It is likely that the study of yeast mutants will allow identification of key molecules involved in constitutive secretion not only in yeast but also in mammalian cells. Studies on the role of the mammalian homologues of the yeast G proteins will be essential to determine the extent to which molecular mechanisms present in yeast have been conserved during evolution. Also of interest is the question of whether the same G proteins are involved in both constitutive and regulated secretion or whether particular G proteins are involved in the regulation of the targeting of secretory vesicles to the correct pathway. Finally, what is the mode of action of G proteins in the secretory pathway? Do they function in signal transduction to generate second messengers as do the well studied G proteins at the plasma membrane (G_s and G_i) or do the ras-like G proteins have other modes of action? In this context it should be remembered that GTP binding and hydrolysis is not confined to proteins involved in signal transduction but is also a property of elongation factors in protein synthesis and tubulin.

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Features

Talking Point

How epithelia grease their microvilli

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All body cavities in mammalian organisms are lined by epithelia. In most cases, for example the gastro-intestinal and urinary tracts, the epithelium consists of a monolayer of cells. The cells are sealed to their neighbors by zones of cell–cell contacts, the tight junctions, which surround the apex of each cell and thereby divide the plasma membrane into two domains. The apical plasma membrane borders the lumen, while the basolateral aspect of the cells faces the basal lamina and the underlying tissue. A number of investigators have reported[1–8] that in rat, dog and man the apical membranes of these epithelial cells are much more viscous (less fluid) than their basolateral counterparts (for a discussion of the term 'fluidity' the reader is referred to Refs 2, 8 and 9). Since fluidity has been positively correlated with passive membrane permeability and mechanical instability, the lower fluidity of apical membranes may be functionally translated into lower passive permeability and increased mechanical stability, which are useful properties for these types of membranes. The lower fluidity of the apical membrane has been attributed exclusively to its lipid component as liposomes prepared from a lipid extract of these membranes share this property[3–8]. More specifically, the supposedly enhanced concentrations of cholesterol and sphingomyelin in the apical as compared to the basolateral plasma membrane of these cells have been held responsible for the effect[2,6–8]. The correlation is, however, deceptive. The levels of cholesterol and sphingomyelin in the apical membrane may well be identical to those in the basolateral membrane. As was discussed 20 years ago[10], it all depends on how these levels are expressed. An evaluation of the problem shows that the increased viscosity of apical membranes most likely originates from the high level of glycosphingolipids. Since the glycosphingolipids have been localized exclusively to the outer leaflet of the plasma membrane bilayer, the increase in viscosity can, in that case, be assigned specifically to the outer leaflet of the apical plasma membrane.

Thirty-five years ago it was suggested[11,12] that cholesterol promotes rigidity in cell membranes. As phospholipids are the major components of cell membranes, cholesterol concentrations have usually been expressed by the cholesterol: phospholipid ratio. A rise in the cholesterol : phospholipid ratio of apical, as compared with basolateral, membranes was observed experimentally and this property was thought to be responsible for the enhanced apical viscosity[2,3,7,8]. This ratio, however, may not be a relevant parameter in epithelial cells. As much as a third of the total number of lipid molecules in the apical membrane of intestinal[2,10,14] and urinary tract[15–17] epithelium consists of glycosphingolipids. Therefore, it seems more realistic[10] to compare the ratios of cholesterol to total polar lipids in the two types of membrane instead of the ratios of cholesterol to phospholipid alone. The significance of this point is well illustrated by data from five studies of the epithelia of rodent intestine and

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calf urinary bladder[2,10,13–15]. The mean cholesterol : phospholipid ratio for apical and basolateral plasma membranes observed in these studies was 1.24 ± 0.46 and 0.61 ± 0.15, respectively, suggesting an enrichment of cholesterol in the apical plasma membrane of 2.1 ± 0.6 times. However, when the glycosphingolipids are entered into the calculation the cholesterol concentration in the two membranes turns out to be identical: cholesterol constitutes 33 ± 5 mol% in the apical versus 31 ± 7 mol% in the basolateral membrane. Thus, the mean ratio of the apical to basolateral cholesterol concentration in these studies is 1.1 ± 0.1!

Similar arguments apply to the sphingomyelin concentration in the two plasma membrane domains. With regard to the discussion of membrane fluidity, sphingomyelin content has been expressed routinely as the sphingomyelin : phosphatidylcholine ratio, and an increase in this ratio in the apical membrane has been related to its enhanced rigidity[2,6–8]. The difference between the apical and basolateral sphingomyelin : phosphatidylcholine ratios is indeed remarkable: a 2.9 ± 1.7-fold higher ratio is found for the apical membrane[2,6,7,14,15,18,19]. Once again, however, the studies that include an analysis of the glycosphingolipids expose the deception: while on the basis of sphingomyelin : phosphatidylcholine ratios the sphingomyelin in these studies was enriched 1.9-fold in the apical membrane, the actual sphingomyelin concentration in the apical membrane, expressed as mol% of the total membrane lipids, is *less* (80%) than that in the basolateral membrane[2,14,15].

From the arguments above, it is obvious that the major difference in lipid composition between apical and basolateral membranes is not an enrichment of cholesterol and sphingomyelin in the apical membrane. In fact, the striking feature is a replacement in the apical membrane of most of the phosphatidylcholine by glycosphingolipids. While the concentration of glycosphingolipids is enhanced two- to four-fold in the apical membrane, the concentration of phosphatidylcholine is three-fold lower[2,14,15]. In these latter studies, the glycosphingolipids accounted for 34 ± 3 mol% of the apical membrane lipids. This becomes even more interesting when one takes into account the fact that glycosphingolipids have always been localized to the outer, non-cytoplasmic leaflet of cellular membranes[20]. With the assumption of a 50:50 distribution of cholesterol across the membrane, this implies that the outer leaflet of the apical membrane bilayer is covered by glycosphingolipids exclusively, while the phospholipids populate the cytoplasmic leaflet. Owing to their extensive hydrogen-bonding capacity, glycosphingolipids are much more highly ordered than phospholipids[20]. The prediction is, therefore, that one will find the glycosphingolipids to be the cause of the increased rigidity of apical membranes. The higher viscosity will then be an exclusive property of the outer leaflet of the apical membrane bilayer. This fits perfectly with the notion that the structure that separates the apical from the basolateral plasma membrane domain, the tight junction, serves as a barrier to lipid diffusion in the outer but not the cytoplasmic leaflet of the plasma membrane bilayer[19]: the lipid composition and the fluidity of the cytoplasmic leaflets may be expected to be very similar between the two domains. It is the exterior of apical membranes that makes them special. The grease does it!

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