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Sigma L Is Important for Cold Shock Adaptation of *Bacillus subtilis*

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Although sigma factor-dependent transcriptional regulation was shown to be essential for adaptation to different environmental stimuli, no such sigma factor has been related to the regulation of the cold shock response in *Bacillus subtilis*. In this study, we present genetic evidence for participation of σ^L (σ^{54}) and the two σ^L-dependent transcriptional enhancers BkdR and YplP in the cold shock response of *Bacillus subtilis* JH642. Single-gene deletion of either *sigL*, *bkdR*, or *yplP* resulted in a cold-sensitive phenotype.

It has been shown that sigma factors allow the bacterial cell to regulate gene expression in response to different environmental stimuli (11, 14). The alternative sigma factor σ^B (12) is responsible for the induction of genes encoding general stress proteins following heat, ethanol, salt, and acid stress and during energy depletion in *Bacillus subtilis* (13). More than 150 general stress proteins or genes belong to the σ^B regulon (13), which provides nongrowing cells with a nonspecific, multiple, and preventive stress resistance (2, 10, 18). Interestingly, the *sigB* operon is also induced in cells continuously exposed to low temperatures (4) but not, however, after cold shock, which is defined as a sudden temperature shift from 37°C to 15°C (3). To explore the possible involvement of an alternative sigma factor in cold shock adaptation, we analyzed data from earlier transcriptional studies of *B. subtilis* (3). The cold-induced transcriptional regulator YplP was identified, which shares significant sequence similarity to σ^L-dependent transcriptional activators. The Δ*yplP* deletion mutant was shown to be cold sensitive in *B. subtilis* JH642 (3). The *sigL* gene encodes a homolog of the σ^{54} subunit of RNA polymerase and requires regulator proteins of the NtrC/NifA family to activate gene transcription (5, 9). Four homologs of the transcriptional regulator YplP in *B. subtilis* have been genetically characterized. AcoR, LevR, and RocR are involved in activation of acetoin, carbohydrate, and amino acid metabolism, respectively (1, 6, 8), while BkdR regulates the *bkd* operon. The strongly cold-induced *bkd* operon (16) is involved in the synthesis of precursor molecules for branched-chain fatty acids (7), which were shown to be essential for membrane adaptation after cold shock (3, 16). As both σ^L-dependent BkdR and YplP transcriptional regulators are linked to the cold shock response, we have investigated the role of σ^L in cold shock adaptation by monitoring the growth rates of Δ*sigL*, Δ*bkdR*, and Δ*yplP* deletion mutants after a sudden temperature shift from 37°C to 15°C.

Strain construction. For the construction of strain Δ*sigL* (FW06) a DNA fragment was PCR amplified from chromosomal DNA of *B. subtilis* QB5505 (8) containing *sigL* disrupted with an *aphA3* kanamycin resistance cassette with primers 5′*sigL* (TATTATCAAGGCTTTAGAGAGAAAATCGTC) and 3′*sigL* (ATGTTTTGTCAGCTCTTGTTTCAATGGCT). *B. subtilis* JH642 was transformed with the DNA fragment of 4,844 bp that was obtained, resulting in kanamycin-resistant strain Δ*sigL* (FW06).

For the construction of strain Δ*bkdR* (FW10), a DNA fragment was PCR amplified from chromosomal DNA of *B. subtilis* QB7512 (7) containing *bkdR* disrupted with an *aphA3* kanamycin resistance cassette using primers 5′*bkdR* (ATTGCAACGGAATAAATAGGT) and 3′*bkdR* (ATGTTTGCGTTTATTCTGCAA). *B. subtilis* JH642 was transformed with the DNA fragment of 2,325 bp obtained, resulting in strain Δ*bkdR* (FW10). All strains used in this study are listed in Table 1.

Growth analysis of JH642 deletion strains. Prior to any further experiments, possible polar effects arising from the described gene deletions of *yplP* and *bkdR* were analyzed. The growth phenotype resulting from the deletion of *yplP* could be complemented in *trans* by introducing a copy of *yplP* in the *amyE* site under control of an inducible promoter (data not shown). The analogous experiment for the Δ*bkdR* mutant was described by Debarbouille et al. (7). Therefore, we conclude that the deletion of either *yplP* or *bkdR* does not have any polar effects.

The deletion strains Δ*bkdR* (FW10) and Δ*yplP* (CB15) were grown in Spizizen's minimal medium (SMM) at 37°C and shocked to 15°C at an optical density at 600 nm (OD_{600}) of 0.5 (Fig. 1A). Both Δ*bkdR* (FW10) and Δ*yplP* (CB15) lysed after cold shock, indicating that BkdR and YplP are important for the cold shock adaptation. In strain Δ*bkdR* (FW10), the transcriptional activator BkdR is not present any more to enhance the transcription of the *bkd* operon. Consequently, isoleucine is not converted to α-keto acids, and no branched-chain fatty acids are synthesized de novo to lower the melting point of the membrane (17). The cells lysed, due to the insufficient membrane adaptation in strain Δ*bkdR* (FW10). The observed lysis of Δ*yplP* (CB15) confirms the results of an earlier study (3); however, the underlying mechanism is still unknown.

As both BkdR and YplP were shown to be important for cold shock adaptation, we investigated the role of the remaining three σ^L-dependent transcriptional activators, AcoR, LevR, and RocR. However, the analysis of the deletion mutant

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TABLE 1. *B. subtilis* strains in this study

Strain	Genotype	Reference or source
JH642	*pheA1 sfp*^{0} *trpC2*	15
Δ*yplP* (CB15)	*pheA1 sfp*^{0} *trpC2 yplP*::*kan*	3
Δ*sigL* (FW06)	*pheA1 sfp*^{0} *trpC2 sigL*::*aphA3*	This work
Δ*bkdR* (FW10)	*pheA1 sfp*^{0} *trpC2 bkdR*::*aphA3*	This work
Δ*acoR* (FW13)	*pheA1 sfp*^{0} *trpC2 acoR*::*aphA3*	This work
Δ*levR* (FW14)	*pheA1 sfp*^{0} *trpC2 levR*::*aphA3*	This work
Δ*rocR* (FW15)	*pheA1 sfp*^{0} *trpC2 rocR*::*aphA3*	This work
Δ*yplP2* (FW19)	*pheA1 sfp*^{0} *trpC2 yplP*::*kan amyE*::*yplP*	This work
Δ*yplP3* (FW20)	*pheA1 sfp*^{0} *trpC2 yplP*::*kan amyE*::*yplP yplQ*	This work

strains Δ*acoR* (FW13), Δ*levR* (FW14), and Δ*rocR* (FW15) did not show cell lysis or cold-dependent growth retardation after a shift from 37°C to 15°C. This implies that AcoR, LevR, and RocR are not essential for cold shock adaptation (data not shown).

As σ^L interacted with the cold-relevant transcriptional regulators YplP and BkdR (Fig. 2), we examined its influence on cold shock adaptation. The deletion strain Δ*sigL* (FW06) was grown in SMM at 37°C and then shifted to 15°C at an OD_{600} of 0.5 (Fig. 1A). Strain Δ*sigL* (FW06) showed cell lysis after cold shock. This is the first demonstration that an alternative

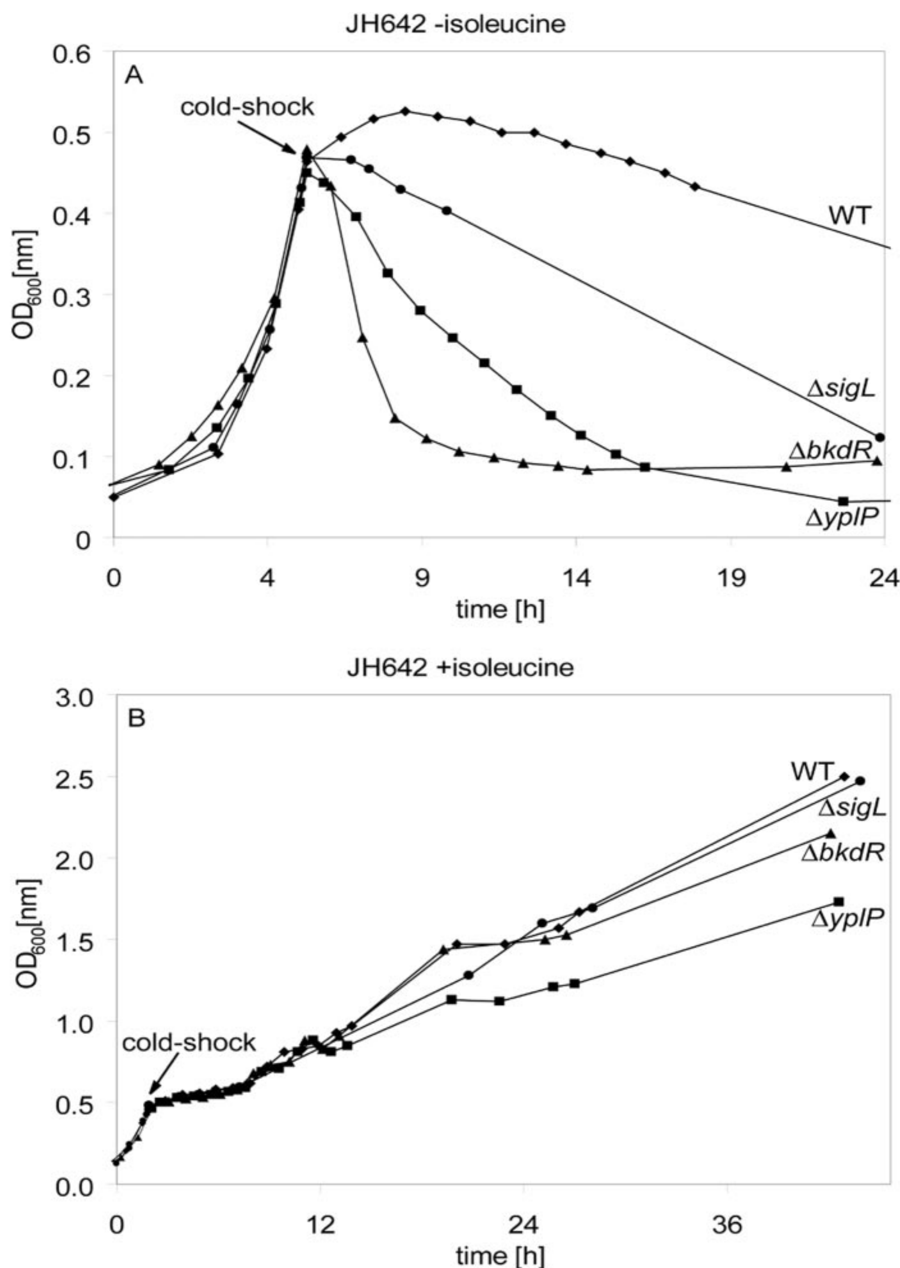

FIG. 1. Growth curves of *B. subtilis* JH642 (diamonds), Δ*yplP* (CB15) (squares), Δ*bkdR* (FW10) (triangles), and Δ*sigL* (FW06) (circles) in the absence (A) and presence (B) of isoleucine (50 μg/ml). Cells were grown in 200 ml SMM supplemented with 0.5% (wt/vol) glucose, 50 μg/ml tryptophan, 50-μg/ml phenylalanine, and trace elements at 37°C to an OD_{600} of 0.45 and then subjected to cold shock (15°C) (19). All experiments were repeated at least three times.

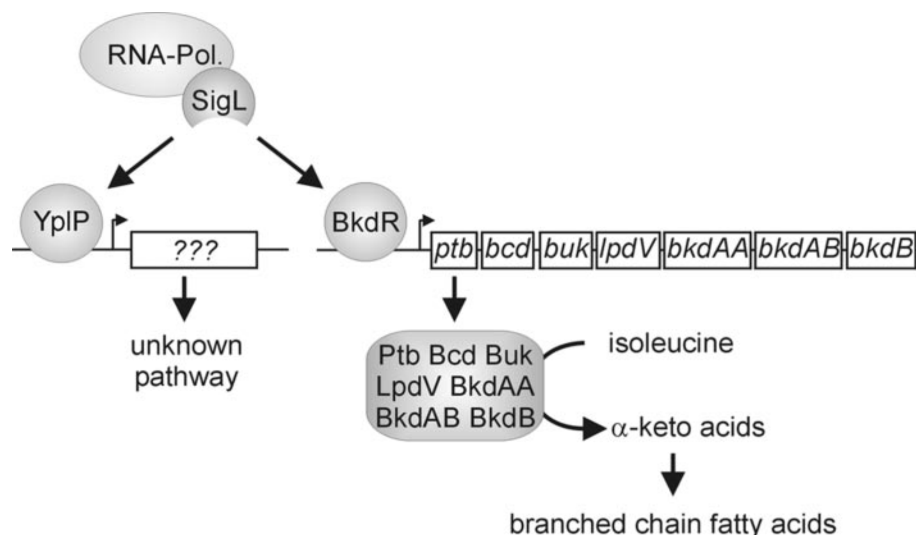

FIG. 2. A diagram showing σ^L-dependent transcriptional enhancers BkdR and YplP, which are associated with cold shock adaptation and the downstream-regulated genes. The σ^L/BkdR regulation pathway is responsible for the cold shock adaptation of the membrane by the degradation of isoleucine to α-keto acids for branched-chain fatty acid synthesis. σ^L/YplP downstream-regulated cold-relevant genes are still unknown.

sigma factor is important for cold shock adaptation (Fig. 1A). However, the cold-sensitive phenotype of the Δ*sigL* mutant was not as severe as that observed for Δ*bkdR* and Δ*yplP*.

To evaluate if both transcriptional activators BkdR and YplP are involved in membrane adaptation, Δ*sigL* (FW06), Δ*bkdR* (FW10), and Δ*yplP* (CB15) were grown in SMM in the presence of isoleucine after a temperature shift from 37°C to 15°C (Fig. 1B). Under these conditions, the growth of all strains was improved after cold shock. While the addition of isoleucine supported the growth of Δ*sigL* (FW06) and Δ*bkdR* (FW10) to the level of the wild type, growth of Δ*yplP* (CB15) was significantly retarded (Fig. 1B). This suggests a membrane-dependent function of the σ^L/BkdR complex and a membrane-independent role of the σ^L/YplP complex for cold shock adaptation.

The σ^L/BkdR complex activates the *bkd* operon, which is responsible for the conversion of isoleucine to α-keto acids that are used as precursors for branched-chain fatty acid synthesis (Fig. 2). Although the transcriptional activator BkdR was missing in the Δ*bkdR* (FW10) mutant, sufficient amounts of α-keto acids were synthesized by residual amounts of the *bkd* operon-encoded enzymes if a large excess of isoleucine was present. This high substrate concentration compensated for the reduced amount of enzymes in the Δ*bkdR* (FW10) mutant. The residual amounts of *bkd*-encoded enzymes resulted from the basal transcription level of the *bkd* operon. Therefore, the observed growth rate of the Δ*bkdR* (FW10) mutant can be fully rescued by the addition of isoleucine. This is in full agreement with the published findings of Klein et al. (17), who showed that isoleucine serves as a switch in the fatty acid branching pattern for membrane adaptation to low temperatures. In contrast, the growth of the Δ*yplP* (CB15) mutant was not restored to wild-type levels. This suggests that the σ^L/YplP-regulated pathway contains at least one additional element, which is not involved in membrane adaptation.

We conclude that σ^L is involved in the regulation of at least two cold shock adaptation pathways in *B. subtilis* JH642 (Fig. 2). The first is the adaptation of the bacterial membrane by σ^L/BkdR-mediated activation of the *bkd* operon. The second is the σ^L/YplP-dependent pathway, with as-yet-unknown functions. Further investigations are in progress to reveal the nature of the σ^L/YplP-activated genes.

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