Biochem. J. (1983) **216**, 1–9
Printed in Great Britain

REVIEW ARTICLE

Mutations that influence the secretory path in animal cells

Alan M. TARTAKOFF
Pathology Department, University of Geneva School of Medicine, 1 rue Michel Servet, 1211 Geneva 4, Switzerland

Introduction

The overall paths of intracellular transport of secretory and membrane proteins from the RER to the cell surface and of lysosomal proteins to the lysosome have been clear for some time (Palade, 1975). It is generally agreed that after exit from the RER, via its transitional elements, these proteins gain access to smooth-surfaced membranes, among which the best-characterized component is the stack of cisternae of the Golgi complex, itself a structurally, histochemically and functionally composite organelle (Tartakoff, 1980, 1983*a*; Farquhar & Palade, 1981; Rothman, 1981). Exit from this stack of cisternae is followed by transport via additional vesicular carriers whose nature may depend on the destination in question. It is becoming increasingly clear that the precise itineraries followed are not known and that very little mechanistic information is available. The impressive efficiency and specificity of these transport events have not been accounted for.

Contemporary methods of subcellular fractionation have not yet proven capable of cleanly resolving all the vesicular and cisternal components along those transport paths. However, other experimental approaches have provided partial resolution: for example, there has been a search for pharmacological means of perturbing transport (Tartakoff, 1983*a*,*b*,*c*), as well as the raising of antibodies to the organelles in question and the use of electron-microscopic immunocytochemistry (Bergmann *et al.*, 1981; Burke *et al.*, 1982; Louvard *et al.*, 1982; Roth & Berger, 1982), all in the context of the increasingly well-characterized sequences of post-translational modifications which accompany transport (Tartakoff, 1983*a*). Among these approaches, the analysis of naturally occurring and induced mutant phenotypes has been important in rather disparate situations.

The present text assembles the data for animal cells which pertain to post-synthetic steps in transport, i.e. from the cisternal space of the RER to the ultimate destination. In most cases the mutation alters the structure of the product undergoing transport. In other cases the alteration lies in the machinery of transport itself or in the ability of the cell to accomplish certain post-translational modifications. One might expect that a genetic approach would serve to dissect the sequence of events of transport, and, ultimately, identify gene products which act at selected steps. Nevertheless, constitutive mutations that are globally inhibitory should be lethal. Constitutive trichocyst discharge mutants of *Paramecium* (Cohen & Beisson, 1980; LeFort-Tran *et al.*, 1981) and a set of temperature-sensitive yeast transport mutants (Novick *et al.*, 1980; Schekman & Novick, 1982) have been characterized.

Since the prospect for selective genetic manipulation of animal cells has obviously become a real one, it is certain that studies related to those described will be of increasing importance in the near future.

Abbreviations used: RER, rough endoplasmic reticulum; Ig, immunoglobulin; VSV, vesicular stomatatis virus.

Defective transport of secretory proteins

It has been recognized over the years that a surprisingly large number of myeloma variants arise which synthesize Ig heavy and/or light chains which are degraded intracellularly rather than being secreted. Indeed, one can screen for the non-secretor phenotype by a number of means (Coffino & Scharff, 1971; Lynch *et al.*, 1972; Secher *et al.*, 1973, 1977; Morrison & Scharff, 1975; Cook & Scharff, 1977; Liesegang *et al.*, 1978). Furthermore, although a cell may synthesize only heavy chains and assemble them to dimeric structure, no myelomas secrete only normal heavy chains. If secretion occurs, either (1) intact Ig is secreted, or (2) the cell secretes aberrant heavy chains, with or without light chains, or (3) only light chains are secreted (Franklin & Frangione, 1975; Morrison, 1978; Dunnick *et al.*, 1980; Morrison & Scharff, 1981). Hence the idea that a cell which attempts to secrete free heavy chains finds them toxic, perhaps because of insolubility (Köhler, 1980), or that normal heavy chains unassociated with light chains exhibit a

'marker' which forces them to follow a degradative path.

The approach of cell fusion has provided observations consistent with the latter idea. For example, myelomas which synthesize but do not secrete variant heavy chains (MOPC-315LV-1 or P3-NSII/1) can be fused with a myeloma which secretes light chains. Chain complementation occurs and the formerly non-secreted chain is recovered in secreted Ig (Sonnenshein *et al.*, 1978; Wilde & Milstein, 1980). A similar example is given by studies of the fusion of pre-B lymphocytes with myeloma fusion partners. The pre-B cells contain intracellular heavy (μ) chains which are not secreted, lack surface μ, and do not synthesize light chains. Only if the fusion partner synthesizes light chains do the resulting hybridomas secrete Ig (Burrows *et al.*, 1979; Kloppel *et al.*, 1981). An example of inverse complementation by cell fusion of a myeloma whose rather insoluble light chains normally fail to be secreted (P3-NSI/1) has also been reported (Köhler *et al.*, 1976; Wilde & Milstein, 1980).

This idea might be further explored by transfer of light chain genes to cells that fail to secrete their heavy chains. A closely related line of experimentation has proven feasible: a gene encoding the light chain of a trinitrophenyl-specific hybridoma (Sp603) has been transferred to a mutant of that hybridoma which lacks the light chain in question. Successful transformants secrete anti-trinitrophenyl antibody (Ochi *et al.*, 1983).

A remarkable further access to study of chain complementation has become available through the use of *Xenopus* oocytes injected with mRNA. For example, injection of mRNA coding for either the light or heavy chain of MOPC-21 results in their synthesis without secretion. If both mRNAs are injected, either at once, or with a delay of many hours between injections, intact Ig is secreted (Colman *et al.*, 1982). Complementation leading to secretion is also observed when the MOPC-21 heavy chain mRNA is injected along with mRNA encoding a λ chain variant, which would not be secreted if the heavy chain were not present (Valle *et al.*, 1983).

Although the structure of a number of the human and mouse chain variants has been studied, the presently available information does not lead to generalizations with respect to those covalent or conformational aspects of heavy chain structure which facilitate or impair their ability to be secreted. Such information may be forthcoming with the aid of a recently developed powerful selection procedure for myelomas which produce chain variants. The procedure relies on the ability of an anti-trinitrophenyl-secreting hybridoma (Sp6, an IgM) to kill itself in the presence of complement when the cell surface has been derivatized with trinitrophenol (Köhler & Shulman, 1980; Köhler *et al.*, 1982). Survivors either fail to synthesize or secrete heavy or light chains or produce Ig with altered antigen-binding or complement-binding sites. The study of several of these variants has provided partial information concerning heavy chain domains whose presence and carbohydrate units influence the rate and extent of secretion versus intracellular degradation (Sidman *et al.*, 1981).

A study of variants of a λ-producing subline derived from MOPC-315 has also produced some such information concerning alterations in primary structure which correlate with secretion (Mosmann & Williamson, 1980). In one case a single amino acid replacement in the variable region can determine whether or not a given λ chain is secreted (Wu *et al.*, 1983).

Among the myelomas that synthesize but fail to secrete a given chain there is no case in which the intracellular site(s) of arrest of transport and degradation are known, although in several cases it is clear that such Ig chains are vectorially transported to the cisternal space of the RER and lose their signal sequence. In one case dilation of the RER has been reported (Winberry *et al.*, 1980).

In an earlier line of investigation lymphomas bearing surface IgM were fused with IgG-secreting myelomas (Levy & Dilley, 1978; Laskov *et al.*, 1979; Raschke *et al.*, 1979). In several cases, the fusion products secreted IgM (as well as IgG). In retrospect, now that it is known that the μ_m and μ_s heavy chains, which distinguish membrane IgM from secretory IgM, are generated by different splicing of a common precursor mRNA (Early *et al.*, 1980) these data should most likely not be ascribed to complementation between chains or subtleties along the secretory path (post-translational modifications characteristic of one of the two classes of Ig) but rather to a shift in the μ mRNA precursor splicing pattern contributed by the myeloma partners.

A related example of synthesis of an Ig-derived chain which is not necessarily secreted is that of the J chain. In IgM- and IgA-secreting myelomas the J chain is covalently bound to the secretory Ig. In IgG-secreting cells J is synthesized but not secreted (Mosmann *et al.*, 1978).

Another source of mutant cells that fail to secrete a given protein may be found in the human disease α_1-proteinase inhibitor deficiency. The Z variant allele encodes an inhibitor with a single amino acid replacement. Interference with its intracellular transport is suggested by the accumulation of substantial deposits of inhibitor within the RER of the hepatocyte and a 90% reduction of circulating levels of the protein. The RER inclusions have been recovered from the liver of ZZ homozygotes. The contained antitrypsin has been shown to lack one of four *N*-linked oligosaccharides, to bear an excess of

mannose and to lack terminal sugars, by comparison with the secreted normal protein. These high-mannose units have undergone considerable glucose and mannose trimming (Hercz *et al.*, 1978; Hercz & Harpaz, 1980; Gadek & Crystal, 1983).

In each of the cases of synthesis without secretion, as well in the cases discussed below pertaining to membrane proteins, the existence of the non-secretory phenotype is dependent on the ability of the cell to eliminate the aberrant product. The study of the incidence of chain loss among myeloma variants suggests that certain free heavy chains, once synthesized, are toxic (Köhler, 1980), possibly because they can neither be secreted nor degraded. A discussion of the mechanisms, significance, and modulation of secretory protein degradation is given in a recent review by Bienkowski (1983).

Defective transport of cell membrane proteins

A variety of membrane protein transport defects have been detected. For example, in fibroblasts of individuals suffering from familial hypercholesterolaemia, several low density lipoprotein receptor-defective phenotypes have been identified (Goldstein & Brown, 1982; Tolleshaug *et al.*, 1983). A receptor may be synthesized but not delivered to the surface, it may reach the surface but not bind lipoprotein, or it may bind lipoprotein yet fail to cluster to coated pits after doing so. In the first-mentioned case, where transport does not occur, it is not known at what station along the secretory path the receptor is arrested; however, a major post-translational modification which increases the apparent size of the wild-type receptor from 120 to 150kDa fails to occur. This modification is suspected to reflect elongation of *O*-linked oligosaccharides, an event known to occur within the Golgi complex of other cells (Tartakoff, 1983*a*). These processing and transport defects are not corrected in heterozygotic fibroblasts. Hence, the defects are to be ascribed to structural alterations in the lipoprotein receptor itself.

Transport defects have also been observed for Class I histocompatibility antigens which are composed of heavy and light (β_2-microglobulin) chains. In the human Daudi lymphoma and among immunoselected HLA-A2-negative lymphoma variants, the heavy chains are synthesized but are not present at the cell surface (Ploegh *et al.*, 1979; Krangel *et al.*, 1982). Consistent with the transport defect is the observation, in both cases, that the heavy chain *N*-linked oligosaccharides are not processed to their complex, endoglucosaminidase H-resistant structure. A role in transport for association between the heavy chains and β_2-microglobulin is suggested (1) by the observation that the heavy chain of the HLA-A2 variant in question fails to bind β_2-microglobulin and (2) by fusion experiments using the Daudi cells, which fail to synthesize β_2-microglobulin (Rosa *et al.*, 1983). By analogy with the Ig chain complementation experiments described above, when the Daudi cell is fused with a cell which does synthesize β_2-microglobulin, the heavy chain of the histocompatibility antigen is transported to the cell surface along with it (Klein *et al.*, 1977).

Similar rescue of thymic leukaemia (TL) and histocompatibility (H2) antigen transport has been reported following fusion of a TL^-, $H2^-$ immunoselected thymoma (which fails to synthesize β_2-microglobulin) with a β_2-microglobulin$^+$ cell line (Hyman & Stallings, 1977; Parnes & Seidman, 1982).

Studies of murine class II histocompatibility antigens also suggest that interaction between two different chains is essential for their transport (Murphy *et al.*, 1980). Moreover, by analogy with secretory Ig, studies of murine lymphomas indicate that the presence of light chains is essential for transport of μ_m (Mains & Sibley, 1983).

By far the most extensive of such investigations are, however, those concerned with the expression of the Thy-1 surface antigen by mouse lymphomas. These studies stand as a prototype for all immunoselection experiments resulting in reduced expression of surface markers.

Thy-1 is an 18kDa membrane glycoprotein which bears three *N*-linked oligosaccharides. It associates with the cell surface via its *C*-terminal hydrophobic extremity. Since the most *C*-terminal amino acids are not themselves hydrophobic, it is possible that a tightly or covalently bound lipid is present. Thy-1 is found on the neurons of many species and the T-lymphocytes of selected species. In the mouse, it is the most abundant surface protein of the T cell (Williams, 1982).

In first studies, naturally occurring mouse lymphoma variants were detected which bore less than 0.1% as much surface Thy-1 as did the wild-type cells. Subsequently, with the use of polyclonal antisera and complement, and making use of mutagenized cells, additional stable variants lacking surface Thy-1 were selected. Several clones were identified that continued to synthesize Thy-1. Hence, transport and/or processing defects are involved (Hyman, 1973; Hyman & Trowbridge, 1976).

Drug-resistant variants of such cell lines were produced so as to perform complementation analysis by cell fusion. The mutations in question proved to be recessive and to fall into several complementation classes, fusion between representatives of any two resulting in appreciable surface Thy-1 expression. Each class might therefore correspond to a distinct essential post-translational modification

or step in transport of Thy-1. Recently, however, an additional possible explanation has been suggested by the observation that Thy-1 mRNA ends eight amino acids earlier than is predicted from the protein sequence and is followed directly by poly(A) (Moriuchi *et al.*, 1983). Some subleties in Thy-1 gene transcription and/or RNA processing might therefore lie at the basis of some of the Thy-1$^-$ phenotypes.

Further studies have shown that the Thy-1$^-$ cells incorporate [^{3}H]mannose (but not [^{3}H]galactose) into a species of Thy-1 which has a slightly accelerated gel mobility relative to the wild-type molecule. The rate of turnover of these species is abnormally rapid (Trowbridge *et al.*, 1978*a*). In the case of the mutants belonging to complementation group E the analysis has been taken considerably further. These cells synthesize a dolichol-linked intermediate (the intermediate precursor of *N*-linked oligosaccharide units) which contains five rather than the usual nine mannose residues (Chapman *et al.*, 1979; Trowbridge & Hyman, 1979). The Thy-1$^-$ class E cells can process such units to characteristic complex structures after their transfer to nascent polypeptides (for example in the case of the glycoproteins of Sindbis virus when the mutant cell is infected). The defect in the structure of the dolichol-linked oligosaccharides has been proven to lie not in the absence of the mannosyl transferase activity normally responsible for elongation of dolichol-*P*-*P*-$(GlcNAc)_2Man_5$ to dolichol-*P*-*P*-$(GlcNAc)_2Man_9$, but in the inability of these cells to synthesize the dolichol-*P*-mannose which is the substrate for this enzyme activity (Chapman *et al.*, 1980).

The irony of the situation is that, despite this detailed and successful pursuit of the biochemical lesion characteristic of these class E mutants, the Thy-1$^-$ phenotype still has not been adequately explained. It is not clear why Thy-1, and at most only selected other lymphoma glycoproteins (Horton & Hyman, 1983), fails to be transported. Nevertheless, it is surely due to the selectivity of the defect that the mutant cell is viable. First immunocytochemical studies of class E cells show Thy-1 to be detectable in the RER and smooth-surfaced cytoplasmic membranes which are considered to be of both Golgi and lysosomal origin (Bourguignon *et al.*, 1982). The Thy-1$^-$ phenotype has also been observed in a concanavalin A-resistant mouse lymphoma (BW5147) (Trowbridge *et al.*, 1978*b*). Extensive immunoselection and complementation analysis of other lymphoma surface glycoproteins (TL, H2, Ly-6, T200) has also been reported (Hyman & Trowbridge, 1981; Horton & Hyman, 1983).

An extensive literature exists concerning the generation of constitutive lectin-resistant cell lines, some of which are deficient in selected oligosaccharide processing enzyme activities (Hughes *et al.*, 1980; Stanley, 1980; Briles, 1982). Generalized transport defects have not been identified in these cells, however, and they are therefore discussed here only in the context of lysosomal enzyme transport. Evidently, the extent to which a particular oligosaccharide structure is required for secretory or plasma membrane glycoprotein transport varies greatly from case to case (Sidman *et al.*, 1981; Chatis & Morrison, 1981).

Viral and cellular mutants exhibiting defective transport of viral envelope glycoproteins

A potential source of numerous mutated proteins that follow the secretory path is provided by mutants of enveloped viruses bearing alterations in genes coding for their surface glycoproteins. Such material is especially promising since temperature-sensitive (ts) mutants can be produced. In these cases one might expect to be able to simulate lesions of cellular origin which would be lethal if constitutive. Several distinct and intriguing phenotypes have been described, yet the analysis is necessarily incomplete since the primary structural changes in the viral glycoproteins have not been reported.

For VSV the temperature-sensitive (ts) mutations are classified in five complementation groups. In group V the mutations can be assigned to the single transmembrane glycoprotein of the virus, the G protein (Flamand, 1970; Pringle, 1970; Lafay, 1974). A procedure exists for substantially enriching in ts group V mutants through generation of Rous sarcoma virus–VSV pseudotypes (Rous sarcoma virus envelope surrounding a VSV nucleocapsid) at the restrictive temperature (40°C) in the presence of G-specific antiserum and complement (Lodish & Weiss, 1979).

Among several hundred VSV ts mutants produced in several laboratories, the most interesting group V mutants are ts 045 (or ts L513, or ts M501) and ts L511. In the former mutants G protein fails to exit from the RER at the restrictive temperature, as judged by subcellular fractionation, electron microscopic immunocytochemistry, study of G oligosaccharide structure and palmitate addition to G protein (Knipe *et al.*, 1977*a*,*b*,*c*; Bergmann *et al.*, 1981; Lodish & Kong, 1983). When the temperature is lowered to 32°C, G protein can be traced in its migration across the Golgi stack to the cell surface. In ts L511 at 40°C G protein does exit from the RER, and acquires nearly normally processed *N*-linked oligosaccharides (fucose is lacking at both 32°C and 40°C). Some G protein even reaches the cell surface, but it does not enter virions (Zilberstein *et al.*, 1980; Lodish & Kong, 1983). By contrast, at 32°C G protein also picks up covalently bound palmitate, an event which

is known in the wild type to occur after polypeptide chain termination but before completion of oligosaccharide maturation (Schmidt & Schlesinger, 1980), and does enter into virions.

Protein–protein interactions may underly the transport defects in both cases. In the viral envelope G protein is thought to exist as a multimeric unit (Dubovi & Wagner, 1977) and interacts with a peripheral viral membrane protein, M, which in turn binds the nucleocapsid. Defective G–G or G–M interactions might be anticipated to alter the ability of G protein to diffuse in the plane of the membrane, thereby retarding its exit from the RER (ts 045) or its gaining access to virions (ts L511). In ts 045 the immunocytochemical data show that G protein is distributed throughout the RER at 40°C. It does not grossly accumulate in the transitional elements of the RER.

ts transport mutants have also been characterized for other viruses. The simplicity of VSV, nevertheless, makes it an ideal candidate for further study. For example, in the case of Semliki Forest virus, Sindbis virus, and influenza virus, more than one surface glycoprotein exists, and in the case of Semliki Forest virus and Sindbis the two glycoproteins (pE2, E1) are known to associate intracellularly before arrival at the cell surface (Bracha & Schlesinger, 1976; Ziemiecki *et al.*, 1980).

For two of the Sindbis mutants, ts-10 and ts-23, carbohydrate maturation and fatty acid addition are interrupted at the non-permissive temperature, suggesting that the glycoproteins in question have not gained access to the Golgi complex. These observations are not, however, altogether in accord with the corresponding subcellular fractionation data (Erwin & Brown, 1980). In another Sindbis mutant (ts-20), glycoprotein transport to the cell surface does occur and fatty acid is added, but oligosaccharide maturation and cleavage of pE2 to E2 fail to occur. The original literature should be consulted for further information on these complex phenotypes (Brown & Smith, 1975; Smith & Brown, 1977; Bell & Waite, 1977; Schmidt & Schlesinger, 1979; Kääriänen *et al.*, 1980; Saraste *et al.*, 1980*a*,*b*; Pesonen *et al.*, 1981).

A set of fowl plague influenza ts mutants has been generated in which transport of the haemagglutinin is defective. In several cases the haemagglutinin is retained in the RER at 42°C (ts-1, ts-227) while in others the haemagglutinin acquires the terminal sugars galactose and fucose and has presumably gained access to the Golgi complex (ts-482, ts-532, ts-651) (Lohmeyer & Klenk, 1979; Klenk *et al.*, 1981). In such mutant-infected cells the synthesis and transport of another viral glycoprotein, the neuraminidase, proceeds normally.

For both the G protein and the influenza haemagglutinin the spectacular consequences of extreme structural alterations of the surface glycoproteins have been studied. Cloned cDNA corresponding to a truncated G protein sequence which stops 79 amino acids short of the *C*-terminus has been transfected into host cells. Similarly, influenza virus–SV40 recombinants bearing deletions which reach into the hydrophobic *C*-terminal region of the haemagglutinin gene have been used for infection. In both cases the shortened glycoproteins are synthesized, but instead of residing at the cell surface, they are secreted, as anticipated from knowledge of the role of their *C*-terminal domains in spanning the membrane (Rose & Bergmann, 1982; Sveda *et al.*, 1982).

An intriguing and complex set of transport variants has been identified among naturally-occurring and immunoselected cells infected with murine leukaemia virus. The Friend and Rauscher erythroleukaemia viruses are both complexes of a spleen focus-forming virus and a helper murine leukaemia virus. The former encodes a 55 kDa surface glycoprotein (gp55) and the latter an immunologically related 70 kDa glycoprotein (gp 70) which is generated from a 90 kDa precursor ($gp\,90^{env}$). gp 55 appears to play an important role in leukaemogenesis.

Among the Rauscher erythroleukemia virus mutants is one (ts-26) which is temperature-sensitive with respect to $gp\,90^{env}$ transport and cleavage (Ruta *et al.*, 1979; Kabat *et al.*, 1980). An immunoselected cell line (H-4) derived from Friend–murine leukaemia virus-infected cells has a defect in transport and cleavage of both $gp90^{env}$ and other viral glycoproteins. Although two-dimensional maps of total iodinated cell-surface proteins reveal only minor differences from the wild type, this mutation has been shown to be of cellular origin (Fitting *et al.*, 1981). By immunoselection using anti-gp55 serum and complement an additional class of transport mutants has been generated. These are of particular interest since in the wild type only a few percent of gp55 bears complex *N*-linked oligosaccharides and residues on the cell surface (most of the protein remains intracellular and bears immature, high-mannose *N*-linked oligosaccharides). In the mutants, gp55 oligosaccharides undergo even less processing; even glucosyl residues appear to persist and none of the gp55 is delivered to the surface. In some of the mutants smaller gp55-related polypeptides are detected in addition to gp55. Some of these mutants are of viral and others are of cellular origin (Ruta *et al.*, 1982).

Viral mutants defective in glycoprotein intracellular transport have also been detected in several other viruses, e.g. herpes simplex virus (Machtiger *et al.*, 1980), and Rous sarcoma virus (Mason & Yeater, 1977; Lineal *et al.*, 1980; Steiner & Boettiger, 1980).

Transport of lysosomal enzymes

The exclusive targeting of newly synthesized lysosomal enzymes to lysosomes is upset in a number of naturally occurring situations. For example, in the mouse liver and kidney the exclusive localization of β-glucuronidase to the lysosome is dependent on the absence of egasyn, a hydrophobic glycoprotein of the endoplasmic reticulum which binds β-glucuronidase (Lusis *et al.*, 1976; Paigen, 1979; Brown *et al.*, 1981). In mouse strains in which egasyn is present, the enzyme is roughly equally distributed between the endoplasmic reticulum and lysosomes. The amount of egasyn recovered in microsomal fractions is considerably in excess of the amount of enzyme; however, other lysosomal enzymes apparently do not associate with it (Lusis *et al.*, 1977).

A set of mouse pigment mutants has also proved interesting with respect to lysosomal function and enzyme localization. In the beige mouse (an analogue of the Chediak–Higashi syndrome), for example, much smaller quantities of lysosomal enzymes are released from kidney epithelial cells than in the wild type (Novak & Swank, 1979). Ultrastructural studies of these cells illustrate an impressive hypertropy of lysosomes and GERL (the acid phosphatase-positive set of smooth membranes often interposed between the stacked Golgi cisternae and lysosomes) (Chi *et al.*, 1978; Novikoff *et al.*, 1978); however, it is not understood why discharge of their content is reduced. The several pigment mutants exhibiting such phenotypes are recessive and map to altogether different chromosomal sites. In the case of the Chediak–Higashi syndrome there are suggestions of an underlying defect in cyclic nucleotide metabolism or microtubule function (Witkop *et al.*, 1983).

The role of the phosphorylated carbohydrate of lysosomal enzymes in governing their transport has recently been much publicized. In fibroblasts, but not in several other cell types, their mannose phosphate 'recognition marker' and its corresponding receptor are the key units responsible for targeting (Hasilik, 1980; Kornfeld, 1982; Neufeld & Robbins, 1982; Sly & Fischer, 1982).

It is now well-established that *N*-linked oligosaccharides of lysosomal enzymes normally are subject to a sequence of post-translational modifications during their transit through the Golgi complex (addition of 1-phospho-*N*-acetylglucosamine, followed by removal of the blocking *N*-acetylglucosamine to generate a phosphomonoester). The result is that several oligosaccharides per polypeptide may bear several phosphodiester and/or phosphomonoester units. The phosphomonoesters confer high affinity for the mannose phosphate receptor (Creek & Sly, 1982; Fischer *et al.*, 1982). That this interaction is essential for targeting is inferred from study of fibroblasts from two human storage syndromes (mucolipidosis II and III) in which *N*-acetylglucosamine phosphotransferase activity is missing. In these conditions, lysosomal enzymes are not phosphorylated and instead of being delivered to lysosomes many of them rapidly exit from the cell.

Only a limited number of cell fusion studies have suggested the correctability of the mucolipidosis II phenotype (Champion & Shows, 1977); however, dramatic complementation has been obtained by co-cultivation of fibroblasts from different storage syndromes in which individual hydrolase activities are missing. Because of the presence of the mannose phosphate receptor at the cell surface, any extracellular phosphomonoester-bearing hydrolases in such a co-culture are recognized and internalized, thereby providing mutual complementation, for example between Hunter and Hurler fibroblasts (Fratantoni *et al.*, 1968).

The availability of several mutant cell lines that synthesize aberrant *N*-linked oligosaccharides has added further information concerning the degree to which the typical phosphorylated structures are necessary for targeting. For example, in a phytohaemagglutinin-resistant mouse lymphoma ($Pha^{R2.7}$) the activity of the second glucosidase responsible for trimming two (α1-3)-glucosyl residues from the parent dolichol-derived oligosaccharide is missing (Gabel & Kornfeld, 1982). In such cells, whose glycoproteins retain an altogether unprecedented titre of glucosyl residues, phosphorylation of the lysosomal oligosaccharides does occur. The product is, however, underphosphorylated by comparison with wild type and the balance between phosphodiester and phosphomonoesters is shifted toward the phosphodiester structure. Nevertheless, the cells do not secrete abnormal amounts of the lysosomal enzymes and the β-galactosidase which can be released from cells treated with NH_4Cl is efficiently endocytosed by other fibroblasts.

A similar situation is encountered in the class E Thy-1^- lymphoma cells discussed above (Gabel & Kornfeld, 1982). Phosphorylation is more efficient than in $Pha^{R2.7}$, but no more than a single phosphate is introduced per oligosaccharide and predominantly phosphomonoesters are found. Similarly, a CHO cell line (B4-2-1) also defective in dolichol-*P*-mannose synthesis has been reported to synthesize an acid hydrolase (α-L-iduronidase) that can be efficiently and specifically endocytosed by wild-type cells (Stoll *et al.*, 1982).

Studies have also been reported of a concanavalin A-resistant CHO cell line (B 211) which fails to add glucose to its dolichol-linked oligosaccharides (Krag & Robbins, 1982). Although the explanation is by no means clear, the oligosaccharides of the β-

hexosaminidase and α-L-iduronidase of these cells lack phosphate and are converted to complex structures. Intracellular enzyme activities are low, but there is no accumulation of extracellular hydrolytic activity, conceivably because the acid hydrolases are unstable.

A further examination of the role of the mannose 6-phosphate receptor has recently been undertaken. CHO cell mutants have been selected for resistance to mannose 6-phosphate–ricin conjugates (in the presence of lactose) (Robbins & Myerowitz, 1981; Robbins *et al.*, 1981). Several clones are grossly defective in specific internalization of lysosomal enzymes. Moreover, in biosynthetic labelling experiments selected clones have been shown to release much more labelled precursor α-L-iduronidase and β-hexosaminidase than wild-type cells, thus providing strong evidence that the receptor implicated in the selection protocol (presumably at the cell surface) is functional in intracellular traffic. A similar situation is encountered with certain macrophage-related cell lines (e.g. p388D_1) which hypersecrete lysosomal enzymes bearing mannose 6-phosphate (Jessup & Dean, 1980). These cells lack the mannose 6-phosphate receptor (Gabel *et al.*, 1983).

In an attempt to obtain mutations that concern the underlying common mechanisms of receptor-mediated endocytosis, mutant CHO cells resistant to diphtheria toxin, which is thought to enter cells by endocytosis, have been screened for uptake of lysosomal enzymes (Robbins *et al.*, 1983). In one uptake-deficient clone (DTF 1-5-1) it is striking that the function of the low density lipoprotein receptor appears to be normal.

Conclusion

The investigations described above constitute a heterogeneous group from the point of view of cell type and proteins studied. However, there are several emerging common features pertaining to the mechanism of transport which should be underlined.

Many cases demonstrate the extent to which the structure of a given protein may determine its eligibility for transport. For the variant α_1-proteinase inhibitor, for certain Ig chains, the low density lipoprotein receptor and many of the viral glycoproteins, alterations in primary structure (more or less well-defined according to the particular case) can result in the protein no longer being eligible for transport to the cell surface. In a closely related class of examples, a determinative event governing transport is interaction between proteins, e.g. between Ig chains, between HLA, H2 or TL antigens and β_2-microglobulin, between at least one lysosomal enzyme and egasyn. In a third class, covalently bound oligosaccharides are implicated (Thy-1$^-$ class E cells, mucolipidosis II and III cells).

The example of the lysosomal enzymes is of particular interest because it illustrates in precise terms that the proteins undergoing transport serve as a set of probes of the machinery of transport. That is, if fibroblasts are to direct their lysosomal enzymes to lysosomes, the enzymes must bear the proper signal and they must encounter the mannose-*P* receptor. In each of the above-mentioned case where transport is interrupted, a possibly comparable recognition event may be occurring which leads to lysosomal degradation, although there are no data to implicate phosphorylated oligosaccharides.

There are few data that implicate cellular components in transport other than those enzymes responsible for post-translational modifications. Possibly certain of the erythroleukaemia virus-infected cell variants fall into this class. The most direct search for such components is that using diphteria toxin in conjunction with other ligands endocytosed by receptor-mediated endocytosis, yet there is to date no comparable effort pertaining directly to the secretory path.

Perhaps, were one to start with a non-secreting cell variant and were it feasible to generate a selective pressure for reacquisition of transport, one might obtain phenotypic revertants in which identifiable cellular components could be implicated. As an ultimate goal one would seek to identify such components, the site(s) at which they act, and the means by which they function in intracellular transport.

I thank Drs. Y. Roth and B. Thorens for their critical comments. My work is supported by grant no. 3.059.81 of the Swiss National Science Foundation.

References

Bell, J. & Waite, M. (1977) *J. Virol.* **21**, 788–791
Bergmann, J., Tokuyasu, K. & Singer, S. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1746–1750
Bienkowski, R. (1983) *Biochem. J.* **214**, 1–10
Bourguignon, L., Balazowich, K., Trowbridge, I. & Hyman, R. (1982) *Cell Biol. Int. Rep.* **6**, 745–755
Bracha, M. & Schlesinger, M. (1976) *Virology* **74**, 441–449
Briles, E. (1982) *Int. Rev. Cytol.* **75**, 101–166
Brown, D. & Smith, J. (1975) *J. Virol.* **15**, 1262–1266
Brown, J., Jahneis, G. & Swank, R. (1981) *Biochem. Biophys. Res. Commun.* **99**, 691–699
Burke, B., Griffiths, G., Reggio, H., Louvard, D. & Warren, G. (1982) *EMBO J.* **1**, 1621–1628
Burrows, P., Le Jeune, M. & Kearney, J. (1979) *Nature (London)* **280**, 838–840
Champion, M. & Shows, T. (1977) *Nature (London)* **270**, 64–66
Chapman, A., Trowbridge, I., Hyman, R. & Kornfeld, S. (1979) *Cell* **17**, 509–515

Chapman, A., Fujimoto, K. & Kornfeld, S. (1980) *J. Biol. Chem.* **255**, 4441–4446
Chatis, P. & Morrison, T. (1981) *J. Virol.* **37**, 307–316
Chi, E., Ignazio, E. & Lagunoff, D. (1978) *J. Histochem. Cytochem.* **26**, 131–137
Coffino, P. & Scharff, M. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 219–223
Cohen, J. & Beisson, J. (1980) *Genetics* **95**, 797–818
Colman, A., Besley, J. & Valle, G. (1982) *J. Mol. Biol.* **160**, 459–468
Cook, W. & Scharff, M. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5687–5691
Creek, K. & Sly, W. (1982) *J. Biol. Chem.* **257**, 9931–9937
Dubovi, E. & Wagner, R. (1977) *J. Virol.* **22**, 500–509
Dunnick, W., Rabbitts, T. & Milstein, C. (1980) *Nature (London)* **286**, 669–675
Early, P., Rogers, J., Davis, M., Calame, K., Bond, M., Wall, R. & Hood, L. (1980) *Cell* **20**, 313–319
Erwin, C. & Brown, D. (1980) *J. Virol.* **36**, 775–786
Farquhar, M. & Palade, G. (1981) *J. Cell Biol.* **91**, 77s–103s
Fischer, H., Creek, K. & Sly, W. (1982) *J. Biol. Chem.* **257**, 9936–9943
Fitting, T., Ruta, M. & Kabat, D. (1981) *Cell* **24**, 847–858
Flamand, A. (1970) *J. Gen. Virol.* **8**, 187–195
Franklin, E. & Frangione, B. (1975) *Contemp. Top. Mol. Immunol.* **4**, 89–126
Fratantoni, J., Hall, C. & Neufeld, E. (1968) *Science* **162**, 570–572
Gabel, C. & Kornfeld, S. (1982) *J. Biol. Chem.* **257**, 10605–10612
Gabel, C., Goldberg, D. & Kornfeld, S. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 775–779
Gadek, J. & Crystal, R. (1983) in *Metabolic Basis of Inherited Disease* (Stanbury, J., Wyngaarden, S., Fredrickson, D., Goldstein, J. & Brown, M., eds.), 5th edn., pp. 1450–1467, McGraw-Hill, New York
Goldstein, J. & Brown, M. (1982) *Med. Clin. N. Am.* **66**, 335–362
Hasilik, A. (1980) *Trends Biochem. Sci.* **5**, 237–240
Hercz, A. & Harpaz, N. (1980) *Can. J. Biochem.* **58**, 644–648
Hercz, A., Katona, E., Cutz, E., Wilson, J. & Barton, M. (1978) *Science* **201**, 1229–1231
Horton, H. & Hyman, R. (1983) *Immunogenetics* **17**, 261–270
Hughes, R., Pena, S. & Vischer, P. (1980) in *Cell Adhesion and Motility* (Curtis, A. & Pitts, J., eds.), pp. 329–356, Cambridge University Press
Hyman, R. (1973) *J. Natl. Cancer Inst.* **50**, 415–422
Hyman, R. & Stallings, V. (1977) *Immunogenetics* **4**, 171–181
Hyman, R. & Trowbridge, I. (1976) *Cold Spring Harbor Symp. Quart. Biol.* **41**, 407–415
Hyman, R. & Trowbridge, I. (1981) *Immunogenetics* **12**, 511–523
Jessup, W. & Dean, R. (1980) *Biochem. J.* **190**, 847–850
Kääriäinen, L., Hashimoto, K., Saraste, J., Virtanen, I. & Perttinen, K. (1980) *J. Cell Biol.* **87**, 783–791
Kabat, D., Ruta, M., Murray, M. & Polonoff, E. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 57–61
Klein, J., Terasaki, P., Billing, R., Honig, R., Jondal, M., Rosen, A., Zeuthen, J. & Clements, G. (1977) *Int. J. Cancer* **19**, 66–76
Klenk, H.-D., Garten, W., Keil, W., Nieman, H., Bosch, F., Schwarz, R., Scholtissek, C. & Rott, R. (1981) in *Genetic Variation among Influenza Virus: ICN-UCLA Symp. Mol. Cell. Biol.* vol. 21, (Nayak, D., ed.), Academic Press, New York
Kloppel, T., Kubo, R., Cain, P., Browne, C., Colon, S., Kearney, J. & Grey, H. (1981) *J. Immunol.* **126**, 1345–1350
Knipe, D., Baltimore, D. & Lodish, H. (1977*a*) *J. Virol.* **21**, 1121–1127
Knipe, D., Lodish, H. & Baltimore, D. (1977*b*) *J. Virol.* **21**, 1140–1148
Knipe, D., Lodish, H. & Baltimore, D. (1977*c*) *J. Virol.* **21**, 1149–1158
Köhler, G. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2197–2199
Köhler, G. & Shulman, M. (1980) *Eur. J. Immunol.* **10**, 467–476
Köhler, G., Howe, S. & Milstein, C. (1976) *Eur. J. Immunol.* **6**, 292–300
Köhler, G., Potash, M., Lehrach, H. & Shulman, M. (1982) *EMBO J.* **1**, 555–563
Kornfeld, S. (1982) in *The Glycoconjugates* III (Horowitz, M. & Pigman, W., eds.), vol. 3, pp. 3–23, Academic Press, New York
Krag, S. & Robbins, A. (1982) *J. Biol. Chem.* **257**, 8414–8431
Krangel, M., Pious, D. & Strominger, J. (1982) *J. Biol. Chem.* **257**, 5296–5305
Lafay, F. (1974) *J. Virol.* **14**, 1220–1228
Laskov, R., Kim., K. & Asofsky, R. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 915–919
LeFort-Tran, M., Aufderheide, K., Pouphile, M., Rossignol, M. & Beisson, J. (1981) *J. Cell Biol.* **88**, 301–311
Levy, R. & Dilley, J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2411–2415
Liesegang, B., Radbruch, A. & Rajewsky, K. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3901–3905
Lineal, M., Fenno, J., Burnette, W. & Rohrschneider, L. (1980) *J. Virol.* **36**, 280–290
Lodish, H. & Kong, N. (1983) *Virology* **125**, 335–348
Lodish, H. & Weiss, R. (1979) *J. Virol.* **30**, 177–189
Lohmeyer, J. & Klenk, H.-D. (1979) *Virology* **93**, 134–145
Louvard, D., Reggio, H. & Warren, G. (1982) *J. Cell. Biol.* **92**, 92–107
Lusis, A., Tomino, S. & Paigen, K. (1976) *J. Biol. Chem.* **251**, 7753–7760
Lusis, A., Tomino, S. & Paigen, K. (1977) *Biochem. Genet.* **15**, 115–122
Lynch, R., Graff, R., Sinisinka, S., Simms, E. & Eisen, H. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1540–1544
Machtiger, N., Pancake, B., Eberle, R., Courtney, R., Tevethia, S. & Schaffner, P. (1980) *J. Virol.* **34**, 336–346
Mains, P. & Sibley, C. (1983) *J. Biol. Chem.* **258**, 5027–5033
Mason, W. & Yeater, C. (1977) *Virology* **77**, 443–456
Moriuchi, H., Chang, C., Denome, R. & Silver, J. (1983) *Nature (London)* **301**, 80–81
Morrison, S. (1978) *Eur. J. Immunol.* **8**, 194–199

Morrison, S. & Scharff, M. (1975) *J. Immunol.* **114**, 655–659
Morrison, S. & Scharff, M. (1981) *C.R.C. Crit. Rev. Immunol.* **3**, 1–22
Mosmann, T. & Williamson, A. (1980) *Cell* **20**, 283–291
Mosmann, T., Gravel, Y., Williamson, A. & Baumal, R. (1978) *Eur. J. Immunol.* **8**, 94–101
Murphy, D., Jones, P., Loken, M. & McDevitt, H. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5404–5408
Neufeld, E. & Robbins, A. (1982) in *Human Genetics, Part B, Medical Aspects* (Bonne-Tamir, B., ed.), pp. 177–185, Alan Liss, New York
Novak, E. & Swank, R. (1979) *Genetics* **92**, 189–204
Novick, P., Field, C. & Schekman, R. (1980) *Cell* **21**, 205–215
Novikoff, A., Quintana, N. & Mori, M. (1978) *J. Histochem. Cytochem.* **26**, 83–93
Ochi, A., Hawley, R., Shulman, M. & Hozumi, N. (1983) *Nature (London)* **302**, 340–342
Paigen, K. (1979) *Annu. Rev. Genet.* **13**, 417–466
Palade, G. (1975) *Science* **189**, 347–358
Parnes, J. & Seidman, J. (1982) *Cell* **19**, 661–669
Pesonen, M., Saraste, J., Hashimoto, K. & Kääriäinen, L. (1981) *Virology* **109**, 165–173
Ploegh, H., Cannon, L. & Strominger, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2273–2277
Pringle, C. (1970) *J. Virol.* **5**, 559–570
Raschke, W., Mather, E. & Koshland, M. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3469–3473
Robbins, A. & Myerowitz, R. (1981) *J. Biol. Chem.* **256**, 10623–10627
Robbins, A., Myerowitz, R., Youle, R., Murray, G. & Neville, D. (1981) *J. Biol. Chem.* **256**, 10618–10622
Robbins, A., Peng, S. & Marshall, D. (1983) *J. Cell Biol.* **96**, 1064–1071
Rosa, F., Berosso, H., Weissenbach, J., Maroteaux, L., Fellous, M. & Revel, M. (1983) *EMBO J.* **2**, 239–243
Rose, J. & Bergmann, J. (1982) *Cell* **30**, 753–762
Roth, J. & Berger, E. (1982) *J. Cell Biol.* **93**, 223–229
Rothman, J. (1981) *Science* **213**, 1212–1220
Ruta, M., Murray, M., Webb, M. & Kabat, D. (1979) *Cell* **16**, 77–88
Ruta, M., Clarke, S., Boswell, B. & Kabat, D. (1982) *J. Biol. Chem.* **257**, 126–130
Saraste, J., von Bonsdorff, C.-H., Hashimoto, K., Kääriäinen, L. & Keränen, S. (1980*a*) *Virology* **100**, 229–245
Saraste, J., von Bonsdorff, C.-H., Hashimoto, K., Kääriäinen, L. & Keränen, S. (1980*b*) *Cell. Biol. Int. Rep.* **4**, 279–286
Schekman, R. & Novick, P. (1982) in *Molecular Biology of the Yeast, Sacchasomyces, Vol. II, Metabolism and Gene Expression* (Strathesn, G., Jones, E. & Broach, J., eds.), Cold Spring Harbor Press, NY
Schmidt, M. & Schlesinger, M. (1979) *Cell.* **17**, 813–819
Schmidt, M. & Schlesinger, M. (1980) *J. Biol. Chem.* **255**, 3334–3339
Secher, D., Cotton, R. & Milstein, C. (1973) *FEBS Lett.* **37**, 311–316
Secher, D., Milstein, C. & Adetugbo, K. (1977) *Immunol. Rev.* **36**, 51–72
Sidman, C., Potash, M. & Köhler, G. (1981) *J. Biol. Chem.* **256**, 13180–13190
Sly, W. & Fischer, D. (1982) *J. Cell. Biochem.* **18**, 67–85
Smith, J. & Brown, D. (1977) *J. Virol.* **22**, 662–678
Sonnenshein, G., Siekevitz, M., Siebert, G. & Gefter, M. (1978) *J. Exp. Med.* **148**, 301–312
Stanley, P. (1980) in *Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., ed.), pp. 161–189, Plenum, New York
Steimer, K. & Boettiger, D. (1980) *J. Virol.* **36**, 883–888
Stoll, J., Robbins, A. & Neufeld, E. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2296–2300
Sveda, M., Markoff, L. & Lai, C.-J. (1982) *Cell* **30**, 649–656
Tartakoff, A. (1980) *Int. Rev. Exp. Pathol.* **22**, 227–251
Tartakoff, A. (1983*a*) *Int. Rev. Cytol.*, **85**, 221–252
Tartakoff, A. (1983*b*) *Cell* **32**, 1026–1028
Tartakoff, A. (1983*c*) *Methods Enzymol.* **98L**, 47–59
Tolleshaug, H., Hobgood, K., Brown, M. & Goldstein, J. (1983) *Cell* **32**, 941–951
Trowbridge, I. & Hyman, R. (1979) *Cell* **17**, 503–508
Trowbridge, I., Hyman, R. & Mazauskas, C. (1978*a*) *Cell* **14**, 21–32
Trowbridge, I., Hyman, R., Ferson, T. & Mazauskas, C. (1978*b*) *Eur. J. Immunol.* **8**, 716–723
Valle, G., Besley, J., Williamson, A., Mosmann, T. & Colman, A. (1983) *Eur. J. Biochem.* **132**, 131–140
Wilde, C. & Milstein, C. (1980) *Eur. J. Immunol.* **10**, 461–467
Williams, A. (1982) *Science* **216**, 696–671
Winberry, L., Marks, A. & Baumal, R. (1980) *J. Immunol.* **124**, 1174–1180
Witkop, C., Quevado, W. & Fitzpatrick, T. (1983) in *Metabolic Basis of Inherited Disease* (Stanbury, J., Wyngaarden, J., Fredrickson, D., Goldstein, J. & Brown, M., eds.), 5th edn., pp. 301–346, McGraw-Hill, New York
Wu, G., Hozumi, N. & Murialdo, H. (1983) *Cell* **33**, 77–83
Ziemiecki, A., Garoff, H. & Simons, K. (1980) *J. Gen. Virol.* **50**, 111–123
Zilberstein, A., Snider, M., Porter, M. & Lodish, H. (1980) *Cell* **21**, 417–427