MINIREVIEW

Control of Membrane Lipid Fluidity by Molecular Thermosensors

María C. Mansilla, Larisa E. Cybulski, Daniela Albanesi, and Diego de Mendoza*

Instituto de Biología Molecular y Celular de Rosario (IBR) and Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

Bacteria can encounter a wide range of environments and must adapt to new conditions in order to survive. As the selective barrier between living cells and their environment, the plasma membrane plays a key role in cell viability. The barrier function of the cytoplasmic membrane is known to depend critically on the physical state of lipid bilayers (17), making it susceptible to changes in environmental temperature. 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. However, at lower temperatures, membrane lipid bilayers undergo a reversible change of state from a fluid (disordered) to a nonfluid (ordered) array of the fatty acyl chains (21, 56). The temperature at the midpoint of this transition is called the transition temperature, and the change of state accompanying an increase in temperature is called the lipid phase transition, the gel-liquid crystalline transition, or most properly, the order-disorder transition. The transition temperature is a function of the membrane lipid composition and, in organisms deficient in cholesterol, mainly depends on the fatty acid composition of the membrane lipids (21, 56). The (overly simplified) rule of thumb is that phospholipids that contain unsaturated fatty acids (UFAs) have much lower transition temperatures than those lipids made of saturated fatty acids (SFAs). The effect is due to different packing of the two types of phospholipid acyl chains. SFA acyl chains can pack tightly, but the steric hindrance imparted by the rigid kink of the *cis* double bond results in much poorer chain packing of UFAs, even below the phase transition temperature (16, 17).

From these considerations, it seems clear that bacteria and most (if not all) poikilothermic organisms must regulate their phase transition in response to temperature. Without regulation, an organism shifted from a high to a low temperature would have membrane lipids with suboptimal fluidity, resulting in subnormal membrane function. The mechanism of regulation in all of the cases examined seems to occur via the incorporation of proportionally more UFAs (or fatty acids of analogous properties) as the temperature decreases. This regulatory mechanism system, called thermal control of fatty acid synthesis, seems to be a universally conserved adaptation response allowing cells to maintain the appropriate fluidity of membrane lipids regardless of the ambient temperature. 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. The question arises, how do cells sense a change in temperature and adjust the fluidity of the membrane lipid bilayer accordingly?

Here, we discuss the basic features of thermal regulation of membrane lipid fluidity in *Escherichia coli* and *Bacillus subtilis*, in which the proposed mechanisms are firmly based on both genetic and biochemical evidence. Although the physiological consequences of this regulation are the same in both organisms, the mechanisms involved are entirely different.

TEMPERATURE-DEPENDENT CHANGES IN THE CONTENT OF UFAs OF GLYCEROPHOSPHOLIPIDS IN *E. COLI*

The molecular mechanism of regulation of UFA synthesis by growth temperature in *E. coli* was elucidated more than 20 years ago and has been discussed in a number of reviews (18, 19, 21). Here, we will briefly review this paradigmatic mechanism with the purpose of contrasting it with recent progress in the elucidation of the pathway controlling the synthesis of UFAs in *B. subtilis*.

E. coli has one of the simplest membrane bilayer phospholipid compositions found in nature, consisting of three phospholipids having only three different fatty acyl chains (19). The three fatty acids are an SFA, palmitic (hexadecanoic) acid, and two UFAs, palmitoleic (*cis*-9-hexadecenoic) acid and *cis*-vaccenic (*cis*-11-octadecenoic) acid. Marr and Ingraham first noted that *E. coli* adjusts its fatty acid composition in response to a decrease in temperature by increasing the amount of *cis*-vaccenic acid and reducing the amount of palmitic acid incorporated into membrane phospholipids (47). Lower growth temperatures result in an increase in the number of diunsaturated phospholipids in the membrane. At 37°C, palmitic acid occupies position 1 of the phospholipid backbone, whereas palmitoleic acid is found only at position 2. As the growth temperature is lowered, *cis*-vaccenic acid competes with palmitic acid for position 1 of the newly synthesized phospholipids (7, 16). This mechanism is thought to allow an organism to regulate the membrane fluidity to optimize its function at various growth temperatures. In *E. coli*, synthesis of the normal UFA content requires three enzymes, the products of the *fabA*, *fabB*, and *fabF* genes (Fig. 1A). FabA, β-hydroxydecanoyl dehydrase, is the key enzyme of the classic anaerobic pathway of UFA synthesis and introduces a *cis* (or Z) double bond into a 10-carbon intermediate (9). The *cis* intermediate is

---

* Corresponding author. Mailing address: Suipacha 531, Universidad Nacional de Rosario, S2002LRK Rosario, Argentina. Phone: 54-341-4350661. Fax: 54-341-4390465. E-mail: diegonet@citynet.com.ar.
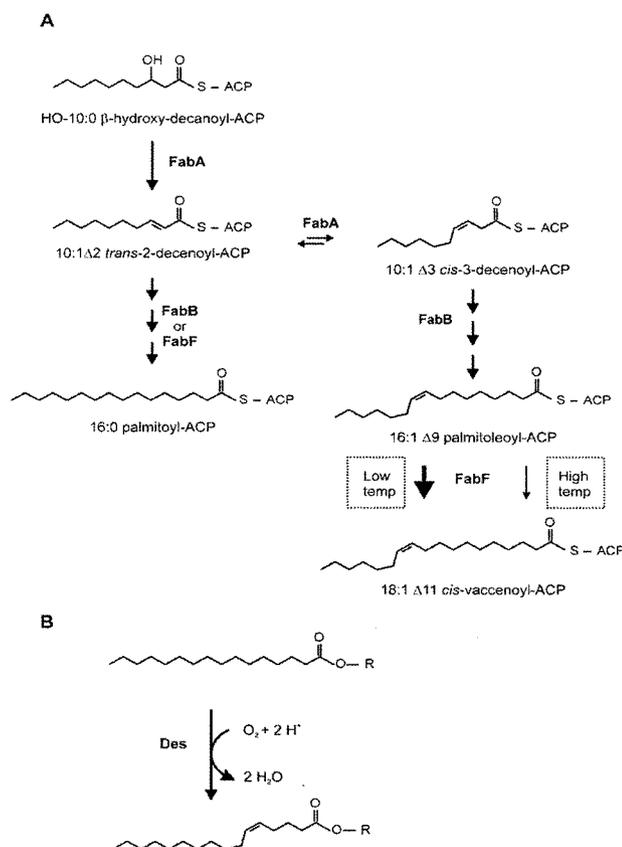
FIG. 1. UFA biosynthesis in *E. coli* and *B. subtilis*. (A) In *E. coli*, FabA catalyzes the key step in UFA production, introducing the double bond into the acyl chain at the 10-carbon intermediate. FabA is a bifunctional enzyme that catalyzes both the removal of water to generate *trans*-2-decenoyl-ACP and the isomerization of this intermediate to *cis*-3-decenol-ACP. The FabB enzyme is required for the elongation of these unsaturated acyl-ACP intermediates, and FabF participates in SFA synthesis and in the elongation of 16:1 Δ9 palmitoleoyl-ACP to 18:1 Δ11 *cis*-vaccenoyl-ACP. The reactivity of this enzyme toward 16:1 Δ9 palmitoleoyl-ACP is increased after a temperature (temp) downshift. (B) Reactants and products of a representative fatty acid desaturase reaction. This is a 2e⁻- and O_2-dependent dehydrogenation at an unactivated position of the fatty acyl chain, resulting in *cis*-double-bond formation. For stearoyl-CoA desaturase from mycobacteria (49), R is CoA; for acyl lipid desaturases from *Bacillus* (1, 32) and cyanobacteria (50), R is a phospholipid.

then elongated by FabB and FabF to form the UFAs found in the membrane phospholipids (21, 22, 29) and, at low growth temperatures, in lipid A (11). FabB and FabF are 3-ketoacyl-acyl carrier protein (ACP) synthase (KAS) enzymes (often referred to as condensing enzymes) (36). They catalyze fatty acid elongation by the addition of two-carbon units, originally derived from acetyl coenzyme A (CoA), until the ACP-bound chains become 12, 14, 16, or 18 carbons in length and are then substrates for incorporation into complex lipids (Fig. 1) (36). The *fabB* gene is defined by a class of mutants defective in UFAs and encodes KAS I (15, 29, 30). The *E. coli fabF* gene, encoding KAS II, was found to be defective in a class of mutants impaired in the elongation of palmitoleic acid to *cis*-vaccenic acid, a phenotype expected for a defect in chain elongation (i.e., KAS) activity (30, 31). Therefore, FabB and FabF

have distinct and nonoverlapping roles in *E. coli* UFA synthesis. FabB is thought to elongate the product of the FabA enzyme to the C_{12} unsaturated intermediate (19), whereas FabF is required to convert the C_{16} unsaturated species to the C_{18} species (Fig. 1) (30). The elucidation of the mechanism of temperature adaptation response in *E. coli* involved a number of independent observations, e.g., (i) deficiency in the synthesis of *cis*-vaccenic acid was due to mutations in the *fabF* gene, and these mutants were unable to control the fatty acid composition by growth temperature (29), and (ii) the increased rate of synthesis of *cis*-vaccenic acid observed at low growth temperatures was independent of de novo synthesis of mRNA and proteins (28). These lines of evidence indicated that thermal regulation of membrane fluidity in *E. coli* was an intrinsic property of the activity, rather than the synthesis, of the FabF condensing enzyme. In fact, it was demonstrated that palmitoleyl-ACP is an excellent substrate for FabF but not for FabB (25, 30). In vivo, the reactivity of FabF toward the substrate increases at low temperatures, leading to increased amounts of *cis*-vaccenic acid in membrane phospholipids (29).

LESSONS OF THE *E. COLI* PARADIGM

E. coli strains with *fabF* null mutants are unable to regulate their UFA contents, but they are viable at low growth temperatures (18). However, the FabF condensing enzyme is essential for survival at elevated hydrostatic pressure in the deep-sea bacterium *Photobacterium profundus* strain SS9 (5). This strain, harboring a disruption of the *fabF* gene, displays growth impairment at elevated hydrostatic pressure concomitant with diminished *cis*-vaccenic acid production. Moreover, its growth ability at elevated pressure can be restored to wild-type levels by the addition of *cis*-vaccenic acid (5). Since (i) high pressure causes a physical change in membrane lipids similar to a drop in temperature (44) and (ii) an *E. coli fabF* strain was as susceptible to elevated pressure as wild-type cells (5), it has been proposed that SS9 FabF has evolved novel pressure-responsive characteristics which facilitate SS9 growth at high pressure (5).

Although the *E. coli* FabB condensing enzyme poorly catalyzes the elongation of palmitoleic acid to *cis*-vaccenic acid, overproduction of the enzyme produces an appreciable increase in the *cis*-vaccenate contents of membrane phospholipids (22). Overexpression of FabB directed by a plasmid results in the accumulation of *cis*-vaccenic acid even in the absence of FabF expression. However, this increase is independent of the growth temperature (22), demonstrating that the soluble enzyme FabF is the sole protein responsible for thermal modulation of membrane phospholipid fluidity in *E. coli*. Notably, as expected from the *E. coli* FabF function, *Haemophilus influenzae*, which contains a sole FabB condensing enzyme and lacks a FabF homologue, is unable to thermally control membrane lipid composition (58). It has been suggested that the loss of this regulatory mechanism is likely to be due to the lack of environmental selection, since *H. influenzae* exists in nature only in extremely close association with warm-blooded animals (58).

EFFECT OF COLD SHOCK ON LIPID A BIOSYNTHESIS IN *E. COLI*

In addition to the temperature-dependent regulation of elongation of palmitoleic acid to *cis*-vaccenic acid by the FabF condensing enzyme, there is evidence for a second temperature-controlled reaction that modifies the fatty acid composition of lipid A, the hydrophobic membrane anchor of *E. coli* lipopolysaccharide. While palmitoleate is not present in lipid A from cells grown at 37°C, it comprises ~11% of the fatty acyl chains of lipid A in cells grown at 12°C (54). This increase in palmitoleic acid is counterbalanced by a decline in lauric acid. Recently, it has been reported that transcription of *lpxP*, which encodes a novel palmitoleoyl-ACP-dependent acyltransferase, is induced when cells are shifted from 30 to 12°C. This enzyme transfers palmitoleate from palmitoleoyl-ACP to Kdo_2-lipid IV_A, which is also the acceptor for the lauroyl transferase encoded by *lpxL* (13, 14). Although extracts of wild-type *E. coli* cells grown at 12°C contain both lauroyl and palmitoleoyl transferase activities, the mechanism by which the cells determine the amount of palmitoleate versus laureate that is incorporated into lipid A will require further study.

The acylation of lipid A with palmitoleate instead of laurate might therefore function to adjust outer membrane fluidity in *E. coli* cells shifted to low temperatures. In agreement with this hypothesis, it has been proved that the integrity of the outer membrane in a mutant lacking *lpxP* is altered during cold shock, as evidenced by its increased sensibility to antibiotics at low temperature (57). Moreover, genes encoding *lpxP* homologues are present in many gram-negative bacteria that survive for long periods outside of animal hosts, including *E. coli*, all serovars of *Salmonella enterica*, and *Klesbiella pneumoniae*, but are absent in pathogens that are transmitted from animal to animal without having to persist in the environment (57).

Elucidation of the mechanism that regulates the expression and activity of the palmitoleoyl transferase would be of particular interest, since LpxP is the first protein in *E. coli* specifically induced by cold shock whose activity has effects on the outer membrane that are beneficial to the cell.

THERMAL REGULATION OF MEMBRANE FATTY ACID COMPOSITION IN *B. SUBTILIS*

In *E. coli*, the protein encoded by the *fabA* gene anaerobically introduces the double bond, and FabB channels the metabolic intermediate into the mainstream of the fatty acid synthetic pathway (Fig. 1A). In contrast, eukaryotes (53) and certain bacteria, such as bacilli (24, 27), mycobacteria (49), and cyanobacteria (50), have completely separate systems for the synthesis of SFAs and UFAs. These organisms use fatty acid desaturase enzymes, which require molecular oxygen and reducing equivalents, to introduce the double bond into previously synthesized SFAs (Fig. 1B).

Bacillus cells respond to a decrease in the ambient growth temperature by increasing the proportion of low-melting-point fatty acids in the membrane lipids. This can be accomplished by desaturating the fatty acids of their membrane lipids (for a recent review, see reference 23) or by increasing the proportion of anteiso-branched-chain fatty acids (40, 41). Phospholipids with anteiso-branched-chain fatty acids have lower melt-

ing points than those with the corresponding iso-branched-chain fatty acids (43, 45, 46). Therefore, it has been proposed that this adaptive response could be important in providing an appropriate degree of membrane fluidity for growth at low temperatures (41). The "primer" carbons for the synthesis of branched-chain fatty acids are α-keto acids derived from valine, leucine, and isoleucine (39, 40) (Fig. 2). While isoleucine is the precursor of anteiso-branched-chain fatty acids, leucine and valine give rise to the primers for iso-branched-chain fatty acids (39, 40). In consequence, it has been hypothesized that the increased synthesis of anteiso-branched-chain fatty acids at low growth temperatures could be due to temperature regulation of the primer selection specificity of FabH, the condensing enzyme involved in the first step of fatty acid elongation (12), or to changes in the cellular primer pools for fatty acid synthesis (41). However, the biochemical mechanism responsible for adjusting the ratio of iso- to anteiso-branched-chain fatty acids is unknown. In contrast to the poorly studied mechanism of cold adjustment of branched-chain fatty acids in *Bacillus* spp., the temperature dependence of UFA biosynthesis has been extensively characterized in vivo in *Bacillus megaterium*. Fulco demonstrated, more than 30 years ago, that there is induction of a fatty-acid-desaturating system when the cultures of this bacterium are grown at low temperatures (27). It was also found that the levels of desaturation of cultures of *B. megaterium* transferred from 35 to 20°C far exceeded the levels of desaturation of cultures growing at 20°C (26). To explain the dramatic change in the lipid composition of bacilli shifted from 35 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 and Fulco (26) 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, our research group 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 almost exclusively synthesizes SFAs (32). However, when a culture grown at 37°C is transferred to 20°C, the synthesis of UFAs is induced (32, 33). As with *B. megaterium*, the desaturation system of *B. subtilis* requires de novo synthesis of RNA and proteins, since it is completely abolished by rifampin or chloramphenicol added before a downward temperature shift (32).

The *des* gene, encoding the sole desaturase from *B. subtilis*, was isolated, before the *B. subtilis* genomic sequence was available, by complementation of *E. coli* strains with mutations in either the *fabA* or *fabB* genes, which are essential for UFA synthesis (1). The *des* gene encodes a polytopic membrane-bound desaturase containing a tripartite motif of His essential for catalysis, located on the cytoplasmic side of the membrane
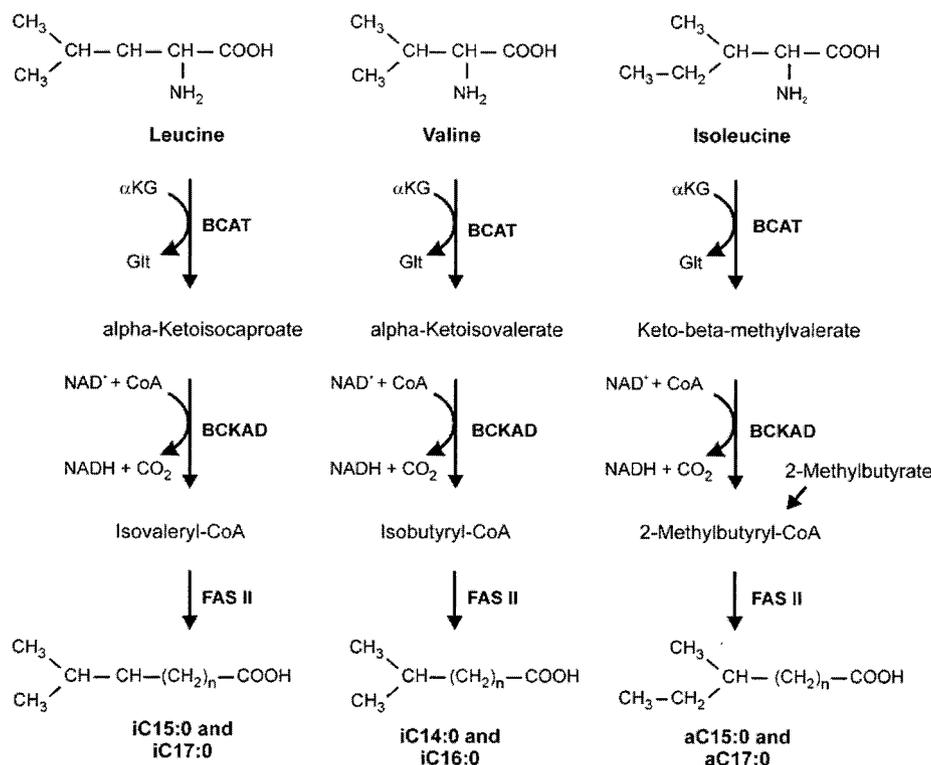
FIG. 2. Branched-chain fatty acid biosynthesis in *B. subtilis*. Branched-chain amino acids are converted to branched-chain 2-keto-acid by a branched-chain amino acid transaminase (BCAT). Acyl-CoA intermediates are then synthesized by a branched-chain α-ketoacid dehydrogenase complex (BCKAD). Isovaleryl-CoA, isobutyryl-CoA, and 2-methylbutyryl-CoA are substrates of the fatty acid synthase (FAS II), yielding iso-branched-chain $C_{15:0}$ and $C_{17:0}$, iso-branched-chain $C_{14:0}$ and $C_{16:0}$, and anteiso-branched-chain $C_{15:0}$ and $C_{17:0}$ fatty acids, respectively. α-KG, α-ketoglutarate; Glt, glutamate.

(24). The *B. subtilis* desaturase catalyzes the introduction of a *cis* double bond at the Δ5 position of a wide range of SFAs, and this protein was named Δ5-Des (6).

A detailed transcriptional analysis demonstrated that the *des* gene is tightly regulated during cold shock. While the *des* transcript is barely detectable at 37°C, the production of *des* mRNA is transiently induced upon a temperature downshift (2). Induction of *des* occurs exclusively at the level of transcription in a promoter-dependent fashion (2) and is not caused by stabilization of *des* mRNA, which was reported to be a major cause of the induction of cold-shock-inducible genes in bacteria (34). Moreover, it was demonstrated by expressing the *des* gene under the control of an IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible promoter that the limiting factor in introducing a double bond into fatty acids at 37°C is the desaturase enzyme, while the other desaturation cofactors, such as the electron donor or components of the electron transport chain, appear to be present at the restrictive temperature (2). 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 resources already existing in the cell at the time of the temperature downshift (2). 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 (2). This finding indicates that the transient induction of the wild-type *des* gene at low growth temperatures was due

to shutoff of transcription rather than to instability of the *des* mRNA. This would explain why, similar *to 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 (32).

HOW DO *B. SUBTILIS* CELLS "TURN ON" AND "TURN OFF" *des* TRANSCRIPTION?

Since the transcriptional induction of *des* at low temperatures does not involve de novo protein synthesis, it follows that the mechanism includes a transcriptional regulator (activator or repressor) which is present at all temperatures and whose activity could be determined by a temperature-dependent change in protein conformation. Thus, it was hypothesized that transcription of the *des* gene could be regulated by a two-component system, which is one of the major mechanisms of detection and transduction of environmental signals in bacteria, leading to specific activation or repression of gene expression (37). To test this hypothesis, the *B. subtilis* genomic sequence was searched for potential two-component regulatory gene pairs involved in *des* environmental regulation. This analysis revealed that a two-gene operon of unknown function formed by the *yocF* and *yocG* open reading frames was located immediately downstream of the *des* gene (42). The predicted products of *yocF* and *yocG* exhibited structural similarity to the histidine kinases and response regulators of two-component

regulatory systems, respectively. Inactivation of either *yocF* or *yocG* inhibited the induction of the *des* promoter and UFA synthesis at low temperatures (3). Since both the histidine kinase, YocF, and its cognate regulator, YocG, are required for *des* induction, the genes coding for these two regulatory proteins were named *desK* and *desR*, respectively.

The *B. subtilis* DesK protein features four transmembrane segments that define the sensor domain and a long cytoplasmic C-terminal tail harboring the histidine predicted to be the site of autophosphorylation. The C-terminal kinase domain of DesK (DesKC) undergoes autophosphorylation in the presence of ATP in the conserved His 188, which is the target residue of this autokinase activity (4). Autophosphorylated DesKC serves as a phosphodonor of the effector protein DesR, which becomes phosphorylated in the predicted Asp 54 residue (4). The phosphorylated form of DesR binds to a DNA sequence extending from the −28 to −77 positions relative to the start site of the temperature-regulated *des* gene (3, 20a). Therefore, the DesR protein is a transcriptional regulator directly involved in activation of the *des* gene at low temperatures.

Another histidine kinase, Hik33, involved in regulation of cold-inducible genes has also been described in *Synechocystis* (51). Mutation of Hik33 leads to diminished induction of several cold-inducible genes, including *desD*, which encodes a Δ6 desaturase (52). In contrast to the *B. subtilis* DesK/DesR system, which seems to regulate the expression of the *des* gene only (8; G. E. Schujman, unpublished data), Hik33 is a global cold sensor involved in the cold expression of ~17 genes (38). A response regulator, Rer1, was also identified by screening of a library of *Synechocystis* cells with random mutations that affected regulation of the transcription of the *desB* gene, which encodes a ω3 desaturase (51). Although the phosphorylation relay between Hik33 and Rer1 and the subsequent regulation of transcription of the *desB* gene by Rer1 remain to be characterized, the cold-regulated induction of gene expression in *Synechocystis* seems to be similar to the pathway controlled by DesK/DesR in *B. subtilis*. The mechanism of regulation of UFA synthesis at low temperatures, mediated by a sensor histidine kinase and its cognate response regulator, could be common to prokaryotes using the aerobic pathway of desaturation of fatty acids.

The shutoff of *des* transcription after continuous growth at low temperature is not due to downregulation of the levels of *desKR* mRNA, since transcription of this operon is independent of temperature (3). Therefore, the transient induction of *des* is due to the inhibition of the pathway that senses or transduces low-temperature signals. Experiments showing that (i) in the absence of UFA synthesis, the transcription of the desaturase promoter (*desp*) is not downregulated by prolonged incubation of cells at low temperatures (3) and (ii) the addition of UFAs to the culture media of cells shifted from 37 to 20°C specifically inhibits the transcriptional activity of *desp* (3), suggested that UFAs negatively regulate *des* transcription. The fact that DesK is required to dephosphorylate DesR-P (3, 4), ensuring its deactivation, suggested that UFAs might interact in some way with the sensor kinase, favoring its phosphatase activity.

THE Des PATHWAY OF *B. SUBTILIS*

Genetic and biochemical experiments demonstrated that the level of phosphorylation of DesR is determined by the balance of the two activities possessed by DesK, a phosphate donor for DesR and a phosphatase of phosphorylated DesR (3, 4). Since the activity of DesR as a transcriptional activator is modulated by the level of phosphorylation (20a), the output of signal transduction is determined by switching of DesK activity between kinase-biased and phosphatase-biased forms. The bifunctional nature of DesK strongly suggests that its kinase and phosphatase activities are reciprocally regulated by changes in the growth temperature that, in turn, adjust the degree of desaturation of membrane phospholipids. This raises two important questions regarding how DesK responds to changes in growth temperature. First, what is the ligand or physical signal that reflects changes in growth temperature if it is not temperature per se? Second, which portion of DesK is involved in temperature sensing?

Evidence that membrane fluidity, rather than growth temperature, controls the transcription of the *des* gene was obtained by experiments in which the proportion of anteisobranched-chain fatty acids of *B. subtilis* membranes was varied, controlling the provision of exogenous fatty acid precursors (20). The anteiso-branched-chain fatty acids, which are synthesized by using ketoacids derived from isoleucine as primers (Fig. 2), are essential to decrease the transition temperature of *B. subtilis* membrane phospholipids to maintain the appropriate fluidity. Limiting the amount of exogenous isoleucine allows the amounts of anteiso-branched-chain fatty acids of plasma membrane lipids to be reduced dramatically (20, 41). Thus, the order of membrane lipids of *B. subtilis* can be increased in vivo under isothermal conditions. Growth of cells in the absence of isoleucine results in activation of *des* transcription at 37°C using a DesK/DesR-dependent mechanism (20). Moreover, a downward shift in temperature in the absence of isoleucine had additive effects on the expression of the desaturase gene (20). Thus, a decrease in the content of membrane isoleucine-derived fatty acids at constant temperature mimics a drop in the growth temperature, and both stimuli can induce UFA synthesis (20). The most straightforward interpretation of these experiments is that, in response to either a temperature downshift or restricted membrane fluidity at 37°C, induction of *des* transcription is brought about via the ability of DesK to sense a decrease in membrane fluidity.

Experimental evidence suggesting that membrane fluidity might be involved in the sensing of low temperatures was also described for *Synechocystis*. Expression of the *desA* gene, encoding the Δ12 desaturase in *Synechocystis*, is induced by reduction of the double bonds of membrane lipids by Pd-catalyzed hydrogenation of lysozyme-treated cells (55). Moreover, disruption of the *desA* and *desD* genes of *Synechocystis*, encoding the Δ12 and Δ6 fatty acid desaturases, respectively, produces rigidity of membrane lipids at physiological temperatures and enhances the cold inducibility of a number of genes (38).

The purified C-terminal domain of DesK (DesKC), which lacks the transmembrane segments, contains both the kinase and phosphatase activities of the sensor protein (4). However, DesKC does not function as a phosphatase in vivo, implying
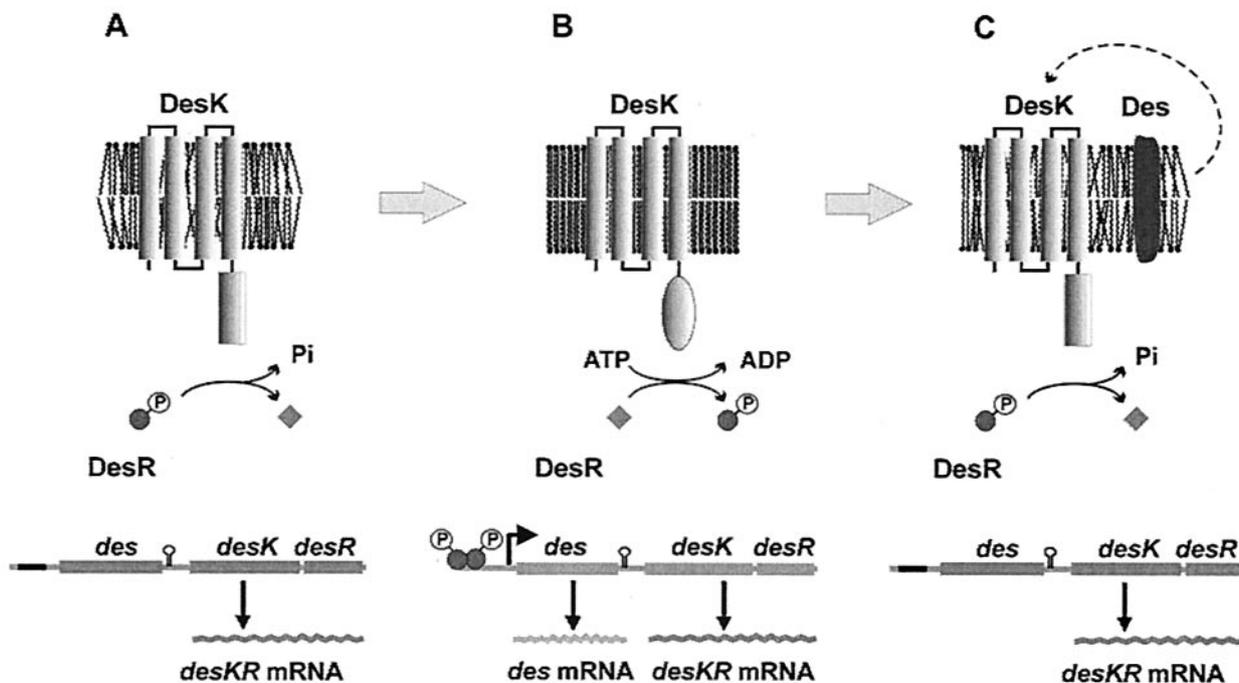
FIG. 3. Signal transduction pathway leading to membrane fluidity optimization in *B. subtilis*. It is proposed that DesK could assume different signaling states in response to changes in membrane fluidity. This is accomplished by regulating the ratio of kinase activity to phosphatase activity. (A) A phosphatase-dominant state is present when membrane lipids are disordered. (B) A kinase-dominant state of DesK predominates upon an increase in the proportion of ordered membrane lipids. This could be accomplished either after a sudden drop in temperature, which results in a drastic reduction in membrane fluidity regardless of the availability of anteiso-branched-chain fatty acid precursors (ABFP), or at 37°C when cells are growing in a medium devoid of ABFP. DesK-mediated phosphorylation of DesR results in transcriptional activation of *des*. (C) Activation of *des* results in synthesis of Des, which desaturates the acyl chains of membrane phospholipids. These newly synthesized UFAs inhibit *des* transcription by favoring the dephosphorylation of DesR.

that the truncated protein might be locked in a kinase-dominant state (4). In strains expressing DesKC, the *des* gene is constitutively expressed and its transcription is not affected by the growth temperature or UFAs (4). Thus, the transmembrane segments of DesK are essential to sense changes in membrane fluidity and for regulating the ratio of kinase activities to phosphatase activities of the cytoplasmic C-terminal domain.

Our present model to explain the signal transduction pathway controlling the low-temperature induction of Δ5-Des is shown in Fig. 3. We envision that one or more of the transmembrane domains of DesK could sense a change in the ordering of the acyl chains of membrane phospholipids and transmit this information to the cytoplasmic domain of the sensor kinase. This would result in adjustment of the ratio of kinase activities to phosphatase activities so that a phosphatase-dominant state is present when membrane lipids are disordered, resulting in dephosphorylation of the cognate response regulator, DesR (Fig. 3A). The unphosphorylated regulator is unable to bind *desp* and, as a consequence, *des* transcription is turned off. Upon an increase in the proportion of ordered membrane lipids, a kinase-dominant state of DesK is favored (Fig. 3B), so that the sensor protein undergoes autophosphorylation, and subsequently, the phosphoryl group is transferred to DesR, which activates transcription of *des*. Activation of *des* results in synthesis of Δ5-Des which specifically introduces a double bond in the acyl chains of membrane

lipids. These newly synthesized UFAs decrease the phase transition temperature of the phospholipids and inhibit the transcriptional activity of *desp*, presumably by favoring the phosphatase activity of DesK. This results in hydrolysis of phosphorylated DesR and the shutoff of *des* transcription (Fig. 3C). This metabolic pathway, called the Des pathway, therefore generates a regulatory loop that optimizes membrane fluidity.

FUNCTION OF UFAs DURING TEMPERATURE ADAPTATION IN *B. SUBTILIS*

The introduction of double bonds by Δ5-Des directly into membrane lipids (1, 32, 33) provides the cell with a rapid mechanism for decreasing the fluidity of preexisting membranes upon a temperature decrease. The old lipids can be retailored to suit the new temperature. However, the *B. subtilis* membrane contains a high percentage of anteiso-branched-chain fatty acids (6, 20, 40, 41). Since the physicochemical effect of a methyl branch in these long-chain fatty acids is similar to that of a *cis* double bond (39), anteiso-branched-chain fatty acids fulfill the role of UFAs in bacteria that synthesize branched-chain fatty acids. Thus, it seemed possible that the Δ5-Des function would not be essential for *B. subtilis* viability at low growth temperatures. In agreement with this hypothesis, a *B. subtilis des* null mutant strain growing in rich medium did not show a cold-sensitive phenotype (1). However,

a *des* deletion mutant of *B. subtilis* underlay a dramatic reduction of viability during cold shock in the absence of exogenous isoleucine sources (59). Induction of *des* expression from a copy integrated in *trans* in the *des* deletion mutant not only cured the observed cold-sensitive phenotype in the absence of isoleucine but allowed growth comparable with that of the parental strain in the presence of isoleucine (59). These results demonstrate that UFA production can entirely mediate cold shock protection of the membrane without varying the anteiso side chain branching pattern. Thus, it was concluded that *des* expression has a crucial role in cold adaptation (59). The most straightforward interpretation of the physiological experiments discussed here (1, 20, 59) is that a functional *B. subtilis* membrane requires that the composition of the membrane phospholipids be within the limits of the phase transition. Many experiments performed with *E. coli* UFA auxotrophs have demonstrated that if all of the phospholipids are in either the ordered state or the disordered state, the membrane is not functional (for a review, see reference 18). Moreover, quite wide variations in fluidity are tolerated; that is, the cells do not have to maintain a precise ratio of fluid to nonfluid lipids to have functional membranes (18). However, there does seem to be an optimal fluidity which is most advantageous for cell growth. Thus, the Des pathway seems designed to optimize the fluidity within the tolerated range rather than to extend the range.

CONCLUSIONS

There are two fundamental differences between the *E. coli* and the *B. subtilis* models that account for the formation of UFAs and the regulation by growth temperature of the synthesis of these fatty acids. First, *B. subtilis* lacks the *fabB* and *fabA* genes that are essential for UFA synthesis in *E. coli*, and this property accounts for the absence of UFA production by the gram-positive fatty acid synthase. Instead, *B. subtilis* makes UFAs via an oxygen-dependent desaturase that uses existing phospholipids as substrates to introduce a double bond at the 5 position of the fatty acyl chain. Second, *B. subtilis* uses a two-component system to uniquely and stringently control the transcription of the gene coding for the desaturase. Induction of the Des pathway under a temperature downshift is brought about via the ability of the DesK histidine kinase to sense a decrease in membrane fluidity. In contrast, the temperature control of UFAs in *E. coli* does not involve changes in gene expression, and the increase in the amounts of UFAs at low growth temperatures is carried out by a direct effect of temperature on the activity of the FabF condensing enzyme. Thus, a cytosolic thermosensor governs the temperature-dependent adjustment of membrane fluidity in *E. coli*, and probably in related bacteria, while in *B. subtilis* and cyanobacteria this control is exerted by a membrane-associated thermosensor. Gaining insight into the mechanism by which changes in membrane fluidity regulate the signaling state of sensor kinases involved in the expression of *des* genes will be a major issue for future research. Genes with significant similarity to fatty acid desaturase genes have been found in the genomes of many bacteria. Although their functions in most cases have not yet been experimentally demonstrated, fatty acid desaturases may be more widespread than originally thought. Future research

will be necessary to determine if the expression of these fatty acid desaturases is regulated by membrane-bound thermosensors.

Finally, the classical pathway of anaerobic UFA synthesis is not widely distributed in bacteria (10, 35). Genomic analysis indicates that only the alpha and gamma proteobacteria encode the proteins of this pathway. Most other bacteria, including many pathogens, synthesize UFAs under anaerobic conditions but lack *fabA* and *fabB* homologues. Several pathways may exist, because *Streptococcus pneumoniae*, which lacks FabA and FabB homologues, has an enzyme called FabM that performs the key FabA isomerization reaction in vitro (48). However, FabM seems to be specific for streptococci and thus does not provide a general answer for organisms that make UFAs anaerobically but lack the FabA-FabB pathway. Thus, a challenge for the future is to discover how these organisms synthesize UFAs anaerobically and if their synthesis is subjected to thermal regulation.

REFERENCES

1. **Aguilar, P. S., J. E. Cronan, Jr., and D. de Mendoza.** 1998. A *Bacillus subtilis* gene induced by cold shock encodes a membrane phospholipid desaturase. J. Bacteriol. **180:**2194–2200. .
2. **Aguilar, P. S., P. López, and D. de Mendoza.** 1999. Transcriptional control of the low-temperature-inducible *des* gene, encoding the delta 5 desaturase of *Bacillus subtilis*. J. Bacteriol. **181:**7028–7033.
3. **Aguilar, P. S., A. M. Hernandez-Arriaga, L. E. Cybulski, A. C. Erazo, and D. de Mendoza.** 2001. Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*. EMBO J. **20:**1681–1691.
4. **Albanesi, D., M. C. Mansilla, and D. de Mendoza.** 2004. The membrane fluidity sensor DesK of *Bacillus subtilis* controls the signal decay of its cognate response regulator. J. Bacteriol. **186:**2655–2663.
5. **Allen, E. E., and D. H. Bartlett.** 2000. FabF is required for piezoregulation of *cis*-vaccenic acid levels and piezophilic growth of the deep-sea bacterium *Photobacterium profundum* strain SS9. J. Bacteriol. **182:**1264–1271.
6. **Altabe, S. G., P. Aguilar, G. M. Caballero, and D. de Mendoza.** 2003. The *Bacillus subtilis* acyl lipid desaturase is a δ5 desaturase. J. Bacteriol. **185:**3228–3231.
7. **Baldassare, J. J., K. B. Rhinehart, and D. F. Silbert.** 1976. Modification of membrane lipid: physical properties in relation to fatty acid structure. Biochemistry **15:**2986–2994.
8. **Beckering, C. L., L. Steil, M. H. Weber, U. Volker, and M. A. Marahiel.** 2002. Genomewide transcriptional analysis of the cold shock response in *Bacillus subtilis*. J. Bacteriol. **184:**6395–6402.
9. **Bloch, K.** 1963. The biological synthesis of unsaturated fatty acids. Biochem. Soc. Symp. **24:**1–16.
10. **Campbell, J. W., and J. E. Cronan, Jr.** 2001. Bacterial fatty acid biosynthesis: targets for antibacterial drug discovery. Annu. Rev. Microbiol. **55:**305–332.
11. **Carty, S. M., K. R. Sreekumar, and C. R. Raetz.** 1999. Effect of cold shock on lipid A biosynthesis in *Escherichia coli*. Induction at 12°C of an acyltransferase specific for palmitoleoyl-acyl carrier protein. J. Biol. Chem. **274:**9677–9685.
12. **Choi, K. H., R. J. Heath, and C. O. Rock.** 2001. Beta-ketoacyl-acyl carrier protein synthase III (FabH) is a determining factor in branched-chain fatty acid biosynthesis. J. Bacteriol. **182:**365–370.
13. **Clementz, T., J. J. Bednarski, and C. R. Raetz.** 1996. Function of the *htrB* high temperature requirement gene of *Escherichia coli* in the acylation of lipid A: HtrB catalyzed incorporation of laurate. J. Biol. Chem. **271:**12095–12102.
14. **Clementz, T., Z. Zhou, and C. R. Raetz.** 1997. Function of the *Escherichia coli msbB* gene, a multicopy suppressor of *htrB* knockouts, in the acylation of lipid A. Acylation by MsbB follows laurate incorporation by HtrB. J. Biol. Chem. **272:**10353–10360.
15. **Cronan, J. E., Jr., C. H. Birge, and P. R. Vagelos.** 1969. Evidence for two genes specifically involved in unsaturated fatty acid biosynthesis in *Escherichia coli*. J. Bacteriol. **100:**601–604.

16. **Cronan, J. E., Jr., and E. P. Gelmann.** 1973. An estimate of the minimum amount of unsaturated fatty acid required for growth of *Escherichia coli.* J. Biol. Chem. **248:**1188–1195.

17. **Cronan, J. E., Jr., R. B. Gennis, and S. R. Maloy.** 1987. Cytoplasmic membrane, p. 31–55. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella:* cellular and molecular biology. American Society for Microbiology, Washington, D.C.

18. **Cronan, J. E., Jr., and C. O. Rock.** 1987. Biosynthesis of membrane lipids, p. 474–497. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella:* cellular and molecular biology. American Society for Microbiology, Washington, D.C.

19. **Cronan, J. E., Jr., and C. O. Rock.** 1996. Biosynthesis of membrane lipids, p. 612–636. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella:* cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.

20. **Cybulski, L. E., D. Albanesi, M. C. Mansilla, S. Altabe, P. S. Aguilar, and D. de Mendoza.** 2002. Mechanism of membrane fluidity optimisation: isothermal control of the *Bacillus subtilis* acyl-lipid desaturase. Mol. Microbiol. **45:**1379–1388.

20a. **Cybulski, L. E., G. del Solar, P. O. Craig, M. Espinosa, and D. de Mendoza.** *Bacillus subtilis* DesR functions as a phosphorylation-activated switch to control membrane lipid fluidity. J. Bio. Chem., in press.

21. **de Mendoza, D., and J. E. Cronan, Jr.** 1983. Thermal regulation of membrane lipid fluidity in bacteria. Trends Biochem. Sci. **8:**49–52.

22. **de Mendoza, D., A. Klages Ulrich, and J. E. Cronan, Jr.** 1983. Thermal regulation of membrane fluidity in *Escherichia coli.* Effects of overproduction of beta-ketoacyl-acyl carrier protein synthase I. J. Biol. Chem. **258:**2098–2101.

23. **de Mendoza, D., G. S. Schujman, and P. S. Aguilar.** 2001. Biosynthesis and function of membrane lipids, p. 43–55. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and its relatives: from genes to cells. ASM Press, Washington, D.C.

24. **Díaz, A. R., M. C. Mansilla, A. J. Vila, and D. de Mendoza.** 2002. Membrane topology of the acyl-lipid desaturase from *Bacillus subtilis.* J. Biol. Chem. **277:**48099–48106.

25. **Edwards, P., J. S. Nelsen, J. G. Metz, and K. Dehesh.** 1997. Cloning of the *fabF* gene in an expression vector and in vitro characterization of recombinant *fabF* and *fabB* encoded enzymes from *Escherichia coli.* FEBS Lett. **402:**62–66.

26. **Fujii, D. K., and A. J. Fulco.** 1977. Biosynthesis of unsaturated fatty acids by bacilli. Hyperinduction and modulation of desaturase synthesis. J. Biol. Chem. **252:**3660–3670.

27. **Fulco, A. J.** 1969. The biosynthesis of unsaturated fatty acids by Bacilli. I. Temperature induction of the desaturation reaction. J. Biol. Chem. **244:**889–895.

28. **Garwin, J. L., and J. E. Cronan, Jr.** 1980. Thermal modulation of fatty acid synthesis in *Escherichia coli* does not involve de novo enzyme synthesis. J. Bacteriol. **141:**1457–1459.

29. **Garwin, J. L., A. L. Klages, and J. E. Cronan, Jr.** 1980. Beta-ketoacyl-acyl carrier protein synthase II of *Escherichia coli.* Evidence for function in the thermal regulation of fatty acid synthesis. J. Biol. Chem. **255:**3263–3265.

30. **Garwin, J. L., A. L. Klages, and J. E. Cronan, Jr.** 1980. Structural, enzymatic, and genetic studies of β-ketoacyl-acyl carrier protein synthases I and II of *Escherichia coli.* J. Biol. Chem. **255:**11949–11956.

31. **Gelmann, E. P., and J. E. Cronan, Jr.** 1972. Mutant of *Escherichia coli* deficient in the synthesis of *cis*-vaccenic acid. J. Bacteriol. **112:**381–387.

32. **Grau, R., and D. de Mendoza.** 1993. Regulation of the synthesis of unsaturated fatty acids by growth temperature in *Bacillus subtilis.* Mol. Microbiol. **8:**535–542.

33. **Grau, R., D. Gardiol, G. C. Glikin, and D. de Mendoza.** 1994. DNA supercoiling and thermal regulation of unsaturated fatty acid synthesis in *Bacillus subtilis.* Mol. Microbiol. **11:**933–941.

34. **Gualerzi, C. O., A. M. Giuliodori, and C. L. Pon.** 2003. Transcriptional and post-transcriptional control of cold-shock genes. J. Mol. Biol. **331:**527–539.

35. **Heath, R. J., S. W. White, and C. O. Rock.** 2001. Lipid biosynthesis as a target for antibacterial agents. Progr. Lipid Res. **40:**467–497.

36. **Heath, R. J., and C. O. Rock.** 2002. The Claisen condensation in biology. Nat. Prod. Rep. **19:**581–596.

37. **Hoch, J. A.** 2000. Two-component and phosphorelay signal transduction. Curr. Opin. Microbiol. **3:**165–170.

38. **Inaba, M., I. Suzuki, B. Szalontai, Y. Kanesaki, D. A. Los, H. Hayashi, and N. Murata.** 2003. Gene-engineered rigidification of membrane lipids enhances the cold inducibility of gene expression in *Synechocystis.* J. Biol. Chem. **278:**12191–12198.

39. **Kaneda, T.** 1977. Fatty acids of the genus *Bacillus:* an example of branched-chain preference. Bacteriol. Rev. **41:**391–418.

40. **Kaneda, T.** 1991. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol. Rev. **55:**288–302.

41. **Klein, W., M. H. Weber, and M. A. Marahiel.** 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:**5341–5349.

42. **Kunst, F., N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessieres, A. Bolotin, S. Borchert, R. Borriss, L. Boursier, A. Brans, M. Braun, S. C. Brignell, S. Bron, S. Brouillet, C. V. Bruschi, B. Caldwell, V. Capuano, N. M. Carter, S. K. Choi, J. J. Codani, I. F. Connerton, A. Danchin, et al.** 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis.* Nature **390:**249–256.

43. **Lewis, R. N., and R. N. McElhaney.** 1985. Thermotropic phase behavior of model membranes composed of phosphatidylcholines containing iso-branched fatty acids. 1. Differential scanning calorimetric studies. Biochemistry **24:**2431–2439.

44. **Macdonald, A. G., and A. R. Cossins.** 1985. The theory of homeoviscous adaptation of membranes applied to deep-sea animals. Symp. Soc. Exp. Biol. **39:**301–322.

45. **Mantsch, H. H., C. Madec, R. N. Lewis, and R. N McElhaney.** 1985. Thermotropic phase behavior of model membranes composed of phosphatidylcholines containing iso-branched fatty acids. 2. Infrared and 31P NMR spectroscopic studies. Biochemistry **24:**2440–2446.

46. **Mantsch, H. H., C. Madec, R. N. Lewis, and R. N McElhaney.** 1987. Thermotropic phase behavior of model membranes composed of phosphatidylcholines containing dl-methyl anteisobranched fatty acids. 2. An infrared spectroscopy study. Biochemistry **26:**4045–4049.

47. **Marr, A. G., and J. I. Ingraham.** 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli.* J. Bacteriol. **84:**1260–1267.

48. **Marrakchi, H., K. H. Choi, and C. O. Rock.** 2002. A new mechanism for anaerobic unsaturated fatty acid formation in *Streptococcus pneumoniae.* J. Biol. Chem. **277:**44809–44816.

49. **Phetsuksiri, B., M. Jackson, H. Scherman, M. McNeil, G. S. Besra, A. R. Baulard, R. A. Slayden, A. E. DeBarber, C. E. Barry III, M. S. Baird, D. C. Crick, and P. J. Brennan.** 2003. Unique mechanism of action of the thiourea drug isoxyl on *Mycobacterium tuberculosis.* J. Biol. Chem. **278:**53123–53130.

50. **Sakamoto, T., and N. Murata.** 2002. Regulation of the desaturation of fatty acids and its role in tolerance to cold and salt stress. Curr. Opin. Microbiol. **5:**208–210.

51. **Suzuki, I., D. A. Los, Y. Kanesaki, K. Mikami, and N. Murata.** 2000. The pathway for perception and transduction of low-temperature signals in *Synechocystis.* EMBO J. **19:**1327–1334.

52. **Suzuki, I., Y. Kanesaki, K. Mikami, M. Kanehisa, and N. Murata.** 2001. Cold-regulated genes under control of the cold sensor Hik33 in *Synechocystis.* Mol. Microbiol. **40:**235–244.

53. **Totcher, D. R., M. J. Leaver, and P. A. Hodgson.** 1988. Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. Progr. Lipid Res. **37:**73–117.

54. **Van Alphen, L., B. Lugtenberg, E. T. Rietschel, and C. Mombers.** 1979. Architecture of the outer membrane of *Escherichia coli* K12. Phase transitions of the bacteriophage K3 receptor complex. Eur. J. Biochem. **101:**571–579.

55. **Vigh, L., D. A. Los, I. Horvath, and N. Murata.** 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:**9090–9094.

56. **Vigh, L., B. Maresca, and J. L. Harwood.** 1998. Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem. Sci. **23:**369–374.

57. **Vorachek-Warren, M. K., S. M. Carty, S. Lin, R. J. Cotter, and C. R. Raetz.** 2002. An *Escherichia coli* mutant lacking the cold shock-induced palmitoleoyltransferase of lipid A biosynthesis: absence of unsaturated acyl chains and antibiotic hypersensitivity at 12°C. J. Biol. Chem. **277:**14186–14193.

58. **Wang, H., and J. E. Cronan.** 2003. *Haemophilus influenzae* Rd lacks a stringently conserved fatty acid biosynthetic enzyme and thermal control of membrane lipid composition. J. Bacteriol. **185:**4930–4937.

59. **Weber, M. H. W., W. Klein, L. Muller, U. M. Niess, and M. Marahiel.** 2001. Role of the *Bacillus subtilis* fatty acid desaturase in membrane adaptation during cold shock. Mol. Microbiol. **39:**1321–1329.