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Membrane Physical State and Function

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Membranes compartmentalize biological functions and separate the cell from its environment. Membranes from various sources display a wide variety of specialized functions, but all contain proteins and lipids as basic constituents.

With rare exceptions, phospholipids constitute the bulk of membrane lipids, and the "fluid" lipid membrane is essential for maintenance of the living state. The thermotropic (melting) behavior of phospholipids is dictated by the constituent fatty acid side chains, and also by head-group composition and degree of hydration. In general, the melting point of a phospholipid is inversely related to the extent of unsaturation and directly related to the chain length of its fatty acid chains.

Microbial fatty acid composition can be modified by growth conditions. For example, most microorganisms contain more unsaturated fatty acids in membrane lipids when grown at lower temperatures.[1-8] This observation stimulated interest in the relationship between the physical state of membrane lipids and membrane function. The first concrete evaluation of the effects of fatty acid composition on the physical state of membrane lipids in the intact cell and also in the membranes of these cells or in the lipids extracted from them was performed by Steim and coworkers who used differential scanning calorimetry to study a thermotropic change in state of *Mycoplasma* membrane lipids.[9]

Auxotrophs (mutants with a nutritional requirement) of *Escherichia coli,* which require an unsaturated fatty acid supplement for growth, provide another system in which the membrane lipid fatty acid composition can be manipulated experimentally. The *E. coli* auxotrophs, initially described by Silbert and Vagelos,[10] have the additional advantage of being well characterized with respect to membrane-localized phenomena such as sugar and amino acid transport. These transport systems bring material from outside the cell across the membrane to the cell interior, and their components must therefore traverse the membrane.

Using unsaturated fatty acid auxotrophs of *E. coli,* two laboratories independently reported observations indicating a dependence of membrane function on membrane lipid physical state. Fox and coworkers[11,12] reported that slope intercepts in Arrhenius plots (logarithm of rate vs. reciprocal of absolute temperature) of sugar transport occurred at "characteristic" temperatures that increased as the melting temperature of the unsaturated fatty acid supplied for growth increased. The same characteristic temperatures were observed for two independent sugar transport systems in cells grown with the same fatty acid supplement, suggesting that both were influenced by the same physical event.

Overath et al.[13] showed that monolayers of phosphatidylethanolamine extracted from cells grown with single unsaturated fatty acid supplements underwent collapse at the same temperatures at which a slope intercept in an Arrhenius plot of transport was observed, thus supplying the first direct correlation between these physical and physiological phenomena. These studies suggested that membrane functions are altered at the point where membrane lipids as-

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sumed a gel, or crystalline, state but neither rigorously defined the behavior of the lipids. Using a variety of physical techniques, several groups undertook a study of this problem.

The techniques of electron spin resonance (ESR) and X-ray diffraction are particularly useful for evaluating the physical state of membrane lipids, both in the membrane and in aqueous dispersions of extracted lipids. Several reviews treat the application of other techniques to this problem.[14-19] This Account focuses on the results of multidisciplinary approaches to the effects of membrane physical state on membrane activities, membrane assembly, and the manifestation of altered surface properties in cultured animal cells. By way of introduction, we first summarize selected physical studies with model membrane systems.

Melting Behavior of Model Lipid Systems

A single, pure phospholipid species undergoes a transition between the solid and liquid states over a narrow temperature range. Since biological membranes contain several species of phospholipids, however, the behavior of mixed lipid systems in aqueous environments is obviously more relevant.

The use of phase diagrams to describe the behavior of binary lipid systems evolved naturally as these systems were investigated by means of various physical techniques. Phillips and coworkers[20,21] used differential scanning calorimetry (DSC) in the first detailed study of the melting behavior of binary phospholipid mixtures in aqueous dispersion. Ideal mixtures such as dipalmitoylphosphatidylcholine–dimyristoylphosphatidylcholine or dipalmitoylphosphatidylcholine–distearoylphosphatidylcholine yield single, broad endothermic transitions upon melting and broad exothermic freezing patterns upon cooling. Nonideal mixtures, including those where the fatty acid substituents on each phospholipid differ in chain length by more than two carbons, where both fatty acids are saturated on one phospholipid, but unsaturated on the other, or where the phospholipids differ in polar head-group composition, yield two endothermic or exothermic deflections at temperatures displaced from the melting temperatures of each pure component. A phase diagram of this type of mixture has a *solidus* curve characteristic of systems where solid-phase immiscibility of the two components occurs.

In both ideal and nonideal mixtures, the onset and termination of melting occur at lower (t_l) and upper (t_h) characteristic temperatures, respectively. Plots of the lower and upper characteristic temperatures for all mixtures of an ideal, binary phospholipid system in aqueous dispersion vs. mole per cent of the higher melting component define the solidus and liquidus curves for a phase diagram of this system.[15,20,22,23]

The spin label Tempo (2,2,6,6-tetramethylpiperidinyl-1-oxyl) was initially studied by Hubbell and McConnell[24] who reported that its distribution between aqueous and hydrocarbon phases of a membrane or aqueous lipid dispersion can be estimated from its ESR spectrum. The ratio of the fraction of Tempo in the hydrocarbon phase to the total Tempo present in both hydrocarbon and aqueous phases has been termed the spectral parameter. Changes in the value of the spectral parameter indicate an alteration in the partitioning of Tempo between the aqueous and hydrocarbon compartments, and can be used to quantitate the amounts of lipid in solid and liquid states.

Shimshick and McConnell measured the Tempo spectral parameter as a function of temperature for several binary phospholipid mixtures.[22] They were able to construct phase diagrams for each system and determine t_h and t_l. Their results agreed with the DSC analyses of similar mixtures reported by Phillips et al.[20,21] ESR has the advantage that both t_l and t_h can be determined in a single ascending or descending temperature program, whereas DSC accurately detects only t_l during an ascending scan (melting) and only t_h during a descending scan (freezing). A detailed discussion of the properties of binary lipid systems and the use of phase diagrams in their analysis can be found in a recent review.[24]

Kornberg and McConnell had previously demonstrated that the rate of vertical lipid exchange between monolayers of a bilayer membrane is far too slow to account for the rapidity of equilibrium mixing which occurs during the melting of model lipid bilayer systems.[25] McConnell and coworkers thus concluded that the mixing and redistribution of lipids in a binary system must occur by lateral movement.[22,26] This movement has been termed lateral diffusion, and is observed in the melting or freezing of a binary or higher order lipid system where the lipids must undergo lateral phase separations. A lipid molecule can exchange positions with a neighboring molecule in a liquid lipid phase at a frequency of greater than 10^7 sec^{-1} at 30°.[26] This rate more than adequately accounts for the rapid kinetics of melting and freezing of aqueous lipid dispersions observed by X-ray diffraction.[27]

Freeze-fraction and electron microscopy have been used to examine lipid mixtures used in the DSC and ESR experiments.[28,29] Aqueous dispersions of phospholipid mixtures are equilibrated at a chosen temperature and quenched rapidly to −210°. The sample is then fractured to expose the internal surfaces of

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Table I
Characteristic Temperatures for Glucoside Transport (t_h*, t_l*) and Tempo Partitioning (t_h, t_l)[a]

	t_h*	t_h	t_l*	t_l
Elaidic acid	38.6–38.8	37.7	32.1	30.7
Oleic acid	26.0 and 21.8	31	14.4	15.8
Linoleic acid	27.1	28.5	6.8	8.9

[a] Cells of an unsaturated fatty acid auxotroph were grown at 37° for four generations with the indicated fatty acid supplement (from ref 40).

the bilayer and prepared for observation by electron microscopy. The fracture face of lipid quenched from a fluid state has a smooth surface appearance, whereas lipid quenched from a solid state has a rippled, banded pattern, with band spacings that are characteristic of the fatty acid substituents on the phospholipids. The results of these experiments are consistent with the results obtained by DSC or ESR.

X-Ray diffraction studies of model lipid systems show that frozen and melted hydrocarbon chains have characteristic diffraction bands at 4.2 and 4.6 Å, respectively.[30–32] Using appropriate sample preparation techniques and equipment, the relative amounts of frozen and melted fatty acid chains in a biological membrane can be measured by X-ray diffraction.[30,33–35]

Melting Behavior of Bacterial Membranes

Some biological membranes have lipid compositions that approximate a binary phospholipid mixture, e.g., those isolated from an unsaturated fatty acid auxotroph of *E. coli* grown with particular fatty acid supplements.[36] These membranes provide convenient systems for comparing the physical properties of model and biological membranes. Nearly 90% of the membrane phospholipids in the *E. coli* auxotroph used in the authors' laboratory are phosphatidylethanolamine (PE).[37–39] The saturated:unsaturated species of PE accounted for over 70% of the phospholipid in cells grown with an oleic acid supplement, the diunsaturated species accounting for nearly all the rest. In the case of cells grown with an elaidic acid supplement, 70% of the phospholipid was the diunsaturated species, dielaidoyl-PE, and the remainder was the saturated:unsaturated species.[36–37]

Cytoplasmic membranes isolated from bacteria grown with oleic, linoleic, or elaidic acid supplements were examined with the spin-label probe Tempo. Two slope intercepts were observed in plots of log spectral parameter vs. $1/K$.[40] By analogy with spin-label studies of binary lipid systems, these intercepts

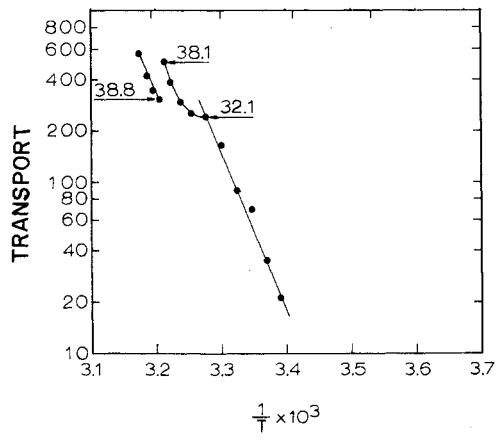

Figure 1. From Linden et al.[40] Arrhenius plots for β-glucoside transport by cells of an *E. coli* unsaturated fatty acid auxotroph grown at 37° in medium supplemented with elaidic acid. The units for transport of *p*-nitrophenyl β-glucoside are nmol min^{-1} per l. × 10^8 cells. In cells grown with an elaidic acid supplement there is a dramatic increase in transport rate as temperature is lowered in the vicinity of t_h. At t_h, thermodynamic principles predict an enhancement of lateral lipid compressibility. Increased lateral compressibility could facilitate a conformational alteration of the transport protein leading to enhanced transport rate.

were designated upper and lower temperature boundaries of the lateral phase separations in the membrane lipids, i.e., the lipid phase transition. Furthermore, the shape of the curves indicated that the membrane lipids have ideal melting behavior. The characteristic temperatures observed for the lipids in membranes and in aqueous dispersion following extraction were nearly identical, although the t_l values were commonly a few degrees lower in the case of the extracted lipids. Although the lipids of cells grown with a linoleic acid supplement composed a ternary system,[36] two distinct characteristic temperatures were observed in both transport and spin-label studies. Thus the rationale for the behavior of membrane lipids in a binary system applies to more complex systems as well.

Examination of two independent transport systems revealed that there are at least two discontinuities in slope of Arrhenius plots for each system and that these intercepts correlate with the t_h and t_l identified in the spin-label studies.[6,40,41] Figure 1 shows an Arrhenius plot of β-glucoside transport mediated by the unsaturated fatty acid auxotroph grown with an elaidic acid supplement. An Arrhenius plot of β-galactoside transport by the same strain of cells is similar to that shown here.[40,41]

A comparison of the characteristic temperatures determined by studies of β-glucoside transport by cells and Tempo partitioning in membranes isolated from these cells is presented in Table I. The correlation between these characteristic temperatures clearly indicates that the changes in transport rate visualized in Arrhenius plots reflect events occurring in the membrane lipids at t_l and t_h. Not surprisingly, the temperature interval between t_h and t_l is greater in the membranes of cells with lipid species most different in physical properties. In membranes derived from cells grown with an elaidic acid supplement, t_h and t_l are separated by approximately 8°, whereas in membranes derived from cells grown with an oleic

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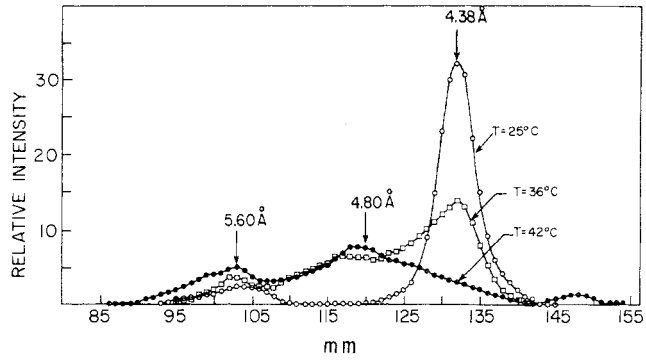

Figure 2. Corrected densitometer tracings of the high angle diffraction arising from a dispersion of cytoplasmic membranes obtained from cells grown with an elaidic acid supplement. Densitometer tracings were corrected for background intensity prior to digitizing the data. Intensity and spacings are expressed in arbitrary units. Units on the abscissa are millimeters on the densitometer tracing. Exposures were obtained over a period of approximately 4 hr using a toroid camera and nickel-filtered Cu Kα vacuum radiation. Temperature was controlled by circulating water from a temperature-controlled bath through the aluminum sample holder.

acid supplement, the separation is approximately 15° (Table I).

X-Ray diffraction and freeze-fracture followed by electron microscopy have been used to corroborate the conclusions derived from transport and ESR studies performed with the *E. coli* unsaturated fatty acid auxotroph. X-Ray diffraction has a number of obvious advantages over other techniques, e.g., no probe is introduced into the system under observation. We examined the effects of temperature on the high-angle diffraction arising from membranes isolated from cells grown with oleic or elaidic acid supplements.[35] Characteristic reflections were identified as arising from frozen and melted phospholipid fatty chains. Figure 2 presents the relative intensities and spacings of the high-angle reflections from a dispersion of elaidate-rich membranes. In these experiments, frozen hydrocarbon chains give rise to the reflection at approximately 4.4 Å and melted chains appear at 4.8 Å. At 25° (below t_l), the only significant intensity is at 4.38 Å, indicating the presence of frozen fatty acid chains. At 42° (above t_h), a broad peak is observed at 4.8 Å, indicating the presence of melted fatty acid chains. At 36°, between t_h and t_l, a small peak is observed at 4.38 Å which broadens into a dominant peak at 4.8 Å, indicating the simultaneous presence of frozen and melted hydrocarbon chains. The characteristic temperatures defined by X-ray diffraction were in excellent agreement with those determined by transport and ESR studies.

Kleemann and McConnell[42] used freeze-fracture followed by electron microscopy to examine the inner surface of the cytoplasmic membranes of an unsaturated fatty acid auxotroph grown in medium supplemented with elaidic acid. Proteins, which appear as particles in the fracture plane, have a regular netlike distribution in the membrane at temperatures where the lipid is in an entirely fluid state. Below t_h the particles become aggregated into large patches, and some areas are totally devoid of particles. These experiments provide dramatic evidence that membrane proteins undergo lateral movement and rearrangement as the result of lateral lipid phase separations.

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Table II
Physical Events Occurring at the Upper and Lower Characteristic Temperatures of the Phase Transition[a]

Upper characteristic temperature	Lower characteristic temperature
Onset of lateral phase separations	End of lateral phase separations
Begin formation of frozen patches of lipid	All lipids assume the S state
Lateral phase separation of proteins into F state ("fluid") lipid	All membrane proteins visualized by freeze-fracture electron microscopy are present in dense patches surrounded by regions of free lipid
Onset of enhanced lateral compressibility of membrane lipids (above t_h all lipids are in the liquid expanded state)	End of enhanced lateral compressibility of membrane lipids (below t_l all lipids are in the condensed state)
Begin thickening of membrane (the vertical dimensions of the bilayer increase at regions of S state ("solid") lipid)	Membrane reaches maximal thickness

[a] The description of events which would occur as a consequence of taking a membrane preparation through a decreasing temperature program starting from above the upper characteristic temperature of the lipid phase transition.

Additional phenomena that respond to changes in the physical state of membrane lipids have been characterized. β-Glucoside and β-galactoside transport are the only two functions as yet identified as responding to an event(s) occurring at t_h. These two systems also respond to an event(s) occurring at t_l. Phenomena observed to be affected primarily or exclusively at t_l are: bacterial growth,[43,44] bacterial membrane assembly,[43] arrest of lateral mixing of membrane components,[45] and chemotaxis.[46] Other investigators have identified proline transport[34] and succinate-ubiquinone reductase[47] as responding to t_l, but their experiments were not conducted in a manner that would detect a response to t_h. The actual physical events which may give rise to these physiological phenomena are described in Table II. While the correlation between membrane physical state and function is of interest in and of itself, it can also be exploited in the formulation of experiments designed to examine certain aspects of membrane growth and behavior.

Many bacterial transport systems are absent from cells or present only in low level until an inducer is added to the growth medium. Tsukagoshi and Fox[43]

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examined induction of the lactose transport system in an *E. coli* unsaturated fatty acid auxotroph. They showed that induction of transport proceeded normally with decreasing temperatures until a critical temperature was reached. This critical temperature was determined by the fatty acid supplement used to support cell growth and correlated in each case with the t_l determined for membranes of cells grown with that supplement. Corollary experiments showed that cellular growth also ceased at t_l,[43] a conclusion also reached by McElhaney in a more extensive study of effects of lipid physical state on the growth of *A. laidlawii*.[44]

The inducibility of the lactose transport system was used in another set of experiments to test for coordinated introduction of newly synthesized lipid and protein into membrane. In these experiments, cells were first grown with one fatty acid supplement and then transferred to medium containing a second prior to induction of the transport system. When induction took place at a temperature above the lower characteristic temperatures of lipids derived from either supplement, only one lower characteristic temperature for transport was observed, and its value was intermediate between those of lipids derived exclusively during growth of cells with one or the other of these supplements.[45,48] This indicated that the lipids derived from both supplements mixed under this condition, and that transport was thus affected by a mixed bulk lipid phase. On the other hand, when induction took place at a temperature between the lower characteristic temperatures of lipids derived exclusively from either supplement, a lower characteristic temperature for transport which was a property of lipids synthesized from the fatty acid supplement present during induction was observed consistently. This indicated that coordinated introduction of newly synthesized lipids and transport proteins occurs under this condition.[45] In addition, when cells induced under the latter condition were subsequently incubated above the lower characteristic temperatures for lipids derived from either fatty acid supplement, a new characteristic temperature for transport was observed, and its value indicated that the apparent unique domains of "new" and "old" lipid had mixed.

The coordinated introduction of newly synthesized lipid and protein into membrane suggested by this experiment was tested further by experiments where the membrane density label bromostearic acid was employed.[49-51] Though it has been possible to isolate membrane fragments enriched for lipids containing brominated or nonbrominated fatty acids following the appropriate density and temperature shifts, interpretation of these experiments has been complicated by the property of such lipids to form immiscible fluid phases.[35] A more comprehensive treatment of microbial membrane assembly appears elsewhere.[52]

Melting Behavior of Animal Cell Membranes

The presence of cholesterol in membranes of animal cells presents complications not ordinarily encountered with bacterial membranes, and the effect of cholesterol and other sterols on the membrane lipid-phase transition has been a subject of debate. Using DSC, Chapman and coworkers showed that increasing amounts of cholesterol added to aqueous dispersions of phospholipids proportionally broaden and decrease the endothermicity of the phase transition up to molar ratios of 1:1 phospholipid:cholesterol.[53,54] When the concentration of cholesterol reached 50%, the endothermic property of the phase transition was completely obscured except when the phospholipid used contained fatty acid moieties with long chain lengths (e.g., C = 22). Similar results were obtained by Hinz and Sturtevant.[55]

Shimshick and McConnell[56] used Tempo to study the phase transition in cholesterol–DMPC and cholesterol–DPPC mixtures. They obtained phase diagrams which indicate solid-phase immiscibility of components of the mixtures at or below 20% cholesterol. Distinct boundaries of lateral phase separations were observed at cholesterol concentrations of up to 50%, at which point cholesterol crystallizes from the mixture. Other investigators have obtained similar data.[29,57-60] Phase transitions are observed in mixed cholesterol–phospholipid systems until cholesterol reaches a relatively high concentration. Cholesterol apparently restricts both the cooperative melting and the freezing of phospholipids, thus broadening but not necessarily obscuring the phase transition. Animal cell surface membranes contain cholesterol,[61,62] and the molar ratio of cholesterol to phospholipid generally falls within the range of 0.5 to 0.8.[63]

Wisnieski et al. have examined the physical state of lipids in cell surface membranes from cultured mouse fibroblasts and in membranes of Newcastle disease virus (NDV).[64] NDV acquires its envelope from the host cell surface membrane. Four characteristic temperatures were consistently observed in NDV membranes for partitioning of a spin-label probe between the membrane lipids and the surrounding aqueous environment. Only two characteristic temperatures for spin-label partitioning were

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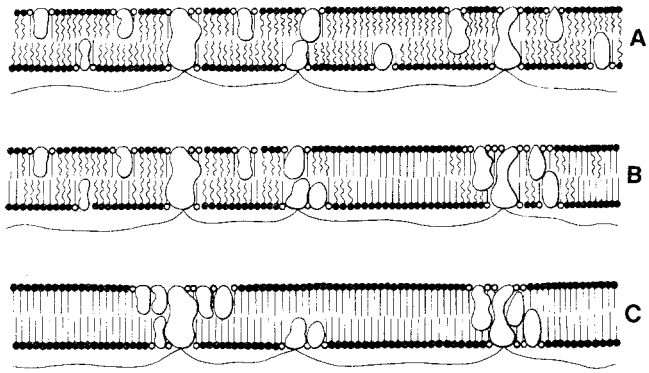

Figure 3. A diagrammatic representation of a cross section through a biological membrane containing proteins and lipids. The lipids are indicated by circles with tails, and the proteins as larger irregularly shaped bodies. Some proteins have been drawn as having points of attachment to a cytoskeletal structure. See text for a discussion of the consequences of this attachment. Lipids immediately adjacent to proteins (boundary lipids) are indicated by open rather than closed circles. This class of lipid may have properties unique from the bulk bilayer lipid as discussed in the text. (A) All the lipid in the membrane is in a fluid state, as indicated by the wavy tails. The proteins not restricted by cytoskeletal attachment are free to diffuse in the plane of the membrane. (B) The membrane is at a temperature between t_h and t_l. Some lipids have begun to freeze (straight tails), thus excluding the proteins into regions containing fluid lipid. As a consequence of their exclusion from regions containing frozen lipid, the proteins have begun to aggregate in patches. (C) The membrane is at a temperature below t_l. At this point all the membrane lipids are frozen, and the proteins have become maximally aggregated.

observed in lipids extracted from the virus, indicating that some aspect of lipid organization in the membranous coat of the intact virus was destroyed by the extraction procedure.

The same four characteristic temperatures observed in the NDV envelope, approximately 15, 21, 31, and 37°, were observed in mouse fibroblast surface membranes and for two catalytic functions localized in these membranes: transport of the amino acid analog α-aminoisobutyric acid and Na^+/K^+-stimulated ATPase activity. An internal membranous structure from mouse fibroblasts, the endoplasmic reticulum (er), had four characteristic temperatures nearly identical with those observed for the cell surface membrane of the fibroblasts.[65] As with the viral system, lipids extracted from er membranes exhibited only two characteristic temperatures. Although mouse fibroblasts and the chicken host cells used for propagation of NDV are evolutionarily divergent, they are both derived from organisms that regulate their body temperature. It is interesting that membranes from these two sources have such similar physical properties.

Quantitative treatment of the ESR data and correlation of the results of this treatment with data on the effects of temperature on a number of physiological parameters[66-68] suggest that 21 and 37° might define the boundaries for the phase transition in the inner monolayer of the cell surface membrane and 15 and 31° the boundaries for the phase transition in the outer monolayer. The physical asymmetry suggested by these studies[64,65] has precedent in studies which indicate asymmetry of phospholipid headgroup distribution in model and biological membranes.[69-71]

The physical state of animal cell membrane lipids is of concern to those studying cell surface receptors, cell surface antigens, and cell–cell interactions. The mobility of cell surface proteins is inhibited or arrested at critical temperatures[66-68,72] that most likely represent the t_l for one of the membrane monolayers.[64] Workers in this laboratory and in Burger's have shown that the distribution and binding properties of the cell surface receptors for concanavalin A (Con A), a plant lectin, are altered as a function of the physical state of lipids in the cell membrane.[68,69,72] Alterations in the membrane lipid composition of mouse fibroblasts also give rise to different characteristic temperatures for Con A mediated agglutination,[67,72] thus suggesting a regulatory role of the physical state of membrane lipids in the display of cell surface receptors.

Other factors, such as submembranous cellular structures, are thought to regulate the movement of membrane localized proteins. Such structures, composed of microfilaments, are illustrated in Figure 3 as closely apposing the cell membrane and having points of attachment to proteins which span the membrane. The properties of cell surface proteins and their possible attachment to such structures have been treated in depth.[73-75]

Concluding Remarks

The effects of physical state of cell membrane lipids on membrane function can be determined with precision through the judicious use of physical techniques in conjunction with a suitable biological assay. The events occurring at boundaries of the lipid phase transition, t_h and t_l, affect membrane functions in all cellular systems, including those where the membranes contain cholesterol. Membrane lipid physical state can be regulated by factors other than temperature, which happens only to be a convenient laboratory variable. Polar head-group and ion interactions clearly affect the physical properties of lipids and in fact might regulate membrane physical state.[76] This is pertinent in view of our current understanding of the asymmetric distribution of membrane components[70,71] and ion flux which is known to occur in excitable membranes.

We do not know precisely how membrane proteins respond to the behavior of membrane lipids. Figure 3A depicts the membrane where the lipids are in a totally fluid state and the proteins seem to be randomly distributed and free to diffuse. The proteins partition preferentially into a fluid lipid phase as lipids freeze.

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This is shown diagrammatically in Figure 3B. One consequence of this behavior is the aggregation of proteins in patches. If all membrane lipids assume a frozen state, membrane proteins become aggregated in large patches. Figure 3C shows this condition. Some proteins are depicted as having points of attachment to a cytoskeletal structure. The movement of these proteins is probably restricted.

We have taken into account only the lateral movements of proteins in the plane of the membrane. As membrane lipids freeze and the bilayer thickens, proteins normally exposed at the membrane surface may become buried, or they may be extruded as a consequence of their exclusion from frozen lipid regions.

Jost and coworkers[77-79] and others[80,81] have performed experiments which suggest that a finite amount of lipid is intimately associated with the surface of a membrane protein. This lipid is immobilized in comparison with "free lipid" in a fluid phase, and is depicted in Figure 3 as lipid with open circles for head groups. Several investigators have successfully extracted and purified certain membrane proteins. A defined lipid requirement for full activity of some proteins has been demonstrated,[82,83] but the mechanistic nature of the lipid requirement is not yet understood.

To date, there is no compelling evidence to indicate that frozen or partly frozen lipid phases play a physiological role. The ability of divalent cations to trigger lipid phase transitions through interactions with polar head groups and the importance of such cations in many membrane mediated functions suggest, however, that localized changes in membrane lipid physical state could be physiologically significant.

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Histone Interactions and Chromatin Structure

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One of the most perplexing mysteries in molecular biology is how the deoxyribonucleic acid (DNA) is organized in the nuclei of eukaryotic cells. A typical cell contains an amount of DNA which, if extended, would be roughly 1 m long, somehow folded into a nucleus about 10^{-3} cm in diameter. The problem is the more acute because this DNA is not inert, but must be capable of being faithfully replicated, and transcribed (at least in part) into ribonucleic acid (RNA). The mystery becomes even deeper when we realize that these processes must be under subtle and complex control, for while all of the different kinds of cells in a eukaryotic organism contain the same nuclear DNA, only a small fraction of that DNA is expressed by transcription in any given tissue.[1] It seems likely that these two facets of the problem, packaging and the control of transcription, are related. However, we do not yet know enough to be able to state that this is so.

It has long been known that the DNA does not exist in eukaryotic nuclei as the free polynucleotide, but as a complex with proteins and some RNA. This complex contains only a portion of the total protein and RNA of the nucleus and is referred to as *chromatin*. Operationally, chromatin represents that portion of the nuclear content that may be extracted as a unit and be accessible to in vitro study. The assumption is made that this complex is also of significance in vivo; this belief is supported by the fact that isolated chromosomes have a DNA:protein ratio not greatly different from that of chromatin.[2,3]

The proteins of chromatin are of two general classes. First, there are a group of basic proteins called *histones*. In most eukaryotic nuclei there are

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