Does the membrane's physical state control the expression of heat shock and other genes?

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Membranes provide the structural framework that divides cells from their environment and that, in eukaryotic cells, permits compartmentation. They are not simply passive barriers that are liable to be damaged during environmental challenge or pathological states, but are involved in cellular responses and in modulating intracellular signalling. Recent data show that the expression of several genes, particularly those that respond to changes in temperature, ageing or disease, is influenced and/or controlled by the membrane's physical state.

TEMPERATURE IS AN important environmental factor that, when changed, requires adaptive responses from cells. For example, sudden drops in temperature lead to a drastic reduction in membrane fluidity (see Box 1) that, in turn, inhibits normal membrane functions. Unless corrected rapidly, such alterations cause physiological damage and, ultimately, death. For mammals, shivering or non-shivering thermogenesis are strategies that are used to combat cold, whereas heat loss through mechanisms such as sweating allows the animal to cope with elevated temperatures. For poikilotherms (organisms that are unable to regulate their body temperature), fluctuations in environmental temperatures are often more serious.

Several essential cellular activities depend on proper membrane function. Thus, membrane lipid composition – in particular the amount of unsaturated fatty acids (UFAs) – and membrane lipid dynamics have received significant attention in the past several years[1,2]. Membranes have fluidity properties that permit the cell to sense changes in temperature, pH, osmotic and atmospheric pressure, etc. Abrupt changes in the environment, and pathological states, can cause modifications of membrane structure and, consequently, loss of physiological functions[3]. However, following a temperature change, cells compensate for stress-induced cellular disturbances through physiological and biochemical mechanisms of *homeoviscous*[3] or *homeophasic* adaptation[2] that allow them to maintain a homeostatic equilibrium.

Multiple changes in membrane lipid composition occur during adaptation to temperature shift, pathological conditions (such as cancer[4] or degenerative diseases[5]) or alterations in diet[6], and during the ageing process[7,8]. Growth at low temperature, for example, causes one or more changes in membrane lipid composition, including increases in fatty acid unsaturation, changes in the proportions of lipid classes and changes in the lipid : protein ratio[3].

Each of these changes leads to membrane remodelling and to alterations in the organization and dynamic properties of membrane lipids. The activities of many membrane-associated enzymes and transporters also change dramatically[9–11]. These changes in protein activity follow or are concurrent with expression of specific genes – among which, the best studied are heat-shock genes and fatty acid desaturase genes that are involved in temperature adaptation. To date, the physical state of the membrane and changes in gene transcription have been studied separately, and little attention has been given to possible connections between the two.

Responses to cold shock

In recent years, considerable attention has been paid to organisms that can adapt to low temperature. Organisms commonly adapt to low temperature by increasing the proportion of *cis*-unsaturated fatty-acyl groups in their membrane lipids and thus increasing membrane fluidity[1,2]. Such adaptation involves the induction of fatty acid desaturase activities. We now know the molecular mechanisms of this induction in detail in cyanobacteria[3], *Tetrahymena*[12], *Acanthamoeba*[11] and fish[3]. Acyl-lipid desaturases introduce double (unsaturated) bonds at defined positions in fatty acids that are linked to membrane glycerolipids.

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Box 1. Glossary

Homeophasic adaptation. An adaptive response that maintains the membrane biophysical structure (i.e. lipid phases) constant.

Homeoviscous adaptation. A natural phenomenon where an organism subjected to different growth temperatures adjusts its membrane lipid composition to maintain fluidity.

Membrane fluidity. A widely used but subjective term that describes the relative diffusional motion of molecules within membranes. Fluidity is used in preference to viscosity, because membranes are planar, asymmetric structures, and their properties are not directly comparable to bulk phases. The term fluidity is meant to convey the impression of lateral diffusion, molecular wobbling and chain flexing, which are found in functional membranes where the lipids are in the fluid-crystalline lamellar phase.

Membrane order. The motional freedom of molecules or molecular domains within the membrane assembly. Membrane order can be quantitated by estimating the motion of paramagnetic probes and calculating an order parameter from the ESR or NMR spectrum.

Non-lamellar phases. Non-bilayer arrangements of lipids in aqueous media. These can be hexagonal (HI) or inverted hexagonal (HII) arrangements; however, the H_I phase is seldom (never?) found in membranes, because lysophospholipids do not accumulate there.

Non-lamellar propensity. The property of certain lipids that makes them form non-lamellar phases when dispersed in dilute salt solutions.

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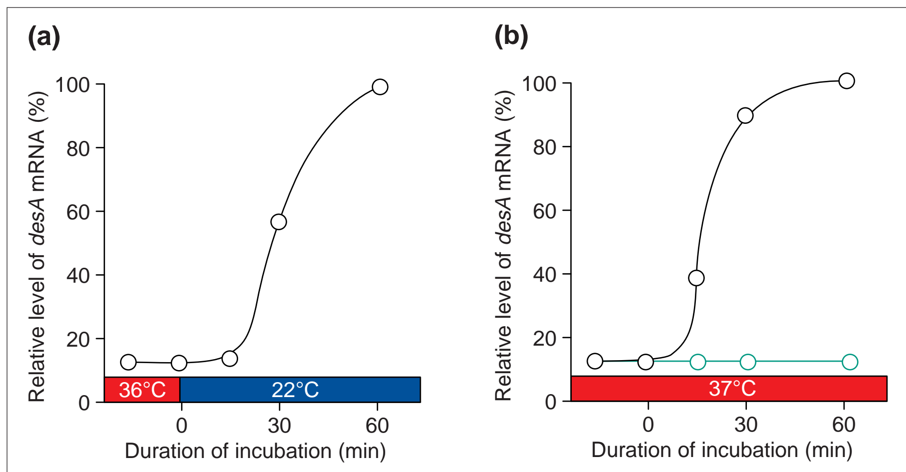

Figure 1
Changing the membrane's physical state induces transcription of a fatty acid desaturase gene in *Synechocystis* sp. PCC 6803. **(a)** Temperature-induced accumulation of *desA* mRNA. Figure adapted, with permission, from Ref. 17. **(b)** Synthesis of *desA* mRNA in *Synechocystis* following plasma-membrane-directed hydrogenation (black plot). The green plot shows the level of *desA* mRNA in the absence of hydrogenation. Figure adapted, with permission, from Ref. 16.

The mechanism of desaturase induction and its physiological importance have been studied in most detail in cyanobacteria[13]. Wada *et al.*[14] produced cyanobacteria that display increased chilling tolerance by transforming them with the *desA* gene, which encodes a Δ12-desaturase[14]. On the basis of targeted mutagenesis of acyl-lipid desaturases (single and double mutants of *Synechocystis*), Tasaka *et al.*[15] have presented evidence for important roles for polyunsaturated fatty acids (polyUFAs) in growth, respiration and photosynthesis. Gene disruption experiments showed that sequential desaturation of stearate to linolenate could be blocked at each step and that replacement of all the polyUFAs with monounsaturated fatty acids suppressed growth and decreased the tolerance of cells to photoinhibition at low temperatures[15]. In the protozoon *Acanthamoeba castellanii*, chilling also induces a Δ12-desaturase that, in turn, increases membrane unsaturation and restores physiological function (in this case phagocytosis)[11].

The ability of cyanobacteria to adapt to chilling begged the question: how could they sense environmental change? Membrane order (fluidity) appeared to be important, and decreasing fluidity (increasing order) induced desaturase genes[16]. Lowering the growth temperature of *Synechocystis* sp. PCC 6803 markedly increased the level of *desA* transcription (Fig. 1)[17]. In addition, saturation of a small pool of plasma membrane UFAs by *in vivo* catalytic hydrogenation in *Synechocystis* sp. PCC 6803 activated transcription of *desA* and thus mimicked the increased transcription seen when

the growth temperature was lowered (Fig. 1)[16]. The conditions used for the catalytic saturation only affected the plasma membrane; this implies that this membrane acts as a sensor and that increased saturation activates a mechanism whose ultimate effect is to enhance *desA* transcription[16]. How the signal is transduced from the membrane to the *desA* gene is unknown.

Membrane physical state and heat-shock-gene transcription

At higher temperatures, some of the problems encountered during chilling also occur. The cell must sense the elevated temperature and couple detection to induction of gene expression – in this case, of heat-shock genes.

Responses to heat shock can occur over a wide range of temperatures, depending on the organism. For example, Antarctic fish respond at about 4°C (Ref. 18), whereas hyperthermophilic bacteria react in the range 95–102°C (Ref. 19). Moreover, in many cases, the critical response temperature is not genetically programmed but depends on the normal growth temperature[20–22]. In addition, evidence suggests that exposure of cells to an abrupt increase in temperature causes their membranes to undergo a rapid decrease in molecular order (i.e. fluidity increases)[23,24].

Modulation of the membrane's physical state following heat shock influences the expression of heat-shock genes by resetting the temperature at which the optimum heat-shock response occurs[25]. Carratu *et al.*[25] complemented a *Saccharomyces cerevisiae* mutant that possesses a disruption in its *OLE*1 (Δ9-desaturase) gene (and hence cannot produce UFAs) with constructs that expressed the *OLE*1 coding region under the control of different promoters. They were therefore able to alter the expression of Δ9-desaturase and, hence, the fatty acid composition of the cell's membranes. Genetic manipulation of saturated fatty acid (SFA) : UFA ratios had a significant effect on *hsp70* and *hsp82* transcription[25]; this supports the idea that changes in membrane order are an important factor in determining heat-shock-gene expression. Further support came from Chatterjee *et al.*[26], whose alteration, by thermal acclimation, of the lipid composition of *S. cerevisiae* set a different temperature threshold for the heat-shock response. The signal transduction mechanism that links rises in temperature to increased expression of heat-shock proteins (HSPs) was not so clear.

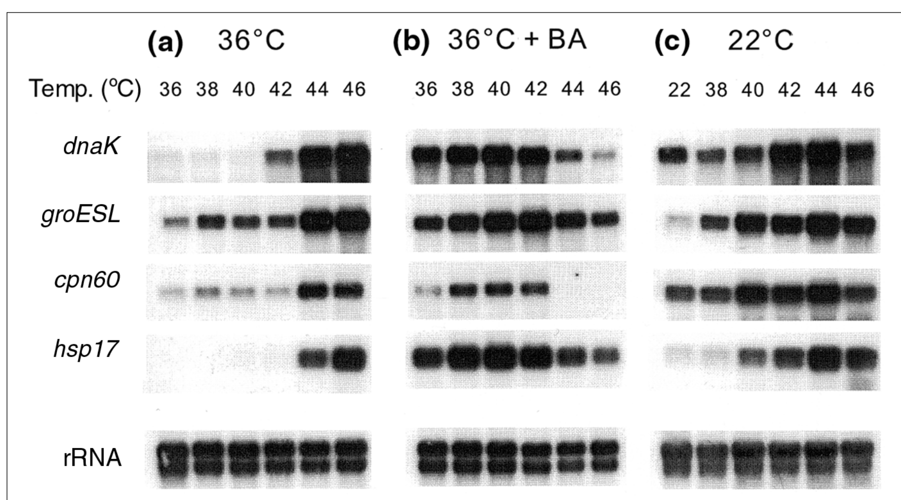

Figure 2
Effect of membrane modulations on the temperature (Temp.) profile of heat-shock-protein expression in *Synechocystis* sp. PCC6803. Cells were incubated at the indicated temperatures, and the purified RNA was subjected to Northern analysis. **(a)** Cells acclimatized to 36°C. **(b)** Cells acclimatized to 36°C and treated with 30 mM benzyl alcohol (BA). **(c)** Cells acclimatized to 22°C. Figure adapted, with permission, from Ref. 20.

The unicellular cyanobacteria *Synechocystis* sp. PCC 6803 is a powerful model system for studying the molecular mechanism of the heat-stress response in photosynthetic organisms. Heat shock induces four HSPs (Hsp70, Hsp60, Hsp17 and Hsp14) in *Synechocystis*[27]. The cyanobacterial DnaK corresponds to eukaryotic HSP70 (Ref. 27), whereas HSP60 are encoded by the *groESL* operon and the *cpn60* gene (which does not process groES) in the neighbouring region. In *Synechocystis*, synthesis of these HSPs is dependent on growth temperature[28] and might be due to membrane fluidity changes.

To test the validity of the hypothesis that membranes can act as temperature sensors, we altered the fluidity of *Synechocystis* membranes *in vivo* by temperature acclimation, by addition of a fluidizer (benzyl alcohol) or by catalytic lipid hydrogenation. The decreased membrane order in thylakoids that was obtained either by temperature acclimation or by administration of benzyl alcohol enhanced the thermosensitivity of the photosynthetic membrane[20,29] and altered the cellular heat-shock response. There was a close correlation between membrane order in thylakoids (monitored by steady-state DPH anisotropy) and threshold temperatures required for maximal activation of all heat-shock genes investigated, including *dnaK*, *groESL*, *cpn60* and *hsp17* (Fig. 2). The causal relationship between thylakoid membrane order and the temperature setpoints for transcriptional activation and *de novo* protein synthesis was most striking for the novel HSP Hsp17, which associated mostly with thylakoid membranes. These findings, together with the fact that the *in vivo* modulation of lipid saturation within the plasma membrane has no effect on heat-shock response, suggest that the thylakoids act as a cellular thermometer from which a cellular signal that activates heat-shock-gene transcription is transduced[20].

Other compounds that modify membrane order, such as short-chain fatty acids or alcohols, also affect the synthesis of HSPs[30], which, in turn, might have a direct effect on membrane fluidity (see below). Alcohols lower the temperature required for maximal activation of heat-shock genes, and the concentration of alcohol required decreases as the alcohol's hydrophobicity increases[31]. Furthermore, heat and ethanol stress cause similar changes to plasma-membrane protein composition in *S. cerevisiae*, reducing the levels of plasma membrane H^+-ATPase protein and inducing the plasma-membrane-associated Hsp30p (Ref. 32). This suggests that alcohols can induce membrane perturbation in a manner analogous to that of heat shock. Bimoclomol®, a new drug that could help alleviate conditions such as neuropathy and angiopathy, or help in ischemia, might induce membrane non-lamellar phases (see Fig. 3). The drug enhances accumulation of all major classes of stress proteins, including HSP60, HSP70, HSP90 and GRP94 (Ref. 33).

Compounds such as chlorpromazine (CPZ), dibucaine, lignocaine, imipramine, tetracaine and procaine, which are

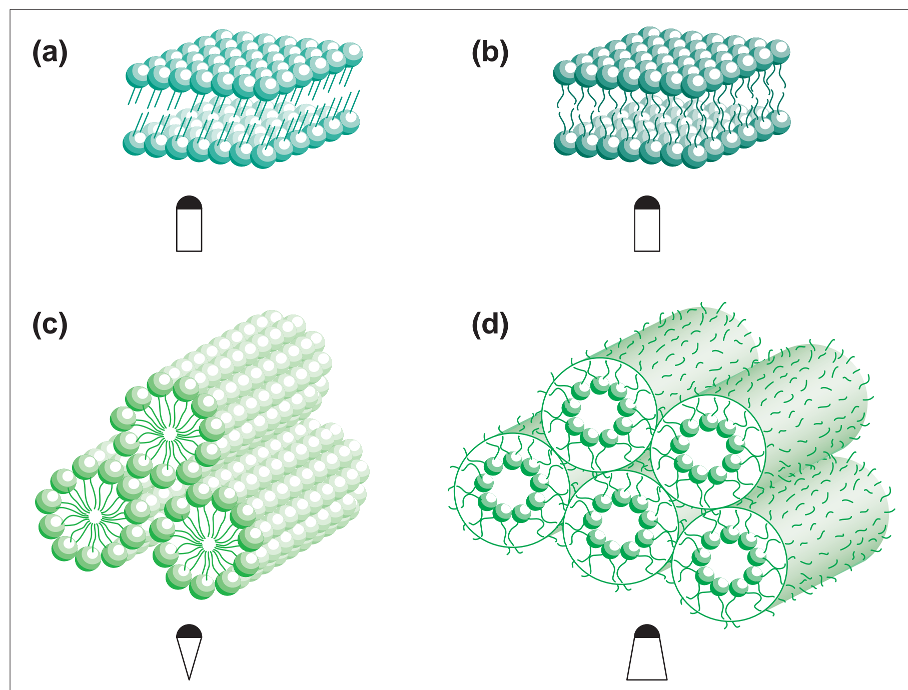

Figure 3
Possible arrangements of amphipathic membrane lipids. **(a)** Bilayer (lamellar phase) in which the acyl chains of the lipids are in the gel phase. **(b)** Bilayer (lamellar phase) in which the acyl chains of the lipids are in the liquid phase. At the transition temperature (Tc), membrane lipids change from the gel-crystalline to the liquid-crystalline state. **(c)** The hexagonal (non-lamellar) H_I phase is characterized by cylinders of lipids in which the latter's polar groups are exposed on the surface; a core of hydrocarbon chains adopt a disordered liquid-like conformation. The cylinders are hexagonally arranged in an aqueous medium. **(d)** The hexagonal (non-lamellar) H_{II} phase is the reverse of H_I (i.e. water forms cylinders and hydrocarbon chains form the continuous medium, and the polar groups lie at the water–hydrocarbon interface). H_{II} phases are adopted by membrane lipids that have small headgroups and whose acyl moieties occupy a large volume (e.g. unsaturated phosphatidylethanolamines). H_I phases are formed by lipids that have large headgroups (e.g. monoacylphosphatidylcholine); because such molecules seldom occur *in vivo*, only the H_{II} phases are common non-lamellar arrangements. The shapes of lipids forming the different phases are shown below each diagram.

used as psychotropic drugs or local anaesthetics, also induce transcription (Table I). These compounds interact with membranes, and CPZ, in particular, causes membrane deformation or fluidization (Table I). The compounds induce transcription of a small number of genes, which implies that they interact directly with distinct membrane domains that initiate a signalling cascade that targets specific genes – rather than associating indiscriminately with membranes and thereby altering transcription of a large number of genes. CPZ and other drugs (such as those in Table I) are currently used in pharmacology; other compounds that possess a partition coefficient for membranes could have similar effects.

Other examples where changes in membrane order seem to be important for regulation of gene expression exist. The yeast *PKC1* gene (whose product is implicated in the regulation of a MAP-kinase pathway) could be activated when cells detect a stretch in their plasma membranes after thermal stress[34]. Moreover, mutant *Escherichia coli* strains that have modified membrane lipid compositions show altered expression of genes that are involved in osmoregulation[35]. These experiments illustrate the link – and cross-talk – between the membrane's physical properties and gene regulation.

Interactions between heat-shock proteins and membranes

While the membrane's physical properties appear to regulate HSP expression, proteins such as the GroEL chaperonins and their eukaryotic analogues, in turn, associate with membranes in many organisms[36]. In *Synechocystis* cells, following heat shock, both GroEL and Hsp17 associate with thylakoids[20,37]. In *Leuconostoc oenos*, an analogous protein, Hsp18, which can be induced by either heat or ethanol, associates with the membrane periphery[38]. Membrane-bound HSPs might act like their soluble counterparts and prevent denaturation of membrane-localized enzymes[39,40]. Support for this idea comes from experiments that show selective binding of GroEL chaperonins, through a specific C-terminal region, to model membranes[40]; thus, for example the testis-specific HSP70 possesses a specific sulphatide-binding site[41]. The binding of GroEL depends on the physical state of the membrane; moreover, the association of chaperonins with membranes in itself caused rigidity and stabilization[40]. In addition, proton NMR measurements have shown that the yeast integral plasma-membrane protein Hsp30 reduces membrane fluidity at higher temperatures (K. Obuchi, pers. commun.). Thus, a cycle of heat-induced membrane perturbation causes induction of HSPs, which then associate with membranes and dampen the induction signal. This cross-talk, ending in feedback inhibition, accounts for the temporary nature of the heat-stress response[42].

The membrane's physical state can control gene expression

The above findings suggest that membranes can sense environmental changes and, as a consequence of changes in their phase state and microdomain organization, transmit signals that activate transcription. Whereas membrane-bound receptors are activated by external molecules and transmit signals across the membrane, here we deal primarily with alterations of lipid phase. Most lipids in cellular membranes – even those with a non-lamellar propensity (see Fig. 3) – are generally in bilayer structures, but heat shock or membrane-perturbing agents (such as alcohol) can induce non-lamellar [inverted hexagonal phase (H_{II})] structures. The latter exert a relatively high lateral pressure on proteins in the membrane's core and therefore modify their function[43]. In fact, Slater *et al.*[44] have suggested that, in a number of cases, such non-lamellar lipid phases affect signal transduction pathways.

The exact molecular details of lamellar–H_{II} transitions (Fig. 3) are a subject of considerable interest[45]. Compared to the enthalpy change in the liquid-crystalline-to-gel transition that is promoted by chilling, the enthalpy necessary for the formation of H_{II} phases is much lower and is easily supplied by heat. The mechanism of non-lamellar-phase formation might involve transient inverted intramembrane micelles or, alternatively (or in addition), the so-called extended conformation – in which the hydrocarbon chains of a lipid are not parallel but

Table I. Genes induced by pharmacological compounds thought to perturb membranes

Drugs	Effect	Gene induced	Organism/cell	Refs
Chlorpromazine (CPZ), procaine dibucaine, lignocaine imipramine, tetracaine	Membrane fluidization		*Escherichia coli*	50
CPZ, dibucaine, lignocaine, imipramine, tetracaine, procaine		DnaK,GroEL (21 kDa, 20 kDa, 17 kDa)	*E. coli*	51
CPZ	Mimics hypotonic stress membrane deformation	*FOS*	Cardiac myocytes	52
CPZ, procaine ethanol	Mimic osmolarity	*kdpD*	*E. coli*	53
CPZ, sulfonamide, (W7) calmidazolium, A23187, trifluoperazine		Calmodulin inhibitors, metallothionein	Liver, Wistar-rat kidney	54
CPZ		Nitric oxide synthase	Mouse, rat	55
CPZ		Interleukin 2, TNF-α, interferon-α, *MYC*	Human thymocytes	56
Clozapine		*c-fos*	Thalamus (rat)	57
Clozapine, loxapine, haloperidol		*FOS*	Thalamic paraventricular nucleus	58
Clozapine, haloperidol		*FRAs*	Cortex striatum medial prefrontal	59
Clozapine		fras, *fosB, junB, junD, Krox24*	Nucleus accumbens of rat brain	60
Clozapine		*FRAs, JUNB, Krox24*	Striatum	60
Haloperidol, fluphenazine		*junB, junD*	Rat striatal tissue	61

extend in opposite directions from the head group. Such a conformation might provide a novel mechanism for membrane attachment of proteins such as protein kinase C or could function as a general growth signal in cells[45,46].

Increasing evidence suggests that membranes are more complex structures than we previously believed. In fact, different lipid species are not only arranged asymmetrically across the bilayer but also organized in lateral domains that display short- or long-range order and form rafts onto which proteins are attached[47]. Such rafts include dynamic clusters of sphingomyelin and cholesterol, which allow calveolin-induced invaginations (calveolae) to trap proteins that are involved in signal transduction pathways. Thus, membrane proteins involved in signal transduction could be restricted to distinct lipid microdomains that have different physical properties controlled by temperature, growth conditions, pathological disorders, etc. In turn, the nature of the microdomains could control the capacity of heat shock and other factors to induce transcription.

The heat-shock response is transient and lasts only a few hours[42]. Accumulation of specific HSPs at (or in) the membrane causes a rigidification of the heat-fluidized membrane so that the membrane resembles that existing prior to heat shock[40]. After re-establishing optimal lateral-packing pressure, membranes recover a pre-stress state and inactivate the membrane-perturbation signal, thus turning HSP synthesis off. This membrane protection is only temporary, and the bilayer structure is re-established only after lowering of the temperature or removal of the perturbing agent.

This model has important clinical implications because, in several well-described pathological disorders, alteration of the membrane's physical state is associated with a decrease in HSP synthesis. Thus, if our hypothesis is widely applicable, it may open new perspectives for how we treat such diseases.

Concluding remarks

Although the membrane's physical state might not be the only factor that regulates the expression of heat-shock and desaturase genes, its importance has implications beyond adaptation to shifts in temperature. HSPs are present at abnormal levels in several pathological conditions, such as neurodegenerative disease, cancer and kidney disease, and during ageing[48]. Furthermore, pre-induction of HSPs is beneficial in a number of diseases. For example, increased levels of HSP70 increase resistance of the heart to ischemic injury[49]. HSPs could therefore be induced by drugs that change the physical properties of membranes. Fundamental work on the temperature adaptation of poikilotherms could thus have far-reaching significance in the development of pharmaceuticals for human use.

Acknowledgements

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Ceramide in apoptosis – does it really matter?

Kay Hofmann and Vishva M. Dixit

During recent years, ceramide has received a lot of attention as a possible mediator of the cellular responses to a variety of apoptotic stimuli. In a manner analogous to generation of its sibling diacylglycerol, ceramide is generated by a phospholipase-C-type reaction from its lipid precursor sphingomyelin. Two observations led to the proposal that ceramide plays a role in apoptosis: (1) treatment of cells with tumor necrosis factor or other inducers of apoptosis leads to activation of sphingomyelinases and to an increase in cellular ceramide levels; (2) ectopic generation or administration of ceramide can mimic apoptotic cell death. Recently, several observations have challenged the notion that ceramide is an important cell-death mediator and have prompted a re-evaluation of previously published results.

SPHINGOMYELIN HYDROLYSIS (Fig. 1), triggered by the binding of extracellular ligands to cell-surface receptors or by agents that induce cellular stress, is the major source of the putative intracellular second messenger ceramide[1–3]. Several sphingomyelin-specific phospholipase C (PLC) activities, termed sphingomyelinases (SMases), exist in mammalian tissues. These isoenzymes differ in their catalytic properties and subcellular localizations, and probably also in their modes of regulation. A lysosomal acidic sphingomyelinase (aSMase) was the first SMase to be cloned and characterized on a molecular basis[4]. Initially, this ubiquitous enzyme appeared to function only in the metabolic degradation of sphingomyelin. Defects in the aSMase gene, which cause the hereditary Niemann Pick disease types A and B, result in a massive accumulation of sphingomyelin in the lysosomes and death in early childhood[5]. Mice carrying an artificially disrupted aSMase gene display a similar phenotype[6,7].

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A secreted form of aSMase is also encoded by the gene that encodes the lysosomal aSMase (Ref. 8). A Mg^{2+}-dependent neutral SMase (nSMase) that resides in the plasma membrane and is expressed mainly in the brain was initially thought to provide most of the ceramide used as a second messenger[9]. This enzyme resisted all attempts to purify it for several years; however, Tomiuk and co-workers[10] recently identified and cloned it by using sequence motifs shared with bacterial sphingomyelinases. A soluble, Mg^{2+}-independent nSMase, which resides in the cytoplasm[11], and a secreted alkaline SMase (Ref. 12) still await molecular characterization. In addition, the *de novo* synthesis of ceramide (from sphingosine) could also be a regulated event that leads to the inducible generation of this putative second messenger molecule[13].

Signaling to sphingomyelinases

A number of stimuli have been reported to increase cellular ceramide levels (Fig. 2). Among the more prominent inducers are tumor necrosis factor (TNF), Fas-ligand (FasL), 1,25-dihydroxyvitamin D, ionizing radiation and various chemotherapeutic agents[1–3]. In some cases, the increase in ceramide levels is due to enhanced *de novo* synthesis[14]; in the majority, however, one or more of the cellular SMases are activated. The most extensively studied system is the activation of aSMase and nSMase that follows binding of TNF to its receptor TNF-R55 (also known as TNF-R1).

The aSMase appears to be ill positioned for a key enzyme in signal transduction. Residing in the lumen of the lysosome, it is not readily accessible by classical mechanisms of receptor-induced signal transduction. Nevertheless, the region of TNF-R55 that is critical for aSMase activation has been

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