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Review

Sensing membrane thickness: Lessons learned from cold stress☆

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ABSTRACT

The lipid bilayer component of biological membranes is important for the distribution, organization, and function of bilayer spanning proteins. These physical barriers are subjected to bilayer perturbations. As a consequence, nature has evolved proteins that are able to sense changes in the bilayer properties and transform these lipid-mediated stimuli into intracellular signals. A structural feature that most signal-transducing membrane-embedded proteins have in common is one or more α-helices that traverse the lipid bilayer. Because of the interaction with the surrounding lipids, the organization of these transmembrane helices will be sensitive to membrane properties, like hydrophobic thickness. The helices may adapt to the lipids in different ways, which in turn can influence the structure and function of the intact membrane proteins. We review recent insights into the molecular basis of thermosensing *via* changes in membrane thickness and consider examples in which the hydrophobic matching can be demonstrated using reconstituted membrane systems.
This article is part of a Special Issue titled: The cellular lipid landscape, edited by Tim Levine and Anant K. Menon.

1. Introduction

The function of the cell membrane as a barrier and a matrix for biological activities relies on the properties imparted by lipids. Most biological membranes contain a complex mixture of lipid molecules that differ in their polar regions and fatty acid chains with diverse structures of lipid molecules among different organisms. Furthermore, the lipid composition of a membrane is not held constant, and, for instance, in animals varies with diet and in bacteria with growth conditions such as temperature.

Notably, although the structure and function of proteins are conserved in different organisms, tissues and intracellular organelles, the specific chemical structures of the lipid molecules in biological membranes have not been conserved. What is indeed maintained is an amphipathic structure capable of forming bilayers in water, with some conserved features. One such feature, true for most if not all membranes, is that the bilayer is fluid, a state achieved if the lipid molecules predominantly contain unsaturated or branched fatty acyl chains. A second conserved feature is the hydrophobic thickness of the bilayer (the thickness of the fatty acyl chain region), which, although not identical for all membranes, in physiological conditions does not vary much from ~25 to 30 Å (1 Å = 0.1 nm).

Much of the communication and exchange of material between the inside and outside of the cell is mediated by transmembrane proteins that enable a variety of biological phenomena, from small molecule and ion transport to cell signaling. Membrane-spanning helices found in these membrane proteins can be divided in two regions: a transmembrane (TM) segment mainly composed of hydrophobic amino acids, which are energetically compatible with the hydrophobic environment of the membrane bilayer core, and the residues flanking the membrane-spanning segments that are located at the membrane–water interfacial region. A still growing amount of evidence support the notion that the interplay among membrane lipids and transmembrane segments is crucial for the regulation of the activity of a diverse group of proteins. Given the diversity of lipid composition in biological membranes, it is not surprising that lipid regulation of membrane protein function has been formulated with different descriptors: bilayer fluidity [1], bilayer compression or bilayer protein hydrophobic mismatch [2], intrinsic lipid curvature [3] or curvature frustration [4], bilayer deformation energy [5], acyl chain packing [6], bilayer-free volume [7], lateral pressure profile [8], lipid packing stress [9], or bilayer stiffness [10].

Of particular interest are the lipid-induced changes in physical membrane properties such as fluidity and thickness where lipids can either expand or compress around the hydrophobic surface of proteins resulting in a hydrophobic mismatch. The energetic cost of exposing hydrophobic groups to water is high, most probably implying that in bilayers with a non-optimal thickness the activity of these proteins could be altered by small changes in the structure or orientation of their transmembrane segments seeking to match the new hydrophobic thickness of the lipidic bilayer [4,11]. There are many possible ways of relieving the energetic constraints imposed by a mismatch between

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the hydrophobic length of membrane proteins and the bilayer thickness (reviewed in [11–16]). In brief, when the protein is too large to match the hydrophobic bilayer thickness, the protein might oligomerize or aggregate in the membrane to minimize the exposed hydrophobic area. Alternatively, TM helices could tilt to reduce their effective hydrophobic length, or they could adopt another conformation. Another possibility is that the helices adjust their hydrophobic length by changing the orientation of hydrophilic side chains near the interface. When the hydrophobic part of a TM protein is too small to match the hydrophobic bilayer thickness, again this might result in protein aggregation, or changes in backbone conformation and/or side chain orientation.

Many experimental and theoretical studies have been performed to gain insight into the consequences of hydrophobic mismatch, using different systems varying from relative simple model membranes to complex biomembranes (reviewed in [11–13,15,16]). From these studies, it seems clear that many membrane proteins have an optimal bilayer thickness for functioning and that the hydrophobic mismatch can strongly affect protein and lipid organization, but that the precise consequences depend on the individual properties of the proteins and lipids.

The aim of this article is to provide a brief overview of how the TM thickness of the membrane bilayer can influence the functioning of some well-characterized membrane proteins. The scope of the article is largely founded on the present knowledge and ideas about the basic biochemical, structural, and biophysical principles governing the lipid-mediated driving force for signal sensing and transduction of the thermosensory protein DesK from *Bacillus subtilis*. For more detailed information on structure and mechanics of the lipid bilayer, as well as lipid interaction with other membrane proteins, the reader is directed to several excellent reviews describing wide ranging lipid–protein effects [4,11,16–20].

2. The importance of lipid–protein interactions in mechanosensitive channels function

Mechanosensitive (MS) channels fulfill a major role in the responses of living organisms to mechanical stimuli by catalyzing the transfer of ions and other solutes across the membrane. These channels function as mechano-electric switches in such diverse physiological processes such as touch, hearing, proprioception, turgor control in plants, and osmoregulation in bacteria [21]. Interestingly, the trigger for this response appears to be a change in the physical properties of the bilayer [21–23]. Thus, MS channels are able to transduce deformations of the bilayer into a sequence of structural rearrangements that lead to changes in the flux through the channel. This mechanism of channel gating mediated by membrane lipids is today referred to as the "force-from lipids paradigm" or FFL principle [24]. The best studied MS channels are the *Escherichia coli* mechanosensitive channel of large conductance (MscL) and mechanosensitive channel of small conductance (MscS), which open in response to increased turgor pressure and thereby save the cell from rupture during osmotic shock [21,25,26].

The currently accepted oligomeric structure of MscL is that of a homopentamer [21,27]. MscL monomer consists of two TM helices TM1 and TM2, with N-terminal and C-terminal domains located at the cytoplasmic side of the bacterial cell membrane and a periplasmic loop [27]. The fact that MscL can be activated by pressure gradients after reconstitution in defined lipid systems indicates that gating is indeed triggered by tension transmitted through the bilayer [28]. The question of whether MS channels are activated by hydrophobic mismatch or lateral pressure changes was addressed by using site-directed spin labeling (SDSL), patch clamp, and a variety of lipid compositions to specifically alter lipid–protein interaction in reconstituted MscL [28]. Focusing around the hydrophobic gate in TM1, hydrophobic mismatch was evaluated by reconstituting spin-labeled channels into vesicles formed by posphatidylcholines (PC) containing monounsaturated acyl chains between 10 and 22 carbons long, whereas the lateral pressure profile was modified by the incorporation of the cone-shaped lipid lysophosphatidylcholine (LPC) into the external leaflet of the membrane [28]. Compared to the control conditions with dioleoylphosphatidylcholine [di(C18:1)PC], where the channel is in the closed conformation, incorporation of MscL into thinner bilayers lowered the threshold of activation in patch clamp experiments [28]. These conditions produced relatively small structural changes as reported by electron paramagnetic resonance (EPR) that might correspond to a closed intermediate. Thus, hydrophobic mismatch alone, although clearly affecting opening energetics, does not seem to provide the primary triggering element in MscL gating [28]. By contrast, LPC incorporation into channel containing liposomes was accompanied by a large increment in probe mobility and a simultaneous decrease in inter-subunit proximity. This would be expected if the channel adopts its fully open conformation. In patch clamp experiments, LPC incorporation was accompanied by spontaneous single channel activity in the absence of an external applied transbilayer pressure [28]. These experiments provide good evidence pointing toward distortions in the lateral pressure profile as the main physical parameter transduced by MscL channels during gating. In summary, these experiments indicate that (i) hydrophobic mismatch is one of the main determinants of the MS channel conformation in the lipid bilayer, favoring opening of MscL in thinner compared to thicker bilayers and (ii) bilayer curvature and/or changes in transbilayer pressure profile are the second major global factors regulating MscL channel structural conformation between the closed and open states [29].

Unlike MscL, MscS channel activity is not affected significantly by hydrophobic mismatch [30]. Because of their molecular structure [21], lipid–protein interaction appears to be different for the two channels. In the case of MscL pentamer, the reduction of the thickness of the TM portion of the channel following the reduction of the lipid bilayer thickness would be attained by a significant tilt of the TM1 and TM2 helices resulting in the opening of the very large pore of 30 Å in diameter [21,28]. In the case of the MscS heptamer, a lesser tilt of the TM1 and TM2 helices is required to accommodate the smaller MscS open channel of 14–16 Å [31]. This suggests that an overall smaller conformational change is required for the closed-open transition in MscS compared with MscL, resulting in MscS being much less sensitive to bilayer thinning [21,30]. Thus, the molecular mechanism underlying the mechanosensitivity of MscL and MscS differs for the two channels as discussed in recent contributions [21,23,32].

Remarkably, recent biochemical and structural studies have convincingly shown that the "force of lipids" model is also applicable to mammalian activated ion channels [21,23]. For example, the mechanosensitivity of the K_{2P}-type channels TREK-1 and TRAAK was shown to be mediated directly through the lipid bilayer in the absence of other cellular components [23,33,34]. However, the molecular mechanism of K_{2P} channel gating and the influence of the lipid bilayer remain unclear. Thus, understanding the molecular mechanism of mechanotransduction not only requires a quantitative narrative of the physical forces, but also a thorough investigation of lipid–protein interaction.

3. The importance of hydrophobic mismatch in the activity of the Ca^{2+} ATPase

The importance of the various properties of the lipid bilayer has been studied for a relatively small number of membrane proteins that can be purified and reconstituted into bilayers of defined lipid composition. One such protein is the P-type Ca^{2+} ATPase from skeletal muscle sarcoplasmic reticulum (SR), which is an integral membrane protein of relative molecular mass 110,000 that pumps Ca^{2+} against a concentration gradient [35]. This protein was reconstituted into PCs in the liquid crystalline phase [36–38]. Highest activity is seen with a fatty acyl chain length of about C18, with lower activities in bilayers of lipids with shorter or longer fatty acids. The mechanism by which membrane

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thickness affects the activity of the Ca^{2+} ATPase is incompletely understood but it seems clear that the reasons for activities being low in short-chain lipids are different from the reasons for them being low in long-chain lipids. For example, the low rate of ATP hydrolysis in dimyristoleoylphosphatidylcholine [di(C14:1)PC] follows in a large part from a low rate of the phosphorylation of the Ca^{2+} ATPase by ATP [39]. In contrast, in dinervonylphosphatidylcholine [di(C24:1)PC], the rate of phosphorylation is the same as in di(C18:1)PC (a control membrane), and the low rate of ATP hydrolysis by the Ca^{2+} ATPase in di(C24:1)PC follows from a low rate of the dephosphorylation of the phosphorylated intermediate [39,40]. The possible explanation for the observed effects of lipid chain length on Ca^{2+} ATPase function appears to be that hydrophobic mismatch results in novel conformations resulting in changes in the stoichiometry of the binding of Ca^{2+} to the enzyme [36,41,for a review,see 35]. Interestingly, the Ca^{2+} binding sites on the Ca^{2+} ATPase are located between the TM α-helices of the protein [42], and it is possible that changes in the packaging of the helices, caused by a change in bilayer thickness, lead to changes in the Ca^{2+} binding sites.

The results with the Ca^{2+} ATPase show that the effects of lipid structure on a membrane protein are likely to be complex and unique for each case. No simple model will describe adequately all the possible effects. Rather, what is required is a detailed structural model for each membrane protein, defining in molecular detail, how particular lipids interact with it and what effects these interactions have on the structure and function of this particular membrane protein.

4. The importance of membrane thickness on DesK thermosensing

4.1. Temperature regulation of membrane thickness

How cells respond to changes in temperature is a fundamental issue in biology [43–45]. Changes in ambient temperature affect nearly all cellular and biochemical processes and drive adaptive responses to maintain cellular homeostasis. For example, up- or downshifts in temperature decrease or increase the packing (or the fluidity) of the acyl chain of membrane lipids, respectively [46,47]. The fluidity of the lipid bilayer is essential for the normal function of the cellular membrane, which relies heavily upon dynamic interactions between its lipid and protein components [48]. Membranes become less fluid when either the temperature or the unsaturated lipid content decrease. To maintain membrane fluidity within an optimal range for normal biological activity, lipid desaturases in the cell convert saturated fatty acids into unsaturated fatty acids to increase lipid desaturation and thus membrane fluidity in response to temperature downshifts [17,43,49–51]. Unsaturated double bonds in lipids generate kinks into the otherwise straightened acyl hydrocarbon chain and thereby increase membrane fluidity. This fundamental process of maintaining membrane fluidity, first identified in bacteria, is called homeoviscous adaptation (HVA) [52]. Cold-induced HVA is a phenomenon common to most (if not all) poikilothermic organisms [52–55], although its regulation mechanism in eukaryotes remains largely unknown [56]. Several biophysical evidences support the idea that upon lowering the environmental temperature lipid bilayers will become more ordered and as a consequence they will become thicker [57–60], directly linking variations in membrane fluidity to changes in membrane thickness.

4.2. A sensor of membrane thickness in bacteria

In addition to altering protein conformation directly, temperature changes can affect protein activity as a secondary consequence of structural alterations in intimately associated molecules. In the case of the bacterial cold sensor DesK, for example, temperature-dependent changes in the lipid membrane appear to be the primary mediators of the protein's thermal responsiveness (for a recent review, see [43]). 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. 1). *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 [61,62]. The phosphoryl group is then transferred to the receiver aspartic acid in the DNA-binding response regulator DesR [62,63]. The phosphorylation of DesR triggers the reorganization of its quaternary structure [63,64], a key event necessary to activate the transcription of the *des* gene that encodes the acyl lipid desaturase Δ5-Des [65]. 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 [61]. Consequently, the concentration of phospho-DesR declines and transcription of *des* is terminated [61–63].

The activation of DesK upon cooling appears to be intimately related to changes in membrane structure. Among other effects, cooling causes an increment in membrane thickness by decreasing disorder (see Section 4.1). Indeed, several lines of evidence support the idea that DesK senses the thickening of the membrane as the temperature drops: (i) the protein exhibits increasing kinase activity when reconstituted into vesicles of longer acyl chains [66], and (ii) DesK kinase activity is stimulated in bacteria with a higher proportion of long-chain fatty acids in their membranes, whereas an increment in the amount of short-chain fatty acids stimulates its phosphatase activity [67].

In summary, while the precise structural changes within DesK's TM region remain fragmentary known (see Sections 4.3 and 4.4), the model depicted in Fig.1 not only provides a compelling scenario for how DesK senses temperature but also links DesK activation to its homeostatic function in maintaining membrane fluidity. In this scenario, as membrane fluidity decreases, the membrane thickens, activating DesK and inducing Δ5-Des. Δ5-Des activity then increases disorder, helping restore appropriate fluidity and thickness. This in turn turns off DesK kinase activity and turns on DesK phosphatase activity terminating the response. Such a regulatory loop is well suited to maintaining levels of membrane fluidity within an optimal range.

4.3. Toward a signal sensing and transduction mechanism for DesK

4.3.1. The minimal sensor

A systematic deletion analysis of the TM segments of DesK suggested that TM segment 1 (TM1) harbors a temperature-sensing motif, as the sole deletion of TM1 produced a variant with constitutive autokinase activity unresponsive to temperature shifts [68]. Further deletions of TM2 to TM4 produced DesK variants also insensitive to temperature variations but with diminished or minimum autokinase activity [68], and deletion of the entire TM domain generated the soluble variant DesKC, with exacerbated and constitutive autokinase activity *in vivo* and *in vitro* [62,69]. These results suggested that TM1 senses the temperature signal and transmits this information to TM5, which is directly connected to the catalytic domain to control its output activity. On this basis, a chimeric protein with a single spanning membrane segment, consisting of the first 17 N-terminal amino acids of DesK fused to the 14 C-terminal amino acids of TM5, was created [68]. This chimeric protein, dubbed minimal sensor (MS-DesK), worked almost as well as full-length DesK activating or repressing expression of *des* gene upon temperature shifts.

4.3.1.1. The sunken buoy motif. Genetic and biochemical experiments obtained with MS-DesK suggested for the first time that DesK appears to sense the thickening of the membrane as the temperature drops [68]. This is thought to force a cluster of charged residues [named the "sunken buoy" motif (SB)] into a more apolar environment resulting in a conformational change that triggers the autokinase activity of DesK [68]. The SB motif, the polar patch formed by Lys10 and Asn12 (Fig. 2A), is located in the extracellular water–lipid interface acting as

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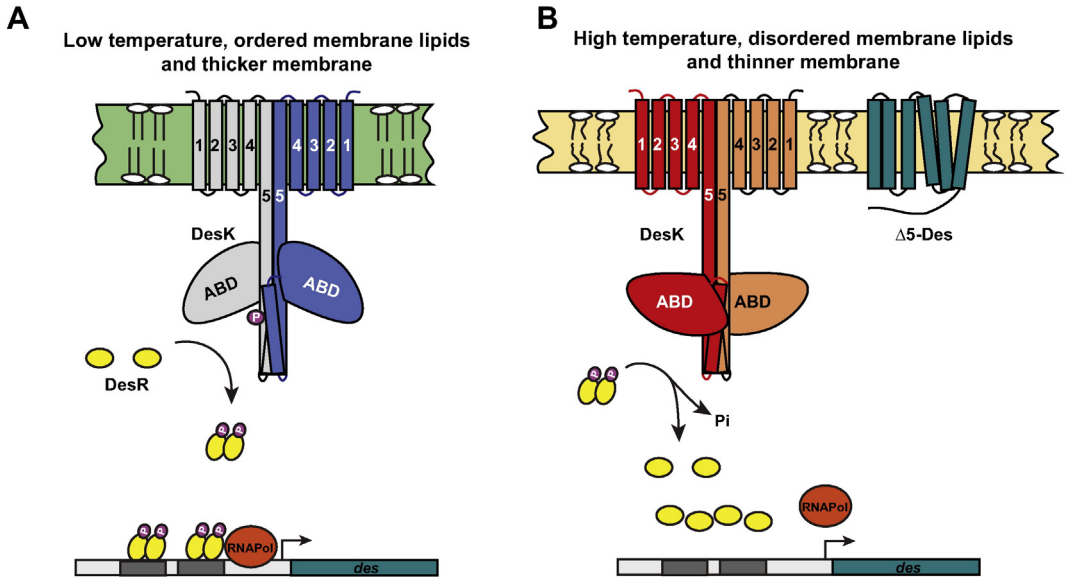

Fig. 1. Membrane fluidity optimization by the Des pathway of *Bacillus subtilis*. (A) An increment in the proportion of ordered membrane lipids promotes a kinase-dominant state of DesK, which autophosphorylates and transfers the phosphate group to DesR. Two DesR ~ P dimmers interact with the *des* promoter 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. The decrease in the order of membrane lipids favors the phosphatase-dominant state of DesK, leading to DesR ~ P dephosphorylation and turning off the transcription of the *des* gene.

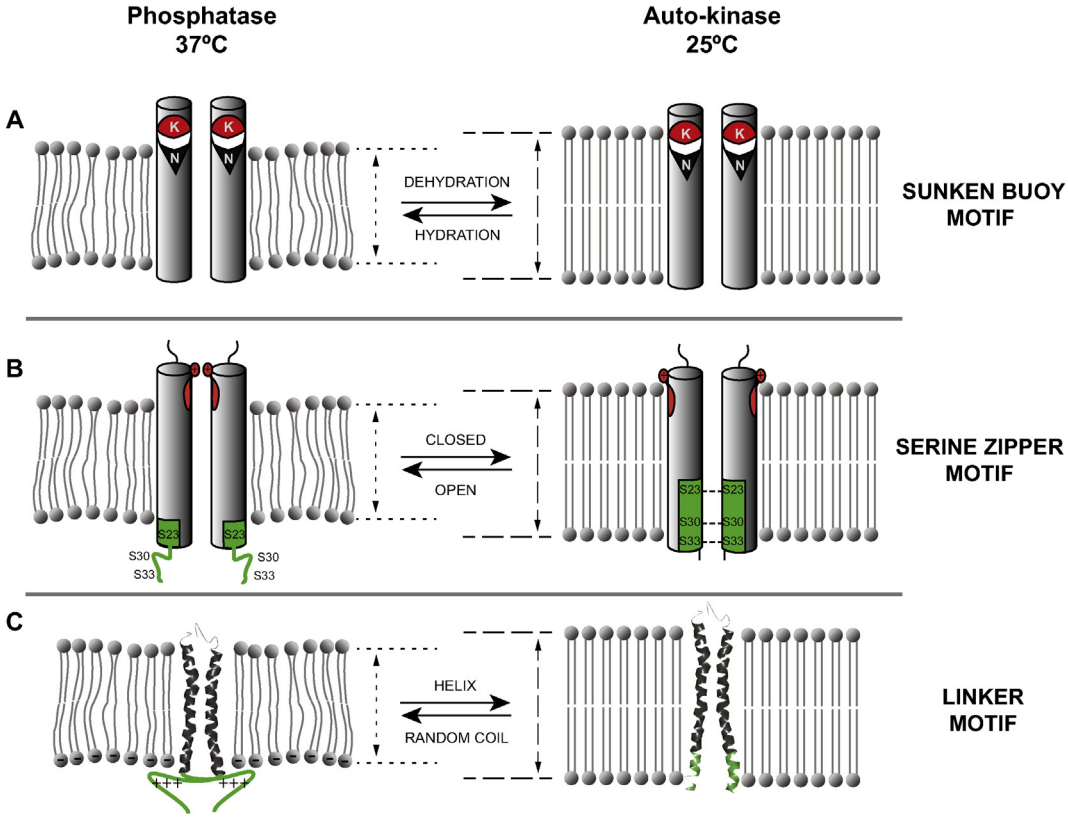

Fig. 2. Proposed temperature sensing mechanisms for MS-DesK. (A) The "sunken buoy" model. At high temperatures, corresponding to the phosphatase-competent state, the polar patch of residues (Gln9, Lys10, and Asn12) locates at the water–lipid interface in a hydrated/stabilized state (left). When the temperature drops this polar patch gets buried into the membrane suffering dehydration/destabilization, constituting the initial event for the conformational change to the autokinase-competent state (right). (B) The serine zipper model. Under standard conditions in a fluid membrane, three serine residues (Ser23, Ser30, and Ser33) located at the C-terminus of the TM segment interact with the hydrophilic interface (Left). With decreasing temperature, the membrane thickens, and an extra helix turn folds inside the membrane, resulting in formation of a serine zipper motif (Right). This motif can form intrahelical hydrogen bonds that shield the hydroxyl groups from the hydrophobic core of the membrane. The reorientation, necessary for this interaction, will lead to a change in the C-terminal catalytic domain (DesKC) from the phosphatase to the kinase state. (C) The linker model. The connecting segment between the TM domain and the catalytic domain is able to adopt a dual conformation: random coil and helix. The increment of the bilayer thickness prevents the interaction of the linker region (green) with the lipid bilayer and contributes to stabilize and favor the helical continuity between the transmembrane segment and the intracellular domain. Reversible interaction of the linker with the lipid bilayer surface would allow DesK to alternate between the kinase and phosphatase states. Figure adapted from references [70,71].

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a sensor of membrane thickness fluctuations in response to temperature shifts. The sensing capacity of this motif can potentially be related to its hydration or dehydration degree, which is dictated by membrane thickness: a thinner membrane will allow exposure of the SB motif to the aqueous environment favoring its hydration while a thicker membrane will bury it into the lipid acyl chains promoting its dehydration [68]. According to this model, high temperatures (thinner membrane scenario) promote hydration/stabilization of the SB motif favoring the phosphatase conformation of MS-DesK, whereas lower temperatures (thicker membrane scenario) promote dehydration/destabilization of the SB motif, displacing the conformational equilibrium toward the autokinase conformation of MS-DesK [68].

4.3.1.2. The linker motif. Another structural element described as a possible regulator of the output activity of DesK is the linker region (K^{152}SRKERERLEEK^{163}) connecting the TM and catalytic domains, which is highly charged [70]. Based on biophysical and biochemical studies with synthetic peptides, it was proposed that this linker region is involved in electrostatic protein–lipid interactions that can modulate the kinase/phosphatase activity ratio of the sensor [70]. Furthermore, when mutant linker variants with net negative (A3: AEAEALEEK) or positive (Q3: KQRQRLQEK) charge were introduced into MS-DesK or full-length DesK *in vivo*, both proteins acquired high (A3) and low (Q3) kinase activity, respectively [70]. On the basis of these results and taking into account the differential helicity propensity of the WT, Q3, and A3 synthetic peptides, it was suggested that DesK's linker has the ability to adopt a dual conformation: helix and random coil. Based on these data, a provocative model was proposed (Fig. 2C): in thinner membranes, i.e., at higher temperatures, the interaction of the linker with the lipid bilayer interface causes helix disruption and the random coil conformation would be stabilized by electrostatic interactions, favoring the phosphatase-competent conformation. Conversely, in thicker membranes, i.e., at lower temperatures, the lipid bilayer tends to bury not only the SB motif of the N-terminus described above, but also the N-terminal part of the linker motif (proximal to the membrane), imposing a stress along the whole transmembrane region, forcing the linker to line up with the membrane normal and adopt a more helical structure to protect the linker backbone from the hydrophobic membrane core. This structural reorganization of the linker was proposed as the basis of transmission and amplification of the signal sensed by the TM domain to the catalytic domain of DesK [70].

4.3.1.3. The serine zipper motif. More recently, biophysical studies on a synthetic peptide corresponding to the TM segment of MS-DesK were combined with mutagenesis and functional assays. These studies led to the description of a third structural element that modulates DesK's activities in which three serine residues (Ser23, Ser30, and Ser33) have a relevant role. Here it was suggested that (i) the helicity of the MS-DesK's TM segment is affected by the bilayer thickness, varying from 55% in thinner membranes to 65% in thicker membranes (what corresponds to approximately one helix turn), (ii) MS-DesK's TM segment dimerizes and rotates in response to changes in membrane thickness, and (iii) Ser23, Ser30, and Ser33 are located at the TM segment dimerization surface in the conformation reached at low temperatures while these serine residues rotate toward the lipids at high temperatures [71]. On the other hand, a subsequent *in vivo* mutagenesis study showed that each of the single replacements Ser23Ala, Ser30Ala, and Ser33Ala reduce the kinase activity of MS-DesK, while addition of a fourth serine residue into the TM dimerization surface (Leu27Ser) increases its autokinase activity [71]. Moreover, it was shown that at low temperatures, the corresponding serine residues of two MS-DesK monomers locate close enough to form inter-helical S-S covalent bonds in *B. subtilis* membranes, as observed with single-point replacements Ser23Cys, Ser30Cys, or Ser33Cys [71]. Based on the *in vitro* and *in vivo* results, it was proposed that MS-DesK's TM segment acts as a dimer at both high and low temperatures, switching between two different conformations according to the physical state of the lipid bilayer (Fig. 2B). In response to a temperature decrease, the lipid acyl chains will become more ordered, leading to an increment in bilayer thickness and a decrease in fluidity and hydration. In this context, MS-DesK would react elongating its transmembrane helix in order to exclude the hydrophilic residues from the apolar environment, resulting in the location of the three serine residues (Ser23, Ser30, and Ser33) at the same face of the TM helix. This "serine zipper" motif, situated at the C-terminal region of the TM segment, would represent the lowest energy state of the dimer at low temperatures, becoming the dominant dimerization motif, and promoting a reorientation of the helices that result in a switch in the activity of DesK and its activation as a kinase [71].

Although the models described above elegantly uncovered essential regulatory regions of DesK, it is difficult to glimpse how the three motifs depicted in Fig.2 would act in concert to activate the autokinase activity of the sensor. In other words, an integrated model should explain how the SB motif buried into the anhydrous environment of the membrane promotes a rotation of the serine zipper motif to in turn induce the random coil to helix transition of the linker region. In order to solve this puzzle, we resorted to the available structural information of the cytoplasmic domain of DesKC in different functional states [69]. On this basis, further *in vivo* and *in vitro* experiments were conducted leading to a possible integrated mechanism for DesK functioning, where a 2-helix coiled coil switch has a crucial role, that allows us to reinterpret the experimental results sustaining the SB motif, the serine zipper and the random coil/helix transition hypothesis (for details, see below and [72]).

4.3.2. Structural snapshots along DesK sensing

The elucidation of a set of X-ray crystal structures of the catalytic core of DesK (DesKC) in different functional sates has provided valuable structural insight into the regulation of the catalytic activities of the protein [69]. DesKC displays the characteristic homodimeric structure observed in other proteins of the family. Each DesKC monomer consists of an N-terminal antiparallel 2-helix hairpin (helices α1 and α2) that includes the phosphorylatable histidine (His188), connected by a short loop to a C-terminal ATP-binding domain (ABD) [69]. The helical hairpins of two monomers interact with each other to form a central four-helix-bundle (4-HB) domain, known as the dimerization and histidine phosphotransfer (DHp) domain. In each monomer, the N-terminal end of helix α1 extends beyond the 4-HB, connecting the catalytic core with the TM sensor domain through a helical linker [69]. The amino acid sequences of helices α1 and α2 follow a heptad repeat pattern (with amino acids occupying positions *a* through *g*) with hydrophobic residues at positions *a* and *d* [69], characteristic of coiled coil domains [73]. Comparison of all DesKC structures identified three distinct conformational states of the protein (phosphatase, kinase, and phosphotranferase). Notably, DesKC structure in the phosphatase-competent state (Fig. 3A) shows a more compact and rigid conformation than the corresponding autokinase-competent state (Fig. 3B). This is due to extensive intramolecular interactions involving both a tighter DHp–ABD interaction in each monomer and the formation of a parallel 2-helix coiled coil (2-HCC), which continues the 4-HB toward the TM domain that is absent in the autokinase-competent conformation. Secondary structure predictions suggest that the 2-HCC would also extend all the way up to and through the membrane (Fig. 3C) [72]. The 2-HCC has been observed in many HKs, typically preceding the 4-HB [74], and corresponds more generally to the signaling helix, or "S-helix". The "S-helix" is a conserved feature that connects sensor and effector domains across a wide range of signaling proteins as a general signal transduction element of long persistence length that can allow for the propagation of rotations and displacements over long distances [75,76]. The S-helix was proposed to organize into coiled coils that work as a stability switch that prevents constitutive activation of the catalytic domains [75].

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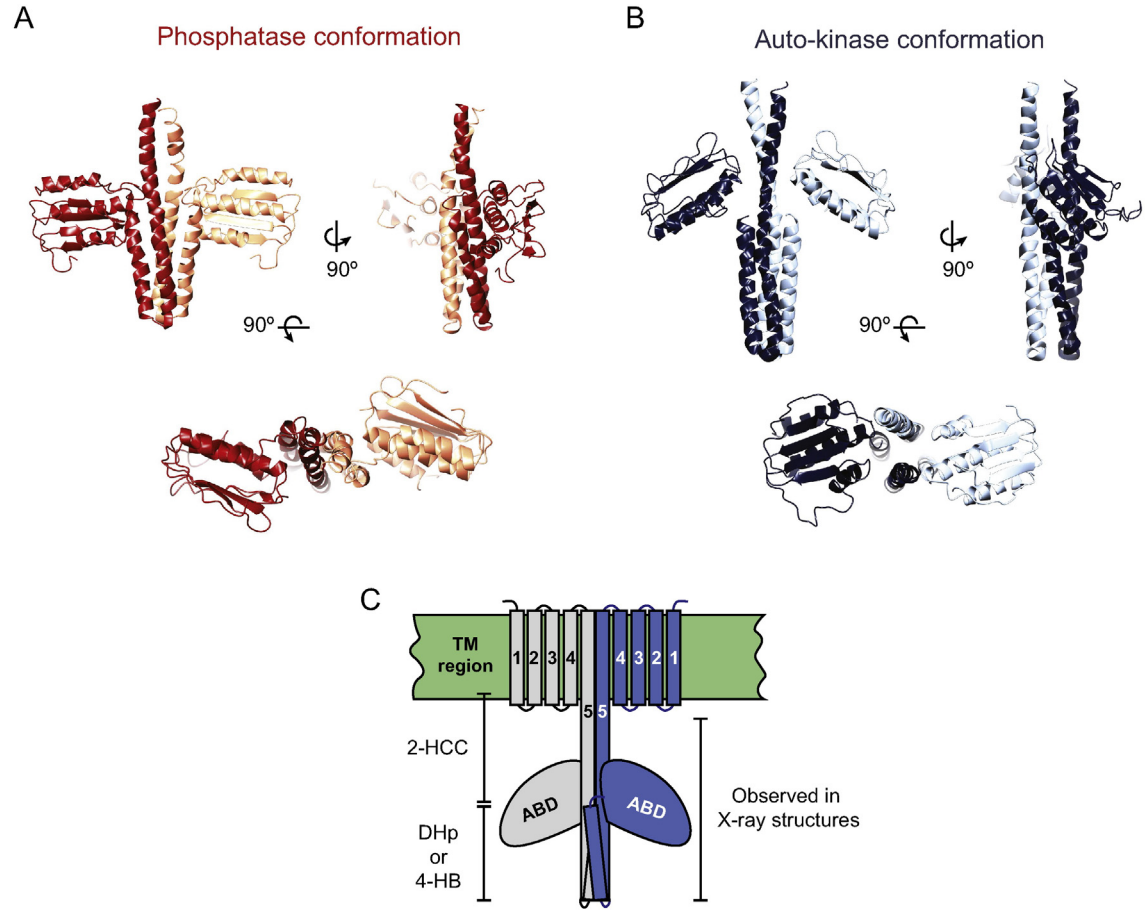

Fig. 3. DesK's topology. Crystal structures are available for DesKC constructs mimicking (A) the phosphatase state (PDB ID 3EHJ) and (B) the autokinase-competent state (3GIE). (C) DesK's topology as determined from known structures and sequence-based predictions of secondary structure and coiled coil formation.

4.3.3. A 2-helix coiled coil switch modulates DesK activities

Comparison of DesKC in the phosphatase and kinase conformations (Fig. 3A, B) suggested that the contacts seen between the DHp and ABD domains, as well as the parallel 2-HCC, support a labile association to be released for autophosphorylation and maintained for the phosphatase activity under control of the sensor domain [69]. Thus, the 2-helix coiled coil (2-HCC) was proposed to constitute an essential structural motif for DesK's phosphatase activity [69]. Its relevant role in the control of DesK's activity was tested *in vivo* using structure-based mutants designed to weaken or favor the formation of the 2-HCC [72]. The destabilization of the 2-HCC produced a DesK variant (named $DesK_{DEST}$) with constitutive and exacerbated autokinase activity at 25 °C or 37 °C, *in vivo*. By contrast, the stabilization of the 2-HCC resulted in a variant (named $DesK_{STA}$) with *in vivo* and *in vitro* minimum autokinase activity at either low or high temperatures, but with phosphatase activity levels at both temperatures comparable to WT DesK at 37 °C, meaning that $DesK_{STA}$ is in a kinase-off/phosphatase on state. These *in vivo* and *in vitro* data provided compelling evidence supporting a model in which a reversible formation of the membrane-connecting 2-HCC regulates the balance of phosphatase and kinase activities of full-length DesK [72]. Moreover, *in silico* atomistic models and molecular dynamics (MD) simulations of WT DesK, starting from its fifth TM segment (TM5-DesKC), as well as of the two 2-HCC variants (TM5-$DesKC_{STA}$ and TM5-$DesKC_{DEST}$), embedded in atomistic membranes, suggested that stabilization/destabilization of the 2-HCC is linked to the hydration state of the region where the protein inserts into the membrane [72].

Further mechanistic insights on DesK were obtained from *in silico* models of the minimalist but fully functional DesK surrogate, MS-DesK (see Section 4.3.1), in its phosphatase and autokinase-competent states (Fig. 4A). On the basis that DesK would be activated by membrane thickening, these models were built and relaxed through MD simulations in di(C18:1)PC and dierucoylphosphatidylcholine [di(C22:1)PC] membranes, the latter being ~4.5 Å thicker [77–79]. The phosphatase model relaxed in di(C18:1)PC membranes shows that Gly13 acts as a hinge that allows the small N-terminal helical segment to fully hydrate exposing the internal polar residues to the solvent, while the rest of the TM segment is organized into a 2-HCC. In contrast, in the thicker and less fluid membrane composed of di(C22:1)PC, no opening of the N-terminal region is observed and the 2-HCC remains paired also in the first half of the transmembrane region. On the contrary, the initial autokinase-competent model adopts stretched, nearly parallel TM helices, instead of a coiled coil arrangement, as observed in two nearly full structures of histidine kinases in their kinase-competent forms [80,81]. Upon relaxation in either kind of membrane, this model experiences asymmetric distortions and cracking of one helix close to the 4-HB, which reflects a stressed, dynamic conformation of the parallel coil in this activated state, as very recently proposed for other histidine kinases [80–84].

Comparison of helical wheel diagrams of the two relaxed models highlighted the rotation of about 90° of each helix in the autokinase conformation with respect to the phosphatase conformation (Fig. 4B). Such rotations provoke the exposure of several hydrophobic residues and the burial of large charged residues inside the coil in the autokinase state, which is precisely opposite to what is expected for proteins in solution, implying a stressed conformation. These data indicate that interconversion of DesK functional states implies the modulation of the 2-HCC stabilization/destabilization by rotation of its helices roughly 90° (Fig. 4C). The simulations further suggested that the highly charged segment just

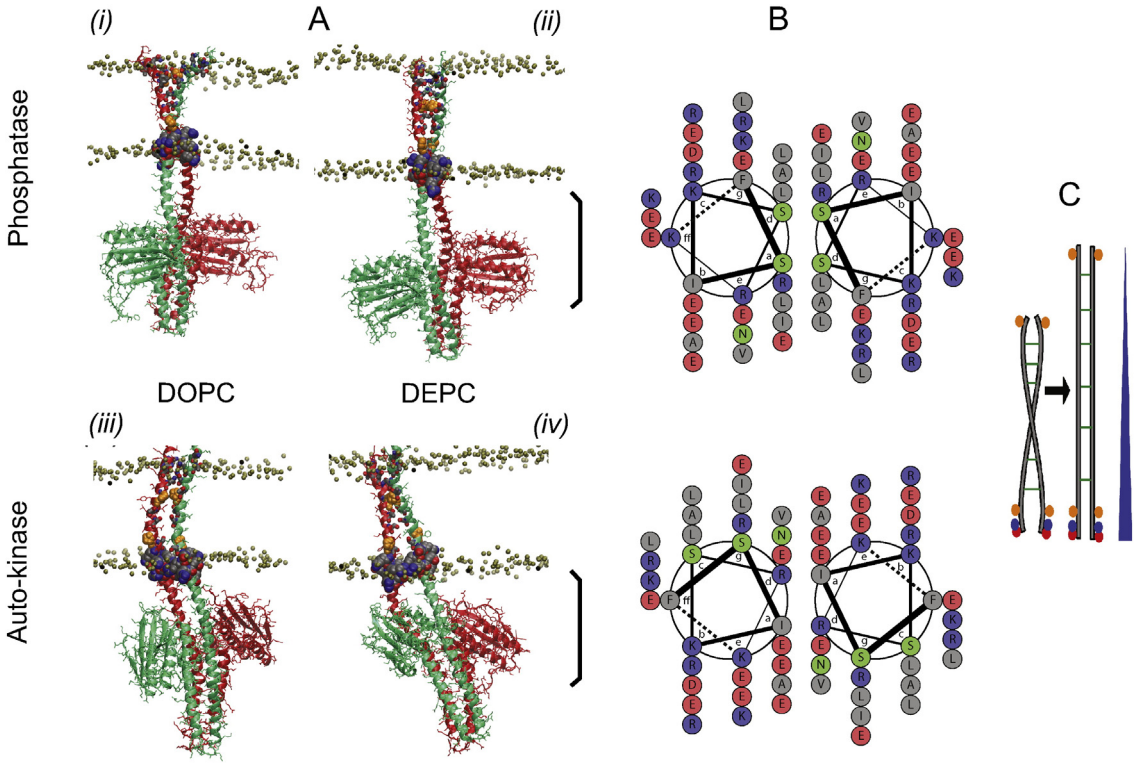

Fig. 4. Models of MS-DesK in the phosphatase and kinase states, relaxed in two membranes of different thickness. Models were built from the X-ray structures of DesKC in the phosphatase and autokinase-competent states (PDB ID 3EHJ and 3GIE, respectively) by extending their helices toward their N-termini following the sequence of MS-DesK. Each model was relaxed through 120 ns of MD simulation in a di(C18:1)PC or di(C22:1)PC membrane as indicated, in explicit solvent. Heavy atoms from the KERER segment are rendered as blue, red, and gray spheres (N, O, and C, respectively), while all heavy atoms of the two prolines of the TM region (Pro135 and 148) were rendered in orange. Panel (B) shows helical wheel diagrams [87] between Phe149 and the entry to the 4-HB, with polar uncharged residues in green, negative residues in red, and positive residues in blue. Panel (C) sketches the idea that unwinding of the 2-HCC results in rotation of the helices between the two Pro residues (in orange) as the membrane thickens, highlighting also that the KERER segment (red and blue spheres) does not shift along the membrane normal when it changes thickness. Figure adapted from reference [72].

C-terminal to the TM region (K^{152}SRKERERLEEK^{163}) is strongly anchored to the cytoplasmic polar side of the membrane. This anchor could be important to limit movements of the TM and 2-HCC helices when the membrane properties change, allowing them to rotate and possibly hinge, tilt, and stretch, but preventing them from sliding along the membrane normal. The functioning of the linker as an anchor does not require a helix/random coil transition, as previously suggested in the linker model by Inda and coworkers [70], and is consistent with all the evidence supporting the role of the S-helix in histidine kinases [72,75].

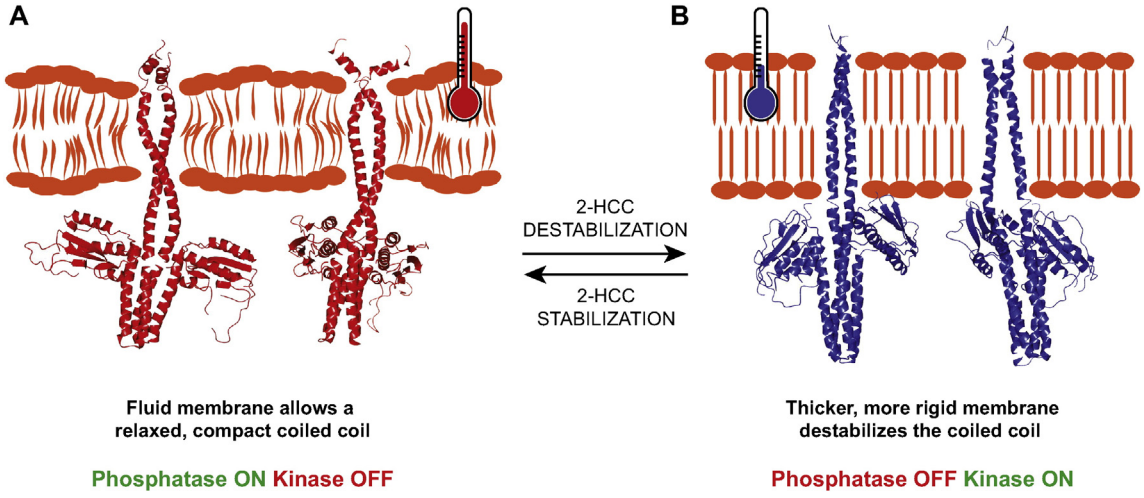

Fig. 5. The coiled coil switch mechanism of temperature sensing and transduction by DesK. High temperatures promote thin and fluid membranes allowing a relaxed structure of DesK, characterized by the formation of a 2-HCC through the entire TM segments and its cytoplasmic extensions toward the 4-HB, plus the tight interactions between the DHp and ABD domains, corresponding to the characteristic structure of the phosphatase-competent state. Upon a temperature drop the membrane thickens, stretching the TM segments, inducing the unwinding of the 2-HCC and a gradual rotation of its helices up to approximately 90°, bringing large charged residues to the interior of the 2-HCC and forcing its destabilization. These rotations are transmitted to the 4-HB, which now hides the residues that were generating the extensive interaction surface with the ABDs, inducing their release to reach the autokinase conformation. Figure adapted from reference [72].

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4.4. A possible sensing mechanism for DesK

The results and concepts presented in recent work [72] and discussed above provide a tempting framework to suggest the following model for DesK sensing (Fig.5). A temperature drop results in thickening of the membrane. As the membrane thickens, the 2-HCC pairs in its outer side, the TM segments are stretched from their ends and the helices tilt, as required to maintain hydrophobic matching to the membrane. Stretching induces unwinding of the 2-HCC and a gradual rotation of its helices increasing downstream toward the cytoplasmic side, reaching 90° by the KERER segment where the 2-HCC exits the TM region. The 90° rotation is transmitted through the 2-HCC bringing large charged residues to its interior, which forces its destabilization and the separation of its helices. The resulting helix rotation and separation are transmitted to the 4-HB domain, which now hides the residues that were generating an extensive interaction surface with the ABDs in the phosphatase form [69], thus releasing the ABD domains and promoting the autokinase-competent state. Previous results sustaining the SB motif, the serine zipper and the random coil/helix transition models could be reinterpreted in terms of this newly proposed mechanism, giving to it further experimental support [73]. Briefly, the opening/closing or hydration/dehydration of the N-terminal external segment of MS-DesK has been independently postulated from mutational evidence that led to the SB hypothesis [68], now explained to be related to the hinge at Gly13 by our new proposal [72]. Moreover, the effect on DesK kinase activity of both the substitutions on the linker K^{155}ERERLE segment on the cytoplasmic side [70] and the single-point substitution on the serine zipper of the TM region [71] can be related to the stabilization/destabilization of the 2-HCC, which has a profound impact on the output activity of the sensor, as shown by Saita and coworkers [72]. Altogether, these observations agree with the idea that a delicate balance must exist in the surface of a transduction element, i.e., the 2-HCC, to allow for the conformational transition to occur upon signal detection [75,85]. A too stable or a too weak coiled coil would lock the protein in a permanent OFF or ON state, respectively, making it insensitive to the signal. Thus, the control of DesK function, and possibly of many integral membrane proteins, by the lipid bilayer becomes primarily a question of energetics of changes in the equilibrium distribution among different conformations.

It is worth noting that the thickness difference of the lipid bilayers used in the simulation modeling [77–79] shown in Fig. 4 and in previous *in vitro* experimental studies [66,68] is ~4.5 Å. This does not necessarily mean that a change of this magnitude could take place in a bacterial membrane when it transitions from 37 °C to 25 °C. However, recent work suggested that the fluid phase-to gel phase transition may contribute to the switch in activity of DesK *in vivo* [66,86]. If the lipids approach their fluid to gel phase transition on cooling, this could result in a greater increment in bilayer thickness [86]. Thus, it is possible that other changes in membrane physical properties related to the phase transition may contribute to the activity switch of DesK.

In summary, the mechanism proposed by Saita et al. [72] suggests that membrane inputs related to cold-induced thickening trade off with the protein's internal mechanics, particularly those concerning 2-HCC stability and its helical rotations. Upon a drop in temperature, the membrane becomes thicker and more structured, imposing on the 2-HCC a stress that results in its conversion from the relaxed phosphatase state (Fig. 5A) into the autokinase-competent state (Fig. 5B). Such mechanism explains several observations on DesK and MS-DesK and puts together essential components of signal transduction by histidine kinases, providing in turn new elements to address the fundamental problem of dynamic helix interactions in transmembrane signaling.

5. Conclusions/future directions

An increased amount of evidence highlights the fact that TM segments of proteins can adapt in different ways to the lipid environment into which they are embedded. It is also clear that even a minor change in, for example, the orientation of the TM segments in the lipid bilayer or their mode of association can have important structural and functional consequences for the intact membrane protein. How exactly a particular TM segment responds to the surrounding lipids will depend not only on its amino acid composition and the nature of the lipids it is in contact with but also on its interaction with other TM helices. Bearing in mind the complex lipid composition and organization of biological membranes and the highly varied amino acid composition found in natural proteins, we conclude that a complete understanding of lipid–protein interactions is still far from being accomplished.

The key challenges to establishing the molecular mechanisms of regulation by membrane thickness of MscL, Ca^{2+} ATPase, and DesK consist in revealing how these proteins, with different TM architectures, are able to efficiently transduce hydrophobic mismatch into protein conformational changes. Although an experimentally based signal sensing mechanism for DesK, provoked by membrane thickness as a driving force, is not yet fully established, dynamic rearrangements supported by structure-guided mutagenesis and predicted by computational modeling and simulation have provided an exciting framework to formulate a speculative model for DesK thermosensing. It should be noted, however, that most of the information about how DesK TM segments sense the lipid environment comes from studies of the chimerical single-membrane-spanning MS-DesK. Nevertheless, it is clear that the situation is much more complex for the full-length DesK protein, where interactions between TM segments also play an important role. It can be expected that spectroscopic methods such as EPR and solid-state NMR will be important tools in investigating how DesK is affected by the lipid environment. However, despite recent developments, such experiments are still far from trivial, and understanding in detail how larger proteins are influenced by the lipid environment *via* their TM segments still remains an important future challenge.

Conflict of interest

There is not conflict of interest.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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References

[1] M.P. Sheetz, S.J. Singer, Biological membranes as bilayer couples. A molecular mechanism of drug–erythrocyte interactions, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 4457–4461.
[2] O.G. Mouritsen, M. Bloom, Mattress model of lipid–protein interactions in membranes, Biophys. J. 46 (1984) 141–153, http://dx.doi.org/10.1016/S0006-3495(84)84007-2.
[3] S.M. Gruner, Intrinsic curvature hypothesis for biomembrane lipid composition : a role for nonbilayer lipids, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 3665–3669.
[4] A.G. Lee, How lipids affect the activities of integral membrane proteins, Biochim. Biophys. Acta 1666 (2004) 62–87, http://dx.doi.org/10.1016/j.bbamem.2004.05.012.
[5] H.W. Huang, Deformation free energy of bilayer membrane and its effect on gramicidin channel lifetime, Biophys. J. 50 (1986) 1061–1070, http://dx.doi.org/10.1016/S0006-3495(86)83550-0.
[6] D.R. Fattal, A. Ben-shaul, A Molecular Model for Lipid–Protein Interaction in Membranes : The Role of Hydrophobic Mismatch, 651993http://dx.doi.org/10.1016/S0006-3495(93)81249-9.

[7] D. Mitchell, M. Straume, J. Miller, B. Litman, Modulation of metarhodopsin formation by cholesterol-induced ordering of bilayer lipids, Biochemistry 29 (1990) 9143–9149.
[8] R.S. Cantor, The lateral pressure profile in membranes: a physical mechanism of general anesthesia, Biochemistry 36 (1997) 2339–2344, http://dx.doi.org/10.1021/bi9627323.
[9] S.M. Bezrukov, Functional consequences of lipid packing stress, Curr. Opin. Colloid Interface Sci. 5 (2000) 237–243.
[10] J.A. Lundbæk, P. Birn, S.E. Tape, G.E.S. Toombes, R. Søgaard, R.E.K. Ii, et al., Capsaicin regulates voltage-dependent sodium channels by altering lipid bilayer elasticity, Mol. Pharmacol. 68 (2005) 680–689, http://dx.doi.org/10.1124/mol.105.013573.
[11] A.G. Lee, Lipid–Protein Interactions in Biological Membranes: A Structural Perspective, 2003http://dx.doi.org/10.1016/S0005-2736(03)00056-7.
[12] J.A. Killian, Hydrophobic Mismatch Between Proteins and Lipids in Membranes, 13761998http://dx.doi.org/10.1016/S0304-4157(98)00017-3.
[13] T.K.M. Nyholm, S. Ozdirekcan, J.A. Killian, How protein transmembrane segments sense the lipid environment, Biochemistry 46 (2007) 1457–1465, http://dx.doi.org/10.1021/bi061941c.
[14] O.S. Andersen, R.E. Koeppe, Bilayer thickness and membrane protein function: an energetic perspective, Annu. Rev. Biophys. Biomol. Struct. 36 (2007) 107–130, http://dx.doi.org/10.1146/annurev.biophys.36.040306.132643.
[15] A. Holt, J.A. Killian, Orientation and dynamics of transmembrane peptides: the power of simple models, Eur. Biophys. J. 39 (2010) 609–621, http://dx.doi.org/10.1007/s00249-009-0567-1.
[16] A.G. Lee, Biological membranes: the importance of molecular detail, Trends Biochem. Sci. 36 (2011) 493–500, http://dx.doi.org/10.1016/j.tibs.2011.06.007.
[17] J.C.M. Holthuis, A.K. Menon, Lipid landscapes and pipelines in membrane homeostasis, Nature 510 (2014) 48–57, http://dx.doi.org/10.1038/nature13474.
[18] R. Phillips, T. Ursell, P. Wiggins, P. Sens, Emerging roles for lipids in shaping membrane-protein function, Nature 459 (2009) 379–385, http://dx.doi.org/10.1038/nature08147.
[19] W. Dowhan, M. Bogdanov, Lipid–protein interactions as determinants of membrane protein structure and function, Biochem. Soc. Trans. 39 (2011) 767–774, http://dx.doi.org/10.1042/BST0390767.Lipid.
[20] A.W. Smith, Biochimica et biophysica acta lipid–protein interactions in biological membranes : a dynamic perspective ☆, BBA - Biomembr. 1818 (2012) 172–177, http://dx.doi.org/10.1016/j.bbamem.2011.06.015.
[21] A.R. Battle, P. Ridone, N. Bavi, Y. Nakayama, Y.A. Nikolaev, B. Martinac, Lipid–protein interactions: lessons learned from stress, Biochim. Biophys. Acta (2015)http://dx.doi.org/10.1016/j.bbamem.2015.04.012.
[22] E. Perozo, D.C. Rees, Structure and mechanism in prokaryotic mechanosensitive channels, Curr. Opin. Struct. Biol. 13 (2003) 432–442, http://dx.doi.org/10.1016/S0959-440X(03)00106-4.
[23] S.S. Ranade, R. Syeda, A. Patapoutian, Mechanically activated ion channels, Neuron 87 (2015) 1162–1179, http://dx.doi.org/10.1016/j.neuron.2015.08.032.
[24] J. Teng, S. Loukin, A. Anishkin, The force-from-lipid (FFL) principle of mechanosensitivity, at large and in elements, Pflugers Arch. - Eur. J. Physiol. (2014)http://dx.doi.org/10.1007/s00424-014-1530-2.
[25] S. Sukharev, B. Martinac, V.Y. Arshavsky, C. Kung, Two Types of Mechanosensitive Channels in the *Escherichia coli* Cell Envelope : Solubilization and Functional Reconstitution, 651993 177–183, http://dx.doi.org/10.1016/S0006-3495(93)81044-0.
[26] S. Sukharev, P. Blount, B. Martinac, F. Blattner, C. Kung, A large-conductance mechanosensitive channel in *E. coli* encoded by mscL alone, Nature 368 (1994) 265–268, http://dx.doi.org/10.1038/368265a0.
[27] G. Chang, R.H. Spencer, A.T. Lee, M.T. Barclay, D.C. Reesf, Structure of the MscL homolog from *Mycobacterium tuberculosis*: a gated mechanosensitive ion channel, Science 282 (1998) 2220–2226 (80-.). (doi:10.1126/science.282.5397.2220).
[28] E. Perozo, L. Cuello, C. DM, Y. Liu, P. Sompornpisut, EPR approaches to ion channel structure and function, Novartis Found. Symp. 245 (2002) 146–168.
[29] B. Martinac, Cellular physiology and biochemistr y biochemistry bacterial mechanosensitive channels as a paradigm for mechanosensory transduction, Cell. Physiol. Biochem. 28 (2011) 1051–1060, http://dx.doi.org/10.1159/000047793.
[30] T. Nomura, C.G. Cran, E. Deplazes, D.M. Owen, A. Macmillan, A.R. Battle, et al., Differential effects of lipids and lyso-lipids on the mechanosensitivity of the mechanosensitive channels MscL and MscS, PNAS 109 (2012) 8770–8775, http://dx.doi.org/10.1073/pnas.1200051109.
[31] I.R. Booth, M.D. Edwards, S. Black, U. Schumann, S. Miller, Mechanosensitive channels in bacteria : signs of closure ?, 52007 431–440, http://dx.doi.org/10.1038/nrmicro1659.
[32] C. Pliotas, A.C.E. Dahl, T. Rasmussen, K.R. Mahendran, T.K. Smith, P. Marius, et al., The role of lipids in mechanosensation, Nat. Struct. Mol. Biol. (2015) 1–11, http://dx.doi.org/10.1038/nsmb.3120.
[33] S.G. Brohawn, E.B. Campbell, R. Mackinnon, Physical mechanism for gating and mechanosensitivity of the human TRAAK K + channel, 516, 2014 126–130, http://dx.doi.org/10.1038/nature14013.
[34] C. Berrier, A. Pozza, A.D.L. De Lavalette, S. Chardonnet, A. Mesneau, C. Jaxel, et al., The purified mechanosensitive channel TREK-1 is directly sensitive to membrane tension, 2882013 27307–27314, http://dx.doi.org/10.1074/jbc.M113.478321.
[35] A.G. Lee, Ca 2+ − ATPase structure in the E1 and E2 conformations: mechanism, helix–helix and helix–lipid interactions, Biochim. Biophys. Acta 1565 (2002) 246–266.
[36] A. Starling, J. East, A. Lee, Effects of phosphatidylcholine fatty acyl chain length on calcium binding and other functions of the (Ca(2+)-Mg2+)-ATPase, Biochemistry 32 (1993) 1593–1600, http://dx.doi.org/10.1021/bi00057a025.
[37] R. Froud, C. Earl, J. East, A. Lee, Effects of lipid fatty acyl chain structure on the activity of the (Ca2+ + Mg2+)-ATPase, Biochim. Biophys. Acta 860 (1986) 354–360.
[38] R. Froud, J. East, O. Jones, A. Lee, Effects of lipids and long-chain alkyl derivatives on the activity of (Ca2 + −Mg2+)-ATPase, Biochemistry 25 (1986) 7544–7552.
[39] A.P. Starling, J.M. East, A.G. Lee, Effects of phospholipid fatty acyl chain length dephosphorylation of the Ca2 + −ATPase phosphorylation and, 879, 1995 875–879.
[40] A.P. Starling, J.M. East, A.G. Lee, Separate effects of long-chain phosphatidylcholines on dephosphorylation of the Ca 2+ ATPase and on Ca 2 + binding, Biochem. J. 788 (1996) 785–788.
[41] F. Michelangeli, S. Orlowski, P. Champeil, E. Grimes, J. East, A. Lee, Effects of phospholipids on binding of calcium to (Ca2(+)-Mg2(+)-ATPase, Biochemistry 29 (1990) 8307–8312.
[42] J.V. Møller, C. Olesen, A.L. Winther, P. Nissen, The sarcoplasmic Ca 2 + −ATPase : design of a perfect chemi-osmotic pump, Q. Rev. Biophys. 43 (2010) 501–566, http://dx.doi.org/10.1017/S003358351000017X.
[43] D. de Mendoza, Temperature sensing by membranes, Annu. Rev. Microbiol. 68 (2014) 101–116, http://dx.doi.org/10.1146/annurev-micro-091313-103612.
[44] P. Sengupta, P. Garrity, Sensing temperature, Curr. Biol. 23 (2013) R304–R307, http://dx.doi.org/10.1016/j.cub.2013.03.009.
[45] S. Jordt, D.D. Mckemy, D. Julius, Lessons from peppers and peppermint : the molecular logic of thermosensation, Curr. Opin. Neurobiol. (2003) 487–492, http://dx.doi.org/10.1016/S0959-4388(03)00101–6.
[46] J.E. Cronan, E.P. Gelmann, Physical properties of membrane lipids: biological relevance and regulation, Bacteriol. Rev. 39 (1975) 232–256.
[47] D. de Mendoza, A. Klages Ulrich, J.E. Cronan, Thermal regulation of membrane fluidity in *Escherichia coli*. Effects of overproduction of beta-ketoacyl-acyl carrier protein synthase I, J. Biol. Chem. 258 (1983) 2098–2101.
[48] M. Mansilla, C. Banchio, D. de Mendoza, Signalling pathways controlling fatty acid desaturation, Subcell. Biochem. 49 (2008) 71–99, http://dx.doi.org/10.1007/978-1-4020-8831-5_3.
[49] Y.-M. Zhang, C.O. Rock, Membrane lipid homeostasis in bacteria, Nat. Rev. Microbiol. 6 (2008) 222–233, http://dx.doi.org/10.1038/nrmicro1839.
[50] M.T. Flowers, J.M. Ntambi, Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism, 192014 248–256, http://dx.doi.org/10.1097/MOL.0b013e3282f9b54d.
[51] M.T. Nakamura, T.Y. Nara, Structure, Function, and Dietary Regulation of Δ6, Δ5, and Δ9 Desaturases, 2004http://dx.doi.org/10.1146/annurev.nutr.24.121803.063211.
[52] M. Sinensky, Homeoviscous adaptation-a homeostatic, Process that regulates the viscosity of membrane lipids in *Escherichia coli*, 71 1974, pp. 522–525.
[53] E. Svensk, M. Ståhlman, C.-H. Andersson, M. Johansson, J. Borén, M. Pilon, PAQR-2 regulates fatty acid desaturation during cold adaptation in *C. elegans*, PLoS Genet. 9 (2013), e1003801http://dx.doi.org/10.1371/journal.pgen.1003801.
[54] A.E. Murray, J.J. Grzymski, Diversity and genomics of Antarctic marine microorganisms, Philos. Trans. R. Soc. Lond. B 362 (2007) 2259–2271, http://dx.doi.org/10.1098/rstb.2006.1944.
[55] P.E. Tiku, A.Y. Gracey, A.I. Macartney, R.J. Beynon, A.R. Cossins, Cold-Induced Expression of Δ9-desaturase in carp by transcriptional and posttranslational mechanisms, Science 271 (1996) 815–818 (80-.).
[56] M.K. Dengke, L. Zhijie, A.Y. Lu, F. Sun, S. Chen, M. Rothe, et al., Acyl-CoA dehydrogenase drives heat adaptation by sequestering fatty acids, Cell 161 (2015) 1152–1163, http://dx.doi.org/10.1016/j.cell.2015.04.026.
[57] Z.V. Leonenko, E. Finot, H. Ma, T.E.S. Dahms, D.T. Cramb, Investigation of temperature-induced phase transitions in DOPC and DPPC phospholipid bilayers using temperature-controlled scanning force microscopy, Biophys. J. 86 (2004) 3783–3793, http://dx.doi.org/10.1529/biophysj.103.036681.
[58] J. Pan, S. Tristram-Nagle, N. Kucerka, J.F. Nagle, Temperature dependence of structure, bending rigidity, and bilayer interactions of dioleoylphosphatidylcholine bilayers, Biophys. J. 94 (2008) 117–124, http://dx.doi.org/10.1529/biophysj.107.115691.
[59] P. Szekely, T. Dvir, R. Asor, R. Resh, A. Steiner, O. Szekely, et al., Effect of temperature on the structure of charged membranes, J. Phys. Chem. B 115 (2011) 14501–14506, http://dx.doi.org/10.1021/jp207566n.
[60] J.P.M. Jämbeck, A.P. Lyubartsev, An extension and further validation of an all-atomistic force field for biological membranes, J. Chem. Theory Comput. 8 (2012) 2938–2948, http://dx.doi.org/10.1021/ct300342n.
[61] P.S. Aguilar, a.M. Hernandez-Arriaga, L.E. Cybulski, a.C. Erazo, D.d. Mendoza, Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*, EMBO J. 20 (2001) 1681–1691, http://dx.doi.org/10.1093/emboj/20.7.1681.
[62] D. Albanesi, M.C. Mansilla, D. De Mendoza, The membrane fluidity sensor DesK of *Bacillus subtilis* controls the signal decay of its cognate response regulator, Society 186 (2004) 2655–2663, http://dx.doi.org/10.1128/JB.186.9.2655.
[63] L.E. Cybulski, G. del Solar, P.O. Craig, M. Espinosa, D. de Mendoza, *Bacillus subtilis* DesR functions as a phosphorylation-activated switch to control membrane lipid fluidity, J. Biol. Chem. 279 (2004) 39340–39347, http://dx.doi.org/10.1074/jbc.M405150200.
[64] F. Trajtenberg, D. Albanesi, N. Ruétalo, H. Botti, A.E. Mechaly, M. Nieves, et al., Allosteric activation of bacterial response regulators: the role of the cognate histidine kinase beyond phosphorylation, MBio 5 (2014)http://dx.doi.org/10.1128/mBio.02105–14.
[65] P.S. Aguilar, J.E. Cronan, D. de Mendoza, A *Bacillus subtilis* gene induced by cold shock encodes a membrane phospholipid desaturase, J. Bacteriol. 180 (1998) 2194–2200.
[66] M. Martín, D. de Mendoza, Regulation of *Bacillus subtilis* DesK thermosensor by lipids, Biochem. J. 451 (2013) 269–275, http://dx.doi.org/10.1042/BJ20121825.
[67] L. Porrini, L.E. Cybulski, S.G. Altabe, M.C. Mansilla, D. de Mendoza, Cerulenin inhibits unsaturated fatty acids synthesis in *Bacillus subtilis* by modifying the input signal of DesK thermosensor, Microbiologyopen 3 (2014) 213–224, http://dx.doi.org/10.1002/mbo3.154.

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[68] L.E. Cybulski, M. Martın, M.C. Mansilla, A. Fernandez, D.D. Mendoza, Report membrane thickness cue for cold sensing in a bacterium, Amino Acids (2010) 1–6, http://dx.doi.org/10.1016/j.cub.2010.06.074.
[69] D. Albanesi, M. Martín, F. Trajtenberg, M.C. Mansilla, A. Haouz, P.M. Alzari, et al., Structural plasticity and catalysis regulation of a thermosensor histidine kinase, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 16185–16190, http://dx.doi.org/10.1073/pnas.0906699106.
[70] M.E. Inda, M. Vandenbranden, A. Fernández, D. de Mendoza, J.-M. Ruysschaert, L.E. Cybulski, A lipid-mediated conformational switch modulates the thermosensing activity of DesK, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 3579–3584, http://dx.doi.org/10.1073/pnas.1317147111.
[71] L.E. Cybulski, J. Ballering, A. Moussatova, M.E. Inda, D.B. Vazquez, T.a. Wassenaar, et al., Activation of the bacterial thermosensor DesK involves a serine zipper dimerization motif that is modulated by bilayer thickness, Proc. Natl. Acad. Sci. 112 (2015) 6353–6358, http://dx.doi.org/10.1073/pnas.1422446112.
[72] E. Saita, L.a. Abriata, Y.T. Tsai, F. Trajtenberg, T. Lemmin, A. Buschiazzo, et al., A coiled coil switch mediates cold sensing by the thermosensory protein DesK, Mol. Microbiol. 98 (2015) 258–271, http://dx.doi.org/10.1111/mmi.13118.
[73] F.H.C. Crick, The Packing of [Alpha]-Helices: Simple Coiled-Coils, 1953http://dx.doi.org/10.1107/S0365110X53001964.
[74] M. Singh, B. Berger, P.S. Kim, J.M. Berger, A.G. Cochran, Computational learning reveals coiled coil-like motifs in histidine kinase linker domains, Comput. Sci. 95 (1998) 2738–2743.
[75] V. Anantharaman, S. Balaji, L. Aravind, The signaling helix: a common functional theme in diverse signaling proteins, Biol. Direct 1 (2006) 25, http://dx.doi.org/10.1186/1745-6150-1-25.
[76] A. Möglich, R.a. Ayers, K. Moffat, Design and signaling mechanism of light-regulated histidine kinases, J. Mol. Biol. 385 (2009) 1433–1444, http://dx.doi.org/10.1016/j.jmb.2008.12.017.
[77] J.C. Mathai, S. Tristram-Nagle, J.F. Nagle, M.L. Zeidel, Structural determinants of water permeability through the lipid membrane, J. Gen. Physiol. 131 (2008) 69–76, http://dx.doi.org/10.1085/jgp.200709848.
[78] Q. Xu, M. Kim, K.W.D. Ho, P. Lachowicz, G.E. Fanucci, D.S. Cafiso, Membrane hydrocarbon thickness modulates the dynamics of a membrane transport protein, Biophys. J. 95 (2008) 2849–2858, http://dx.doi.org/10.1529/biophysj.108.133629.
[79] C. Muhle-goll, S. Hoffmann, S. Afonin, S.L. Grage, A.A. Polyansky, D. Windisch, et al., Hydrophobic matching controls the tilt and stability of the dimeric platelet-derived growth factor receptor (PDGFR) B transmembrane segment, 2872012 26178–26186, http://dx.doi.org/10.1074/jbc.M111.325555.
[80] R.P. Diensthuber, M. Bommer, T. Gleichmann, A. Möglich, Full-length structure of a sensor histidine kinase pinpoints coaxial coiled coils as signal transducers and modulators, Structure 21 (2013) 1127–1136, http://dx.doi.org/10.1016/j.str.2013.04.024.
[81] C. Wang, J. Sang, J. Wang, M. Su, J.S. Downey, Q. Wu, et al., Mechanistic insights revealed by the crystal structure of a histidine kinase with signal transducer and sensor domains, PLoS Biol. 11 (2013), e1001493http://dx.doi.org/10.1371/journal.pbio.1001493.
[82] A.E. Dago, A. Schug, A. Procaccini, J.A. Hoch, M. Weigt, H. Szurmant, Structural basis of histidine kinase autophosphorylation deduced by integrating genomics, molecular dynamics, and mutagenesis, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) E1733–E1742, http://dx.doi.org/10.1073/pnas.1201301109.
[83] P. Casino, L. Miguel-Romero, A. Marina, Visualizing autophosphorylation in histidine kinases, Nat. Commun. 5 (2014) 1–11, http://dx.doi.org/10.1038/ncomms4258.
[84] A.E. Mechaly, N. Sassoon, J.-M. Betton, P.M. Alzari, Segmental helical motions and dynamical asymmetry modulate histidine kinase autophosphorylation, PLoS Biol. 12 (2014), e1001776http://dx.doi.org/10.1371/journal.pbio.1001776.
[85] J. Liu, J. Yang, J. Wen, Y. Yang, X. Wei, X. Zhang, et al., Mutational analysis of dimeric linkers in peri- and cytoplasmic domains of histidine kinase DctB reveals their functional roles in signal transduction, Open Biol. 4 (2014) 140023, http://dx.doi.org/10.1098/rsob.140023.
[86] E. Saita, D. de Mendoza, Thermosensing via transmembrane protein–lipid interactions, Biochim. Biophys. Acta Biomembr. 1848 (2015) 1757–1764, http://dx.doi.org/10.1016/j.bbamem.2015.04.005.
[87] G. Grigoryan, A.E. Keating, Structural specificity in coiled-coil interactions, Curr. Opin. Struct. Biol. 18 (2008) 477–483, http://dx.doi.org/10.1016/j.sbi.2008.04.008. Structural.

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