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Protein localization in the plant Golgi apparatus and the *trans*-Golgi network

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Abstract. This review presents plant-specific characteristics of the Golgi apparatus and discusses their impact on retention of membrane proteins in the Golgi or the *trans*-Golgi network (TGN). The plant Golgi consists of distinct stacks of cisternae that actively move throughout the cytoplasm. The Golgi apparatus is a very dynamic compartment and the site for maturation of N-linked glycans. It is also a factory for complex carbohydrates that are part of the cell wall. The TGN is believed to be the site from where vacuolar proteins are sorted by receptors towards each type of vacuole. To maintain the structure and specific features of the Golgi, resident proteins ought to be maintained in the proper Golgi cisternae or in the TGN. Two families of membrane proteins will be taken as examples for Golgi/TGN retention: (i) the enzymes involved in *N*-glycosylation processes and (ii) a vacuolar sorting receptor. Although the number of available plant proteins localized in Golgi/TGN is low, the basis of retention appears to be shared over all kingdoms and may result from pure retention and recycling mechanisms. In this review, we will summarize the characteristics of a plant Golgi and will discuss especially their consequences on on the study of this highly dynamic structure. We then choose membrane proteins with a single transmembrane domain to illustrate the signals and mechanisms involved in plants to localize and maintain proteins in the Golgi and the TGN.

Key words. Plant Golgi; TGN; glycosyltransferase; glycosidase; BP-80.

Characteristics of the plant Golgi

As in animal cells, the plant Golgi is made of distinct cisternae (six on average) with a typical *cis-trans* organization [1] as shown in figure 1 A. However, in contrast to the Golgi apparatus in animal cells, the plant Golgi appears smaller (fig. 1 B; compare its size with the animal Golgi presented in Ward and Brandizzi, in this issue). Another typical characteristic of plant Golgi is its mobility [2]. As a consequence, intermediate compartments at the *cis*- and *trans*-faces of Golgi, if they exist, should be far less developed. The plant Golgi is the site of protein N-glycan processing as for its animal counterpart, but it also generates unique types of complex glycans [3]. Finally, plant Golgi is implicated in synthesizing part of the cell wall that surrounds each cell.

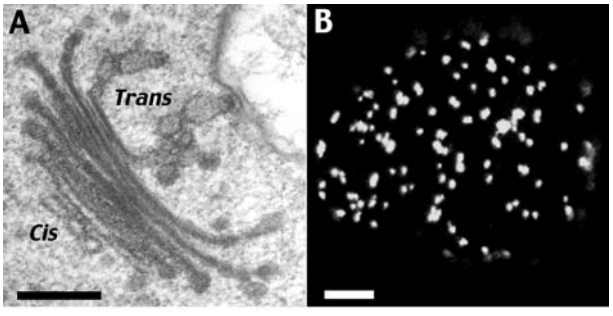

Figure 1. Golgi apparatus in plant cells expressing a Golgi-localised fusion protein made of a portion of the rat sialyl-transferase to the GFP. (*A*) Using freeze-substitution EM on root tips from 2-week-old tobacco plantlets, a Golgi stack appears subdivided in cisternae from *cis* (left) to *trans* (right). At the *trans*-face, a network-like structure can occasionally be seen that resembles an expected *trans*-Golgi network. This image, kindly provided by Dr Ulla Neumann, was obtained using high-pressure freezing. (*B*) Using confocal microscopy on tobacco BY2 suspension culture cells, Golgi stacks appear as 1-µm structures with independent mobility and occasionally aggregate in specific areas of the cortical region of the cell. Scale bar, 100 nm (*A*) or 10 µm (*B*).

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Plant Golgi is fragmented

The number of dictyosomes per cell is extremely variable. For example, small meristematic cells contain 20 stacks, while root tip cells have several hundred and giant cells such as cotton fibres have over 10,000 stacks [1]. Recently, Golgi fragmentation has been largely documented using a reporter, the green fluorescent protein (GFP), fused to various Golgi resident proteins (fig. 1B). So far, what regulates the number of Golgi stacks per cell is unknown, but presumably this number could reflect the secretion activity. In tobacco Bright Yellow (BY-2) suspension cultured cells, up to thousands of Golgi stacks can be seen per cell when expressing GFP fused to the *Glycine max* alpha1, 2 mannosidase I (GmMan I-GFP), and their number may also vary together with the volume of the cell [4]. It is possible that the large amount of membrane proteins to be stored in the Golgi may also increase the number of stacks. As an illustration of membrane flexibility, plant cells overexpressing a plasma membrane-localized GFP chimera often show extra material as punctate structures internal to the membrane [5]. We proposed that these fluorescent spots correspond to extra membrane made by the cell to store the highly expressed GFP marker [5]. Even multi-vesiculated compartments associated with the plasma membrane were occasionally visualised [N. Paris, unpublished results]. This means that cells are able to increase the amount of a given membrane to store extra material, which would result in an increase of stacks in the case of Golgi localized proteins. In fact, the amount of GFP-labelled Golgi stacks that are seen with a confocal microscope in tobacco leaf epidermal cells is surprisingly high compared with the difficulty to find a Golgi stack in untransformed tissue by electron microscopy (EM). The hypothesis that new stacks are being formed to store Golgi resident proteins could easily be tested at the EM level by comparing the frequency of Golgi between transformed and nontransformed cells.

More than 20 years ago, it was observed that the number of Golgi stacks doubles prior to mitosis in onion root tip cells [6], but how the extra Golgi material is produced during cytokinesis is still a matter of debate. Three main models are described in systems other than plants [7]: (i) the 'disintegration and reassembly' model used by most animal Golgi; (ii) the 'fission' model used in Algae and Protozoa and (iii) the 'de novo' construction model used by *Pichia pastoris*. Since plant Golgi is already fragmented into stacks, there may be necessity for disintegration as in animal cells. Golgi stack distribution was studied in BY-2 cells expressing the fusion protein GmMan I-GFP [8]. Stacks were found next to the phragmoplast, presumably to build the new cell wall. This repartition on each side of the phragmoplast also equilibrates the number of stacks in the two daughter cells and requires microtubules to be maintained. Using the same material, these authors also attempted to document the doubling of stacks prior to cytokinesis. Unfortunately, given the fact that the stacks are mobile and can easily be more than a thousand per cell, the approach appeared unworkable. Many research groups are using GFP fusion proteins to label Golgi, but so far no one has ever described stacks undergoing fission. The de novo model, which postulates that new Golgi stacks would form from the ER, was recently detailed using four-dimensional (4D) confocal microscopy in *Pichia* expressing fluorescent markers to the Golgi and the transitional endoplasmic reticulum (ER); the specific site where the coatemer protein II (COPII) complex forms [9]. We do not know whether this de novo model applies to plants, but in our view it is the most probable model to generate new stacks prior to mitosis.

Plant Golgi mobility

The most striking property of plant Golgi is its mobility. The use of GFP targeted to various compartments of the secretory pathway revolutionised our view of the plant cell and brought a new dynamic dimension to it [10]. For example, Boevink et al. studied tobacco leaf epidermal cells transiently expressing a fusion of a rat sialyltransferase transmembrane domain to GFP [2]. The fluorescent stacks travel along actin filaments, with which the ER is associated, with speeds varying from 0 to 0.76 µm/s in cortical areas of the cell to 2.2 µm/s in cytoplasmic strands [2]. In BY-2 suspension cultured cells, the typical saltatory movement of fluorescent Golgi was further described by Staehelin's group [4] who showed that the stop phase is fairly short and often occurs in specific areas of the ER; possible ER export sites. It is hard to imagine that the Golgi-transport vesicles form and bud in less than a second. Therefore, it is suggested that the exchange of material between ER and Golgi may occur mostly, if not exclusively, during pauses in Golgi movement. Plant Golgi stacks depend on actin for movement and accumulate in specific areas of the ER when actin is disrupted using cytochalasin treatment. Interestingly, even when the actin cytoskeleton is destroyed, transport between the ER and the Golgi is maintained [11]. These results indicate that although plant and animal cells contain the same actin and tubulin cytoskeleton, their use in secretory pathway transport is drastically different.

As a consequence of Golgi saltatory movement, a plant equivalent of the animal *cis*-Golgi network which reflects protein transport between the ER and the Golgi may be detectable only while Golgi stacks are not mobile. What is known of the *cis* Golgi network will be further developed elsewhere in this multi-author review [T. Y. Ward and F. Brandizzi, this issue]. Interestingly, a network typical of what we would expect for a plant TGN is not always seen at the *trans*-face of the Golgi (fig. 1A) when using optimal fixation procedures such as high-pressure

freezing (HPF). This may also indicate that post-Golgi transport is not continuous. When less preservative treatments are chosen to increase epitope stability for further immunolabelling, a strict morphological-based identification of the TGN is no longer possible. Instead, we often assume that a given membrane structure is the TGN because of its proximity to the *trans*-face of the Golgi. Given the rarity of classical TGN seen using HPF electron microscopy and the high mobility of Golgi, this is probably a risky assumption.

Plant Golgi and vacuolar sorting

The TGN is defined in animal cells as a sorting station for secreted versus lysosomal proteins. Lysosomal enzymes are sorted at the level of the TGN through their interaction with the mannose-6-phosphate receptor and are packed into clathrin-coated vesicles that are transported to the late endosome [12]. In plants, the sorting events are even more complex [13] since two vacuolar destinations can coexist in the same cell [14], a lytic compartment and a storage vacuole as shown on figure 2. It is yet unclear whether most cells are able to generate both types of vacuoles and whether these are maintained as separate entities, as in developing cotyledons. Each type of vacuole contains its own specific pool of soluble proteins that is transported independently from the TGN, via specific shuttle vesicles [15] and specific receptors. A vacuolar targeting signal, most often carried within the propeptide of soluble vacuolar proteins, is recognized within the lumen of the secretory pathway by its specific receptor [16]. The interaction ligand-receptor is then believed to trigger formation of a transport vesicle that buds off the TGN and that is further transported through the cell. The type of shuttle vesicle differs depending on the vacuolar route. Clathrin-coated vesicles transport proteins to the lytic vacuole and contain the specific receptor peabp80 (for binding protein of 80-kDa [17, 18]). These vesicles are made of a layer of adaptor complex surrounded by a cage of clathrin [19]. The original receptor was cloned from pea and sorts soluble proteins such as aleurain to the lytic vacuole [20]. Dense vesicles contain storage proteins such as prolegumin but not peabp80, and mediate transport to the storage vacuole [15]. The ReMember-RingH2 membrane protein (RMR) would be involved in the route to the storage vacuole [N. Paris, unpublished results and 21]. While the lytic pathway starts at the TGN, dense vesicles could often be seen earlier in the *cis*-Golgi cisternae [22] meaning that the storage route may bypass the TGN.

So far, the only functional evidence for the existence of a sorting TGN therefore comes from the bp80 family of vacuolar sorting receptors as exemplified by peabp80. At the ultrastructural level, the receptor was found in regions budding off the *trans*-face of the Golgi, the TGN [23]. Similarly to the mannose-6-phosphate receptor (MPR46), peabp80 constantly cycles between the TGN and the prevacuole, where it releases its ligand [23]. Unfortunately, since the steady-state location of bp80 family is preferentially the prevacuole [24], anti-bp80 antibodies do not represent an ideal tool to specifically identify the plant TGN.

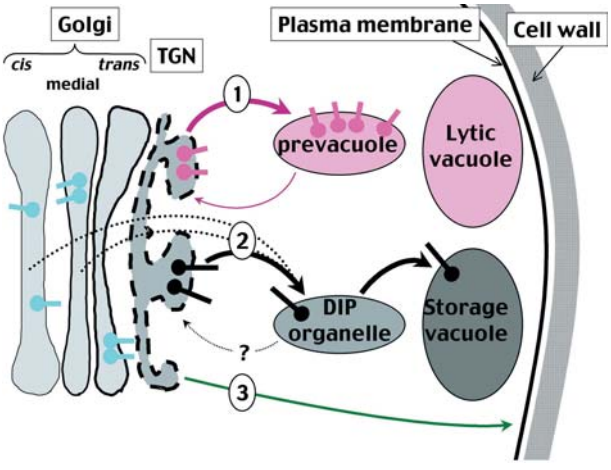

Figure 2. Schematic representation of a Golgi stack and post-Golgi destinations in plant cells. The Golgi apparatus is divided into subcompartments each with different subsets of glycosyltransferases (blue). From the TGN, three pathways are believed to lead to either one of the two vacuolar compartments or to the plasma membrane. The best-described vacuolar pathway (1) involves the receptor BP-80 (pink) and leads to the lytic vacuole through a prevacuolar intermediate. The least-described route (2) may involve specific receptors such as Re Member H2 (RMR) and will lead to a storage compartment via a possible intermediate organelle accumulating dark intrinsic protein (DIP). Finally the secretion route (3) is believed to occur by default for soluble proteins.

Plant N-linked glycans

The Golgi apparatus is the site of maturation of the *N*-glycans born by numerous cargo proteins (recently reviewed in [3, 25]). Among the large number of Golgi enzymes involved in these events, we can distinguish the glycosidases from the glycosyltransferases that are respectively either trimming or adding various sugars to the oligosaccharide precursor. The latter is attached to the protein at the ER level and is initially composed of *N*-acetylglucosamine, mannose and glucose residues, more precisely $GlcNAc_2Man_9Glc_3$. Following the removal of the three terminal glucoses and of one or two mannoses, the glycoprotein is then transported to the Golgi apparatus, where the oligosaccharide side chain is further processed to produce a high variety of oligosaccharide structures from high mannose to complex glycans. Plant complex N-glycans differ from their animal equivalents as they bear β1,2-xylose and α1,3-fucose residues that can be both allergenic and immunogenic in humans

[26–28]. Plant cells can also decorate Golgi and plasma membrane proteins with complex glycans bearing the Lewis^a epitope which contains β1,3-galactose and α1,4-fucose [29].

Most available cloned glycan-processing enzymes come from the animal kingdom, and to date, only 14 plant equivalents have been cloned and characterised. The first Golgi enzymes to be involved in N-glycan maturation are the glycosidases, more precisely α-mannosidase I and II. The soybean GmMan I is the only Golgi glycosidase cloned so far in plants [4]. The second group of enzymes involved in glycan maturation are the glycosyltransferases (for reviews, see [30, 31]). *N*-acetylglucosaminyltransferases I and II (GNT I and GNT II) successively transfer *N*-acetylglucosamine residues to the oligo-mannosyl acceptor $Man_5GlcNAc_2$-Asn. Recently, NAGTI genes were isolated from tobacco [32] potato [33] and *Arabidopsis thaliana* [34], as well as the NAGTII from *A. thaliana* [35].

Among the plant specific enzymes, two α1,3-fucosyltransferase have been cloned, one from mung beans [36] and another from *A. thaliana* [37]. *A* β1,2-xylosyl transferase was cloned from *A. thaliana* [38, 39]. To form a Lewis^a epitope the Golgi requires specific enzymes: (i) the β1,3-galactosyltransferase that has not yet been cloned in plants and (ii) α1,4-fucosyltransferase [40, 41]. Interestingly, this enzyme does not share any sequence similarity with other fucosyltransferases already characterised [41]. Importantly, since cell wall formation also requires galactosyltransferases, it is unclear which of the potential candidates identified by sequence homology with animal equivalents are indeed involved in the maturation of N-glycans in plants.

Subcompartmentation of Golgi enzymes involved in N-glycan maturation

Since N-glycan maturation ought to be performed in a given order and N-glycans are modified while the cargo proteins are transiting from the *cis*- through the *trans*-Golgi, compartmentation of the glycosylation enzymes mirrors the ordered glycan maturation [42, 43]. However, intra-Golgi segregation of enzymes involved in glycan processing often spread over two or more cisternae in a gradient, which itself can vary between cell types for a given enzyme [43]. This wide distribution of enzymes can result from an imprecise segregation in Golgi but more likely reflects intra-Golgi shuttling for enzyme recycling [11].

Since immunodetection of native N-glycan-maturing enzymes is often a challenge due to the low level of available epitopes, sub-Golgi-related compartmentalisation was originally provided by data dealing with immunolocalisation of their products. For example, it has been shown that the addition of β1,2-xylose to plant N-glycans starts in the *cis*-Golgi and reaches its highest level in the medial cisternae, while α1,3-fucosylation occurs mainly in the *trans*-Golgi cisternae and the TGN of suspension-cultured sycamore cells [44, 45]. Finally, antibodies raised against the Lewis^a epitope label the *trans*-Golgi and the TGN in plant cells [46], indicating that Lewis^a biosynthesis is a late Golgi event.

Alpha-mannosidases I and II are most commonly found in medial and/or *trans*-cisternae with cell type-dependent variations in Golgi distribution in animals [47]. In plants, when fused to GFP, GmMan I was shown to localize to the *cis*-side of the Golgi stacks [4]. Based on animal data, the glycosyltransferases which further modify the N-glycan are in majority found in the medial and *trans*-Golgi. We have so far only one plant example to confirm this compartmentation. Using a GFP fusion approach, the *Arabidopsis* β1,2-xylosyltransferase was shown to localise in the medial-cisternae of Golgi of tobacco BY-2 cells [39], a localisation which is consistent with previous immunodetection of the corresponding products [44, 45].

The role of the Golgi in the biosynthesis of noncellulosic cell wall polysaccharides

The cell wall provides a rigid support to plants and a barrier against invading organisms. It is a highly complex structure with importance for growth and development, cell-to-cell communication, and it contains a number of different polysaccharides [48]. Both in the primary and in the secondary cell walls are cellulose microfibrils embedded in an amorphous ground substance, the matrix. Matrix materials are heteropolymers that are traditionally grouped into two families of polysaccharides: pectins and hemicelluloses. Pectins are mainly polygalacturonic acids with different degrees of D-galactosyl, L-arabinosyl and L-rhamnosyl residues. Three classes of pectins can be distinguished based on two different backbone configurations: homogalacturonan (HGA), rhamnogalacturonan I (RGI) and rhamnogalacturonans II (RGII). Pectins are predominant in the middle lamella, the layer between neighbouring cells. Hemicelluloses have short chains and are therefore partially soluble polymers, consisting of xylosyl-, glucosyl-, galactosyl-, arabinosyl- or mannosyl residues. Depending on the dominant constitutive sugar, the polymers are named xylans, galactans or arabinogalactans, for example, when both sugars occur in nearly equal amounts (for reviews see [49, 50]). Heteropolysaccharide biosynthesis can be divided into four steps: chain or backbone initiation, elongation, side-chain addition, and termination and extracellular deposition [51, 52]. Our understanding of the different steps of biosynthesis is still very incomplete. Early labelling studies located the major site of the cell wall polysaccharide synthesis to the Golgi with the exception of cellulosic polysaccharide [49, 53, 54]. Evidence for the compartmentation and subcompartmentation of polysaccharide biosyn-

thesis was first obtained using immunogold location with antibodies specific to the backbone of xyloglucan hemicellulose (XG) or RGI [55, 56], to the terminal fucose of the XG side chain [57], to xylan [58] or to the linkage of pectins [59]. RG-I-type polysaccharides appear to be synthesized in *cis*- and medial cisternae and have the potential to leave from a monensin-insensitive, medial cisternal compartment, while the labelling pattern for XG suggests that it is assembled in the *trans*-Golgi and packed for their cell wall transport from the monensin-sensitive TGN [55, 60]. Moreover, Moore et al. demonstrated that individual Golgi stacks can simultaneously process glycoproteins and complex polysaccharides [55].

Polysaccharide synthases involved in cell wall biosynthesis in plants are relatively difficult enzymes to work with, due to the low yield of purification of plant Golgi and inevitable copurification with other membranes. So although many polysaccharide synthases have been solubilised, good purification of these enzymes has been limited to two arabinosyltransferases and two xylan xylosyltransferases [61, 62], as well as a callose synthase [63], a fucosyltransferase [64] and a galactosyltransferase [65]. These purifications were sufficient in some cases to allow production of antibodies to immunolocalise the corresponding enzymes in the Golgi [66] and to confirm the location of the biosynthesis of cell wall polysaccharides in the Golgi. However, no information is yet available on the subcompartmentation of the enzymes. In a few cases, acquisition of the protein sequence enabled cloning of the glycosyltransferase. This is the case for the fucosyltransferase from *A. thaliana* involved in XG biosynthesis [31, 64] and for the galactomannan galactosyltransferase from fenugreek [65]. In parallel, using the sequence-based classification scheme, Henrissat et al. [67] showed that 351 *A. thaliana* putative glycosyltransferase genes could be involved in cell wall polymer biosynthesis. Among these, Faik et al. [68] recently identified an α1,6-xylosyltransferase involved in xyloglucan biosynthesis. This enzyme is a typical Golgi type II membrane protein, confirming the Golgi location of xyloglucan biosynthesis. Identification and characterisation of major glycosyltransferases involved in pectin and hemicellulose biosynthesis is an 'indispensable' step to get more information on the location and the subcompartmentation of these enzymes in the Golgi, and to build a model of polysaccharide biosynthesis in the Golgi.

Protein localisation in the Golgi or the TGN

Golgi and TGN resident proteins identified so far are exclusively membrane bound. Through available examples of membrane proteins, we will summarize current knowledge regarding plant Golgi retention. We have selected two types of proteins, (i) enzymes involved in N-glycan maturation and (ii) the plant vacuolar receptor peabp80. Only single-pass membrane proteins will be considered, since there are to date only few examples of possible Golgi resident proteins with multiple transmembrane domains (TMDs): a Golgi nucleotide sugar transporter, GONST1 [69], a calcium pump [70] and receptors involved in retrieving proteins to the ER [71, 72].

Golgi localization – what can we learn from mammalian data?

Our knowledge about Golgi retention mainly comes from animal studies. Nevertheless, some animal Golgi enzymes expressed in plant cells accumulate in the same types of cisternae as their native ones, even though these animal enzymes have no plant equivalent. Also, animal N-glycosylation enzymes can functionally replace their plant homologues and vice versa. We can therefore expect basic Golgi retention signals and mechanisms to be similar between the two kingdoms.

Complementation experiments sustain shared mechanisms for Golgi retention between plant and animal cells. For example, an *A. thaliana* mutant (*cgl1*) defective in NAGTI activity does not show any obvious phenotype [73] and could be complemented by a human GNTI complementary DNA (cDNA) [74]. The reverse is also true since the *A. thaliana* NAGTI can complement Lec1 CHO mutant cells deficient in NAGTI activity [34].

Plant cells are able to accumulate a mammalian enzyme which has no equivalent in plants, since a chimeric protein made of the transmembrane domain of rat α2,6-sialyltransferase (α2,6-SialT) and GFP was localised to the Golgi apparatus in tobacco leaf epidermal cells [2], in BY-2 tobacco suspension cultured cells [75] and in *A. thaliana* [C. Saint-Jore and C. Hawes, unpublished results]. EM even revealed that the GFP chimera was sublocated to the *trans*-half of the plant Golgi [2], the native location in animal Golgi. More important, the full-length rat α2,6-SialT expressed in *A. thaliana* was active when using a membrane fraction and the appropriate substrate [76]. This activity was significantly higher when Triton X-100 was added, meaning that the animal enzyme had a native topology with its catalytic domain in the lumen of the plant secretory pathway [76].

When the first 60 amino acids from human β1,4-galactosyltransferase (β1,4-GalT), including the cytosolic tail and the transmembrane domain, were fused to GFP and expressed in HeLa cells, the resulting fluorescence was localized to the Golgi apparatus [77] and the ER [78]. Several groups used a similar construct in plants and found equivalent subcellular locations for the fusion protein, although there is no equivalent enzyme in plant cells. This was shown in *A. thaliana* [79], in *N. tabacum* leaf epidermal cells [75] and in tobacco BY-2 suspension cultured cells [C. Saint-Jore and C. Hawes, unpublished

results]. Finally, full-length human β1,4-GalT is active in vivo when expressed in tobacco plants [80] and BY-2 suspension cultured cells [81].

Golgi localization – sequence required

Golgi N-glycosylation enzymes are type II proteins, with an N-terminal cytosolic tail (CT), a transmembrane domain (TMD) of 16–25 amino acids and a C-terminal luminal domain (LD) which is responsible for catalytic activity (fig. 3). For glycosyltransferases, a loosely defined 'stem' region (SR, fig. 3) separates the transmembrane and the luminal domains and may play a role in positioning the catalytic domain away from the lipid bilayer, therefore facilitating access to the substrate. A common strategy has been employed to identify putative retention signal(s) by analysing the location of hybrid molecules containing limited sequences derived from glycosyltransferases fused to various reporter proteins and expressed in mammalian or in plant cells.

From the numerous results brought by various groups within the last 20 years, we can conclude that there is no consensus amino acid sequence that is shared by most Golgi-resident proteins and that serves as a Golgi retention signal. Instead, retention information resides within the intact constitutive domains. The role of sequences within and adjacent to the TMD is predominant, but the CT and occasionally the SR also participate in Golgi retention.

Many studies investigated the role of the TMD in Golgi retention and are already well reviewed [82–84]. Among the latest examples, constructing various deletion mutants of murine α1,2-mannosidase IB and α1,2-mannosidase/GFP chimeras, Becker et al. [85] found that the TMD is a major targeting determinant in Golgi localisation in COS7 cells. A series of TMD replacements and mutations increased β1,4-GalT secretion in COS cells and highlighted the crucial role of cysteine 29 and histidine 32 that are located in the TMD [86, 87]. However, including these two amino acids in transferrin receptor (TfR) TMD did not confer Golgi retention [86]. Another possible key amino acid within the TMD is the phenylalanine residue (Phe). In mammalian sialyltransferase, there are four Phe residues that clearly help Golgi retention [88], although they are not absolutely required. There are yet too few available cloned plant enzymes to evaluate any conserved amino acid within the TMD.

It is important to note that each research group has its own definition of 'the TMD'. In Nilsson et al.'s experiments, two or three charged amino acids on either side of the hydrophobic stretch were included in the sequence defined as β1,4-GalT's TMD, whereas in Munro's analysis of α2,6-SialT charged residues necessary for anchoring membrane proteins within the lipid bilayer were excluded from the glycosyltransferase TMD [89, 90]. Additionally, most available programs used to estimate the position and the length of the TMD in a novel sequence are optimized for type I membrane proteins (for example, http://www.cbs.dtu.dk/services/TMHMM-2.0 [91]), but are not very suitable for type II proteins. Recently, a program based on a new method [92] was described to predict the TMD of Golgi type II membrane proteins (http://microarray.imb.uq.edu.au/golgi).

Many results also indicate that the CT plays a key role in Golgi retention in animal cells, especially for *trans*-Golgi localised enzymes, and in some cases this CT is required for Golgi retention. For example, Nilsson et al. [89] showed that the TMD and CT of β1,4-GalT were sufficient for the reporter protein to reach the Golgi, while removing most of the CT from the same chimeric protein led to a mistargeting of the reporter to the cell surface. Also, using various regions of rat α2,6-SialT fused to a reporter, it was shown that the presence of CT clearly increases the efficiency of Golgi retention of the TMD and its flanking amino acids [90]. For plants, few data are yet available. The N-terminal first 77 amino acids of *Nt*-NAGTI (which include the CT, TMD and SR) were sufficient to sustain Golgi retention of GFP in *Nicotiana benthamiana* leaf epidermal cells [93]. Detailed studies of arabidopsis β1,2-xylosyltransferase (AtXylT) showed that the CT and TMD were necessary and sufficient to target the GFP chimera to the Golgi apparatus in *N. benthamiana* leaf cells [39, 94]. More precisely, the 36 first amino acids of AtXylT contain sub-Golgi location information since they mediate a preferential accumulation of the chimera to the medial-Golgi cisternae in tobacco BY-2 suspension cultured cells [39].

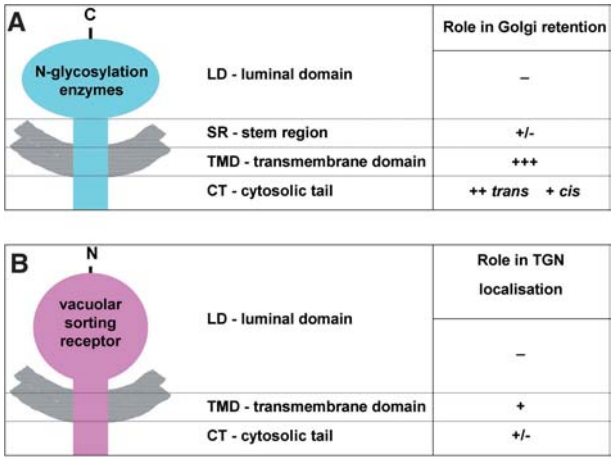

Figure 3. Schematic representation of Golgi or TGN localized proteins and the role of each domain in retention. (*A*) N-glycosylation enzymes (in blue) are type II membrane proteins with a large catalytic domain at the C-terminal end. (*B*) The vacuolar sorting receptors from BP-80 family (in purple) are type I membrane proteins with the ligand-recognizing region located at the N-terminus of the protein.

The stem region was also found to play a role in Golgi retention of NAGTs. For example, the SR of NAGTI is crucial for Golgi retention in HeLa cells [95], and its removal from NAGTV leads to secretion in COS cells [96]. Yet there is no information available from plant proteins.
The relative importance of Golgi retention for the various constitutive domains of type II proteins is summarised in figure 3 A. The essential portion is the TMD. The second most important region is the CT, especially for *trans*-Golgi proteins. Finally, the stem region sometimes has a positive role in Golgi retention, while the luminal domain, whenever its role in retention was tested, was not required for Golgi location.

Golgi localization – mechanisms

An important consideration ought to be taken into account before discussing retention mechanisms of proteins. The Golgi apparatus is an intermediate compartment within the secretory pathway. Numerous secreted proteins are constantly trafficking through the Golgi. It is therefore admitted that *cis*-to-*trans* Golgi fluxes of proteins and membrane generate the main force for protein transport in the direction of secretion. A resident protein of an intermediate compartment will have to present specific information, called retention or recycling signals, to be actively taken off this force. This model is clearly accepted for proteins that are not anchored into the membrane. For example, vacuolar soluble proteins are diverted from the secretion flux and redirected towards the vacuole by the mean of vacuolar sorting signals and their specific receptors. For membrane proteins, the dynamic is not so clear.
To date, there are two proposed mechanisms for Golgi retention, again, mainly substantiated by data originating from animal proteins. One of these models, the kin recognition model, proposes that proteins may associate to form high molecular weight aggregates that will be unable to enter vesicles budding from the Golgi [84]. The second model involves membrane heterogeneity and mostly its variation in thickness. As a consequence of membrane heterogeneity, the interaction between the hydrophobic regions of a protein and the lipidic bilayer will tend to segregate each protein in its ideal membrane [97]. Membrane proteins may therefore be retained in a given sublocation almost exclusively by means of this equilibrium. In this second model, the TMD therefore plays a key role in retention, although it may be overridden by cytosolic signals. The latter recruit cytosolic factors used for controlled transport within the secretory pathway, such as vesicle formation. Most available approaches allow us to address the steady-state sublocation of a protein of interest. The transit of a protein via compartments that differ from its apparent native one can only be demonstrated through the specific modification that this protein will have acquired (such as N-glycan maturation or propeptide cleavage). Such modifications are unfortunately not yet available for all compartments of the secretory pathway. When available, they nevertheless show that protein concentration in a given compartment is ensured by efficient recycling but not strictly by retention [98].

The oligomerisation/kin recognition model

This model proposes that glycosyltransferases in a particular cisternum interact to form structures too large to enter transport vesicles [83, 84, 99]. Aggregation may be regulated by differences within the Golgi cisternae, such as pH and calcium concentration. This model arose initially from work on a viral Golgi protein that forms homooligomers [100] and was extended by the observation that two enzymes of the medial Golgi, GNTI and mannosidase II, are tightly associated in vivo in HeLa cells [99]. More precisely, the association of both enzymes is mediated by charged amino acids within the SR of GNTI [95]. Following the same model, glycolipid glycosyltransferases β1,4-*N*-acetylgalactosaminyltransferase and β1,3-galactosyltransferase associate in the distal Golgi in CHO cells. However, the N-terminal domains (CT, TMD and a few amino acids of the stem) are involved in the interaction [101]. It was recently proposed that medial Golgi (GNTI and II) but not late Golgi glycosyltransferases (β1,4-GalT, α1,2-fucosyltransferase) exist as high molecular weight complexes, the luminal domain being involved in complex formation and localisation [102].
It has been suggested that retention of these enzyme oligomers in specific Golgi cisternae could increase by their binding to a putative Golgi matrix located between the cisternae [103]. Also, it has been shown that galactosyltransferases not only form homodimers in vivo but also interact with α- and β-tubulin, suggesting that such an interaction may be important for Golgi retention [104]. Importantly, at the EM level using cryofixation techniques, a possible Golgi matrix can be detected in plant cells (fig. 1). This matrix is apparently composed of fine fibrillar material and surrounds each stack. Support for the presence of actin associated with the Golgi matrix has come from studies with actin-disrupting agents such as cytochalasins, which cause the clustering of Golgi stacks [105, 106]. Other candidate proteins for the Golgi matrix include myosin-like proteins and spectrins [107, 108].

Lipid bilayer features

The essential role of the TMD in Golgi retention could be due to thickness and composition variations of the lipid bilayers constitutive of the secretory pathway. In mammalian cells there is an increasing gradient of cholesterol and sphingolipids from the ER to the plasma membrane. This variation brought up the raft model, where a subset of lipids including sphingolipid and intercalated cholesterol would concentrate at the TGN, forming platforms where plasma membrane proteins are concentrated while Golgi

proteins are excluded [109]. This platform of lipid would ultimately form a lipidic raft at the plasma membrane. In plants, there is no evidence yet for a similar raft, but the six principal sterols (including cholesterol) are 2.7 times more abundant in the plasma membrane in comparison with the Golgi [110]. These similarities suggest as well a role of lipid bilayer composition in plant cells for Golgi retention. It is difficult to manipulate the sterol content of membranes in plant cells. All of the available sterol-deficient *A. thaliana* mutants are never entirely sterol depleted, since the diminution in a given member of the sterol family will be compensated by an increase of other members (summarized in [111]). Confusingly, insect cells depleted of detectable cholesterol were found to correctly target murine α-mannosidase II and bovine β1,4-GalT to the Golgi apparatus, as in control cells [112]. So the length of the TMDs would determine the subcellular location. Mammalian Golgi enzymes are on average five residues shorter than those of plasma membrane proteins and contain more of the bulky residue phenylalanine [113]. The relevance of this difference in TMD length to retention is supported by mutagenesis studies, which have shown that lengthening the TMD of α2,6-SialT or β1,4-GalT results in reduced retention and that a synthetic TMD of 17 leucines gives Golgi retention [87, 90], whereas one of 23 leucines does not [90]. Nevertheless, not all mutagenesis studies have been interpreted as being consistent with the TMD length being the key signal for retention [83, 114]. Replacement of the 23-amino acid TMD of NAGTI by a series of 19, 23 or 27 leucine residues did not alter enzyme retention in the Golgi but disrupted the Golgi itself in HeLa cells [95]. This curious result suggests a hierarchy within the various factors involved in Golgi retention with signals, presumably within the CT, dominant over TMD length in this case.

In plant cells membrane thickness also varies within the secretory pathway. In monocotyledonous cells, it was shown that the ER membrane is on average 4–5 nm, while the plasma membrane is ~9.5 nm thick [115]. The relationship between membrane thickness and length of the TMD was shown in planta using type I membrane GFP reporter proteins, with TMDs varying between 17, 20 and 23 amino acids [5]. The various membrane proteins were expressed in tobacco leaf epidermal cells, and the site of accumulation was investigated using confocal microscopy. A TMD of 17 amino acids leads to retention of the reporter in the ER, while a 23-amino acid TMD containing chimera accumulated in the plasma membrane. A TMD of 20 amino acids targeted GFP to the Golgi, but unfortunately its exact location within the Golgi was not addressed.

Evidence for recycling within the Golgi

An interesting piece of work was recently provided in favour of intra-Golgi recycling [98]. In this study, human β1,2-*N*-acetylglucosaminyltransferase was modified by the addition of an N-glycosylation site. When expressed in CHO cells, the modified enzyme was indeed glycosylated and was found in the medial-Golgi, like the wild type. The N-glycans carried by the modified transferase were shown to contain sialic acid, a residue added to the TGN in these particular cells. These results support recycling of medial-Golgi enzymes from the TGN back to their steady-state location. To date, there is no evidence for intraplant Golgi recycling, although the ER recycling machinery involving the receptor ERD2 is also present in plants [18].

TGN localisation – peabp80

As an example of TGN resident protein in plant cells, we chose the family of vacuolar receptors (reviewed in [116]) and its best-characterized example, peabpP80.

Peabp80 structure and subcellular location

Peabp80 is a type I membrane protein (fig. 3B) made of a large LD, a single TMD and a CT. The LD recognizes the vacuolar targeting propeptide carried by barley aleurain and other sequence-specific signals [117]. These binding properties defined in vitro were confirmed in vivo using *Saccharomyces cerevisiae*. In more detail, the plant receptor and its specific ligand fused to GFP were coexpressed in a yeast strain disrupted for the gene encoding its own vacuolar receptor, Vps10p. In this mutant strain, expression of peabp80 led to redirecting the GFP reporter fused to the aleurain signal towards the yeast vacuole [20]. There is a very high degree of conservation between the peptide sequence of the *Arabidopsis* family (seven members) and peabp80 (see aligned sequences in [18]), suggesting that all members are as well type I membrane proteins. Nevertheless, it is unclear whether all of the peabp80 homologues are involved in sorting proteins to the lytic vacuole.

In pea cotyledon, EM immunolabelling showed that peabp80 is localized in the TGN and the prevacuole. Li et al. [24] studied the repartition of proteins of the bp80 family by immunofluorescence microscopy using various compartment-marker antibodies. For example, the prevacuole was labelled using antibodies against AtPep12p (later renamed AtSYP21p, [118]). This in an *A. thaliana* homologue of Pep12p, a syntaxin originally identified in yeast and involved in yeast vacuolar transport [119, 120]. Since AtSYP21p can complement the yeast Pep12p, the structures that are labelled with the corresponding antibodies are thought to represent prevacuolar compartments in plants. Jiang's group [24] showed that 80% of the structures labelled with the receptors were also positive for anti-AtPep12p antibodies. This means that the family of bp80 preferentially concentrates in the prevacuolar compartments.

Both TMD and CT are necessary for peabp80 localisation

As for type II proteins, the TMD and the CT of peabp80 are essential for its localisation, since the LD alone is secreted and very unstable in plant cells [23]. Presumably, the LD alone cannot anchor into the membrane and is transported out of the cell by the default route for soluble proteins. Recently, it was shown that a chimeric protein made with the GFP fused to peabp80 TMD (BP19) is membrane bound, meaning that peabp80 TMD alone is sufficient for membrane anchoring [5]. Since BP19 accumulates in structures overlapping with the fluorescent Golgi at the confocal microscopy level, this indicates that the TMD alone is not sufficient to accumulate the reporter in the prevacuole. The calculated length of this chimera's TMD was estimated to be 19 amino acids, using the TMHMM-2.0 program. Lengthening the chimera's TMD to 22 amino acids led to accumulation of the reporter in a plasma membrane and not the prevacuole nor the tonoplast. This means that TGN and prevacuole targeting information do not reside within the TMD and indirectly suggests a dominant role of the CT of peabp80 for localisation. Indeed, when the CT of peabp80 is added to the TMD in the fusion protein with GFP, the reporter is found in structures that are independent from the Golgi stacks using fluorescence microscopy [N. Paris, unpublished results].

Signals within the bp80 CT

The steady-state location of peabp80 in the TGN and the prevacuole results from constant cycling, presumably with no plasma membrane requirement, unlike lysosomal transport in mammalian cells. Cycling of peabp80 is probably ensured by various signals located within the CT as for its animal and yeast equivalents (MPRs and Vps10p, respectively [121]).

For MPR46, cytosolic signals reside in short peptidic sequences that are regulated to favour either one of the two directions of transport, depending on the receptor state. Signals involved in transport towards the late endosome would be turned on when the receptor is bound to its ligand, while another set of signals would be activated to favour the recycling of MPR46 when it is free. One of these signals is called tyrosine motif, the tetrapeptide YxxΦ, where Φ represents a large hydrophobic amino acid. This tyrosine motif serves to retrieve the mannose b-phosphate receptors (MPRs) from the plasma membrane back to the TGN, more precisely to the internalization step (recently reviewed in [121]). Tyrosine motifs were shown to directly interact in vitro with the μ subunit of the adaptin complex involved in the formation of the clathrin-coated vesicle. Another signal involved in MPR trafficking is the acidic cluster dileucine signal (AC-LL), 'EESEERDDHLL' in the case of MPR46. This AC-LL motif is recognized by GGA (Golgi-associated, γ-adaptin homologous, ARF-interacting protein), a multi-partner adaptator used for clathrin-coated vesicle formation at the level of the TGN. These GGAs were recently described in mammals and yeast but apparently have no equivalent in plants.

The CT of peabp80 contains 37 amino acids that are well conserved over the family of homologues (fig. 4). When all the available sequences are aligned, a consensus sequence of 25 amino acids can be proposed for this family of proteins from mono- and dicotyledoneous plants. In contrast, the C-terminal amino acids are the least conserved and may be responsible for trafficking differences between homologues. A tyrosine motif (fig. 4, underlined) is found in this highly conserved region and is present in all the sequences of homologues available so far. The tyrosine motif carried by the family of bp80s was shown to interact in vitro with mammalian adaptins [17, 122]. More precisely, part of the cytosolic domain of *Arabidopsis* homologue Atbp80b was used; between amino acid –25 to the amino-acid at position –12. This peptide, including the conserved tyrosine motif, was a more efficient towards the AP1 complex, used for TGN sorting, than for AP2 complex, used at the plasma membrane level. Recently, in collaboration with the group of David Robinson, it was shown that the entire peabp80 cytosolic domain can bind directly to µA adaptin cloned from *Arabidopsis* [N. Happel et al., unpublished]. This interaction presents a K_d 144 nM, as measured by plasmon resonance, and drops to 690 nM when the tyrosine from the motif is replaced by an alanine. Altogether, these results demonstrate that the tyrosine motif is used for the receptor traffic but they do not imply the direction of transport. The only indirect support for the role of the tyrosine motif in vivo comes from immunolabelling of the steady state µA adaptin in *Arabidopsis* root tip cells. At the EM level µA adaptin was found accumulated in the Golgi. Additionally, using confocal microscopy, compartments labelled µA adap-tin were found juxtaposed with structures that are accumulating Arabidopsis bp80s. This may indicate that µA adaptin is used in vivo by bp80 either for TGN retention or for TGN exit. This would differ from the use of such motifs in mammalian cells for retrieval to the TGN.

Conclusion

As a general conclusion, our knowledge of the plant Golgi and TGN are still fragmented and far behind their animal equivalent. The particularities of the plant Golgi, such as its small size and high mobility, raise questions that are unique to the plant Golgi, and protein retention within these subcellular locations must adapt to these features. This indicates that plant cells have developed some recycling and retention processes

Figure 4. Alignment of the cytosolic tail of peabp80 with all variants identified thus far. Consensus amino acids are represented on the top line with annotation referring to the C-terminus. Fully conserved residues are shown in black, and residues that are found in at least half of the full sequences available (24 listed here) in grey. Underlined is the tyrosine motif found in all bp80 homologues so far. Sequences from *Arabidopis* were taken from already published alignment [18] or sequences [23, 123] and from database information.

unique to the plant kingdom, and therefore novel to cell biology.

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1 Driouich A. and Staehelin A. (1997) The plant Golgi apparatus: structural organization and functional properties. In: The Golgi apparatus, pp. 275–301, Berger E. G. and Roth J. (eds), Birkhäuser, Basel
2 Boevink P., Oparka K., Cruz S. S., Martin B., Betteridge A. and Hawes C. (1998) Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. Plant J. **15:** 441–447
3 Lerouge P., Cabanes-Macheteau M., Rayon C., Fitchette-Lainé A. C., Gomord V. and Faye L. (1998) N-glycoprotein biosynthesis in plants – recent developments and future trends. Plant Mol. Biol. **38:** 31–48
4 Nebenführ A., Gallagher L. A., Dunahay T. G., Frohlick J. A., Mazurkiewicz A. M., Meehl J. B. et al. (1999) Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. Plant Physiol. **121:** 1127–1141
5 Brandizzi F., Frangne N., Marc-Martin S., Hawes C., Neuhaus J.-M. and Paris N. (2002) In plants the destination for single pass membrane proteins is markedly influenced by the length of the hydrophobic domain. Plant Cell **14:** 1077–1092
6 Garcia-Herdugo G., Gonzáles-Reyes J. A., Garcia-Navarro F. and Navas P. (1988) Growth kinetics of the Golgi apparatus during the cell cycle in onion root meristems. Planta **175:** 305–312
7 Munro S. (2002) More than one way to replicate the Golgi apparatus. Nat. Cell Biol. **4:** E223–E224
8 Nebenführ A., Frohlick J. A. and Staehelin L. A. (2000) Redistribution of Golgi stacks and other organelles during mitosis and cytokinesis in plant cells. Plant Physiol. **124:** 135–151
9 Bevis B. J., Hammond A. T., Reinke C. A. and Glick B. S. (2002) De novo formation of transitional ER and Golgi structures in *Pichia pastoris*. Nat. Cell Biol. **4:** 750–756
10 Hawes C., Saint-Jore C. M., Brandizzi F., Zheng H., Andreeva A. V. and Boevink P. (2001) Cytoplasmic illuminations: in planta targeting of fluorescent proteins to cellular organelles. Protoplasma **215:** 77–88
11 Brandizzi F., Snapp E. L., Roberts A. G., Lippincott-Schwartz J. and Hawes C. (2002) Membrane protein transport between the endoplasmic reticulum and the Golgi in tobacco leaves is energy dependent but cytoskeleton independent: evidence from selective photobleaching. Plant Cell **14:** 1293–1309

12 Kornfeld S. (1990) Lysosomal enzyme targeting. Biochem. Scty. Trans. **18:** 367–374
13 Jiang L. and Rogers J. C. (2003) Sorting of lytic enzymes in the plant Golgi apparatus. In: The Golgi Apparatus and the Plant Secretory Pathway, vol. 7, pp. 114–140, Robinson D. G. (ed.), Blackwell, Oxford
14 Paris N., Stanley C. M., Jones R. L. and Rogers J. C. (1996) Plant cells contain two functionally distinct vacuolar compartments. Cell **85:** 563–572
15 Hinz G., Hillmer S., Baumer M. and Hohl I. (1999) Vacuolar storage proteins and the putative vacuolar sorting receptor BP-80 exit the Golgi apparatus of developing pea cotyledons in different transport vesicles. Plant Cell **11:** 1509–1524
16 Bassham D. C. and Raikhel N. V. (2000) Unique features of the plant vacuolar sorting machinery. Curr. Opin. Cell Biol. **12:** 491–505
17 Kirsch T., Paris N., Butler J. M., Beevers L. and Rogers J. C. (1994) Purification and initial characterization of a potential plant vacuolar targeting receptor. Proc. Natl. Acad. Sci. USA **91:** 3403–3407
18 Hadlington J. L. and Denecke J. (2000) Sorting of soluble proteins in the secretory pathway of plants. Curr. Opin. Plant Biol. **3:** 461–468
19 Robinson D. G. (1996) Clathrin-mediated trafficking. Trends Plant Sci. **1:** 349–355
20 Humair D., Hernández Felipe D., Neuhaus J.-M. and Paris N. (2001) Demonstration in yeast of the function of BP-80, a putative plant vacuolar sorting receptor. Plant Cell **13:** 781–792
21 Jiang L., Phillips T. E., Rogers S. W. and Rogers J. C. (2000) Biogenesis of the protein storage vacuole crystalloid. J. Cell Biol. **150:** 755–770
22 Hillmer S., Movafeghi A., Robinson D. G. and Hinz G. (2001) Vacuolar storage proteins are sorted in the *cis*-cisternae of pea cotyledon Golgi apparatus. J. Cell Biol. **152:** 41–50
23 Paris N., Rogers S. W., Jiang L., Kirsch T., Beevers L., Phillips T. E. et al. (1997) Molecular cloning and further characterization of a probable plant vacuolar sorting receptor. Plant Physiol. **115:** 29–39
24 Li Y.-B., Rogers S. W., Tse Y. C., Lo S. W., Sun S. S. M., Jauh G.-Y. et al. (2002) BP-80 and homologs are concentrated on post-Golgi, probable lytic prevacuolar compartments. Plant Cell Physiol. **43:** 726–742
25 Wilson I. B. H. (2002) Glycosylation of proteins in plants and invertebrates. Curr. Opin. Struct. Biol. **12:** 569–577
26 Fötisch K. and Vieths S. (2001) *N*- and *O*-linked oligosaccharides of allergenic glycoproteins. Glycoconjugate Journal **18:** 373–390
27 van Ree R., Cabanes-Macheteau M., Akkerdaas J., Milazzo J.-P., Loutelier-Bourhis C., Rayon C. et al. (2000) β(1,2)-xylose and α(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. J. Biol. Chem. **275:** 11451–11458
28 Bardor M., Faveeuw C., Fitchette A.-C., Gilbert D., Galas L., Trottein F. et al. (2003) Immunoreactivity in mammals of two typical plant glyco-epitopes, core α(1,3)-fucose and core xylose. Glycobiology **13:** 427–434
29 Fitchette-Lainé A. C., Gomord V., Cabanes M., Michalski J. C., Saint Macary M., Foucher B. et al. (1997) *N*-glycans harboring the Lewis a epitope are expressed at the surface of plant cells. Plant J. **12:** 1411–1417
30 Breton C., Mucha J. and Jeanneau C. (2001) Structural and functional features of glycosyltransferases. Biochimie **83:** 713–718
31 Keegstra K. and Rhaikel N. V. (2001) Plant glycosyltransferases. Curr. Opin. Plant Biol. **4:** 219–224
32 Strasser R., Mucha J., Schwihla H., Altmann F., Glossi J. and Steinkellner H. (1999) Molecular cloning and characterisation of cDNA coding for beta 1,2 *N*-acetylglucosaminyltransferase I (GlcNAc-TI) from *Nicotiana tabacum*. Glycobiology **9:** 779–785
33 Wenderoth I. and Schaewen A. (2000) Isolation and characterisation of plant *N*-acetylglucosaminyltransferase I (GntI) cDNA sequences. Functional analysis in Arabidopsis *cgl* mutant and in antisens plants. Plant Physiol. **123:** 1097–1108
34 Bakker D., Lommen A., Jordi W., Stiekema W. and Bosch D. (1999) An *Arabidopsis thaliana* cDNA complements the *N*-acetylglucosaminyltransferase I deficiency of CHO Lec1 cells. Biochem. Biophys. Res. Commun. **261:** 829–832
35 Strasser R., Steinkellner H., Boren M., Altmann F., Mach L., Gossl J. et al. (1999) Molecular cloning of cDNA encoding *N*-acetylglucosaminyltransferase II from *Arabidopsis thaliana*. Glycoconjugate Journal **16:** 787–791
36 Leiter H., Mucha J., Staudacher E., Grimm R., Glössl J. and Altmann F. (1999) Purification, cDNA cloning and expression of GDP-L-Fuc:Asn-linked GlcNAc alpha1,3-fucosyltransferase from mung beans. J. Biol. Chem. **274:** 21830–21839
37 Wilson I. B. H., Rendic D., Dumic J., Freilinger A., Altmann F., Mucha J. et al. (2001) Cloning and expression of α1,3-fucosyltransferase homologues from *Arabidopsis thaliana.* Biochim. Biophys. Acta **1527:** 88–96
38 Strasser R., Mucha J., Mach L., Altmann F., Wilson I. B. H., Glössl J. et al. (2000) Molecular cloning and functional expression of β1,2-xylosyltransferase cDNA from *Arabidopsis thaliana.* FEBS Lett. **472:** 105–108
39 Pagny S., Bouissonié F., Sarkar M., Follet-Gueye M. L., Driouich A., Schachter H. et al. (2003) Structural requirements for Arabidopsis β-1,2-xylosyltransferase activity and targeting to the Golgi. Plant J. **33:** 189–203
40 Wilson I. B. H. (2001) Identification of a cDNA encoding a plant Lewis-type α1,4-fucosyltransferase. Glycoconjugate Journal **18:** 439–447
41 Leonard R., Costa G., Darrambide E., Lhernould S., Fleurat-Lessard P., Carlué M. et al. (2002) The presence of Lewisa epitopes in *Arabidopsis thaliana* glycoconjugates depends on an active α4-fucosyltransferase gene. Glycobiology **12:** 299–306
42 Kornfeld R. and Kornfeld S. (1985) Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **54:** 631–664
43 Roth J. (1997) The Golgi Apparatus, Birkhäuser, Basel
44 Lainé A.-C., Gomord V. and Faye L. (1991) Xylose-specific antibodies as markers of subcompartmentation of terminal glycosylation in the Golgi apparatus of sycamore cells. FEBS Lett. **295:** 179–184
45 Fitchette-Lainé A. C., Gomord V., Chekkafi A. and Faye L. (1994) Distribution of xylosylation and fucosylation in the plant Golgi apparatus. Plant J. **5:** 673–682
46 Fitchette A. C., Cabanes-Macheteau M., Marvin L., Martin B., Satiat-Jeunemaitre B., Gomord V. et al. (1999) Biosynthesis and immunolocalization of Lewis a-containing *N*-glycans in the plant cell. J. Plant Physiol. **121:** 333–344
47 Velasco A., Hendricks L., Moremen K. W., Tulsiani D. R. P., Touster O. and Farquhar M. G. (1993) Cell type-dependent variations in the subcellular distribution of α-mannosidase I and II. J. Cell Biol. **122:** 39–51
48 Reiter W.-D. (2002) Biosynthesis and properties of the plant cell wall. Curr. Opin. Plant Biol. **5:** 536–542
49 Carpita N. C. and Gibeaut D. M. (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J. **3:** 1–30
50 Carpita N. C. and Gibeaut D. M. (1993) Biosynthesis of plant cell wall polysaccharides. FASEB J. **8:** 904–915
51 Waldron K. W. and Brett C. T. (1985) Interaction of enzymes involved in cell wall heteropolysaccharide biosynthesis. In: Biochemistry of Plant Cell Wall, pp. 79–97, Brett C. T. and Hillman J. R. (eds), Cambridge University Press, Cambridge, UK
52 Iiyama K., Lam T. B. T., Meikle P. J., Ng K., Rhodes D. I. and Stone B. A. (1993) Cell wall biosynthesis and its regulation.

In: Forage Cell Wall Structure and Digestibility, pp. 621–683, Jung H. G., Buxton D. R., Hatfield R. D. and Ralph J., Crop Science Society of America, Madison, WI
53 Dupree P. and Sherrier D. J. (1998) The plant Golgi apparatus. Biochim. Biophys. Acta Mol. Cell Res. **1404:** 259–270
54 Mohnen D. (1999) Biosynthesis of pectins and galactomannans. In: Carbohydrates and Their Derivatives including Tannins, Cellulose and Related Lignins, pp. 497–527, Pinto B. (eds), Elsevier, Amsterdam
55 Moore P. J., Swords K. M. M., Lynch M. A. and Staehelin L. A. (1991) Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the Golgi apparatus of plants. J. Cell Biol. **112:** 589–602
56 Lynch M. A. and Staehelin L. A. (1992) Domain-specific and cell type specific localization of two types of cell wall matrix polysaccharides in the clover root tip. J. Cell Biol. **118:** 467–479
57 Zhang F. and Staehelin L. A. (1992) Functional compartmentation of the Golgi apparatus of plant cells. Immunocytochemical analysis of high-pressure frozen- and freeze-substituted Sycamore Maple suspension culture cells. Plant Physiol. **99:** 1070–1083
58 Northcote D., Davey R. and Lay J. (1989) Use of antisera to localize callose, xylan and arabinogalactan in the cell-plate, primary and secondary walls of plant cells. Planta **178:** 353–366
59 Vicre M., Jauneau A., Knox J. P. and Driouich A. (1998) Immunolocalization of beta- (1→4) and beta- (1→6)-D- galactan epitopes in the cell wall and Golgi stacks of developing flax root tissues. Protoplasma **203:** 26–34
60 Driouich A., Faye L. and Staehelin L. A. (1993) The plant Golgi apparatus: a factory for complex polysaccharides and glycoproteins. Trends Biochem. Sci. **18:** 210–214
61 Rodgers M. W. and Bolwell G. P. (1992) Partial purification of Golgi-bound arabinosyltransferase and two isoforms of xylosyltransferase from French bean (*Phaseolus vulgaris L.*). Biochem. J. **288:** 817–822
62 Porchia A. C., Sorensen S. O. and Scheller H. V. (2002) Arabinoxylan biosynthesis in wheat. Characterization of arabinosyltransferase activity in Golgi membranes. J. Plant Physiol. **130:** 432–441
63 McCormack B. A., Gregory A. C., Kerry M. E., Smith C. and Bolwell G. P. (1997) Purification of an elicitor-induced glucan synthase (callose synthase) from suspension cultures of French bean (*Phaseolus vulgaris* L.): purification and immunolocation of a probable M(r)-65,000 subunit of the enzyme. Planta **203:** 196–203
64 Perrin R. M., DeRocher A. E., Bar-Peled M., Zeng W. Q., Norambuena L., Orellana A. et al. (1999) Xyloglucan fucosyltransferase, an enzyme involved in plant cell wall biosynthesis. Science **284:** 1976–1979
65 Edwards M. E., Dickson C. A., Chengappa S., Sidebottom C., Gidley M. J. and Reid J. S. (1999) Molecular characterisation of a membrane-bound galactosyltransferase of plant cell wall matrix polysaccharide biosynthesis. Plant J. **19:** 691–707
66 Bolwell G. P. (2000) Biosynthesis of plant cell wall polysaccharides. Trends Glycosci. Glycotechn. **12:** 143–160
67 Henrissat B., Coutinho P. M. and Davies G. J. (2001) A census of carbohydrate-active enzymes in the genome of *Arabidopsis thaliana*. Plant Mol. Biol. **47:** 55–72
68 Faik A., Price N. J., Raikhel N. V. and Keegstra K. (2002) An *Arabidopsis* gene encoding an alpha-xylosyltransferase involved in xyloglucan biosynthesis. Proc. Natl. Acad. Sci USA **99:** 7797–7802
69 Baldwin T. C., Handford M. G., Yuseff M.-I., Orellana A. and Dupree P. (2001) Identification and characterization of GONST1, a Golgi-localized GDP-mannose transporter in Arabidopsis. Plant Cell **13:** 2283–2295
70 Ordenes V. R., Reyes F. C., Wolff D. and Orellana A. (2002) A thapsigargin-sensitive Ca2+ pump is present in the pea Golgi apparatus membrane. Plant Physiol. **129:** 1820–1828
71 Bar-Peled M., Conceicao A. dS., Frigerio L. and Raikhel N. V. (1995) Expression and regulation of aERD2, a gene encoding the KDEL receptor homolog in plants, and other genes encoding proteins involved in ER-Golgi vesicular trafficking. Plant Cell **7:** 667–676
72 Sato K., Ueda T. and Nakano A. (1999) The *Arabidopsis thaliana* RER1 gene family: its potential role in the endoplasmic reticulum localization of membrane proteins. Plant Mol. Biol. **41:** 815–824
73 von Schaewen A., Sturm A., O'Neill J. and Chrispeels M. J. (1993) Isolation of a mutant *Arabidopsis* plant that lacks *N*-acetyl glucosaminyl transferase I and is unable to synthesize Golgi-modified complex *N*-linked glycans. Plant Physiol. **102:** 1109–1118
74 Gomez L. and Chrispeels M. J. (1994) Complementation of an *Arabidopsis thaliana* mutant that lacks complex asparagine-linked glycans with the human cDNA encoding *N*-acetylglucosaminyltransferase I. Proc. Natl. Acad. Sci USA **91:** 1829–1833
75 Saint-Jore C. M., Evins J., Brandizzi F., Batoko H., Moore I. and Hawes C. (2002) Redistribution of membrane proteins between the Golgi apparatus and endoplasmic reticulum in plants is reversible and not dependent on cytoskeletal networks. Plant J. **29:** 661–678
76 Wee E. G.-T., Sherrier J., Prime T. A. and Dupree P. (1998) Targeting of active sialyltransferases to the plant Golgi apparatus. Plant Cell **10:** 1759–1768
77 Cole N. B., Smith C. L., Sciaky N., Terasaki M., Edidin M. and Lippincott-Schwartz J. (1996) Diffusional mobility of Golgi proteins in membranes of living cells. Science **273:** 797–801
78 Zaal K. J. M., Smith C. L., Polishchuk R. S., Altan N., Cole N. B., Ellenberg J. et al. (1999) Golgi membranes are absorbed into and reemerge from the ER during mitosis. Cell **99:** 589–601
79 Hawes C., Saint-Jore C. M., Martin B. and Zheng H.-Q. (2001) ER confirmed as the location of mystery organelles in *Arabidopsis* plants expressing GFP! Trends Plant Sci. **6:** 245–246
80 Bakker H., Bardor M., Molthoff J. W., Gomord V., Elbers I., Stevens L. H. et al. (2001) Galactose-extended glycans of antibodies produced by transgenic plants. Proc. Natl. Acad. Sci USA **98:** 2899–2904
81 Palacpac N. Q., Yoshida S., Sakai H., Kimura Y., Fujiyama K., Yoshida T. et al. (1999) Stable expression of human β1,4-galactosyltransferase in plant cells modifies N-linked glycosylation patterns. Proc. Natl. Acad. Sci USA **96:** 4692–4697
82 Gleeson P. A., Teasdale R. D. and Burke J. (1994) Targeting of proteins to the Golgi apparatus. Glycoconjugate Journal **11:** 381–394
83 Colley K. J. (1997) Golgi localization of glycosyltransferases – more questions than answers. Glycobiology **7:** 1–13
84 Munro S. (1998) Localization of proteins to the Golgi apparatus. Trends Cell Biol. **8:** 11–15
85 Becker B., Haggarty A., Romero P. A., Poon T. and Herscovics A. (2000) The transmembrane domain of murine α-mannosidase IB is a major determinant of Golgi localization. Eur. J. Cell Biol. **79:** 986–992
86 Aoki D., Lee N., Yamaguchi N., Dubois C. and Fukuda M. N. (1992) Golgi retention of a *trans*-Golgi membrane protein, galactosyl-transferase, requires cysteine and histidine residues within the membrane-anchoring domain. Proc. Natl. Acad. Sci USA **89:** 4319–4323
87 Masibay A. S., Balaji P. V., Boeggeman E. E. and Qasba P. K. (1993) Mutational analysis of the Golgi retention signal of bovine beta-1,4-Galactosyltransferase. J. Biol. Chem. **268:** 9908–9916

88 Munro S. (1995) An investigation of the role of transmembrane domains in Golgi protein retention. EMBO J. **14:** 4695–4704

89 Nilsson T., Lucocq J. M., Mackay D. and Warren G. (1991) The nembrane spanning domain of beta-1,4-galactosyltransferase specifies trans Golgi localization. EMBO J. **10:** 3567–3575

90 Munro S. (1991) Sequences within and adjacent to the transmembrane segment of alpha-2,6-sialyltransferase specify Golgi retention. EMBO J. **10:** 3577–3588

91 Sonnhammer E. L. L., von Heijne G. and Krogh A. (1998) A hidden Markov model for predicting transmembrane helices in protein sequences. In: Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, pp. 175–182, Glasgow J., Littlejohn T., Major F., Lathrop R., Sankoff D. and Sensen C. (eds), AAAI Press, Menlo Park, CA

92 Yuan Z. and Teasdale R. D. (2002) Prediction of Golgi type II membrane proteins based on their transmembrane domains. Bioinformatics **18:** 1109–1115

93 Essl D., Dirnberger D., Gomord V., Strasser R., Faye L., Glossl J. et al. (1999) The N-terminal 77 amino acids from tobacco N-acetylglucosaminyltransferase I are sufficient to retain a reporter protein in the Golgi apparatus of *Nicotiana benthamiana* cells. FEBS Lett. **453:** 169–173

94 Dirnberger D., Bencur P., Mach L. and Steinkellner H. (2002) The Golgi localisation of *Arabidopsis thaliana* β1,2-xylosyltransferase in plant cell is dependent on its cytoplasmic and transmembrane sequences. Plant Mol. Biol. **50:** 273–281

95 Nilsson T., Rabouille C., Hui N., Watson R. and Warren G. (1996) The role of the membrane spanning domain and stalk region of *N*-acetylglucosaminyltransferase I in retention, kin recognition and structural maintenance of the Golgi apparatus in HeLa cells. J. Cell Sci. **109:** 1975–1989

96 Sasai K., Ikeda Y., Tsuda T., Ihara H., Korekane H., Shiota K. et al. (2001) The critical role of the stem region as a functional domain responsible for the oligomerization and Golgi localization of *N*-acetylglucosaminyltransferase V. J. Biol. Chem. **276:** 759–765

97 Killian J. A. (1998) Hydrophobic mismatch between proteins and lipids in membranes. Biochim. Biophys. Acta Rev. Biomembranes **1376:** 401–416

98 Opat A. S., Houghton F. and Gleeson P. A. (2001) Steady-state localization of a medial-Golgi glycosyltransferase involves transit through the *trans*-Golgi network. Biochem. J. **358:** 33–40

99 Nilsson T., Hoe M. H., Slusarewicz P., Rabouille C., Watson R., Hunte F. et al. (1994) Kin recognition between medial Golgi enzymes in HeLa cells. EMBO J. **13:** 562–574

100 Weisz O. A., Swift A. M. and Machamer C. E. (1993) Oligomerization of a membrane protein correlates with its retention in the Golgi complex. J. Cell Biol. **122:** 1185–1196

101 Giraudo C. G., Daniotti J. L. and Maccioni H. J. F. (2001) Physical and functional association of glycolipid *N*-acetylgalactosaminyl and galactosyl transferases in the Golgi apparatus. Proc. Natl. Acad. Sci USA **98:** 1625–1630

102 Opat A. S., Houghton F. and Gleeson P. A. (2000) Medial Golgi but not late Golgi glycosyltransferases exist as high molecular weight complexes. J. Biol. Chem. **275:** 11836–11845

103 Slusarewicz P., Nilsson T., Hui N., Watson R. and Warren G. (1994) Isolation of a matrix that binds medial Golgi enzymes. J. Cell Biol. **124:** 405–413

104 Yamaguchi N. and Fukuda M. N. (1995) Golgi retention mechanism of β-1,4-galactosyltransferase. Membrane-spanning domain-dependant homodimerization and association with α- and β-tubulins. J. Biol. Chem. **270:** 12170–12176

105 Mollenhauer H. H. and Morré D. J. (1976) Transition elements between endoplasmic reticulum and Golgi apparatus in plant cells. J. Cell Sci. **19:** 231–237

106 Satiat-Jeunemaître B., Steele C. and Hawes C. (1996) Golgi-membrane dynamics are cytoskeleton dependent – a study on Golgi stack movement induced by brefeldin A. Protopl. **191:** 21–33

107 Staehelin L. A. and Moore I. (1995) The plant Golgi apparatus: structure, functional organization and trafficking mechanisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. **46:** 261–288

108 Hawes C., Saint-Jore C. M. and Brandizzi F. (2003) Endomembrane and cytoskeleton interrelationships in higher plants. In: The Golgi Apparatus and the Plant Secretory Pathway, vol. 7, pp. 63–75, Robinson D. G. (ed.), Blackwell, Oxford

109 Brown D. A. and London E. (2000) Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J. Biol. Chem. **275:** 17221–17224

110 Moreau P., Bessoule J.-J., Mongrand S., Testet E., Vincent P. and Cassagne C. (1998) Lipid trafficking in plant cells. Prog. Lipid Res. **37:** 371–391

111 Clouse S. D. (2002) *Arabidopsis* mutants reveal multiple roles for sterols in plant development. Plant Cell **14:** 1995–2000

112 Rolls M. M., Marquardt M. T., Kielian M. and Machamer C. E. (1997) Cholesterol-independent targeting of Golgi membrane proteins in insect cells. Mol. Biol. Cell **8:** 2111–2118

113 Bretscher A. (1993) Microfilaments and membranes. Curr. Opin. Cell Biol. **5:** 653–660

114 Dahdal R. Y. and Colley K. J. (1993) Specific sequences in the signal anchor of the β-galactoside α-2,6-sialyltransferase are not essential for Golgi localisation – membrane flanking sequences may specify Golgi retention. J. Biol. Chem. **268:** 26310–26319

115 Morré D. J., Mollenhauer H. H. and Bracker C. E. (1971) Origin and continuity of Golgi apparatus. In: Results and Problems in Cell Differentiation, vol. 2, Origin and Continuity of Cell Organelles, pp. 82, Reinert J. and Ursprung H. (eds), Springer, Berlin

116 Paris N. and Neuhaus J.-M. (2002) BP-80 as a vacuolar sorting receptor. Plant Mol. Biol. **50:** 903–914

117 Cao X., Rogers S. W., Butler J., Beevers L. and Rogers J. C. (2000) Structural requirements for ligand binding by a probable plant vacuolar sorting receptor. Plant Cell **12:** 493–506

118 Sanderfoot A. A., Assaad F. F. and Raikhel N. V. (2000) The *Arabidopsis* genome. An abundance of soluble N-ethylmaleimide-sensitive factor adaptator protein receptors. J. Plant Physiol. **124:** 1558–1569

119 Bassham D. C., Gal S., Conceiçao A. D. and Raikhel N. V. (1995) An *Arabidopsis* syntaxin homologue isolated by functional complementation of a yeast pep12 mutant. Proc. Natl. Acad. Sci. USA **92:** 7262–7266

120 Becherer K. A., Rieder S. E., Emr S. D. and Jones E. W. (1996) Novel syntaxin homologue, PEP12P, required for the sorting of lumenal hydrolases to the lysosome-like vacuole in yeast. Mol. Biol. Cell **7:** 579–594

121 Dell'Angelica E. C. and Payne G. S. (2001) Intracellular cycling of lysosomal enzyme receptors: cytoplasmic tails' tales. Cell **106:** 395–398

122 Ahmed S. U., Rojo E., Kovaleva V., Venkataraman S., Dombrowski J. E., Matsuoka K. et al. (2000) The plant vacuolar sorting receptor AtELP is involved in transport of NH2-terminal propeptide-containing vacuolar proteins in *Arabidopsis thaliana.* J. Cell Biol. **149:** 1335–1344

123 Shimada T., Kuroyanagi M., Nishimura M. and Hara-Nishimura I. (1997) A pumpkin 72-kDa membrane protein of precursor-accumulating vesicles has characteristics of a vacuolar sorting receptor. Plant. Cell. Physiol. **38:** 1414–1420