of elastic strain in the bilayers and that this strain modulates the function of certain membrane proteins.

The rationale that lipid mesomorphic plasticity plays a role in membrane fusion has been demonstrated in several pure lipid vesicle systems (see Ellens et al., 1989, and references cited therein). An understanding of how mesomorphic plasticity can be manipulated to control vesicle fusion may have technological importance for applications such as drug delivery and chemical processing (Gruner, 1987). The role mesomorphic plasticity may play in living cells is uncertain, largely because of lack of knowledge of the mechanism of the proteins involved in processes such as biomembrane fusion. Because biomembrane fusion occurs under protein control, an understanding of the role mesomorphic plasticity must ultimately involve a better understanding of the interactions of protein with lipid. Consequently, the rationale of mesomorphic plasticity is really a subset of the notion that lipid curvature strain affects the function of specific proteins.

Nonlamellar phases, as explained earlier in this chapter, may be understood on the basis of the competition between curvature elasticity and the constraints of uniform packing of the hydrocarbon chains. In the bilayer phase, lipid mixtures characterized by small, spontaneous curvature radii result in the buildup of a curvature strain, which is released upon undergoing nonlamellar phase transitions, such as the Lα-HII transition. Other mixtures, characterized by larger R0 values at the given temperature, are more elastically relaxed in the bilayer phase. What are the biological implications of the buildup of a bilayer curvature strain energy? To choose a concrete example, bilayers rich in unsaturated PEs tend to have small R0 values, and those rich in PCs tend to have larger R0 values. Thus, at physiological temperature, bilayers of the former composition will have a built-in strain, whereas the PC-rich bilayers will be more relaxed. How will this physical difference affect, for instance, the functioning of imbedded membrane proteins?

One can conceive of experiments that directly test the hypothesis that protein function is modulated by the spontaneous curvature of the membrane lipid monolayers (Gruner, 1985). It would be necessary to reconstitute the protein, typically a membrane protein, in lipid bilayers in which the composition is varied in a way so as to attain a given value of the spontaneous curvature. This is possible because the spontaneous curvature is a colligative quantity that can be adjusted to a given value by many different lipid compositions. If the activity of the protein were correlated most directly with the spontaneous curvature (and not the compositions used to achieve the spontaneous curvature), then the experiment would be very strong evidence that the spontaneous curvature modulates lipid activity. In practice, such experiments are very difficult. Simply purifying and reconstituting integral membrane proteins is often experimentally difficult. When lipid mixtures are used for reconstitutions, some of the lipid does not end up in the reconstituted vesicles, so the final lipid compositions must be determined by assay. Also, membrane proteins are complex conformational engines that may be subject to many simultaneous requirements, such as the bilayer thickness and charge, as well as to specific auxiliary lipid requirements. In varying the compositions, it will be important to keep all the other requirements constant as well, if the dependence on R0 is to be unambiguously measured. Even given these difficulties, it will be important to perform experiments whereby correlations with R0 are examined if the interactions between protein and nonlamellar-prone lipid are to be examined. Investigation of such correlations is an important area for future research.

5.5 CONCLUSION

Although nonlamellar lipid phases have been known for decades, modern investigations that have sought to elucidate the relationship between nonlamellar-prone lipid and biomembranes have mostly been performed since the late 1970s. There was a time when research on lipid mesomorphism was regarded by many biologically oriented scientists as a peripheral activity to the study of biomem-
branes because nonlamellar lipid phases were rare in living systems. It is now understood that study of the appearance of nonlamellar phases under nonphysiological conditions is not divorced from biological concern. This is because the phase boundaries are frequently very close to the physiological conditions of biomembranes, suggesting that biomembranes regulate their compositions near the edge of instability (Gruner, 1985). There must be advantages to doing so, because it is not hard to select lipid compositions that are far removed from the phase boundaries. Attention is now being focused on understanding what these advantages might be.

Interest in the biological roles of nonlamellar-prone lipid has resulted from an improved understanding of the physical basis of lipid mesomorphism. In particular, it is now understood that a competition between monolayer spontaneous curvature and hydrocarbon packing dominates the mesomorphic behavior of lipid systems. Significantly, it is now recognized that the forces that drive lipid mesomorphism are present in both lamellar and nonlamellar phases and that the focus of study of the biological importance of lipid mesomorphism should be on understanding these forces. It is especially important to investigate the interaction of these forces with biomembrane proteins. Recent experiments have suggested that the activities of at least some important proteins are strongly modulated by the nonlamellar-prone characteristics of the imbedding lipid bilayer.

Interest in nonlamellar-prone lipid has also been catalyzed by studies that have shown that the presence of such lipid is carefully regulated in at least some cellular systems. A major difficulty in performing studies of this kind has been in identifying physical properties that relate to, indeed, define the nonlamellar tendency of a given lipid composition. In the past, the temperature of the Lα-nonlamellar phase transition has most commonly been used as a measure of the nonlamellar tendency. Physical studies on isolated lipid systems have demonstrated that the transition temperature is a result of the balance between curvature and other competing forces and that the temperature can be shifted by very small levels of specific impurity molecules. The spontaneous curvature is, itself, a better measure of the nonlamellar tendencies of a given mixture, but few studies have yet correlated biological behavior with this measure. Another problem that limits progress is the fact that most measures of the nonlamellar tendencies of a system require extraction of the lipid. Physical probes are needed that can measure curvature stress in bilayers in the presence of protein.

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