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Review

Functions of reticulons in plants: What we can learn from animals and yeasts

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Abstract. Reticulons (RTNs) are membrane-spanning proteins sharing a typical domain named reticulon homology domain (RHD). RTN genes have been identified in all eukaryotic organisms examined so far, and the corresponding proteins have been found predominantly associated to the endoplasmic reticulum membranes. In animal and yeast, in which knowledge of the protein family is more advanced, RTNs are involved in numerous cellular processes such as apoptosis, cell division and intracellular trafficking. Up to now, a little attention has been paid to their plant counterparts, *i.e.*, RTNLBs. In this review, we summarize the data available for RTNLB proteins and, using the data obtained with animal and yeast models, several functions for RTNLBs in plant cells are proposed and discussed.

Keywords. Reticulon, membrane shaping, intracellular trafficking, endoplasmic reticulum, chloroplast-endoplasmic reticulon interaction.

Introduction

Reticulons (RTNs) are endoplasmic reticulum (ER)-localized proteins (Fig. 1A) that have recently attracted much attention. They are characterized by the presence of the RTN homology domain (Fig. 2) [1], a 188-amino acid protein domain consisting of two hydrophobic regions spaced by a 60–70-amino acid residue loop located at the C-terminal side.
The first known RTN protein, RNT1, was identified from a cDNA isolated from mammalian neural tissue [2]. Since this pioneer work, the RTN family has enlarged considerably and contains at present more than 300 members.

RTNs are ubiquitous proteins present in all eukaryotic organisms examined so far ranging for plants to humans, including yeasts. The nomenclature used to designate the RTN subfamilies was proposed by Oertel *et al.* [3]. The proteins of chordate taxa are called RTNs. RTN homologs from nonchordate taxa have been classified into six RTN-like (RTNL) subfamilies including the plant subfamily, termed RTNLB. Several aspects of RTNs such as cellular localization, topology and functions have been reviewed so far [1, 4, 5], but no review has been dedicated to plants.
Gene search across fully sequenced genomes revealed that the RTN gene family is more diverse in plants than in animals and yeasts. For instance, the genomes of *Arabidopsis thaliana* and *Oryza sativa* contain 21 and 17 genes, respectively, while yeast, human and zebra fish genomes host 2, 4 and 7 genes, respectively.

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The genomic structure of RTN genes in plant systems shares a common feature with their mammalian counterparts: it spans a region of considerable length and contains multiple large introns. Intron loss and gain over the courses of evolution have given rise to the large and diverse RTN genes of the different RTN subfamilies. In animal cells, the differential expression and/or the alternative splicing that occurs in the RTN gene family increase the protein number to 16–18 [3, 4]. Although this process has not yet been reported to occur in plant cells, the finding of different ESTs deriving from a single AtRTNLB gene [6] suggests such a possibility. Altogether, these features increase the number of protein members within a subfamily and may allow functional specificity for specific RTNs in plant cells.

The forty-one plant protein sequences from *A. thaliana* (20), *Glycine max* (1), *Hordeum vulgare* (1), *Solanum tuberosum* (1), *Oryza sativa* (17) and *Vitis vinifera* (6) found in databases are phylogenetically clustered in four groups (Fig. 3). Group I proteins, except Q306I3 (*H. vulgare*) and Os01g12650, comprise proteins with a N-terminal domain with 43–93 amino acid residues and a short C-terminal domain. Group II contains members with short C- and the shortest N-terminal domains. Group III contains only AtRTNLB19 and AtRTNLB20 that have the largest amino-terminal region and a short C-terminal domain. Finally, group IV is enriched in RTNs with long N- and C-terminal ends. The fact that *A. thaliana* and *O. sativa*, the most represented species in the tree in term of sequences, have protein members in each group, except group IV, suggests a similar evolution story of RTN genes. The presence of heterogeneous duplicates with *A. thaliana* and *O. sativa* sequences may indicate orthologous proteins and therefore suggests similarity of functions.

In this review, we summarize the data available on RTNs in plants. Using these data, and those available from other taxa, several additional possible functions for RTNs in plant cells are proposed and discussed.

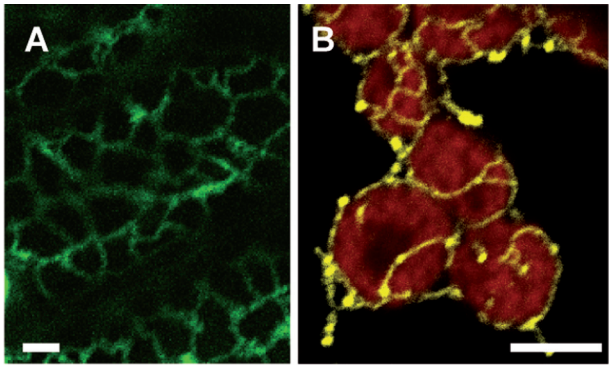

Figure 1. Subcellular localisation of AtRTNLB2 and AtRTNLB4 proteins in stably transformed *Arabidopsis thaliana* plants. (*A*) Tubular endoplasmic reticulum (ER) membranes labeled by AtRTNLB2-GFP in leaf epidermal cell. (*B*) AtRTNLB4-YFP protein labels the ER forming a basket-like structure around chloroplasts of leaf mesophyll protoplasts. Scale bars 5 μm.

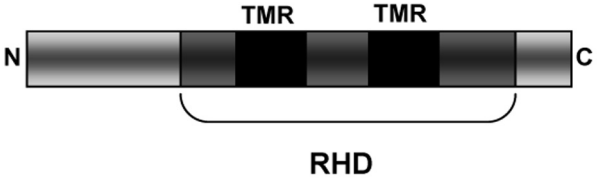

Figure 2. Scheme of the primary structure of the reticulon (RTN) protein family. Most of the RTN proteins have an RTN homology domain (RHD) of ~180 amino acid at the C terminus, consisting of two transmembrane regions (TMR). The flanking regions at the N and C termini are less conserved in length and amino content.

Structure of RTN proteins

The number of amino acids of RTN proteins ranges from 200 to 1200. All the members of the RTN family have a conserved region located at the C-terminal end of the protein and contain two hydrophobic regions flanking a hydrophilic loop (Fig. 2). This domain is called the RTN homology domain (RHD). Across phyla, the second hydrophobic region of the RHD is the most highly conserved, followed by the first hydrophobic region; the N terminus is the least conserved region in animals [4] and *A. thaliana* [6]. In the Pfam database, less than 1% of all metazoan RTN, 11% of RTNLB and 100% of the yeast RTN proteins display a C-terminal region [6]. Therefore, if RTN proteins contain one of several domains carrying cellular functions, it is likely that it is located at the N-terminal side of the protein. For instance, it can be deduced from the studies using genetic knockout of different regions and isoforms of RTN4 that the divergent N-terminal domains carry out species and cell-specific roles, whereas the RHD would have more basic cellular functions [1].

N- and C-terminal ends. In contrast to the highly conserved C terminus and the RHD, the N-terminal regions of RTNs display little or no sequence similarity in animals [3, 4] and *A. thaliana* [6], suggesting wide possibilities of interaction with other proteins or for anchoring or modulating enzymatic activity [3, 4]. For instance, AtRTNLB19 contains a 3β-hydroxysteroid dehydrogenase isomerase domain that functions *in vitro* as a 3β-hydroxysteroid dehydrogenase/C-4 decarboxylase activity when the RHD has been removed [7]. Beside the structured sequences, the C-

Table 1. The plant reticulon (RTNLB) protein subfamily.

Organism	Name	aa length	Motifs		Organism	Locus	aa length	Motifs	
			K(X)KXX	YXX∅				K(X)KXX	YXX∅
A . thaliana	AtRTNLB1[a,c]	275	+	4 (71^N)	O. sativa	Os01g04640	213	–	3 (60^R)
	AtRTNLB2 [a,b]	271	+	5 (67^N)		Os01g12650	279	+	3 (125^R)
	AtRTNLB3	255	+	3 (46^N)		Os01g49660	202	+	3 (36^R)
	AtRTNLB4 [a,b]	257	+	4 (50^N)		Os01g53520	253	+	5 (50^R)
	AtRTNLB5	255	+	4 (50^N)		Os01g63240	203	+	3 (58^R)
	AtRTNLB6 [c]	253	+	4 (50^N)		Os02g16620	230	+	3 (75^R)
	AtRTNLB7	242	–	3 (50^N)		Os02g19990	450	–	1 (336^R)
	AtRTNLB8	247	+	3 (100^R)		Os03g21840	231	–	2 (198^R)
	AtRTNLB9	225	+	3 (70^R)		Os03g56900	247	+	4 (92^R)
	AtRTNLB10	201	+	4 (45^R)		Os04g29920	213	–	7 (55^R)
	AtRTNLB11	200	+	2 (53^R)		Os04g57420	199	+	2 (135^R)
	AtRTNLB12	203	+	3 (55^R)		Os05g24770	251	+	–
	AtRTNLB13 [a]	206	+	–		Os05g45050	251	+	5 (48^N)
	AtRTNLB14	215	–	3 (23^N)		Os05g47690	220	+	3 (66^R)
	AtRTNLB15¤	179	–	4 (*)		Os06g30750	265	+	3 (111^R)
	AtRTNLB16	226	+	3 (23^R)		Os07g04910	246	+	4 (91^R)
	AtRTNLB17	431	–	1 (286^R)		Os08g17870	694	+	5 (65^R)
	AtRTNLB18	457	+	2 (238^R)					
	AtRTNLB19	564	+	6 (32^N)	*V. vinifera*	CAO68105	212	+	2 (57^R)
	AtRTNLB20	561	–	8 (69^N)		CAO65886	252	+	4 (48^N)
	AtRTNLB21	487	+	3 (272^R)		CAO42195	197	+	4 (46^R)
						CAO69968	248	+	5 (54^N)
G. max	Q9ZTZ3	212	+	3 (77^R)		CAO39687	255	+	4 (100^R)
						CAO49539	254	–	4 (50^R)
H. vulgare	Q306I3	204	+	3 (50^R)					
S. tuberosum	Q2PYX0	255	+	3 (51^R)					

RTNLB protein sequences were identified in *Arabidopsis thaliana*, *Glycine max*, *Hordeum vulgare*, *Oryza sativa* and *Vitis vinifera. A. thaliana* genes have been already reported [6], sequences for *G. max*, *H. vulgare*, *O. sativa* and *V. vinifera* were identified in databases. The symbol ¤ indicates that the NTR sequence of the putative protein is missing [6]. RTNLB localized at (a) the cell periphery [23], (b) endoplasmic reticulum [6, 15] and (c) plasma membrane [33]. aa length indicates the amino acid length of proteins. The dilysine sorting signal, K(X)KXX (X=any amino acid), is known to permit the ER localization of many membrane-bound proteins [12]. The tyrosine-based sorting signal, YXX∅ (Y=tyrosine residue, X=any amino acid and ∅=a bulky hydrophobic residue), is responsible for interaction with mu subunit of adaptor protein (AP) complex [61]. The number in parentheses indicates the position of the leading YXX∅ motif and, (N) and (R) indicated the motif localization at the N-terminal and RHD, respectively.

and/or N-terminal domains can be highly unstructured, possibly due to the presence of proline and alanine residues [8]. This property seems important to form multiprotein complexes [9], and possibly to carry out alternative functions [10] as they can fold upon binding to their partners [11]. The C-terminal tail of many RTNs has the canonical dilysine signal that is known to direct the ER localization of some transmembrane proteins (Table 1) [1, 6, 12–15]. Since some RTNLB proteins do not have the dilysine signal (Table 1), it would be interesting to know whether they still localize in ER. In animal cells, the C-terminal region of RTN proteins contains two large hydrophobic sequences that have been suggested to form calcium-permeable pores in the ER membrane [1, 13, 14].

RHD domain. The RHD consists of two hydrophobic regions, each of 28–39 amino acids long, which are thought to be membrane-embedded regions (see below). They are separated by a hydrophilic loop of 60–70 amino acids, and followed by a C-terminal tail of around 50 amino acids (Fig. 2). Despite the fact that much amino acid identity has been lost over the course of evolution, the overall structure of the RHD has been preserved from plants to yeast to mammals,

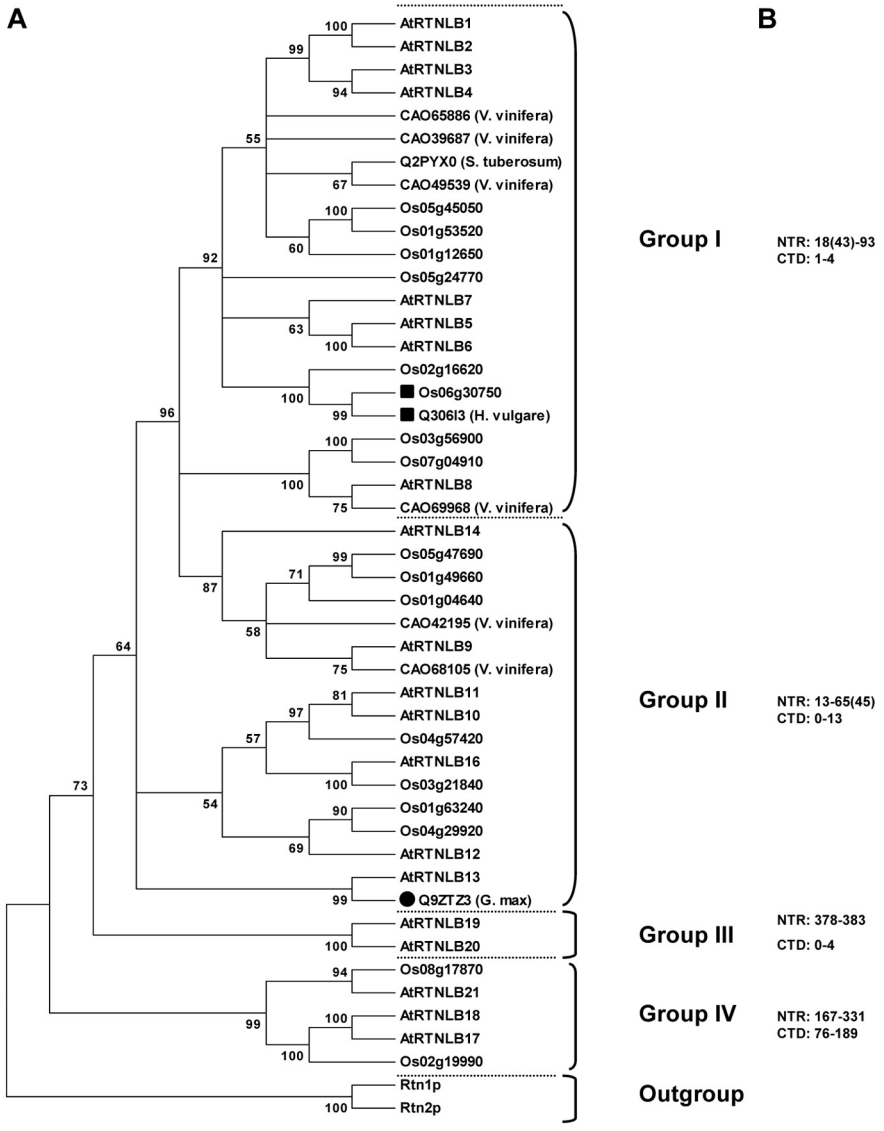

Figure 3. Phylogenetic relationships of plant RTNs. (*A*) The full sequences of the putative RTNLB proteins from *A. thaliana* (At), *Glycine max*, *Hordeum vulgare*, *Oryza sativa*, *Solanum tuberosum* and *Vitis vinifera* were identified in databases. Sequences from *A. thaliana*, *O. Sativa* and *V. vinifera* derived from genomic sequences, whereas those from *G. max*, *H. Vulgare* and *S. tuberosum* derived from cDNA sequences. The tree was done using MEGA 4.1 program by neighbor-joining method with 500 boostrap repetitions. *Saccharomyces cerevisiae* RTNs Rtn1p and Rtn2p were used as the outgroup. (*B*) Range of amino acid length of N-terminal region (NTR) and C-terminal domain (CTR) flanking the RHD. Numbers in parentheses indicate that the NTR length without Q306I3 (*H. vulgare*) and Os0630750 for group I (■) and Q9ZTZ3 (*G. max*) sequence for group II (●).

including humans. This strongly suggests that the 3-D structure of the proteins is of greater importance than individual residues for RHD function. In animal cells, the RHD loop region has been detected on the surface of cells and intracellularly. One key to the understanding of the structure-function relationship of RTN protein lies in the determination of the insertion mode of the protein in the membrane and the conformation adopted there. The RHD hydrophobic regions are unusually long for transmembrane domains: ~28–39 amino acids, whereas most transmembrane domains are about 22 amino acids in length. This particularity raises the question of the real topology of these regions within the membranes.

RTN conformation in the membrane. Taking into account the number of putative membrane-spanning segments lying within the two hydrophobic regions, and experimental results using antibodies against specific parts of the RTN protein, several topologies for the RHD hydrophobic regions in the membrane are possible. They vary from a horseshoe topology [3, 16, 17] to a ω topology, in which the α-helixes forms two successive hairpin structures separated by a short link (Fig. 4). Structures combining both possibilities have been also proposed [18–20]. Using prediction tools, Nziengui et al. [6] found that all *A. thaliana* RTNs would adopt either a combined or the ω topology, except AtRTNLB8 that would adopt the horseshoe configuration. These predictions have still to be confirmed experimentally. Lacking signal peptide, RTNs are typical for type I membrane proteins in which the N-terminal region is highly negatively charged [21]. With this type of proteins the first transmembrane segment of the RHD governs membrane integration and defines the protein topology.

Using directed mutagenesis, He et al. [19] demonstrated that cells expressing *rtn3* mutation at the region corresponding to the first transmembrane segment fails to insert the mutant proteins into membranes, and the produced proteins are eliminated. Mutation in the second transmembrane segment does not prevent RTN3 insertion but may affect the proper folding, which, in turn, has an impact on the interaction with protein partners [19]. Importantly, the RTN conformation might be different if plasma membrane or ER are used as host membranes [22]. Therefore, in mammalian cells, RTNs may adopt different topologies depending on the membrane in which they are inserted. This possibility might be important for carrying different functions such as the anchoring of an enzyme or imposing a curvature to the host membrane (see below). Like the mammal counterparts [19, 22], RTNLB proteins are able to bind to different kind of proteins. For instance, AtRTLNB1, AtRTLNB2 and AtRTLNB4 have been found to interact *in vitro* with each other, with AtRABE1a and with the *Agrobacterium* VirB2 [23]. Although, the importance of RTN protein-protein interactions still need to be investigated, it is obvious that the RTN conformation might be important for carrying different functions such as providing anchorage for an enzyme, imposing a curvature to the host membrane, T-DNA transfer, or signaling (see below). Key for protein organization is the involvement of the different cysteine residues in disulfide bonds. To our knowledge, there is only fragmented data on this aspect. For instance, the implication of the six cysteine residues in the folding of bacterial-overexpressed soluble large extramembrane domain located at the N terminus of human RTN4A has been studied using CD and NMR spectroscopies [8, 21]. The results showed that two cysteine residues (Cys699 and Cys912) out of the six, are not involved in a structurally disulfide bound. The interpretation of the CD spectra suggested the absence of an α-helix in this peptide, while the prediction software predicted a content of 23 % of α-helix [21]. The conclusions arising from such studies should, however, be taken with certain care; indeed, it could be possible that the conformation adopted by the polypeptide is different from that adopted in the whole protein, *i.e.*, when the conformation is constrained by the other protein part. The cysteine residues not engaged in disulfide bound could be the target of SH inhibitors that inhibit membrane network formation from vesicles *in vitro* [17].

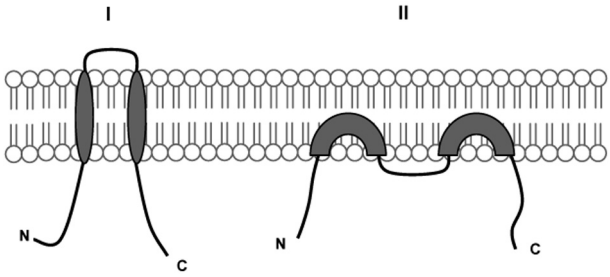

Figure 4. Possible insertion modes of RTNLB proteins. Two main insertion modes of hydrophobic regions of the RTNLB proteins have been described. The usual insertion mode with TMR spanning the membrane (I) [16], and the insertion in which the TMR does not span the membrane but forms hairpin-loop domains (II) [16, 17].

From protein conformation to membrane design or how RTNs shape membranes

Many RTN proteins share with some other proteins, such as caveolin-1 and reggie-1, the unusual property to adopt single or multiple hairpin-like topologies when inserted in the membrane (reviewed in [18, 20]). Similar to the proteins involved in vesicle budding, in which initial membrane curvature is generated by the action of cytosolic proteins such as endophilin and amphiphysin [24–26], RTN insertion in the membrane would cause curvature by inserting α-helix(es) constituting the RHD domain into the outer leaflet of the lipid bilayer [17]. RTNs, like caveolin-1 and reggie-1, are able to oligomerize through the soluble N-terminal domain [27]. By homology with the oligomerization model of caveolin-1, it was proposed that RTN oligomerization would be favored by protein phosphorylation and through particular lipid binding [18]. Because RTNs are localized in cell compartments presenting a high positive curvature, such as ER, it was hypothesized that this curvature is induced by a local concentration of several hairpin-like structure, inserted into the external membrane leaflet [18]. Consistent with this hypothesis, RTN overexpression allows the creation of more ER tubules. The direct implication of RTN proteins in shaping organelles with a high curvature has recently received considerable support. Using an *in vitro* approach, Hu et al. [28] demonstrated that the yeast RTN Rtn1p is, alone, able to induce tubule formation when mixed with lipids. A further step in the understanding of ER shaping was the discovery by Voeltz et al. [17] that the mammalian RTN RTN4A is required for ER tubule formation *in vitro*. Searching for the potential RTN partner in this process, Voeltz et al. [17] found that RTN4A is associated with DP1, another integral membrane protein, and a homolog of the yeast Yop1p, a Rtn1p-binding protein. Both DP1 and

Yop1p are able to induce tubule formation when mixed with lipids [28]. Thus, Rtn1p and RTN4A are involved in ER tubule formation. The high curvature adopted by the membrane is likely induced by the conjunction of several factors, *i.e.*, (i) a particular protein conformation such as single or multiple hairpins in the outer leaflet membrane, (ii) oligomerization, a likely energy-dependent process, and (iii) binding to protein partners such as DP1/Yop1p [17]. The oligomerization and binding of partner could generate an arc-like structure that may shape the membrane [28]. Using a model, these authors showed that the arc-like structure may be distributed randomly along a tubule. Such a distribution would favor arc assembly and disassembly processes, and would allow for ER dynamics. Interestingly, plant genomes, including *A. thaliana* (11 genes), *O. sativa* (15), *H. vulgare* (1) and *S. tuberosum* (1), contain putative homologs to *DP1/Yop1* genes. The plant proteins that are predicted to be the most closely homologs are 20–31 % identical to DP1/Yop1p; an average similar to the identity between DP1 and Yop1p (Fig. 5A). The conserved amino acid residues are mainly located within the TB2/DP1/HVA22 domain (Fig. 5B). The plant-derived proteins belong to the HVA22 abscisic acid (ABA)-induced protein family [29], which has been suggested to be regulatory proteins of ABA action. Overexpression of HVA22 proteins inhibits vacuolation in plant cells, and delays coalescence of protein storage vacuoles by inhibiting vesicular trafficking [30]. The finding of an ABA-induced DP1/Yop1p homolog in plant suggests a possible implication of RTN in stress response. This is in line with the macroarray analyses data, from the Genevestigator database [31], revealing that expression of plant RTN genes is regulated by ABA. The fact that RTN proteins were not found in membranes that are not highly curved, such as the ER membrane sheets, the ER-derived membranes or the outer nuclear envelope constitutes an additional argument in favor of the specific implication of RTN proteins in a biochemical mechanism aiming to confer high curvature of membranes.

The use of fluorescent-labeled RTNs clearly established that AtRTNLB2, AtRTNLB4 [6] and AtRTNLB13 [15] are localized in the ER tubules and not in the plasma membrane. The ER localization was confirmed by subcellular proteomic analyses [32]. Consistent with a role in ER shaping, the overexpression of *AtRTNLB13* gene remodels lumen of cortical ER [15]. Interestingly, AtRTNLB13 has one of the shortest N-terminal regions [6, 15]. In the case of AtRTNLB4, the localization in ER-cisternae and Golgi apparatus could be a consequence of overexpression. The exclusive localization of these RTNs in ER does not, however, exclude the possibility that RTNs could be present in other cell compartments. For instance, AtRTNLB1 and AtRTNLB6 have been found by proteomic analyses in a plasma membrane-enriched preparation [33].

RTNLBs mediate plant transformation by *Agrobacterium tumefaciens*

Three members of the RTNLB family from *A. thaliana*, RTNLB1, RTNLB2 and RTNLB4 (also called as BTI1, BTI2 and BTI3, respectively), have been identified as binding partners of VirB2, a pilin protein of *A. tumefaciens* [23]. Using genetic tools, Hwang and Gelvin [23] showed that the antisense and RNA interference plant lines of theses RTNLBs were less susceptible to being transformed by *Agrobacterium.* In contrast, transgenic lines overexpressing AtRTNLB1 were hypersusceptible to *Agrobacterium*-mediated transformation. Taken together, these data have demonstrated the involvement of AtRTNLB1, AtRTNLB2 and AtRTNLB4 in plant transformation by *A. tumefaciens.* The precise role of each RTNLB in this process remains to be elucidated. Such an event may involve an interaction with proteins of the cytoskeleton. In animal cells, the rat RTN4A is associated with α-tubulin [34].

RTNs and cellular trafficking: Proteins and other molecules

In mammalian cells, RTN proteins have been shown to be involved in intracellular transport [27, 35]. Many cellular processes involve cell compartments other than ER with high-curvature membrane. For instance, the vectorial protein transport between ER and the Golgi apparatus requires two types of vesicles. Coated protein II (COPII) vesicles are formed at the ER and travel through the anterograde pathway to the Golgi apparatus, while COPI vesicles that bud at the Golgi apparatus travel back to the ER through the retrograde pathway (reviewed in [36]). Many steps of the process are controlled by Rab proteins that are small GTP-binding proteins of the Ras superfamily, each controlling distinct aspects of protein sorting, vesicle formation and fusion process [37–40]. These GTP-binding proteins share common mode of action involving the recruitment of cytosolic protein complexes to localized domains on membrane surface [41, 42]. These proteins complexes are important for directed vesicle movement and target recognition. To do this, these proteins cycle between a GDP-bound inactive state and a GTP-bound membrane-associat-

(A)

Plant proteins and their homolog DP1	Yop1p (Yeast)	DP1 (Human)
AtHV22E	23%	25%
HVA22	27%	31%
Os11g30500	21%	23%
StHVA22	20%	21%
DP1	28%	-

(B)

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YOP1        LGFVGFYFILIFLNIGGIGQLLSNIAGLVIPGYYSLLALETPGKADDTQYLTYWVVFATLNVFEFWSKAILYWVPF 116
DP1         LGVI--GLVALYLVFGYGASLLCNLIGFGYPAYISIKAIESPNKEDDTQWLTYWVVYGVFSIAEFFSDIFLSWFPF 112
AtHVA22E    MT-------KLWTSLSALHSLAGPVVMLLYPLYASVIAIESPSKVDDEQWLAYWILYSFLTLSELILQSLLEWIPI  69
Os11g30500  MG-------KLWTILTHVHSLAGPTVMLLYPLYASVQAMESPSKLDDEQWLAYWILYSFITLVEMLLESLIYWIPI  69
HVA22       MG-------KSWALLTHLHSVAGPSITLLYPLYASVCAMESPSKVDDEQWLAYWILYSFITLLEMVAEPVLYWIPV  69
StHVA22     MGSD---NNVLAVIAKNIDVLALPLVSLVYPLYASIKAIETKSRADDRQWLTYWVLYSLITLFELSFSKLIEWFPI  73
Consensus   :                    :      :  * * *: *:*: .: ** *:*:**:::. :.: *:  . .: *.*.

YOP1        YYLFKTAFLLYIGLP-QYGGAELVYKAIVKPLAQKLVN----------------IQPHGGPSDSL 164
DP1         YYMLKCGFLLWCMAPSPSNGAELLYKRIIRPFELKHES----------------QMDSVVKDL 159
AtHVA22E    WYTAKLVFVAWLVLP-QFRGAAFIYNKVVREQFKKYG---------------------------- 105
Os11g30500  WYELKLLFIAWLALP-NFRGAAFIYNRFVREQLRKHGLAGAGAGAAASVGKKDKSSPSSSPKDK 132
HVA22       WYPVKLLFVAWLALP-QFKGASFIYDKVVREQLRKYR----------------------GRNR 109
StHVA22     WSYAKLGAICWLVLP-YFNGACYVYENFIRPFYRNPLVKIWYVPLKKDIFSKPDDVLTAAEKYI 136
Consensus   :   *   : :   *    **  :*. .::    :
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Figure 5. Plant protein sequences homologous to that of the human DP1 and the yeast Yop1p. The plant sequences were identified by data mining in protein databases. Only the most homologous protein sequences – AtHVA22E (*A. thaliana*, At5g50720), HVA22 (*H. vulgare*), Os11g30500 (*O. sativa*) and StHVA22 (*S. tuberosum*, A4UV42) – are displayed. (*A*) Table shows percent identity of plant proteins to DP1/Yop1p. (*B*) The multiple sequence alignments were performed with the ClustalW 2.0.8 software (http://www.ebi.ac.uk/Tools/clustalw2/index.html). In the alignment, the colors indicate the subsets of amino acids: red for small, hydrophobic residues and tyrosine, blue for acidic; magenta for hydroxyl, amine and basic residues. The numbers at the right side indicate the amino acid position. The boxes display the start and the end of the TB2/DP1/HVA22 domain (Pfam 03134). In the consensus sequence, the asterisk (*) indicates that the residues are conserved in all sequences, the double dots (:) means that the amino acid residues are conserved in the column and the single dot (.) mentions the semi-conserved substitutions.

ed active state [43, 44]. It is this GTP-bound state that makes specific interactions with the so-called effector complexes and thus promotes their recruitment to membrane. This activation-inactivation cycle is therefore under the control of GDP/GTP exchange factors, *i.e.*, GEF and GAP [37, 43]. RabGAPs are characterized by a conserved catalytic domain, the Tre2/Bub2/Cdc16 (TBC) domain [45–47] that promotes GTP hydrolysis [46, 48–50]. By searching which specific RabGAPs are of general importance for the anterograde transport in mammalian cells, Haas et al. [51] identified the TBC1D20 protein as a RabGAP for Rab1 and Rab2, two Rab proteins acting sequentially in tethering and fusion reactions at the Golgi membranes [52–56]. Rab1 is also involved in regulating the exit of secreted cargo from ER [57]. In human cells, Rtn1 is a partner of TBC1D20 and both are localized in the ER. Using mapping experiments, Haas et al. [51] found that Rtn1 interacts with TBC1D20 through the C-terminal fragment between amino acids 336 and 403. This fragment contains a single transmembrane domain and is localized in the ER [51]. So far the necessity of TBC1D20/RTN interaction remains unknown. Two nonexclusive roles could be proposed for RTN proteins. The RTN moiety could be either used to shape the COPII vesicles or used as "pilot" to drive TBC1D20 specialized ER domain where vesicle budding and/or cargo secretion occur(s). The findings that Rtn1-A and Rtn1-B interact with AP50 [58], a component of the adaptator protein complex AP-2 (which maintains the clathrin coat on the coated vesicles), and that RTN2B binds to proteins control-

ling the ER protein exit [59] are in favor of the first role. The amount of RTN proteins available in the ER seems important because the anterograde and retrograde transports are impaired when Rtn3 is overexpressed in mammalian cells [27].

In plants, a similar scheme of protein trafficking can be drawn (reviewed in [36]). Proteins belonging to the RabGAP family have been recently identified in plant genomes [60]. In *Nicotiana tabacum* leaves, the overexpression of AtRTLNB13 alters the ER morphology without major effect on the anterograde pathway [15]. Furthermore, most of the putative RTNLBs possess a tyrosine sorting signal (YXX$\varnothing$) at the N-terminal end (Table 1) that, like in animal systems, may serve as a potential link with the AP-2 complex [61] of clathrin-coated vesicles (CCVs), *e.g.*, through an interaction with the AP50. Interestingly, considering that AtRTNLB1, 2 and 4 are able to interact to VirB2 and AtRABE1a [23], which is a member of Ras family that regulates tubulovesicular trafficking [62–64], one can suggest that these three RTNLBs could mediate plant transformation by mediating the endocytosis event (of the T-DNA) *via* CCVs.

Plant cells can be distinguished from other eukaryotic types of cells by the presence of unique organelles such as plastids, which, according to the endosymbiotic theory, have derived from a cyanobacterial cell. During the course of evolution, parts of the bacterial genome that were originally encoded by the symbiont DNA were transferred to the nucleus DNA. In modern organisms, plastids are semiautonomous organelles that, to function, require the import of nuclear-encoded proteins. It has recently been shown that beside the canonical TIC/TOC machinery, a pathway using the endomembrane system can be used to import glycoproteins to plastids (reviewed in [65]). Chloroplasts are organelles in which different types of lipids are synthesized [66] and exchanged against the lipids synthesized in ER and/or the cytoplasm [67]. These lipids are transported to their target membranes in bulk form as vesicle constituents or as monomers at membrane contact sites. With target membranes outside the secretory pathway, lipid trafficking occurs exclusively *via* membrane contact sites (reviewed in [68]). Both protein import through a noncanonical pathway and lipid trafficking may require a close association between ER and plastids, a possibility quite well documented [6, 69–72]. Taking advantage of the fact that chloroplasts emit autofluorescence, Nziengui et al. [6] confirmed the close proximity between ER and chloroplasts in protoplasts isolated from *A. thaliana* leaves overexpressing the *AtRTLNB4-YFP* gene fusion (Fig. 1B). In fact, the ER network labeled by the RTNLB4-YFP protein formed a basket-like structure around the plastids. This close association was not observed in plants overexpressing the *AtRTLNB2-GFP* gene fusion [6]. These results suggest that AtRTLNB4, but not AtRTLNB2, may play a role in ER-chloroplast traffic, for instance in the formation of appositional contacts between plastid envelope and ER. These observations may constitute an additional argument in favor of the existence of functional ER domain [73]. Interestingly, RTN proteins have been shown to be involved in ER-mitochondria interactions through modulation of calcium concentration [74, 75].

Nuclear envelope growth

The nuclear envelope consists of two membranes connected at nuclear pore complexes (reviewed in [76, 77]). Although the outer membrane is continuous with the rough ER, RTN proteins are usually excluded from this cell region [6, 15, 76, 78]. During cell division, the nuclear envelope is dismantled and the membrane disperses into the cytyoplasm [79–82]. RTN4A plays an essential role in this process in *Caenorhabditis elegans* [83]. During telophase, the nuclear envelope is reassembled around the newly formed chromosomes through a multistage process involving vesicles and ER [81, 84–87]. From these materials, tubules and sheets are formed, which, in turn, are converted to a large flattened double sheet during nuclear envelope reassembly. At the prophase stage, the *Xenopus* oocytes contain extraneous ER-like membranes [88] resembling a string of vesicles. Using an immunogold-labeling approach, Kiseleva et al. [89] showed localization of RTN4A at the vesicles interconnections. The precise role(s) of the RTNs in these regions is so far not known, but one may postulate a structural role because intermembrane fusion implies transitory extreme curvature with stalk formation [90, 91]. When RTN4A is perturbed, the ER membranes are converted to large sacs, which can attach to the nuclear envelope but fail to contribute to nuclear envelope expansion [89].

In plants, the breakdown and reassembly of the nuclear envelope takes place according to a scenario similar to the one described with animal cells. Indeed, the nuclear envelope can form from cytosolic extracts of plant cell suspensions around demembranated *Xenopus* sperm chromatin [92, 93], and conversely, animal extracts are able to assemble a nuclear envelope structure around plant chromatin [82, 94]. Therefore, one may postulate that RTN will also intervene in the reassembly of the nuclear envelope in plant cells as they do in animal cells.

Conclusions and perspectives

In animal cells, RTNs have been proved to be implicated in many cellular and physiological processes such as programmed cell death [14, 95, 96], cellular traffic [27, 35, 97], inhibition of axon regeneration [21, 98–100] and regulation of atherosclerosis and Alzheimer's disease [19, 101–103]. The study on the functions of RTNs in plants is much less advanced, but possible roles can be envisioned from the data obtained with animal and yeast models.

The localization of some RTNs in plant cells is now clearly established, *i.e.*, in the tubules forming the ER [6, 15]. Quite convincing data on their structural role have been obtained with animal cells. On the basis of the homology between the cellular processes arising in animal and plant cells, one can infer that RTNs play crucial roles in the reassembly of the nuclear envelope during cell division and in cell traffic. Data confirming the implication of RTLNB proteins in this last process have been already published [15]. The understanding of the regulation of cell traffic mechanism is of particular importance not only for the academic science but also for applied science and daily life because, for instance, cell traffic is a major process involved in the filling of seeds that ultimately are used as food.

Some physiological processes occurring in animal cells involve the binding of RTNs to other proteins, such as those of the cytoskeleton (reviewed in [104]) and may also involve RTN receptor [105]. The hydrophilic loop of human RTN Nogo-66 binds to an axonal protein, the Nogo-66 receptor (NgR), a leucine-rich-repeat protein that mediates Nogo-66 inhibition of axonal regeneration after injury [106]. Nogo receptor gene homologs have been described in human and mouse [107]. A blast search for NgR protein homologs in plant databases reveals no candidate with a significant homology over the length of the sequence. Nevertheless, many candidates shared the leucine-rich-repeat domain characteristic of NgR. Fournier et al. [108] demonstrated that in the case of NgR, this domain is necessary and sufficient for binding to Nogo-66. It is, however, doubtful that every plant protein exhibiting a leucine-rich repeat binds the RHD domain of RTNLB proteins. Experiments on protein array technology would certainly be useful in the quest of a putative RHD domain receptor in plants. The absence of a significant plant homolog may also mean that the 3-D organization of the protein is more important than the individual amino acid residue homology. Thus, the study of RTN in plants will surely reveal crucial role for these proteins, especially from the physiological point of view. Considering the orchestrated movements of cellular membranes and the mobile interactions between the different cell compartments, analysis of the connections between RTNs and molecular motors will provide exciting data in a near future.

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