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THERMAL ADAPTATION IN BIOLOGICAL MEMBRANES: Is Homeoviscous Adaptation the Explanation?

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INTRODUCTION

The phase behavior and physical properties of lipids in biological membranes are exquisitely sensitive to changes in temperature (50). Because membranes (*a*) act as physical barriers to solute diffusion, (*b*) mediate the transmembrane movement of specific solutes, (*c*) regulate the utilization of energy stored in transmembrane ion gradients, (*d*) provide an organizing matrix for the assembly of multicomponent metabolic and signal transduction pathways, and (*e*) supply precursors for the generation of lipid-derived second messengers, temperature-induced perturbations in membrane organization pose a serious challenge to the maintenance of physiological function in poikilotherms. However, poikilotherms exploit the diversity of lipid structure to fashion membranes with physical properties appropriate to their thermal circumstance and, in this way, restore membrane function following thermal challenge. Based on the finding that membrane lipids of *Escherichia coli* grown at 43 and 15°C displayed similar physical properties when compared at their respective growth temperatures, Sinensky concluded that membrane fluidity was defended as growth temperature changes and referred to this cellular homeostatic response as homeoviscous adaptation (HVA) (94). Since the original exposition of this hypothesis, HVA has emerged as the most commonly employed paradigm to assess the efficacy of thermal adaptation in biological membranes and to explain patterns of temperature-induced change in membrane lipid composi-

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tion (23). The goals of this review are to assess critically the role of HVA in the thermal adaptation of biological membranes and to explore alternative explanations for patterns of thermotropic lipid restructuring.

MEMBRANE CONSTRAINTS TO GROWTH AND FUNCTION AT DIFFERENT TEMPERATURES

Thermal Perturbation of Membrane Structure and Function

One consequence of poikilothermy is perturbation of membrane organization when cell or body temperature changes. Effects of temperature are most evident as altered properties of the acyl chain domain in the bilayer interior. At physiological temperatures, gauche rotamers (rotations about carbon-carbon single bonds) freely propagate up and down the length of the fatty acyl chains, which results in a relatively fluid, disordered liquid-crystalline phase (Figure 1) (72). However, acyl chain motion is moderately constrained for 8–10 carbon atoms extending from the membrane surface primarily by the covalent attachment and parallel alignment of the hydrocarbon chains (11). When temperature drops below the physiological range, acyl chains, at some defined point (the gel/fluid or chain-melting transition temperature, T_m), adopt the all-*trans* conformation and pack efficiently to form a highly ordered gel phase (Figure 1). However, in biological membranes, a region of phase separation (consisting of coexisting domains of fluid and gel phase lipids) may extend over a temperature range of 10–15°C due to the diversity of lipid species present (59). Conversely, when temperature exceeds the physiological range, some lipids [most notably phosphatidylethanolamine (PE)] assume the inverted hexagonal (H_{II}) phase (Figure 1), which results in a loss of bilayer integrity (67, 91). The transition to the H_{II} phase (occurring at T_h) is driven, in part, by a temperature-induced change in phospholipid molecular geometry from a cylindrical to a conical shape (refer to Figure 1). Although conical lipids can accommodate increased disorder in the acyl domain, they cannot alone form a lamellar or bilayer phase. Finally, even in the absence of lipid phase transitions, rising temperature increases the rate and extent of acyl chain motion (111).

Thermal perturbation of lipid phase state has a profound impact on membrane structure and function. For example, transition from the fluid to gel phase (*a*) induces the clustering of integral membrane proteins, which are largely excluded from domains of gel phase lipids (54); (*b*) reduces the activity of many membrane-associated enzymes (52, 108); (*c*) slows the rate of lateral protein diffusion within the plane of the bilayer, thereby reducing the efficiency of diffusion-coupled processes (45); and (*d*) markedly increases the permeability to cations and water, presumably because of packing defects that form at boundaries between microdomains of gel and fluid phase lipids (10, 95).

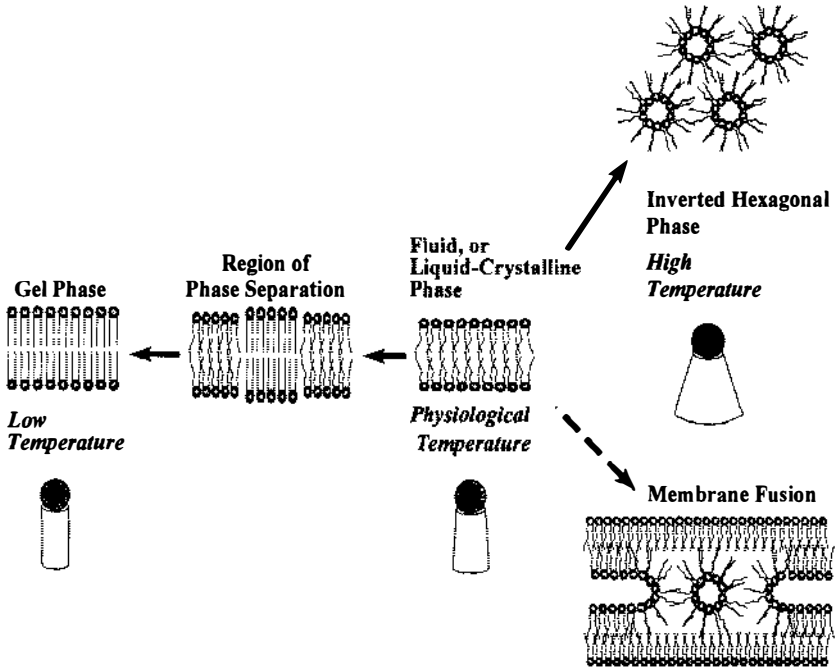

Figure 1 Solid arrows indicate the effects of either a rise or drop in temperature on the phase behavior and molecular geometry of membrane phospholipids. The physiological temperature refers to the temperature at which an organism is either adapted or acclimated. The dashed arrow illustrates the presumed involvement of the inverted hexagonal phase in membrane fusion.

Furthermore, temperature effects too slight to induce phase transitions can also significantly affect membrane function. Activities as diverse as Na^+/K^+-ATPase in lamb kidney (47), chloride transport in secretory granules of rat pancreas (40), the binding, uptake, and degradation of LDL by rat hepatocytes (61), collision coupling between components of the β-adrenergic signal transduction pathway (46), the rotational mobility of sarcoplasmic reticular Ca^{2+}-ATPase (99), and the passive permeability of fluid phase membranes (26) are all positively correlated with membrane fluidity, which is, in turn, determined by temperature.

Membrane Constraints to Growth and Function

Considerable evidence indicates that the phase state and/or physical properties of membrane lipids contribute to the definition of the thermal limits for growth and function. For example, in *Acholeplasma laidlawii* enriched in relatively high melting point fatty acids, growth is inhibited at low temperatures when more than 50% of the membrane lipid is present in the gel phase and ceases

entirely when the proportion of gel phase lipids reaches 90%, which indicates a growth requirement for fluid phase lipids (70). Similarly, loss of photosynthetic activity at cold temperature in the cyanobacterium *Anacystis nidulans* coincides with the onset of phase separation in the plasma membrane (77). In addition, butylated hydroxytoluene, a perturber of lipid bilayers, improves the survival of mammalian cells at temperatures below the fluid/gel transition of their membrane lipids, which further supports a functional requirement for fluid phase lipids (60).

Whether membranes also constrain growth and function at elevated temperatures is less clear. The leakage of K^+ from muscle fibers at elevated temperatures has been implicated as a cause of heat death in crayfish (42), and in *Acholeplasma,* the maximum growth temperature is decreased in cells grown on low melting point fatty acids (70). Furthermore, *E. coli* regulate the phospholipid composition of their membranes so that the H_{II} phase transition occurs approximately 10°C above the growth temperature (85). Thus either increases in membrane permeability or formation of nonlamellar phases, with the consequent loss of bilayer integrity, may constrain growth and function at high temperatures, just as formation of the gel phase does at low temperatures.

Membrane properties may also influence physiological performance in the interval between T_m and T_h. For example, in *Acholeplasma* grown on various fatty acid mixtures containing perdeuterated palmitate (a nonperturbing ^{2}H-NMR probe of membrane order), maximal growth rates were restricted to a range of average molecular order parameters (S_{mol}—a measure of the time-averaged orientation of the ^{2}H-C bond vector relative to the bilayer normal) between 0.140 and 0.177 (74).

MEMBRANE REMODELING: The Basis of Thermal Adaptation

The inherent sensitivity of the phase behavior and physical properties of membrane lipids to changes in temperature restricts the thermal range over which a designated set of membrane constituents can function effectively. Consequently, to function over a broad range of environmental temperatures, poikilothermic organisms must restructure their membranes so that lipids of appropriate physical properties are matched to the prevailing thermal conditions. Accordingly, the most commonly observed cellular response to altered temperature is a remodeling of biological membranes. Growth at low temperature invariably leads to one or a combination of the following adjustments to membrane lipid composition: (*a*) increased proportions of *cis* unsaturated fatty acids (UFA), particularly long-chain polyunsaturated fatty acids (PUFA) in the most cold-tolerant animals (49), or branched-chain fatty acids in some

microorganisms (103); (*b*) elevated proportions of PE relative to PC in animal cell membranes (82) or, in higher plants, monoglucosyldiglyceride (MGDG) to diglucosyldiglyceride (DGDG) (105) so that the ratio of bilayer-stabilizing to bilayer-destabilizing lipids increases with growth temperature; and (*c*) reduced proportions of plasmalogens (i.e. alk-1-enyl ether) compared to diacyl phospholipids, particularly in nervous tissue (68). The interested reader is referred to Reference 50 for a more thorough discussion of these compensatory responses.

ADAPTIVE EXPLANATIONS FOR MEMBRANE REMODELING

Homeoviscous Adaptation

The paradigm most widely invoked to explain the temperature-induced remodeling of membrane lipid composition is homeoviscous adaptation. According to this hypothesis, optimal membrane function is restricted to a limited range of membrane fluidities. As temperature is raised acutely, fluidity is increased beyond the optimal range and the membrane becomes "hyperfluid." Conversely, as temperature drops, fluidity falls below the optimal range and membrane activities are constrained. Consequently, persistent exposure to temperatures either above or below those required to maintain optimal fluidity initiates acclimatory (within the lifetime of an individual) or adaptational (over evolutionary time) alterations in lipid composition that largely offset the direct effects of temperature on membrane lipid fluidity.

In assessing the extent of HVA, it is important to recognize that, due in part to the range of motions displayed by lipid molecules (from rotamer formation within an acyl chain to the lateral diffusion of a phospholipid within the plane of the bilayer), membrane fluidity cannot be defined with rigor. No single technique for estimating fluidity is sensitive to the entire range of motions available to membrane lipids and, as a result, estimates of fluidity are biased by the type(s) of motions sensed. The steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) has been most widely used in the comparative literature to assess membrane fluidity. However, because DPH is an asymmetrical molecule and does not undergo isotropic rotation within a membrane, the average extent of acyl chain motion, or membrane order, contributes more to the observed anisotropy than do acyl chain dynamics (65). Thus for the purposes of this review, fluidity measurements derived from the steady-state anisotropy of DPH are described in terms of membrane order, and HVA implies the conservation of lipid order (a static description of the time-averaged disposition in space of membrane constituents) rather than rates of molecular motion.

THE EVIDENCE FOR HOMEOVISCOUS ADAPTATION The most compelling evidence in support of HVA is derived from interspecific comparisons by Cossins and colleagues of membrane order in synaptosomal preparations of various vertebrates (14, 21). Recently these original studies were expanded to include a broader range of species comparisons, a better-characterized membrane fraction, and the application of time-resolved anisotropy measurements, which permit the unambiguous separation of rate and order effects (4). As illustrated in Figure 2 (*solid symbols*), membrane order, i.e. the anisotropy value, when measured at a common temperature (20°C), is lowest in synaptosomes of Antarctic fish (of the genus *Notothenia*) and highest for homeothermic vertebrates (rat and pigeon), with values for temperate fish being intermediate between these extremes. Consequently, the rank sequence of membrane order [Antarctic fish (–1°C) < perch (15°C) < convict cichlid (28°C) < rat (37°C) < pigeon (42°C)] correlates directly with body or habitat temperature, which indicates that evolutionary adaptation to cold environments produces membranes of significantly lower order. Conversely, when compared at the respective cell or body temperatures (*open symbols*), membrane order is roughly equivalent in all species, which illustrates the phenomenon of HVA. Similar trends in lipid order have been reported for comparisons between Arctic and tropical copepods (34), sarcoplasmic reticular membranes of rabbit and winter flounder (111), and mitochondrial membranes of warm- and cold-water abalones (25). Regression of the temperature required to produce a specified anisotropy on body temperature (slope = 1.0 for perfect compensation) for the data reported in Figure 2 indicates that interspecific differences in lipid anisotropy compensate for only 70% of the direct effects of temperature on membrane order. However, use of *trans*-parinaric acid, a probe more closely resembling the acyl chains of membrane phospholipids than DPH, indicates nearly perfect compensation of membrane order. In addition, time-resolved anisotropy measurements indicate that it is those features of membrane structure that influence the amplitude of probe motion, i.e. membrane order, rather than its reorientational rate that are under adaptive regulatory control (4). Efficacies of interspecific HVA generally fall between 0.7 and 1.0 (20).

HVA is also a common outcome of temperature acclimation in eurythermal poikilotherms, as illustrated in Figure 3 (*left*) for basolateral membranes of rainbow trout enterocytes. Although an excursion by 20°C-acclimated trout into 5°C water orders the membrane to the extent indicated by a rise in polarization from point A to B, subsequent acclimation to 5°C disorders the membrane by an amount equivalent to the drop in polarization between points B and C. Because lipid order is similar in 20°C- and 5°C-acclimated trout when compared at the respective acclimation temperatures, membrane order is conserved. Recent application of Fourier transform infrared (FT-IR) spectroscopy to living cells of *Acholeplasma laidlawii* B (75) and time-resolved

fluorescence polarization techniques to isolated membranes of *Bacillus subtilis* (51) confirm that conformational order rather than the rate of lipid motion is the feature of membrane organization subject to regulation when temperature changes. Furthermore, in *Acholeplasma,* the point of regulation corresponds to the occurrence of 1.5 gauche rotamers per acyl chain regardless of growth temperature (75). Although reports of perfect compensation are not unusual [other examples include the plasma membranes of fish lymphocytes (1, 6) and thylakoid membranes of oleander (83)], more commonly the efficacies of intraspecific HVA range between 20 and 50% (reviewed in 20, 23), depending on membrane type. For example, mitochondria commonly exhibit higher efficacies of HVA (0.5–0.75) than do other membranes (18, 22).

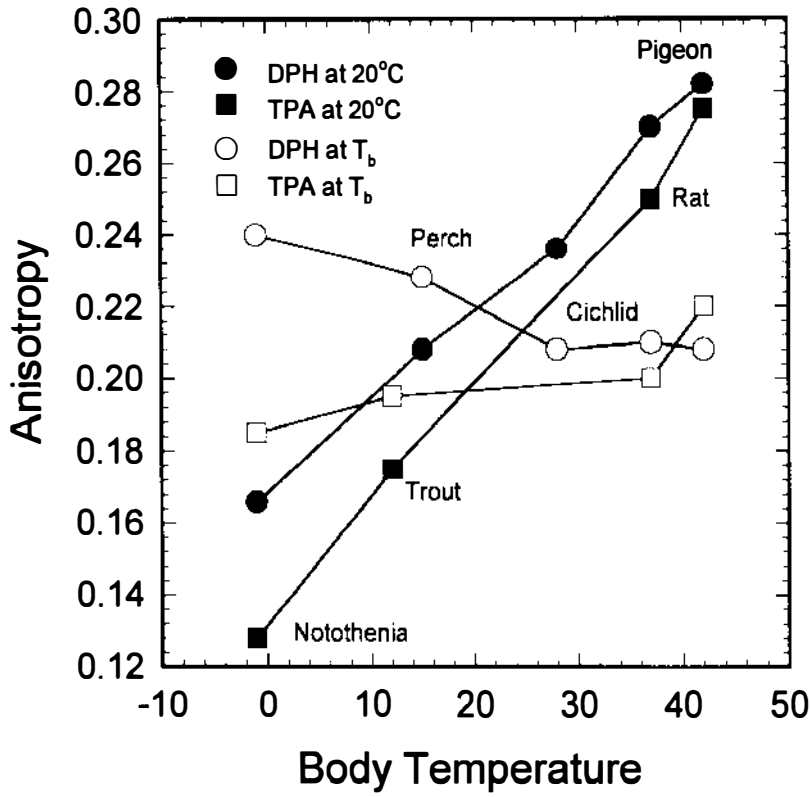

Figure 2 Anisotropy values for the fluorescent probes DPH and *trans*-parinaric acid (TPA) as a function of body temperature for several vertebrates, when measured either at a constant temperature of 20°C (*solid symbols*) or at the body temperature of the respective species (*open symbols*). The species illustrated include the Antarctic fish *Notothenia neglecta*; the rainbow trout *Oncorhynchus mykiss*; the perch *Perca fluviatilis*; the convict cichlid *Cichlasoma nigrofaciatum*; the rat *Rattus rattus*; and the feral pigeon *Columbia livia* (data are from 4).

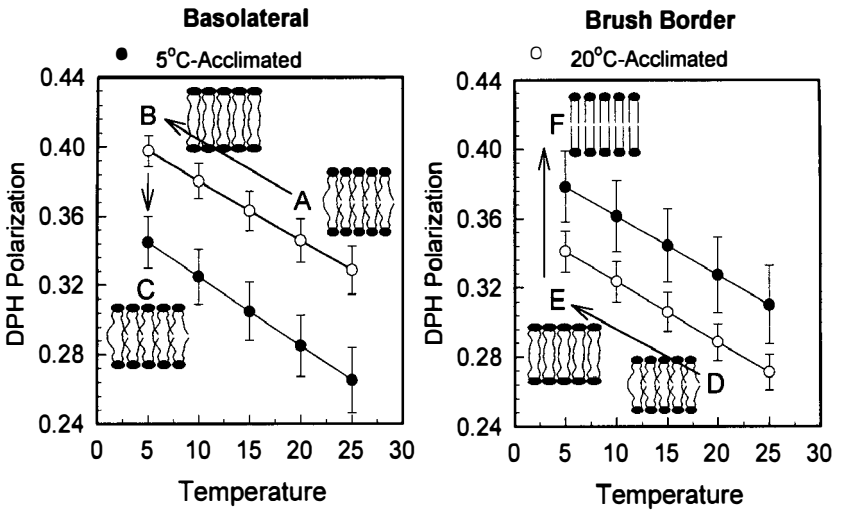

Figure 3 The effect of temperature on membrane order in basolateral and brush border plasma membrane domains of enterocytes isolated from 5°C- and 20°C-acclimated rainbow trout (data are from 24).

Interestingly, the capacity for acclimatory HVA appears to be a basic cellular response displayed not only by microorganisms (56, 75, 88), but also by cells of vertebrate poikilotherms. For example, in vitro acclimation experiments employing either cell lines established in tissue culture (7, 106), or cells [either red blood cells (29) or hepatocytes (114)] freshly isolated from temperate fish document efficacies of HVA varying from 34 to 100%.

ADAPTIVE SIGNIFICANCE OF HVA The conservation of membrane order over a wide range of vertebrate species differing in body temperature provides strong circumstantial evidence for the adaptive significance of HVA, yet there is remarkably little direct evidence to support this view. Robust correlations between membrane order and the activity of Na^+/K^+-ATPase (47) indicate that the lipid environment of the enzyme can constrain protein motions required for catalysis. Thermal compensation of membrane order could therefore conceivably stabilize and/or optimize the active conformations of integral membrane proteins. Adaptational and acclimatory shifts in the sensitivity to thermal denaturation by membrane-associated enzymes provide the strongest evidence in support of this view. For example, the LT_{50} (the temperature required to reduce enzyme activity by 50% in a 15 min period) of Na^+/K^+-ATPase is nearly 3°C lower (44.9 vs 47.7°C) in the less-ordered synaptosomes of 6°C- than 28°C-acclimated goldfish, and perturbation of membrane order by the

addition of *n*-hexanol also reduces thermostability (15). Furthermore, for a variety of both inter- and intraspecific comparisons, differences in LT_{50} for synaptosomal Na^+/K^+-ATPase co-vary closely with differences in the extent of HVA, which suggests a causal relationship between membrane order and the susceptibility of the enzyme to thermal denaturation (19). Similarly, thermal denaturation of chlorophyll in thylakoid membranes of oleander (83) and vertebrate rhodopsin in comparisons among fish, amphibians, and mammals (107) commences at equivalent values of membrane order regardless of thermal history, although denaturation occurs at higher temperatures in warm- than cold-acclimated or adapted organisms. Acclimatory shifts in membrane order and the temperature at which cytochrome c oxidase is inactivated in mitochondrial membranes of abalone (25), coupled with the demonstration that reductions in membrane order induced either by low temperature acclimation or the application of *n*-hexanol reduce the heat resistance of ciliary activity in *Anodonta* gill (62), further support a role for HVA in modulating protein thermostability.

Although the above data implicate HVA in the resistance adaptation of membrane function, there is little direct evidence to support a role for HVA in the capacity adaptations (i.e. modulation of the rates of enzyme activity) of poikilothermic organisms. In a few instances, reductions in membrane order following cold acclimation have been correlated with higher rates of Na^+/K^+-ATPase activity (84, 90), but in no case has a causal relationship between catalytic rate and membrane order been independently established. More commonly, reductions in membrane order, although not actually measured, have been invoked to explain increased activities of membrane-associated enzymes in cases where enzyme titres were not increased by cold acclimation (9, 48, 115). Nevertheless, in cold-acclimating goldfish, compensatory adjustments in the cold-block temperature of spinal reflexes are strongly correlated with temporal changes in the order of synaptosomal membranes (17), which suggests a role for HVA in the acclimation of neural function. Furthermore, isothermal catalytic hydrogenation of plasma membrane lipids in the cyanobacterium *Synechocystis* PCC6803 stimulates the expression of a Δ^{12} desaturase, just as does a drop in temperature, which suggests that hydrocarbon order is the membrane attribute subject to adaptive regulation (110).

Limitations of HVA as an Adaptive Paradigm

There is little doubt that in many poikilotherms temperature-induced restructuring of membrane lipid composition results in some degree of HVA. This, coupled with the intuitive appeal of such a straightforward mechanism of cellular homeostasis, has frequently resulted in HVA being the only paradigm considered when interpreting the pattern of temperature-induced changes in membrane lipid composition and the extent of thermal compensation in bio-

logical membranes. Yet there are numerous examples of membrane responses to altered growth temperature that are either inconsistent with or difficult to explain in terms of HVA, which suggests that mechanisms other than the defense of lipid order may also contribute to the thermal compensation of membrane function. The limitations of HVA as a general paradigm for membrane adaptation are further explored within the context of specific observations that are difficult to reconcile with the hypothesis.

VARIABILITY IN THE EXTENT AND OCCURRENCE OF HVA As noted above, the efficacy of HVA varies widely, ranging from 20 to 100% for acclimatory HVA, and averaging ~ 70% for evolutionary HVA. However, comparisons between several species of cold (5–10°C, from the North Atlantic/Pacific and Baltic Sea)- and warm (20-27°C, South China Sea)-adapted teleost fish indicate only a 7–10% compensation of lipid order in hepatic phospholipids (28). In addition, a total lack of HVA has been reported for both sarcoplasmic reticular membranes and the apical plasma membrane domains of enterocytes in teleost fish (16, 108). Furthermore, as illustrated in Figure 3 (*right*), apical membrane domains isolated from trout enterocytes display a highly significant inverse compensation in lipid order. Not only does an acute drop in temperature from 20 to 5°C order the membrane by an amount equivalent to the interval between D and E, but acclimation to 5°C further orders the membrane to an extent indicated by the interval from E to F; consequently, the apical membrane domain is significantly more ordered in 5°C- than in 20°C-acclimated trout. Collectively, these data indicate that there is no consistent relationship between either the direction or magnitude of HVA and the thermal stress. Thus, although it is possible that moderate degrees of HVA could result in perfect compensation of function in some membranes, or that variable degrees of HVA could compensate function to different extents in different membranes, the tendency of cold exposure to disorder some membranes, while not influencing or ordering others, argues against the regulation of membrane order as a generally applicable paradigm of membrane adaptation.

THERMAL COMPENSATION OF MEMBRANE FUNCTION WITHOUT HVA Thermal compensation of membrane function and the capacity for HVA are not tightly linked. For example, although rates of calcium uptake are consistently higher in sarcoplasmic reticular membranes of cold- compared with warm-acclimated fish (108), these membranes consistently reveal no (16) or only limited capacity (108) for HVA. In addition, although increased rates of sodium pump activity in red blood cells of 3°C- compared with 20°C-acclimated trout coincided with reduced lipid order at certain times of the year, at other times no compensation in pump activity could be demonstrated in spite of HVA (efficacy ~ 30–40%) (84). Furthermore, in Arctic charr, *Salvelinus alpinus,* neither the activity nor

thermal stability of Na^+/K^+-ATPase in basolateral membranes of kidney was altered by cold acclimation in spite of substantial HVA (efficacy ~ 78%); instead a reduction in passive ion fluxes (which were 60% lower in 5°C- than 20°C-acclimated fish) appears to be the major acclimatory adjustment responsible for the maintenance of cation gradients at low temperature (90). In contrast, in the roach *Rutilus rutilus,* which coexists with Arctic charr in subalpine lakes of central Europe, the density of sodium pump sites was increased fourfold by cold acclimation, whereas the efficacy of HVA was relatively modest (~ 20%). Thus, although *R. rutilus* and *S. alpinus* maintain ion gradients at low temperature by fundamentally different mechanisms (an acclimatory increase in transport capacity for *R. rutilus* as opposed to diminished passive dissipation of ion gradients in *S. alpinus*) in neither species does HVA play an essential role in the acclimatory response. Finally, the low temperature suppression of immune function in poikilothermic vertebrates, which in channel catfish is due primarily to an inhibitory effect on the activity of helper T cells (5), cannot be attributed to a lack of HVA, because compensation of membrane order is nearly perfect in the plasma membranes of both B and T cells (6). Interestingly, oleic acid (18:1*n*9), but not linoleic acid (18:2*n*6), can rescue ~ 60% of the con A-induced T cell proliferation normally inhibited at low temperatures (5). However, since 18:2 is no less effective than 18:1 in fluidizing membranes, it is difficult to explain the fatty acid specificity of these immune rescue experiments in terms of modulation of lipid order. These few examples clearly illustrate that thermal compensation of membrane function can occur in the absence of HVA and vice versa, thus calling into question a consistent causal relationship between modulation of lipid order and the conservation of membrane function.

MEMBRANE REMODELING NOT CONSISTENT WITH HVA Two aspects of temperature-induced membrane restructuring are particularly difficult to explain in terms of HVA. One is a preference for the accumulation of long-chain PUFA at low temperature, and the other is a positive correlation between growth temperature and the ratio of bilayer-stabilizing to bilayer-destabilizing lipids.

The low-temperature accumulation of PUFA Increased unsaturation of membrane lipids promotes survival at cold temperatures. For example, desaturase mutants of the cyanobacterium *Synechocystis* PCC6803 are more sensitive than wild-type to low-temperature inhibition of photosynthesis (44), and strains of *Arabidopsis* deficient in fatty acid desaturation fail to grow and eventually die at 6°C, whereas wild-type plants grow and develop normally at this temperature (73). Furthermore, the transfer of a desaturase gene from chilling-resistant *Synechocystis* into chilling-sensitive *Anacystis nidulans* lowers the T_m of plasma membrane lipids in the transformed cells by 4–8°C and increases the

tolerance of the latter to low temperatures (112). Conversely, reduced levels of lipid unsaturation promote survival at warm temperatures because hydrogenation of thylakoid membranes in pea seedlings increases their resistance to heat stress (109); however, the complete loss of PUFA in desaturase mutants of *Synechocystis* actually reduces heat tolerance (43). Because *cis* double bonds introduce a kink into the acyl chain, UFAs pack less compactly and thus offset, to a significant degree, the increase in membrane lipid order caused by a drop in temperature. However, from the standpoint of modulating lipid order, it is unclear why most winter-active poikilotherms accumulate PUFA rather than monoenes in their membrane lipids when grown at low temperature (113) because it is well established that not all double bonds in a fatty acid have an equivalent impact on membrane physical properties. For example, substituting oleic acid (18:1*n*9) for palmitic acid (16:0) at the *sn*-2 position of dipalmitoyl-PC (to form 16:0/18:1-PC) reduces the melting point by 50°C, whereas the incorporation of a second double bond to form 16:0/18:2-PC lowers the melting point by an additional 22°C. However, introduction of a third double bond, resulting in the formation of 16:0/18:3-PC, actually increases the melting point slightly (by 3°C) (12). Furthermore, T_m values for 16:0/16:1- and 16:0/22:6-PC do not differ significantly (–12 vs –10°C, respectively) (101). Thus from the standpoint of altering membrane physical properties, monoenoic fatty acids are superior to PUFAs with respect to the magnitude (expressed on a per double bond basis) of the changes they produce and the lower metabolic cost of their production. Consequently, if lipid order is the membrane parameter subject to regulation, monoenes are expected to play a more prominent role than they do in the restructuring process. The fact that they do not implies that other aspect(s) of membrane architecture must be conserved during the acclimation process.

The balance between bilayer-stabilizing and bilayer-destabilizing lipids A second compositional adjustment difficult to reconcile with HVA is the increased abundance of bilayer-destabilizing lipids such as PE in membranes of cold-adapted poikilotherms because elevated proportions of PE (relative to PC) commonly order rather than fluidize a membrane. For example, gel/fluid transition temperatures for PE are generally about 20°C higher than those for PCs of similar acyl chain composition due, in part, to the reduced hydration and stearic bulk of the ethanolamine compared to the choline headgroup and the capacity for hydrogen bonding between the headgroups of PE but not PC (92). In addition, because bilayer-destabilizing lipids are conically shaped (53), they increase the lateral pressure within the plane of the bilayer and the tendency of the bilayer to curve, thus displacing the phase behavior of the membrane to a point in closer proximity to the H_{II} phase transition (91). Thermal modulation of headgroup composition (reflected in altered PE/PC and

MGDG/DGDG ratios) may thus have a greater adaptive impact on membrane phase behavior than on hydrocarbon order.

THE LACK OF CORRELATION BETWEEN MEMBRANE FUNCTION AND ACYL CHAIN ORDER In spite of many correlative data supporting a causal link between lipid order and membrane function (see above), there is an equally compelling body of evidence indicating that many aspects of membrane organization can influence function to a greater extent than changes in lipid order (64). For example, although activity of the reconstituted glucose transporter of human erythrocytes increases markedly at the gel/fluid transition in bilayers of PC (a zwitterionic phospholipid), activity was unaffected as the membrane passed through this same transition in bilayers of phosphatidic acid, phosphatidylglycerol, or phosphatidylserine (all acidic phospholipids), which suggests that membrane surface charge can stabilize the transporter against the most extreme changes in membrane order (104). Furthermore, activity of the transporter in gel phase bilayers of distearoyl (di-$C_{18:0}$) PC (at 10°C) was similar to that in fluid phase bilayers of dimyristoyl (di-$C_{14:0}$) PC (at 60°) and greater than the activity supported by dioleoyl (di-$C_{18:1}$) PC, even though the latter has a much lower melting point (i.e. is significantly less ordered) (10). Similarly, the activity of rat brain protein kinase C, although insensitive to lipid order, is dependent on the packing arrangement of phospholipid headgroups in the interfacial region of the bilayer (98). Activity of the sarcoplasmic reticular Ca^{2+}-ATPase also varies little with degree of acyl chain unsaturation (64), but does depend on the chain length of the reconstituting phospholipid (with optimal activity being supported by C_{16-20} acyl chains) (100), which indicates that an appropriate bilayer thickness is required to prevent a mismatch between the hydrophobic thickness of the bilayer and the transmembrane span of the protein (13, 100). Furthermore, the nicotinic acetylcholine receptor displays an obligate requirement for cholesterol (36) and a lipid compositional dependence of ion channel activity (102) that cannot be explained by modulation of lipid order (35). Finally, the activities and regulatory properties of several membrane-associated enzymes are more sensitive to the balance between conically and cylindrically shaped phospholipids than to lipid order per se (55, 78, 98). These few examples illustrate the diversity of variables that influence membrane function and suggest that an adaptive focus confined to effects of lipid order is too restrictive to be of general use.

A Dynamic Phase Behavior Model of Membrane Adaptation

The concept of HVA is an adaptational extension of the fluid mosaic membrane model, which emphasizes the lack of long-range order in membranes and the functional importance of maintaining an appropriate lipid fluidity (97). Al-

though this model has been extremely useful in guiding membrane research (96), the failure of HVA to explain apparently fundamental patterns of lipid remodeling in poikilotherms, the lack of a consistent relationship between altered growth temperature and either the extent or direction of adjustments in lipid order, and the failure of membrane function and compensations of membrane function to be consistently correlated with changes in acyl chain order suggest that features of membrane organization other than lipid order are subject to regulation when environmental conditions change.

One feature of membrane organization, only recently appreciated, is the existence of discrete membrane domains. Not only do the apical and basolateral domains of epithelial cell plasma membranes differ significantly with respect to lipid composition, morphology, protein content, and function (93), but microdomains of protein and lipid have been directly demonstrated in plasma membranes of cells lacking epithelial polarity by fluorescence recovery after photobleaching (30) and fluorescence digital imaging microscopy techniques (86, 87). Microdomains of cholesterol (32, 58) and phospholipids (39, 81) have also been inferred from less direct measurements. Furthermore, the activation of lipases by signal transduction pathways may create specific microdomains of regulatory significance to the activity of colocalized membrane-associated processes because of local accumulation of reaction products (76). The extent to which microdomain heterogeneity is perturbed by temperature or conserved by the processes of thermal acclimation remains to be determined. In this context, the adaptive significance of temperature-induced alterations in membrane lipid composition may relate to the conservation of dynamic membrane properties, including the maintenance of an appropriate balance between membrane microdomains and the ability to regulate intracellular membrane traffic, i.e. the dynamic phase behavior of a membrane, rather than to the fine tuning of lipid order.

McElhaney (69, 70) is a leading proponent of the significance of regulating lipid phase state rather than membrane physical properties at altered growth temperatures because structural rearrangements are most extensive and functional perturbations most severe when the phase state of a membrane changes. Since many microorganisms can grow and function normally with membranes of widely different fluidities (37), whereas growth is impaired when a critical proportion (in *E. coli,* 50%) of the membrane lipid is present in the gel phase, McElhaney has proposed the term homeophasic adaptation (HPA) to describe this pattern of thermal adaptation in microorganisms. HPA thus extends the effective range of growth temperatures by preventing transition to the gel phase. There are numerous examples among microorganisms of adaptive alterations in the phase behavior of membrane lipids following a period of growth at altered temperature. For example, in plasma membranes of *Anacystis nidulans,* the onset of phase separation occurs at 5 and 16°C in cells grown at

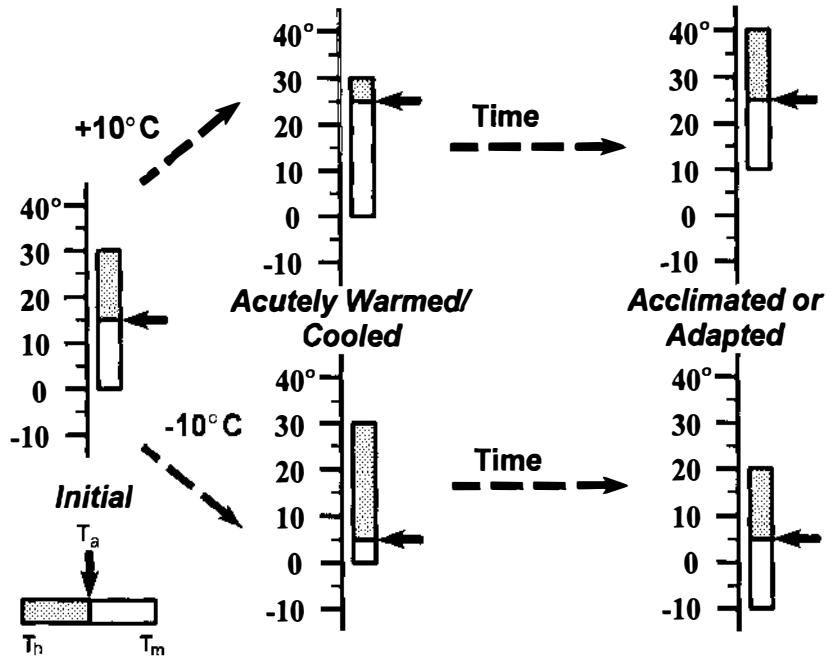

Figure 4 A dynamic phase behavior model of thermal adaptation in biological membranes. An acute rise or drop in temperature (*diagonal arrows*) alters the relationship between the ambient or body temperature (T_a, i.e. the temperature at which the membrane is functioning) and the transitions to the gel (T_m) and H_{II} (T_h) phases (a rise in temperature decreases the interval between T_a and T_h, while increasing the interval between T_m and T_a, whereas a drop in temperature has the opposite effects). Acclimation or adaptation to an altered temperature (*horizontal arrows*) restores the proximity of T_a to T_h and T_m.

28 and 38°C, respectively (38); similar results have been widely reported among other microorganisms and plants (see 50).

A dynamic phase behavior model of membrane adaptation has been developed by broadening the concept of HPA to encompass the full range of membrane phase behavior and to emphasize the dynamic (i.e. the propensity to form nonbilayer phases) rather than the static phase behavior (i.e. the lipid phase actually present) of a membrane. According to this model, it is the relationship between the ambient temperature (T_a) and the temperatures of the gel/fluid and H_{II} phase transitions that is conserved when growth temperature changes. As illustrated in Figure 4, a rise in temperature decreases the interval (*shaded bar*) between T_a and the temperature of the H_{II} phase transition (T_h), while simultaneously moving the membrane farther away from the gel/fluid transition (T_m); a drop in temperature has the opposite effects. Accordingly,

temperature acclimation or adaptation, by altering the chemical composition of the membrane, modifies both T_m and T_h so that the operational temperature (T_a) remains at a suitable interval above T_m, yet below T_h.

The proximity of a membrane to the H_{II} phase transition may be a particularly important functional attribute because the H_{II} phase has been postulated to be an intermediate in membrane fusion (33), and regulation of fusion events is central to the control of intracellular membrane traffic (via exo- and endocytosis). Although membrane fusion is a protein mediated and regulated process, it is clearly influenced by lipids intrinsic and extrinsic to the bilayer (79, 116). The concept of dynamic phase behavior predicts that at physiological temperatures a membrane must be positioned close enough to the H_{II} transition, i.e. be sufficiently unstable, to permit the fusion events associated with normal membrane traffic, yet be stable enough to prevent these processes from occurring in an unregulated fashion (66). Alternatively, cell function will be compromised at low temperature because the interval between T_a and T_h is increased, and membrane traffic is thereby inhibited. A rise in temperature, on the other hand, destabilizes the lamellar phase by decreasing the interval between T_a and T_h, thereby increasing the probability of unregulated fusion events and the loss of bilayer integrity. The marked temperature sensitivity of membrane traffic supports the dynamic phase behavior concept. For example, the transport of cholesterol from its site of synthesis in the endoplasmic reticulum to its primary cellular location in the plasma membrane ceases at temperatures below 15°C in a variety of mammalian cell lines (27, 57, 63). Furthermore, in the yeast *Saccharomyces cerevisiae*, the maintenance of Golgi secretory activity requires a high ratio of bilayer-destabilizing (PI) to bilayer-stabilizing (PC) lipids, which is maintained, in part, by the activity of a PI/PC transfer protein (71). Conversely, membranes that do not engage in vesicular commerce with the plasma membrane possess relatively large amounts of bilayer-stabilizing lipids such as PC (2).

Interspecific conservation of dynamic phase behavior is suggested by the observation that rates of endocytosis by the absorptive epithelium of poikilothermic goodeid fish embryos are maintained down to 5°C but, in mammalian cells, are blocked by moderate cold exposure (~ 15°C) (89). Similarly, the critical temperature, T^*, at which membrane lipid extracts from bacteria, squid axon, and red blood cell and brain membranes of mammals assemble spontaneously into unilamellar structures corresponds to the physiological temperature of the cells from which the lipids were extracted (41).

The dynamic phase behavior model also explains some aspects of membrane restructuring not consistent with HVA. In particular, the positive correlation between growth temperature and the PC/PE ratio in animal cell membranes can be viewed as a homeostatic mechanism to restore (by increasing the proportions of bilayer-stabilizing phospholipids, i.e. PC) the appropriate inter-

val between T_a and T_h, which is otherwise reduced as temperature rises. Indeed, the balance between bilayer-stabilizing and destabilizing lipids may be the principle means of regulating dynamic phase behavior. For example, in mutant strains of *E. coli* lacking the ability to synthesize PE (a bilayer-destabilizing phospholipid), which normally accounts for 70–80% of membrane phospholipids in wild-type strains, PE is replaced by cardiolipin (CL) and phosphatidylglycerol (PG) (85). Both CL and PG can form the H_{II} phase, but only in the presence of divalent cations. Interestingly, mutant strains, unlike the wild-type, display an obligate requirement for high concentrations of divalent cations. Furthermore, although the relative proportions of PG and CL varied widely in membrane lipids depending on the type (Ca^{2+}, Mg^{2+}, or Sr^{2+}) and concentration of divalent cation, all cultures experienced a transition to a nonbilayer phase at approximately 10°C above the growth temperature. As illustrated in Figure 5, despite substantial differences in membrane lipid composition (see figure inset), cells grown with either $MgCl_2$ or $CaCl_2$ display remarkably similar phase behavior, characterized by an H_{II} transition midpoint at 55°C when measured in the presence of the divalent cation present during cell growth (*solid symbols*). However, phase behaviors diverge when tested in

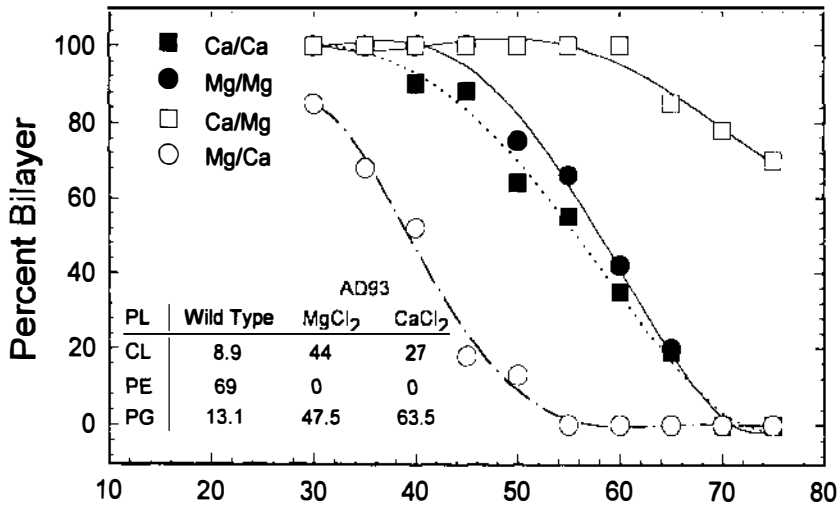

Figure 5 The temperature dependence of the bilayer to hexagonal (H_{II}) phase transition in phospholipids of mutant strains of *E. coli* unable to synthesize PE and grown in the presence of either 50 mM $MgCl_2$ or $CaCl_2$. Phase behavior of the isolated lipids was determined in the presence of either 50 mM $MgCl_2$ or $CaCl_2$. The designation Mg/Mg indicates phase behavior tested in the presence of $MgCl_2$ for phospholipids isolated from cells grown in $MgCl_2$; conversely, the designation Mg/Ca indicates phase behavior of phospholipid extracts from cells grown in $MgCl_2$, but tested in the presence of $CaCl_2$. The inset shows the phospholipid composition (in mol%) of wild-type and mutant strains (redrawn from 85).

the presence of the alternate cation (Figure 5, *open symbols*), which indicates that the differences in lipid composition do indeed influence lipid phase behavior. These data imply that the propensity to form a nonbilayer phase is regulated by variations in membrane lipid composition so that the dynamic phase behavior of the membrane remains independent of growth conditions. Although a monolayer containing high levels of conically-shaped lipids will spontaneously curve to minimize the headgroup packing energy, and in an extreme case collapse to form the hexagonal phase, a monolayer prevented from curving by the presence of other phospholipids that prefer a lamellar phase will be internally stressed (or frustrated) and possess an associated potential energy expressed as the propensity to form the hexagonal phase (91). The available evidence suggests that it is the balance between these opposing forces within a membrane, i.e. the dynamic phase behavior, rather than the actual phase state of a membrane, that is subject to regulation when growth conditions change.

Finally, differences in the dynamic properties of PUFA and monoenes derived from molecular simulations of the ^{2}H-NMR spectra of isolinoleic acid (18:2) (6, 9) provide a mechanistic explanation for the preferential accumulation of PUFA at low temperatures that is more consistent with the concept of dynamic phase behavior than modulation of lipid order (3). Although monoenes markedly reduce membrane order, they cannot pack regularly to form a tightly sealed bilayer that restricts cation permeability. In contrast, the multiple double bonds of PUFA, because they increase the extent of acyl chain motion and yet order the membrane in their immediate vicinity (8), simultaneously maintain an appropriate dynamic state of bilayer and permeability characteristics compatible with biological function.

CONCLUSIONS

The available evidence indicates that the HVA hypothesis does not adequately reflect the specificity of lipid-protein interactions, the microdomain heterogeneity of biological membranes, or the diversity of membrane attributes that can influence function, and cannot explain several consistently observed patterns of temperature-dependent changes in membrane lipid composition. Furthermore, even though effects of temperature on the physical properties and phase behavior of membrane lipids pervade all aspects of membrane function, modification of membrane lipid composition should be acknowledged as but one component of a broader repertoire of adaptive responses to altered temperatures. For example, proliferation of mitochondrial and sarcoplasmic reticular membranes is an important acclimatory response of many poikilotherms to cold temperatures (31, 80). Alternatively, altered expression of membrane proteins appears to contribute to the thermal adaptation of membrane function

in some cases. Thus the diversity of membrane adaptations to temperature is unlikely to be captured by lipid-based adjustments alone. Nevertheless, the widespread and generally similar effects of growth temperature on the membrane lipid composition of microorganisms, fungi, plants, and animals provide compelling evidence that some attribute(s) of membrane lipid organization other than, or in addition to, lipid order are subject to physiological regulation. An adaptational perspective emphasizing the dynamics of lipid phase behavior as developed in this review may provide insights into those properties that must be conserved in order for membrane function to be maintained at extremes of environmental temperature.

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