Membrane Negative Spontaneous Curvature as an Ancient Signal for Cell Growth

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Abstract

Biomembranes have been described as platforms which integrate and control entire metabolic pathways in a cooperative manner, the activities of the proteins being dependent on the physical (phase) state of the given membrane, the latter further being controlled by the liquid crystalline properties of the constituent lipids. Accordingly, there is a correlation between the physiological state of the cell (or cell organelle in a eukaryote) and the physical state of the membrane (Kinnunen et al., 1994; Kinnunen, 1996a). A good example is provided by the control of growth in prokaryotes and eukaryotes (Kinnunen, 1996b). In brief, a unifying feature in rapidly growing cells appears to be the increase in the content of lipids which in isolation form inverted non-lamellar phases (INLs). On a mechanistic level the activities of membrane proteins in these cells can be controlled by the lateral pressure profile which in addition to possible direct effects on the conformation of integral proteins can also induce the attachment of peripheral membrane proteins by the so-called extended lipid anchorage. Similarly, INLs may also induce peripheral, secondary interactions for integral membrane proteins.

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Inverted Non-lamellar (INL) Phases

Both prokaryote and eukaryote cells contain in their membranes substantial amounts of lipids forming, in isolation and at proper conditions (e.g. temperature, presence of ions, protons, proteins, and impurities) inverted non-lamellar phases (INL), such as hexagonal phase HII and inverted cubic phases (Tate et al., 1991). Notable examples of INLs are phosphatidylethanolamine (PE), in particular its plasmalogen form, diacylglycerol (DAG) containing unsaturated acyl chains, and cardiolipin, the latter in the presence of protons or divalent cations. We will here refer to these lipids as INLs. These lipids have a smaller polar head group when compared to the relative size of their

hydrophobic part and, accordingly, at the free energy minimum they spontaneously adopt 3-D structures with negative curvature. When present in sufficiently small quantities in mixed membranes with lipids forming lamellar phases, INLs increase the tendency of the bilayer for adopting negative curvature, even though the membrane remains lamellar. Such membranes are frustrated and there is increasing packing strain towards the interior of the hydrocarbon phase of the bilayer. In contrast, the packing closer to the bilayer surface becomes more loose. In other words, compared to a relaxed bilayer, the free volume distribution within the bilayer has changed. The magnitude of the pressure difference in the interior of the bilayer and the surface can be considerable (Cantor, 1997). A parameter Q can be calculated, giving the free energy difference for the membrane in frustrated and relaxed states, i.e. in lamellar and non-lamellar states, the latter corresponding to the organization after adoption of the spontaneous negative curvature.

INLs and Cellular Growth

In eukaryotes the contents of INLs vary in different tissues as well as in different organelles. Likewise, their content varies in the cell cycle (for recent review, see Kinnunen, 1996b). More specifically, the content of PE increases towards the end of the G1 phase of cell cycle, preceeding the entry of the cell into the S-phase, where the duplication of the cellular macromolecular machineries, such as replication of DNA, takes place. DAG is used in eukaryotes as a lipidic second messenger for cellular proliferation, and is generated from both phosphatidylcholine (PC) and -inositol (PI) by specific phospholipases C (PLC) which become activated in signalling cascades following the activation of plasma membrane growth factor receptors by their agonists. Increasing contents of DAG are present also in ras-transformed cells. In mutants at restrictive temperatures, the contents of DAG remain low but increase at permissive temperatures. Overexpression of PC-PLC results in the transformed phenotype. Notably, increasing the content of DAG in cells by choline deficiency results in epigenetic transformation, i.e. in the absence of mutation. Accordingly, there is strong evidence linking both normal as well as malignant growth of eukaryotes to increasing contents of INL and thus an increase in Q.

As demonstrated by Lindblom and his coworkers (e.g. Lindblom and Rilfors, 1989) optimal growth conditions for the mycobacterium *Acholeplasma laidlawii* require their plasma membrane to contain INL, i.e. to have a proper negative spontaneous curvature and thus proper value for Q. This may be achieved at appropriate temperature, ions, or proper lipids or membrane-partitioning additives. Likewise, recent data for *E. coli* indicate that no specific lipid structure such as PE is necessary but membrane negative spontaneous curvature in general is needed (see Kinnunen, 1996b, for a review). Correlation between lipid packing and the activity of one of the rate-limiting enzymes of lipid synthesis in *A. laidlawii* has recently been demonstrated *in vitro* (Karlsson et al., 1996) and is discussed in more detail by Wieslander in the present volume.

To conclude, it is argued that INLs and more specifically their manifestation in a particular physical property of the membrane, given by Q, represent an ancient signal activating cellular growth both in eukaryotes and prokaryotes. Yet, exceeding growth-promoting values for Q causes destabilization of the membrane and ultimately their transition into non-lamellar phases, thus resulting in cell death. Efficient feedback mechanisms (synthesis and degradation) are required for maintaining the correct membrane lipid composition yielding under given conditions (e.g. temperature, pressure, presence of membrane partitioning 'impurities'), both membrane stability as well as correct signalling for growth.

To this end, it important to notice that Q can be regulated independently in the different or-

ganelle membranes of eukaryote cells and thus can be used to activate or inactivate specific metabolic pathways independently. Yet, in the S-phase Q should increase in all organelles harboring machineries needed for replication. Aberrant control of the content of INL will result in malignant transformation and cancer.

INLs and Lipid-Protein Interactions

In both eukaryotes as well as prokaryotes, INLs activate enzymes needed for replication as well as acting in cascades promoting growth (Kinnunen, 1996a). Prominent examples of peripheral proteins activated by INLs are protein kinase, CTP:phosphocholine cytidyl transferase, dnaA protein, and cytochrome c. The molecular-level mechanisms have remained poorly understood. We have propagated the concept of INL causing, due to the strain in the membrane, anchoring of proteins to lipid surfaces by so-called extended lipid anchorage (Kinnunen et al., 1994; Rytömaa and Kinnunen, 1995). In this mechanism, one of the acyl chains of a lipid becomes intercalated within a hydrophobic cavity of a protein, while the other chain remains in the bilayer. Accordingly, there is reduction in Q and hydrophobic lipid-protein interaction is established in the absence of penetration of the protein into the membrane. The extent of this interaction is regulated by factors controlling the overall magnitude of Q of the membrane and may further be linked to specific lipid headgroup-proteins as well as specific lipid acyl chain-protein interactions. In this regard, the role of hydroxy-fatty acids and eicosanoids is of particular interest.

Towards the Functioning Biomembrane

The modern view of biomembranes emphasizes coupling between their organization and function (Mouritsen and Kinnunen, 1996). The supramolecular membrane assemblies of proteins and lipids exist on different length- and timescales and represent both:

(i) spontaneously forming self-organizing assemblies due to intermolecular forces at thermodynamic equilibrium as well as

(ii) dissipative non-equilibrium structures, maintained by energy input.

These organizates are highly dynamic and apt to regulation by a number of membrane binding ligands such as hormones and growth factors, metabolites, ions, pH, drugs, proteins, as well as membrane potential, osmotic forces, pressure, temperature, and hydration (Kinnunen, 1991). As an inherent feature of all liquid crystalline materials is their ability to undergo phase changes, and accompanying these phase changes are alterations in the physical state of the membrane. Inherent to phase transitions are also changes in the lateral distribution of the membrane constituents, as recently demonstrated (Jutila and Kinnunen, 1997).

The central issue advocated by the present author is that life has adopted these phase changes for the regulation of both metabolism as well as replication of cells, with phase transitions in the membranes and the intracellular polymers corresponding to changes in the physiological states of the cell, such as those represented by apoptosis and the distinct phases in the cell cycle.

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