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# The role of cholesterol in membrane fusion

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ABSTRACT

Cholesterol modulates the bilayer structure of biological membranes in multiple ways. It changes the fluidity, thickness, compressibility, water penetration and intrinsic curvature of lipid bilayers. In multi-component lipid mixtures, cholesterol induces phase separations, partitions selectively between different coexisting lipid phases, and causes integral membrane proteins to respond by changing conformation or redistribution in the membrane. But, which of these often overlapping properties are important for membrane fusion?—Here we review a range of recent experiments that elucidate the multiple roles that cholesterol plays in SNARE-mediated and viral envelope glycoprotein-mediated membrane fusion.



## 1. Introduction

Cholesterol is an essential component of mammalian cells. It is synthesized in a complex series of enzymatic steps in the endoplasmic reticulum and is eventually transported through the Golgi to the plasma membrane where its concentration is much higher than in other cellular compartments. Large reservoirs of cholesterol also reside in blood serum in the form of lipoproteins, which are taken up by cells through endocytosis and recycled into the intracellular pool of cholesterol. Thus cholesterol cycles within cells and in and out of cells with many of these transport functions involving fission and fusion between different membranes. Because cholesterol has profound physical effects on the membranes in which it resides, it is not surprising that membrane cholesterol also dramatically affects membrane fusion and membrane fission. In this review, we first recapitulate briefly some of the unique effects that cholesterol imparts on the host lipid bilayer and some common modes of how cholesterol interacts with integral membrane proteins. This sets the stage to discuss a host of relatively recent discoveries on how cholesterol influences membrane fusion in intracellular membrane traffic, particularly in exocytosis of secretory vesicles, and in cell entry of enveloped viruses whose membranes typically are also highly enriched in cholesterol.

## 2. Cholesterol orders lipids and induces phase separation and curvature changes in fluid lipid bilayers

Cholesterol has a unique structure of four fused hydrocarbon rings with a polar hydroxyl group at one end and an eight-carbon branched aliphatic tail at the other end. The ring structure is rigid with an almost flat front face and a more corrugated back face, whereas the tail is flexible and able to undergo *trans-gauche* isomerizations like the hydrophobic tails of the phospholipids of the bilayer in which cholesterol resides. The small hydroxyl group is the only polar group in the molecule; the remainder is highly apolar and therefore deeply immersed in the host lipid bilayer.

The polar hydroxyl group aligns approximately with the ester carbonyl groups of the phospholipids in which it is perpendicularly embedded (Smondyrev and Berkowitz, 1999; Heftberger et al., 2015). The rigid ring structure considerably reduces the *trans-gauche* isomerizations of the neighboring lipid acyl chains and therefore orders them and reduces their dynamics and fluidity. Hence, this mixed phospholipid/cholesterol phase has been termed a liquid-ordered (Lo) phase (Ipsen et al., 1989; Sankaram and Thompson, 1990), in contrast to the liquid-disordered (Ld) phase of phospholipid bilayers in the absence of cholesterol above their chain melting phase transition. The ordering effect of cholesterol on fluid lipid bilayers does not only reduce the

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dynamics of the individual lipids, it also alters the continuum properties of the lipid bilayer like bending and compressibility moduli (Chen and Rand, 1997). The increased viscosity of cholesterol-containing membranes slows down the lateral and rotational diffusion of lipids and embedded membrane proteins (Rubenstein et al., 1979; Kahya et al., 2003; Crane and Tamm, 2004). A summary of multiple effects that cholesterol can exert on membranes and membrane proteins is shown in Fig. 1.

In multi-component lipid mixtures, cholesterol interacts preferentially with long, saturated phospho- and sphingo-lipids, which on their own exhibit high gel-to-liquid-crystalline phase transition temperatures. Therefore, lipid bilayers composed of a low- and a high-melting lipid and cholesterol laterally segregate over a wide range of proportions into cholesterol-rich Lo and cholesterol-poor Ld domains (Brown and London, 1998; Feigenson, 2006). Such domains, termed lipid rafts and enriched in cholesterol and sphingolipids, are hypothesized to also exist in the membranes of living cells (Simons and Ikonen, 1997; Lingwood and Simons, 2010) where they seem to be small, dynamic and transient, which makes their study difficult and controversial (Shaikh and Edidin, 2006). In model membrane systems, some of the factors that lead to sub-microscopic, dynamic domains in cells can be eliminated so that phase separation occurs on a larger scale, exhibiting micrometer-size domains that can be easily observed by fluorescence microscopy. Lo phase domains surrounded by continuous Ld phase lipid bilayers can be used as simple models for lipid rafts, which permit studies of the relevant physical interactions under controlled conditions (Veatch and Keller, 2005; Feigenson, 2006; Jacobson et al., 2007; Crane and Tamm, 2007; Sezgin et al., 2012; Kiessling et al., 2015).

Cell membranes are highly asymmetric in terms of lipid composition. Phospho- and sphingo-lipid, but not cholesterol flip-flop across the membrane is slow in the absence of “flippases” in healthy, non-apoptotic cells. Such lipid asymmetry can also be generated and maintained in some model systems with and without lipid rafts (Crane et al., 2005; Garg et al., 2007; Lin and London, 2014). It has been shown that sphingomyelin-rich domains in the outer leaflet of the plasma membrane can couple and induce domains in the inner leaflet of the membrane, which otherwise might not be present (Kiessling et al., 2006; Collins and Keller, 2008; Wan et al., 2008; Kiessling et al., 2009; Chiantia et al., 2011; Wan et al., 2011). All intracellular membrane fusion processes take place by initial contact of inner leaflet lipids and thus induced phase heterogeneity in these leaflets of the membrane may be mechanistically important.

Since cholesterol straightens out the lipid tails in cholesterol-rich Lo phase lipid bilayers, such bilayers are usually thicker than cholesterol-poor Ld phase bilayers (Pan et al., 2008). When both phases coexist in membranes, a discontinuity of the bilayer width arises at the phase boundary, which results in a line-tension that has direct implications on membrane curvature at the interface between the Lo and Ld phases (Baumgart et al., 2003). Line-tension at Lo-Ld interfaces has been recognized as an essential parameter that controls the kinetics of phase separation and the sizes of lipid domains (Garcia-Saez et al., 2007). Since line-tension also induces membrane bending it can drive the budding and formation of new vesicles at domain boundaries (Julicher and Lipowsky, 1993; Garcia-Saez et al., 2007).

Due to the very small size of the polar headgroup compared to the cross-sectional area of the apolar portion, cholesterol generates intrinsic negative curvature in lipid bilayers. Cholesterol thereby has the potential of promoting highly curved membrane structures such as lipid stalks that have been proposed as lipid intermediates in membrane fusion (Yang and Huang, 2002; Chernomordik and Kozlov, 2008; Aeffner et al., 2012). The resistance that lipid bilayers exhibit towards bending into curved

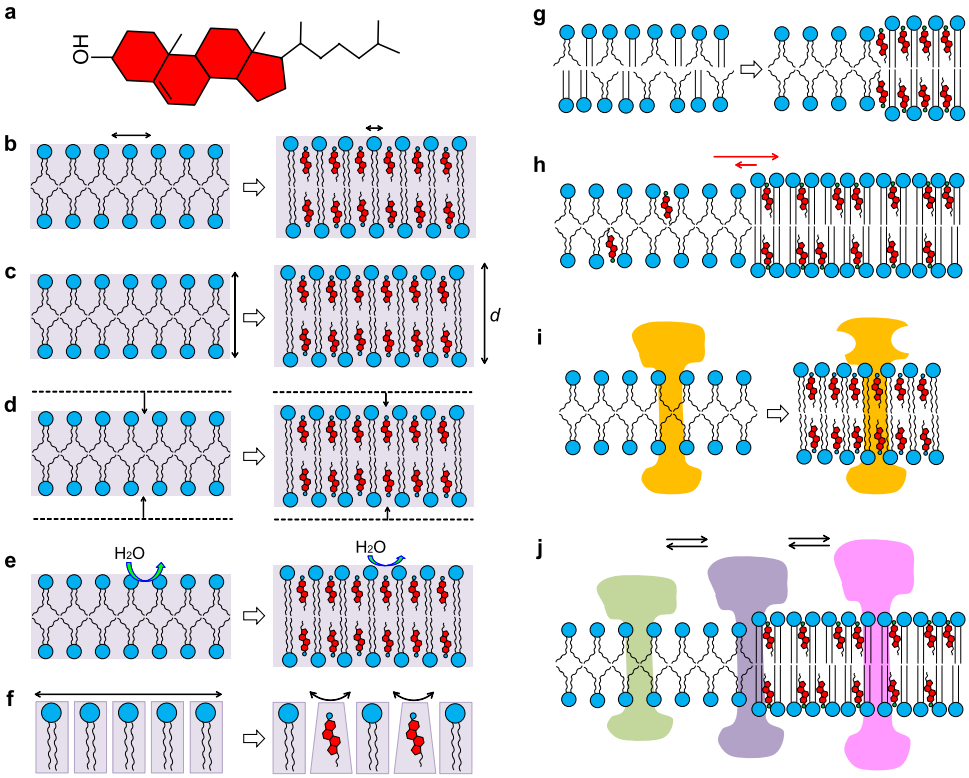


**Fig. 1.** Cholesterol has multiple effects on lipid bilayers. Cholesterol (a) changes the fluidity (b), thickness (c), compressibility (d), water penetration (e), and intrinsic curvature (f) of lipid bilayers. Cholesterol also induces phase separations in multicomponent lipid mixtures (g), partitions selectively between different coexisting lipid phases (h), and causes integral membrane proteins to respond by changing conformation (i) or redistribution (j) in the membrane.