Cholesterol and the Golgi Apparatus

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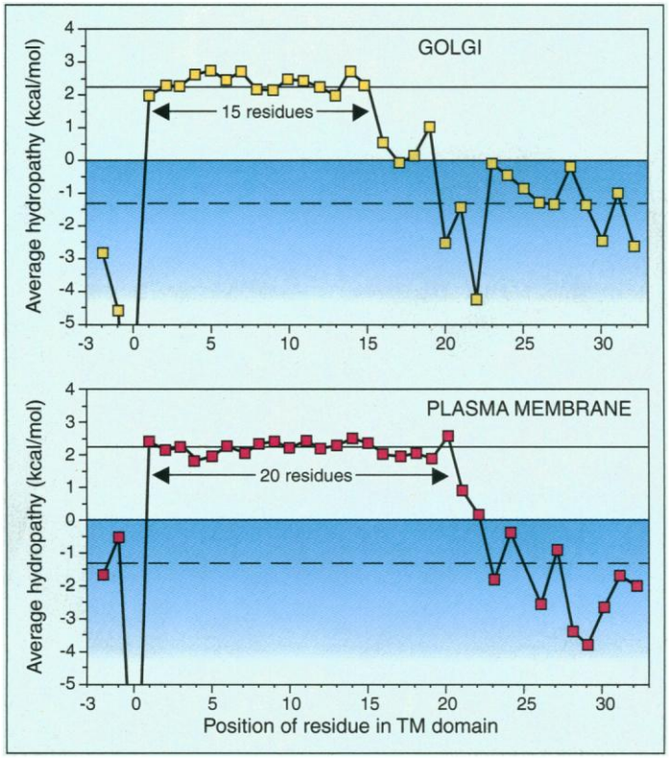
Cholesterol and closely related sterols are not uniformly distributed among the membranes of eukaryotic cells. Here we consider the effects of these flat disc-like molecules on lipid bilayers and suggest why the nonuniform distribution exists. In an interesting twist, consideration of how the cell maintains the uneven cholesterol distribution points to a mechanism for the sorting of other membrane components, in particular the membrane proteins of the Golgi apparatus.

Cholesterol and its analogs (*1*) occur in the plasma membranes of eukaryotic cells in amounts roughly equimolar to the sum of all other lipids. Sterols are clearly not required for membrane integrity since bacteria do not contain them, and the endoplasmic reticulum and mitochondria of eukaryotes contain very low amounts, so, why are they there? The principal reason for having cholesterol in the plasma membrane is to make this bilayer less permeable to small molecules. Most natural phospholipids contain one or more *cis* double bonds in their acyl chains; this makes it difficult to pack them together and hence they are liquids at ambient temperatures. This flexibility of the fatty acid chains also allows transient cavities to form, which in turn allow the transbilayer passage of small water-soluble molecules, like glucose. Introduction of cholesterol has two main effects on a bilayer: (i) It makes deformation of those parts of the fatty acid chains that abut the fused ring system of cholesterol more difficult. These parts of the acyl chains—methylene groups 2 to 10 approximately—become ordered and more tightly packed, causing the reduction in permeability. (ii) The cholesterol-ordered segments of the acyl chains now lie perpendicular to the bilayer, causing it to thicken; in egg phosphatidylcholine, the most widely studied phospholipid, the thickness of the hydrocarbon region typically increases from about 25 Å to 31 Å on addition of an equimolar amount of cholesterol (*2*).



Hydropathy in transmembrane segments of membrane proteins: a mechanism of segregation? Plots of the average hydropathy at each amino acid position in the transmembrane (TM) segments of 15 Golgi enzymes and of 17 plasma membrane proteins [all with a Type II orientation (*18*)]. The proteins are aligned such that the first residue of the hydrophobic section at the amino terminal (cytoplasmic end) is number 1. The solid lines (at 2.2 kcal/mol) indicate the average hydropathy per residue in an analysis of the transmembrane domains of 115 plasma membrane proteins with a single membrane span and the broken lines (–1.4 kcal/mol) indicate the average hydropathy per residue of the nonmembrane residues in these 115 proteins (*19*). At the carboxyl-terminal end of the transmembrane domain, the mean hydrophobicity of several of the residues is significantly less for the Golgi proteins than for the plasma membrane proteins [for positions 17 and 20, $P < 0.005$; for 18 and 22, $P < 0.025$; for 16 and 21, $P < 0.1$ (one-tailed t test at each position)]. That the hydrophobic length of the transmembrane domain may be a contributing factor to Golgi localization has also been suggested elsewhere (*12*).

The decrease in permeability that occurs as cholesterol is introduced into the bilayer has a biological consequence. Membrane proteins are inserted into eukaryotic cell membranes in the endoplasmic reticulum (ER), a membrane with, at most, a few percent of the cholesterol of the plasma membrane. For membrane proteins to enter into, and fold up in, such a hydrophobic environment (*3*), the transient cavities that enable small molecules to diffuse across a bilayer are likely to be needed—even with translocational chaperones to aid these assembly processes. In other words, a membrane into which proteins are inserted has to be readily deformable and hence be cholesterol-poor. A eukaryotic cell is thus faced with the dilemma of needing a cholesterol-rich plasma membrane, but also requiring a cholesterol-poor site for membrane protein assembly.

Cholesterol is synthesized in the ER. Therefore, as it is made it has to be continuously removed. But the situation is actually more dire than this because cholesterol—unlike phospholipids—can rapidly flip spontaneously from one side of a bilayer to the other and, more slowly, can hop between membranes. The off-rate of cholesterol from a bilayer (for example, the surface of a red cell) has a $T_{1/2}$ of about 2 hours at 37°C (*4*). Thus, in 2 hours about 50% of the plasma membrane cholesterol diffuses out into the cytoplasm; the same would hold for endosomal and other cholesterol-rich membranes inside the cell. In most cells, the ER exists as a large extended network that would be efficient at capturing this cholesterol. Indeed, for a cell with a doubling time of 24 hours, arrival of cholesterol in the ER by diffusion from other membranes is perhaps some sixfold greater than its maximal rate of biosynthesis.

Cells clearly need a mechanism for removing cholesterol from the ER. In principle, this could be done in one of two ways: The cell could pluck out individual cholesterols and transport them to the plasma membrane, a possibility that cannot be excluded at present, but for which there exists no clearly defined mechanism. Or the cell could progressively concentrate cholesterol in the membrane as it moves along the secretory pathway. Membrane travels from the ER to the plasma membrane through the Golgi apparatus. The Golgi is seen in many cells as a stack of flattened membranes and has long been thought to be a "countercurrent" fractionation system to separate proteins destined for the plasma membrane from those to be retained in the ER (*5*). In those protein-sorting processes so far examined, specific labels on the proteins are used to segregate them (*6*). But sorting lipids is a different matter, and their physical properties could allow a counter-

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current mechanism to drive the net forward transport of molecules such as cholesterol. Indeed, the original function of the Golgi apparatus may have been to effect this very separation. The Golgi apparatus contains substantial amounts of cholesterol, and there is some evidence that it forms a gradient (*7*).

The Golgi apparatus is composed of multiple compartments, with continuous and selective vesicular membrane exchange occurring between them. These compartments, which go from "cis" to "trans," are conventionally defined by the presence of particular enzymes (glycosidases, glycosyltransferases, and other enzymes involved in protein modification or sphingolipid synthesis) that are localized to one or more compartments, often in a graded manner (*8*). How are these proteins—usually having a single transmembrane domain—localized to the Golgi apparatus? Several groups have shown that the Golgi "retention signal" lies in the bilayer-spanning transmembrane domain (*9, 10*). This is especially surprising since these portions of Golgi enzymes do not show sequence homology. Furthermore, attempts to identify key residues required for retention have revealed a surprising lack of sequence dependence (*10, 11*). In the most extreme case, sialyltransferase remained localized to the Golgi even when its 17–amino acid transmembrane domain was replaced by 17 leucine residues (*10*). A longer stretch of leucines (23) did not provide efficient retention and, furthermore, a four-residue insertion into the transmembrane domain of a second Golgi enzyme, galactosyltransferase, causes retention to be reduced (*12*). This suggests that the physical properties of the transmembrane segment are responsible for its retaining activity and so forces consideration of the lipid bilayer in the Golgi.

There is clearly something unusual about Golgi membranes: The fluorescent lipid probe C_6-NBD ceramide preferentially binds to the Golgi in amounts suggesting a lipid interaction; this binding is lost if cholesterol is removed (*13*). Moreover, the bilayer changes across the Golgi as two different processes occur. The cholesterol concentration increases, and sphingolipids (both sphingomyelin and glycolipds) are synthesized in the cis Golgi (*14*). Like cholesterol, sphingolipids favor thicker bilayers (*15*) and are concentrated in the plasma membrane, strengthening the view that the membrane exiting the Golgi for the cell surface is thicker than that entering it from the ER.

There are two features of a single transmembrane polypeptide that may affect its behavior in such a changing bilayer: the length of the hydrophobic domain and the packing arrangements that its side chains can make with the adjacent fatty acyl chains. A shorter transmembrane domain would make it energetically unfavorable for a protein to enter a domain where the bilayer is thicker (*16*). And large projecting side chains could also be energetically unfavorable in a cholesterol-rich domain, because fitting them into a more ordered bilayer might require the introduction of permanent cavities—a process with a high energy cost (*17*). Examination of the transmembrane domains of known Golgi proteins shows that their properties are indeed consistent with exclusion from cholesterol-rich membranes. First, their transmembrane domains are consistently shorter than those of plasma membrane proteins (see figure). The average difference is about five residues, which represents approximately 7.5 Å of α helix. There are both experimental and theoretical reasons to believe that it is easier to accommodate a long transmembrane domain in a thinner bilayer than a short transmembrane domain in a thicker bilayer (*16*). Thus the plasma membrane and Golgi proteins could coexist in low-cholesterol bilayers, but the plasma membrane proteins would segregate away as the cholesterol-sphingolipid concentration rises. Second, the Golgi transmembrane domains contain more of the bulky residue phenylalanine than do plasma membrane transmembrane domains [13.7% (*18*) versus 5.3% (*19*)], consistent with a preference of the former proteins for a more disordered bilayer.

An increase in concentration of a particular lipid along the secretory pathway suggests that forward-moving vesicles contain a higher concentration of that lipid than the compartments they are leaving. This may occur either because the constraints of vesicle budding affect the composition of the bilayer in the bud or because budding occurs specifically from distinct lipid microdomains in the compartmental bilayer. The latter possibility would be consistent with physical studies that show that cholesterol-rich and cholesterol-poor domains can coexist within an entirely fluid bilayer (*20*). The notion of protein sorting by lateral partitioning between coexisting lipid domains has the particular appeal that it is inherently self-organizing, thereby escaping the requirement for further proteins that would themselves have to be retained in place. Regardless of the actual mechanism of lipid segregation, the physical properties of the transmembrane domain of a Golgi protein could serve to exclude it from a cholesterol-sphingolipid–rich vesicle as it is forming and hence retain it in that compartment.

Given that the post-Golgi membranes of a eukaryotic cell contain high concentrations of cholesterol, that cholesterol can hop between membranes, and that the ER must be relatively free of cholesterol for membrane protein assembly, a process must exist to continuously remove cholesterol from the ER. The agent for this process is probably the Golgi apparatus; the changing nature of the lipid bilayer as the Golgi is crossed may have enabled different Golgi enzymes to evolve so that they become hung up there in the order in which they are required. How cholesterol is concentrated in the Golgi is presently unknown, but the co-existence of cholesterol-poor and cholesterol-rich fluid phases within a contiguous bilayer points to a possible mechanism.

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18. Mean residue hydropathies (*3*) were calculated for the 15 published Golgi enzymes (having excluded species homologs and very close sequence relatives). GenBank accession numbers: M18679, M97753, M97754, J03880, J04989, J05175, M35531, X53578, M58596, M83651, D10852, M55621, M97347, M92042, X61172. The 17 plasma membrane proteins were chosen for their Type II orientation. GenBank accession numbers: M12029, J04591, X13276, M29961, M15944, X01060, M10058, V01090, M15059, J02700, M11505, M34461, M54992, X03339, J02939, M25812, X54867, M77676. The observed minimum length of the hydrophobic span (20 residues) is similar to that seen for Type I plasma membrane proteins [21 residues (*19*)].
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