Biochimica et Biophysica Acta 1848 (2015) 1757–1764

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Thermosensing via transmembrane protein–lipid interactions☆

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ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 8 April 2015
Accepted 9 April 2015
Available online 20 April 2015

Keywords:
Membrane protein
Temperature sensing
Transmembrane signaling
Lipid interaction

ABSTRACT

Cell membranes are composed of a lipid bilayer containing proteins that cross and/or interact with lipids on either side of the two leaflets. The basic structure of cell membranes is this bilayer, composed of two opposing lipid monolayers with fascinating properties designed to perform all the functions the cell requires. To coordinate these functions, lipid composition of cellular membranes is tailored to suit their specialized tasks. In this review, we describe the general mechanisms of membrane–protein interactions and relate them to some of the molecular strategies organisms use to adjust the membrane lipid composition in response to a decrease in environmental temperature. While the activities of all biomolecules are altered as a function of temperature, the thermosensors we focus on here are molecules whose temperature sensitivity appears to be linked to changes in the biophysical properties of membrane lipids. This article is part of a Special Issue entitled: Lipid–protein interactions.

Contents

1. Introduction

Lipids are among the most abundant cellular metabolites, with an overwhelming diversity in structure and function. A challenging problem is to understand how this enormous diversity is exploited at the biological level. Besides their fundamental role in membrane assembly and architecture, lipids can serve as signaling molecules in a multitude of cellular processes. Of particular interest are the lipid-induced changes in physical membrane properties such as bilayer thickness, lipid packaging density, membrane fluidity and membrane curvature that regulate the function of membrane-associated proteins. These alterations are conserved from bacteria to human and provide specific cues for membrane proteins to exert control over numerous molecular events.

In this review we first describe the properties of lipids and lipid membranes, and give several examples of how these properties lead to the regulation of protein activity. Next, we describe how membrane-mediated

☆ This article is part of a Special Issue entitled: Lipid–protein interactions.

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http://dx.doi.org/10.1016/j.bbamem.2015.04.005
0005-2736/

structural changes of a bacterial cold sensor in particular are accomplished and how a eukaryotic membrane-bound sensor may be a regulator of membrane fluidity during cold adaptation.

2. Membrane lipids: Structure and properties

Lipids found in biomembranes fall into three main classes: glycerophospholipids, sphingolipids and sterols. The most common class in bacteria is glycerophospholipids, also named simply as phospholipids (PL). Esterification of fatty acyl chains to carbons 1 and 2 of L-glycerol 3-phosphate form phosphatidic acid (PA), and esterification with another alcohol creates the phospholipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidylinositol (PI). In addition, PG can link through its glycerol headgroup to PA to form diphosphatidylglycerol (CL for its common name, cardiolipin) (Fig. 1) [1]. The phosphate group and headgroups are the polar portions, and the acyl chains are the nonpolar regions of these amphiphilic molecules. In order to study the functionality of biological membranes it is important to understand the diversity of chemical, biological and physical properties of its constituent phospholipids. For example, anionic PLs (PS, PI, PG and CL) have a net negative charge at physiological pH, while zwitterionic PLs (PE and PC) are neutral. PE and PS contain reactive amines that can participate in hydrogen bonding, while PI, PC and CL are relatively bulky, which affects their packing in bilayers. Additionally, lysophospholipids (PLs that lose one acyl chain through activity of phospholipase enzymes) have surfactant properties which alter the lipid membrane structure [1]. Moreover, the degree of unsaturation of the acyl chains of PLs contributes to the elasticity of the membrane, a fundamental property for the insertion and sequestration of proteins.

The diversity in lipid structure and properties not only influences the capability of bilayers to adjust, integrate and favor the correct folding and functionality of proteins, but also has effects on the formation of many lipid aggregates with particular characteristics. Bilayers are only one of the possible lipid aggregates that form spontaneously when amphiphilic lipids are mixed with water. Lipid structures can be classified in three main families: lamellar (includes the familiar bilayer), hexagonal and cubic. The most common states of lamellar phase are L_α (liquid crystalline, or liquid disordered) and L_β (lamellar gel, or ordered solid) [1,2]. Hexagonal phases consist of hexagonally packed arrays of lipids in long cylindrical tubes with two topologies, H_I (nonpolar chains in the center and polar headgroups and water in the outside) and H_{II} (polar headgroups and water molecules in the center and nonpolar chains in the outside). Like hexagonal phases, cubic phases can be either type I (positive curvature, acyl chains inside) or type II (negative curvature, acyl chains outside), but present a much greater variety of three-dimensional structures as they are formed from cubic packing of rod-like elements, resulting in discontinuous phases [1–3].

2.1. Membrane curvature

The formation of either lamellar or hexagonal phases is determined by the molecular shape of the constituent lipids. Lipid molecules that present either cone shape or inverted cone shape tend to form non-lamellar phases [3–5]. A concept related to the shape of lipid molecules is the intrinsic radius of curvature (R_0), i.e. the curvature of a lipid–water interface in a situation where the lipid monolayer is elastically relaxed [4,5]. By definition, R_0 is positive for type I structures and negative for type II structures and is inversely proportional to the curvature of the lipid monolayer [5]. Temperature and unsaturation are factors that increase the separation between the lipid tails decreasing R_0,

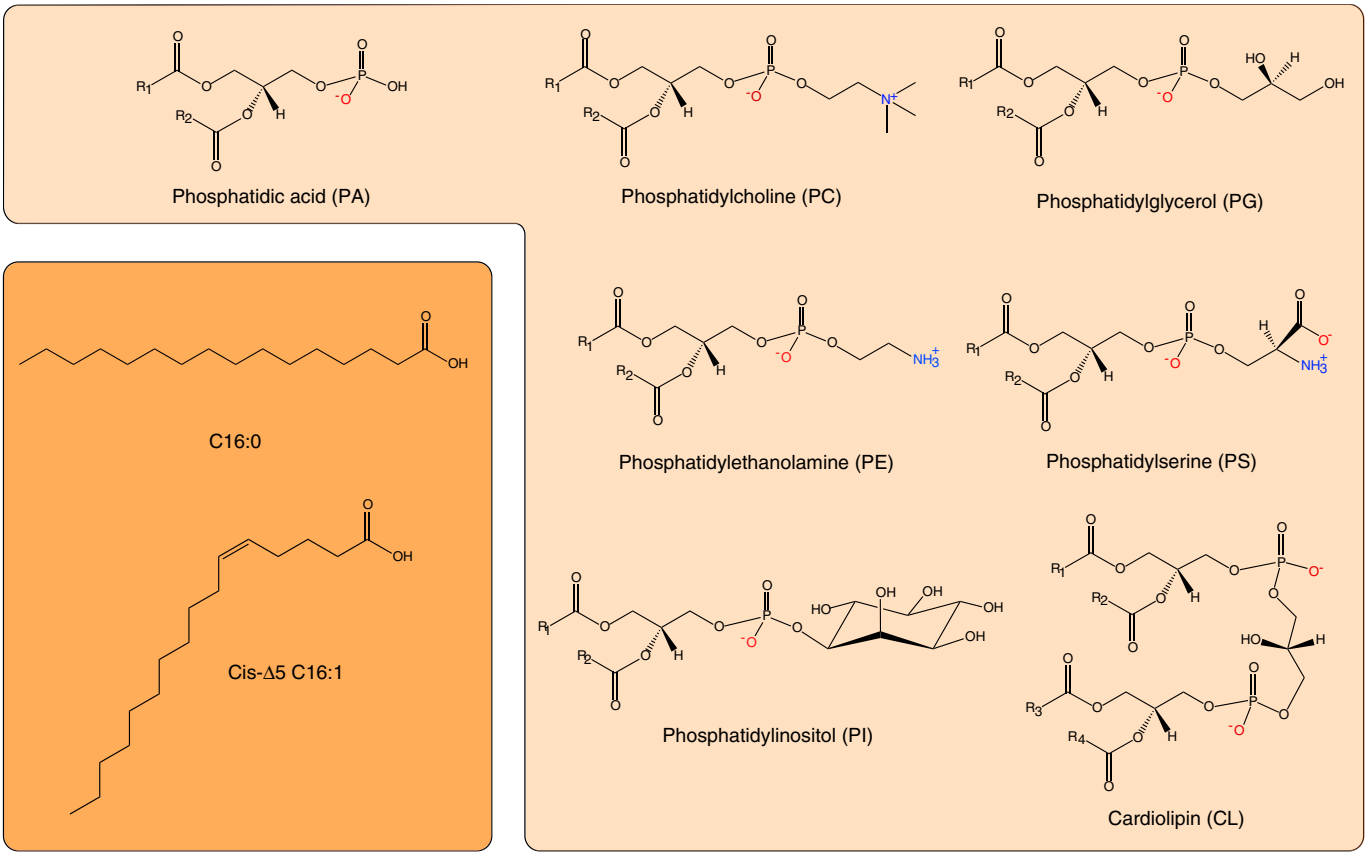

Fig. 1. Chemical structure of lipid molecules. Several polar headgroups are shown highlighted in light orange, where positions R1 and R2 denote the binding sites of hydrocarbon chains. Fatty acid saturated and unsaturated hydrocarbon chains are highlighted in dark orange.

while the size and charge of the headgroups raise the effective headgroup area increasing R_0. When non-bilayer lipids are forced to constitute a bilayer, they are in a state known as "curvature frustration". Hexagonal and cubic phases are formed if curvature frustration exceeds the hydrophobic interactions of the chains. Curvature frustration also depends on temperature, pH, salt concentration, concentration of divalent cations, and hydration of headgroups [1,2].

One of the best examples of an enzyme that is directly coupled to intrinsic curvature strain is CTP:phosphocholine cytidylyltransferase (CCT) which catalyzes the formation of cytidine diphosphate choline, the rate limiting step in the biosynthesis of PC [6,7]. CCT contains an amphipathic helix, called domain M, that silences the activity of the enzyme in its soluble form [8]. In the presence of PC bilayers with a high content of conical lipids such as diacylglycerol or PE, domain M undergoes a conformational switch that creates a hydrophobic face for membrane binding. Association with membranes relieves self-inhibition and drastically increases the affinity of CCT for its substrate CTP. By contrast, membrane association and activity of CCT is reduced when diacylglycerol/PC or PE/PC ratios are low. Thus, CCT seems to act as a general sensor of lipid packing defects that signals a demand for PC biosynthesis to allow membrane expansion or to prevent its transition into a porous state [7].

2.2. Hydrophobic mismatch

A hydrophobic mismatch occurs when the hydrophobic patch of a membrane protein does not match the hydrophobic thickness of the membrane in which it is inserted, i.e. the non-polar region of the bilayer. To be more specific, a positive hydrophobic mismatch describes the extreme situation in which membranes are much too thin, and a negative hydrophobic mismatch the situation in which membranes are much too thick [9]. The hydrophobic thickness of a membrane depends on the lipid composition of the bilayer, mainly on the length, degree of saturation and fluidity state of the hydrocarbon chains. For pure PC bilayers in liquid phase, the hydrocarbon thickness increases linearly with the chain length of saturated lipids, as observed for dimyristoylphosphatidylcholine [di(14:0)PC] (25.7 Å), dipalmytoylphosphatidylcholine [di(16:0)PC] (28.5 Å) and distearoylphosphatidylcholine [di(18:0)PC] (31.9 Å) [10,11]. However, one double bond diminishes the thickness substantially as observed for dioleoylphosphatidylcholine [di(18:1)PC], where hydrophobic thickness is 28.8 Å at 30 °C [10,11]. If the hydrophobic regions of proteins and lipids do not match, the lipid bilayer must either stretch or compress, or the protein must change its structure by tilting helices or rotating side chains, in order to compensate such hydrophobic mismatch. For example, gramicidin A is a 15-aminoacid antibiotic peptide which forms bilayer-spanning monovalent cation channels in biological membranes and synthetic bilayers. Molecular dynamics simulations showed that di(14:0)PC membranes become thinner by 2.6 Å upon insertion of the gramicidin A dimer, while dilauroylphosphatidylcholine [di(12:0)PC] membranes thicken 1.3 Å in the presence of the antibiotic [12]. The simulations show that the channel structure varied little with changes in hydrophobic mismatch, and that the lipid bilayer adapts to the bilayer-spanning channel to minimize the exposure of hydrophobic residues [12]. Generally, alpha helices do not perturb the membrane as much as β-barrel proteins, as helices can adapt by tilting with respect to the bilayer normal, as is observed for the monomer of gramidicin A [12–17]. However, although proteins and lipids can adapt to compensate hydrophobic mismatches, proteins seem to prefer to bind lipids that do not require changing. For example, covalently spin-labeled rhodopsin reconstituted in PC lipids with different chain lengths showed activity in di(14:0)PC, but segregated into protein-rich domains in di(12:0)PC, and aggregated in di(18:0)PC. These results suggest that the protein partitioned into the gel phase with the shorter acyl chains and into the liquid crystalline phase with the longer acyl chains, since the bilayer is thicker in gel phase than in fluid phase [1].

The hydrophobic mismatch effect is also related to the curvature of the membrane, as stretching of lipid molecules near the protein produces a local negative curvature and compression generates a local positive curvature, while the overall structure of the membrane is still a straight bilayer. Therefore, the presence of non-bilayer lipids such as PE is expected to influence the ability of the membrane to adapt to a mismatch situation. These effects might be of relevance for transmembrane peptides or proteins that coexist in an equilibrium between conformations with different hydrophobic lengths, such as gramicidin A and rhodopsin [18–20].

2.3. Lateral pressure

Besides curvature and membrane thickness, a mechanical coupling between membrane proteins and lipid bilayers by using the so-called lateral pressure profile has been proposed. The lateral pressure profile describes an inhomogeneous stress across the bilayer arising from hydrophobic, electrostatic and steric interactions. Although lateral pressures must be balanced to give a stable planar bilayer, the distribution of these forces in the membrane varies with depth [21,22]. The cohesive hydrophobic interfacial tension between the acyl chains and headgroups, produces a large negative pressure, which is balanced by a positive pressure due to repulsion between the hydrocarbon chains. In addition, electrostatic interactions between headgroups lead to mainly positive lateral pressures [21,23,24]. This profile of lateral pressures is proposed to be sensitive to changes in the structures of the fatty acyl chains, as the presence of *cis* double bonds produces marked shifts of pressure, especially if the double bond is close to the headgroup [25]. In addition, non-bilayer lipids can either increase the lateral pressure in the acyl chain region or among the lipid headgroups, if they are cone-shaped or inverted-cone-shaped, respectively [26–28]. Changes in lateral pressure affect the stability of membrane proteins, as described for KcsA, the potassium channel of *Streptomyces lividans*, reconstituted into liposomes. Integration of small alcohol molecules into the proteoliposomes leads to an increase in the lateral pressure on the headgroup region, and a compensating decrease in lateral pressure on the hydrocarbon chains, which destabilizes the active tetramer conformation of KcsA [29].

Given the case in which the activation/deactivation of a membrane protein requires a conformational change at a particular depth of the bilayer, such change would be affected by lateral pressures. Then, anything that modifies the lateral pressure profile would also affect the conformational equilibrium of the protein, and therefore would be an important determinant of the protein functions [21,30]. Molecular dynamics simulations of a di(16:0)PC bilayer, in a state of gel–liquid phase coexistence, show a lateral pressure profile of the liquid phase similar to that of a homogeneous fluid bilayer, while the profile of the gel phase is strikingly different, with reminiscence of more rigid bilayers like those with large amounts of cholesterol [31,32]. These results imply that membrane lipids exert phase-dependent lateral pressures over proteins, which can be significant to overcome the energy barrier between conformational states, leading to the activation/deactivation of the proteins. Perozo et al. found that MscL can be opened by adding lysophosphatidylcholine molecules asymmetrically to only one of the two leaflets of a bilayer, but remains closed upon symmetrical addition to both leaflets [33]. Therefore, membrane lipids could act as allosteric regulators of protein activity by altering lateral pressure profiles.

2.4. Gel to liquid crystalline phase transition

Phase transition not only affects lateral pressure profiles, as described above, but also the hydrophobic thickness of the membrane and the possibility of a mismatch with the hydrophobic patch of the proteins. If proteins are in a situation of less than optimum hydrophobic

match, a slight change in membrane thickness can produce a significant effect on protein activity. This is observed for diacylglycerol kinase, whose activity decreases when inserted into membranes of di(16:0)PC in gel phase state, and increases at the same temperatures in membranes of di(16:1)PC, i.e. in liquid phase state. When lipid environment is closer to the optimum thickness (in di(14:0)PC membranes) no effect on diacylglycerol kinase activity was observed upon liquid-gel phase transition [34]. Therefore, if a membrane protein is sensitive to hydrophobic thickness, the fluid state of the membrane lipids is an effective and precise way to modulate its activation/deactivation. This is the case of DesK, the *Bacillus subtilis* cold sensor, whose characteristics and functional properties are described extensively in the third section of this review.

3. DesK, a bacterial lipid composition sensor

In addition to altering protein conformation directly, temperature changes can affect protein activity as a secondary consequence of structural alterations in the lipid environment. A paradigmatic example is the well-studied thermosensor DesK from *B. subtilis* in which temperature-dependent changes in the lipid membrane appear to be the primary mediators of this protein's thermal responsiveness [35]. DesK is an integral membrane-associated histidine kinase which is at the top of the signaling cascade of a regulatory pathway that controls the synthesis of unsaturated fatty acids in *B. subtilis* (Fig. 2). In vivo and in vitro experiments have demonstrated that DesK acts as a kinase at cold temperatures, autophosphorylating a conserved histidine residue within its kinase domain [36]. The phosphoryl group is then transferred to the receiver aspartic acid in the DNA-binding response regulator DesR [36,37]. Phosphorylation of DesR triggers the reorganization of its quaternary structure [38], a key event necessary to activate the transcription of the *des* gene that encodes the acyl lipid desaturase Δ5-Des [39]. Unsaturated fatty acids, the end products of Δ5-Des activity, promote a more fluid membrane that appears to switch DesK from the kinase to the phosphatase state [36]. Consequently, the concentration of phosphoryl DesR declines and transcription of *des* is terminated [37]. Two key discoveries that helped to the molecular elucidation of the pathway are (i) the crystallographic study of the DesK catalytic core (DesKC) which has revealed how the domains of this protein can interact to assemble the three active sites that determine its regulatory state, providing an excellent baseline for understanding the mechanism by which DesK functions as a molecular switch that transduces bilayer deformations into protein motions [40] (Fig. 3) and (ii) the establishment that DesK retains its functionality even when reconstituted in pure vesicles and hence that no other protein components are involved in either sensing or signaling [41,42]. Thus, later structural and biochemical approaches showed that DesK is cold-activated through specific interhelical rearrangements in its central four helix bundle domain, known as DHp (for Dimerization and Histidine phosphotransfer) [42]. It was proposed that in a fluid membrane, the transmembrane (TM) domain would stabilize a connecting parallel 2-helix coiled-coil (2-HCC) and the catalytic core in a rigid conformation with the ATP binding domains attached to the DHp domain [42]. This conformation inhibits autokinase activity and the DHp surface is competent to interact with DesR-phosphate, resulting in a phosphatase signaling state (Fig. 3). Upon cold signal reception, the 2-HCC is thought to be disrupted and the ensuing structural reorganization releases the ATP-binding domains for histidine phosphorylation (kinase state; Fig. 3) [42]. Phosphorylation of DesKC would then induce an as yet different, asymmetric conformation capable of interacting with DesR (phosphotranferase state; Fig. 3). These structural changes of the catalytic domain are promoted by the sensor domain of DesK [42,43]. One or more of the five TM segments in DesK undergo a conformational change, in the form of helix rotations and asymmetric helical bends, induced by a modification in the physical state of the membrane lipid bilayer. This information is transmitted to the cytoplasmic domain by the membrane-connecting 2-HCC, ultimately controlling the alternation between output autokinase and phosphatase activities.

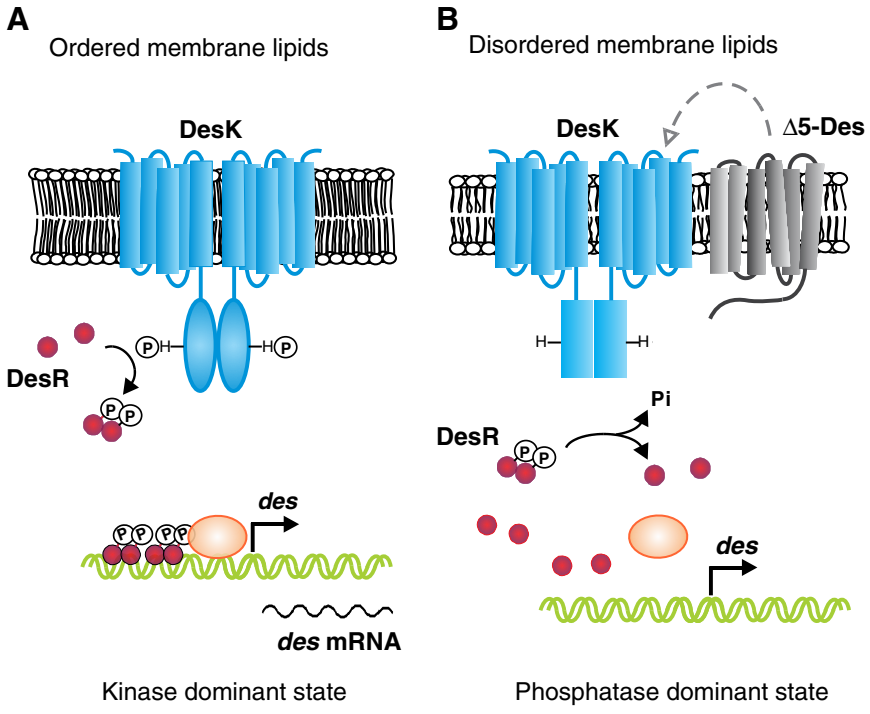

Fig. 2. Membrane fluidity optimization by the Des pathway of *Bacillus subtilis*. (A) An increase in the proportion of ordered membrane lipids promotes the kinase-dominant state of DesK, which autophosphorylates and transfers the phosphate group to DesR. Two DesR-P dimers interact with the des promoter and the RNA polymerase, resulting in the transcriptional activation of *des*. (B) Δ5-Des is synthesized and desaturates the acyl chains of membrane phospholipids increasing the fluidity of the membrane. A decrease in the order of membrane lipids favours the phosphatase-dominant state of DesK, leading to DesR-P dephosphorylation and turning off the transcription of *des*.

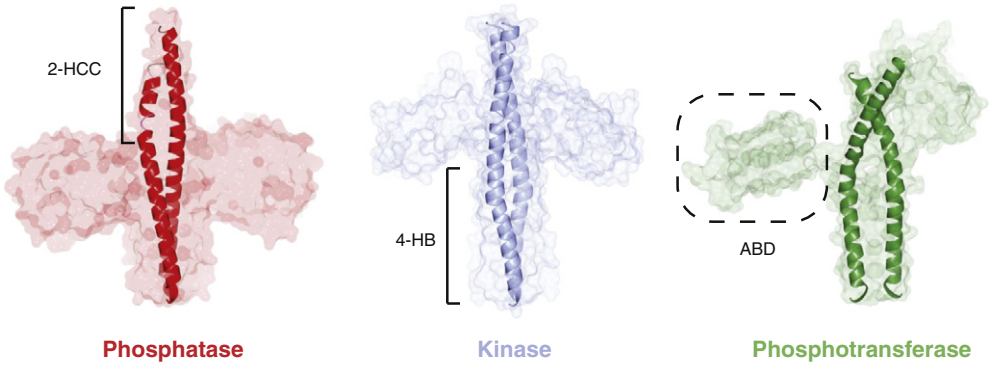

Fig. 3. Structural bases of DesK catalysis regulation. DesKC structures in phosphatase (red), kinase (blue) and phosphotransferase (green) competent states corresponding to Protein Data Bank files 3HJH, 3GIE and 3GIG respectively. In the phosphatase competent state the N-terminal portion of the 4-HB is forming a 2-helix coiled-coil (2-HCC) and the ABDs are packed close to the 4-HB. In the kinase competent state a rotation in the alpha helices of the DHp domain produces the disruption of the 2-HCC and the loosening of the interaction of the ABDs with the 4-HB domain. In the phosphotransferase competent state a pronounced bending of the α1 helix occurs just below the phosphorylated histidine, leaving room for the entrance of DesR.

3.1. A molecular model for thermosensing

The crystal structure of the entire cytoplasmic portion of DesK has provided an appropriate molecular framework to interpret the basic mechanistic principles by which the sensor protein alternates between autokinase and phosphatase activities [42]. Nevertheless, given the critical role membrane lipids play in DesK function, a fundamental question arises: Which are the TM helix rearrangements underlying the catalytic transitions along the signal transduction pathway? This enormous challenge recently became more accessible through the discovery that the multimembrane-spanning domain of DesK can be simplified into a chimerical single-membrane-spanning minimal sensor (MS) that is still able to respond to changes in membrane fluidity [44]. A systematic deletion analysis of the five-pass TM domains of DesK revealed that deletion of just the first TM region (TM1) abolished the ability of the sensor to respond to lower temperatures and resulted in a constitutively kinase-active state, suggesting that TM1 harbors a temperature-sensing motif [44]. Based on this finding, a model was envisioned in which TM1 would be able to detect a drop in temperature and transmit this information to TM5, which is connected to the catalytic core of DesK. A chimeric TM region was therefore created, consisting of N-terminal residues of TM1 and C-terminal residues of TM5, fused to the catalytic domain of DesK (Fig. 4). Remarkably, this minimal thermosensor (MS-DesK) harboring a single engineered transmembrane segment worked almost as well as wild-type DesK, harboring five membrane-spanning helices, to activate the expression of the *des* gene after a cold shock shift from 37 °C to 20 °C [44]. What membrane properties could MS-DesK be sensing? It is well documented that the physical state of the bilayer affects the barrier properties of membranes and the location and activities of their proteins [45]. At low temperature, the acyl chains are in the closely packed, ordered array of the rigid gel state in which molecular motion is highly restricted [46,47]. Upon warming, the membrane undergoes an endothermic transition and has much higher permeability to small molecules than gel-phase bilayers [45]. The melted lipids in the liquid–crystalline state remain in lamellar structure, but the bilayer is thinner because the acyl chains are less often in their fully extended conformation. For example, in fully hydrated DMPC bilayers at 10 °C (gel phase) the area per lipid molecule is 47.2 $Å^2$, whereas in fully hydrated DMPC bilayers at 30 °C (fluid phase) the area per lipid increases up to 60.6 $Å^2$ [48,49]. Membrane thickness variations have been shown to affect the activity of several membrane-embedded proteins [50,51], and given the strong correlation between temperature and area per lipid, it is likely that a temperature decrease will increase membrane thickness, generating a mismatch between the hydrophobic transmembrane helices of DesK and the surrounding lipid environment. Thus, it was hypothesized that such temperature-mediated bilayer perturbation could regulate the signaling state of DesK. Intriguingly, two hydrophilic amino acids (K-10 and N-12) near the amino terminus of DesK's first TM domain are critical for its cold activation [44]. Presumably these residues are located within the TM region just below the

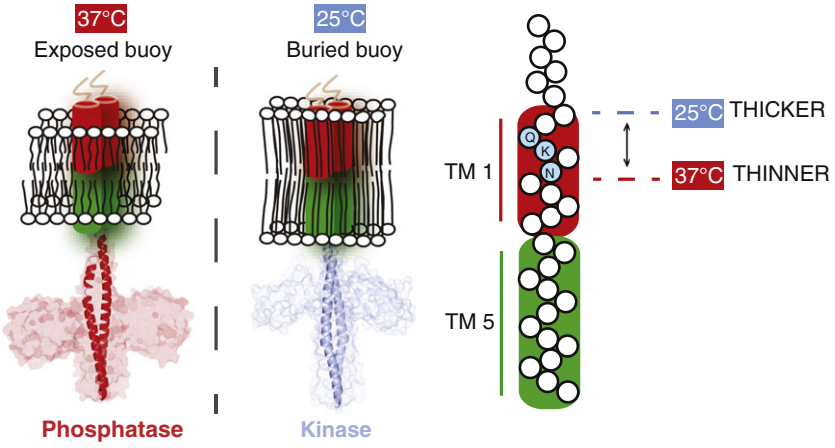

Fig. 4. Depiction of an engineered minimal signal-sensing domain of DesK that detects changes in environmental temperature. The minimal thermosensor harbors a cluster of hydrophilic amino acids (blue circles) near its amino-terminus that floats like a buoy near the lipid–water interface at high temperatures (37 °C) when the membrane is thinner (left). At lower temperatures (25 °C, right), an increase in lipid ordering results in a thicker membrane, forcing the 'buoy' into the hydrophobic lipid bilayer, thereby activating the autokinase domain. DesK therefore responds to changes in temperature by directly measuring membrane thickness.

lipid–water interface. Given that their side chains should be able to snorkel to the hydrophilic membrane–water interface, these amino acids could act as a buoy, stabilizing the position of the transmembrane segment. For this reason this region has been called the sunken-buoy (SB) motif [44] (Fig. 4). The SB model of thermosensing poses that an unstable state with deprivation of hydration of the polar cluster caused by membrane expansion is associated with kinase activity, whereas hydration during membrane narrowing promotes phosphatase activity. Several molecular tests, using chimeric MS-DesK, support this model. For example, shifting the critical K-10 one position inward into the hydrophobic lipid phase, a manipulation that should enhance SB dehydration at all temperatures, increases kinase activity. Conversely, an MS length mutant containing four extra valines in the nonpolar region that lies towards the carboxy-terminus of the SB motif, a manipulation that should help unbury the cluster of hydrophilic residues, decreases autokinase activity [44].

A segment linking the TM5 with the 4-HB domain of DesK was recently identified and called the linker region. This segment is located in close proximity to the inner leaflet of the lipid bilayer and probably interacts with negatively charged lipid head groups. Based on biophysical and biochemical studies performed with MS-DesK, it was proposed that protein–lipid interactions primarily involving amino acid side chains of the linker region appear to be important to modulate the kinase/phosphatase activity ratio of the sensor. Further experiments are necessary to establish whether this hypothesis is congruent with the model predicting a reversible formation of the membrane-connecting 2-HCC to regulate the signaling state of DesK [52].

3.2. Regulation of DesK by lipids

Although the minimalist approach performed with MS-DesK shed light on the role of the N-terminal TM domain of DesK on signal transduction, the sensor domain of DesK is nonetheless composed of five TM-spanning segments. Thus, the mechanism by which full-length DesK discriminates the surrounding lipid environment to adjust its signaling state may be much more complex. To address the role of lipids in the activity of full-length DesK, cell membrane complexity was bypassed by reconstituting DesK into proteoliposomes with defined lipid composition and this simplified system was employed to study the role of lipid environment on autokinase activity [53]. Reconstitution of DesK into bilayers of di(16:1)PC, di(18:1)PC and di(20:1)PC gave almost equivalent autokinase activities. Nevertheless, when DesK was reconstituted in bilayers of di(14:1)PC, its activity decreased significantly compared with DesK reconstituted into PCs containing longer acyl chains [53]. Thus, as reported for MS-DesK [44], the thickness of the bilayer is an important parameter regulating the activity of full-length DesK. This conclusion was supported by in vivo studies in which the length of *B. subtilis* membrane fatty acids was manipulated by the inhibition of fatty acid or phospholipid synthesis [54]. These studies showed that inhibition of fatty acid synthesis by the addition of cerulenin, a potent and specific inhibitor of the type II fatty acid synthase, results in increased levels of short-chain fatty acids in membrane phospholipids, which in turn leads to inhibition of the transmembrane-input thermal control of DesK. Furthermore, reduction of phospholipid synthesis by conditional inactivation of the PlsC acyltransferase (the enzyme that acylates acyl-glycerol phosphate) causes a significantly elevated incorporation of long-chain fatty acids into membrane lipids, leading to constitutive upregulation of the desaturase gene. Together, these studies are consistent with the hypothesis that native DesK regulation, at least in part, is linked to changes in membrane thickness that could trigger buoy-dependent conformational changes in its multispanning sensor domain. However, recent results suggest that in addition to membrane thickness, phase separation into coexisting lipid domains could exert a profound regulatory effect on kinase domain activation at low temperatures [53]. The role of lipid environment on DesK activity was examined by reconstitution of the sensor in bilayers containing lipids that display different phase states at the assay temperature. To this end, DesK was reconstituted into selected PCs that undergo phase transitions between the liquid-disordered and solid-ordered states at −18.3 °C (di(18:1)PC), 24 °C (di(14:0)PC) or 42 °C (di(16:0)PC) [55–58]. The highest autokinase activity was observed in di(14:0)PC, whereas reconstitution of DesK into di(18:1)PC and di(16:0)PC leads to a gradual decrease in this activity (Fig. 5A). Further experiments, which analyzed the autokinase activity of DesK as a function of temperature in lipid bilayers composed of either di(14:0)PC or di(18:1)PC, showed that DesK activity levels in these proteoliposomes were almost the same as those observed at 20 °C. However, at 25 °C the autokinase activity of

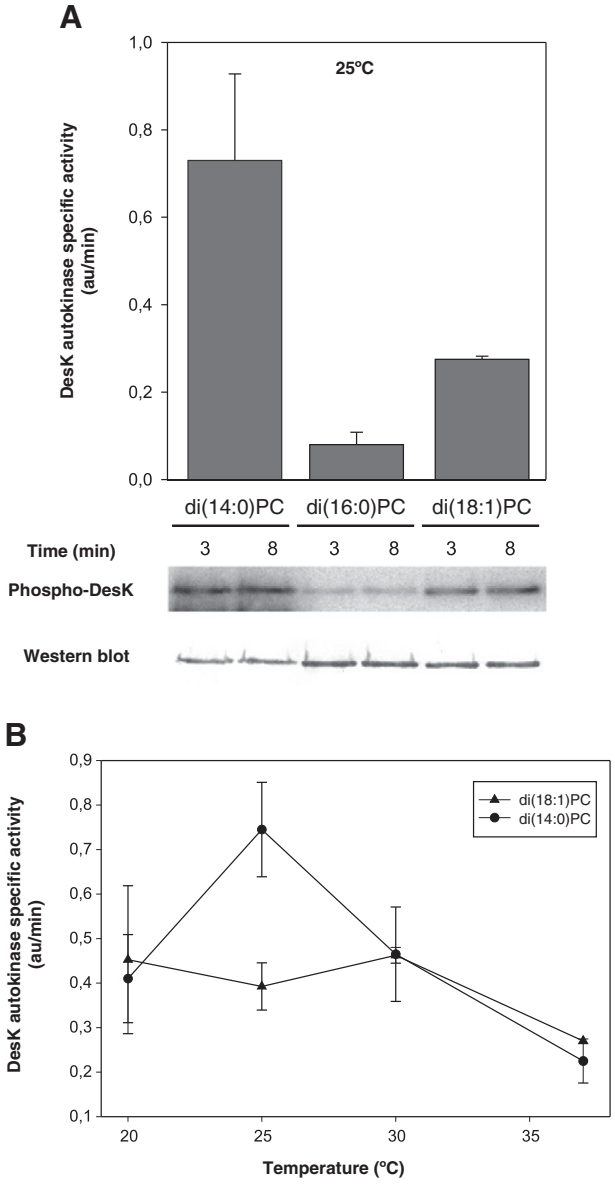

Fig. 5. Effect of phospholipid phase state on DesK autophosphorylation activity. (A) DesK was reconstituted in bilayers of di(18:1)PC (Tm: −18.3 °C), di(16:0)PC (Tm: 42 °C) and di(14:0)PC (Tm: 24 °C) and the autophosphorylation of proteoliposomes was assayed at 25 °C. Quantification of the level of phosphoprotein (Phospho-DesK autoradiography) as well as of protein (Western blot using anti-His antibodies) was performed by densitometry and plotted as AU against time in order to calculate specific activities. Bars show the DesK autokinase-specific activities obtained and error bars represent the SD for the values obtained. (B) Effect of temperature on DesK activity in di(18:1)PC or di(14:0)PC lipids. DesK was reconstituted in bilayers of di(18:1)PC or di(14:0)PC and autophosphorylation of the proteoliposomes assayed at different temperatures. Specific DesK activities were calculated as described in (A). The values are presented as means ± SD for least two independent experiments. The figure was taken from reference [63].

DesK in di(14:0)PC increased 2-fold, whereas in di(18:1)PC, this activity remained similar to the one assayed at 20 °C (Fig. 5B). Since at this assay temperature (25 °C) liposomes of di(14:0)PC are close to a temperature where the fluid phase and the gel phase coexist [59,60], it is tempting to speculate that DesK is activated by phase separated lipids. How this finding is connected to DesK's behavior when *B. subtilis* living cells are exposed to low temperatures is not clear. One possibility is that DesK could preferentially associate in a dynamic fashion with nanoscale membrane domains induced during *B. subtilis* cold adaptation. Evidence of lipid-dependent functional domains has been reported for *B. subtilis* [61] and several other bacteria [62]. Thus, we could speculate that the compartmentalization of DesK in phase-separated areas might facilitate its activation. For example, cooling could favor the oligomerization of DesK into microdomains stimulating its autokinase activity. Recent work has demonstrated that cool temperatures enhance the oligomerization and enzymatic activity of receptor guanylyl cyclase-G, a thermosensory protein expressed in neurons of the Grueneberg ganglion [63]. Applying live cell microscopy to study the organization of DesK in the membrane would be of particular interest, as this approach could give a hint about whether DesK is in fact recruited into microdomains during cold adaptation.

4. Regulation of membrane fluidity during cold adaptation in a multicellular organism

How multicellular organisms maintain an optimal level of phospholipid desaturation remains to be established, but recent evidence indicates that the underlying mechanisms of these phenomena are interconnected with the metabolism of PC [64,65]. The integral membrane protein PAQR-2 is a *Caenorhabditis elegans* homolog of the mammalian adiponectin receptors. Recent work has demonstrated that PAQR-2 is essential for the ability of *C. elegans* to grow at its lower temperature range, i.e., 15 °C, and that, similarly to DesK, the likely role of PAQR-2 during cold adaptation is to regulate membrane fluidity by promoting fatty acid desaturation [64]. Worms containing the *paqr-2* mutation are unable to grow at 15 °C. The requirement of PAQR-2 to grow at low temperatures can be bypassed by several suppressor mutations [64]. Whole genome sequencing followed by experimental confirmation of the role of the suppressor mutations allowed identifying the metabolic pathways regulated by PAQR-2. These experiments showed that PAQR-2 decreases PC biosynthesis. A reduction in the levels of PC enhances the transcription of Δ9 desaturases, eventually activating the biosynthesis of unsaturated fatty acids and increasing their relative abundance in membrane phospholipids (Fig. 6). These findings suggest that decreasing PC biosynthesis may be essential for cold adaptation by allowing adaptive remodeling of the structural lipids via Δ9-desaturases [65]. However, the direct point of interaction between PAQR-2 and its target pathway is not immediately apparent. One interesting hypothesis is that PAQR-2 acts on fatty acid metabolism by regulating PC abundance via an associated phospholipase activity. It will be of great interest to determine whether PAQR2 has phospholipase activity and if the role of PAQR-2 is conserved in homeothermic organisms such as mammals.

5. Outlook

Although the propagation of a signal through TM segments represents one of the initial stages in many complex signaling life processes, very little is known about the molecular nature of this important step. We can only begin to understand how membrane proteins perform their function if they are studied within the context of the lipids that surround them and often regulate their activities. Therefore, our knowledge of temperature sensors with the ability to measure membrane viscosity in model organisms may ultimately contribute to clarify how proteins and lipids interact with each other. Gaining insight into how the TM segments of DesK sense the lipid environment and transmit this information to the membrane-connecting 2-HCC will be a major issue for future research. Surprisingly, the recently identified PAQR-2 lipid composition sensor in the multicellular organism *C. elegans* has turned out to be similar to bacterial DesK thermometer, in the sense that both proteins are necessary to adjust the biosynthesis of unsaturated fatty acids at low temperatures. The mechanism by which PAQR-2 controls membrane lipid homeostasis in the nematode remains enigmatic and warrants further investigation.

Conflict of interest

There is no conflict of interest.

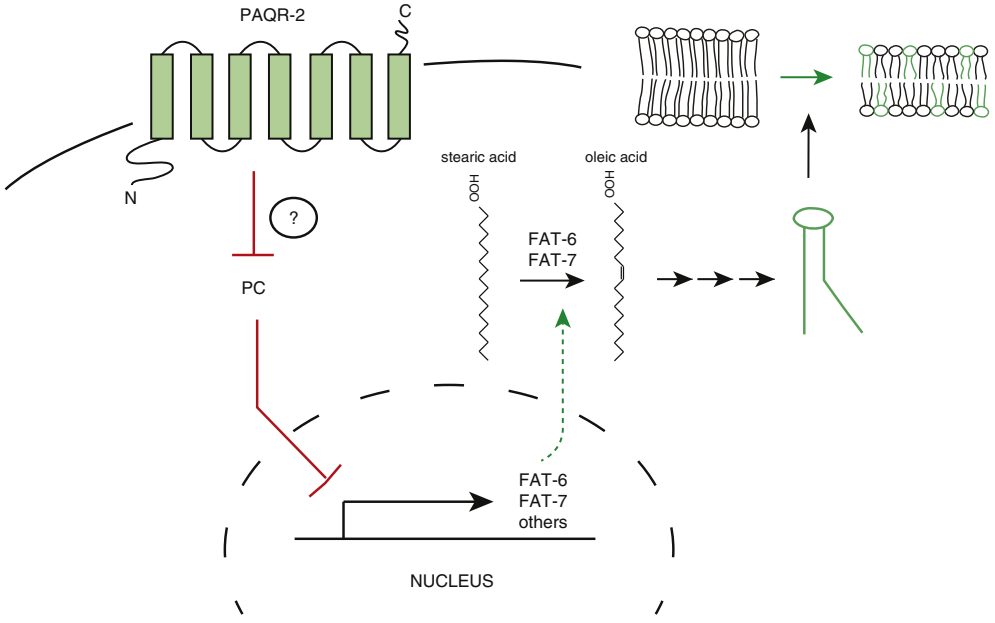

Fig. 6. Hypothetic model for the mode of action of PAQR-2. At 15 °C PAQR-2 decreases the levels of PC by an unknown mechanism. Lowering PC biosynthesis enhances the transcription of *fat-6* and *fat-7* genes encoding Δ9 desaturases, resulting in higher synthesis of unsaturated fatty acids and their incorporation into membrane phospholipids, ultimately modifying membrane fluidity.

Acknowledgments

We thank Celina Galles for critically proofreading the manuscript and Diego Sastre for help in generating illustrations. E.S. is a fellow from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and D. de M. is a Career Investigator of the same institution. This research was partially supported by grant PICT 2678 from Agencia Nacional de Promoción Científica y Tecnológica (FONCYT).

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