Temperature Sensing by Membranes

Diego de Mendoza

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET) and Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, 2000-Rosario, Argentina; email: demendoza@ibr-conicet.gov.ar

Annu. Rev. Microbiol. 2014. 68:101–16

First published online as a Review in Advance on May 7, 2014

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi: 10.1146/annurev-micro-091313-103612

Keywords

membrane fluidity, temperature sensing, transmembrane signaling, dynamic bundle

Abstract

Bacteria remodel the fluidity of their membrane bilayer precisely via the incorporation of proportionally more unsaturated fatty acids (or fatty acids with analogous properties) as growth temperature decreases. This process, termed homoviscous adaptation, is suited to disrupt the order of the lipid bilayer and optimizes the performance of a large array of cellular physiological processes at the new temperature. As such, microbes have developed molecular strategies to sense changes in membrane fluidity, provoked by a decrease in environmental temperature, and initiate cellular responses that upregulate the biosynthesis of unsaturated fatty acids. This review focuses on the architecture of a membrane fluidity communication network; how thermal information is integrated, processed, and transduced to control gene expression; how membrane-mediated structural changes of a cold sensor are accomplished; and the intriguing possibility that temperature-induced deformations of the cell membrane act as allosteric regulators of protein function.

Contents

INTRODUCTION

The cytoplasmic membrane is the boundary between the cytoplasm and the environment and is involved in almost every aspect of bacterial growth and metabolism. The bacterial cytoplasmic membrane is built on the familiar lamellar phospholipid bilayer, an extended two-dimensional surface of two opposed monolayers (73). Although there is a considerable diversity of phospholipid structures in the bacterial world, most membrane phospholipids are glycerolipids that contain two fatty acid chains (73). The lipid bilayer of the cytoplasmic membrane forms a hydrophobic barrier that prevents the uncontrolled movement of polar molecules and allows the accumulation and retention of metabolites and proteins (23). The lipids also provide a suitable environment for the proper functioning of the membrane protein complexes involved in bioenergetics and biosynthetic functions. The lipid composition determines the dynamics and interactions of lipid molecules, which in turn determine the barrier and permeability properties of the membrane and influence the topology, interactions, and functions of the membrane proteins (14, 53). The functions of the cytoplasmic membrane are known to depend heavily on the physical state of lipid bilayers, making it susceptible to changes in environmental temperature (15, 51, 59). In fact, it has been established that normal cell function requires membrane lipid bilayers that are largely fluid; indeed, the bilayers of most organisms are entirely or mostly fluid at physiological temperatures (21, 73). However, at low temperatures membrane bilayers undergo a reversible change of state from a fluid (disordered, liquid crystalline) to a nonfluid (ordered, gel) array of the fatty acyl chains (17, 21). The temperature at the midpoint of this transition is called the transition temperature (T_m), and the change of state accompanying an increase in temperature is called the liquid-gel transition (21). The T_m is a function of the membrane lipid composition and, in organisms lacking cholesterol, mainly depends on the fatty acid composition of the membrane lipids (17, 21). A disordered state is imparted by the presence of either unsaturated or terminally branched fatty acids, both of which act to offset the closely packed, ordered arrangement of the lipid bilayer acyl chains that is due to straight, saturated acyl chains (17, 21, 73) (**Figure 1*a***). Straight-chain, saturated fatty acids, such as hexadecanoic acid (C16:0), are linear and pack together efficiently to produce a bilayer that has a high T_m and low permeability properties (**Figure 1*a***). The *cis* unsaturated fatty acids (UFAs) introduce a pronounced kink in the chain, which disrupts the order

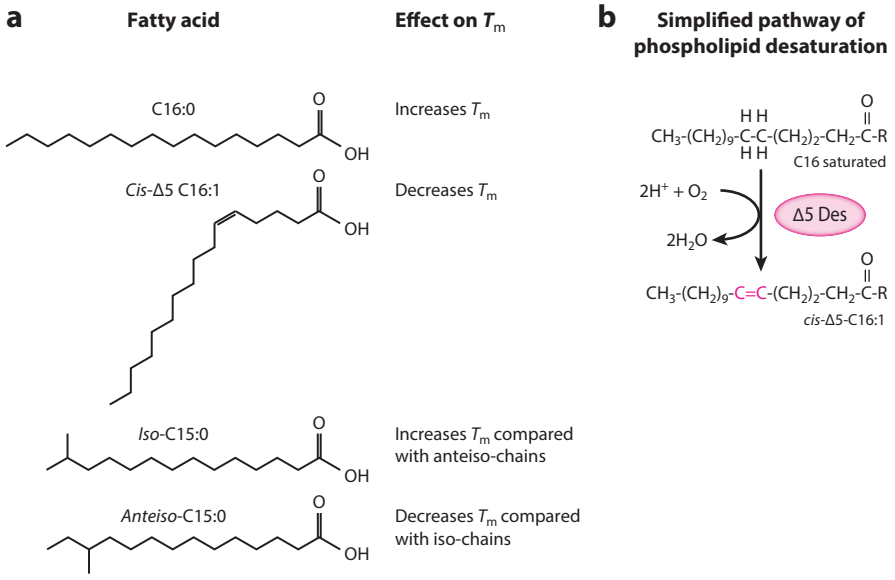

Figure 1

Chemical structure and physical properties of lipids. (*a*) Diagram of the hydrocarbon portion of fatty acids of membrane phospholipids and its effects on T_m. (*b*) Postsynthetic desaturation of a fatty acid mediated by the Δ5-Des acyl lipid desaturase. The R group is the 1-acyl lysophospholipid moiety of a membrane phospholipid. Panel *a* was modified from Reference 69, copyright 2008, with permission from Nature Publishing Group.

of the bilayer and results in lower T_m and higher permeability (**Figure 1*a***). The composition of the branched-chain fatty acids affects membrane fluidity owing to the disruptive effect of the methyl group on acyl chain packing. The anteiso-branched-chain fatty acids (a-BCFA) promote a more fluid membrane than the iso-branched-chain fatty acids (**Figure 1*a***), because the methyl branch is further away from the end of the fatty acid (36, 43, 73, see **Figure 1*a***).

From these considerations, it seems clear that bacteria and most (if not all) organisms unable to maintain thermal homeostasis must regulate their plasmatic membrane phase transition in response to temperature (60). Without regulation, an organism shifted from a high to a low temperature would have membrane lipids with suboptimal fluidity, resulting in subnormal membrane function. This membrane lipid homeostasis that maintains the biophysical properties of membranes is referred to as homoviscous adaptation and is interpreted as a mechanism that modifies the viscosity and permeability of the phospholipid bilayer to minimize energy expenditure and optimize growth (73).

The mechanism of homoviscous adaptation in all cases examined seems to occur via the incorporation of proportionally more UFAs (or fatty acids with analogous properties, such as a-BCFAs) as the temperature decreases (42). This regulatory mechanism system seems to be a universally conserved adaptation response allowing cells to maintain the appropriate fluidity of membrane lipids regardless of the ambient temperature (17, 21, 42, 56). This means that cells must process temperature signals to adjust enzyme activities or to activate unique genes necessary to adapt the membranes to the new temperature (60). Whereas the activities of all biomolecules are altered as a function of temperature, the thermosensors I focus on here are proteins that sense changes in the lipid bilayer environment triggered by a sudden decrease in temperature. These sensors mediate the cold transcriptional induction of acyl lipid desaturase enzymes that introduce *cis* double

Branched-chain fatty acids: fatty acids that contain methyl branches on the penultimate carbon atom (iso) or the antepenultimate carbon atom (anteiso)

a-BCFA: anteiso-branched-chain fatty acid

bonds into preexisting fatty acids, thus optimizing membrane fluidity at the new temperature. Here, I initially provide an overview of the work on bacterial acyl lipid desaturases in *Bacillus* spp. and, to some extent, in cyanobacteria. Then, the focus is set mainly on the recent biochemical and structural insights into the paradigmatic DesK thermosensor of the model soil bacterium *Bacillus subtilis*. The *B. subtilis*–centric tone of this review reflects the fact that most of the work on control of membrane fluidity by a molecular thermometer has been carried out on this model gram-positive bacterium.

Acyl-acyl-carrier protein: a fatty acid chain attached to the prosthetic group of the low-molecular-mass acyl carrier protein

UNSATURATED FATTY ACID BIOSYNTHESIS

Konrad Bloch, a Nobel Laureate chemist, and coworkers determined that UFAs are synthesized in two different ways: One occurs only in aerobic organisms and requires molecular oxygen, whereas the other pathway is used in anaerobic conditions (16, 58). The anaerobic pathway of UFA biosynthesis was extensively studied in the *Escherichia coli* model system and has been covered in several comprehensive reviews (41, 42, 73). Here, I briefly review the aerobic pathway of UFA synthesis, which functions in all eukaryotic forms of life and certain bacteria (7, 42, 43). In this pathway the double bond is introduced postbiosynthetically into saturated fatty acids by desaturase enzymes (7, 61) (**Figure 1*b***). Desaturase enzymes perform dehydrogenation reactions that result in the introduction of double bonds into fatty acids through a mechanism initiated by the energy-demanding abstraction of a hydrogen atom from a methylene group (**Figure 1*b***) (61). To achieve this, desaturase enzymes recruit and activate molecular oxygen with the use of a diiron cluster located in their active sites (7, 43, 61). The diiron center is common to a variety of proteins, including methane monooxygenase, ribonucleotide reductase, rubrerythrins, and a variety of oxidase enzymes (61). Desaturase enzymes have evolved independently twice; the acyl-acyl-carrier protein desaturases are soluble enzymes found in the plastids of higher plants (62), whereas the more widespread class of integral membrane desaturases is found in endomembrane systems in prokaryotes (39, 42) and eukaryotes (47, 65). The membrane-bound desaturases, which introduce the double bond in fatty acids sterified to glycerolipids (**Figure 1*b***), are named acyl lipid desaturases (7, 39). All these enzymes utilize reducing equivalents obtained from an electron transport chain and are able to introduce the double bond in a chemo-, regio-, and stereoselective manner (61). Three main types of specificities for the introduction of the double bond (regioselectivity) have been observed for fatty acid desaturases that would reflect differences in the position of the active site relative to the features of the substrate-binding pocket associated with substrate recognition: The Δx desaturases introduce a double bond x carbons from the carboxyl end; $\omega - x$ desaturases dehydrogenate x carbons from the methyl terminus; and $v + x$ desaturases use a preexisting double bond as a reference point and dehydrogenate x carbons from the nearest olefinic carbon (7, 72).

Transcriptional Regulation of Lipid Desaturation in *Bacillus* Species

Konrad Bloch and coworkers were the first to describe the existence of an oxidative pathway for the biosynthesis of long-chain UFAs by microorganisms, thus initiating the study of bacterial desaturases (57). Fulco and Bloch demonstrated desaturation in several bacteria, including *Bacillus megaterium* (30–32). One of the most significant findings of these researchers, at least for understanding thermosensing via changes in membrane properties, was the remarkable discovery of temperature dependence of the desaturation reaction in *B. megaterium* (32) (for a historical perspective see Reference 29). Thus, at a growth temperature of 23°C, radioactive palmitate added to the medium was almost completely desaturated to a UFA containing a *cis* double bond at position 5 relative to the carboxyl end of the fatty acid ($\Delta 5$) (**Figure 1*b***), whereas at 30°C desaturation was negligible (32). Presumably, this inverse relationship between temperature and desaturation

functioned in the regulation of membrane fluidity in response to fluctuations in growth temperature, but the mechanism was totally unknown. After this initial discovery, Fulco and coworkers went on with the characterization of this adaptive response, demonstrating that the fatty acid desaturation system was induced when cultures of *B. megaterium* were grown at low temperatures (25, 26; for reviews see 27, 28). It was also found that the levels of desaturation of cultures of *B. megaterium* transferred from 35°C to 20°C far exceeded the levels of desaturation of cultures growing at 20°C (38). To explain the dramatic change in the lipid composition of bacilli shifted from 35°C to 20°C, it was proposed that transcription of the fatty acid desaturase gene occurs only at low growth temperatures. To account for the initial degree of desaturation seen immediately after a downward temperature shift, Fujii & Fulco (24) postulated the existence of a modulator protein whose synthesis also proceeds at lower temperatures but only following a brief delay. Thus, the rapid desaturation taking place in freshly downshifted cells would soon be moderated to a rate yielding the steady-state level of fatty acid unsaturation characteristic at that temperature. However, no direct experimental evidence supported this proposed on-or-off transcriptional regulatory model of desaturase synthesis. To further explore the molecular mechanism of cold induction of UFA biosynthesis and how a change in growth temperature regulates the expression of the *Bacillus* desaturase, I decided to study this phenomenon in *B. subtilis*, which is an excellent experimental model because of its general experimental tractability. Like *B. megaterium*, *B. subtilis* growing in rich medium at 37°C synthesizes saturated fatty acids almost exclusively (34). However, when a culture grown at 37°C is transferred to 20°C, the synthesis of UFAs is induced (34). As with *B. megaterium*, the desaturation system of *B. subtilis* requires de novo synthesis of RNA and proteins, given that it is completely abolished by rifampin or chloramphenicol added before a downward temperature shift (34). During a short visit to the laboratory of John Cronan at the University of Illinois at Urbana-Champaign I succeed in isolating the *des* gene, which encodes the sole desaturase of *B. subtilis* (1), by complementation of *E. coli* strains with mutations in either the *fabA* gene or *fabB* gene, both of which are essential for UFA synthesis (41). The *des* gene encodes a polytopic membrane-bound desaturase (22) and catalyzes the introduction of a *cis* double bond at the Δ5 position of a wide range of saturated fatty acids (6) (**Figure 1*b***) using either ferredoxin or flavodoxin (13) as electron donors. This protein was named Δ5-Des (6). A detailed transcriptional analysis demonstrated that the *des* gene is tightly regulated during cold shock (3). The induction of *des* mRNA takes place in the absence of new protein synthesis, indicating that the desaturase transcript can be produced upon cold shock by using already existing resources at the time of the temperature downshift (3). In addition, the level of the *des* transcript produced in a *B. subtilis* strain in which the wild-type *des* promoter was exchanged with the *spac* promoter was not decreased after continuous growth at 20°C (3). This finding indicates that the transient induction of the wild-type *des* gene at low growth temperatures was due to the shutoff of transcription rather than to the instability of the *des* mRNA. This would explain why, similar to the case of *B. megaterium*, the level of UFAs synthesized by *B. subtilis* during the first growth division cycle was much higher than those of cultures growing for several generations at 20°C (33).

A Two-Component Signal Transduction Regulatory Thermometer in *Bacillus subtilis*

Targeted mutagenesis of a two-gene operon of unknown function located immediately upstream of the *des* gene led to the identification of a histidine kinase (DesK) and a response regulator (DesR) that are involved in the increased expression of *des* in response to low temperatures in *B. subtilis* cells (2). DesK features an N-terminal sensor domain (~150 residues) composed of five transmembrane (TM) segments connected to a C-terminal cytoplasmic domain (DesKC,

TM: transmembrane

~220 residues). DesKC undergoes autophosphorylation in the presence of ATP in the conserved His-188, which is the target residue of its autokinase activity (4). Autophosphorylated DesKC transfers the phosphoryl group to the effector protein DesR, which becomes phosphorylated in the predicted Asp-54 residue (4). Phosphorylated DesR (DesR-P) is the active form of the protein that binds DNA, promoting expression of the *des* gene (19).

Autophosphorylation: a reaction where a protein kinase covalently attaches the γ phosphoryl group from ATP to itself

Phosphatase: a histidine kinase that also stimulates the dephosphorylation of a cognate response regulator

Genetic and biochemical experiments demonstrated that the level of phosphorylation of DesR is determined by the balance of the two functions of DesK, a phosphate donor for DesR and a phosphatase of DesR-P (2, 4). Since the activity of DesR as a transcriptional activator is modulated by the level of phosphorylation (19), the output of signal transduction is determined by switches of DesK activity between kinase- and phosphatase-competent forms. These activities would be reciprocally regulated by changes in growth temperature that, in turn, adjust the unsaturation of membrane phospholipids.

Evidence that membrane fluidity, rather than growth temperature, controls transcription of the *des* gene was obtained from experiments in which the proportion of a-BCFAs of *B. subtilis* membranes was varied by controlling the provision of branched-chain acyl-CoA molecules, used as primers (18). *B. subtilis* has a branched-chain keto acid dehydrogenase (BKDH) that produces anteiso-branched-chain acyl-CoA precursors from isoleucine (12, 36). The a-BCFAs are essential to decrease the transition temperature of *B. subtilis* membrane phospholipids to maintain the appropriate fluidity (36). Limiting the supply of isoleucine dramatically reduces the amount of a-BCFAs of plasma membrane lipids (37), resulting in ordered membrane lipids. Growth of *B. subtilis* cells in the absence of isoleucine results in activation of *des* transcription under isothermal conditions, using a DesK/DesR-dependent mechanism (18). Moreover, in *lipA* mutants, which are unable to synthesize BCFA precursors, because of BKDH's lack of function in the absence of lipoic acid synthesis (46), transcription of the *des* gene reaches levels about fourfold greater than those observed in wild-type strains growing in an isoleucine-free medium at 37°C. These physiological and transcriptional data give further support to the hypothesis that DesK senses membrane fluidity, given that the membrane lipids of *lipA*-deficient cells contain a much higher proportion of high-melting-point saturated fatty acids than those of cells expressing LipA (46). Thus, an increase in the order of membrane lipids due to a lower content of isoleucine-derived fatty acids at constant temperature or a decrease in temperature at constant lipid composition can be sensed by DesK, leading to induction of UFA synthesis. Furthermore, when membrane fluidity is restored the transcription of *des* is shut off by deactivation of the flux of phosphate from DesK to DesR (2, 19).

Membranes of *B. subtilis* cells grown at 40°C are still completely fluid at 25°C, as determined by spectroscopic and calorimetric techniques (10). Lipid composition is adjusted during growth at different temperatures to maintain the membrane in the fluid liquid-crystalline state by a considerable margin. Several lines of evidence demonstrated that membrane function (defined as cell growth) at low temperatures requires the presence of both a-BCFAs and UFAs (11, 37, 46, 71). Whereas long-term membrane adaptation employs an increase in a-BCFA, by a mechanism far from being understood, the introduction of double bonds by Δ5-Des provides the cell with a rapid mechanism for decreasing the fluidity of preexisting membranes upon a temperature decrease (10, 71).

A GLOBAL COLD SENSOR IN CYANOBACTERIA

Another pathway for the perception and transduction of low-temperature signals to optimize membrane lipid fluidity, similar to the one used by *B. subtilis*, was reported in cyanobacteria. It was early shown that when catalytic hydrogenation under isothermal conditions in vivo induced a decrease in the degree of unsaturation of fatty acids in the plasma membrane of the cyanobacterium

Synechocystis PCC6803, the expression of the *desA* gene encoding a Δ12 acyl lipid desaturase, which is normally inducible by a low-temperature shift, was enhanced (70). Murata and his coworkers later used selective gene inactivation in *Synechocystis* PCC6803 to identify two histidine kinases (membrane-bound Hik33 and soluble Hik19) together with a response regulator (Rer1) as key components of the signal cascade in cold shock (67). Hik33 was able to regulate the expression of the *desB* and *desD* genes coding for the cold-inducible ω-3 and Δ6 desaturases, respectively (67, 68). Comparative DNA microarray analyses of the wild type and the corresponding *hik33* deletion strain finally demonstrated that the membrane-bound Hik33 kinase indeed controls the expression of ~30 cold-inducible genes (66). Thus, in contrast to the DesK/DesR system, which seems to regulate the expression of the *des* gene only (9), Hik33 is a global cold sensor controlling an important subset of cold-shock-induced genes (40). Although considerable efforts are currently underway to biochemically characterize Hik33 (63), the phosphorylation relay between Hik33 and Rer1 and the subsequent regulation of transcription of the *desB* and *desD* gene by Rer1 remain to be characterized. Nevertheless, the molecular logic of cold-regulated induction of gene expression in *Synechocystis* spp. seems to be similar to that of the pathway controlled by DesK/DesR in *B. subtilis*.

DesK-SIGNALING MECHANISMS

Input Transmission via the Transmembrane Segments

DesK contains multiple TM segments and lacks obvious extracellular domains. These features, combined with genetic studies and mutant phenotypes, suggested that the DesK sensory region could lie within the TM segments and that the thermosensor could assume different signaling states in response to the order of the lipids (42, 43). This hypothesis was verified by reconstitution of full-length DesK into liposomes composed of *E. coli* lipids (5, 44). These lipids undergo a reversible change of state from a fluid to a nonfluid array of fatty acyl chains when the temperature is decreased from 37°C to 25°C (17, 21). In this reconstituted system the autokinase activity of DesK was upregulated (~50-fold) when temperature was decreased (5). Truncation of the TM segments resulted in a constitutive autokinase state, supporting the notion that the TM domain of DesK stabilizes a kinase-repressed state at higher temperatures (5). In proteoliposomes, DesK-stimulated dephosphorylation of phospho-DesR was significantly lower at 25°C than at 37°C (5). This thermal response also required the TM domain, ruling out a simple temperature effect on enzyme activity. In contrast, temperature changes did not induce changes in the initial rates of phosphotransferase activity, which result in phosphoryl migration from DesK to DesR. Taken together these experiments demonstrated that (*a*) the temperature stimuli come directly from the membrane lipid bilayer, with no other proteins involved in the sensing or signaling mechanism; (*b*) the cold signal regulates the autokinase and phosphatase activities of DesK in opposite directions; and (*c*) the TM domain plays a crucial role, because the cytoplasmic domain alone (DesKC) is unresponsive to the cold signal. In this scenario as growth temperature decreases, the order of membrane lipids increases, activating DesK and inducing desaturase expression. Desaturase activity increases lipid disorder, helping restore appropriate membrane fluidity. This, in turn, turns off DesK kinase activity and turns on DesK phosphatase activity, terminating the response (**Figure 2**). Such a regulatory loop is well suited to maintain levels of membrane fluidity within an optimal range.

A Structural Model of DesK Catalysis Regulation

The recent elucidation of a set of X-ray crystal structures of the catalytic core of DesK (DesKC) has provided valuable structural insight into the regulation of the catalytic activities of the protein

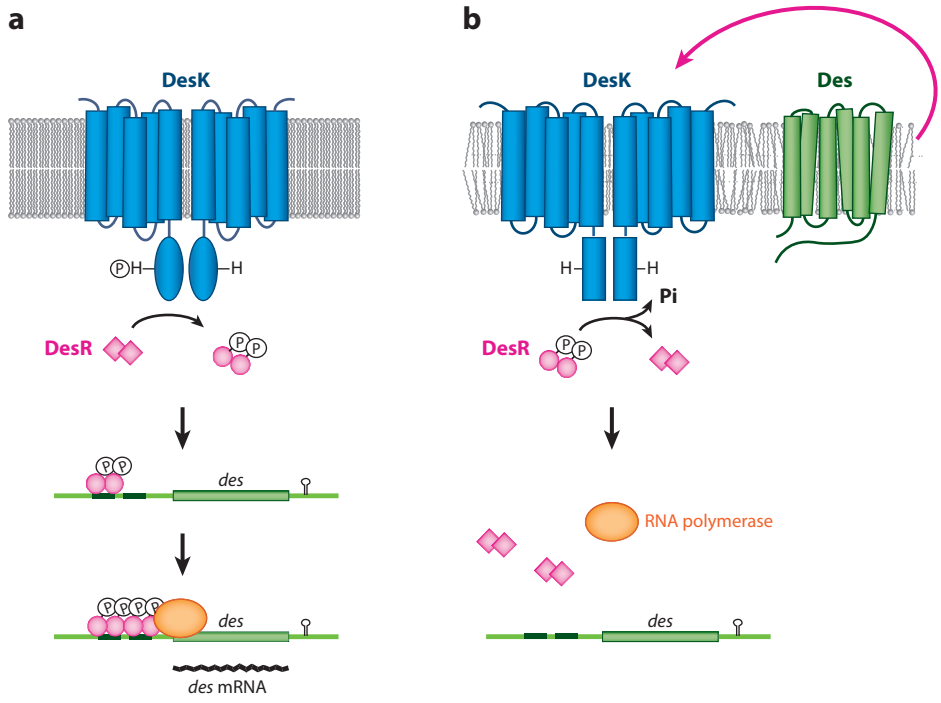

Figure 2

Model for regulation of unsaturated fatty acid (UFA) synthesis in *Bacillus subtilis*. (*a*) DesK could assume different signaling states in response to changes in membrane fluidity. An increase in the order of the acyl chains of membrane lipids promotes a kinase-dominant state of DesK, which autophosphorylates and transfers the phosphate group to DesR. DesK-mediated phosphorylation of DesR enables interaction of DesR-P dimers with the *des* promoter and RNA polymerase, resulting in transcriptional activation of *des*. (*b*) Δ5-Des is synthesized and desaturates the acyl chains of membrane phospholipids. These newly synthesized UFAs cause a decrease in the order of membrane lipids favoring a phosphatase-dominant state of DesK, leading to dephosphorylation of DesR and thus turning off *des* transcription.

(5). DesKC shows the characteristic homodimeric structure observed in other histidine kinases. Each monomer consists of an N-terminal antiparallel 2-helix hairpin (helices α1 and α2) that includes the phosphorylatable histidine (H-188), connected by a short linker region to a C-terminal ATP-binding domain (ABD). The helical hairpins of two monomers interact with each other to form a central four-helix-bundle (4-HB) domain, known as the DHp (dimerization and histidine phosphotransfer) 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 (5). Comparison of all DesKC structures identifies three distinct conformational states of the protein (phosphatase, kinase, and phosphotransferase), which differ significantly in quaternary organization and internal flexibility. The structures differ in the interhelical packing of the DHp domain and the mobility and relative orientation of the ABDs. These structural studies, combined with in vitro studies of DesK reconstituted into proteoliposomes, suggest that the thermosensor is cold-activated through specific interhelical rearrangements in its central 4-HB domain. This helical domain has a remarkable plasticity that influences the different catalytic activities, either by modifying the mobility of the ATP-binding domains, required for autokinase activity, or by modulating binding of the cognate response regulator, crucial for sustaining the phosphotransferase and phosphatase activities.

4-HB: four-helix bundle, a protein structure stabilized mainly through packing interactions between the hydrophobic faces of amphiphilic helices

A Model of DesK Signaling

A replacement of H-188 by valine provides a suitable model for the phosphatase-competent state of DesK because $DesKC_{H188V}$ was shown to retain specific phosphatase activity toward DesR-P at levels comparable to those of wild-type DesK, both in vivo and in vitro. $DesKC_{H188V}$ has a more compact and rigid conformation than the kinase-competent state of DesKC (**Figure 3*a***, phosphatase state). This is due to extensive intramolecular interactions involving both a tighter DHp-ABD interaction in each monomer (**Figure 3*a***, phosphatase state) and the formation of a parallel 2-helix coiled coil, which continues the 4-HB toward the TM domain (**Figure 3*c***, phosphatase state), whereas in the structure of the kinase-competent state of DesKC the two helices continuing toward the TM domain are not packed together (**Figure 3*c***, kinase state). Comparison of the phosphatase and kinase states (**Figure 3*a–c***) suggests that the contacts seen between the DHp domain and the ABD, as well as the parallel 2-helix coiled coil, support a labile association to be released for autophosphorylation and maintained for the phosphatase activity under control of the sensor domain (5). Thus, in a fluid membrane the TM domain would stabilize the connecting coiled coil and the catalytic core into a rigid conformation with the ABDs attached to the DHp domain (**Figure 3*a–c***, phosphatase state). This conformation inhibits autokinase activity, and the DHp surface is competent to interact with phospho-DesR. Upon cold-signal reception, the parallel 2-helix coiled coil (**Figure 3*c***, kinase state) would be disrupted and the ensuing structural reorganization would release the ATP binding for histidine phosphorylation and produce a kinase-on state (**Figure 3*a*,*b***, kinase state). Phosphorylation of DesKC induces an as yet different, asymmetric conformation with a pronounced bending of helix α1, capable of binding and transferring the phosphoryl group to the conserved aspartate of DesR, activating its output domain (**Figure 3*a*,*b***, phosphotransferase state).

Coiled coils: a bundle of α helices that are wound into superhelical structures

A three-state dynamic-bundle-signaling model best accounts for the signaling properties of the catalytic domain of DesK and may apply to other prokaryotic and eukaryotic signal receptors of diverse biological activities. In this dynamic model the kinase-on output state corresponds to multiple helical rearrangements in the 4-HB dynamic range (5). Alpha helices are indeed common information-transducer elements, and different types of helical movements performing mechanical work have been proposed to convey input/output signals (64). Furthermore, incoming signals in the form of helix rotations are congruent with mechanistic models derived from the study of upstream elements in different signal-transducing elements (33, 49, 50). Regardless of the precise way by which the cold signal reaches DesK 4-HB, the structural data suggest that it relies on rotation of the TM segment(s), which in turn forces the membrane connecting two helices to leave the coiled-coil conformation favoring a kinase-on state of DesK (**Figure 3*a–c***).

A segment linking TM5 with the 4-HB domain of DesK called the linker region was recently identified (35). The linker region 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, 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-helix coiled coil to regulate the signaling state of DesK.

A MOLECULAR MODEL OF THERMOSENSING

The crystal structure of the entire cytoplasmic portion of DesK has provided a molecular framework to interpret the basic mechanistic principles by which the sensor protein alternates between

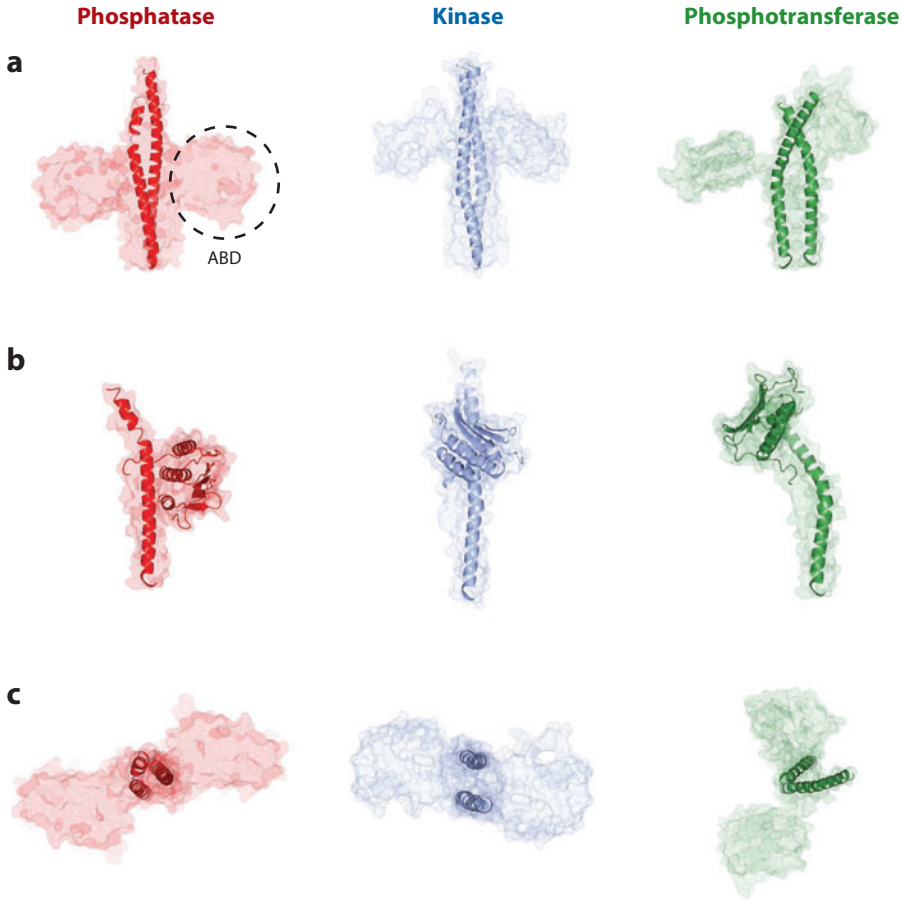

Figure 3

Structural bases of DesK catalysis regulation. The observed structures of DesKC homodimers (shown as surface models with helix α1 highlighted) are aligned to illustrate, from three different views, each conformation considered to be competent for the phosphatase, kinase, and phosphotransferase states of DesKC. DesK structures correspond to Protein Data Bank files 3HJH (*left*), 3GIE (*middle*) and 3GIG (*right*). One of the ATP-binding domains (ABDs) is highlighted with a dotted circle. All structures were aligned from residues 180 to 211 corresponding to the carboxy terminus of the α1 helices. This alignment facilitates the observation and comparison of the relative motions of each domain, as DesKC changes its conformation from a phosphatase-competent to the kinase- and phosphotransferase-competent states. (*a*) The alignment of the structures shows how the ABDs lose their strong interaction with the four-helix-bundle domain (shown in the phosphatase-competent state) along the DesK catalytic cycle (for a detailed explanation see main text). (*b*) Comparison of the structures illustrates the rotation of the ABDs around an axis perpendicular to the α1 helix. A remarkable feature of this structural transition is a pronounced bending of the α1 helix just below H-188, which thus leaves room for the entrance of DesR and its interaction with phosphorylated H-188 (phosphotransferase-competent state). (*c*) DesKC structures are viewed from the membrane, showing the rotation of the ABDs around the main α1-helices axis. The formation of a parallel coiled coil at the amino terminal ends of α1 helices, observed only in the phosphatase state, is proposed to play a key role in the conformation adopted by DesK in response to changes in membrane fluidity (for further details see main text).

output autokinase and phosphatase activities. Nevertheless, given the critical role that 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 from DesK can be simplified into a chimerical single-membrane-spanning minimal sensor (MS) that is still able to respond to changes in membrane fluidity (20). 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 temperature and resulted in a constitutively active protein, suggesting that TM1 harbored a temperature-sensing motif. Based on this finding, a model was envisioned in which TM1 would detect a drop in temperature and transmit this information to TM5, which is connected to the catalytic core of DesK (**Figure 2**). A chimeric TM region was therefore created, consisting of N-terminal residues of TM1 and C-terminal residues of TM5, fused to the DesK catalytic domain. 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 from 37°C to 20°C (20).

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 (73). 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 (17, 21). Upon warming, the membrane undergoes an endothermic transition and has much higher permeability to small molecules than gel-phase bilayers (73). 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. The average area occupied by each phospholipid molecule increases upon melting, from ~0.47 nm^2 to around 0.67 nm^2, and the distance between polar head groups across the bilayer decreases, from 4.7 nm to 4.2 nm (54, 55). Membrane thickness variations have been shown to affect the activity of several membrane-embedded proteins (8, 48), 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 (20). Presumably these residues are located within the TM region just below the 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 it has been called the sunken-buoy (SB) motif (20). 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 a kinase activity, whereas hydration during membrane narrowing would promote phosphatase activity. There are some molecular tests, using chimeric MS-DesK, that 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 toward the carboxy terminus of the SB motif, a manipulation that should help unbury the cluster of hydrophilic residues, decreases autokinase activity (20).

However, the most compelling evidence for membrane thickness as a regulator of the signaling state of DesK comes from (*a*) biochemical studies in which thermosensors (MS-DesK and full-length DesK) were reconstituted into vesicles of defined lipid composition (20, 45) and (*b*) in vivo

studies in which the length of *B. subtilis* membrane fatty acids was manipulated by inhibition of fatty acid or phospholipid synthesis (52). When either MS-DesK (20) or full-length DesK (45) was reconstituted into a series of phosphatidylcholines containing fatty acids of different chain lengths that were in the liquid-crystalline phase at the assay temperature, the longer the fatty acyl chains (the thicker the bilayer), the greater DesK's autokinase activities were (20, 45). The in vivo experiments showed that inhibition of fatty acid synthesis by the addition of cerulenin, a potent and specific inhibitor of the type II fatty acid synthase (51, 69), results in increased levels of short-chain fatty acids in membrane phospholipids that lead to inhibition of the transmembrane-input thermal control of DesK (52). Furthermore, reduction of phospholipid synthesis by conditional inactivation of the PlsC acyltransferase (the enzyme that acylates acyl-glycerol phosphate) (51, 73) causes significantly elevated incorporation of long-chain fatty acids into membrane lipids, leading to constitutive upregulation of the *des* gene (52). Together, these studies are comfortingly consistent with the hypothesis that native DesK regulation is linked to changes in membrane thickness that could trigger buoy-dependent conformational changes in its multispanning sensor domain. Nevertheless, the delicate balance of protein conformation and communication between DesK protein subdomains must always be kept in mind. It seems unlikely that artificial, chimeric constructs (such as MS-DesK) retain all of the properties of their intact progenitors. It is therefore advisable to be cautious about overinterpreting results from experiments utilizing such unnatural constructs.

THE MEMBRANE AS AN ALLOSTERIC REGULATOR OF DesK FUNCTION

It has been noted that single amino acid replacements of several MS-DesK residues favor either a kinase or a phosphatase state regardless of the environmental temperature (20, 35). On the one hand, this means that the free energies of both signaling states are similar at around 30°C, with a small excess of the phosphatase-on or kinase-on populations at higher or lower temperatures, respectively. On the other hand, the energetic barrier separating both signaling states is expected to be very large because the associated conformational change entails large-scale motions in the greater part of the protein, as evidenced by the X-ray structures of the catalytic domain (**Figure 3**). Thus, it is tempting to speculate that temperature-associated bilayer deformations drive conformational changes by subtly altering their relative free energies and overcoming the overall free-energy barrier between different DesK conformations. Thus, the lipid bilayer with its associated thermal properties serves as an allosteric regulator of DesK function.

CONCLUDING REMARKS

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. Temperature sensing is essential for the survival of almost all living cells (60). However, despite the great efforts to understand the fundamental process of thermosensation, the molecular mechanisms underlying this process are yet to be discovered. Therefore, our knowledge of temperature sensing by bacterial sensors of membrane viscosity may ultimately contribute to the understanding of the thermosensing mechanisms of higher organisms' cold sensors. Because almost all eukaryotic cold sensors are integral membrane proteins, it is possible that the motion of α helices in response to membrane structure is involved in the signal transduction mechanisms used by these sensor proteins to guarantee the maintenance of thermal homeostasis.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Many thanks to the following colleagues, who provided ideas and/or comments on a draft of this article: Luciano Abriata, Daniela Albanesi, Larisa Cybuslki, Celina Galles, Maria C. Mansilla, Rafael G. Oliveira, and Emilio Saita. I thank Dr. Luciana Paoletti, Dr. Maria C. Mansilla, and Emilio Saita for help in generating illustrations. I am a Career Investigator from CONICET, and this research was partially supported by grant PICT 2678 from Promoción Científica y Tecnológica (FONCYT).

LITERATURE CITED

1. Aguilar PS, Cronan JE, de Mendoza D. 1998. A *Bacillus subtilis* gene induced by cold shock encodes a membrane phospholipid desaturase. *J. Bacteriol.* 180:2194–200
2. Aguilar PS, Hernandez-Arriaga AM, Cybulski LE, Erazo AC, de Mendoza D. 2001. Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*. *EMBO J.* 20:1681–91
3. Aguilar PS, Lopez P, de Mendoza D. 1999. Transcriptional control of the low-temperature-inducible *des* gene, encoding the Δ5 desaturase of *Bacillus subtilis*. *J. Bacteriol.* 181:28–33
4. Albanesi D, Mansilla MC, de Mendoza D. 2004. The membrane fluidity sensor DesK of *Bacillus subtilis* controls the signal decay of its cognate response regulator. *J. Bacteriol.* 186:2655–63
5. Albanesi D, Martín M, Trajtenberg F, Mansilla MC, Haouz A, et al. 2009. Structural plasticity and catalysis regulation of a thermosensor histidine kinase. *Proc. Natl. Acad. Sci. USA* 106:16185–90
6. Altabe SG, Aguilar P, Caballero GM, de Mendoza D. 2003. The *Bacillus subtilis* acyl lipid desaturase is a Δ5 desaturase. *J. Bacteriol.* 185:3228–31
7. Altabe SG, Mansilla MC, de Mendoza D. 2013. Remodeling of membrane lipids by bacterial desaturases. In *Stearoyl-CoA Desaturase Genes in Lipid Metabolism*, ed. JM Ntambi, pp. 209–231. New York: Springer
8. Andersen OS, Koeppe RE. 2007. Bilayer thickness and membrane protein function: an energetic perspective. *Annu. Rev. Biophys. Biomol. Struct.* 36:107–30
9. Beckering CL, Steil L, Weber MHW, Volker U, Marahiel MA. 2002. Genomewide transcriptional analysis of the cold shock response in *Bacillus subtilis*. *J. Bacteriol.* 184:6395–402
10. Beranová J, Jemioła-Rzemińska M, Elhottová D, Strzałka K, Konopásek I. 2008. Metabolic control of the membrane fluidity in *Bacillus subtilis* during cold adaptation. *Biochim. Biophys. Acta* 1778:445–53
11. Beranová J, Mansilla MC, de Mendoza D, Elhottová D, Konopásek I. 2010. Differences in cold adaptation of *Bacillus subtilis* under anaerobic and aerobic conditions. *J. Bacteriol.* 192:4164–71
12. Butterworth PH, Bloch K. 1970. Comparative aspects of fatty acid synthesis in *Bacillus subtilis* and *Escherichia coli*. *Eur. J. Biochem.* 12:496–501
13. Chazarreta-Cifre L, Martiarena L, de Mendoza D, Altabe SG. 2011. Role of ferredoxin and flavodoxins in *Bacillus subtilis* fatty acid desaturation. *J. Bacteriol.* 193:4043–48
14. Coskun U, Simons K. 2011. Cell membranes: the lipid perspective. *Structure* 19:1543–48
15. Cronan JE. 1978. Molecular biology of bacterial membrane lipids. *Annu. Rev. Biochem.* 47:163–89
16. Cronan JE. 2006. A bacterium that has three pathways to regulate membrane lipid fluidity. *Mol. Microbiol.* 60:256–59
17. Cronan JE, Gelmann EP. 1975. Physical properties of membrane lipids: biological relevance and regulation. *Bacteriol. Rev.* 39:232–56
18. Cybulski LE, Albanesi D, Mansilla MC, Altabe S, Aguilar PS, de Mendoza D. 2002. Mechanism of membrane fluidity optimization: isothermal control of the *Bacillus subtilis* acyl-lipid desaturase. *Mol. Microbiol.* 45:1379–88

19. Cybulski LE, del Solar G, Craig PO, Espinosa M, de Mendoza D. 2004. *Bacillus subtilis* DesR functions as a phosphorylation-activated switch to control membrane lipid fluidity. *J. Biol. Chem.* 279:39340–47
20. Cybulski LE, Martín M, Mansilla MC, Fernández A, de Mendoza D. 2010. Membrane thickness cue for cold sensing in a bacterium. *Curr. Biol.* 20:1539–44
21. de Mendoza D, Cronan JE. 1983. Thermal regulation of membrane lipid fluidity in bacteria. *Trends Biochem. Sci.* 8:49–52
22. Diaz AR, Mansilla MC, Vila AJ, de Mendoza D. 2002. Membrane topology of the acyl-lipid desaturase from *Bacillus subtilis*. *J. Biol. Chem.* 277:48099–106
23. Dowhan W, Bogdanov M. 2009. Lipid-dependent membrane protein topogenesis. *Annu. Rev. Biochem.* 78:515–40
24. Fujii DK, Fulco AJ. 1977. Biosynthesis of unsaturated fatty acids by bacilli: hyperinduction and modulation of desaturase synthesis. *J. Biol. Chem.* 252:3660–70
25. Fulco AJ. 1967. The effect of temperature on the formation of Δ^5-unsaturated fatty acids by bacilli. *Biochim. Biophys. Acta* 144:701–3
26. Fulco AJ. 1969. The biosynthesis of unsaturated fatty acids by bacilli. I. Temperature induction of the desaturation reaction. *J. Biol. Chem.* 244:889–95
27. Fulco AJ. 1974. Metabolic alterations of fatty acids. *Annu. Rev. Biochem.* 43:215–41
28. Fulco AJ. 1983. Fatty acid metabolism in bacteria. *Prog. Lipid Res.* 22:133–60
29. Fulco AJ. 2002. My Bloch years: 1961–1963 and beyond. *Biochem. Biophys. Res. Commun.* 292:1221–26
30. Fulco AJ, Bloch K. 1962. Cofactor requirements for fatty acid desaturation in *Mycobacterium phlei*. *Biochim. Biophys. Acta* 63:545–46
31. Fulco AJ, Bloch K. 1964. Cofactor requirements for the formation of Δ^9-unsaturated fatty acids in *Mycobacterium phlei*. *J. Biol. Chem.* 239:993–97
32. Fulco AJ, Levy R, Bloch K. 1964. The biosynthesis of Δ^9 and Δ^5 monosaturated fatty acids by bacteria. *J. Biol. Chem.* 239:998–1003
33. Gao R, Lynn DG. 2007. Integration of rotation and piston motions in coiled-coil signal transduction. *J. Bacteriol.* 189:6048–56
34. Grau R, de Mendoza D. 1993. Regulation of the synthesis of unsaturated fatty acids by growth temperature in *Bacillus subtilis*. *Mol. Microbiol.* 8:535–42
35. Inda ME, Vandenbranden M, Fernandez A, de Mendoza D, Ruysschaert JM, Cybulski L. 2014. A lipid-mediated conformational switch modulates the thermosensing activity of DesK. *Proc. Natl. Acad. Sci. USA* 111:3579–84
36. Kaneda T. 1991. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiol. Rev.* 55:288–302
37. Klein W, Weber MH, Marahiel MA. 1999. Cold shock response of *Bacillus subtilis*: isoleucine-dependent switch in the fatty acid branching pattern for membrane adaptation to low temperatures. *J. Bacteriol.* 181:5341–49
38. Lombardi FJ, Fulco AJ. 1980. Temperature-mediated hyperinduction of fatty acid desaturation in preexisting and newly formed fatty acids synthesized endogenously in *Bacillus megaterium*. *Biochim. Biophys. Acta* 618:359–63
39. Los DA, Murata N. 2004. Membrane fluidity and its roles in the perception of environmental signals. *Biochim. Biophys. Acta* 1666:142–57
40. Los DA, Zorina A, Sinetova M, Kryazhov S, Mironov K, Zinchenko VV. 2010. Stress sensors and signal transducers in cyanobacteria. *Sensors* 10:2386–415
41. Magnuson K, Jackovski S, Rock CO, Cronan JE Jr. 1993. Regulation of fatty acid biosynthesis in *Escherichia coli*. *Microbiol. Rev.* 57:522–42
42. Mansilla MC, Cybulski LE, Albanesi D, de Mendoza D. 2004. Control of membrane lipid fluidity by molecular thermosensors. *J. Bacteriol.* 186:6681–88
43. Mansilla MC, de Mendoza D. 2005. The *Bacillus subtilis* desaturase: a model to understand phospholipid modification and temperature sensing. *Arch. Microbiol.* 183:229–35
44. Martín M, Albanesi D, Alzari PM, de Mendoza D. 2009. Functional in vitro assembly of the integral membrane bacterial thermosensor DesK. *Protein Expr. Purif.* 66:39–45

45. Martín M, de Mendoza D. 2013. Regulation of *Bacillus subtilis* DesK thermosensor by lipids. *Biochem. J.* 451(2):269–75
46. Martin N, Lombardía E, Altabe SG, de Mendoza D, Mansilla MC. 2009. A *lipA* (*YutB*) mutant, encoding lipoic acid synthase, provides insight into the interplay between branched-chain and unsaturated fatty acid biosynthesis in *Bacillus subtilis*. *J. Bacteriol.* 191:7447–55
47. Ntambi J. 2004. Regulation of stearoyl-coA desaturases and role in metabolism. *Prog. Lipid Res.* 43:91–104
48. Nyholm TKM, Ozdirekcan S, Killian JA. 2007. How protein transmembrane segments sense the lipid environment. *Biochemistry* 46:1457–65
49. Ottemann KM, Xiao W, Shin YK, Koshland DE. 1999. A piston model for transmembrane signaling of the aspartate receptor. *Science* 285:1751–54
50. Parkinson JS. 2010. Signaling mechanisms of HAMP domains in chemoreceptors and sensor kinases. *Annu. Rev. Microbiol.* 64:101–22
51. Parsons JB, Rock CO. 2013. Bacterial lipids: metabolism and membrane homeostasis. *Prog. Lipid Res.* 52:249–76
52. Porrini L, Cybulski L, Altabe SG, Mansilla MC, de Mendoza D. 2014. Cerulenin inhibits unsaturated fatty acids synthesis in *Bacillus subtilis* by modifying the input signal of DesK thermosensor. *MicrobiologyOpen*. 3:213–24
53. Quinn PJ. 2012. Lipid-lipid interactions in bilayer membranes: married couples and casual liaisons. *Prog. Lipid Res.* 51:179–98
54. Ranck JL, Letellier L, Shechter E, Krop B, Pernot P, Tardieu A. 1984. X-ray analysis of the kinetics of *Escherichia coli* lipid and membrane structural transitions. *Biochemistry* 23:4955–61
55. Sackman E. 1995. Physical basis of self-organization and function of membranes: physics of vesicles. In *Handbook of Biological Physics*, ed. R Lipowsky, E Sackmann, pp. 215–303. Amsterdam: Elsevier
56. Sakamoto T, Murata N. 2002. Regulation of the desaturation of fatty acids and its role in tolerance to cold and salt stress. *Curr. Opin. Microbiol.* 5:208–10
57. Scheuerbrandt G, Bloch K. 1962. Unsaturated fatty acids in microorgansims. *J. Biol. Chem.* 237:2064–68
58. Scheuerbrandt G, Goldfine H, Baranowsky PE, Bloch K. 1961. A novel mechanism for the biosynthesis of unsaturated fatty acids. *J. Biol. Chem.* 236:PC70–71
59. Seelig J, Seelig A. 1980. Lipid conformation in model membranes and biological membranes. *Q. Rev. Biophys.* 13:19–61
60. Sengupta P, Garrity P. 2013. Sensing temperature. *Curr. Biol.* 23:R304–7
61. Shanklin J, Guy JE, Mishra G, Lindqvist Y. 2009. Desaturases: emerging models for understanding functional diversification of diiron-containing enzymes. *J. Biol. Chem.* 284:18559–63
62. Shanklin J, Somerville C. 1991. Stearoyl-acyl-carrier-protein desaturase from higher plants is structurally unrelated to the animal and fungal homologs. *Proc. Natl. Acad. Sci. USA* 88:2510–14
63. Shimura Y, Shiraiwa Y, Susuzuki I. 2012. Characterization of the subdomains in the N-terminal region histidine kinase Hik33 in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.* 53:1255–66
64. Strickland D, Moffat K, Sosnick TR. 2008. Light-activated DNA binding in a designed allosteric protein. *Proc. Natl. Acad. Sci. USA* 105:10709–14
65. Stukey JE, McDonough VM, Martin CE. 1990. The *ole1* gene of *Saccharomyces cerevisiae* encodes the Δ9 fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. *J. Biol. Chem.* 265:20144–49
66. Suzuki I, Kanesaki Y, Mikami K, Kanehisa M, Murata N. 2001. Cold-regulated genes under control of the cold sensor Hik33 in *Synechocystis*. *Mol. Microbiol.* 40:235–44
67. Suzuki I, Los DA, Kanesaki Y, Mikami K, Murata N. 2000. The pathway for perception and transduction of low-temperature signals in *Synechocystis*. *EMBO J.* 19:1327–34
68. Suzuki I, Los DA, Murata N. 2000. Perception and transduction of low-temperature signals to induce desaturation of fatty acids. *Biochem. Soc. Trans.* 28:628–30
69. Trajtenberg F, Altabe S, Larrieux N, Ficarra F, de Mendoza D, et al. 2014. Structural insights into bacterial resistance to cerulenin. *FEBS J.* 10:2324–38
70. Vigh L, Los DA, Horváth I, Murata N. 1993. The primary signal in the biological perception of temperature: Pd-catalyzed hydrogenation of membrane lipids stimulated the expression of the *desA* gene in *Synechocystis* PCC6803. *Proc. Natl. Acad. Sci. USA* 90(19):9090–94

71. Weber MH, Klein W, Müller L, Niess UM, Marahiel MA. 2001. Role of the *Bacillus subtilis* fatty acid desaturase in membrane adaptation during cold shock. *Mol. Microbiol.* 39:1321–29
72. Yadav NS, Wierzbicki A, Aegerter M, Caster CS, Pérez-Grau L, et al. 1993. Cloning of higher plant ω-3 fatty acid desaturases. *Plant Physiol.* 103:467–76
73. Zhang Y-M, Rock CO. 2008. Membrane lipid homeostasis in bacteria. *Nat. Rev. Microbiol.* 6:222–33