Minireview

Lectins and traffic in the secretory pathway

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Abstract Evidence is accumulating that intracellular animal lectins play important roles in quality control and glycoprotein sorting along the secretory pathway. Calnexin and calreticulin in conjunction with associated chaperones promote correct folding and oligomerization of many glycoproteins in the endoplasmic reticulum (ER). The mannose lectin ERGIC-53 operates as a cargo receptor in transport of glycoproteins from ER to Golgi and the homologous lectin VIP36 may operate in quality control of glycosylation in the Golgi. Exit from the Golgi of lysosomal hydrolases to endosomes requires mannose 6-phosphate receptors and exit to the apical plasma membrane may also involve traffic lectins. Here we discuss the features of these lectins and their role in glycoprotein traffic in the secretory pathway.

Key words: Calnexin/calreticulin; Endoplasmic reticulum; ER–Golgi intermediate compartment; ERGIC-53; Golgi; Lectin; Mannose 6-phosphate receptor; Membrane traffic; Quality control; VIP36

1. Introduction

Lectins are defined as non-enzymatic, sugar binding proteins. While plant lectins have been invaluable tools in the molecular analysis of animal glycoproteins for decades, animal lectins have raised interest only recently. A long-standing enigma has been the role of *N*-linked glycans attached to many proteins in the endoplasmic reticulum (ER) and their co- and posttranslational remodelling along the secretory pathway. The early discovery that one of these glycan modifications, mannose 6-phosphate (Man-6-P), serves as a lysosomal targeting signal that is recognized by mannose 6-phosphate receptors (MPRs [1]) has led to the notion that lectins may play more general roles in exocytotic protein trafficking. Only recently though has there been substantial evidence in support of this notion. Here we discuss the role of intracellular lectins in exocytotic glycoprotein transport and their role in understanding the mechanisms underlying protein traffic in the secretory pathway.

2. ER quality control

During and after translocation into the ER many newly synthesized secretory proteins acquire posttranslational modifications, including *N*-glycosylation, as well as chaperone-assisted folding and oligomerization that lead to a transport-competent conformation. Elaborate quality control mechanisms sort incorrectly from correctly folded proteins in the ER [2]. Incorrectly folded proteins are retained and, if folding is unsuccessful, degraded by the proteasome after retrotranslocation into the cytosol [3]. In both the folding and degradation processes lectins play an important part.

Two homologous lectins are known to be localized in the ER, the type I membrane protein calnexin and the soluble protein calreticulin [4–6] (Table 1). Both proteins share the same glycan specificity and bind monoglucosylated *N*-linked oligosaccharides with a $Glc_1Man_9GlcNAc_2$ structure [7]. The terminal glucose is crucial for binding but some mannose residues also contribute to the specificity of oligosaccharide binding. Calnexin and calreticulin operate as chaperones in the quality control of newly synthesized glycoproteins in two opposing ways. They ensure that correctly folded glycoproteins leave the ER, but they can also mediate glycoprotein degradation in cases where correct protein folding is impossible. The lectin-assisted glycoprotein folding involves a remarkable cycle of de- and reglucosylation [2,4] (Fig. 1). Newly synthesized glycoproteins carrying three glucose residues on each glycan are co-translationally trimmed by the sequential action of ER glucosidases I and II. Monoglucosylated glycoproteins bind calnexin and/or calreticulin and are presented to the thiol oxidoreductase chaperone ERp 57 [8] and presumably other chaperones. Glucosidase II then removes the remaining glucose residue that abolishes lectin–glycoprotein interaction. If the glycoprotein is correctly folded it will leave the ER. If not, it will be recognized by the ER enzyme UDP-glucose:glycoprotein glucosyltransferase (GT) and reglucosylated, thereby allowing it to reassociate with the quality control machinery. GT operates as a folding sensor, because it recognizes glycoproteins in their non-native but not in their native conformation [9]. GT is able to distinguish between different non-native conformations with a distinct preference for partially structured folding intermediates [10]. The deglucosylation and reglucosylation cycle continues until correct folding is achieved. While there is general agreement that the lectin function of calnexin and calreticulin is important for glycoprotein folding, the question of whether the two proteins function solely as lectins ('lectin-only' hypothesis [4]) or both as a lectin and as a classic chaperone ('dual binding' model [11]) remains controversial. In the dual binding model the lectins would possess a second site that binds to polypeptide segments of unfolded or partially folded proteins. Recent in vitro studies with purified components lend support to this notion. Both lectins were found to prevent aggregation of glycoproteins bearing monoglucosylated oligosaccharides but also proteins lacking *N*-glycans and this effect was enhanced

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by ATP [12,13]. It remains to be shown if the non-lectin function of calreticulin and calnexin is productive in vivo.

Calreticulin was also isolated from Golgi fractions of rat liver and it co-fractionated with Golgi endomannosidase to comparable amounts [14]. It is possible therefore that in some cells calreticulin-mediated quality control includes post-ER compartments in which deglucosylation to dissociate the chaperone–glycoprotein complexes would involve endomannosidase instead of glucosidase II.

Calnexin appears to also play a role in glycoprotein degradation. Proteasomal degradation of misfolded human α_1-antitrypsin requires the physical interaction with calnexin [15], and mannose trimming by ER mannosidase I in conjunction with GT-mediated reglucosylation, leading to monoglucosylated $Man_8GlcNAc_2$, delays dissociation of misfolded α_1-antitrypsin from calnexin. This suggests that calnexin in conjunction with mannosidase I selects misfolded glycoproteins for degradation by the proteasome (Fig. 1). It is not known if calreticulin can also participate in the degradative pathway.

ER degradation of misfolded carboxypeptidase Y in the yeast *Saccharomyces cerevisiae* is determined by a similar oligosaccharide structure that requires the action of ER mannosidase I, and an ER lectin was postulated to specifically recognize the $Man_8GlcNAc_2$ structure and target the malfolded protein to the degradation pathway [16]. However, the unknown lectin is neither calnexin nor calreticulin since the calnexin/calreticulin cycle of reglucosylation has not been found in *S. cerevisiae* [17,18].

After exit from the calreticulin/calnexin cycle correctly folded glycoproteins can undergo some mannose trimming by ER mannosidases I and II leading to $Man_{7-8}GlcNAc_2$ glycans [19].

3. From ER to ERGIC and Golgi

Correctly folded proteins, also termed 'cargo', are sorted from ER-resident proteins and leave the ER at the transitional elements both near the Golgi and in more peripheral sites where they are packaged into COPII-coated vesicles [20]. The polymerization of the COPII coat onto ER membranes drives vesicle budding in vitro. This occurs by sequential binding of the COPII components Sar1 GTPase and the heterodimeric protein complexes Sec23p/Sec24p and Sec13p/Sec31p [21]. The incorporation of cargo is believed to be selective, i.e. signal-mediated [22–24], and perhaps non-selective to some extent, i.e. by bulk flow [25,26]. Soon after formation, COPII vesicles shed their coat and, according to the maturation hypothesis, are believed to fuse with one another to form the ER–Golgi intermediate compartment (ERGIC). According to the stable compartment model the vesicles would fuse with preexisting ERGIC elements. The ERGIC is a collection of highly mobile tubulovesicular clusters located both near the Golgi and in the cell periphery. It is the first sorting station for forward (anterograde) and backward (retrograde) traffic [27,28]. The 53 kDa type I lectin membrane protein ERGIC-53 (Table 1) is the most popular marker for the ERGIC [29]. ERGIC-53 is expressed in all cells of multicellular organisms and there is a non-essential yeast homolog known as Emp47p [30].

Selective protein transport from the ER requires transport receptors. Recent evidence indicates that ERGIC-53 serves as a transport receptor for some glycoproteins. ERGIC-53 possesses all the hallmarks of such a cargo receptor. First, although predominantly localized to the ERGIC at steady state [31], it constitutively recycles between ERGIC and ER [27,28,32]. Second, ERGIC-53 possesses a $\sim$200 amino acid segment in its luminal domain that shares homology with the carbohydrate recognition domain of several plant lectins and with the mammalian protein VIP36. ERGIC-53 and VIP36 were therefore proposed to define a new class of animal lectins [33]. Mannose column chromatography showed that ERGIC-53 is a mannose-selective lectin [34] but its precise lectin specificity remains to be established. Third, ERGIC-53 possesses an ER exit determinant in its cytosolic domain that mediates binding to COPII coats [35] (Fig. 2).

What are the cargo glycoproteins recognized by ERGIC-53? If a cycling-impaired mutant of ERGIC-53 is mislocalized to the ER of HeLa cells in a tetracycline-inducible manner, intracellular transport of the lysosomal enzyme cathepsin C is inefficient suggesting that this enzyme is a ligand for ERGIC-53 [36]. Interestingly, several mutations in human ERGIC-53 cause the bleeding disorder combined deficiency of coagulation factors V and VIII [37–39]. Like cathepsin C, factors V and VIII are inefficiently transported if expressed in the ERGIC-53 cycling-impaired HeLa cell line [40] arguing that these two coagulation factors are also ligands for ERGIC-53. A mutant of ERGIC-53 in *Drosophila*, dubbed rhea, causes a β-integrin-related developmental defect (J. Fristrom, personal communication). Rhea is autosomal recessive and disrupts integrin-dependent junctions in *Drosophila* both between wing surfaces and between muscle and tendon cells resulting in a blistered phenotype [41]. ERGIC-53 may therefore directly or indirectly affect β-integrin traffic. Inactivating ERGIC-53 by the RNAi approach in *Caenorhabditis elegans* leads to an oocyte maturation defect (unpublished). It appears

Table 1
Lectins of the secretory pathway

Lectin	Major localization	Sugar specificity	Ca^{2+} dependence	Function
Calnexin	ER	Gluc+Man	+	folding and degradation
Calreticulin	ER	Gluc+Man	+	folding and degradation
Man_8 lectin[a]	ER	Man_8	?	degradation
ERGIC-53	ERGIC	Man	+	ER-to-ERGIC transport
VIP36	*cis*-Golgi/ERGIC	Man_{6-9}	−	retrieval?
Apical lectin(s)[a]	TGN?	Man?	?	Golgi-to-plasma membrane transport
IGF-II/CI-MPR[b]	late endosome	Man-6-P	−	Golgi-to-endosome and plasma membrane-to-endosome transport
CD-MPR[c]	late endosome	Man-6-P	+	Golgi-to-endosome transport

[a]Such lectins have been postulated, but their identity is unknown.
[b]Insulin-like growth factor-II/cation-independent mannose 6-phosphate/receptor.
[c]Cation-dependent mannose 6-phosphate receptor.

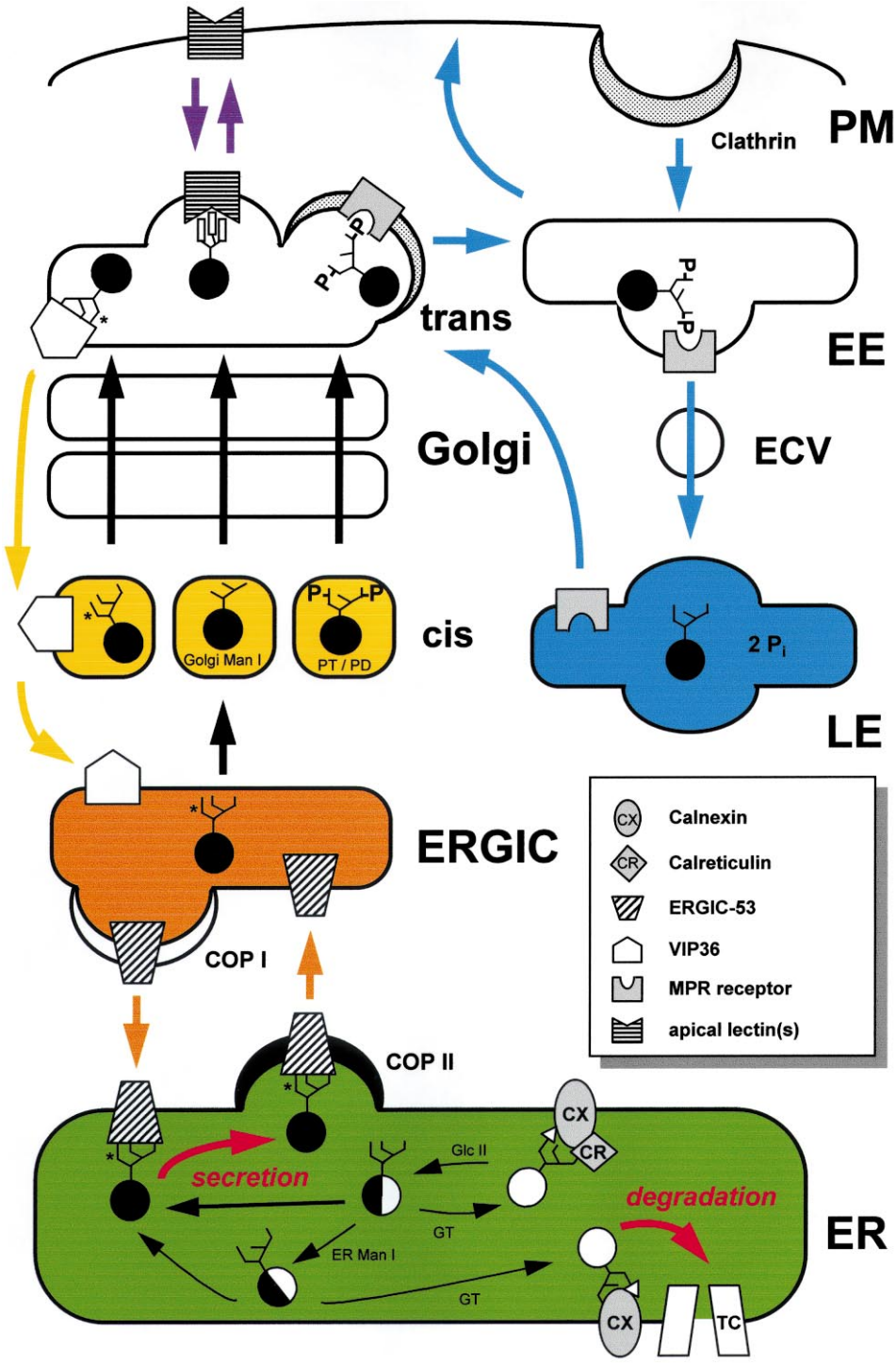

Fig. 1. Lectin-mediated glycoprotein transport in the secretory pathway. After synthesis and removal of the two outermost glucose residues of their *N*-glycans many glycoproteins bind to the ER lectins calnexin and/or calreticulin which recognize monoglucosylated *N*-glycans. Subsequently the glycoproteins are trimmed by glucosidase II (GlcII) and, if still incompletely folded (marked in white), reglucosylated by GT which redirects them to another cycle of quality control. After prolonged residence time in the ER, ER mannosidase I (ER Man I) removes one mannose residue of the middle branch of the *N*-glycan. Incompletely folded, and thus reglucosylated, Man_8 glycoproteins are targeted for calnexin-dependent retrotranslocation to the cytosol and subsequent degradation by the proteasome. By contrast, correctly folded proteins (marked in black) are no longer recognized by GT after deglucosylation by GlcII and are transport-competent. They may or may not undergo some additional trimming by mannosidase I and II before leaving the ER. Some of these Man_{7-9} glycan-bearing proteins (*) now bind to the lectin ERGIC-53 which recruits them to COPII buds and thereby facilitates transport to the ERGIC. Dissociation of ERGIC-53 and its glycoprotein ligand occurs in the ERGIC and free ERGIC-53 recycles to the ER via COPI vesicles. In the *cis*-Golgi, glycoproteins are either trimmed to Man_5 prior to reglucosylation by Golgi glycosyltransferases, or tagged with the lysosomal signal Man-6-P by sequential action of phosphotransferase (PT) and phosphodiesterase (PD). Some glycoproteins escape *cis*-Golgi trimming but may be recognized by VIP36 in the *trans*-Golgi and recycled to the *cis*-Golgi for another trimming attempt. Proteins carrying Man-6-P residues are recognized by MPRs in the *trans*-Golgi and sorted to endosomes via clathrin-coated vesicles. Secreted Man-6-P-bearing glycoproteins can also be internalized from the plasma membrane by the large MPR. *N*-Glycans also serve as signals for Golgi exit and apical targeting in epithelial cells. These processes may also be mediated by lectins that are, however, unknown. ECV, endosomal carrier vesicles; EE, early endosome; LE, late endosome; TC, translocation channel.

likely that in *C. elegans* ERGIC-53 affects the transport of yet other glycoproteins. Direct evidence for a transport receptor function was obtained in hamster cell cultures by chemical crosslinking [42]. These experiments identified a glycoprotein related to human cathepsin Z as a ligand for ERGIC-53. Cloning confirmed that this protein is indeed the hamster homolog of human cathepsin Z, a close relative of cathepsin C (unpublished). Thus, ERGIC-53 is the first identified cargo receptor for protein transport from ER to ERGIC.

Collectively the experiments with ERGIC-53 suggest the following mechanism (Fig. 1). ERGIC-53 recognizes glycoproteins carrying high-mannose *N*-glycans in the ER in a calcium-dependent manner. Studies with deoxymannojirimycin indicate that mannose trimming down to Man_7 is not required for binding to ERGIC-53. Although ERGIC-53 is an oligomeric protein forming disulfide-linked dimers and hexamers the monomer can bind cargo glycoproteins in vivo [42]. Efficient ER exit of ERGIC-53 is determined by a complex interplay of numerous determinants including oligomerization [42], particular amino acids in the transmembrane domain (unpublished) and a C-terminal diphenylalanine ER exit determinant (Fig. 2) [35]. Peptide binding studies in vitro indicate that the diphenylalanine motif mediates binding to COPII coat proteins which is the likely mechanism by which ERGIC-53 is selectively recruited to COPII vesicles [35]. In *C. elegans* the most C-terminal phenylalanine is replaced by tyrosine and in yeast Emp47p the diphenylalanine motif is replaced by two leucines suggesting that other hydrophobic amino acids also mediate efficient ER exit (Fig. 2). Dissociation of cargo occurs in the ERGIC by a mechanism that remains to be elucidated, but mannose trimming is not required for dissociation [42]. After dissociation, ERGIC-53 recycles to the ER in COPI vesicles, a process that is blocked by numerous agents including brefeldin A [29]. ERGIC-53 possesses a dilysine ER targeting signal [43] that binds COPI proteins [35,44] (Fig. 2).

While ERGIC-53 null mutations in humans apparently lead to no other phenotype than reduced levels of the blood coagulation factors V and VIII, the above mentioned experiments with *C. elegans* and *Drosophila*, as well as with mammalian cell cultures, indicate that ERGIC-53 also affects the transport of an increasing number of other glycoproteins. This raises the question if other lectins can compensate for the lack of ERGIC-53. The only known homolog of ERGIC-53 is VIP36 (see below) but due to its different lectin properties and localization it appears less likely that VIP36 can replace ERGIC-53. If other lectins are responsible for this compensation they would in all likelihood not be homologous to ERGIC-53. Extrapolating from quality control in the ER in which calnexin/calreticulin-dependent processes can be compensated to some extent by non-lectin chaperones it is conceivable that the lack of ERGIC-53 can be compensated by other non-lectin transport receptors mediating ER-to-Golgi transport. The only other currently known transport receptor is Emp24p in yeast [45]. A knock-out of the corresponding gene in yeast delays transport of invertase and Gas1p and deficiency of the Emp24p-related protein p23 in mice is embryonically lethal [46]. Yet another possibility is that in normal cells efficient transport of many glycoproteins is dependent on ERGIC-53 and that ERGIC-53-defective cells would largely bypass this requirement by increasing the synthesis of the ERGIC-53-dependent glycoproteins. Close to normal amounts of these proteins could therefore leave the ER by bulk flow. In that sense, ERGIC-53, and selective transport from the ER in general, would function as an energy saver.

ERGIC-53	-10									-1	
Human, monkey, Xenopus	R	S	Q	Q	E	A	A	A	K K	**F**	**F**
Rat	R	T	Q	Q	E	A	A	A	K K	**F**	**F**
Drosophila	K	N	R	S	E	A	Q	A	K K	**F**	**F**
C.elegans	R	S	K	Q	D	-	-	K	A K	**F**	**Y**
Yeast (Emp47)	R	I	R	Q	E	I	I	K	T K	**L**	**L**
Yeast(Emp47 homolog)	R	I	N	Q	D	I	K	K	V K	**L**	**L**
VIP36											
Human, dog	Q	K	R	Q	E	R	N	-	K R	**F**	**Y**
C.elegans	Q	K	K	N	E	R	Q	R	K R	**F**	**Y**

Fig. 2. Targeting motifs in the cytosolic domain of the traffic lectins ERGIC-53 and VIP36. ER exit determinants are bold-faced and the ER-targeting dilysine signal is underlined. Numbering is from the carboxy-terminal end with the last amino acid indicated as −1. Note that both proteins have an ER exit determinant but only ERGIC-53 also possesses a dilysine signal operating in COPI vesicle-mediated retrograde transport.

4. Sorting in the Golgi

The Golgi is the next sorting station in the secretory pathway after the ERGIC. Sorting occurs all along the *cis–trans* axis. It involves sorting of anterograde and retrograde traffic in *cis*-, medial-, and *trans*-cisternae as well as sorting to post-Golgi compartments in the *trans*-Golgi network (TGN) [47,48]. A putative sorting lectin is the type I integral membrane glycoprotein VIP36 (Table 1). VIP36 is homologous to ERGIC-53, and was initially thought to cycle between TGN and plasma membrane [49]. More recent immunofluorescence studies suggest, however, localization to *cis*-Golgi and some ERGIC elements, and previous cell surface localization is now believed to be non-physiological due to overexpression [50]. At steady state there is overlap but not identity with the localization of ERGIC-53, but the patterns of the two proteins entirely merge after brefeldin A treatment suggesting that VIP36 recycles between Golgi and ERGIC [50]. Distribution of VIP36 more to the Golgi may be due to the fact that VIP36 lacks a cytosolic dilysine ER retention/retrieval signal [49,51] (Fig. 2). Unlike (artificially) glycosylated ERGIC-53, VIP36 rapidly acquires endoglycosidase H resistance in pulse-chase experiments indicating that the protein has access to the medial-Golgi and presumably recycles from all levels of the Golgi. However, no recycling motif has been identified yet. The lectin specificity of VIP36 has been studied in detail [52,53]. VIP36 shows specificity for high-mannose type glycans of the $Man_{6-9}GlcNAc_2$ structure but efficient binding requires the additional presence of an α-substituted asparagine residue. Contrary to ERGIC-53, VIP36 does not require calcium for ligand binding [50], and it may also recognize GalNAc residues [54].

The function of VIP36 is currently unknown. Collectively, the features of VIP36 suggest a function in quality control in the Golgi. We speculate (Fig. 1) that VIP36 recognizes glyco-

proteins that have escaped glycan trimming by *cis*-Golgi mannosidase I to the $Man_5GlcNAc_2$ structure and recycles them back for an additional round of trimming. Due to the slightly acidic pH optimum VIP36 binding to incorrectly trimmed cargo, and hence retrieval efficiency, would increase from *cis*- to *trans*-Golgi. This would make sense because correct trimming by Golgi mannosidase I may be possible at least up to the medial-Golgi [55]. Once correctly trimmed, the glycoproteins can no longer bind to VIP36 and move on in the secretory pathway.

Lectins also play a role in glycoprotein exit from the Golgi. As mentioned in Section 1, MPRs are the paradigm for traffic lectins in the secretory pathway [56,57]. Two MPRs are known (Table 1). Both are type I integral membrane proteins and constitute the P-type family of animal lectins. The 275 kDa cation-independent MPR also binds insulin-like growth factor (IGF) II. The 46 kDa receptor requires cations for ligand binding. Both receptors recognize Man-6-P-modified lysosomal hydrolases in the TGN and sort them into clathrin-coated vesicles destined for endosomes (Fig. 1). In the acidified endosomes the enzymes dissociate and undergo dephosphorylation. Hydrolases bearing glycans with two Man-6-P residues show about 10-fold higher binding than those with a single Man-6-P residue which is in line with the dimeric nature of the receptors [58,59]. Both receptors have access to the plasma membrane but only the large MPR can internalize lysosomal enzymes and IGF II. Knock-out experiments in mice indicate that each MPR has distinct but overlapping functions for the targeting of lysosomal enzymes in vivo [57,60]. Trafficking of the MPRs depends on numerous sorting signals in the cytosolic domain of the proteins that determine Golgi exit, internalization at the plasma membrane and recycling from endosomes [57,61–63]. MPR recycling from endosomes to the TGN is clathrin-independent but the nature of the transport carrier is unknown.

Studies with polarized epithelial cells suggest that lectins may be involved in glycoprotein transport from the Golgi to the apical cell surface [64]. Apical targeting has been attributed to different types of sorting signals, including *N*-linked and *O*-linked carbohydrates [65]. Introduction of *N*-glycosylation sites into non-glycosylated soluble or membrane proteins can target them to the apical cell surface indicating that *N*-glycans operate as apical targeting signals [66,67]. Basolateral targeting determinants are generally dominant over the apical glycan signal. In the absence of specific sorting signals, transmembrane proteins accumulate in the Golgi suggesting that exit from the Golgi is signal-mediated. It is interesting to note that *N*-glycans also mediate Golgi exit in non-polarized cells [67]. It is conceivable therefore that membrane lectins sort glycoproteins into transport vesicles in the TGN (Fig. 1), but such lectins remain to be discovered. Because this sorting process does not require complex glycosylation (K. Matter, unpublished) one would predict that the postulated Golgi lectins recognize the Man-GlcNAc core of glycoproteins. VIP36 was initially postulated as a candidate for apical glycoprotein sorting [49,53] but because VIP36 is localized to the early rather than the late secretory pathway [50] such a function now appears less likely.

5. Conclusions

Lectins play important roles in quality control and protein traffic along the secretory pathway. The studies with ERGIC-53 point to a novel function of lectins in the secretory pathway: facilitation of glycoprotein transport from ER to ERGIC. Although lectin-dependent transport from the ER would be a conceptually striking general solution for selective transport of all glycoproteins it appears that only a limited set of glycoproteins depend on ERGIC-53. It is currently unknown if there are other lectins that can also operate as receptors for glycoprotein transport from ER to Golgi. Other challenges for the future include the elucidation of the mechanism by which ERGIC-53 recognizes glycoprotein cargo in a selective way, the determination of the role of VIP36 in the secretory pathway and the search for the postulated lectins mediating Golgi exit. This research is likely to have an impact on understanding fundamental mechanisms underlying selective protein transport and sorting in the secretory pathway in health and disease. An increasing number of inherited or acquired diseases are due to inefficient secretion of key molecules. Understanding the molecular basis of these defects may lead to the development of new strategies for the treatment of these diseases.

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