deamidation. Methylation may act primarily as a backup mechanism to allow cells to reprogram their receptors in cases of excessive deamidation. It is only when cells are exposed to dramatic increases in attractant concentration that methylation becomes rate limiting. Under these conditions swimming is perturbed until the methylating enzyme has had sufficient time to introduce a new receptor program.

## Conclusion

When bacteria migrate in chemical gradients they are adapting to their environment and every component of the chemotaxis system in some way serves an adaptive function. Receptor methylation probably does not provide the adaptive mechanism by which cells compare their immediate past with the present to sense temporal changes, nor is it entirely responsible for maintaining an optimal steady state balance between runs and tumbles. Methylation does, however, appear to provide an important mechanism by which receptor sensitivities can be tuned to a particular environmental setting.

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# Talking Point

# Plasma membrane cholesterol pools

Gerrit van Meer

Cholesterol is an amphipathic lipid molecule. In higher eukaryotes it occurs as a major component of cellular membranes[1] and of lipoproteins, in which most of it is present esterified to long chain fatty acids. Besides its function in membrane structure and in lipid transport in the circulation, cholesterol serves as the precursor for bile acids and steroid hormones. In human pathophysiology cholesterol is best known for the correlation that exists between high plasma cholesterol levels and atherosclerosis[2]. The vigorous search for the structure and biosynthesis of cholesterol and its fate in the human body has, in the past 25 years, been honoured with several Nobel prizes, to Konrad Bloch in 1964 and to Michael Brown and Joseph Goldstein in 1985. However, despite the vast amount of literature on cholesterol, there is still a severe difference of opinion on such a basic issue as the location of this cholesterol in the mammalian cell. While experimental evidence assigns 85–90% of the cholesterol to the plasma membrane, model calculations suggest that this cannot be true and that in fact most of the cholesterol is present in the membranes of intracellular organelles.

Cholesterol is known as a typical plasma membrane lipid. The ratio of cholesterol to the other plasma membrane lipids, phospholipids and glycosphingolipids, is about 0.7–0.8 (mol/mol), while in intracellular membranes it may be as low as 0.1–0.2 (Refs 3 and 4). The mechanism by which this enrichment in the plasma membrane is established and maintained is far from clear. The variation in cholesterol content may be unexpected in view of the general assumption that cholesterol rapidly equilibrates between membranes by monomeric exchange through the aqueous phase. However, the spontaneous exchange of cholesterol between membranes is in fact slow ($t_{1/2} > 2$ h, see for example Ref. 5) compared to the vesicular transport in the cell[6]. Experimental data[7–9] indeed suggest that newly synthesized cholesterol is transported to the plasma membrane by vesicles rather than by exchange.

It would seem that the picture of the organization of cholesterol in the cell is hereby complete. However, hidden in the literature lies the inconsistency already mentioned, which seriously questions the validity of the individual approaches. Although there is agreement about the ratio of cholesterol to the other lipids in the plasma membrane being 4- to 8-fold higher than that of the intracellular membranes, the various data in the literature have highly different implications for the fraction of the total cellular cholesterol that is situated

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in the plasma membrane. On the one hand there is the evidence from experiments in which the amount of plasma membrane cholesterol was established by either the accessibility to the enzyme cholesteroloxidase or by comparison of the composition of isolated plasma membranes with that of intact cells These approaches[7–10] led to the conclusion that the plasma membrane contained the bulk of the total cellular cholesterol (80–95%) On the other hand, calculations combining the published lipid compositions of the various intracellular organelles[3,4,13] with morphometric measurements[11,12,14] suggest that less than 40% of the total cholesterol is situated in the plasma membrane Let us look into these calculations in a little more detail

In the rat liver cells, which had also been studied with cholesteroloxidase[10], the molar ratio of cholesterol to phospholipid was determined to be 0 76 for the plasma membrane and 0 12 for the ER plus the outer mitochondrial membrane[3] With a surface area of 37 Å$^2$ for a cholesterol molecule and 65 Å$^2$ for a phospholipid molecule[1], cholesterol occupied 30% of the surface area of the plasma membrane and 6% of the ER plus the outer mitochondrial membrane. The surface areas of these membranes were determined to be 8% and 59% of the total cellular membranes, respectively[11] Thus the plasma membrane cholesterol covered a fraction of 30/100 × 8% of the total cellular membrane surface area, versus 6/100 × 59% for the ER plus outer mitochondrial membrane cholesterol, a ratio of 40 to 60. If one would include the Golgi complex, endosomes and lysosomes in the calculation this would almost certainly reduce the fraction of the total cellular cholesterol present in the plasma membrane below 40%

A similar calculation for BHK cells, from the lipid compositions of the plasma membrane and the ER and their surface areas, suggests that maximally 24% of the total cholesterol is present in the plasma membrane of these cells The molar ratios of cholesterol to phospholipid were 0.63 and 0.25 for the plasma membrane and the ER, respectively[4], while their surface areas were 3400 μm$^2$ and 22 170 μm$^2$ per cell (Ref 12)

These low values for the fraction of the cholesterol that is present in the plasma membrane are supported by an independent calculation for yet another cell type, the epithelial MDCK cells In this calculation the amount of cholesterol per cell is simply compared to the surface area of the plasma membrane. The surface area of the two plasma membrane bilayer leaflets was 2464 μm$^2$ per MDCK strain II cell (Ref. 14, similar results are obtained for a different MDCK cell line, strain I [Refs 13, 14]). MDCK II cells contained 6 × 10$^9$ cholesterol molecules per cell[13]. With a surface area of 37 Å$^2$ per cholesterol molecule[1], the total cholesterol of a particular MDCK cell covers a surface area of 2220 μm$^2$ If 90% of this cholesterol would be situated in the plasma membane, it would occupy 81% of the plasma membrane surface area, resulting in a molar cholesterol to phospholipid ratio for the plasma membrane of 7 5 This is quite unrealistic Clearly, far less than 90% of the cellular cholesterol can be present in the plasma membrane if this membrane is to have a cholesterol to phospholipid ratio of around 0 8, a value generally found in plasma membranes[1] In fact, if one assumes that the latter value represents the actual molar ratio of cholesterol to phospholipid plus glycolipid in the MDCK plasma membrane, cholesterol would cover 31% of the plasma membrane A calculation shows that, in that case, only 34% of the total cellular cholesterol is located in the plasma membrane. The presence of membrane proteins would reduce this number even further

The question whether almost all (80–95%) or less than half (24–40%) of the cellular cholesterol is located in the plasma membrane is of obvious significance for the interpretation of results concerning the uptake and transport pathways of cholesterol and the regulation of its metabolism in the cell. The discrepancy will have to be resolved in order to evaluate the various approaches used and to provide a reliable set of tools with which to study the disposition and dynamic properties of cellular cholesterol It should be stressed that two more aspects of the organization of cholesterol in cellular membranes, its transbilayer distribution and transbilayer translocation rate, are still subject to controversy.

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