. Membrane receptor level, secondary messengers such as PKCα [127, 128] or ligand concentration can affect this response. This later parameter appeared in netrin-1 gradient where lower concentrations are attractive and higher repulsive [129]. This could be explained by the higher affinity of netrin-1 for DCC homodimer compared to DCC/UNC5 heterodimer [126] which would, therefore, bind repulsive heterodimer only at highest concentrations. Intracellular signalling of both receptors relies on similar actors like SFK and Rho GEFs. DCC homodimer drives to Rac1 activation [130] whereas DCC/UNC5 heterodimer drives to RhoA activation [131] (For extensive review on netrin-1 signalling, see [132]). This regulation of RhoA has been shown to be mediated by a Rho GEF in *C. elegans* [133].

In oligodendrocyte lineage, netrin-1 acts similarly and displays a chemorepulsive effect on OPCs mediated by RhoA

activation and its downstream effector ROCK. Interestingly, netrin seems later necessary for differentiated oligodendrocytes branching [134, 135]. This remyelinating effect relies in fact on Fyn recruitment to DCC receptor resulting in RhoA inhibition [136]. Presence of Netrin in MS sample is attested and could regulate OPC recruitment to the lesions [137].

Slit/Robo

Studies on Drosophila embryo revealed that binding of Slit to transmembrane receptor Robo is a chemorepulsive cue stopping midline crossing of the CNS [138, 139]. Slit could display its action by inducing molecular tension in Robo allowed by ECM-immobilization of Slit [140]. This leads to exposure of metalloproteinase cleavage site and ectodomain shedding [141]. This cleavage is required for recruitment of downstream signalling molecules [142]. Slit-Robo signalling involves GSK3β/β-catenin pathways and Rho GTPases. Binding of Slit2 induces the binding of the PI3K subunit p85 and blocks Akt activation. As a consequence, GSK3-β inhibition by Akt is released and β-catenin translocation is blocked [143]. Slit-Robo-GTPase activating proteins (srGAPs) inhibit Rho-GTPases like srGAP2 which inhibits Rac1 [144] (for review: [145]). Interestingly, Slit/Robo pathway can cross react with the other inhibitory pathways. Indeed, SlitC cleaved fragment can interact with PlexinA1 and induces growth cone collapse [146]. In the presence of Slit, Robo1 can silence Netrin/DCC attraction signalling [147]. On the contrary, Robo3 which has lost its ability to bind Slit during evolution, collaborate with DCC for Netrin-1 attraction signalling [148].

Slit2 has been involved in the dispersal of OPCs. Slit2 binding with Robo1 induces Fyn recruitment and inactivation of Fyn as well as RhoA activation [149].

Ephrin/Eph

Ephrin/Eph signalling is involved in direct cell interactions signalling and regulates axon guidance [150]. Ephrin and Eph receptors are both membrane-anchored and from their interaction results a bi-directional signalling with a forward signalling relying on Eph tyrosine phosphorylation and a reverse signalling in ephrin expressing cells. EphrinA binding to EphA4 triggers the RhoA GEF ephexin activation in neurons [151] or Vsm-RhoGEF in vascular smooth cells [152].

Several ephrin and Eph have been found in MS lesion samples [153]. OPC express ephrinB2 and are repealed by EphB2 [154].Ephrin-A1 interaction with OPC EphA4 activates RhoA/ROCK signalling and causes inhibition of oligodendrocyte process extension [155] probably via a RhoGEF. EphrinB3 found in MS lesion is also responsible for RhoA activation in OPCs probably via EphA4 binding, and its antibody-mediated neutralization favours remyelination in an experiment model [156].

Targeting the inhibitory signals to promote remyelination

Few therapeutic approaches are attacking the problem of myelin repair (Fig. 4). The most promising ones are the use of miconazole [157], MD1003 [158] a highly concentrated oral formulation of biotin (also known as Vitamin H or Vitamin B7), the retinoid X receptor Gamma [159], Clemastine fumarate [160] or Sobetirome [161]. The molecules are acting by favouring the differentiation of OPC but do not address the inhibitory molecular barrier. As described above, one common feature of the inhibitory/repulsive molecules signalling overexpressed in MS lesions relies on RhoA activation. This convergence in the signalling pathway mirrors what is seen in CNS nerve lesions where the glial scar being formed upon lesion is also exhibiting a variety of inhibitory factors [162] including ECM components (CSPGs, Tenascin or NG2), myelin-derived growth inhibitors NOGO, MAG, OMgp, and other membrane bound or secreted repulsive cues (Semaphorins, Ephrins). In this situation, many therapeutic options have been developed to antagonize the inhibitory factors and favour neuron-intrinsic regeneration capability to restore axon regrowth [163]. The use of small molecules, antibodies or peptides indeed showed significant improvement of axon regrowth and in some cases retargeting with improved functional recovery [164]. Strikingly, the inhibition of one single inhibitory factor is commonly sufficient to circumvent the deficit of growth in preclinical studies but it so far poorly translated into clear benefits in clinical studies. However, the beneficial effect obtained relies on resetting of the growth capacity by direct or indirect modulation of axon growth-associated signalling pathways. By analogy, it appears interesting in the context of demyelination diseases to modulate the response of OPCs to environmental inhibitory signals in order to promote remyelination. The promotion of pro-migratory/pro-differentiating factors by the selective blockade of inhibitory signals should allow a reinforcement of the intrinsic myelin repair mechanism.

To test this strategy, studies have been conducted with anti-Nogo-A antibody [165] up to clinical phases. On the same line, the antagonism of the Nogo-Receptor subunit -Lingo1 favouring MAPK and glucocorticoid receptors is showing remyelination capability with a quite good tolerance in human [166]. An anti-SEMA4D antibody (VX15/2503) is currently being investigated [167] to counteract SEMA4D inhibitory effects on remyelination. More recently, an experimental therapeutic approach was designed to block Sema3A inhibitory signalling by inhibiting its Plexin-A1 signalling receptor [105]. This approach is based on the use of a peptide targeting the transmembrane domain of Plexin-A1 (MTP-PlexA1) that disrupts the dimerization necessary to trigger Sema3A signalling pathway [168]. This strategy of inhibiting dimerization of the Plexin-A1 receptor has already been

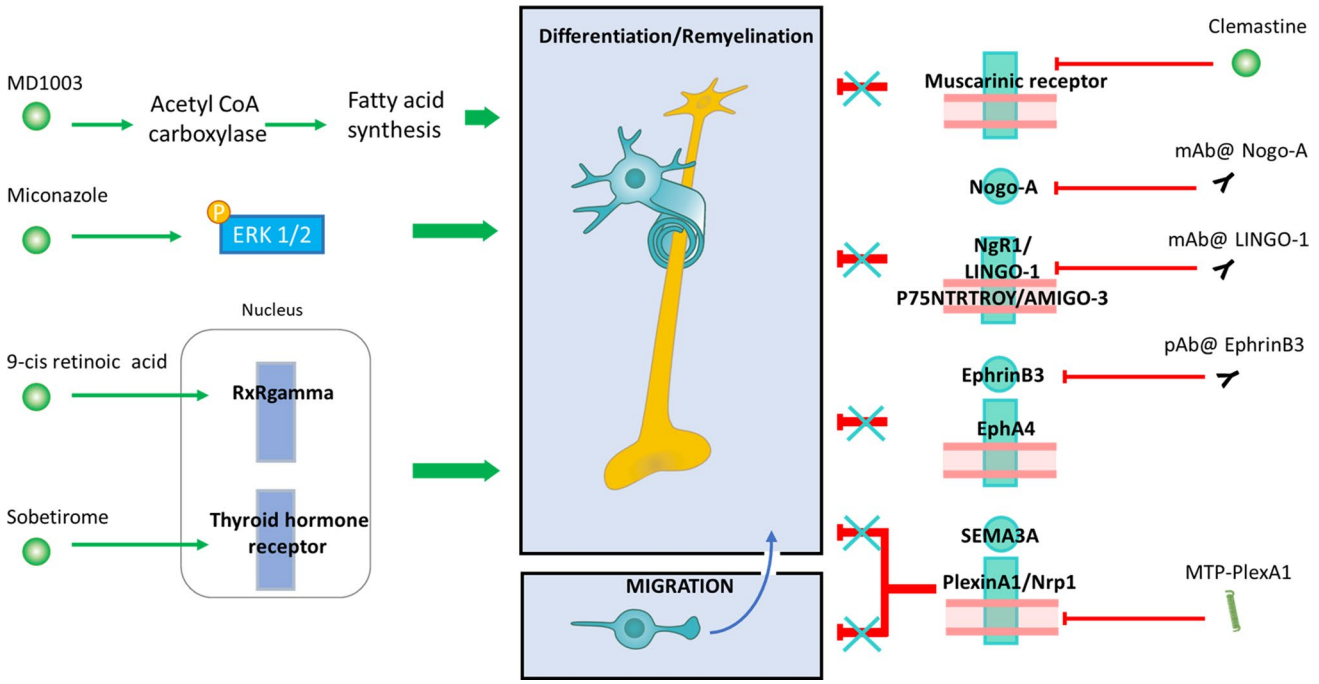

Fig. 4 Therapeutic strategies to promote remyelination. The left panel shows therapeutic strategies to activate OPC differentiation. MD1003 increases fatty acid synthesis trough acetyl CoA carboxylase activation. Miconazole drives ERK phosphorylation. 9-*Cis* retinoic acid and Sobetirome act on nuclear receptor Retinoic X receptor gamma and Thyroid hormone receptor, respectively. The right panel illustrates alternative strategies blocking inhibitory signals. Clemastine drives remyelination by antagonising muscarinic receptor activation. Monoclonal antibodies directed against Nogo-A or the receptor LINGO-1 cancel the inhibitory signalling. A polyclonal antibody directed against ephrinB3 prevents its association with EphA4. MTP-PlexA1 promotes both OPC differentiation and migration by blocking Sema3A-induced NRP1/Plexin-A1 dimerization

used for the inhibition of glioblastoma progression in PDX models [168] and is a validated approach for other receptors such as NRP1 [169–171] or ErbB2 [172]. The choice to target Plexin-A1 is further strengthened by the demonstration of overexpression in the oligodendrocytes of MS patients. This increase is concomitant with transient re-expression of Sema3A in demyelinated regions. The administration of MTP-PlexA1 peptide showed a positive effect on remyelination which results in a restoration of myelin sheaths and the disappearance of locomotor disorders in the cuprizone but also in the EAE demyelinating in vivo models. The observed effects are due to a disruption of the dimerization of NRP1 and Plexin-A1 which counters the anti-migratory and anti-differentiation effect of Sema3A as shown by in vitro and in vivo approaches using in particular the proximity ligation assay (PLA). The therapeutic benefit observed in mice does not appear to be related to modulation of inflammation but to a direct effect on repair capacity.

Thus, it would seem that alteration of the balance between remyelination promoter and inhibitor signals may offer an interesting avenue for the development of what could become, in combination with anti-inflammatory drugs, the beginning of curative therapeutic approaches for MS. However, the exact therapeutic window of anti-inhibitory molecules has to be correctly defined because RhoA activity appears involved in final steps of remyelination. Indeed, RhoA GEF Vav3 knockdown mice display impaired remyelination in lysolecithin and cuprizone demyelination models and produce thinner myelin sheaths [173]. This can be attributed to the necessary fine tuning of actin cytoskeleton during myelination or differentiation program regulation as shown in Schwann cells where RhoA allows differentiation by inhibiting JNK pathway [174]. Generally, it appears that signalling pathways promoted by growth factors and chemokines like Ras, PI3K and Rho GTPases are inversely regulated by repulsive molecules. These inhibitory molecules can, therefore, modulate signalling pathways induced around the lesion and contribute to behavioural transitions. After recruitment of new OPCs thanks to migration and proliferation, cells obviously need a dramatical change of signalling pathways to induce differentiation and produce the myelin sheaths. Repulsive molecules could play a role in this final step. However, we can argue that their pathological secretion in demyelinating pathologies reach a too high level and impairs all steps required for proper remyelination. The next challenge will be to demonstrate the clinical benefit of counteracting the inhibitory molecular barriers to promote remyelination.

Box 1: Negative loop between Rac and RhoA GTPases

Studies addressing specifically cell signalling in oligodendrocytes are still sparse. However, we can hypothesize that ubiquitous intrinsic regulation of RhoGTPases applies in oligodendrocytes. Cytoskeleton dynamics are under the control of RhoGTPases which cycle between an inactive GDP-bound form and an active GTP-bound form. Their activity is tightly regulated by the coordinated action of regulators: guanine nucleotide exchange factors (GEFs) improve GTP loading, GTPase-activating proteins (GAPs) promote GTPase inactivation by enhancing GTP hydrolysis and GDIs inhibit RhoGTPases by sequestering them in the cytoplasm [175]. Rac promotes actin polymerization at migration front while RhoA ensures contractility of actomyosin predominantly through its effector Rho-associated protein kinase (ROCK). The balance between these antagonistic activities is critical for coordination of cell motility.

Rac negatively regulates RhoA through the RhoGAP p190A. Active Rac induces ROS production which inactivates the tyrosine phosphatase LMW-PTP through direct oxidation of cysteines in the catalytic pocket, relieving the inhibition of p190 [176]. P190A GAP specificity for RhoA is enhanced by PKCα which dissociates p190A from plasma membrane acidic phospholipids and blocks its RacGAP activity [177]. Subcellular targeting of p190A controls its spatial regulation of RhoA [178]. Rnd1 [179], Src, FAK, p120-catenin [180] and cortactin [181] allow targeting of p190A to actin protrusions. The polarity complex proteins also contribute to inhibit RhoA at the migration front via Par6/aPKC dependent activation of p190A [182].

RhoA negatively regulates Rac through ROCK. Tiam1, a main Rac GEF, is recruited by the PAR complex proteins Par3 and Par6, resulting in Rac activation at the migration front [183, 184]. ROCK disrupts Par complex by phosphorylating Par3 and impairs Rac activation in the rear of the cell [185]. Other effects of ROCK have been found such as dissociation of the Rac GEF β-PIX from integrin-based adhesions [186] and Rac GAPs activation [187, 188].

It should be noted that interactions between Rac and Rho are more complex since another effector of RhoA, the formin mDia1, antagonizes ROCK effect by stimulating Rac through Src [189] and could drive RhoA activity found in initial events of membrane ruffling [190].

Box 2: The concept of membrane domain targeting peptides

Most of the current drugs target extra- or intracellular domains of membrane receptors to modulate downstream signalling. Alternatively, protein–protein interaction (PPI) inhibitors are conceived to alter the dimerization /oligomerization of receptors to inhibit their activity [191]. Membrane domain targeting peptides (MTP) are indeed a new class of small interfering peptides mimicking the transmembrane domains of receptors to act as decoy acting by direct competitive binding with the target domain leading to abnormal oligomerization of the receptor [192]. The consequence is a partial or total shutdown of downstream signals blocking the subsequent biological functions. This approach turns out to be a potent therapeutic strategy in the context of brain tumor or breast cancer and also recently finds an application in the context of Multiple Sclerosis. More than 20 years' research [193, 194] was needed to solve issues related to the high insolubility of MTPs and to demonstrate the specificity of the approach which is nowadays applicable to GPCR receptors [195–198].

MTPs are 20–30 amino acids long peptides.

MTPs are often containing a GxxxG motif or GAS motif defining the dimerization interface.

MTPs activity is in the range of 0.01–0.1 µM in vitro and µg/kg in vivo.

MTPs exhibit long-lasting biodistribution profiles markedly different from soluble peptides.

MTPs exhibit good tolerance profiles compatible with chronic administrations.

The strength of the strategy is the mechanism of action inducing a prolonged inhibition of receptors which was seen with a single dose up to 48 h in vitro and 72 h in vivo. Initially described as antagonist peptides blocking the dimerization interfaces, studies are now demonstrating a more profound impact by inducing conformational changes of extracellular domains of target receptors. The interference of the dimerization capacity is providing a way to attack both the activation of the receptor but also potential compensatory or redundant pathways when neutralizing the heterodimerization capability of receptors. None of these MTPs reached so far a clinical validation. A global effort is needed to promote the use of MTPs which should not stay at the level of bench tools.

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Declarations

Conflict of interests The authors declare no conflict of interest.

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