REVIEW ARTICLE

Mammalian glycosylation mutants as tools for the analysis and reconstitution of protein transport

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INTRODUCTION

Since the isolation of the first cell lines altered in oligosaccharide assembly in the early 1970s, these mutants have proven to be valuable research tools. Elucidation of the biochemical basis for the defects has led to a greater understanding of the functional significance of glycosylation, the biosynthesis of carbohydrate moieties, and the carbohydrate-binding specificities of various lectins (Briles, 1982; Stanley, 1984, 1987*a,b*). Animal cell glycosylation mutants have also found increasing use as aids to dissect trafficking pathways in the cell. In 1980, Rothman and Fries had constructed the first *in vitro* assay for studying membrane traffic using a glycosylation mutant as an essential component. In the ensuing decade, the availability of defined glycosylation mutants has been crucial for the development of a number of powerful model systems to study protein transport between subcellular compartments of the secretory pathway and made biochemical dissection of them possible. The purpose of this review is to outline aspects of protein traffic in which glycosylation mutants have already contributed new information and to suggest ways in which these cell lines might be exploited in the future.

THE EUKARYOTIC SECRETORY PATHWAY

The secretory pathway of eukaryotic cells consists of a series of distinct membrane-bounded organelles and of transport vesicles that function to transport membrane proteins and lipids between these organelles. These organelles include the endoplasmic reticulum (ER), Golgi compartments, lysosomes, secretory granules and the plasma membrane. Proteins destined for the secretory pathway of eukaryotic cells are synthesized on membrane-bound ribosomes, and inserted co-translationally into the ER membrane. Movement from the ER along the secretory pathway involves a series of consecutive steps involving membrane vesicle budding from one compartment and specific fusion with another (Palade, 1975; Pfeffer & Rothman, 1987). Unless specifically retained as resident proteins, all proteins *en route* traverse the ER and the different compartments of the Golgi complex. Within the *trans*-most Golgi compartment, the TGN, the pathways are thought to diverge (Griffiths & Simons, 1986). A sorting event in the TGN determines whether proteins are constitutively delivered to the cell surface, enter the regulated pathway and are stored in secretory granules, or enter the lysosomal pathway. In simple epithelia, sorting to two distinct cell surface domains, the apical or the basolateral plasma membrane, also occurs in the TGN.

Once delivered to the cell surface, membrane proteins either remain there or undergo endocytosis and recycling (Goldstein *et*

al., 1985; Hubbard, 1989; Gruenberg & Howell, 1989). Internalization of cell surface molecules usually requires segregation into coated pits on the cell surface. Movement to endosomes is then mediated by clathrin-coated vesicles. An alternative clathrin-independent internalization route has also been postulated, but remains as yet ill-defined (van Deurs *et al.*, 1989). In endosomes, three different sorting events can occur. Membrane proteins can be sent for degradation to the lysosomes, return to the cell surface or recycle to the TGN. Epithelial cells offer membrane components the additional possibility to move across the cell to the opposite plasma membrane domain by transcytosis (Mostov & Simister, 1985). Each organelle of the secretory pathway is both a stable as well as a dynamic structure. The compartments are characterized by a set of resident proteins which carry out compartment-specific functions. The extensive flow of proteins and lipids mediated by transport vesicles that continuously bud and fuse illustrate the dynamic nature of the compartments. In recent years, stable cell lines with fibroblast or epithelial characteristics have made it possible to identify and follow proteins travelling along specific pathways, while perforated cells and cell-free systems have emerged to allow the study of the biochemistry of interorganelle protein transport (Featherstone, 1988; Balch, 1989; Goda & Pfeffer, 1989; Wilschut, 1989).

Oligosaccharide chains of glycoproteins are assembled while the newly synthesized proteins are moving through the secretory pathway. Every compartment along the pathway, from ER to the TGN, is equipped with a set of enzymes to carry out the required reactions. Therefore, movement of glycoproteins through the organelles of the secretory pathway can be monitored by analysing the processing and elongation of oligosaccharides. Glycosylation mutants which are defective in a particular step of oligosaccharide synthesis and, thus, contain immature glycoproteins, have turned out to be particularly useful for the development of cell-free systems (Fries & Rothman, 1980; Balch *et al.*, 1984*a*; Beckers *et al.*, 1987; Goda & Pfeffer, 1988) and more recently for monitoring movement between different cellular compartments *in vivo* (Duncan & Kornfeld, 1988; Brändli & Simons, 1989; Brändli *et al.*, 1990).

ASSEMBLY OF N-LINKED GLYCANS AND SUBCELLULAR LOCALIZATION OF GLYCOSYLATION REACTIONS

The usefulness of glycosylation mutants to study protein transport between two intracellular compartments depends on the biochemical pathways that lead to the synthesis of mature carbohydrates. Glycoproteins can carry carbohydrates attached to asparagine (*N*-linked glycans), and to serine or threonine (*O*-linked glycans). Since almost all of the mammalian glycosylation

Abbreviations used: BFA, brefeldin A; ER, endoplasmic reticulum; LCA, *Lens culinaris* agglutinin; MPR, cation-independent mannose 6-phosphate receptor; NEM, *N*-ethylmaleimide; PHA, phytohemagglutinin from *Phaseolus vulgaris*; SA, sialic acid; TGN, *trans*-Golgi network; VSV, vesicular stomatitis virus; WGA, wheat germ agglutinin; GTP γ S, guanosine 5'-[γ -thio]triphosphate.

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Fig. 1. Simplified model of the *N*-linked glycosylation pathway

For simplicity not all of the intermediates are shown. The model is adapted from Carraway & Hull (1989). ●, GlcNAc; ○, Man; ■, Glc; □, Gal; ▼, sialic acid.

mutants characterized to date are affected in the biosynthesis of *N*-linked glycans, this pathway will be described briefly. The details of the *N*-linked and *O*-linked glycosylation have been reviewed previously (Kornfeld & Kornfeld, 1985; Carraway & Hull, 1989). The biosynthesis of *N*-linked glycans is a multistep process which proceeds in a strictly sequential manner. Every step in oligosaccharide synthesis can be localized either to the ER or the Golgi apparatus (Roth, 1987). Two main steps can be distinguished (Fig. 1). The first step occurs in the ER and involves the assembly of a lipid-linked oligosaccharide chain followed by transfer *en bloc* onto the nascent protein. The second step consists of trimming and elongation of the oligosaccharide chains. This step is initiated in the ER and completed in the Golgi apparatus. The final structures can be grouped into high mannose-type, hybrid-type, and complex-type oligosaccharides.

The lipid-linked oligosaccharide chain is formed by a series of complex reactions in the ER. The assembly of the oligosaccharide begins by linking a GlcNAc residue via a pyrophosphate linkage to the lipid carrier, dolichol. By stepwise addition of further sugar residues the precursor oligosaccharide is assembled to Glc₃Man₉GlcNAc₂. This series of reactions occurs partially on the cytoplasmic and partially on the luminal face of the ER. The topography of the individual steps is still unclear (Hirschberg & Snider, 1987; Lennarz, 1987). The assembled precursor oligosaccharide is subsequently transferred *en bloc* to an asparagine residue of a growing polypeptide chain emerging into the lumen of the ER. This reaction is catalysed by oligosaccharyltransferase. The enzyme recognizes asparagines within Asn-Xaa-Ser/Thr sequences (with Xaa being every amino acid except Pro) available for glycosylation. Many, but not all, of the sites containing these consensus sequences are modified. Therefore, other structural features must discriminate between the used and unused sites.

Processing is initiated by the removal of all glucose residues by the sequential action of glucosidase I (for the terminal one) and glucosidase II (for the remaining glucose residues). An ER-specific mannosidase removes a single mannose residue to form the Man₈GlcNAc₂ structure. The newly synthesized glycoproteins destined for later compartments exit from the ER by means of vesicles and are delivered to the *cis*-Golgi cisterna. Recent evidence suggests that there is a vesiculo-tubular compartment, called the salvage compartment (Warren, 1987), located between the ER and the *cis*-Golgi compartment (Saraste & Kuismanen, 1984). It could also be that this tubular network is part of the *cis*-cisterna and forms a *cis*-Golgi network. At present, it is unclear how this compartment functions in respect to *N*-linked oligosaccharide assembly (Pelham, 1989). Newly

synthesized glycoproteins move from the *cis*-side of the Golgi through medial and *trans* cisternae to the TGN (Balch & Rothman, 1985; Pfeffer & Rothman, 1987).

For processing of complex-type oligosaccharides, the Golgi mannosidase I removes three further mannose residues, resulting in a Man₅GlcNAc₂ structure. The addition of a GlcNAc residue yielding GlcNAc₁Man₅GlcNAc₂ is catalysed by *N*-acetylglucosaminyltransferase I and this enzyme has been localized to the medial Golgi cisternae. Subsequently, Golgi mannosidase II removes a further two mannose residues. The addition of a second GlcNAc residue by *N*-acetylglucosaminyltransferase II resulting in GlcNAc₂Man₃GlcNAc₂ terminates the reactions attributed to the medial Golgi. Synthesis of complex glycans is continued by addition of galactose residues by galactosyltransferase in the *trans*-Golgi cisternae and completed by sialyltransferase-mediated transfer of sialic acid residues in the TGN. Fig. 1 depicts the major intermediate structures of complex oligosaccharide synthesis and their subcellular location. Each step in the synthesis of *N*-linked oligosaccharides requires the presence of the appropriate acceptor and nucleotide sugar, as well as the glycosyltransferase enzyme assembled in the correct compartment. Defects in localization of one of the components leads to altered glycosylation (Deutscher *et al.*, 1984; Chaney *et al.*, 1989). All the Golgi glycosyltransferases face the lumen and are supplied with nucleotide sugars by specific antiporter systems. One note of caution is necessary regarding the Golgi subcompartmentalization. Although glycosyltransferases have usually been localized to defined Golgi cisternae, they may have diffuse distributions throughout the Golgi stack in some cells (Roth, 1987; Yuan *et al.*, 1987; Gonatas *et al.*, 1989; Lee *et al.*, 1989). Whether there is a similar diffuse distribution of the corresponding nucleotide sugar antiporters and nucleotide diphosphatases remains to be proven. Detailed immunoelectron-microscopy will be required using simultaneous labelling for two consecutive Golgi markers to substantiate the specialized scheme drawn in Fig. 1, which reflects present interpretations.

ISOLATION OF GLYCOSYLATION MUTANTS

Most glycosylation mutants have been obtained by exposing cell lines to lectins and selecting for those cells that survive this treatment. Lectins are proteins of nonimmune origin capable of non-covalent binding to specific oligosaccharide structures on cell surfaces (Table 1). They were first discovered in plants, but recently have also been found in animals and invertebrates (Barondes, 1981; Lis & Sharon, 1986; Sharon & Lis, 1989; Mandel & Mandel, 1990). Usually lectins exhibit multivalent

Table 1. Commonly used lectins

Source of lectin	Acronym	Sugar inhibitors
<i>Canavalia ensiformis</i>	Con-A	D-Man
<i>Lens culinaris</i>	LCA	D-Man
<i>Ricinus communis</i>	RCA	D-Gal
<i>Bandeirea simplicifolia</i>	BS-II	D-GlcNAc
<i>Triticum vulgaris</i>	WGA	D-GlcNAc, sialic acid
<i>Phaseolus vulgaris</i>	PHA	D-GalNAc
<i>Glycine max</i>	SBA	D-GalNAc
<i>Ulex europaeus</i>	UEA	L-Fuc
<i>Limax flavus</i>	LFA	Sialic acid

binding to oligosaccharides and can therefore agglutinate cells. Differential agglutinability of different cell types by lectins has been useful in fractionating heterogeneous populations of cells (Reisner & Sharon, 1984). Some lectins are mitogenic for specific cell types, allowing the experimental synchronization of mitosis. A number of lectins are toxic to mammalian cells. For example, the galactose-specific lectin ricin is one of the most potent toxins known. Ricin is internalized by endocytosis and after translocation into the cytosol acts by modifying ribosomal 28 S RNA and thus leads to the inhibition of protein synthesis (Endo & Tsurugi, 1987; Endo *et al.*, 1987). Cytotoxic lectins have turned out to be widely useful selective agents to isolate glycosylation-defective mutants.

There are a variety of ways in which mammalian cells may develop resistance to specific cytotoxic lectins. Lectin resistance is usually caused by an alteration of oligosaccharide structures on the cell surface resulting in decreased lectin binding. The biochemical basis of several glycosylation defects has been identified. Mutations affecting enzyme activities at all stages of oligosaccharide synthesis have been isolated. They include mutations affecting formation of activated sugars (Kingsley *et al.*, 1986), the synthesis of the mature oligosaccharide-dolichol (Chapman *et al.*, 1979; Krag, 1979; Hunt, 1980; Rosenwald & Krag, 1990), the activity of glycosidases (Reitman *et al.*, 1982), glycosyltransferases (Gottlieb *et al.*, 1975; Toyoma *et al.*, 1983), and the transport of nucleotide sugars (Deutscher *et al.*, 1984; Deutscher & Hirschberg, 1986; Brändli *et al.*, 1988). Miscompartmentalization of enzymes involved in glycosylation has also been reported recently (Chaney *et al.*, 1989). Several of the mutants that affect the biosynthesis of *N*-linked oligosaccharides are pleiotropic, causing modifications of *O*-linked oligosaccharides as well as oligosaccharides of glycolipids. For example, mutations in the UDP-galactose antiporter reduce the level of UDP-galactose in the lumen of the Golgi complex, thereby affecting all glycoconjugates that contain galactose (Brändli *et al.*, 1988). Some mutants are resistant to lectins for other reasons, and therefore are not referred to as glycosylation mutants. For example, mutants may display normal binding, but be defective in internalization of the lectin. Alternatively, alterations of the primary cellular target of the toxin can result in lectin resistance. Mutant CHO cell lines with both phenotypes have been described (Ray & Wu, 1982; Ono *et al.*, 1982).

Glycosylation mutants have proved to be invaluable tools for analysing the biosynthetic pathway of oligosaccharide assembly. They are also useful in a number of other ways. Mutants displaying defined carbohydrate alterations have been used to determine the binding specificity of novel lectins, monoclonal antibodies directed against cell surface carbohydrates and bacterial toxins (Witvliet *et al.*, 1989). Since adherence of parasites and viruses to host cells can also be mediated by carbohydrates, glycosylation-defective cell lines can be helpful in elucidating the

nature of the host receptor (Li *et al.*, 1988). This strategy has also been successful in identifying carbohydrate ligands of leukocyte-endothelial cell adhesion molecules (Phillips *et al.*, 1990). The role of carbohydrates in determining structural and functional properties of glycoproteins can be investigated by transfection of the cloned gene into an appropriate mutant (Kingsley *et al.*, 1986; Reddy *et al.*, 1989). In addition, glycosylation mutants can serve as convenient factories to synthesize glycoproteins with specifically altered carbohydrate moieties (Robertson *et al.*, 1978; Krieger *et al.*, 1989; Stanley, 1989). Glycosyltransferases are found in small quantities in animal cells and are generally difficult to purify. This has hampered cloning of glycosyltransferase genes by conventional methods. Recently, a dominantly acting glycosyltransferase gene has been successfully isolated by cDNA transfection and plasmid rescue (Larsen *et al.*, 1989). A cell line that does not normally express the glycosyltransferase was used. Similar strategies were employed using glycosylation mutants as host cells to isolate a cDNA clone encoding GlcNAc transferase I (Kumar *et al.*, 1990). Other applications of glycosylation mutants can be found elsewhere (Stanley, 1987b).

GLYCOSYLATION MUTANTS USED IN PROTEIN TRANSPORT STUDIES

Since 1972, several laboratories have isolated stable, glycosylation-defective lines from a variety of different mammalian cells. Mutants have been derived from most of the cell lines frequently used in molecular and cellular biology, such as Chinese hamster ovary (CHO) cells, HeLa, NIH 3T3, and baby hamster kidney (BHK) cells (Briles, 1982; Stanley, 1984, 1987a). Also, two glycosylation mutants of epithelial origin, MDCK II-ConA^r and MDCK II-RCA^r, which differentiate in culture to form polarized epithelial monolayers, have been described (Meiss *et al.*, 1982). For a number of these mutants, biochemical analysis has localized the defect to specific steps in the synthesis of carbohydrates. The precise knowledge of the biochemical basis of the lesions is a prerequisite for the successful use of glycosylation mutants. It should be noted, however, that in no case has the molecular basis of the mutation, e.g. altered gene, been identified. The recent success in cloning and expression of several glycosyltransferases opens up the possibility of addressing such questions in the future (Paulson & Colley, 1989; Kumar *et al.*, 1990). Biochemically defined glycosylation mutants that are useful in the analysis and reconstitution of membrane traffic are listed in Table 2.

Glycosidase-deficient mutants

BW5127 PHA^R2.7. This lectin-resistant cell line displays altered oligosaccharides on the cell surface due to a deficiency in a hydrolytic enzyme of the glycosylation pathway. The PHA^R2.7 variant of the mouse lymphoma cell line was selected for resistance to the cytotoxic lectin PHA (Trowbridge *et al.*, 1978). Oligosaccharide analysis revealed Glc₂Man₆GlcNAc₂ and Glc₂Man₈GlcNAc₂ structures. This suggests a defect in a trimming reaction occurring in the ER. Enzyme assays *in vitro* demonstrated that mutant cells contain active glucosidase I, but are deficient in glucosidase II (Reitman *et al.*, 1982). Therefore, the defect is probably restricted to the assembly of *N*-linked oligosaccharides. Surface appearance of altered ER-type oligosaccharide structures in BW5127 PHA^R2.7 suggests that removal of glucose residues is not required for exit of newly synthesized glycoproteins from the ER.

L CL6. A further glycosylation mutant defective in a glycosidase of the glycosylation pathway of *N*-linked oligosaccharides was selected from mouse L cells. The ricin-resistant variant CL6

Table 2. Biochemical phenotypes of glycosylation mutants used in protein transport studies

The Table was adapted from Stanley (1984, 1987a). The origins of the different parental cell lines are: BW5147, mouse lymphoma; CHO, Chinese hamster ovary (fibroblast); L, mouse lung (fibroblast); MDCK II, canine kidney (epithelial); PC 12, rat pheochromocytoma.

Parental line	Mutant line	Altered step	Mature carbohydrates
BW5147	PHA ^R 2.7	α -Glucosidase II ↓	Glycoproteins possess unprocessed Glc ₂ Man ₆ GlcNAc ₂ . Complex carbohydrates decreased.
L	CL6	α -Mannosidase I ↓	Glycoproteins possess unprocessed Man ₆ GlcNAc ₂ . Complex carbohydrates decreased.
CHO	Clone 15B Lec 1	GlcNAc transferase I ↓	Blocked at the Man ₆ GlcNAc ₂ processing intermediate. Complex carbohydrates decreased.
CHO	Clone 13 Lec 8	UDP-Gal → glycoproteins or glycolipids	Glycoproteins and glycolipids decreased in Gal-sialic acid residues. Complex glycans terminate in GlcNAc.
BW5127 MDCKII PC12	PHA ^R 1.8 RCA ^r A1		
CHO	Clone 1021 Lec 2	CMP-SA → glycoproteins or glycolipids	Glycoproteins and glycolipids decreased in sialic acid and terminate in Gal.

was found to have decreased amounts of membrane-associated sialic acid, galactose and GlcNAc residues, but increased content of mannose (Gottlieb & Kornfeld, 1976). The oligosaccharide chains of vesicular stomatitis virus (VSV) glycoprotein G expressed in L CL6 cells are mainly Man₆GlcNAc₂, and, thus, only partially trimmed. This indicates a deficiency in Golgi mannosidase I (Tabas & Kornfeld, 1978).

Glycosyltransferase-deficient mutants

CHO clone 15B. Clone 15B was isolated from CHO cells on the basis of resistance to ricin (Gottlieb *et al.*, 1974). Several other mutant CHO cell lines displaying the same phenotype have been selected on the basis of their cross-resistance to the lectins WGA, PHA and LCA by Stanley *et al.* (1975a). The best characterized of these mutants is called CHO Lec 1 (for nomenclature, see Stanley, 1983). CHO clone 15B and Lec 1 cells have the same biochemical defect. Carbohydrate analysis revealed significant decreases in the contents of sialic acid, galactose and GlcNAc relative to wild-type cells and a 50% increase in mannose content (Gottlieb *et al.*, 1974). These observations were consistent with the idea that mutant cells are deficient in the synthesis of *N*-linked complex oligosaccharides. Analysis *in vitro* of glycosyltransferases was performed to identify the biochemical basis for the oligosaccharide defects. Gottlieb *et al.* (1975) reported a complete lack of GlcNAc transferase activity in clone 15B cells, while Stanley *et al.* (1975b) observed a 50% decrease in GlcNAc transferase activity in Lec 1 cells. The discrepancy was attributed to a difference in the acceptor molecules used. This suggested that wild-type CHO cells possess at least two GlcNAc transferases with different specificities, and that only one of these is active in mutant cells. Analysis of the endogenous substrate for GlcNAc transferase which accumulates in the mutants revealed, surprisingly, Man₆GlcNAc₂ rather than the expected Man₃GlcNAc₂ structure (Tabas *et al.*, 1978; Robertson *et al.*, 1978). Further studies on the mutants defined two new enzyme activities GlcNAc transferase II (Narasimhan *et al.*, 1977), and mannosidase II (Tabas & Kornfeld, 1978). These findings established that removal of further mannose residues from the Man₆GlcNAc₂ structure cannot occur unless GlcNAc transferase I transfers a GlcNAc to form GlcNAc₁Man₆GlcNAc₂. Mannosidase II will subsequently remove two additional mannose residues. The resulting GlcNAc₁Man₃GlcNAc₂ structure is then the substrate for GlcNAc transferase II (Tabas & Kornfeld, 1978). The accumulation of the Man₆GlcNAc₂ structure in CHO clone 15B cells and Lec 1 is therefore the direct consequence of a GlcNAc

transferase I deficiency. Recently, cDNA clones encoding GlcNAc transferase I have been isolated, and Northern blot analysis have provided evidence that the *lec1* mutation is a point mutation (Kumar *et al.*, 1990; Sarkar *et al.*, 1991).

Mutants defective in nucleotide sugar transport

CHO clone 1021. Two laboratories have isolated and characterized CHO cell lines which have an 80–90% decrease in sialic acids. These cell lines are unable to incorporate sialic acid into glycoproteins and glycolipids. They were isolated for resistance to the lectin WGA and were termed CHO clone 1021 (Briles *et al.*, 1977) and CHO Wga^{RII} (Stanley *et al.*, 1975a; renamed to Lec 2, Stanley, 1983). Glycoprotein and glycolipid sialyltransferase activities and CMP-sialic acid levels of CHO clone 1021 cells were similar to wild-type cells (Briles *et al.*, 1977). Therefore, the alteration might be due to a block in transfer of CMP-sialic acid into the Golgi apparatus. With an assay *in vitro*, Lec 2 cells were unable to translocate CMP-sialic acid across membranes of isolated Golgi complexes. Transport of other nucleotide sugars, however, was comparable to that of wild-type Golgi (Deutscher *et al.*, 1984). These results were important for two reasons. First, they provided genetic evidence for the postulated nucleotide sugar translocations in the Golgi apparatus. Second, they suggested that there are separate translocator proteins for each nucleotide sugar (Perez & Hirschberg, 1986; Hirschberg & Snider, 1987).

CHO clone 13. Another class of lectin-resistant CHO cells has been found to synthesize altered glycoproteins and glycolipids deficient in both galactose and sialic acids. These mutants were isolated independently by selecting for resistance to WGA and were termed CHO clone 13 (Briles *et al.*, 1977) and CHO Lec 8 (Stanley, 1980, 1981, 1985). The defect in these cell lines could not be traced to a reduction in the levels of UDP-galactose or CMP-sialic acid, endogenous acceptors of galactosyl- and sialyltransferase activities (Briles *et al.*, 1977; Stanley, 1980, 1981). Deutscher & Hirschberg (1986) showed that the primary defect in these mutants was due to their inability to translocate UDP-galactose into Golgi preparations, while CMP-sialic acid translocation was not impaired. Therefore, the lack of sialic acid residues in glycoconjugates is an indirect consequence of the mutants not being able to synthesize appropriate galactose-bearing substrates for sialyltransferase. Although UDP-GlcNAc has the UDP moiety in common with UDP-galactose, transport of UDP-GlcNAc appears to be normal in the mutant. This again

Table 3. Model systems for protein transport studies using glycosylation mutants

Transport step	Type of model system	Cells (donor/acceptor)	Assay
ER → <i>cis</i> -Golgi	<i>In vitro</i> /perforated cells	L CL6/CHO clone 15B	Trimming to Man ₅ GlcNAc ₂
<i>Cis</i> → medial Golgi	<i>In vitro</i>	CHO clone 15B/wild type CHO	Transfer of GlcNAc
<i>Trans</i> -Golgi → TGN	<i>In vitro</i>	CHO clone 1021/wild type CHO	Transfer of sialic acid
Prelysosomes → TGN	Perforated cells	CHO clone 1021/wild type hepatocytes	Transfer of sialic acid
Plasma membrane → TGN	Intact cells	CHO clone 13, BW5127 PHA ^R 1.8, PC12A1 or MDCK II-RCA ⁺	Transfer of sialic acid
Transcytosis	Intact cells	MDCK II-RCA ⁺	Exogalactosylation and surface biotinylation

suggests unique translocator proteins must exist for different nucleotide sugars (Perez & Hirschberg, 1986; Hirschberg & Snider, 1987). Several other mutants exhibiting a similar phenotype have been isolated: PHA^R1.8 from the mouse lymphoma cell line BW5127, (Trowbridge *et al.*, 1978; Duncan & Kornfeld, 1988); BSI.B4 from the mouse fibroblast cell line 3T3 (Stanley *et al.*, 1979); MDW4 from the tumour cell line MDAY-D2 (Dennis, 1986), and A1 from the rat pheochromocytoma cell line PC 12 (Green & Kelly, 1990).

MDCK II-RCA⁺. This epithelial cell line was isolated as a ricin-resistant variant of MDCK II cells (Meiss *et al.*, 1982). MDCK II-RCA⁺ cells carry a defect in the assembly of galactosylated and sialylated glycoconjugates resembling the phenotype of CHO clone 13 cells. The alteration affects both *N*- and *O*-linked glycosylation of glycoproteins, as well as glycolipid synthesis (Brändli *et al.*, 1988). Mutant cells possess functional galactosyl- and sialyltransferases. The uptake of UDP-galactose into isolated Golgi vesicles, however, is impaired. As for CHO clone 13 cells, translocation of UDP-GlcNAc and CMP-sialic acid into the Golgi complex is unaffected (Brändli *et al.*, 1988). Although MDCK II-RCA⁺ cells appear to have such a severe defect in the synthesis of glycoconjugates, they form functional epithelial monolayers in culture. This suggests that terminal glycosylation of glycoconjugates is not required to maintain a polarized phenotype (Green *et al.*, 1981; Meiss *et al.*, 1982; Brändli *et al.*, 1988).

MODEL SYSTEMS BASED ON GLYCOSYLATION MUTANTS TO STUDY INTERORGANELLAR PROTEIN TRANSPORT

With the elucidation of the glycosylation defects displayed in various mutant cell lines, it was recognized that these mutants could be used in the analysis of distinct steps of the secretory pathway. Model systems based on biochemically defined glycosylation mutants are summarized in Table 3 and will be discussed in detail.

Protein transport between the *cis*- and medial-compartments of the Golgi complex

The first model system to study early stages of the secretory pathway was pioneered by Rothman and colleagues (Fries & Rothman, 1980; Balch *et al.*, 1984a). A cell-free system was established to study vectorial transport of a viral glycoprotein, vesicular stomatitis virus (VSV) G protein, through compartments of the Golgi complex. During viral infection, G protein will replace the entire population of endogenous, newly synthesized proteins in the secretory pathway. The assay made use of the glycosylation mutant CHO clone 15B that lacks functional

GlcNAc transferase I, normally present in the medial cisternae of the Golgi complex. Therefore, CHO clone 15B cells infected with VSV cannot add GlcNAc residues to G protein in the Golgi. Transfer between two successive Golgi compartments was measured in the following way (Fig. 2). Postnuclear supernatants were prepared from VSV-infected CHO clone 15B cells which had been pulse-labelled with [³⁵S]methionine ('donor') and from uninfected wild-type CHO cells ('acceptor'). Donor and acceptor fractions were mixed and incubated at 37 °C. Following incubation, some of the G protein matured and was observed to contain GlcNAc residues, as measured by resistance to attack by endoglycosidase H. Transport was shown to be dependent on ATP and a soluble, cytosolic fraction (Fries & Rothman, 1980). The observed maturation of G protein implied that the transport process was dissociative, probably mediated by vesicles, and that the specificity *in vivo* of membrane traffic was retained *in vitro*. In further studies, the nature of acceptor and donor fraction were characterized in detail. It was shown that the crude acceptor extract from CHO cells could be replaced by a purified Golgi fraction from rat liver (Rothman & Fries, 1981). The capacity of the pulse-labelled VSV-infected fraction to serve as donor was transient, and indicated together with other independent lines of physical and enzymic evidence that Golgi membranes served as principal donor in the cell-free assay (Fries & Rothman, 1981).

Detailed biochemical analysis of this model system was greatly facilitated by an improved version of the transport assay utilizing highly purified Golgi fractions (Balch *et al.*, 1984a). *Cis*-to-medial-Golgi transport was now measured as a function of [³H]GlcNAc incorporation into the viral G protein, permitting rapid analysis. Electron microscopic studies and autoradiographic methods suggested that G protein was transported in sealed vesicles (Braell *et al.*, 1984). Careful kinetic analysis revealed intermediates in the transfer process which accumulated by *N*-ethylmaleimide (NEM) treatment (Balch *et al.*, 1984b). Further factors were discovered. Fatty acyl coenzyme A was found to act as a cofactor required for transport (Glick & Rothman, 1987; Pfanner *et al.*, 1989). Guanosine 5'-[γ-thio]-triphosphate (GTPγS) irreversibly inhibited transport of the viral G protein, probably by interfering with the processing step between vesicle attachment to the acceptor Golgi stack and the actual fusion step (Melançon *et al.*, 1987). The most remarkable outcome of this biochemical approach was the isolation and cloning of the NEM-sensitive factor (NSF), a cytosolic component that complements the block in transport caused by NEM (Block *et al.*, 1988; Wilson *et al.*, 1989). The amino acid sequence of NSF turned out to be very similar to that of SEC18p, a yeast cytosolic protein required for ER-to-Golgi transport (Novick *et al.*, 1980) and for endocytosis (Riezman, 1985). Both transport steps are dependent on functional NSF also in higher eukaryotic cells (Diaz *et al.*, 1989; Beckers *et al.*, 1989). In order to perform

Fig. 2. Cell-free assay to detect transport of a protein between successive Golgi compartments *in vitro*

A donor fraction containing Golgi membranes is prepared from VSV-infected CHO clone 15B cells, defective in GlcNAc transferase I. This fraction is incubated with an acceptor fraction also enriched in Golgi, but prepared from wild-type CHO cells. Incorporation of GlcNAc into G protein occurs when G protein is transferred in a dissociative process between the two Golgi complexes. Scheme adapted from Balch *et al.* (1984a). ●, GlcNAc; ○, Man.

Fig. 3. Model system to detect transport of VSV G protein from ER to the *cis*-Golgi cisternae

The donor ER is prepared from mouse L CL6 cells defective in the processing of $\text{Man}_8\text{GlcNAc}_2$ oligosaccharide chains of G protein. Alternatively, perforated L CL6 cells can serve as donor. *In vitro*, the donor is incubated in the presence of an acceptor Golgi compartment containing mannosidase I prepared from the CHO clone 15B cell line. Trimming of the oligosaccharide chains to the $\text{Man}_8\text{GlcNAc}_2$ structure occurs only when G is transported between the two distinct compartments. Scheme adapted from Balch *et al.* (1987). ●, GlcNAc; ○, Man.

its function NSF requires both an integral membrane-protein receptor (Weidman *et al.*, 1989) and soluble low-molecular-mass 'SNAP' proteins (Clary *et al.*, 1990). Although the exact role of these components is unknown, it is evident that the cell-free system will permit a detailed biochemical dissection in the future. The most recent developments have been reviewed elsewhere (Balch, 1990; Rothman & Orci, 1990).

Protein transport between late compartments of the Golgi complex

Based on the methodology established for the *cis*-to-medial-Golgi cell-free system by Balch *et al.* (1984a), Rothman (1987) reported the reconstitution *in vitro* of protein transport between the *trans*-compartment of the Golgi and the TGN. Delivery to the TGN was measured as incorporation of [^3H]sialic acid into G

of endo D-sensitive G protein measures transport of the Golgi cisternae (Beckers *et al.*, 1987). In wild-type cells, this structure is transient, and will be modified to GlcNAc₁Man₅GlcNAc₂, which is endo D-resistant. CHO clone 15B cells, however, are not able to generate this structure due to a deficiency of GlcNAc transferase I, and therefore G protein remains permanently sensitive to endo D. CHO clone 15B cells can thus serve as acceptors for G protein. On the other hand, the glycosylation mutant L CL6, deficient in Golgi mannosidase I, accumulates the endo-D-resistant structure Man₅GlcNAc₂ structure. Therefore, these cells were chosen to 'donate' G protein in the cell-free assay (Fig. 3). It turned out to be difficult to construct an efficient cell-free system. Transport-competent ER could not be isolated from interphase cells, probably because intact ER cisternae were required (Balch *et al.*, 1987). The use of ER preparations from mitotic cells as donor fraction was more successful, suggesting that they might fuse to form intact ER cisternae upon incubation (Balch *et al.*, 1987). With such a donor fraction, however, unambiguous differentiation between the components required for ER reassembly from those required for transport of G protein to the Golgi complex was not possible.

Perforated or semi-intact cells can be prepared by selective rupture of the plasma membrane. They become permeable to chemicals, antibodies and even to vesicles or organelles (Simons & Virta, 1987; Bennett *et al.*, 1988). As shown by Beckers *et al.* (1987), viral G protein can be efficiently transferred from the ER of perforated cells to an exogenously supplied Golgi compartment. Perforated ts045 VSV-infected mouse L CL6 cells (as donor) were mixed with a post-nuclear supernatant from CHO clone 15B as acceptor fraction. Transport measured by the conversion of G protein to the endo D-sensitive form was dependent on ATP and cytosol added exogenously (Beckers *et al.*, 1987). Due to the use of glycosylation mutants, the observed trimming could only be explained by transfer of G protein from distinct donor ER to acceptor Golgi compartments *in vitro*. Further biochemical analysis, using a simplified version of the original assay, revealed the requirement for calcium and GTP (Beckers & Balch, 1989) and the involvement of the NEM-sensitive protein, NSF, functioning also in intra-Golgi protein traffic (Beckers *et al.*, 1989). The biochemical details of ER-to-Golgi transport have been reviewed recently (Balch, 1989, 1990; Wilschut, 1989).

Protein traffic from prelysosomal compartments to the TGN

The most recent demonstration of reconstitution of protein traffic *in vitro* using glycosylation mutants was reported by Goda & Pfeffer (1988). They described the construction of a cell-free system in which movement of the cation-independent mannose 6-phosphate receptor (MPR) from prelysosomal compartments to the TGN occurred. MPRs play an important role in the biogenesis of lysosomes by carrying newly synthesized soluble lysosomal enzymes from the TGN to a prelysosomal compartment. After discharging, lysosomal enzymes are delivered to lysosomes, and MPRs return to the TGN to initiate a new round of sorting (von Figura & Hasilik, 1986; Kornfeld, 1987; Kornfeld & Mellman, 1989; Griffiths *et al.*, 1988).

Using extracts from perforated cells, Goda & Pfeffer (1988) described a novel complementation scheme that permitted detection of the transport step of MPRs moving from the prelysosomal compartment to the TGN. The assay took advantage of the fact that sialyltransferase is localized exclusively in the TGN (Roth *et al.*, 1985). Thus, the addition of sialic acid to oligosaccharides of the MPR can be used to mark the arrival in the TGN. In order to obtain suitable substrates for such an assay, the mutant cell line CHO clone 1021 was utilized. In this glycosylation-defective cell line, N-linked oligosaccharides ter-

minate in galactose due to a defect in the uptake of the sialyltransferase substrate CMP-sialic acid into the Golgi complex (Deutscher *et al.*, 1984). The assay was carried out as follows. ³⁵S-labelled CHO clone 1021 cells are osmotically swollen and perforated to prepare cell extracts that serve as donor fraction. Wild-type Golgi complexes (acceptor fraction) were isolated from rat liver homogenates. Donor and acceptor fractions supplemented with ATP and a crude cytosol preparation were mixed and incubated. If ³⁵S-labelled MPRs from CHO clone 1021 cells were transported to wild-type TGN, they should acquire sialic acid (Fig. 4). Sialylation was determined by lectin affinity chromatography. Goda & Pfeffer (1988) demonstrated that transport *in vitro* to the TGN occurred selectively, requiring GTP hydrolysis. Transport was not inhibited by the weak base primaquine. NEM treatment of the cytosolic fraction completely inhibited movement of the MPR. Addition of the purified Golgi factor NSF could not overcome the block, indicating that a distinct NEM-sensitive factor is needed (Goda & Pfeffer, 1989). In contrast to endocytosis, clathrin did not seem to be involved in the recycling of MPRs to the TGN *in vitro* (Draper *et al.*, 1990). Further analysis is needed to reveal which components are shared between the various cell-free systems for protein traffic and which are unique to the particular transport steps and, therefore, are responsible for specificity.

Protein traffic from the cell surface to the TGN

In contrast to cell-free systems reconstituting protein transport *in vitro*, model systems utilizing glycosylation mutants have been recently established to study movement of cell surface glycoproteins to either subcompartments of the Golgi (Duncan & Kornfeld, 1988; Brändli & Simons, 1989; Green & Kelly, 1990) or to other plasma membrane domains in epithelial cells (Brändli *et al.*, 1990). These assays have in common a requirement for intact cells and methods to modify cell surface glycoproteins specifically. They permit detection of movement to the target compartment and the identification of molecules moving along the particular pathway studied. However, they are of limited use for detailed biochemical analysis of the mechanisms involved.

Duncan & Kornfeld (1988) characterized the movement of the MPR from the cell surface to the TGN. Cell-surface-localized MPRs have been implicated in recapturing of secreted lysosomal enzymes (von Figura & Hasilik, 1986; Kornfeld, 1987). Recaptured lysosomal enzymes bound to cell surface MPRs will be internalized and delivered to prelysosomal compartments, where lysosomal enzymes dissociate to reach lysosomes. On the other hand, MPRs are believed to return to the TGN to initiate a new sorting cycle. The experimental approach taken by Duncan & Kornfeld (1988) to demonstrate movement of cell surface MPRs to the TGN was based on the observation that sialyltransferase is localized to the TGN (Roth *et al.*, 1985). Cell surface MPRs modified to be substrates of sialyltransferase would indicate delivery to the TGN by acquisition of sialic acid. The experimental approach used two glycosylation mutants, CHO clone 13 and BW5147 PHA^R1.8, both impaired in galactosylation of glycoconjugates. The mutants are, therefore, enriched in N-linked oligosaccharides terminating with GlcNAc residues which are readily labelled with galactose *in vitro* and serve now as excellent substrates for sialyltransferase. The assay was initiated by treating whole cells with galactosyltransferase and UDP-galactose to label cell-surface-exposed MPRs with galactose. Galactosylated MPRs entering the TGN are sialylated by sialyltransferase (cf. Fig. 5). The process of sialylation of MPRs was shown to be time- and temperature-dependent and proceeded also in the absence of synthesis of lysosomal proteins, indicating

that cell-surface MPRs are indeed recycling to the TGN (Duncan & Kornfeld, 1988).

The cell surface-to-TGN transport assay by Duncan & Kornfeld (1988) was extended recently to permit identification of novel glycoproteins moving along this pathway and recycling back to the cell surface (Brändli & Simons, 1989). Instead of glycosylation mutants of fibroblast origin, the epithelial glycosylation mutant MDCK II-RCA^r was utilized. The apical and basolateral domains of epithelial cells both communicate with the TGN by protein traffic (Brändli & Simons, 1989; Prydz *et al.*, 1990). As CHO clone 13, MDCK II-RCA^r cells display altered cell surface oligosaccharide chains due to a defect in the uptake of UDP-galactose into the Golgi complex. The strategy used to detect delivery to the TGN is shown in Fig. 5. Cell surface glycoproteins were labelled by galactosyltransferase-mediated galactosylation as described by Duncan & Kornfeld (1988). At the end of the incubation, however, sialylated species reappearing on the cell surface were modified by a membrane-impermeable radioactive marker selective for sialic acid residues. Radiolabelled sialoglycoproteins were analysed by two-dimensional gel electrophoresis and compared with the sialoglycoprotein pattern of ungalactosylated control cells. Recycling of cell surface glycoproteins through the TGN was a highly selective process. Only a subset of the apical and the basolateral proteins were found to recycle to the TGN. Of the basolaterally-located glycoproteins, recycling of the cation-independent MPR through the TGN was observed (Prydz *et al.*, 1990). Three apical glycoproteins were found to move to the TGN (Brändli & Simons, 1989).

Transcellular protein transport in epithelial cells

Certain cell surface molecules in epithelial cells can move from one plasma membrane domain to the other by a vesicle-mediated process referred to as transcytosis (Mostov & Simister, 1985). Examples of such molecules include the polymeric immunoglobulin (Ig) receptor shuttling IgA and IgM molecules in basolateral-to-apical direction across mammary gland cells, and the IgG receptor of the intestinal epithelium of new-born rats responsible for transcellular movement of IgG molecules in the opposite direction. The epithelial cell line MDCK has been used to study transcytosis of exogenous proteins introduced by cDNA transfection (Mostov & Deitcher, 1986). Also, the endogenous MDCK glycoproteins undergo transcytosis as shown by an assay based on the ricin-resistant variant of MDCK, MDCK II-RCA^r (Brändli *et al.*, 1990). If MDCK II-RCA^r cells are grown on permeable supports, glycoproteins can be labelled in a plasma membrane domain-specific manner (Brändli *et al.*, 1988). The experimental strategy outlined in Fig. 6 required galactosyltransferase-mediated labelling of cell surface glycoproteins by [³H]galactose on the starting plasma membrane domain. After incubation, glycoproteins delivered to the opposing plasma membrane were reacted with membrane-impermeable biotin derivatives. Cells were solubilized and biotinylated glycoproteins were precipitated with streptavidin-agarose and analysed by SDS gel electrophoresis and fluorography. Glycoproteins carrying both a [³H]galactose and biotin tags were defined as transcytosing molecules. By starting the assay either at the apical or basolateral side of the cell monolayer, both directions of transcytosis could be analysed. Transcytosis was found to be differentially sensitive to the microtubule-depolymerizing agent nocodazole. While transcytosis to the apical cell surface was perturbed, traffic in the opposite direction was not affected (Brändli *et al.*, 1990; see also Breitfeld *et al.*, 1990; Hunziker *et al.*, 1990). Three classes of glycoproteins undergoing transcytosis in MDCK cells were identified. The first two classes of glycoproteins consisted of molecules which transcytose unidirectionally, either in apical-to-basolateral or in basolateral-to-

apical direction, across the cell. The third class of glycoproteins represented a previously undescribed group of molecules. These molecules were able to shuttle back and forth between the two cell surfaces of the epithelial cell (Brändli *et al.*, 1990). Exact functions for the different classes of transcytosing molecules have not yet been defined. However, they have already been useful as marker molecules to study transcytosis in epithelial cells, such as to determine the role of epidermal growth factor (EGF) receptor in the transport of EGF across MDCK cells (Brändli *et al.*, 1991).

FUTURE DIRECTIONS

As an outlook, some areas of membrane traffic will be outlined where novel protein transport assay based on glycosylation mutants might be helpful in the future. Finally, I want to draw attention to the growing number of yeast glycosylation mutants which might provide an attractive alternative to mammalian glycosylation mutants.

Intra-Golgi protein transport

The Golgi apparatus is subdivided into *cis*-, medial and *trans*-Golgi cisternae and the TGN, all of which are connected by vesicle-mediated protein and lipid traffic (Griffiths & Simons, 1986). *In vitro* systems have been established for two of the three intra-Golgi transport steps (Balch *et al.*, 1984a; Rothman, 1987). Transport between medial and *trans*-Golgi cisternae has to date not been reconstituted *in vitro*. A complementation scheme to monitor the transfer of galactose by the *trans*-Golgi-localized galactosyltransferase to a viral glycoprotein such as VSV G protein could be based on Golgi fractions derived from VSV-infected CHO clone 13 or MDCK II-RCA^r cells. Biochemical analysis of the two established assays for *cis*-to-medial-Golgi and *trans*-Golgi-to-TGN transport has been unable to demonstrate differences between the two. It is unlikely that the factors needed for the common budding and fusion reactions of vesicles during transport will differ from the previously established factors, such as the NEM-sensitive factor NSF or GTP. However, the proposed assay might reveal differences in the fusion step, since specificity is required to ensure that transport vesicles are targeted to the correct acceptor compartment.

Backward traffic within the Golgi apparatus

In general, newly synthesized glycoproteins move unidirectionally, from ER to the TGN, along the secretory pathway (Griffiths & Simons, 1986; Pfeffer & Rothman, 1987). This has been elegantly shown for the transport of VSV G protein in cell fusion experiments using glycosylation-defective CHO cell lines (Rothman *et al.*, 1984). Notably, transfer from later to earlier Golgi compartments was not detected. This simple model has, however, been questioned by the recent discovery of resident glycoproteins of *cis*- and medial Golgi compartments bearing sialic acid residues (Yuan *et al.*, 1987; Gonatas *et al.*, 1989). These findings could be explained by postulating that galactosylation and sialylation reactions are not strictly confined to late Golgi compartments. Alternatively, these glycoproteins could first acquire terminal glycosylation in late Golgi compartments during their biosynthesis, and then be transported back to earlier compartments. This would imply that in addition to the forward vesicle-mediated protein traffic a counter-flow of transport vesicles exists. Such a mechanism could be important to ensure a constant size of the Golgi complex despite extensive protein and lipid traffic to the cell surface. In order to demonstrate the existence of backward traffic within the Golgi complex, transport assays based on mutants defective in Golgi glycosylation could be developed.

Fig. 6. Model system to detect transcytosis of cell surface glycoproteins in epithelial cells

The polarized epithelial cell line, MDCK II-RCA⁺, synthesizes altered *N*-linked oligosaccharide chains of complex type terminating with GlcNAc residues. Apical glycoproteins of filter-grown MDCK II-RCA⁺ cells can be selectively labelled with $[^3\text{H}]$ galactose by exogalactosylation ①. Transcellular transport of labelled apical glycoproteins can occur during incubation at 37 °C ②. Delivery to the basolateral cell surface is detected by selective biotinylation ③. Scheme adapted from Brändli *et al.* (1990). ●, GlcNAc; □, Gal; B, biotin.

Recycling from the *cis*-Golgi to the ER

A group of soluble proteins of the ER have the sequence Lys-Asp-Glu-Leu (KDEL) at their *C*-termini which is required for retention in the ER (Pelham, 1990). KDEL proteins are not simply anchored to the ER membrane by a receptor. Instead, the KDEL-bearing proteins reach the nearest compartment of the *cis*-Golgi or the proposed salvage compartment and are then rerouted back into the ER by a receptor-mediated process (Pelham, 1989). Independent evidence for recycling of com-

ponents from the Golgi to the ER can be drawn from experiments using the drug brefeldin A (Doms *et al.*, 1989; Lippincott-Schwartz *et al.*, 1989, 1990). First attempts using yeast homogenates to reconstitute this novel pathway have so far been unsuccessful (Dean & Pelham, 1990). Alternatively, mammalian glycosylation mutants altered in an ER-located oligosaccharide processing step could be used for an *in vitro* system. The mutant cell line BW5127 PHA^R2.7 defective in glucosidase II (Reitman *et al.*, 1982), displaying immature oligosaccharide chains, appears to be an ideal tool for such a task.

Yeast glycosylation mutants

Biosynthesis of *N*-linked oligosaccharides in yeast and mammalian cells proceeds initially along similar pathways. Assembly of the lipid-linked oligosaccharide precursor, transfer to asparagine residues of proteins, and the initial removal of monosaccharides occur in the ER and are identical in yeast and in mammalian cells. Outer chain carbohydrates in yeast, unlike in mammalian cells, are composed principally of mannose residues. They are assembled by different mannosyltransferases located in the Golgi apparatus (Kukuruzinska *et al.*, 1987; Tanner & Lehle, 1987). For the yeast *Saccharomyces cerevisiae*, several mutants have been generated that are defective in the proper assembly and trimming of *N*-linked oligosaccharides (Ballou, 1982; Kukuruzinska *et al.*, 1987; Deshaies *et al.*, 1989). One of these mutants, *mun1*, defective in Golgi $\alpha 1 \rightarrow 3$ -mannosyltransferase, has been used to establish an *in vitro* assay to measure protein transport between the ER and the Golgi apparatus (Haselbeck & Schekman, 1986). Improved assays based on gently lysed or permeabilized yeast spheroplasts have been reported (Baker *et al.*, 1988; Ruohola *et al.*, 1988). Both assays have turned out to be essential tools for the biochemical analysis of yeast secretory mutants that have temperature-sensitive defects in protein transport (for review, Newman & Ferro-Novick, 1990; Hicke & Schekman, 1990).

The analysis of compartmental organization of the yeast Golgi apparatus has lagged behind the mammalian Golgi complex. However, recent reports suggest that the yeast Golgi apparatus is divided into functionally distinct compartments similar to those of higher eukaryotes (Abeijon *et al.*, 1989; Cunningham & Wickner, 1989; Franzusoff & Schekman, 1989). Intra-Golgi transport may now be investigated by simple extension of the existing ER-Golgi assays using Golgi-localized glycosylation reactions to monitor transport (Franzusoff & Schekman, 1989). In this respect, it is important to mention that, unlike *Saccharomyces cerevisiae*, some yeasts, such as *Schizosaccharomyces pombe*, synthesize *N*-linked oligosaccharide chains containing terminal galactose and GlcNAc residues (Kukuruzinska *et al.*, 1987). In addition, *S. pombe* contains a morphologically well defined Golgi apparatus. Galactosyltransferase from *S. pombe* has been recently purified and will allow the generation of specific antibodies and cloning of the gene coding for galactosyltransferase (Chappell & Warren, 1989). In the future, *S. pombe* might be an ideal model system to extend studies in *S. cerevisiae* on intra-Golgi protein transport, especially between late compartments of the Golgi apparatus.

CONCLUSION

The successful reconstitution of protein transport between *cis*- and medial-Golgi cisternae by Rothman and colleagues exemplifies most convincingly how glycosylation mutants can be used in powerful ways for the analysis of membrane traffic in mammalian cells. The introduction of perforated cells as an alternative experimental system to cell-free assays has allowed reconstitution of protein traffic from ER to Golgi and from prelysosomal compartments to the TGN. Cell-free transport assays and systems based on perforated cells allow direct access to surfaces of membranous compartments exposed to the cytosol. The participation of cytosolic factors required for the budding and fusion reactions occurring during vesicle-mediated protein transport can be elucidated. Intermediates common to all transport steps can be defined. For example, all vesicular fusion events studied so far require ATP and are blocked by GTP γ S, suggesting that ATP and GTP-binding proteins are involved. Differences between the systems might reflect factors involved in directing

specificity and correct targeting of transport vesicles. In contrast, model systems based on glycosylation-defective cell lines to study protein traffic from the cell surface to the TGN or other plasma membrane domains are not readily dissected biochemically. However, they have allowed the description of the intracellular fate of internalized glycoproteins and the identification of previously unknown glycoproteins moving along these pathways.

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