Protein Retention in the Golgi Stack

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1 Introduction

The entire output of proteins newly synthesized in the endoplasmic reticulum (ER) is funneled through the Golgi stack and sorted once it reaches the *trans* Golgi network (TGN) (Griffiths and Simons 1986). Up until this point, transport occurs by default, no signals being needed for proteins to move from the ER to the Golgi and from cisterna to cisterna within the stack (Rothman and Orci 1992). This immediately raises the question of how proteins are retained along the secretory pathway; how do they resist transport to the TGN if such transport has no need of specific signals?

Two mechanisms appear to be responsible for retention along the secretory pathway. The best characterized is the retrieval mechanism which acts to recover both soluble (Pelham 1989) and membrane proteins (Jackson et al. 1993) that have been inadvertently lost from the compartment in which they normally function, or to return proteins that are part of a recycling pathway (Sweet and Pelham 1992).

Less well characterized is the retention mechanism which provides the primary means of keeping proteins in the correct compartment. Recent work on both Golgi (Swift and Machamer 1991; Nilsson et al. 1991; Munro 1991; Teasdale et al. 1992; Wong et al. 1992; Colley et al. 1992; Aoki et al. 1992; Russo et al. 1992; Tang et al. 1992; Burke et al. 1992) and ER (Wozniak and Blobel 1992; Smith and Blobel 1993) proteins shows that the retention signal lies in the membrane-spanning domain.

2 The Membrane-Spanning Domain

As an example of the evidence pointing to this domain as the retention signal, we will describe, briefly, the work we have done on the *trans* Golgi enzyme, β 1,4 galactosyl-transferase (GalT) (Nilsson et al. 1991). Parts of this protein were grafted onto a reporter molecule, the human invariant chain, and the location of the hybrid proteins determined using immunofluoresence and immuno-electron microscopy, metabolic labeling and FACS analysis. The results are summarized in Fig. 1.

The human invariant chain (Ii), when transiently expressed in HeLa cells, was mostly found in the plasma membrane and early endosomes. When the cytoplasmic domain was replaced by that from GalT (GalT-I), the same results were obtained

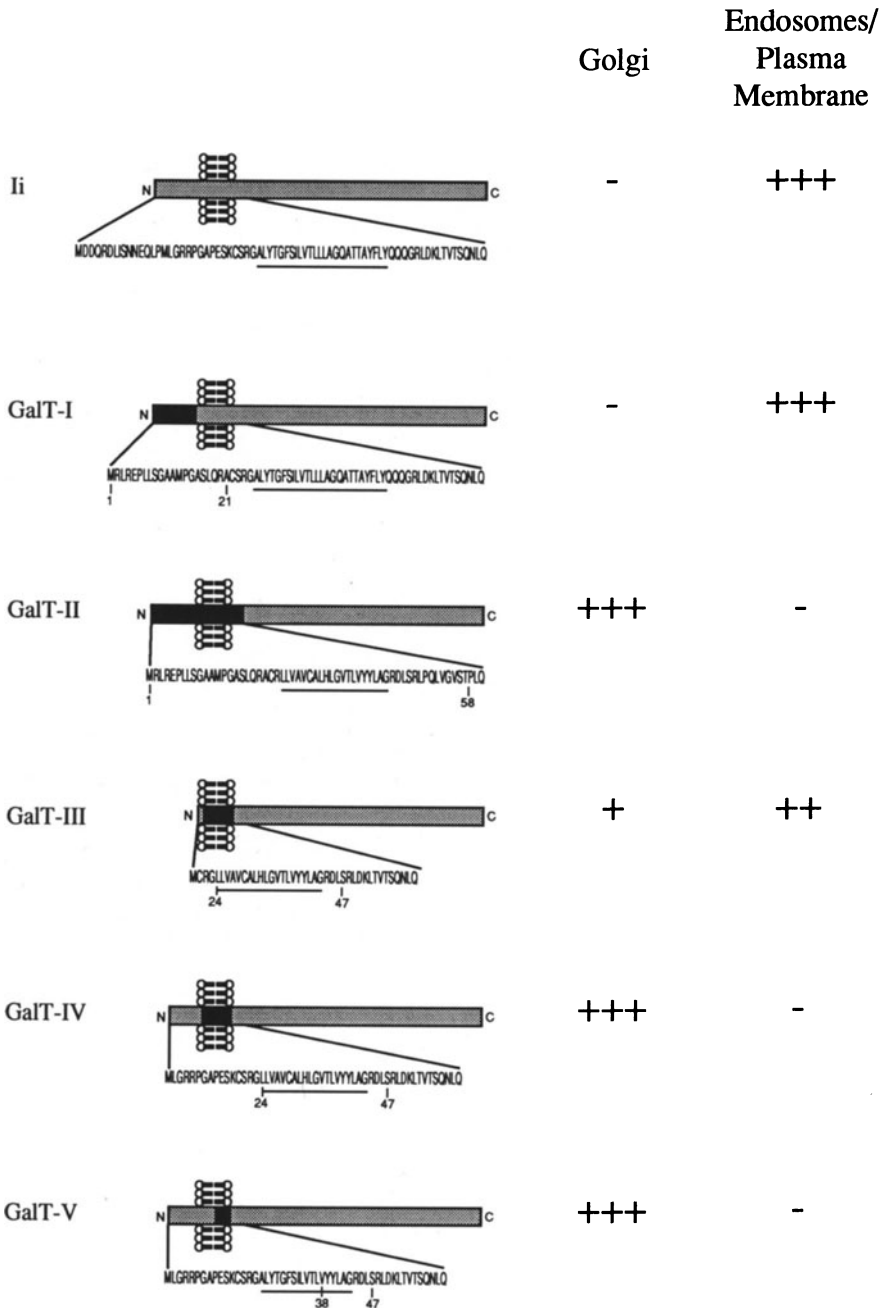|	Golgi	Endosomes/ Plasma Membrane
Ii	-	+++
GalT-I	-	+++
GalT-II	+++	-
GalT-III	+	++
GalT-IV	+++	-
GalT-V	+++	-

Fig. 1. Topology of the reporter molecule (Ii) and hybrid proteins containing parts of the GalT cytoplasmic and/or membrane-spanning domains. The location of these proteins in transiently transfected HeLa cells is shown *on the right*

showing that the tail alone could not specify retention in the Golgi apparatus. When the cytoplasmic and membrane-spanning domains of Ii were replaced by those from GalT (GalT-II), the hybrid proteins were now found only in the Golgi apparatus. Though these results would suggest that the membrane-spanning domain alone should specify retention, truncation of the cytoplasmic domain (GalT-III) caused some of the hybrid protein to appear on the plasma membrane. Grafting on the cytoplasmic domain of Ii (GalT-IV) restored Golgi retention, showing that it does play a role but one which can be provided by the cytoplasmic domain of the reporter molecule. Further analysis showed that only ten amino acids in the spanning region of GalT (GalT-V) were required for retention in the *trans* cisterna, the location being confirmed by immuno-electron microscopy (Nilsson et al. 1991).

Results using such transient expression systems have been confirmed using stable cell lines and, insofar as morphological studies have been carried out (Burke et al. 1992), the morphology of the Golgi apparatus is indistinguishable from that in the parental cell line. Here we report an interesting exception.

3 The 10.40 Cell Line

HeLa cells were stably transfected with the cDNA encoding the hybrid protein detailed in Fig. 2 to generate the stable cell line 10.40. The hybrid protein comprised the cytoplasmic and membrane-spanning domains of murine $\alpha 1,2$ mannosidase II (Mann II) together with part of the stalk region, and the lumenal domain of Ii. As expected, the hybrid protein was present in the Golgi apparatus by a number of criteria, including immunofluorescence microscopy, but the surprise came when the cells were examined by electron microscopy.

In marked contrast to the parental HeLa cell line (Fig. 3) which had typical stacks of closely apposed and flattened cisternae, the Golgi in the 10.40 cell line comprised large numbers of vesicles associated with residual cisternae which formed disorganized stacks (Fig. 4). Careful examination of the associated vesicles (Fig. 5) showed that many had the size and morphology of Golgi transport vesicles (Malhotra et al. 1989). Others had the morphology of clathrin-coated vesicles (Pearse 1987).

The explanation for this morphology is far from clear, but one intriguing possibility is a consequence of the fact that the native Ii appears to be a trimer (Marks et al. 1990). We had earlier suggested that retention occurred because the Golgi enzymes interacted with each other through their membrane-spanning domains, forming oligomers too large to enter the vesicles budding from the dilated cisternal rims
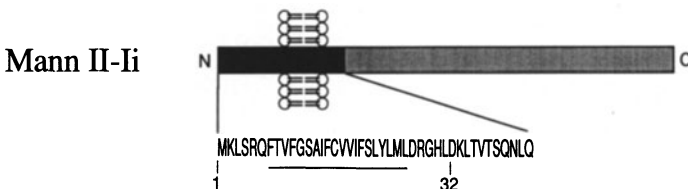
Mann II-Ii

N   C

MKLSRQFTVFGSAIFCVVIFSLYLMLDRGHLDKLTVTSQNLQ

1   32

Fig. 2. Topology of the Mann II-Ii hybrid protein stably expressed by the 10.40 cell line

Fig. 3. Morphology of the Golgi apparatus in the parental HeLa cell line. Note the stacks of closely apposed and flattened cisternae. *Bar* 0.2 μm
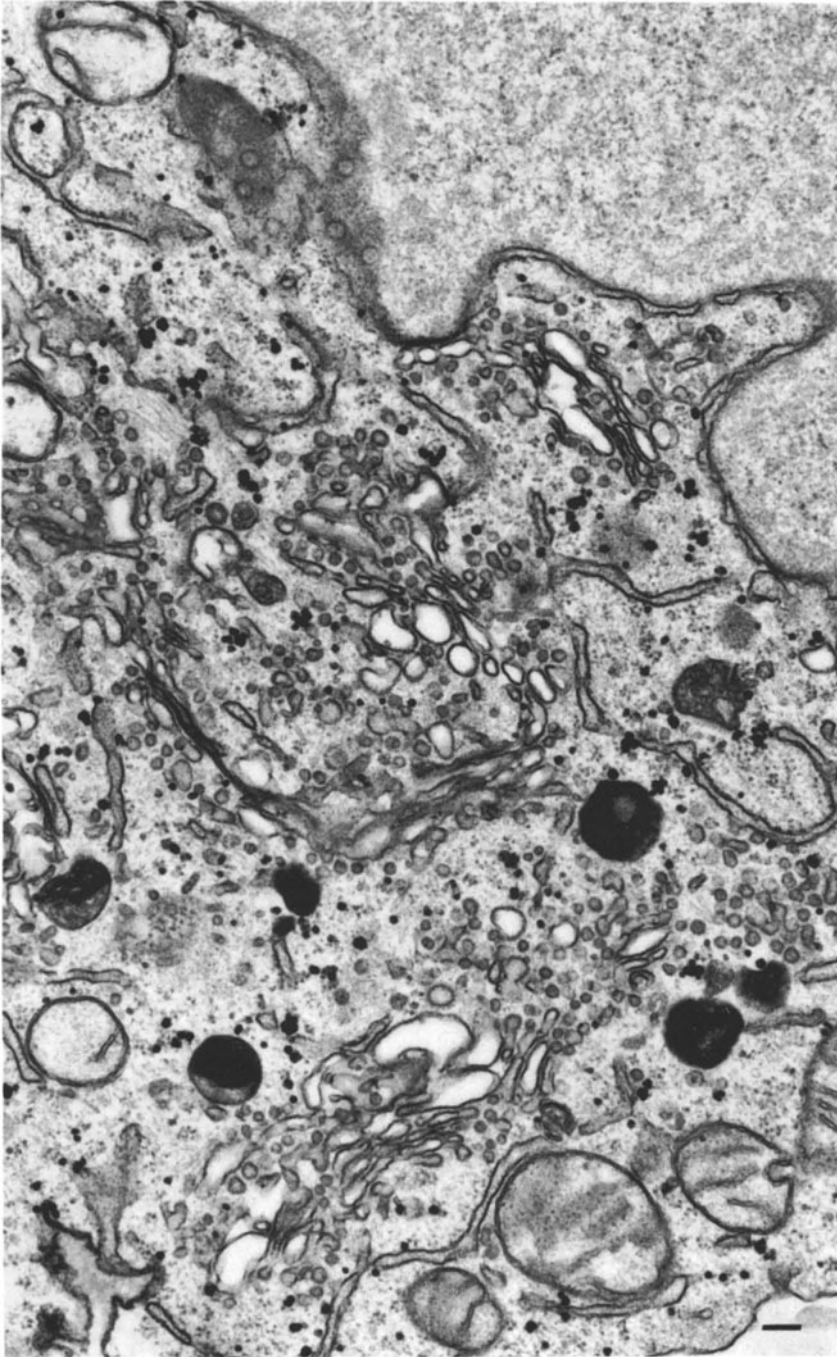
Fig. 4. Morphology of the Golgi apparatus in the 10.40 HeLa cell line. Note the large increase in the number of Golgi-associated vesicles and the disorganized nature of the stacks that remain. *Bar* 0.2 μm
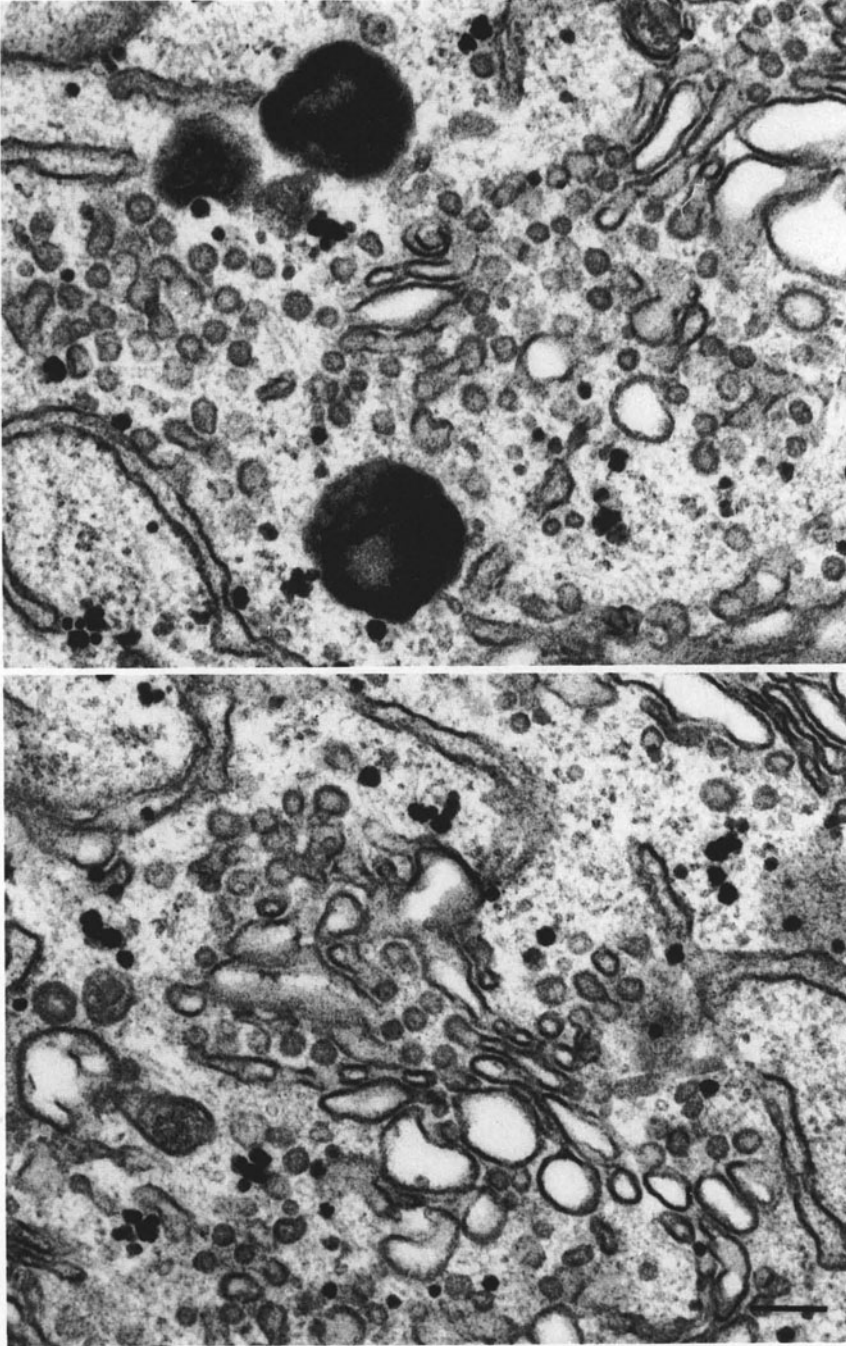
Fig. 5. Morphology of the Golgi apparatus in the 10.40 HeLa cell line. Enlargement of two of the regions in Fig. 4 showing both coated (COP and clathrin) and uncoated vesicles. *Bar* 0.2 μm

(Nilsson et al. 1991). We have recently shown that Golgi enzymes sharing the same cisterna can interact with each other (Nilsson et al. 1994). Since all Golgi enzymes so far analyzed are dimers both in vitro and in vivo (Navaratnam et al. 1988, Khatra et al. 1974; Moremen et al. 1991; Fleischer et al. 1993), this means that the oligomers would be long, linear structures. Though this would obviously aid the action of the Golgi enzymes on their substrates as they passed through the cisterna, it is difficult to see how a linear oligomer, no matter how long, could be prevented from at least partially entering the budding vesicles. We therefore suggested that these oligomers were anchored to an intercisternal matrix and we have recently obtained evidence for such a postulate (Slusarewicz et al. 1994).

If the Mann II-Ii hybrid protein is a trimer, then this could have one of two consequences. First, it could convert the linear oligomers into large, three-dimensional enzyme aggregates. This might be expected to improve retention of Golgi enzymes, but we had earlier obtained evidence without realizing it at the time that such aggregates inhibit intracellular transport. We had micro-injected mRNA encoding the monoclonal antibody 53FC3 (Burke and Warren 1984). This antibody was synthesized in the ER of micro-injected cells and transported to the Golgi apparatus, where it stopped, presumably because it was bound to Mann II. By so doing, however, it blocked transport through the Golgi apparatus of the VSV G protein, suggesting that large aggregates are incompatible with Golgi function. In the 10.40 cell line, transport is normal as shown by pulse-chase experiments following the transport of histocompatability antigens (HLA). Figure 6 compares transport to the medial and *trans* cisternae in both
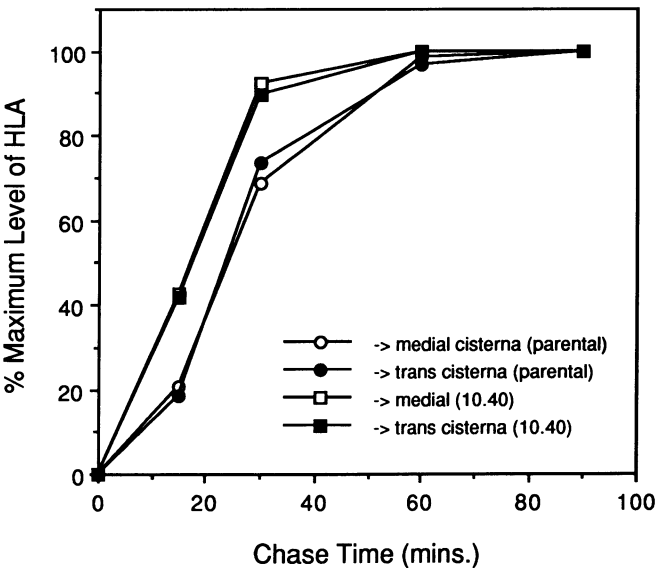

Fig. 6. Intracellular transport of HLA in the parental and 10.40 HeLa cell lines. HLA was pulsed with ^{35}S-methionine and chased for increasing times before immuneprecipitation and fractionation by SDS-PAGE. Transport to the medial cisterna was monitored by the acquisition of resistance to endoglycosidase H and to the *trans* cisterna by the acquisition of sialic acid residues detected by treatment with neuraminidase

the parental HeLa and 10.40 cell lines. If anything, transport of HLA was slightly faster in the 10.40 cell line.

The second and more likely consequence of the trimeric nature of the Mann II-Ii hybrid protein is that it somehow destabilizes the oligomers, allowing Golgi enzymes to enter the budding vesicles (Fig. 7). If this happens, there might be a mechanism to correct this error which causes these vesicles to fuse with the cisternae from which they have just budded. Such fusion events normally occur at the end of mitosis, when thousands of dispersed mitotic Golgi vesicles associate and fuse to re-form the different cisternae in the stack (Lucocq et al. 1989). In interphase cells, however, fusion with the original cisterna would constitute futile cycling, which would slow down the transport of proteins through the Golgi stack. In order to survive, the 10.40 cell line might have compensated by increasing the number of vesicles budding from each cis-

Parental HeLa cell line

10.40 HeLa cell line
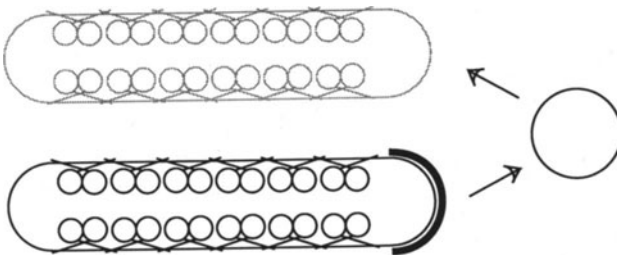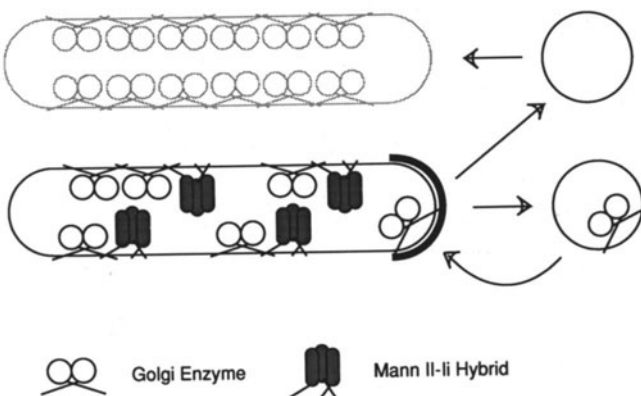

Golgi Enzyme   Mann II-Ii Hybrid

Fig. 7. model to explain the large number of vesicles seen in the 10.40 cell line. The trimeric nature of the Mann II-Ii hybrid protein is assumed to break up the oligomers that normally retain Golgi enzymes in the cisterna. Released enzymes would enter the budding vesicles, which could then no longer fuse with the next cisterna in the stack, but would fuse back with the cisterna from which they had just budded. The simplest way to restore transport to normal levels would be to increase the number of vesicles budding from each cisterna

terna. This would both restore transport to parental levels and explain the large number of transport vesicles observed.

The 10.40 cell line shows yet again that the stacked structure of Golgi cisternae is not essential for efficient transport through it. As more and more Golgi functions are uncovered, this cell line may well prove useful in showing precisely which functions do depend on an intact Golgi stack.

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