

## The role of carbohydrates in vectorial exocytosis

### The secretion of the gp 80 glycoprotein complex in a ricin-resistant mutant of MDCK cells

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In the polarized epithelial Madin-Darby canine kidney (MDCK) cell line an 80 kDa glycoprotein complex (gp 80) is sorted into the apical pathway of exocytosis and is secreted constitutively at the apical cell surface. The unglycosylated form of the protein complex is secreted in a nonpolar fashion at both surface domains [(1987) *J. Cell. Biol.* 105, 2735-2743]. Using ricin resistant MDCK cells the role of the terminal galactose and sialic acid residues in the sorting of the gp 80 complex was analysed. The results suggest that the carbohydrate cores, rather than the ultimate or penultimate sugar residues, play a critical role in the intracellular transport of this protein.

Epithelial cell, Protein sorting, Carbohydrate moiety

#### 1. INTRODUCTION

Polarized epithelial cells are characterized by the differentiation of their plasma membrane into two structurally and functionally distinct domains, the apical and the basolateral membrane [1]. The two domains differ in protein and lipid composition, reflecting the capability of these cells to target different sets of proteins and lipids to either cell surface. The vectorial targeting is not restricted to plasma membrane proteins; secretory proteins are also released in a polar fashion. In analogy to the sorting into other cellular organelles, it has been proposed that the vectorial cell surface transport is determined by the interaction of specific structures in the transported proteins (sorting addresses) with cellular receptors (sorting receptors) [2]. Neither the sorting signals nor the sorting receptors operative in the vectorial exocytosis have been defined.

We are studying the vectorial transport of an 80 kDa *N*-glycosylated protein complex (gp 80) in the polarized Madin-Darby canine kidney (MDCK) epithelial cell line [3]. This protein complex is synthesized as a precursor protein with an apparent  $M_r$  of 65 000 in its high mannose and of 80 000 in its terminally glycosylated form. The 80 kDa precursor protein is cleaved intracellularly into disulfide-linked subunits of 30 and 45 kDa and the

mature complex is secreted constitutively at the apical cell surface [4]. However, in tunicamycin-treated MDCK cells the unglycosylated complex is secreted in a nonpolar fashion at both cell surfaces. This indicates that the carbohydrate moieties play an important role in the sorting of this protein into the apical exocytotic pathway. In an attempt to dissect the role of the complex carbohydrate structures, we now report the analysis of the intracellular transport of this protein complex in a *Ricinus communis* agglutinin (RCA) resistant mutant of MDCK cells.

#### 2. MATERIALS AND METHODS

MDCK cells, strain II (Louvard) were a gift from K. Simons (EMBL, Heidelberg, Germany), RCA<sup>R</sup> mutant cells [5] were a gift from E. Rodriguez-Boulan (Cornell University, New York, USA). The suppliers of the media and the reagents [4], the generation and the characterization of the anti-gp 80 antiserum [4], the methods for culturing MDCK cells on polycarbonate filter [4], the tunicamycin treatment [4], metabolic labeling [5], immunoprecipitation [6], SDS-PAGE [7], and fluorography [8] have been described before.

#### 3. RESULTS

The present study was prompted by our observation that the polarized transport of the gp 80 glycoprotein complex in MDCK cells was abolished in the presence of tunicamycin [3]. Tunicamycin inhibits the synthesis of the dolichol-linked oligosaccharide precursor, and therefore completely blocks *N*-linked glycosylation [9]. A control experiment demonstrating this result is shown in Fig. 1. At 5 min of chase the core glyco-

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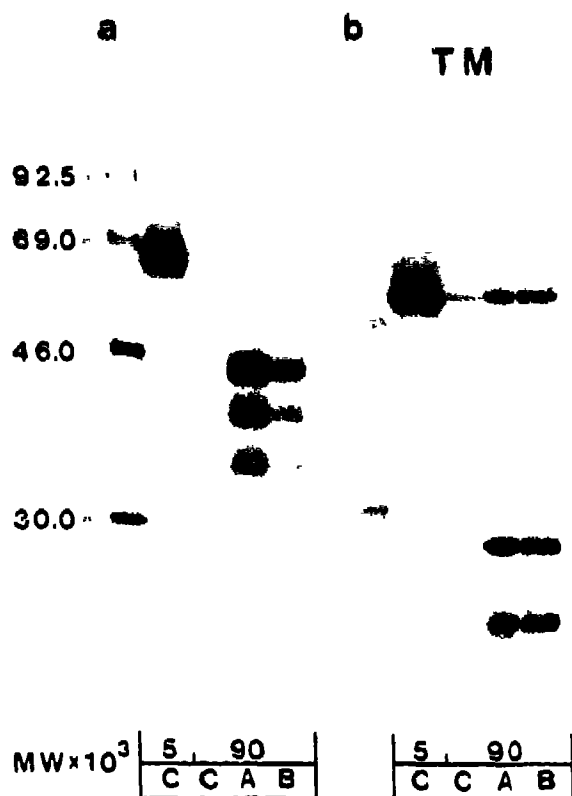


Fig. 1 Analysis of the polarity of gp 80 secretion from MDCK cells in the absence and presence of tunicamycin. Filter-grown monolayers of MDCK cells were pulse-labeled with [ $^{35}$ S]methionine and chased for either 5 or 90 min. Cell lysates, apical and basolateral media were collected, the proteins immunoprecipitated, and analyzed by SDS PAGE and fluorography

sylated 65 kDa precursor protein is detected in the cell lysates. At 90 min after synthesis the glycoprotein complex has been quantitatively secreted. The pulse-labeled protein is predominantly recovered from the apical medium; only a minor proportion is found in the basolateral medium (Fig. 1a).

The analysis of the reduced immunoprecipitable proteins in the media shows three bands. The upper band represents the 45 kDa, the lower band the 30 kDa subunit. As determined by N-terminal amino acid sequence analysis, the intermediate band at 35 kDa represents a mixture of the 30 kDa and the 45 kDa subunits (K. Mann and Cl. Koch-Brandt, unpublished observation). Since this variability in the migration pattern of the two subunits is not observed in tunicamycin-treated MDCK cells (Fig. 1), it is most probably due to differences in the carbohydrate moieties. The gp 80 complex is secreted in a polar fashion irrespective of this heterogeneity.

If the *N*-glycosylation is inhibited by preincubating, pulse-labeling, and chasing MDCK cells in the presence of tunicamycin, the polarity of transport is lost. The unglycosylated 50 kDa precursor protein is cleaved into subunits of 27 and 23 kDa, which appear in equal

amounts in the apical and the basolateral media (Fig. 1b). Neither the kinetics of transport nor the stability of the proteins is affected under these conditions.

On the basis of these data it could not be decided whether the key role is played by individual carbohydrate chain(s) located at (a) particular *N*-glycosylation site(s) and/or by (a) specific sugar component(s) in these side chains. In an attempt to address this question the gp 80 transport was analyzed in a ricin-resistant ( $RCA^R$ ) mutant of MDCK cells [5]. Because of a defect in UDP-galactosyl-transferase, no galactose residues are added when the carbohydrate chains mature in the Golgi complex. Consequently, the addition of sialic acid is also inhibited. Therefore, glycoproteins synthesized in this cell line possess only truncated versions of the mature carbohydrate side chains. When these cells were grown on polycarbonate filters, the monolayer displayed an electrical resistance comparable to that of wild-type MDCK cells grown under identical conditions (data not shown). This demonstrates that despite the glycosylation defect, the cells are able to form a tight monolayer on the filter, a prerequisite for the analysis of vectorial secretion in this cell line.

The analysis of the polarity of gp 80 secretion in this cell line is shown in Fig. 2. As expected, the core glycosylated precursor proteins synthesized in the wild-type and in the  $RCA^R$  mutant cells comigrate in SDS-PAGE (Fig. 2, lanes '5'). At this stage of protein maturation no differences can be detected. However, after the acquisition of complex carbohydrate moieties, distinct differences in the migration pattern are found (Fig. 2, lanes '90'). The subunits generated in the  $RCA^R$  mutant cells display an increased electrophoretic mobility when compared with the corresponding polypeptides present in the wild-type MDCK cells, reflecting the absence of the terminal galactose and sialic acid residues. Secretion of the complex is polar in  $RCA^R$  mutant cells despite the lack of these sugar residues. The pulse-labeled complex is secreted predominantly at the apical cell surface with only a minor component found in the basolateral medium. This result shows that the penultimate and ultimate galactose and sialic acid residues of the *N*-linked carbohydrate side chains do not play a critical role in the function of the oligosaccharide side chains in the vectorial transport of the gp 80 protein complex.

#### 4. DISCUSSION

Although *N*-glycosylation is a common feature of secretory and cell surface proteins, no universal role for the *N*-linked oligosaccharides has yet been defined. Potential functions that have been proposed include roles in polypeptide folding and stability, in cellular recognition, and in intracellular targeting [10]. A role

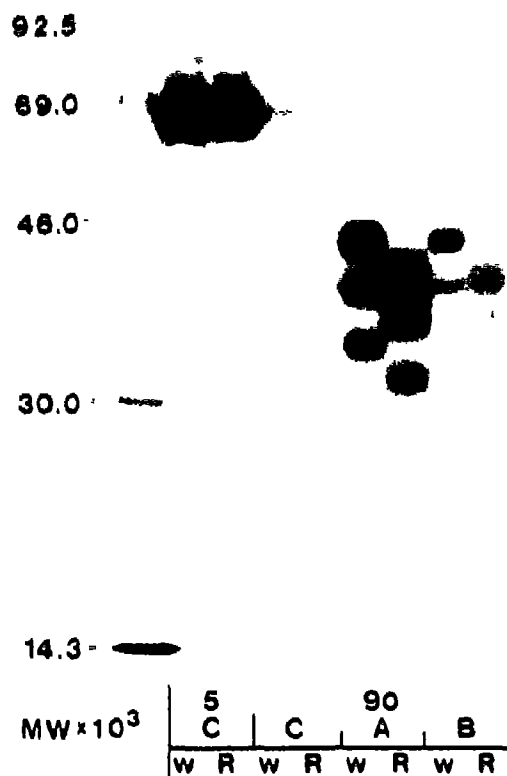


Fig. 2 Analysis of the polarity of the gp 80 secretion in RCA<sup>R</sup> mutant MDCK cells. Wild-type and RCA<sup>R</sup> mutant MDCK cells were pulse-labeled and chased for either 5 or 90 min, cell lysates and media were collected, proteins immunoprecipitated and analyzed by SDS-PAGE and fluorography

for a carbohydrate structure in intracellular targeting has been demonstrated for the mannose-6-phosphate group, which acts as a signal for the delivery of proteins into lysosomes [11]. It has further been suggested that transient glycosylation of protein-bound oligosaccharides could direct glycoproteins to different cellular locations [12]. The role of carbohydrates in cell surface transport is complex. While the transport of some proteins is unaffected if *N*-linked glycosylation is inhibited, the transport of others is completely abolished [13,14,16]. Such results suggest that the carbohydrates do not play a direct role as a transport signal but rather an indirect one by stabilizing – in different proteins to varying extents – a transport competent protein conformation. This notion is also supported by studies of the cell surface transport of proteins with new glycosylation sites introduced by the *in vitro* mutagenesis of the cDNA [17,18], as well as by an investigation of the relationship of *N*-linked glycosylation and heavy chain-binding protein (BIP) association with secretion in fibroblast cells [19]. In respect to the vectorial cell surface transport in polarized epithelial cells, a glycopospholipid structure, glycosyl-

phosphatidyl-inositol (GPI), has recently been shown to act as a targeting signal in the apical transport [20]. It is, however, clear that this glycolipid is not the only structure conferring an apical localization on proteins, since a membrane protein from which this anchor has been deleted is still transported vectorially to the apical cell surface [21].

For the gp 80 glycoprotein complex it has been shown that the *N*-linked carbohydrates play an essential role in the targeting to the apical cell surface. Interestingly, neither the kinetics of transport nor the stability of the protein are affected by the glycosylation block, while the vectorial transport is completely abolished. This suggests that the role of the carbohydrates in the intracellular transport of this protein is highly specific, involving the interaction of the protein with (a) putative sorting receptor(s). We have shown in this paper that this interaction does not depend on the presence of the penultimate and ultimate galactose and sialic acid residues. Two approaches may be envisaged to further dissect the role of the *N*-linked carbohydrates in this process. (i) The role of specific sugar components in the sorting process can be studied by the analysis of the gp 80 transport in MDCK cells resistant to lectins other than RCA or by the use of drugs, such as deoxymannonojirimycin and swainsonine, that inhibit specific steps in *N*-glycosylation. (ii) With the availability of a cDNA probe (Hartmann et al., submitted) it is now possible to analyse the role of individual carbohydrate chains by *in vitro* mutagenesis at particular *N*-glycosylation sites. The combined use of these two approaches should elucidate the role of the carbohydrates in the sorting of the gp 80 complex into the apical pathway of exocytosis.

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