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Role of heterotrimeric G proteins in polarized membrane transport

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SUMMARY

MDCK cells maintain the polarized distribution of surface proteins mainly by sorting the newly synthesized proteins in the *trans*-Golgi network (TGN). In order to identify the components of the putative sorting machinery and to study factors that affect the sorting process, we have developed an in vitro system that reconstitutes the transport of viral glycoproteins from the TGN to the apical or basolateral surface. We have used this system to study effects of membrane impermeable reagents (such as peptides and antibodies) on the polarized transport. We observed that reagents affecting the stimulatory class (Gs) of heterotrimeric GTP binding proteins (G proteins) influenced the apical but not the basolateral transport. In contrast, reagents specific for the inhibitory class of G proteins (Gi) affected the basolateral but not the apical transport. These results show that the heterotrimeric G proteins differentially regulate the two pathways of polarized transport. The G proteins may regulate the process of polarized sorting of proteins in a fashion analogous to their role in signal transduction by providing a communication link with the cytosolic side of the membrane.

Key words: protein sorting, heterotrimeric G proteins, epithelial cells

THE POLARITY PATHWAYS

In polarized epithelial cells, the plasma membrane is divided into two domains, apical and basolateral, which are distinct in their lipid and protein compositions. Most of the early information on polarized sorting and transport of proteins comes from studies on the transport of viral glycoproteins in MDCK cells infected with vesicular stomatitis virus (VSV) or influenza virus (Rodriguez-Boulan and Sabatini, 1978; Simons and Fuller, 1985). Both VSV glycoprotein (VSV-G) and influenza haemagglutinin (HA), like cellular membrane proteins, are inserted in the ER membrane upon synthesis and occupy the same compartments while en route through the Golgi complex to the *trans*-Golgi network (TGN; Rindler et al., 1984; Fuller et al., 1985; Griffiths and Simons, 1986). In the TGN, VSV-G and HA are segregated and packaged in two distinct classes of vesicles and are delivered to basolateral and apical domains, respectively (Wandinger-Ness et al., 1990). Pulse-chase experiments have demonstrated that the endogenous cellular proteins are also transported directly to the desired cell surface. Thus, it appears that MDCK cells sort apical and basolateral proteins in the TGN and deliver them directly to their respective destinations (see Fig. 1; for a review see Rodriguez-Boulan and Powell, 1992).

This is not the strategy adopted by all epithelial cells to achieve polarized distribution of their cell-surface proteins. Hepatocytes, for example, deliver both the apical and basolateral proteins first to the basolateral domain. The apical proteins are then selectively retrieved and are delivered to the apical domain via a transcytotic pathway (Bartles and Hubbard, 1988). Thus, hepatocytes seem to sort their apical proteins not from the TGN but from the basolateral side. Interestingly, the processes of apical sorting from the TGN (vectorial delivery) and from the basolateral surface (transcytotic delivery) share many characteristics such as microtubule-dependence and sensitivity to the drug brefeldin A, a drug that profoundly affects the membrane traffic in non-polarized cells. Some cells, for example CaCo2, seem to use both the direct and the transcytotic pathway to deliver proteins to the apical surface (Le Bivic et al., 1990; Matter et al., 1990). A third mechanism, that of random delivery of proteins to both surfaces followed by selective removal or retention, may also contribute significantly towards the generation of polarity (Nelson and Hammerton, 1989).

It is not clear why epithelial cells adopt different strategies to attain the polarized distribution of proteins. These differences become more enigmatic if one considers the fact that a vectorially delivered apical protein, when expressed in other epithelial cells, may be delivered to the apical domain by the transcytotic pathway. This suggests that both vectorial and transcytotic apical sorting mechanisms share common features (Simons and Wandinger-Ness, 1990).

THE FACTORS THAT AFFECT POLARIZED TRANSPORT

Although most of the molecules responsible for generat-

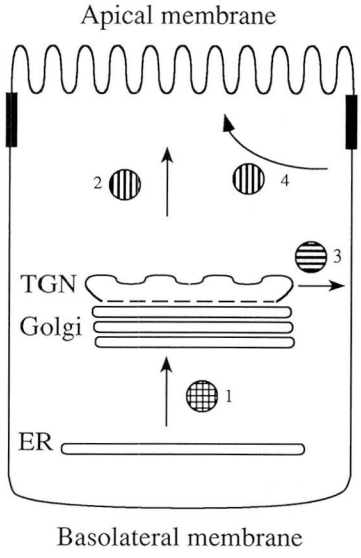

Fig. 1. Schematic representation of exocytic pathways in polarized MDCK cells. Newly synthesized proteins are transported from the ER to the Golgi complex (1). At this stage, the apical and basolateral proteins are not yet sorted from each other and occupy the same compartments. In the TGN, the apical proteins are sorted into apical vesicles and transported directly to the apical membrane (vectorial delivery; 2) wheras the basolateral proteins are delivered directly to the basolateral membrane (3). Apically destined proteins that were delivered to the basolateral membrane (e.g. polymeric Ig receptor) are then retrieved from the surface and transported to the apical surface (transcytotic delivery; 4).

ing and maintaining the polarity in epithelial cells remain unidentified, a wealth of phenomenological information is available on the factors that affect the state of polarity or regulate the polarized delivery of proteins. For example, it is generally observed that both the vectorial (direct) and transcytotic (indirect) delivery of apical proteins is dependent on the presence of intact microtubules, whereas delivery of proteins to the basolateral surface is relatively independent of microtubules (Rindler et al., 1987; Hunziker et al., 1990). Conflicting observations are also reported, however (Salas et al., 1986; van Zeijl and Matlin, 1990). A fungal metabolite, brefeldin A, that has been extensively used to study non-polarized membrane transport, inhibits both the vectorial and the transcytotic pathway to the apical surface but has no effect on the basolateral route (Low et al., 1991; Hunziker et al., 1991). However, we (unpublished data) and others have observed that brefeldin A inhibits both the apical and basolateral pathways (Rodriguez-Boulan and Powell, 1992) and Prydz et al. (1992) have reported that most endocytic and transcytotic events are either unaffected or even stimulated by brefeldin A.

A recent exciting development in the area of membrane traffic has been the appreciation of the role of GTP binding proteins at various steps of intracellular transport. Broadly, the GTP binding proteins can be classified into two families: small, monomeric ras-related proteins (smgp) and the heterotrimeric G proteins (G proteins). In non-polarized cells, the involvement of rab and ARF proteins (two subfamiles of smgp) at various stages of both exocytic and endocytic traffic is well documented (reviewed by Pfeffer, 1992). Recent evidence indicates that rab proteins also play an important role in cell polarity (Luetcke et al., 1993) and polarized protein transport (Huber et al., 1993). Different members of the rab family are localized to specific organelles of the exo- and endocytic pathways (Olkkonen et al., 1993) suggesting that they may be involved in each specific transport step.

INVOLVEMENT OF THE G PROTEINS IN MEMBRANE TRANSPORT

Although the role of G proteins in signal transduction is widely known, their involvement in membrane transport, both in non-polarized and polarized cells, has been recognized only recently (reviewed by Barr et al., 1992; Bomsel and Mostov, 1992). Most of the information about the G proteins comes from studies on their role in signal transduction. These proteins are composed of α, β and γ subunits and it is the α subunit that binds the guanine nucleotides. In the inactive state, the α subunit is bound by GDP, which is replaced by GTP upon activation of an upstream receptor. This activated state of G proteins can also be mimicked by the addition of $AlF_{(3\text{-}5)}$ and affected specifically by toxins and other reagents such as mastoparan. These properties have been exploited to investigate the involvement of G proteins in the process of membrane traffic. Overexpression of Giα-3 in LLC-PK1 cells inhibits the secretion of proteoglycans, and this inhibition can be reversed by pertussis toxin (PTX; Stow et al., 1991). In vitro budding of exocytic vesicles from the TGN is shown to be sensitive to reagents that affect the Gi function (Barr et al., 1991). The same reagents affect the binding of ARF and β-COP, proteins known to be involved in membrane traffic, to the Golgi membranes (Donaldson et al., 1991). These reagents also interfere with the action of brefeldin A on the association of these proteins with the Golgi membranes (Ktistakis et al., 1992). These observations suggest that the G proteins play a role in membrane traffic, perhaps in vesicle formation.

There is a tantalizing possibility that G proteins may regulate specific pathways of polarized traffic. For example, the proteoglycans studied by Stow et al. (1991) are secreted to the basolateral but not to the apical surface. Thus, inhibition of proteoglycan secretion by overexpression of Giα-3 may represent regulation of the basolateral route by a Gi class of G proteins. However, the general effect of Giα-3 overexpression on apical or basolateral secretion is not yet known. On the other hand, it seems that the transcytotic pathway from the basolateral to apical direction is regulated by a Gs class of G proteins (K. Mostov, personal communication). Using an in vitro system that reconstitutes budding of polymeric Ig receptor-containing transcytotic vesicles from early endosomes, Bomsel and Mostov (1992) have observed that Gs-specific reagents influence the budding of transcytotic vesicles whereas the Gi-specific reagents do not affect the system.

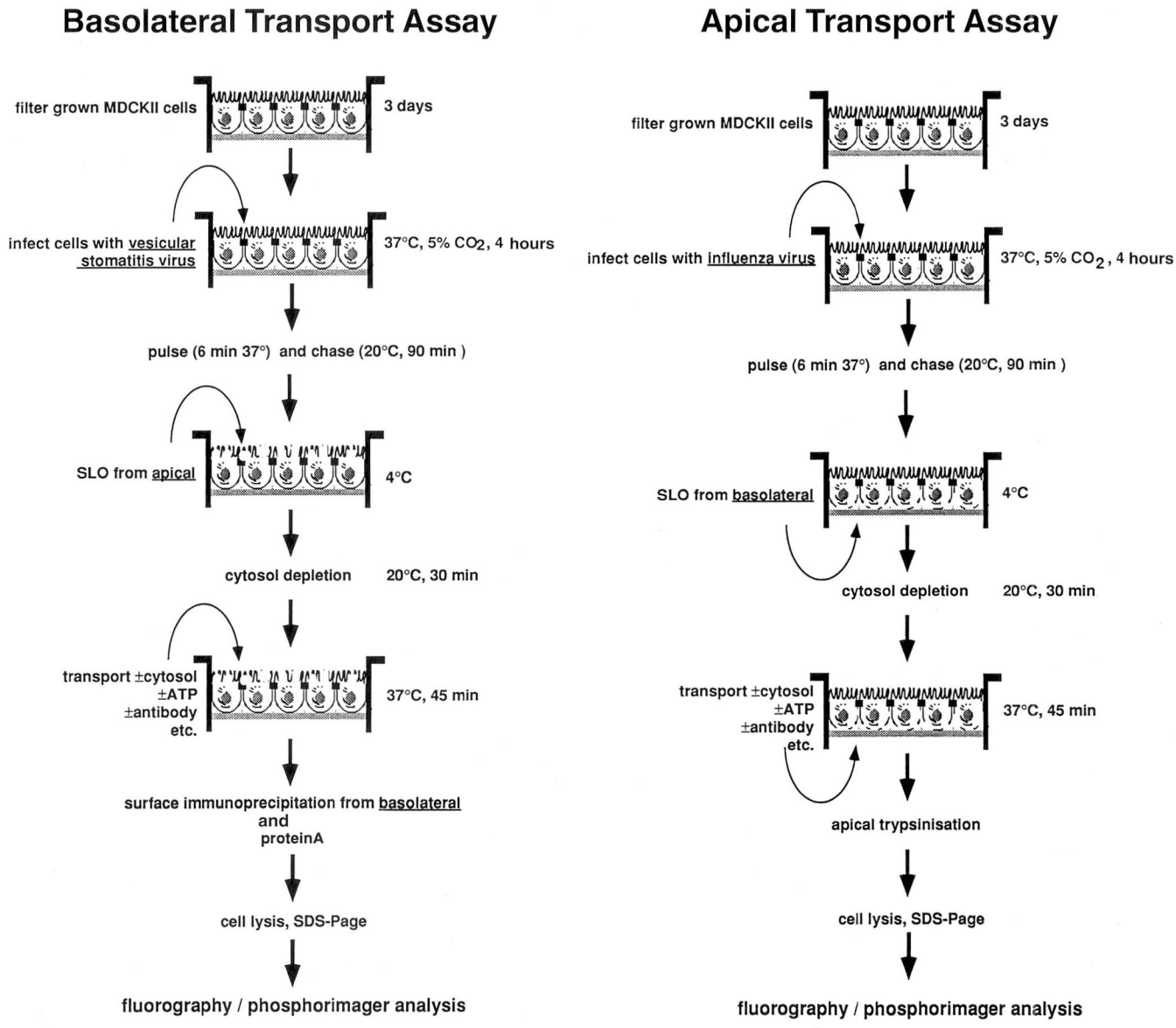

Fig. 2. Schematic representation of the in vitro transport assay in SLO-permeabilized cells. (A) TGN-to-basolateral transport of VSV-G protein. (B) TGN-to-apical transport of HA.

AN IN VITRO SYSTEM TO STUDY POLARIZED TRANSPORT

In order to access the site of polarized sorting directly and to gain a better understanding of the sorting process, we have developed an in vitro system to study the polarized sorting and transport of proteins (Kobayashi et al., 1992; Pimplikar and Simons, 1993). We use the bacterial toxin streptolysin O (SLO) to permeabilise selectively either surface of MDCK monolayers grown on a permeable filter support. The toxin is thought to bind to cholesterol in the cell surface at low temperatures (4°C) but the pores are formed only after raising the temperature (20°C or above). The pores formed are usually in the range of 30 nm and thus large enough to allow leakage of cytosolic proteins but small enough to retain the vesicles. Under these conditions, transport of proteins between two organelles becomes dependent upon addition of exogenous cytosol and energy. An advantage of the SLO permeabilization procedure is that the integrity of the cellular structure and intercellular junctions is mostly maintained. Thus, the SLO permeabilization procedure has proved useful in reconstructing a number of in vitro membrane transport systems (Gravotta et al., 1990; Tan et al., 1992).

Briefly, MDCK cells are grown on a permeable filter support until a tight monolayer is obtained (Fig. 2). The cells are then infected either with influenza virus to study apical transport of HA glycoprotein, or with vesicular stomatitis virus (VSV) to study basolateral transport of VSV-G glycoprotein. After a short pulse of radioactive methionine, the newly synthesized viral glycoproteins are blocked in the TGN at 20°C. At this stage, the opposite cell surface is per-

Table 1. Effects of G protein-specific reagents on polarized transport in MDCK cells

Reagents	Transport step		
	TGN→apical	TGN→basolateral	Transcytotic vesicles
$AlF_{(3-5)}$	↑	↓	↑
Mastoparan	–	↓	–
PTX	–	↑	–
CTX	↑	–	↑
Gs antibodies	↓	?	↓

↑, stimulation; ↓, inhibition; –, no significant effect; ?, not known. The data for the TGN→apical and TGN→basolateral transport are from Pimplikar and Simons (1993) and those for the production of transcytotic vesicles are from Bomsel and Mostov (1992).

meabilized with SLO and the endogenous cytosol is removed by washing. Upon raising the temperature to 37°C and addition of exogenous cytosol and ATP, the appearance of viral glycoprotein at the intact cell surface is monitored. Under these conditions the transport of viral glycoproteins from the TGN to the cell surface is temperature-, cytosol- and energy-dependent. An additional advantage of the SLO-permeabilized cell system is that besides the polarized transport we can also study ER-to-Golgi transport by monitoring acquisition of Endo H resistance by HA. This non-polarized transport step serves as a control to demonstrate the specificity of reagents that affect the polarized transport steps.

In conjunction with studies on intact cells, we have used the in vitro system to study the roles of Gs and Gi classes of G proteins on the apical and basolateral transport (Pimplikar and Simons, 1993). We observed that the basolateral transport of proteins was inhibited by $AlF_{(3-5)}$ and mastoparan and stimulated by PTX. These observations are consistent with the involvement of Gi in basolateral traffic. Recent studies suggest that the Gi class may also regulate ER to Golgi transport (Schwaninger et al., 1992; Wilson et al., 1993). In contrast to the basolateral transport, the apical transport was stimulated by $AlF_{(3-5)}$ and cholera toxin (CTX) but was unaffected by mastoparan. These data suggested involvement of the Gs class of G proteins in the apical transport. Furthermore, antibodies against Gs inhibited the TGN to apical transport of HA but had no effect on its passage from the ER to Golgi. Therefore, these observations (Table 1) suggest that the basolateral and apical transport pathways are specifically regulated by the Gi and Gs class of G proteins, respectively. It is interesting to note that both the vectorial and the transcytotic apical pathway seem to be regulated by the Gs class of G proteins. This further strengthens the idea that the apical sorting machinery at these two sites (TGN and basolateral surface) may be identical (Simons and Wandinger-Ness, 1990). At the present stage it is not known precisely which step of the transport assay (vesicle budding or vesicle fusion) is regulated by G proteins.

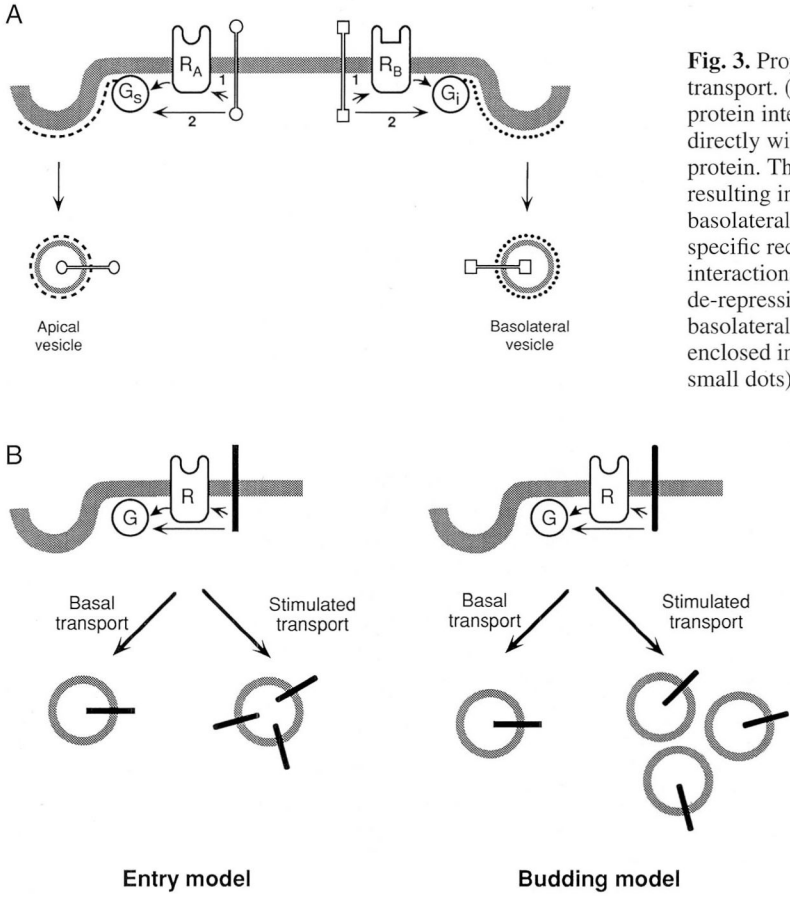

Fig. 3. Proposed role for G proteins in polarized membrane transport. (A) Upon arrival in the TGN, an apical-membrane protein interacts (1) with an apical-specific receptor (R_A) or directly with the Gs protein (2). These interactions activate the Gs protein. The activated Gs stimulates the downstream events resulting in increased apical transport. In an analogous way, a basolateral-membrane protein interacts (1) with a basolateral-specific receptor (R_B) or directly with the Gi protein (2). These interactions inhibit the Gi protein. The inhibition of Gi results in de-repression of the downstream events resulting in increased basolateral transport. The apical and basolateral vesicles are enclosed in specific coat proteins (shown as broken lines and small dots). In the TGN, the apical- and basolateral-secretory proteins could bind to their putative receptors (not shown in the figure), which either directly or via R_A and R_B, will interact with the respective G proteins. (B) There are two ways in which the G proteins can regulate membrane transport. The G proteins could facilitate the *incorporation* of the cargo molecules in vesicles that are being constitutively formed (Entry model). A stimulated transport, therefore, results from the increased number of cargo molecules per vesicle. An alternative possibility is that the G proteins regulate the *formation* of vesicles (Budding model). In this mode of action, a stimulated transport results from the increased number of vesicles. For the sake of simplicity, the coat proteins and the secretory proteins are not shown. Also, the receptors and the G proteins are not specified since this mode of action will be the same for both the apical and basolateral transport.

REGULATION OF POLARIZED TRANSPORT BY TRIMERIC G PROTEINS: A WORKING HYPOTHESIS

What precise role do G proteins play in polarized sorting and transport of membrane proteins? Current evidence from non-polarized and polarized cells favours the idea that G proteins regulate some aspect of vesicle budding (Barr et al., 1991; Bomsel and Mostov, 1992). However, it is possible that the G proteins may also be required for the fusion of vesicles and there is evidence that fusion of secretory granules with the plasma membrane (Gomperts, 1990) and fusion between early endosomes (Colombo et al., 1992) may be regulated by G proteins.

Without excluding the possibility that the G proteins may also be involved in the fusion of apical and basolateral vesicles with the respective domains, we speculate that the G proteins regulate the polarized sorting at the donor site, i.e. in the TGN. Our data suggest that the apical transport is under stimulatory control whereas the basolateral transport is under inhibitory control. We propose that the apical proteins, via an apical-specific receptor or acting alone, stimulate the Gs protein (Fig. 3A). The activated Gs then activates the downstream events resulting in apical transport. In contrast, the basolateral proteins inhibit the Gi protein via a basolateral receptor or acting alone as an antagonist. Inhibition of Gi function results in de-repression of the downstream events resulting in basolateral transport.

There are two possibilities in which the G proteins may control the downstream events and thus regulate the process of polarized transport (Fig. 3B). The G proteins may regulate the efficiency of protein inclusion into the correct vesicles (Entry model). During a given time interval, a constant number of both apical and basolateral vesicles are constitutively released from the TGN. The Gs and Gi class of G proteins could control the entry of newly synthesized apical and basolateral proteins in the respective budding vesicles. Therefore, a treatment that results in the stimulation or inhibition of a given G protein function will result in increased or decreased number of cargo molecules in the budded vesicles. Alternatively, the apical or basolateral proteins may stimulate the formation of vesicles (Budding model; also see Barr et al., 1992; Bomsel and Mostov, 1992). In this case, formation of the apical and basolateral vesicles will be under stimulatory and inhibitory controls, respectively. Stimulation of the Gs by an apical protein will result in an increased number of apical vesicles while inhibition of the Gi by a basolateral protein will increase the budding of the basolateral vesicles.

We assume that the apical and basolateral vesicles are formed by recruiting different cytosolic proteins on the TGN membrane. The nascent apical and basolateral vesicles will be coated with specific coat proteins, which may be released in the cytosol prior to fusion. Consistent with this idea, we have found that a protein associates with VSV-G tail only in the TGN but not in the ER or at the cell surface. Importantly, this protein does not associate with the HA tail in the TGN (unpublished observations). The G proteins could play a part in ensuring that the correct cargo is linked with the correct cytoplasmic coat proteins. It is not yet clear whether inhibiting a given G function results in mis-sorting of the cargo proteins to a 'wrong' pathway or simply in its accumulation in the TGN.

It should be noted that these two models are not mutually exclusive and that the G proteins may regulate both aspects (entry and budding) of vesicle formation. Whatever may be the precise role of G proteins in membrane traffic, it is clear that they play an important role in the polarized membrane transport.

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