Nogo and its paRTNers

Thomas Oertle and Martin E. Schwab

Brain Research Institute, University of Zurich and Dept of Biology, ETH Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Reticulons (RTNs) are a relatively new eukaryotic gene family with unknown functions but broad expression and peculiar topological features. RTNs are widely distributed in plants, yeast and animals and are characterized by a ~200-amino-acid C-terminal domain, including two long hydrophobic sequences. Nogo/RTN4 can inhibit neurite growth from the cell surface via specific receptors, whereas more general, 'ancestral', RTN functions might relate to those of the endoplasmic reticulum – for example, intracellular trafficking, cell division and apoptosis. Here, we review the taxonomic distribution and tissue expression of RTNs, summarize recent discoveries about RTN localization and membrane topology, and discuss the possible functions of RTNs.

The Reticulon/Nogo family of proteins has attracted more and more interest during the past two years, although the functions of most members still remain largely elusive. Over a decade ago, RTN1 was the first member identified, as a cDNA (called C1–13) specific for neural tissue [1]. Shortly thereafter, it was found to be the antigen recognized by two lung-cancer monoclonal antibodies that are specific for small-cell lung carcinoma (SCLC) cell lines and neuroendocrine cells [2]. Owing to its neuroendocrine-specific expression, the gene was called *NSP* (for neuroendocrine-specific protein). Immunocytochemical studies revealed that the protein is a membrane-anchored component of the endoplasmic reticulum (ER), and it was renamed reticulon (RTN) [3].

Nine years later, >300 family members sharing homologies within the C-terminal region of ~200 amino acids, called the reticulon-homology domain (RHD), are known in a variety of organisms (T. Oertle, unpublished). One member, Nogo-A/RTN4-A, has recently been the focus of intense investigation because of its function as an inhibitor of neurite outgrowth and its involvement in restricting the plasticity of the central nervous system (CNS) [4–6]. Another member, Nogo-B/RTN4-B1 has been implicated in the induction of apoptosis, specifically in cancer cells [7]. Several reviews in the past three years have concentrated on Nogo and its possible clinical implications [8–10], but the rest of the RTN family has received little attention. Here, we highlight the shared features of the RTN family. Our purpose is to summarize the relatively few existing data about the RTN proteins, to project possible functions and hence to indicate new directions for future research.

Corresponding author: Martin E. Schwab (schwab@hifo.unizh.ch).

Taxonomic distribution and evolution of the RTN family

Genes for RTN-like proteins have been identified in most eukaryotic taxa and have evolved from an intron-rich ancestor (T. Oertle, unpublished). In prokaryotes, no homologues have been identified so far, suggesting that RTNs emerged relatively recently in eukaryotes, potentially in parallel with the evolution of the endomembrane system (T. Oertle, unpublished). There are four mammalian reticulon genes (*RTN1*, *RTN2*, *RTN3* and *Nogo/RTN4*), each of which can give rise to a range of alternative transcripts (Fig. 1). The four paralogues have a similar gene structure and probably arose during the putative genome-duplication events that occurred after the urochordata–craniata split. There is no homology between the N-terminal regions of paralogues, and the divergence of these segments compared with the C-terminal RHD within orthologues is significant (T. Oertle, unpublished). Nogo-A, which is

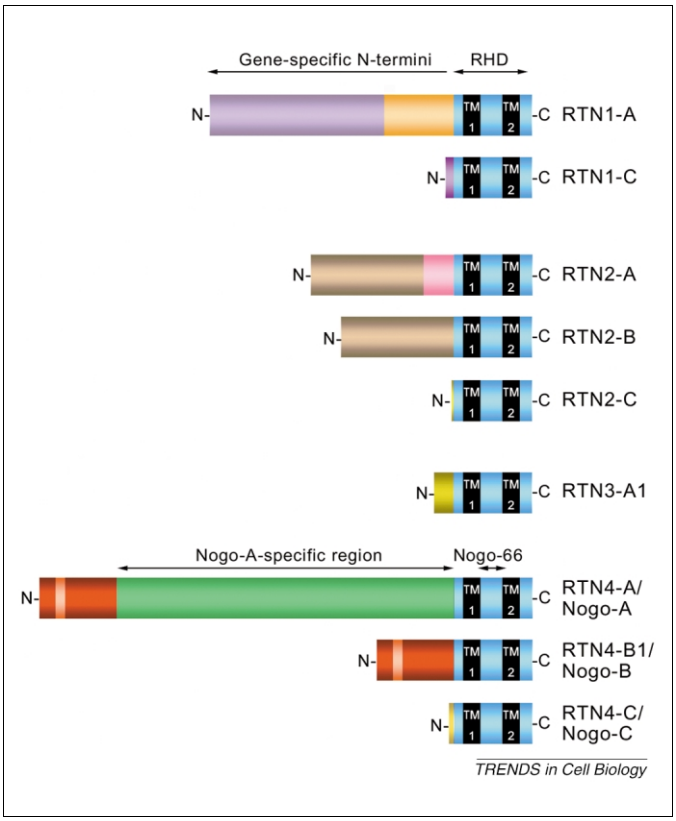

Fig. 1. The nine main transcripts from the four mammalian reticulon genes. The common C-terminus encodes the reticulon-homology domain (RHD), whereas the N-termini are specific for each paralogue and have no obvious sequence homologies to other proteins. The 66-amino-acid loop region between the two putative transmembrane (TM) domains (called Nogo-66) and stretches within the Nogo-A-specific N-terminal region have been found to inhibit neurite outgrowth.

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the largest of these RTN proteins, has sequences essential for neurite growth inhibition encoded by N-terminal exons that occur in amphibians but not in fish. They probably emerged during the transition from fish to land vertebrates (T. Oertle, unpublished).

Tissue expression of mammalian *RTN* genes

The four mammalian *RTN* genes have a broad tissue expression pattern (Table 1) Most transcripts are enriched in nervous tissues (*RTN1-A*, *RTN1-C*, *RTN2-A*, *RTN2-B*, *Nogo-A/RTN4-A*). The *RTN1* transcripts are almost exclusively expressed in neurons and neuroendocrine cells. *Nogo-A/RTN4-A* is expressed by oligodendrocytes, the myelin-forming cells of the adult CNS, and some neuronal subpopulations, heart and testis (Table 1). Two *RTN* transcripts (*RTN2-C*, *Nogo-C/RTN4-C*) are particularly enriched in skeletal muscle and both have E-boxes in their promoters to which muscle-specific transcription factors of the basic-helix–loop–helix family could bind [11,12]. By contrast, *RTN3* and *Nogo-B/RTN4-B1* have a widespread expression pattern (Table 1), as is the case for housekeeping genes. Their promoters lack a canonical TATA box and are GC rich [11,13]. Interestingly, the expression levels of both *RTN2* and *RTN3* seem to cycle in the suprachiasmatic nucleus, with a cycling phase of 22 h and 14 h, respectively [14].

There is evidence from expressed sequence tags that some *RTN* genes are expressed very early in development. It is currently unknown whether there are cells that do not express any *RTN* family members.

Subcellular localization

The shared feature of RTN proteins is their association with membranes of the endoplasmic reticulum (ER) [3] (Fig. 2b). This has been shown for mammalian RTN1 [3,15], RTN3 [16] and Nogo/RTN4 ([4,6,]; T. Oertle, unpublished) as well as for *Drosophila* Rtnl1 [17] and *Caenorhabditis* RTNL [18]. Because all RTNs lack a canonical leader peptide at their N-termini, translocation into the ER is assumed to be directed by internal signals (e.g. transmembrane domains). Alternatively, the ER association could be independent of signal-recognition particle (SRP) and thus occur post-translationally. Deletion of the second hydrophobic region, but not of the C-terminal dilysine ER-retention motif, of Nogo disrupts its ER association and is thus crucially involved in correct membrane association [19].

Some of the RTNs associate with cellular structures other than the ER. In myoblasts, RTN2 colocalizes with the intermediate filament desmin, whereas, in myotubes, it colocalizes with α-actinin and seems to be associated with muscular Z band [20]. Nogo-A localizes with Golgi markers in addition to the ER and is present in small amounts at the plasma membrane of oligodendrocytes and fibroblasts [6] (T. Oertle, unpublished).

The subcellular localization of RTNs might depend on their mRNA distribution. For example, rat *RTN1-C* mRNA is restricted to axonal poles of certain neurons and is enriched in synaptosomal fractions [21]. This compartmentalization is thought to be due to 'zipcodes' present in the 3′-untranslated region of *RTN1* mRNA [22]. Consistent with an enrichment of mRNA in synaptosomal fractions, RTN1-C and Nogo-A are concentrated in growth cones of neuroblastoma cell lines (e.g. SK-N-SH) and olfactory neurons, respectively [23,24]. It is tempting to speculate that this enrichment represents the association of RTNs with synaptic vesicles.

Membrane topology

The membrane topology of RTNs is of specific interest, particularly because the two very large (~35 amino acid) putative transmembrane domains could both span the membrane either once or twice (Fig. 2). Immunofluorescence studies have shown that, in the prevalent ER-associated topology, the N- and C-termini of RTNs face the cytoplasm ([6,16]; M. van der Haar, unpublished) (Fig. 2a). The 66-amino-acid loop between the two hydrophobic domains of Nogo-A cannot be detected by antibodies without prior permeabilization of the ER, suggesting that it assumes a lumenal orientation

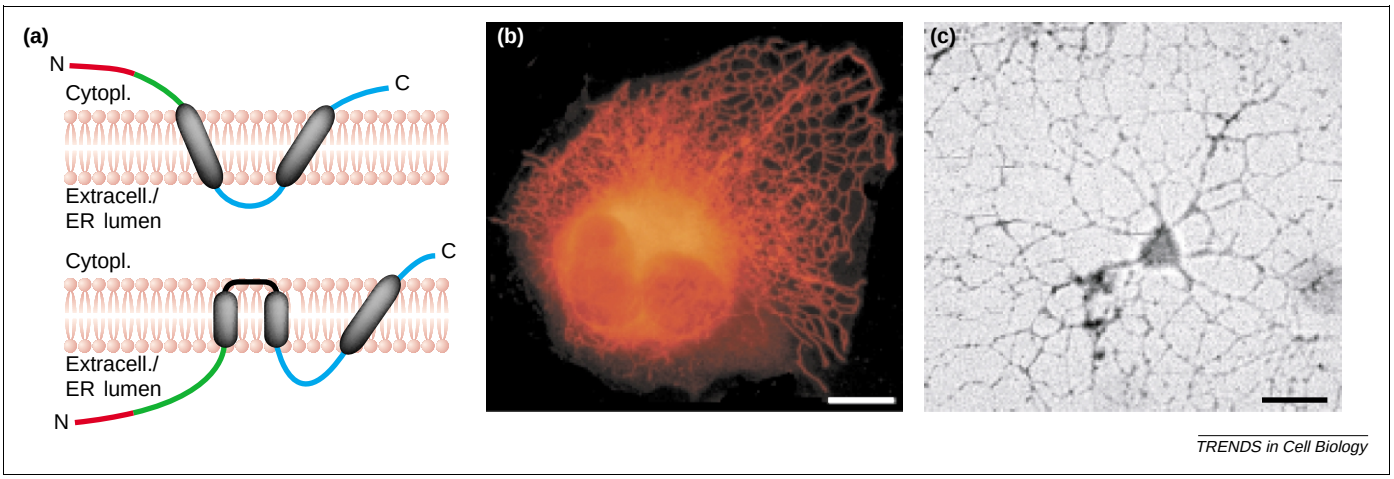

Fig. 2. Membrane topologies and subcellular localization of RTN1 and Nogo/RTN4. (a) Two proposed topologies for the C-terminal hydrophobic regions of RTNs. The lengths of the hydrophobic stretches (~35 amino acids) could allow them to span the membrane once or twice. Because deletion of the second putative TM domain leads to a partial cytoplasmic localization of the protein, it is possible that the first hydrophobic region does not cross the membrane. There are also other possible topological models (not shown). (b) Immunocytochemical localization of RTN1-A in the endoplasmic reticulum (ER) of a transfected COS cell. Scale bar, 4 μm. (c) Surface staining of endogenous Nogo-A on a cultured oligodendrocyte. Scale bar, 10 μm.

Table 1. Summary of published and unpublished data on the expression pattern of the main reticulon transcripts

Tissue/cell	RTN1-A	RTN1-C	RTN2-A/-B	RTN2-C	RTN3	RTN4-A	RTN4-B	RTN4-C	RTN4-D-G
Central nervous system	+ [65]	+ [1,65,72]	+ [12,65]	+/− [12,65]	+ [16,66,A,B]	+ [4,6,7,33,58,67, 68,71,A,D]	+ [4,6,7,33, 58,A,D]	+ [4,6,7,33, 58,70,A]	− [4,7,11, 58,69#,D]
Cerebral cortex	+ [22,23,62,63]	+ [1,22,23,62,63]	+ [12]	+/− [12]	+ [60,A,B]	+ [33,A]	+ [33*,A]	+ [A]; +/− [33]	
Olfactory cortex	+ [64]					+ [24,67]			
Entorhinal cortex	+ [62]				+ [60]				
Piriform cortex	+ [62]					+ [34]			
Hippocampus	+ [22,62]	+ [22]	+ [12]	− [12]	+ [60,66,A,B]	+ [33,34,68,A]	+ [33*]	+ [A]; +/− [33]	
Cerebellum	+ [22,23,62,63]	+ [22,23,62,63]	+ [12]	+/− [12]	+ [A,B]; − [60]	+ [33,67,68,A]	+ [33*]	+/− [33]	
Corpus callosum			+ [12]	− [12]		+ [24,33,68]	+ [33*]	− [33]	
Putamen	+ [62]	+ [1]	+ [12]	+/− [12]	+ [A]	+ [68]			
Amygdala			+ [12]	− [12]	+ [60,A]	+ [34,68,A]			
Nucleus caudatus			+ [12]	+/− [12]		+ [68]			
Thalamus			+ [12]	− [12]		+ [68]			
Substantia nigra	+ [62]		+ [12]	− [12]		+ [68]			
Brain stem	+ [22]	+ [22]			+ [A,B]				
Spinal cord			+ [12]	+/− [12]	+ [A,B]	+ [4,33,34,67,68, 71,D]	+ [4,33,34* *]	+ [4,33,34* *]	
Retina	+ [61]	+ [61]			+ [66,A]	+ [33,A]	+ [33*]	+ [33,73]	
Optic nerve						+ [4,6,24,33,D]	+ [4,6,33,D]	+ [4,33]; − [6]	
Peripheral nervous system	+/− [23]	+ [23]			+ [A]	+/− [4,24,33,68]; − [6,34]	+ [4,33*]; − [6,34* *]	− [4,33]	− [4,58]
Adrenal gland	+/− [23]; − [22]	+/− [23]; − [22]				+ [A]	+ [34* *]		
Lung	− [22]; +/− [23,65]	− [22,65,72]; +/− [23]	+/− [12,65]	+ [12]	+/− [16,66,A]	− [6,7,33,58,68]; + [71,A]	− [6]; + [7,33,58,D]	− [6,7,33, 58,70]; + [A]	− [7,58,69#]
Liver	− [22,23,65]	− [1,22,23,65,72]			+/− [16,66,A]	− [6,7,33,58,68,71]	− [6,7,58,D]; +/− [33]	− [6,33,70]; + [7,58]	− [7,58,69#]
Kidney	− [22,23]	− [1,22,23,72]			+/− [16,66,A]	− [6,7,33,58,68]; + [71,A]	− [6]; + [7,33,58,D]	− [6,70]; +[7,33,58]	− [7,58,69#]
Spleen	− [22]; + [65]	− [22,65,72]	+/− [12,65]	+/− [12,65]	+/− [16,66,A]	− [7,33,58,71]	+ [7,33,58,D]	− [7,33,58,70]	− [7,58]
Stomach	+/− [23]	− [23,72]			+/− [66]				
Small intestine	+/− [65]; − [23]	+/− [65]; − [23,72]	+ [12]	+ [12]	+/− [66]	− [7,58,71]	+ [7,58]	+/− [7,58]	− [7,58]
Colon	+/− [23,65]	+/− [65]; − [23]	− [12]; +/− [65]	+/− [12]	+ [A]	− [7,58]	+ [7,58]	+ [58]; +/− [7]	− [7,58]
Pancreas	+/− [23,65]	− [23,65]	− [12]	+/− [12]	+ [A]	− [7,58]; + [C]	+ [7,58,A]	− [58]; + [7,A]	− [7,58,69#]
Thymus	− [22,65]	− [22,65,72]	− [65]	+/− [65]	+ [A]	− [7,58]	+ [7,58,D]	− [7,58]	− [7,58]
Prostate	+/− [65]; − [23]	+/− [65], − [23]			+/− [66,A]	− [7,58]	+ [7,58]	+ [7,58,A]	− [7,58]
Testis	+/− [22,65]	− [22,72]	+/− [12]	− [12]	+/− [16,66,A]	+ [7,11,33,58,A]	+ [7,11,33,58,D]	− [7,33,58, 70]	+ [7,11,58,69#,D]
Ovary	− [23]; +/− [65]	− [23]	− [12,65]	+/− [12,65]	+ [A]	− [7,58]	+ [7,58]	− [7,58]	− [7,58]
Placenta			− [12]	+ [12,65]	+ [A]	− [7,58]	+ [7,58,A]	− [7,58]; + [A]	− [7,58,69#]
Mammary gland	− [23]	+/− [23]			+ [A]			+ [A]	
Cervix	− [23]	− [23]			+ [A]			+ [A]	
Skin	+/− [23]	− [23,72]			+ [A]		+ [A]	+ [A]	
Adipose tissue			− [12]	+ [12]			+ [A]	+ [70]	
Heart	− [22,23,65]	− [1,22,23,72]	− [12,65]	+ [12,65]	+/− [16,A]	− [6,7,68]; + [33,71,A,D]	− [6]; + [7,33,58]	− [6,7,33,58]; + [70]	− [7,58,69#]
Skeletal muscle	− [23]	− [23,72]		+ [12,65]		− [4,33,68]; +/− [7,24,58,67]	− [4,33]; + [7,58]	+ [4,7,33,58,70]	− [7,58,69#]
Smooth muscle	− [23]	+ [23]		+/− [12]			+ [A]		
Blood cells	+ [23,65]	− [23,65]	+/− [12,65]	+/− [65]	+ [A]	− [7,58]	+ [59* *]; − [7,58]	− [7,58]	− [7,58]

No expression detected is marked with ' − ', very low expression levels with ' +/− ' and clearly detectable signals with ' + '. The data were obtained by analysing reticulon mRNA (by *in situ* histochemistry, northern blotting, reverse-transcription polymerase chain reaction and microarray and expressed-sequence-tag databanks) and protein (western blot, immunohistochemistry). Notice that the expression of *RTN1* transcripts in peripheral tissues has been shown to be due mainly to the presence of peripheral neuroendocrine cells and *RTN1*-positive macrophages.
Abbreviations: *, RTN4-A/-B; * *, RTN4-A/B/C; #, RTN4-E.
A Genbank dbEST
B Gillerion, O *et al.*, unpublished.
C Pot, C. *et al.*, unpublished.
D Merkler, D. *et al.*, unpublished.

([6]; M. van der Haar, unpublished). However, antibodies against the 66-amino-acid loop region and antibodies against the N-terminus of Nogo-A can bind to the surface of differentiated oligodendrocytes in culture ([6]; M. van der Haar, unpublished), which suggests that, at least in these cells, Nogo-A can assume two membrane topologies (Fig. 2a,c).

Therefore, future studies are required to investigate: (a) whether the membrane translocation of RTNs is SRP dependent and, if so, which sequences function as insertion modules; (b) the three-dimensional structure of the transmembrane domains; (c) whether RTNs other than Nogo-A are localized to the Golgi and plasma membrane; and (d) which molecular mechanisms or

chaperones are responsible for the acquisition of the two proposed topologies.

RTNs as marker proteins and their roles in neuronal differentiation

RTN1-A and RTN1-C are both expressed in most neuroendocrine tumours, such as SCLCs, whereas they are absent in atypical carcinoids [25–27]. Only non-SCLCs showing a neuroendocrine immunophenotype also produce RTN1-A [28]. Thus, RTN1 is considered to be a highly sensitive and specific marker of neuroendocrine differentiation in lung cancer to be used in the diagnosis of this disease. Although it has been speculated that the presence of neuroendocrine markers could help to identify patients that are more likely to respond to chemotherapy, most clinical studies have shown that there are no biological markers that can reliably predict the response to chemotherapy in patients with non-SCLC [29].

RTN1-C mRNA is specifically upregulated upon spontaneous and induced neuronal differentiation [30,31]. It has also been proposed as a marker of neuronal differentiation that is reduced (together with Nogo-C) in temporal cortices of Down's-syndrome patients [32]. Nogo-A seems to be downregulated in some neurons and in skeletal muscle after differentiation [33,34]. De-differentiation of skeletal muscle cells (e.g. after denervation) leads to upregulation of Nogo-A and a concomitant downregulation of Nogo-C [35,36]. In line with this observation, the Nogo proteins have been recently proposed as molecular markers for the diagnosis of amyotrophic lateral sclerosis (ALS) and peripheral neuropathies in which changes in Nogo-A and Nogo-C expression levels have been observed [36].

Autoreactive antibodies against Nogo-A have been detected in sera of pilocytic astrocytoma patients [37] and of patients with multiple sclerosis and acute neurological disorders (M. Reindl *et al.*, pers. commun.). Therefore, the presence of anti-Nogo-A antibodies could potentially be used as marker for these diseases.

Possible functions of RTNs

ER function, plasma-membrane formation and cell division

Because RTNs are found in almost all eukaryotic cells and organisms, they would be expected to exert basic functions in the cellular machinery. Single *RTN* genes seem not to be of vital importance, however, because organisms with *RTN* gene disruptions (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Caenorhabditis elegans* and *Drosophila melanogaster*) are viable (e.g. [17,18,40]). On the basis of the (few) existing data, the following hypotheses for cellular functions of RTN proteins can be formulated.

(1) RTNs could function as pore or transporter complexes in the ER. RTN1-A and RTN1-B isoforms form supramolecular aggregates of ~500 kDa composed of homo- and heteropolymers [15], whereas RTN1-A and RTN1-C do not seem to co-polymerize [23] (Table 2). Because the domain involved in multimerization is likely to be the common C-terminus, especially the highly homologous RTNs (RTN1, RTN3 and RTN4) could also form homo- and heteromers if co-expressed together in the same cells. Their unusually long hydrophobic regions might form pore-like structures. The evolutionary conservation of RTNs is clustered to these hydrophobic regions, underlining their relevance (T. Oertle, unpublished). The interaction of (*S. cerevisiae*) RTN2 with SHO1 (Table 2) could also point to a function of RTNs related to osmosensitivity.

(2) RTNs could function in the transport of constituents from the ER to other membrane compartments such as the Golgi, endosomes, synaptic vesicles and the plasma membrane. In the case of *C. elegans* RTNL-C, its N-terminal transcript-specific region interacts with RME-1 [18] (Table 2), a protein localized to the endocytic recycling compartment and involved in the exit of proteins from the endocytic recycling compartment to the plasma membrane or to the *trans*-Golgi network [38]. This N-terminus has no homology to vertebrate RTNs but a stretch of the protein is homologous to the endosomal protein endofin. *S. cerevisiae* RTN2 interacts with SEC27 (Table 2), a protein involved in ER-to-Golgi protein trafficking [39]. The enrichment of RTNs in neuronal growth cones (structures particularly active in endo- and exocytic processes) supports this possibility. In addition, overexpression of *S. pombe rtn* leads to a reduced cell wall lipid content (~20%) and to cell lysis in the absence of sorbitol [40]. The multiplication of *RTN* genes in plants (T. Oertle, unpublished) could be associated with the increased need for specialized proteins involved in the formation and maintenance of the plant cell wall.

(3) RTNs might also play role in structural stabilization of the ER network, for example by linking the ER to the cytoskeleton via protein-protein interactions. Indeed, the proline-rich nature of the N-termini of RTN1, RTN2 and RTN4 with many potential SH3-domain-binding sites [11] suggests that RTNs could interact with a range of cytoskeletal and signalling molecules. In line with this prediction, Nogo-A has been shown to co-immunoprecipitate with α-tubulin and myelin basic protein (MBP) [41] (Table 2).

(4) Last but not least, RTNs could have a role in cell division. Overexpression of *S. pombe rtn* inhibits cell separation after division in the absence of thiamine [40]. During mitotic cell division, both *D. melanogaster* Rtnl1 [42] and mammalian Nogo-A [41] have been shown to be in a ring-like structure surrounding the spindle apparatus. RTNs might be responsible for the distribution of endomembranes to the two daughter cells during mitotic division.

Nogo and inhibition of neurite outgrowth and cell spreading

The main focus of present RTN research is on Nogo-A, a protein that has been purified and cloned as a potent inhibitor of neurite outgrowth, and is thought to be an important negative regulator of the regenerative and plastic capabilities of the adult central nervous system [4–6,43]. Its potential to inhibit nerve growth is shown by the fact that peripheral nerves regenerate well after a lesion, whereas ectopic expression of Nogo-A in Schwann cells leads to delayed recovery of the crushed sciatic nerve [44]. Two main regions of Nogo are likely to be responsible

Table 2. Intracellular proteins known to interact with members of the reticulon family

Reticulon	Interacting partner	Refs
(HUMAN) RTN1-A	(HUMAN) RTN1-A	[15]
(HUMAN) RTN1-A	(HUMAN) RTN1-B	[15]
(HUMAN) RTN1-C	Bcl-x_L	[58]
(HUMAN/RAT) RTN4-A	α-Tubulin	[41]
(RAT) RTN4-A	Myelin basic protein (MBP)	[41]
(HUMAN) RTN4-B	Bcl-2	[58]
(HUMAN) RTN4-B	Bcl-x_L	[58]
(HUMAN) RTN4	NIMP	[71]
(HUMAN) RTN4	UQCRC1, UQCRC2	[71]
(CAEEL) RTNL-C	RME-1	[18]
(YEAST) RTN1	YOR285w	[39]
(YEAST) RTN1	YDL089w	[39]
(YEAST) RTN1	HAT2 (YEL056w)	[73]
(YEAST) RTN2	INP54 [inositol (1,4,5)-trisphosphate 5-phosphatase]	[39]
(YEAST) RTN2	SHO1 (osmosensor)	[39]
(YEAST) RTN2	KAP104 (karyopherin)	[73]
(YEAST) RTN2	SEC27 (endoplasmic-reticulum-to-Golgi trafficking)	[73]
(YEAST) RTN2	YMR110c (aldehyde dehydrogenase III)	[39]

for its neurite-growth-inhibiting effect: the first region includes two stretches located in the N-terminal Nogo-A-specific region; the second region is the 66-amino acid loop-region between the two hydrophobic domains in the C-terminus (Nogo-66) [5,6,45] (T. Oertle, unpublished). Both regions induce the downstream activation of the small GTPase RhoA in responsive neurons [46] (Fig. 3).

Nogo-66, but not the corresponding loop region of the other three RTNs, induces neuronal growth-cone collapse [6] via binding to a glycosylphosphatidylinositol-linked receptor in the plasma membrane called NgR [45]. NgR is also a receptor for two other myelin-associated inhibitory proteins known as MAG [47,48] and OMgp [49]. A co-receptor of NgR, the so-called low-affinity neurotrophin receptor p75, might be involved in signal transduction [50,51]. Indeed, soluble p75, soluble NgR and a peptide antagonist of NgR neutralize the inhibitory activity of myelin *in vitro* [50,52,53] and induce axonal regeneration after spinal-cord injury *in vivo* 52]. This suggests that the NgR–p75 receptor complex has a pivotal role in mediating myelin-associated inhibition. Many neuronal signalling molecules (e.g. neurotrophic factors or the axonal guidance molecules netrin and semaphorins) have complex multi-subunit receptors that include more than one binding site (for example, the neurotrophin receptors p75 and trk, and the semaphorin receptors plexins and neuropilins). Nogo-A might follow the same rules (Fig. 3a). For the potent neurite-outgrowth and cell-spreading inhibitory site in the Nogo-A-specific part, binding to rat brain membranes and responsive cell lines (3T3 fibroblasts) can be shown biochemically (T. Oertle, unpublished). The Rho GTPase activation induced by Nogo-A fragments [46] argues for similar, convergent intracellular pathways as for Nogo-66/p75, or for common signal-transduction-receptor subunits for the different active sites of Nogo-A (Fig. 3a).

The predominant expression of Nogo-A in the myelin-forming cells of the CNS supports the inhibitory effects of CNS myelin on neurite outgrowth *in vitro* and the poor capacity of the adult, myelinated CNS for regenerative and plastic neurite growth following lesions *in vivo* [54,55]. Indeed, *in vivo* application of antibodies against the Nogo-A-specific region induced sprouting of Purkinje cells in the adult cerebellum [56] and improved functional recovery following cortical stroke lesions [57]. The possible functions (perhaps as a repulsive axonal guidance molecule) of Nogo-A in developing neurons remain to be studied. The same is true for Nogo-B and Nogo-C: Nogo-B has an almost ubiquitous tissue expression pattern and both proteins possess the Nogo-66 inhibitory region. Short Nogo isoforms as well as NgR also occur in the lower vertebrate CNS, where regeneration does occur (T. Oertle, unpublished; M. Klinger *et al.*, pers. commun.). In addition to a function as cell-surface signalling molecules, Nogo proteins–like the other RTNs–might also have intracellular roles in neuronal or non-neuronal cells.

Cancer and apoptosis

A recent study has suggested that Nogo-B might function as a pro-apoptotic protein. RTN1-C and Nogo-B interact with antiapoptotic members of the Bcl-2 family (Bcl-2, Bcl-x_L) [58]. When overproduced, Nogo-B and RTN1-C could sequester Bcl-2 and Bcl-x_L from mitochondria to the ER, thus reducing their antiapoptotic effect [58]. Strikingly, Nogo-B was also claimed to be a potent pro-apoptotic protein, specifically in certain cancer cells, when ectopically overproduced [7]. Transient transfection of Nogo-B into carcinoma cell lines (CGL4, SaOS-2) induced apoptosis, and this activity depended on the second hydrophobic region of the common RTN C-terminus [7]. However, these data contrast with other results, which show that neither spontaneous survival nor the extent of apoptosis induced by the chemotherapeutic drugs staurosporine or tunicamycin were enhanced in *Nogo-B*-transfected carcinoma cells (SaOS-2) or CHO cells. This thus questions the interpretation of Nogo-B as a cancer-specific pro-apoptotic protein [19].

Concluding remarks

Research in the field of RTNs has increasingly moved beyond descriptive studies of their expression patterns and genomic structures towards functional enquiries. The role of Nogo in neurite-outgrowth inhibition is currently being studied extensively. By contrast, the roles of RTN1, RTN2 and RTN3 in vertebrates, the possible intracellular role of Nogo/RTN4, and the function of the *RTN* genes in invertebrates, plants and yeast are poorly understood, and represent an exciting subject for future studies. The main questions that remain to be addressed include the exact membrane topology of RTN proteins, the identification of the putative receptor(s) on neuronal and non-neuronal cells transducing the inhibitory activity of the Nogo-A-specific region, the intracellular interaction partners of RTNs, and the physiological roles of *RTN* genes in vertebrate and non-vertebrate species. We are only starting to comprehend the cell biology of RTNs, but their broad taxonomic and tissue distributions are a promising sign for a mesmerizing journey.

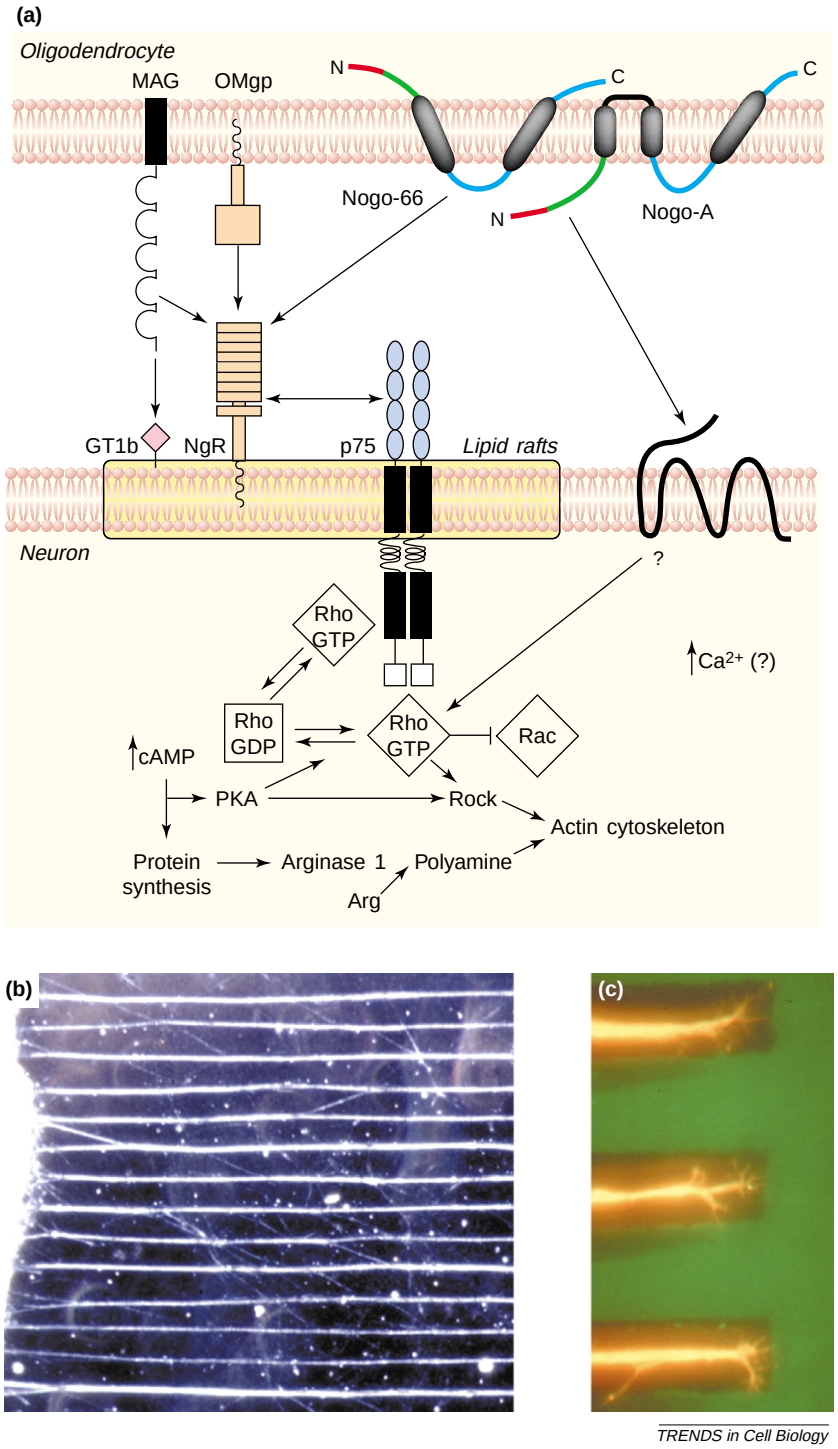

Fig. 3. Molecular basis of Nogo-A mediated neurite outgrowth inhibition. (a) Molecules that are currently known to be involved in the inhibition of neurite outgrowth by myelin-associated proteins. NgR is a common receptor subunit for Nogo-A via Nogo-66, MAG and OMgp, and is complexed with p75 as a probable signal-transduction unit. A concomitant activation of the small GTPase RhoA and inhibition of Rac, as well as an elevation of intracellular Ca^{2+}, are thought to be the main downstream signals of Nogo and other inhibitory proteins. RhoA might be activated by the guanine-exchange factor (GEF) activity of the mastoparan-like domain present in p75. Experimental elevation of intracellular cAMP, which leads to protein-kinase A (PKA) activation, arginase-I upregulation and the synthesis of neurite-growth-promoting polyamines, as well as blockade of RhoA GTPase (with C3 exoenzyme) or of the Rho kinase Rock (e.g. with Y-27632), can together abolish Nogo-mediated neurite outgrowth inhibition. (b) Nogo-A mediated inhibition of chicken retinal ganglion cell axon growth on alternating stripes of laminin and laminin–Nogo-A. Notice the stripe-specific, strongly fasciculated outgrowth of ganglion cell neurites. Only a small proportion of neurites cross the Nogo-A-containing stripes. (c) Detail of an immunofluorescently labelled sample as shown in (b). Nogo-A substrate is shown in green, whereas axons are labelled in red with an anti-neurofilament antibody. The growth cones stop in the 'dead-end street' formed by the Nogo-A containing territory.

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References

1 Wieczorek, D.F. and Hughes, S.R. (1991) Developmentally regulated cDNA expressed exclusively in neural tissue. *Brain Res. Mol. Brain Res.* 10, 33–41
2 Roebroek, A.J. *et al.* (1993) Cloning and expression of alternative transcripts of a novel neuroendocrine-specific gene and identification of its 135-kDa translational product. *J. Biol. Chem.* 268, 13439–13447
3 van de Velde, H.J.K. *et al.* (1994) NSP-encoded reticulons, neuroendocrine proteins of a novel gene family associated with membranes of the endoplasmic reticulum. *J. Cell Sci.* 107, 2403–2416
4 Chen, M.S. *et al.* (2000) Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen form monoclonal antibody IN-1. *Nature* 403, 434–439
5 Prinjha, R. *et al.* (2000) Inhibitor of neurite outgrowth in humans. *Nature* 403, 383–384
6 Grand-Pré, T. *et al.* (2000) Identification of the Nogo inhibitor of axon regeneration as a reticulon protein. *Nature* 403, 439–444
7 Li, Q. *et al.* (2001) Link of a new type of apoptosis-inducing gene *ASY/Nogo-B* to human cancer. *Oncogene* 20, 3929–3936
8 Huber, A.B. and Schwab, M.E. (2000) Nogo-A, a potent inhibitor of neurite outgrowth and regeneration. *Biol. Chem.* 381, 407–419
9 Brittis, P.A. and Flanagan, J.G. (2001) Nogo domains and a Nogo receptor: implications for axon regeneration. *Neuron* 30, 11–14
10 Ng, C.E. and Tang, B.L. (2002) Nogos and the Nogo-66 receptor: factors inhibiting CNS neuron regeneration. *J. Neurosci. Res.* 67, 559–565
11 Oertle, T. *et al.* (2003) Genomic structure and functional characterisation of the promoters of human and mouse Nogo/Rtn-4. *J. Mol. Biol.* 325, 299–323
12 Geisler, J.G. *et al.* (1998) Molecular cloning of a novel mouse gene with predominant muscle and neural expression. *Mamm. Genome* 9, 274–282
13 Roebroek, A.J. *et al.* (1996) Genomic organization of the human NSP gene, prototype of a novel gene family encoding reticulons. *Genomics* 32, 191–199
14 Panda, S. *et al.* (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109, 307–320
15 Senden, N.H. *et al.* (1994) Subcellular localization and supramolecular organization of neuroendocrine-specific protein B (NSP-B) in small cell lung cancer. *Eur. J. Cell Biol.* 65, 341–353
16 Hamada, N. *et al.* (2002) Molecular cloning and characterization of the mouse reticulon 3 cDNA. *Cell. Mol. Biol.* 48, 163–172
17 Morin, X. *et al.* (2001) A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 15050–15055
18 Iwahashi, J. *et al.* (2002) *Caenorhabditis elegans* reticulon interacts with RME-1 during embryogenesis. *Biochem. Biophys. Res. Commun.* 293, 698–704
19 Oertle, T. *et al*. Do cancer cells die because of Nogo-B? *Oncogene* (in press)
20 Geisler, J.G. *et al.* (1999) Nspl1, a new Z-band-associated protein. *J. Muscle Res. Cell Motil.* 20, 661–668
21 Baka, I.D. *et al.* (1996) Intracellular compartmentalization of two differentially spliced *s-rex*/NSP mRNAs in neurons. *Mol. Cell. Neurosci.* 7, 289–303
22 Ninkina, N.N. *et al.* (1997) Rat and chicken *s-rex*/NSP mRNA: nucleotide sequence of main transcripts and expression of splice variants in rat tissues. *Gene* 184, 205–210
23 Senden, N.H. *et al.* (1996) Neuroendocrine-specific protein C (NSP-C): subcellular localization and differential expression in relation to NSP-A. *Eur. J. Cell Biol.* 69, 197–213
24 Tozaki, H. *et al.* (2002) Expression of Nogo protein by growing axons in the developing nervous system. *Brain Res. Mol. Brain Res.* 104, 111–119
25 Senden, N.H. *et al.* (1994) Cluster-10 lung-cancer antibodies recognize NSPs, novel neuro-endocrine proteins associated with membranes of the endoplasmic reticulum. *Int. J. Cancer* 8 (Suppl.), 84–88
26 van de Velde, H.J.K. *et al.* (1994) NSP-encoded reticulons are neuroendocrine markers of a novel category in human lung cancer diagnosis. *Cancer Res.* 54, 4769–4776
27 Senden, N.H. *et al.* (1997) A comparison of NSP-reticulons with conventional neuroendocrine markers in immunophenotyping of lung cancers. *J. Pathol.* 182, 13–21
28 Senden, N. *et al.* (1997) Neuroendocrine-specific protein (NSP)-reticulons as independent markers for non-small cell lung cancer with neuroendocrine differentiation. An *in vitro* histochemical study. *Histochem. Cell Biol.* 108, 155–165
29 Gajra, A. *et al.* (2002) The predictive value of neuroendocrine markers and p53 for response to chemotherapy and survival in patients with advanced non-small cell lung cancer. *Lung Cancer* 36, 159–165
30 Hens, J. *et al.* (1998) Neuronal differentiation is accompanied by NSP-C expression. *Cell Tissue Res.* 292, 229–237
31 Li, Q. *et al.* (2002) Identification of transcripts expressed under functional differentiation in primary culture of cerebral cortical neurons. *Neurochem. Res.* 27, 147–154
32 Kim, S.H. *et al.* (2000) Neuroendocrine-specific protein C, a marker of neuronal differentiation, is reduced in brain of patients with Down syndrome and Alzheimer's disease. *Biochem. Biophys. Res. Commun.* 276, 329–334
33 Huber, A.B. *et al.* (2002) Patterns of *Nogo* mRNA and protein expression in the developing and adult rat and after CNS lesions. *J. Neurosci.* 22, 3553–3567
34 Josephson, A. *et al.* (2001) *Nogo* mRNA expression in adult and fetal human and rat nervous tissue and in weight drop injury. *Exp. Neurol.* 169, 319–328
35 Magnusson, C. *et al.* (2001) Differential expression of *Nogo* (*Reticulon 4*) transcripts in innervated and denervated mouse skeletal muscle. *Soc. Neurosci. Abstr.* 27, 1367
36 Dupuis, L. *et al.* (2002) Nogo provides a molecular marker for diagnosis of amyotrophic lateral sclerosis. *Neurobiol. Dis.* 10, 358–365
37 Michel, A. *et al.* (1999) A reticulon gene localized on chromosome 2 encodes a protein with increased autoimmunogenic properties in pilocytic astrocytoma patients. *Electron. J. Pathol. Histol.* 5, 993–997
38 Wendland, B. (2001) Round-trip ticket: recycling to the plasma membrane requires RME-1. *Nat. Cell Biol.* 3, E133–E135
39 Ito, T. *et al.* (2001) A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4569–4574
40 Godoy, C. *et al.* (1996) Characterization of *cwl1+*, a gene from *Schizosaccharomyces pombe* whose overexpression causes cell lysis. *Yeast* 12, 983–990
41 Taketomi, M. *et al.* (2002) Nogo-A expression in mature oligodendrocytes of rat spinal cord in association with specific molecules. *Neurosci. Lett.* 332, 37–40
42 Kaushik, R. *et al.* (2002) Characterisation of a novel gene CG8895 in *Drosophila melanogaster*. *Annu. Dros. Res. Conf.* 43, 186C
43 Spillmann, A.A. *et al.* (1998) Identification and characterization of a bovine neurite growth inhibitor (bNI-220). *J. Biol. Chem.* 273, 19283–19293
44 Pot, C. *et al.* (2002) Nogo-A expressed in Schwann cells impairs axonal regeneration after peripheral nerve injury. *J. Cell Biol.* 159, 29–35
45 Fournier, A.E. *et al.* (2001) Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature* 409, 341–346
46 Niederost, B. *et al.* (2002) Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. *J. Neurosci.* 22, 10368–10376
47 Domeniconi, M. *et al.* (2002) Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. *Neuron* 35, 283–290
48 Liu, B.P. *et al.* (2002) Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. *Science* 297, 1190–1193
49 Wang, K.C. *et al.* (2002) Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature* 417, 941–944
50 Wang, K.C. *et al.* (2002) p75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature* 420, 74–78
51 Yamashita, T. *et al.* (2002) The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. *J. Cell Biol.* 157, 565–570

52 Grand-Pré, T. *et al.* (2002) Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature* 417, 547–551
53 Fournier, A.E. *et al.* (2002) Truncated soluble Nogo receptor binds Nogo-66 and blocks inhibition of axon growth by myelin. *J. Neurosci.* 22, 8876–8883
54 Schwab, M.E. and Caroni, P. (1988) Oligodendrocytes and CNS myelin are nonpermissive substrates for neurite growth and fibroblast spreading *in vitro*. *J. Neurosci.* 8, 2381–2393
55 Bandtlow, C.E. (1993) Axonal regeneration in the adult vertebrate CNS. *Neuroscience* 5, 391–399
56 Buffo, A. *et al.* (2000) Application of neutralizing antibodies against NI-35/250 myelin-associated neurite growth inhibitory proteins to the adult rat cerebellum induces sprouting of uninjured Purkinje cell axons. *J. Neurosci.* 20, 2275–2286
57 Wiessner, C. *et al.* (2003) Anti Nogo-A antibody infusion 24 hours after experimental stroke improved behavioral outcome and corticospinal plasticity in normotensive and spontaneously hypertensive rats. *J. Cereb. Blood Flow Metab.* 23, 154–165
58 Tagami, S. *et al.* (2000) A novel protein, RTN-XS, interacts with both Bcl-x_L and Bcl-2 on endoplasmic reticulum and reduces their anti-apoptotic activity. *Oncogene* 19, 5736–5746
59 Zhang, Q.H. *et al.* (2000) Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in $CD34^+$ hematopoietic stem/progenitor cells. *Genome Res.* 10, 1546–1560
60 Pavlidis, P. and Noble, W.S. (2001) Analysis of strain and regional variation in gene expression in mouse brain. *Genome Biol.* 2, 00421–004215
61 Fujii, S. *et al.* (2000) Differential expression of neuroendocrine-specific protein in form-deprived chick eyes. *Invest. Ophthalmol. Vis. Sci.* 41, 1533–1541
62 van de Velde, H.J.K. *et al.* (1994) Molecular analysis of expression in rat brain of NSP-A, a novel neuroendocrine-specific protein of the endoplasmic reticulum. *Mol. Brain Res.* 23, 81–92
63 Dowling, A.L. *et al.* (2000) Acute changes in maternal thyroid hormone induce rapid and transient changes in gene expression in fetal rat brain. *J. Neurosci.* 20, 2255–2265
64 Hirata, T. *et al.* (2002) Mosaic development of the olfactory cortex with Pax6-dependent and -independent components. *Brain Res. Dev. Brain Res.* 136, 17–26
65 Roebroek, A.J. *et al.* (1998) cDNA cloning, genomic organization, and expression of the human RTN2 gene, a member of a gene family encoding reticulons. *Genomics* 51, 98–106
66 Moreira, E.F. *et al.* (1999) Cloning of a novel member of the reticulon gene family (RTN3): gene structure and chromosomal localization to 11q13. *Genomics* 58, 73–81
67 Liu, H. *et al.* (2002) Nogo-A expression in mouse central nervous system neurons. *Neurosci. Lett.* 328, 257–260
68 Wang, X. *et al.* (2002) Localization of Nogo-A and Nogo-66 receptor proteins at sites of axon-myelin and synaptic contact. *J. Neurosci.* 22, 5505–5515
69 Zhou, Z.M. *et al.* (2002) Expression of a novel reticulon-like gene in human testis. *Reproduction* 123, 227–234
70 Morris, N.J. *et al.* (1999) Cloning and characterization of a 22 kDa protein from rat adipocytes: a new member of the reticulon family. *Biochim. Biophys. Acta* 1450, 68–76
71 Hu, W.H. *et al.* (2002) Identification and characterization of a novel Nogo-interacting mitochondrial protein (NIMP). *J. Neurochem.* 81, 36–45
72 Yoshikawa, T. *et al.* (2000) Isolation of novel mouse genes differentially expressed in brain using cDNA microarray. *Biochem. Biophys. Res. Commun.* 275, 532–537
73 Ho, Y. *et al.* (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* 415, 180–183