Activation of the bacterial thermosensor DesK involves a serine zipper dimerization motif that is modulated by bilayer thickness

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DesK is a bacterial thermosensor protein involved in maintaining membrane fluidity in response to changes in environmental temperature. Most likely, the protein is activated by changes in membrane thickness, but the molecular mechanism of sensing and signaling is still poorly understood. Here we aimed to elucidate the mode of action of DesK by studying the so-called "minimal sensor DesK" (MS-DesK), in which sensing and signaling are captured in a single transmembrane segment. This simplified version of the sensor allows investigation of membrane thickness-dependent protein–lipid interactions simply by using synthetic peptides, corresponding to the membrane-spanning parts of functional and nonfunctional mutants of MS-DesK incorporated in lipid bilayers with varying thicknesses. The lipid-dependent behavior of the peptides was investigated by circular dichroism, tryptophan fluorescence, and molecular modeling. These experiments were complemented with in vivo functional studies on MS-DesK mutants. Based on the results, we constructed a model that suggests a new mechanism for sensing in which the protein is present as a dimer and responds to an increase in bilayer thickness by membrane incorporation of a C-terminal hydrophilic motif. This results in exposure of three serines on the same side of the transmembrane helices of MS-DesK, triggering a switching of the dimerization interface to allow the formation of a serine zipper. The final result is activation of the kinase state of MS-DesK.

thermosensing | two-component system | lipid–protein interaction | helix–helix interaction | transmembrane helix dimerization

All organisms have to be able to rapidly adapt to a vast variety of external stimuli to survive. In bacteria, two-component signal transduction systems are some of the most abundant mechanisms for sensing and adapting to changes in the extracellular environment. These systems mediate responses in chemotaxis and phototaxis, and regulate feedback to changes in osmolarity, redox state, and temperature (1, 2). However, despite the evident importance of two-component systems for bacterial survival, the molecular mechanisms of signal transduction via these systems have barely begun to be untangled.

The DesKR system is a two-component system first identified in the soil bacterium *Bacillus subtilis* (3). Together with other regulatory systems, it is involved in maintaining membrane fluidity when the environmental temperature changes. The DesKR system works as follows. The actual thermosensor—i.e., the protein that senses the temperature change—is DesK. This protein consists of five transmembrane helices and an intracellular catalytic domain (DesKC) and is believed to function as a dimer (2, 4). In response to decreased environmental temperature, DesKC phosphorylates the response regulator DesR, which in turn controls the expression levels of the effector enzyme, a desaturase. This desaturase is inserted into the membrane, where it can introduce double bonds into preexisting lipids, allowing the recovery of membrane fluidity at this lower temperature (3).

In the present study, we focused on the first step of the signaling pathway, examining how the sensor is able to sense and transmit a temperature-dependent signal. This challenge was recently simplified by the discovery that both the sensing and the signal transduction properties of the membrane-spanning part of DesK can be captured into a single transmembrane segment by fusing the N-terminal part of the first transmembrane segment to the C-terminal part of the fifth transmembrane segment (5). The resulting protein is called the minimal sensor DesK (MS-DesK).

Importantly, MS-DesK shows a temperature-dependent switch in activity comparable to the full-length DesK not only in vivo, but also when reconstituted in protein-free lipid bilayers made from bacterial lipids (5). Therefore, no other membrane proteins are involved in sensing or signal transduction. Furthermore, the activity of the catalytic domain DesKC itself, is not temperature-sensitive (5, 6), and thus it must be the transmembrane segment of MS-DesK that somehow reacts to changes in temperature, most likely by sensing corresponding changes in the physical properties of the lipids.

Which properties of the membrane could be sensed by DesK and MS-DesK? On a decrease in environmental temperature, membrane

Significance

The ability to sense and respond to environmental signals is essential for cell survival. Unraveling the molecular mechanisms underlying signaling processes remains a challenge, however. Here we present a model for the mode of action of a bacterial thermosensor. The physical stimulus for activation appears to be a temperature-induced increase in membrane thickness, to which the sensor responds by elongation of its transmembrane helix. This leads to exposure of three serine residues on one side of the helix, inducing reorientation of adjacent helices to allow the formation of a serine zipper, which then acts as trigger for kinase activation. The reversible formation of a serine zipper represents a novel mechanism by which membrane-embedded sensors may detect and transmit signals.

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Table 1. Amino acid sequences of the peptides used in this study

Peptide	Sequence
MS-TMS	MIKNHFTFQK LNGITPYVIT LISAILLPWS IKSRKERERL EEK
4V-TMS	MIKNHFTFQK LNGITPYVIT LISVVVVAIL LPWSIKSRKE RERLEEK

Sequences of the synthetic peptides corresponding to the transmembrane region of MS-DesK and the 4V extension (yellow) of MS-DesK (MS-TMS and 4V-TMS, respectively) with the SB motif (red) and the C-terminal charged linker (blue). A Trp (green) was incorporated instead of a Phe to allow for fluorescence measurements.

lipids become more ordered, and consequently the membrane becomes thicker (7). Some evidence suggests that such changes in membrane thickness may be a key factor in the regulation of DesK sensing and signaling. First, an MS-DesK length mutant (4V) containing four extra valines in the C-terminal region of its transmembrane segment was found to be inactive and to remain locked in the phosphatase state on a decrease in temperature (5). Second, reconstitution studies showed increasing activity of both DesK and MS-DesK with increasing acyl chain length of the lipids in which the protein is reconstituted (5, 8). Third, increased incorporation of long-chain fatty acids into the membrane lipids was found to stimulate kinase activity of DesK in vivo, whereas increased levels of short-chain fatty acids result in loss of activity (9).

How can membrane thickness regulate the activity of DesK? It has been shown that the N terminus of MS-DesK at the exoplasmic side of the membrane contains a motif that may render the protein sensitive to membrane thickness and interfacial hydration. This motif contains two hydrophilic amino acids, K10 and N12, that are essential for activity (5) and that presumably are located within the transmembrane region just below the lipid–water interface. Because their side chains can snorkel to the hydrophilic membrane–water interface, these amino acids were proposed to act as a buoy, stabilizing the position of the transmembrane segment. For this reason, this has been called the sunken-buoy (SB) motif (5). In addition to the SB motif, a charged linker region at the intracellular membrane–water interface was found to be important for activity (10). It has been proposed that both motifs act together as a molecular gauge that senses membrane thickness and thereby regulates the switching of activity of the intracellular catalytic domain of DesK (10). The molecular details of the mode of action of this molecular gauge have remained elusive, however.

Because the activity of MS-DesK is most likely regulated by direct interactions of its single transmembrane segment with surrounding lipids, as discussed above, this system is ideally suited for studies on relatively simple model membranes. In such model systems, the biological complexity of the host membrane is reduced to allow for systematic studies. Here, to gain insight into the molecular mode of action of the sensor, we studied the behavior of peptides mimicking the membrane-spanning parts of a functional mutant and a nonfunctional mutant of MS-DesK in synthetic lipid bilayers by spectroscopic techniques and molecular modeling. Combined with in vivo functional studies on MS-DesK mutants and cross-linking experiments, our results lead to a new model of thermosensing in which changes in bilayer thickness trigger a switch between distinct dimerization interfaces within the membrane, resulting in activation of the sensor.

Results

The MS-DesK Transmembrane Segment Adopts a Bilayer Thickness-Dependent α-Helical Structure. To study how membrane thickness may modulate the behavior of MS-DesK, we incorporated peptides corresponding to a functional and a nonfunctional MS-DesK transmembrane segment (Table 1) into model membranes of phosphatidylcholine (PC) lipids. These synthetic lipids were chosen because they allow the investigation of changes in membrane thickness by systematically varying the lipid acyl chain length. Furthermore, MS-DesK is active when incorporated into PC lipids and shows higher activity with longer acyl chains (5).

We used circular dichroism (CD) to investigate whether bilayer thickness affects secondary structure. Fig. 1*A* shows the CD spectra of the transmembrane segment of MS-DesK (MS-TMS) in different bilayers. All spectra are characteristic for mainly the α-helical structure; however, in thicker membranes, the negative peak at 208 nm has higher intensity, and the intersection with the *x*-axis is shifted to slightly lower wavelengths. Deconvolution of the spectra shows that helicity increases from ∼55% in thin membranes to ∼65% in thicker membranes (Table 2), which would correspond to approximately four amino acids and thus the folding of approximately one helix turn.

Fig. 1*B* shows that the CD spectra of an elongated peptide corresponding to an inactive minimal sensor (4V-TMS) also exhibit a mostly α-helical character. However, in contrast to MS-TMS, here no change in helicity is observed on increasing bilayer thickness, and the number of residues present as α-helix in 4V-TMS (∼24 amino acids) is similar to that in MS-TMS in thinner membranes (Table 2). A regular helix of this length would correspond to ∼36 Å, which in principle would suffice to span even membranes of 22:1 PC (11). Why then are MS-TMS and 4V-TMS behaving in such a dissimilar manner? An important difference between the two helices is their hydrophobicity. For 4V-TMS, a helix of 24 amino acids between residues 11 and 35 will be hydrophobic and uncharged, whereas for MS-TMS, any helix with a length of 24 residues or more will contain at least one charged Lys residue and also will include more hydrophilic residues (Table 1). This will make it energetically less favorable for MS-TMS to incorporate in thicker membranes. Thus, MS-TMS will have to react to increases in bilayer thickness in a manner that possibly involves helix elongation and that allows for relief of the hydrophobic mismatch.

The MS-DesK Transmembrane Segment Shows a Bilayer Thickness-Dependent Positioning in the Membrane. Further insight into the interaction of MS-DesK with lipids was obtained by fluorescence spectroscopy. In the peptides used in this study, a phenylalanine residue near the C-terminal membrane–water interface was substituted for a tryptophan (Table 1). This allowed for further study of membrane thickness-dependent properties of the peptides by monitoring the Trp emission maximum, which depends on the polarity of the environment (12). Mutagenesis and functional analysis showed that the corresponding MS-DesK mutant is active (Fig. S1).

Trp fluorescence spectra of MS-TMS in model membranes (Fig. 2*A*) show emission maxima at a lower wavelength (near 345 nm) than for a soluble tryptophan (near 356 nm), consistent with a position of the peptide inside the membrane with its Trp residues

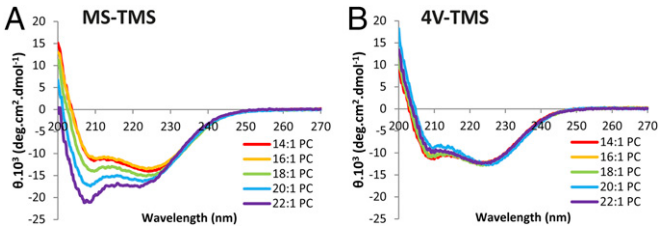

Fig. 1. CD spectra of MS-TMS (*A*) and 4V-TMS (*B*) in lipid vesicles of 14:1 PC (red), 16:1 PC (yellow), 18:1 PC (green), 20:1 PC (blue), and 22:1 PC (purple). The peptide:lipid ratio was 1:50, and measurements were performed at 25 °C. The data are a representative set of two independent experiments.

Table 2. Secondary structural elements in relation to membrane thickness of MS-TMS and 4V-TMS

Lipids		MS-TMS				4V-TMS			
Fatty acid chain length	Hydrophobic thickness, Å	α-helix, %	α-helix, no. of residues	Sheet, %	Random coil, %	α-helix, %	α-helix, no. of residues	Sheet, %	Random coil, %
14:1 PC	23	56 ± 2	24 ± 1	9 ± 1	35 ± 1	50 ± 3	24 ± 1	9 ± 2	41 ± 1
16:1 PC	26	55 ± 3	24 ± 1	9 ± 1	36 ± 3	52 ± 3	24 ± 1	8 ± 2	40 ± 1
18:1 PC	30	59 ± 2	25 ± 1	8 ± 1	33 ± 2	50 ± 1	24 ± 1	9 ± 1	41 ± 1
20:1 PC	33	63 ± 1	27 ± 1	6 ± 2	31 ± 1	52 ± 2	24 ± 1	10 ± 2	38 ± 1
22:1 PC	36	67 ± 2	29 ± 1	7 ± 3	26 ± 3	50 ± 3	24 ± 1	10 ± 1	40 ± 2

Percentages of secondary structural elements are averages with SDs of the three deconvolution programs of CD-Pro based on two independent experiments. The number of residues in the α-helix is calculated from the percentage and the total number of residues in the peptide. In the case of a regular α-helix, one residue corresponds to 1.5 Å α-helix length. The hydrophobic thicknesses are carbonyl-carbonyl distances derived from previous work (11).

close to the lipid–water interface (12). However, careful analysis of the emission maxima of MS-TMS as function of bilayer thickness shows complex behavior (Fig. 2*C*, blue line). The maximum shifts to slightly lower wavelength when the acyl chain length increases from C14 to C18, suggesting a more hydrophobic environment, but on a further increase in chain length from C18 to C22, the maximum shifts back to a somewhat higher wavelength. This behavior clearly differs from that of 4V-TMS (Fig. 2 *B* and *C*, red line), which is more straightforward. In thinner membranes, the emission maximum of 4V-TMS is shifted to a higher wavelength, in line with positioning of Trp closer to the aqueous phase owing to the longer transmembrane segment of 4V-TMS. More importantly, the spectrum shows a blue shift of the emission maximum with increasing chain length, as expected for a Trp residue that simply becomes located in a more hydrophobic environment on thickening of the bilayer. Qualitatively comparable results for MS-TMS and 4V-TMS were obtained with quenching experiments using the aqueous quencher acrylamide (Fig. S2 and Table S1).

How can we understand the complex behavior of MS-TMS? A tentative interpretation is that two events are involved with opposite outcomes. The first event could be a localization of Trp deeper into the membrane on increasing bilayer thickness, leading to a more hydrophobic environment. This event would then be dominant from 14:1 PC to 18:1 PC, whereas in thicker membranes a second event would become dominant, which places Trp in a more hydrophilic environment. What could this second event be? One possibility is that the elongation of the helix, as suggested by CD experiments, results in a change in polarity of the environment of the tryptophan residue. Alternatively, it is possible that the increase in membrane thickness, perhaps in combination with helix elongation, leads to additional events, such as dimerization of the peptide or reorientation of helices within a dimer, which then might result in a more hydrophilic environment of the Trp residue. This latter possibility seems realistic, given that all histidine kinases of two-component systems studied to date have been seen to function as a dimer (2) and that the crystal structure of the intracellular domain of DesK was found to be a dimer in both its functional state and a nonfunctional state (4). Taken together, these results raise the immediate questions of whether MS-TMS indeed does prefer to form dimers and, if so, what the conformations of these dimers would be. We addressed these questions next.

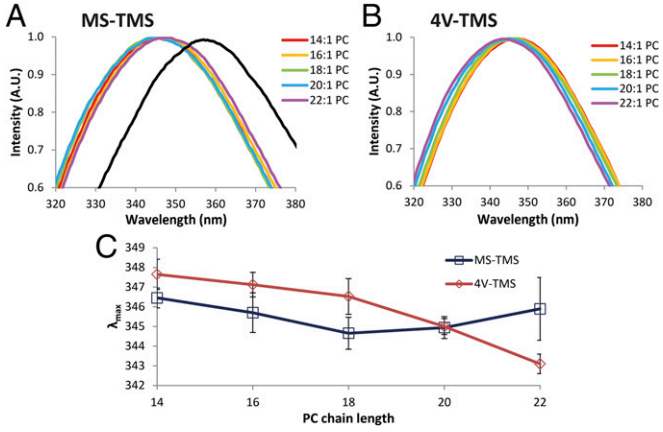

Fig. 2. Comparison of MS-TMS and 4V-TMS emission spectra in lipid vesicles with varying acyl chain lengths. (*A* and *B*) Emission spectra of soluble Trp (black), MS-TMS (*A*), and 4V-TMS (*B*) in lipid vesicles of 14:1 PC (red), 16:1 PC (orange), 18:1 PC (green), 20:1 PC (cyan), and 22:1 PC (magenta). (*C*) Emission maxima for MS-TMS (blue squares) and 4V-TMS (red diamonds) as a function of acyl chain length. The peptide:lipid ratio was 1:50, and measurements were performed at 25 °C. Error bars indicate the SD for three independent experiments.

Molecular Dynamics Simulations. To gain insight into the dimerization behavior of the transmembrane region of MS-DesK, we carried out large-scale coarse-grained (CG) molecular dynamics (MD) simulations on the MS-TMS sequence. In the absence of structural data, we chose the CG approach because it enables efficient screening of a large number of different dimer conformations; however, the nature of the CG model does not support changes in the secondary structure of the proteins, and thus it must be decided beforehand. As a first approximation, we modeled the MS-DesK sequence as a canonical α-helix for the transmembrane segment. Our goal here was not only to identify TMS dimer conformations, but also to establish shifts as a function of temperature. Specifically, we expected that the most populated dimer configuration at low temperature should become less populated at the higher temperature, and vice versa. With this goal in mind, we carried out MD simulations in a fully solvated 18:1 PC lipid bilayer as a function of temperature. We selected this bilayer because it has been well characterized with CG-MD simulations (13), and because it allowed us to detect possible shifts in dimer populations simply by varying temperature, while keeping the composition constant.

The data were collected at three different temperatures from 1,000 simulations for each temperature, providing a large pool of potential dimer conformations. The results thus obtained were interpreted by applying cluster analysis without using any experimental input, to avoid bias. To gain insight into the shifts between different cluster populations, we performed cluster analysis using all dimers found across all temperatures (Fig. S3). Overall, our cluster analysis suggests that MS-TMS behaves as a dimer, and that two distinct dimer conformations dominate at different temperatures. These two dimer conformations are shown in the corresponding lipid bilayer at 310K and 290K in Fig. 3. The dominating dimer conformation found at 300K is similar to the conformation at 310K, with an rmsd value of 0.28 nm between the central structures, and thus is not shown here. At high temperature, the residues S23 and S30 are found facing the lipid environment. At low temperature, the opposite situation is found, with residues S23 and S30 facing toward the dimer interface (Fig. S4). In this case, S33 also faces the interface and may contribute to helix–helix interactions (Figs. S4 and S5). The

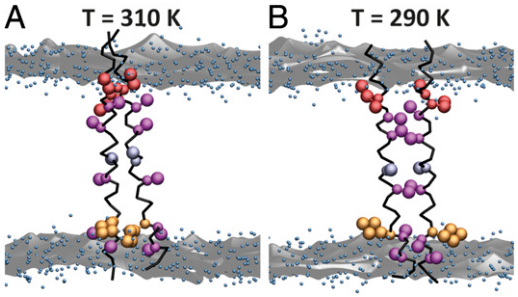

Fig. 3. Interfaces formed by MS-DesK dimer as found in MD simulations at 310K (*A*) and at 290K (*B*). The central structures of the major clusters are shown as representative conformations for each temperature. The peptide backbone is shown in black and key amino acids are highlighted as spheres. The amino acid residues are color-coded as follows: W29 in orange; T20 in light blue; T15, S23, S30, S33, and N12 in magenta as the residues forming the signal dimer interface; and Q9 and K10 in red. The location of lipid PO_4 groups is shown as a gray surface to indicate the position of the dimer in the bilayer. Water at the interface is shown in blue spheres.

opposite is observed for W29. At high temperature, W29 is distributed around the dimer interface, whereas at low temperature, it is oriented toward the water–lipid interface in most of the cluster members. Unfortunately, owing to the different setups and the fixed helix constraint, the MD simulations do not allow straightforward comparison with the fluorescence results on MS-TMS. Residue T20 does not appear to contribute to helix–helix interactions in either orientation.

Our simulations also suggest changes in the dimer interface structure at the N-terminal end. Residues Q9 face the dimer interface at 310K, whereas N12 faces the dimer interface at 290K, suggesting opposite rotations. The position of K10 remains the same at both temperatures, always facing the lipids and located close to the bilayer–water interface, which supports its role as an SB.

Thus, we can observe that at low temperature, MS-TMS is capable of forming an energetically favorable dimer interface defined by three serine residues located at the same face of the helix, which would form a serine zipper. Structural motifs formed by small residues such as serine have been previously suggested to promote strong and specific transmembrane helix–helix associations (14). In particular, the spatial arrangement of repeating serine residues recognized through analysis of interhelical H-bonds has been termed the "serine zipper" in analogy to the coiled-coil leucine zipper heptad motif (15). The structural arrangement of three serine residues (S23, S30, and S33) in the MS-TMS dimer forms a similar serine zipper motif and could be capable of driving helix–helix associations at low temperature. Our results suggest a model of reorientation of the dimer as a possible mechanism for activation of the thermosensor, which would be consistent with the fluorescence data.

Mutational Studies Confirm an Essential Zipper Motif. To directly test the importance of the serine zipper dimerization motif for activation of the MS-DesK in vivo, we introduced a series of point mutations that would either weaken or favor the putative dimerization interface. According to the MD model, mutations that eliminate serine residues at the C terminus would destabilize the serine zipper dimerization motif and thereby weaken the driving force for rotation, resulting in stabilization of the phosphatase state. Indeed, we found that the mutations S23A, S30A, and S33A all result in decreased kinase activity at 25 °C, favoring the phosphatase state of the sensor regardless of the temperature signal (Fig. 4 and Fig. S6). In accordance with the molecular modeling data, T20A seems to have no effect on MS-DesK signaling (Fig. 4).

As an additional control, we analyzed the effect of introducing an extra serine at position 27 of the postulated helix–helix interface. This would favor the kinase state owing to stabilization of the dimerization interface now constituted by four serines localized one turn apart in the helix: S23, S27, S30, and S33. We found that this construct indeed strongly stabilizes the kinase conformation (Fig. 4). We noted the same effects when these mutations were introduced in full-length DesK (Fig. S7), supporting the importance of a specific dimer interface for functional activity.

If a serine zipper connects the two helices at the C terminus of the transmembrane segment at low temperature, then the helices should be close enough to allow capture by cysteine cross-linking. The presence of a cysteine residue in the dimerization interface can serve as a label to detect the proximity of the monomers by formation of a disulfide bond (16). Cultures of *B. subtilis* strain carrying MS-DesK or MS-DesK with the mutation S30C were incubated at 25 °C with diamide (a membrane-permeable oxidant). Western blot analyses were performed to analyze the presence of monomers and dimers. As shown in Fig. 5, in MS-DesK S30C, cysteine residues are cross-linked, leading mainly to dimer formation, whereas MS-DesK without cysteine residues in the transmembrane region forms monomers. We obtained similar results when using variant MS-DesK S23C, S33C, or L27C (Fig. S8). As a control, we introduced a cysteine at position 31 (I31C). In this mutant, cysteine is located on the other side of the helix, and the ratio dimer to monomer is decreased, as expected. Taken together, these results confirm that the interface delineated by the serine zipper motif at the C terminus of the transmembrane segment is responsible for dimer stabilization, a requirement for stabilizing the kinase conformation.

Discussion

The Model: A Serine Zipper as a Kinase-Regulatory Motif. In this work, our specific aim was to decipher in molecular detail how the thermosensor DesK reacts to temperature changes by alternating between two opposite activities, kinase and phosphatase. By making use of a fully active but minimal version of DesK with only one membrane-spanning segment, and using complementary approaches, we obtained a detailed picture that allows us to propose a novel mechanism for thermosensing, as illustrated schematically in Fig. 6.

This model suggests that MS-DesK acts as a dimer at both high and low temperatures, but that it switches between two different conformations according to the physical state of the lipid bilayer. In response to decreasing temperature, the lipid acyl chains become more ordered, leading to an increase in bilayer thickness and decreases in fluidity and hydration (7). MS-DesK reacts to these physical changes by elongation of its transmembrane helix, resulting in location of three serine residues (S23, S30, and S33) at the same face of the helix. This serine zipper motif, located at the C-terminal region of the transmembrane segment, now becomes the dominant dimerization motif at low temperature, so that the helices will reorient to achieve the new lowest energy state of the

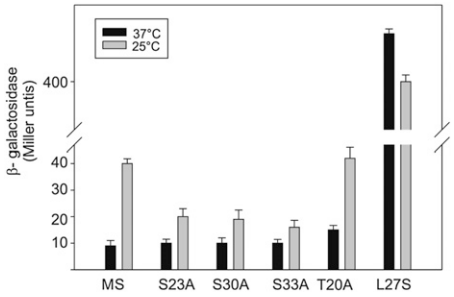

Fig. 4. Activity of MS-DesK variants with an altered hydrophilic motif at the C terminus of the TM segment. *B. subtilis* desK$^-$ cells harboring each of the MS-DesK point mutants were grown at 37 °C to an OD of 0.3 at 525 nm and divided into two samples. One sample was transferred to 25 °C (gray), and the other was kept at 37 °C (black). Aliquots were taken every hour, and β-galactosidase activity was determined. The values are representative of three independent experiments and correspond to 4 h after the shift.

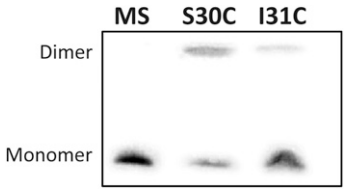

Fig. 5. Disulfide cross-linking of cysteine reporters. Cells expressing MS-DesK, MS-DesK S30C, or MS-DesK I31C were treated with diamide at 25 °C, and disulfide–cross-linked molecules were analyzed by Western blot analysis, as detailed in *Materials and Methods*.

dimer. In vivo, such a reorientation could be a mechanism by which a signal is transmitted to the cytoplasmic domain, resulting in a switch in the activity of DesK.

Our model is fully consistent with the results reported herein, as well as with data in the literature. The CD data suggest elongation of the transmembrane helix in thicker membranes for MS-TMS. This elongation most likely occurs at the C terminus, because the N-terminal SB motif can be pulled into the membrane with the lysine residue (K10) snorkeling toward the interface (5), but this same motif will prevent the N-terminal part from being pulled further into the membrane on further increases in bilayer thickness. On elongation of the C-terminal part of the helix, three hydrophilic serine residues (S23, S30, and S33) cluster together on one side of this helix, thereby constituting a potential serine zipper dimerization motif (15, 17, 18). These serines would be able to form interhelical H-bonds when located inside the membrane. Furthermore, dimerization of the transmembrane region of MS-DesK is consistent with the fluorescence data, and MD data confirm that MS-TMS is prone to form dimers inside the membrane, showing two dominant dimer interfaces. Moreover, the interface that includes the serine zipper motif is formed at low temperature, indicating that the serine zipper is associated with the active MS-DesK. Finally, our mutational and cross-linking studies confirm that the transmembrane part of MS-DesK forms mainly dimers in its active form with the serine zipper at the interface.

The model is also consistent with the data obtained for the 4V length mutant. The straightforward behavior of this mutant in model membranes may be related to its longer hydrophobic length, which takes away the driving force for reorientation of the helices on increasing bilayer thickness. In addition, it should be noted that the 4V extension interrupts the serine zipper motif. Consequently, S23 is spatially separated from S30 and S33 and also is not located on the same face of the helix. This would inhibit formation of the active dimer conformation.

Finally, in our model studies, the length of the acyl chains of PC varied from 14:1 to 22:1, corresponding to hydrophobic thicknesses of 23–36 Å (11). This does not necessarily mean that such large differences in thickness are required for the actual temperature switch of DesK in vivo, however. Indeed, changes in membrane thickness as a function of temperature are likely to be quite small, particularly when lipids remain in a fluid phase. However, it was recently suggested that the fluid phase-to-gel phase transition may contribute to the switch in activity of DesK in vivo (19). If the lipids approach their fluid-to-gel transition temperature on cooling, this could result in a greater increase in bilayer thickness. Thus, it is possible that other changes in membrane physical properties related to the phase transition may contribute to the activity switch of DesK. Nevertheless, regardless of whether exclusively thickness is sensed or is a combination of physical properties, our model remains valid: a molecular gauge consisting of the SB motif on one side of the membrane and the serine zipper motif on the other side, with the serine zipper acting as a reversible helix–helix association motif that is ultimately responsible for triggering the switch in DesK activity in response to temperature-induced changes in bilayer properties.

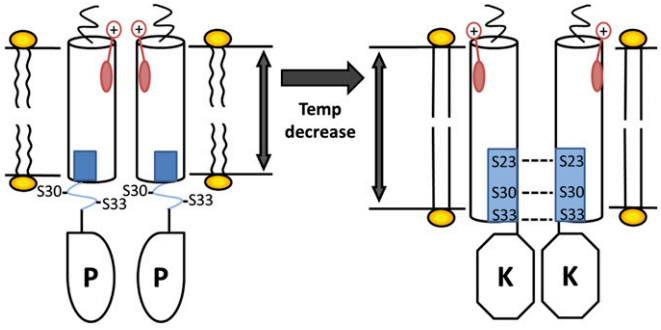

Fig. 6. Schematic representation of the mode of action of MS-DesK. Its transmembrane segment (MS-TMS) contains an N-terminal charged hydrophilic motif (K10 and N12; red), the so-called SB motif. The side chains of these residues can snorkel to the hydrophilic membrane interface and limit further downward vertical movement of the MS-TMS. The C terminus of the transmembrane part contains a hydrophilic motif of three serine residues (S23, S30, and S33; blue). Under standard conditions in a fluid membrane, these serines interact with the hydrophilic interface (*Left*). With decreasing temperature, the membrane thickens, and an extra turn of the helix 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 domain (DesKC) from the phosphatase to the kinase state.

Perspective. It has been shown that serine zippers represent a structural motif that tightly connects TM helices in several membrane proteins, including halorhodopsin, Ca-transporting ATPase, and cytochrome *c* oxidase (15). Here we present data for on the reversible formation of an intramembrane serine zipper motif subjected to regulation by physical properties of membrane lipids. Based on the level of conservation between two-component signal transduction systems (TCS) (1), and on the notion that our general model of switching between two different interfaces is consistent with other models for TCS (20, 21), we suggest that the reversible formation of a serine zipper represents a possibly more general mechanism by which membrane-embedded sensors may detect and transmit signals.

Materials and Methods

Materials. 1,2-Dimyristoleoyl-sn-glycero-3-phosphocholine [14:1 (Δ9-Cis) PC], 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine [16:1 (Δ9-Cis) PC], 1,2-dioleoyl-snglycero-3-phosphocholine [18:1 (Δ9-Cis) PC], 1,2-dieicosenoyl-sn-glycero-3-phosphocholine [20:1 (Δ11-Cis) PC], and 1,2-dierucoyl-sn-glycero-3-phosphocholine [22:1 (Δ13-Cis) PC] were obtained from Avanti Polar Lipids. Routine quality controls of the lipid stocks by TLC confirmed the purity of the lipids and the absence of degradation products. Synthetic peptides were obtained from Eurogentec as >95% pure peptides. Identity and purity were confirmed with mass spectrometry and analytical HPLC. Water was deionized and purified with a Milli-Q Gradient Water purification system (Millipore). All chemicals used were of analytical grade.

Sample Preparation. Samples were prepared as described previously (22). In brief, peptides dissolved in 0.5 mL of trifluoroethanol were added to 0.5 mL of the desired phospholipid dispersion. Excess water was added, and the samples were lyophilized after rapid freezing in liquid nitrogen. Vesicles were prepared by rehydrating the dry film at room temperature in 10 mM Pipes, 150 mM NaCl, and 1 mM EDTA (pH 7.4) for the fluorescence experiments and in 10 mM phosphate (pH 7.4) for the CD measurements. After 10 freeze–thaw cycles, the vesicles were extruded 10 times through 0.2-μm filters in an Avanti hand-held extrusion device. The final peptide concentration was 25 μM for the fluorescence experiments and 15 μM for the CD measurements, with a molar peptide:lipid ratio of 1:50 for all samples. Peptide concentrations were quantified by the average absorbance of tryptophan at 280 nm using a molar extinction coefficient of ε = 5,600 (23). Lipid concentrations were determined by a phosphorus titration according to the method of Rouser (24).

Fluorescence Measurements. Tryptophan fluorescence was measured on a Varian Cary Eclipse fluorescence spectrophotometer, using a 10-mm quartz cuvette, 5-mm excitation slit, 5- mm emission slit, 0.5-nm resolution, 1-s

averaging time, and 30-nm/min scan speed. Temperature was controlled with a Peltier device at 25 °C. The tryptophan residues were excited at 295 nm, and emission in the 300–500 nm region was recorded. The contribution of pure lipid samples was subtracted from the signal thus obtained. Because the optical densities of the samples were all <0.05, correction for the inner filter effect was unnecessary (25). The recorded spectra were fitted to a lognormal distribution using SigmaPlot software as described previously (12), normalized, and smoothed using a moving-average filter with a window size of 5. Acrylamide quenching of tryptophan fluorescence was performed by adding acrylamide in aliquots from a 3 M stock solution to each sample up to a concentration of ∼180 mM. The results were analyzed using Stern–Volmer plots (25).

CD. Measurements were carried out on a Jasco J-810 spectropolarimeter, with 1-mm path length quartz cuvette, 1-nm bandwidth, 0.2-nm resolution, 4-s response time, and 20-nm/min scan speed. Temperature was controlled with a Peltier device at 25 °C. For each measurement, 10 scans were recorded over a wavelength range of 200–270 nm. These spectra were averaged and normalized to molar ellipticity per residue. The spectra were deconvoluted with CDPro software (26).

Molecular Modeling. We carried out large-scale CG MD simulations on the MS-DesK sequence with the Martini 2.2 force field (27) and standard Martini settings in GROMACS 4.5.x (28). Martini is a widely used CG force field (29), and this type of approach has been proven successful in modeling helix–helix interactions in membrane environments (21, 30–34). Here we used a recently developed high-throughput method, the docking assay for transmembrane components (DAFT) (35), to screen MS-TMS dimers. For modeling, we used a sequence corresponding to MS-TMS in Table 1, but in which the six C-terminal residues were left out to reduce the size of the system. Further details are provided in *SI Materials and Methods*.

Plasmid and Strain Constructions. The *MS-DesK* gene and its variants were PCR-amplified from plasmid TM1/5-DesKC-pHPKS or from DesK-pHPKS for full-length DesK (5, 6). Site-directed mutagenesis was performed to introduce the mutations (T20A, S23A, S30A, S33A, and L27S for MS-DesK and T140A, S143A, S150A, and L147S for full-length DesK). The resulting plasmids were used to transform CM21 or AKP20 *B. subtilis* cells (3, 6). These strains lack *desK* and contain a transcriptional fusion between the reporter gene β-galactosidase and the promoter of the desaturase (gene up-regulated by DesK-DesR at low temperature), which allows for monitoring of DesK activity. The expression of DesK variants was induced by adding 0.1% xylose to the growth medium. All mutations were confirmed by DNA sequence analysis.

Bacterial Strains and Growth Conditions. *B. subtilis* JH642 strains were grown at 37 °C or 25 °C under 250 rpm gyration in Spizizen salts supplemented with 0.1% glycerol, 50 μg/mL each tryptophan and phenylalanine, 0.05% cas amino acids, and trace elements (36, 37). β-galactosidase activity was assayed in independent triplicates. The results shown are the average of three independent experiments and correspond to 4 h after the cold shock. For the disulfide cross-linking experiments, *B. subtilis* strain CM21 (desK$^-$) (6) carrying plasmid pHPKS with MS-DesK cysteine variants was grown at 37 °C in SPI medium supplemented with 0.8% xylose to induce MS-DesK expression. At $OD_{600} = 0.5$, cells were shifted to 25 °C and treated with 1 mM diamide (Sigma-Aldrich) for 30 min. Reactions were quenched by the addition of 10 mM *N*-ethylmaleimide. Cells were pelleted and then lysed by lysozyme treatment and sonication. After ultracentrifugation, the membrane proteins were analyzed by nonreducing SDS/PAGE and visualized by immunoblotting with the specific antiserum anti-DesK.

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