JOURNAL OF BACTERIOLOGY, Feb. 1997, p. 445–452 Vol. 179, No. 2
0021-9193/97/$04.00+0

Heat Shock Regulation of σ^S Turnover: a Role for DnaK and Relationship between Stress Responses Mediated by σ^S and σ^{32} in *Escherichia coli*

ANDREA MUFFLER, MECHTHILD BARTH, CHRISTOPH MARSCHALL, AND REGINE HENGGE-ARONIS*

Department of Biology, University of Konstanz, 78434 Konstanz, Germany

Received 17 July 1996/Accepted 1 November 1996

The cellular level of the *rpoS*-encoded σ^S subunit of RNA polymerase increases in response to various stress situations that include starvation, high osmolarity, and shift to acid pH, and these different stress signals differentially affect *rpoS* translation and/or σ^S stability. Here we demonstrate that σ^S is also induced by heat shock and that this induction is exclusively due to an interference with σ^S turnover. Some σ^S-dependent genes exhibit similar heat shock induction, whereas others are not induced probably because they need additional regulatory factors that might not be present under conditions of heat shock or exponential growth. Despite its induction, σ^S does not seem to contribute to heat adaptation but may induce cross-protection against different stresses. While σ^S is not involved in the regulation of the heat shock sigma factor σ^{32}, the heat shock protein DnaK has a positive role in the posttranscriptional control of σ^S. The present evidence suggests that DnaK is involved in the transduction of two of the signals that result in reduced σ^S turnover, i.e., heat shock and carbon starvation. Heat shock induction of σ^S also clearly indicates that a cessation of growth or even a reduction of the growth rate is not a prerequisite for the induction of σ^S and σ^S-dependent genes and underscores the importance of σ^S as a general stress sigma factor.

The heat shock response consists of increased expression of a set of universally conserved proteins with mainly chaperone and protease functions. In enteric bacteria such as *Escherichia coli*, this response is mediated by the *rpoH*-encoded alternate sigma factor σ^{32}, whose rate of synthesis and stability and therefore cellular concentration rapidly increase upon temperature upshift (7, 44). Alternatively, above all gram-positive bacteria, e.g., *Bacillus subtilis*, use a regulatory mechanism based on repression at an inverted repeat operator site located upstream of major heat shock genes (45). While these control mechanisms seem to be specific for heat shock, there are also more general regulatory mechanisms that respond to a number of environmental stress conditions that include heat shock as well as starvation, stationary phase, and high osmolarity (9). In *B. subtilis*, this general stress response is mediated by the alternate sigma factor σ^B, whose activity is regulated in a complex and differential way by all of these signals (36, 37, 39). This suggests that to some extent the heat shock response may overlap with a more general stress response or that multiple stress-inducible genes may also respond to heat shock.

In *E. coli*, the general stress response is mediated by σ^S, which is encoded by the *rpoS* gene (19). While σ^S is best known for its crucial role in gene regulation during entry into stationary phase (10, 12, 16), it also serves as a global regulator in cells exposed to hyperosmotic stress (11, 14) or acid stress (18). The cellular σ^S level increases in response to all of these conditions. High osmolarity (17, 26), low temperature (31), and late-exponential-phase conditions (17) stimulate *rpoS* translation. The degradation of σ^S protein, which under nonstress conditions is highly unstable, is inhibited by carbon starvation (17, 34), high osmolarity (26), and probably a shift to acid pH (2). The details of this complex regulation are not yet understood, but the RNA-binding protein HF-I has recently been shown to be crucial for *rpoS* translation (25), whereas σ^S turnover requires the response regulator RssB (2, 24, 27) and ClpXP protease (29).

Since σ^S responds to many rather different environmental stress signals, we investigated whether σ^S and σ^S-dependent genes also react to heat shock. While our work was in progress, moderate σ^S induction in response to temperature upshift was shown in a Western blot experiment (15), but no mechanistic analysis was provided. Which mechanism in the transcriptional or posttranscriptional regulation of σ^S is affected by heat shock? What is the physiological function of σ^S induction under heat shock conditions? Is there a physiological or regulatory connection between the σ^S-mediated general stress response and the σ^{32}-mediated heat shock response? In the present study, we try to elucidate these various aspects of thermal regulation of σ^S.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are derivatives of MC4100 and are listed in Table 1. P1 transduction (23) was used for the construction of strains. *dnaK* mutants were freshly constructed by P1 transduction to avoid the occurrence of second-site suppressor mutations.

For distinguishing effects on *rpoS* transcription and translation as well as on σ^S degradation, a set of *rpoS*::*lacZ* fusions has been used (17, 26). All fusion constructs are present in single copy on λ phages located at the *att* site in the chromosome and contain more than 1 kb of the DNA upstream of *rpoS* which includes all the promoters that contribute to *rpoS* expression. The translational and transcriptional *rpoS742*::*lacZ* fusions are inserted after nucleotide 742 in *rpoS*. The only difference between these two constructs is the presence in the latter fusion of a 90-bp insertion upstream of the eighth codon of *lacZ*, which contains stop codons in all three reading frames, a ribosomal binding site, and an initiation codon followed by six additional codons (17). The translational *rpoS379*::*lacZ* fusion is similar to the translational *rpoS742*::*lacZ* fusion, with the only difference being that it is inserted after nucleotide 379 within *rpoS*. Since the longer hybrid protein contains a turnover element located approximately in the middle of σ^S, it is subject to σ^S-like turnover, whereas the shorter hybrid protein is stable (26).

Cultures were grown at various growth temperatures under aeration in mini-

* Corresponding author. Mailing address: Department of Biology, University of Konstanz, P.O. Box 5560, 78434 Konstanz, Germany. Phone: (49)7531-88-2039. Fax: (49)7531-88-2966. E-mail: Regine.Hengge-Aronis@unikonstanz.de.

TABLE 1. Bacterial strains

Strain	Relevant genotype	Reference or source
MC4100	F⁻ Δ(*arg-lac*)*U169 araD139 rpsL150 ptsF25 flbB5301 rbsR deoC relA1*	30
RH90	MC4100 *rpoS359*::Tn*10*	16
AM106	MC4100 *rssB*::Tn*10*	24
AM125	MC4100 *clpP1*::*cat*[a]	This study
AM111	MC4100 *hfq1*::Ω	25
RO200	MC4100 (λRZ5:*rpoS742*::*lacZ*)	17
RO90	MC4100 (λRZ5:*rpoS379*::*lacZ*[hybr])	26
RO91	MC4100 (λRZ5:*rpoS742*::*lacZ*[hybr])	17
BB1553	MC4100 Δ*dnaK52*::*cat sidB1*[b]	4
MB300	MC4100 Δ*dnaK52*::*cat*	This study
MB305	RO200 Δ*dnaK52*::*cat*	This study
MB303	RO90 Δ*dnaK52*::*cat*	This study
MB304	RO91 Δ*dnaK52*::*cat*	This study
RO151	MC4100 Φ(*csi-5*::*lacZ*)(λp*lac*Mu55)[c]	38
MB302	RO151 Δ*dnaK52*::*cat*	This study
FF2032	MC4100 Φ(*otsA*::*lacZ*)(λp*lac*Mu55)	6
DW12	MC4100 Φ(*csiD*::*lacZ*)(λp*lac*Mu15)	38
DW16	MC4100 Φ(*csiE*::*lacZ*)(λp*lac*Mu15)	38

[a] The *clpP1*::*cat* allele was described by Maurizi et al. (21).
[b] *sidB1* is a mutant allele of *rpoH* (4).
[c] This fusion is located in *osmY* (38)

mal medium M9 (23) containing 0.1% glucose or 0.4% glycerol as carbon sources. Growth was monitored by determining the optical density at 578 nm (OD_{578}). For temperature shift experiments, cultures were grown at 30°C to mid-exponential phase (to an OD_{578} of approximately 0.3) and divided into two aliquots, one of which was rapidly shifted to 42.5°C.

SDS-PAGE and immunoblot analysis. Sample preparation for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis was performed as previously described (17). Fifteen micrograms of total cellular protein was applied per lane (this amount of total cellular protein yields σ^S bands in the linear range for densitometric quantitation). A polyclonal serum against σ^S (17), a goat anti-rabbit immunoglobulin G alkaline phosphatase conjugate (Sigma), and a chromogenic substrate (5-bromo-4-chloro-3-indolylphosphate tolindinium [BCIP]–nitroblue tetrazolium [NBT]; Boehringer Mannheim) were used for visualization of σ^S bands.

Pulse-labeling of cells and immunoprecipitation. The procedure used for pulse-labeling of cells with L-[^{35}S]methionine and immunoprecipitation with the polyclonal antiserum against σ^S was previously described (17). The pulse-labeling time was 1 min, and chase times varied as indicated in the figure legends. As a σ^S-deficient control, strain RH90 was used (labeled in exponential-phase samples harvested at an OD_{578} of 0.3). Immunoprecipitated proteins were quantified on the dried gels with a PhosphorImager (Molecular Dynamics) relative to bands of weakly cross-reacting but stable proteins present in the cellular extracts used for immunoprecipitation.

For assaying changes in the rate of expression of major heat shock proteins in response to heat shock, mid-exponential-phase cells grown at 30°C were labeled at 30°C, and 5 and 30 min after a shift to 42°C, they were labeled with L-[^{35}S]methionine (1 min pulse-labeling, 1 min chase time, labeling temperature identical to growth temperature); extracts were prepared as described above. Cellular extracts were directly subjected to SDS-PAGE followed by autoradiography.

β-Galactosidase assay. β-Galactosidase activity was assayed by using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate and is reported as micromoles of *o*-nitrophenol per minute per milligram of cellular protein (23).

RESULTS

σ^S is induced upon heat shock. The cellular level of σ^S increases not only during entry into stationary phase (12) but also during exponential-phase growth in response to high osmolarity (17, 26) or acid pH (18). Here we demonstrate that heat shock, i.e., a shift of the growth temperature from 30 to 42.5°C, is also a σ^S-inducing exponential-phase stress condition and results in three- to fivefold-elevated levels of σ^S (Fig. 1, lanes 2 and 3). Increased σ^S levels persisted for at least 2 h after heat shock (data not shown) (see also data obtained with *rpoS*::*lacZ* fusions described below). Unlike σ^{32} induction (33), heat shock induction of σ^S is thus not a transient response.

Thermal induction of σ^S cannot be observed in *rssB* or *clpP* mutants (Fig. 1, lanes 4 to 7), i.e., in strains in which σ^S is not subject to turnover and therefore present in large amounts already in nonstressed exponentially growing cells (24, 29). A null mutation in *hfq*, the structural gene for the RNA-binding protein HF-I which is crucial for *rpoS* translation (25), results in extremely low σ^S levels and no apparent heat shock induction (Fig. 1, lanes 8 and 9).

Heat shock does not alter σ^S expression but interferes with σ^S turnover. Since various environmental stress signals differentially affect *rpoS* transcription, *rpoS* mRNA translation, and σ^S turnover (17, 26), we wanted to know which level of control was affected by heat shock. Temperature upshift had no effect on the expression of the transcriptional *rpoS742*::*lacZ* fusion (Fig. 2A) (see Materials and Methods for a detailed description of the fusions used here). In contrast, the corresponding translational *rpoS742*::*lacZ* fusion exhibited approximately 10-fold induction (Fig. 2B). Therefore, heat shock induction of σ^S is mediated by a posttranscriptional mechanism.

β-Galactosidase activities measured with the translational *rpoS742*::*lacZ* fusion reflect not only *rpoS* transcription and translation but also σ^S turnover, since the hybrid protein is subject to regulated degradation similar to that of σ^S itself. Degradation seems to require a turnover element located approximately in the middle of σ^S, i.e., in a region present in the RpoS742::LacZ hybrid protein, but not in the stable shorter RpoS379::LacZ hybrid protein (26). Comparing activities of these two translational fusions thus allows differentiation between effects on *rpoS* expression and σ^S turnover. The expression of the shorter *rpoS379*::*lacZ* fusion was not altered by heat shock (Fig. 2C), indicating that heat shock does not affect σ^S synthesis.

This result was confirmed by directly assaying σ^S synthesis and turnover before and after heat shock in a pulse-chase experiment followed by immunoprecipitation (Fig. 3). The intensity of pulse-labeled σ^S bands, i.e., the relative rate of σ^S synthesis, did not increase after temperature upshift, but σ^S half-life increased from approximately 1.5 to 7 min, as determined before and 40 min after heat shock. When half-life determinations were performed only 10 min after temperature upshift, σ^S half-life seemed to increase during the course of the experiment (i.e., during the 10-min chase time) (Fig. 3).

Based on the results with *rpoS*::*lacZ* fusions as well as on the direct pulse-chase measurements, we conclude that increased cellular levels of σ^S observed after heat shock are exclusively due to σ^S stabilization, whereas σ^S synthesis remains unaffected. Moreover, the heat shock-triggered change in σ^S sta-

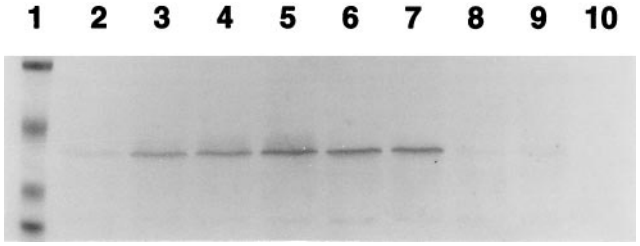

FIG. 1. Cellular levels of σ^S protein before and after heat shock. MC4100 (lanes 2 and 3) and its derivatives carrying *rssB*::Tn*10* (lanes 4 and 5), *clpP1*::*cat* (lanes 6 and 7), *hfq1*::Ω (lanes 8 and 9), and *rpoS359*::Tn*10* (lane 10) were grown at 30°C in M9 minimal medium with 0.4% glycerol. At an OD_{578} of 0.3, the cultures were divided into two aliquots, one of which was shifted to 42.5°C (lanes 3, 5, 7, and 9), whereas the other remained at 30°C (lanes 2, 4, 6, 8, and 10). Thirty minutes after temperature shift, samples were taken and subjected to immunoblot analysis with an antiserum against σ^S as described in Materials and Methods. Size standard proteins (80, 49.5, 32, and 27 kDa) are shown in lane 1.

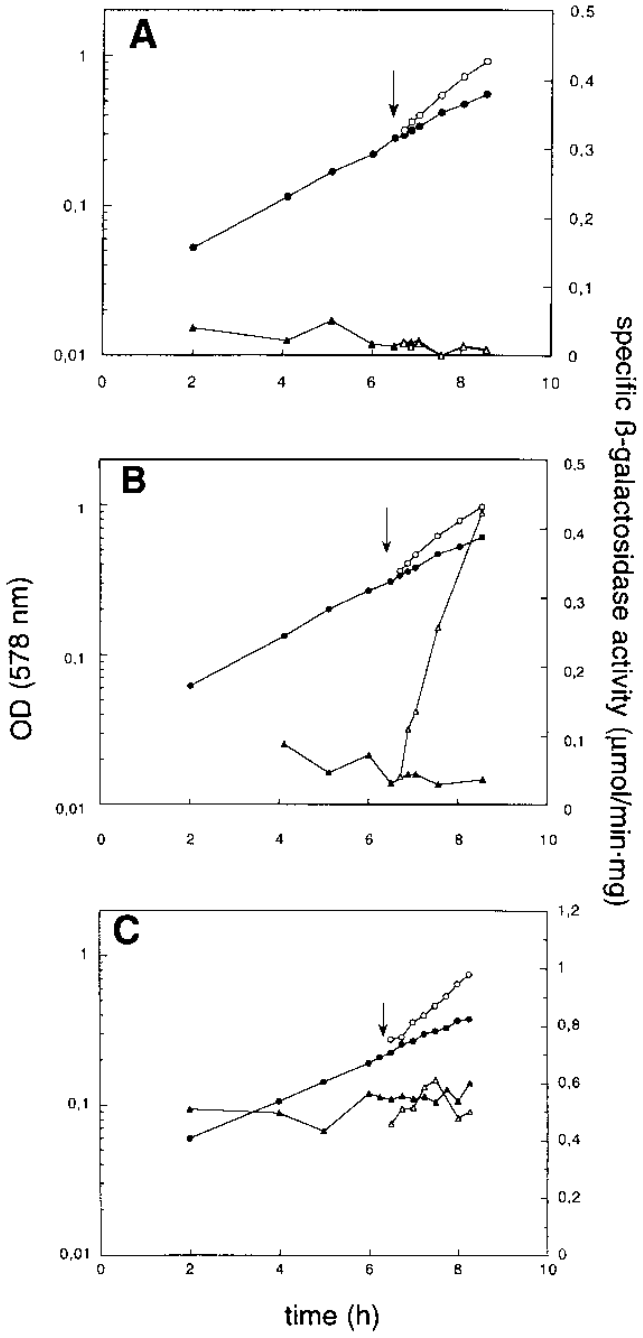

FIG. 2. Expression of transcriptional and translational *rpoS*::*lacZ* fusions before and after heat shock. MC4100 derivatives carrying the transcriptional *rpoS742*::*lacZ* fusion (A) or the translational fusions *rpoS742*::*lacZ* (B) and *rpoS379*::*lacZ* (C) were grown and subjected to heat shock (arrows) as described in the legend to Fig. 1. ODs (circles) and specific β-galactosidase activities (triangles) were determined for the cultures growing at 30°C (closed symbols) and 42.5°C (open symbols).

bility occurs with a significantly slower kinetics than that observed for the heat shock sigma factor σ^{32} (33) and is a lasting response rather than being transient, as for σ^{32}.

Differential heat shock induction of σ^S-dependent genes. If thermal induction of σ^S ought to be of any physiological consequence, it should be reflected in induction of σ^S-regulated genes, which was tested with *lacZ* fusions to the σ^S-dependent genes *osmY*(*csi-5*) (16, 38), *otsA* (6, 13), *csiD* (38), and *csiE* (20, 38). *osmY* as well as *otsA* exhibit heat shock induction (Fig. 4A and B), although induction is not as strong as during entry into stationary phase in Luria-Bertani medium (13, 38) or in response to high osmolarity (6, 13, 14, 42, 43), which is consistent with σ^S levels also not increasing as strongly as under the other stress conditions mentioned above (compare the data presented in Fig. 1 and 2 to data published in references 17 and 26). The equally σ^S-dependent genes *csiD* and *csiE*, however, are not induced by heat shock (Fig. 4C and D). We conclude that heat shock induction of σ^S is reflected in heat shock induction of a subset of σ^S-dependent genes but that there is a differential response within the large σ^S regulon.

The heat shock protein DnaK is involved in the posttranscriptional regulation of σ^S. Since σ^{32} and σ^S are both heat shock induced, we wondered whether the two sigmas influence each other's regulation. However, as observed in Western blot experiments, the *rpoS*::Tn*10* mutant does not contain altered levels of σ^{32}, and heat shock induction of σ^{32} in the *rpoS* mutant is similar to that in the corresponding wild-type strain (data not shown). Moreover, in pulse-chase experiments (with total cell extracts being analyzed by SDS-gel electrophoresis and autoradiography), the major heat shock proteins exhibited

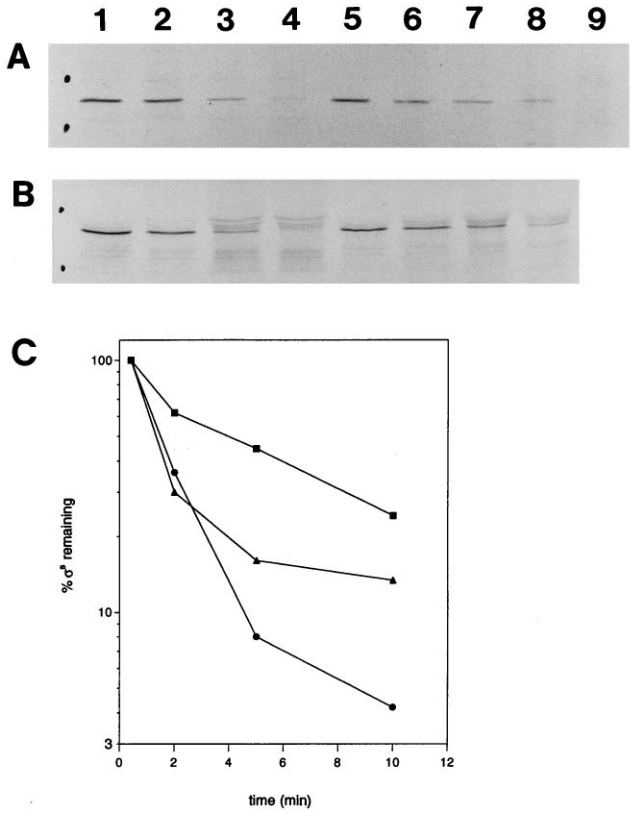

FIG. 3. Heat shock results in increased σ^S half-life. Strain MC4100 was grown and subjected to heat shock as described in the legend to Fig. 1. Samples taken from the culture growing at 30°C (lanes 1 to 4 in panels A and B and lane 9 in panel A) and the culture that had been incubated for 10 min (A) and 40 min (B) at 42.5°C (lanes 5 to 8) were pulse-labeled with [^{35}S]methionine as described in Materials and Methods. Chase times were 25 s (lanes 1, 5, and 10), 2 min (lanes 2 and 6), 5 min (lanes 3 and 7), and 10 min (lanes 4 and 8). Samples were processed for immunoprecipitation as described in Materials and Methods. Autoradiographs (A and B) and quantitated data (C [circles, 30°C, data taken from panel B, lanes 1 to 4; triangles, 10 min after heat shock, data taken from panel A, lanes 5 to 8; squares, 40 min after heat shock, data taken from panel B, lanes 5 to 8]) are shown.

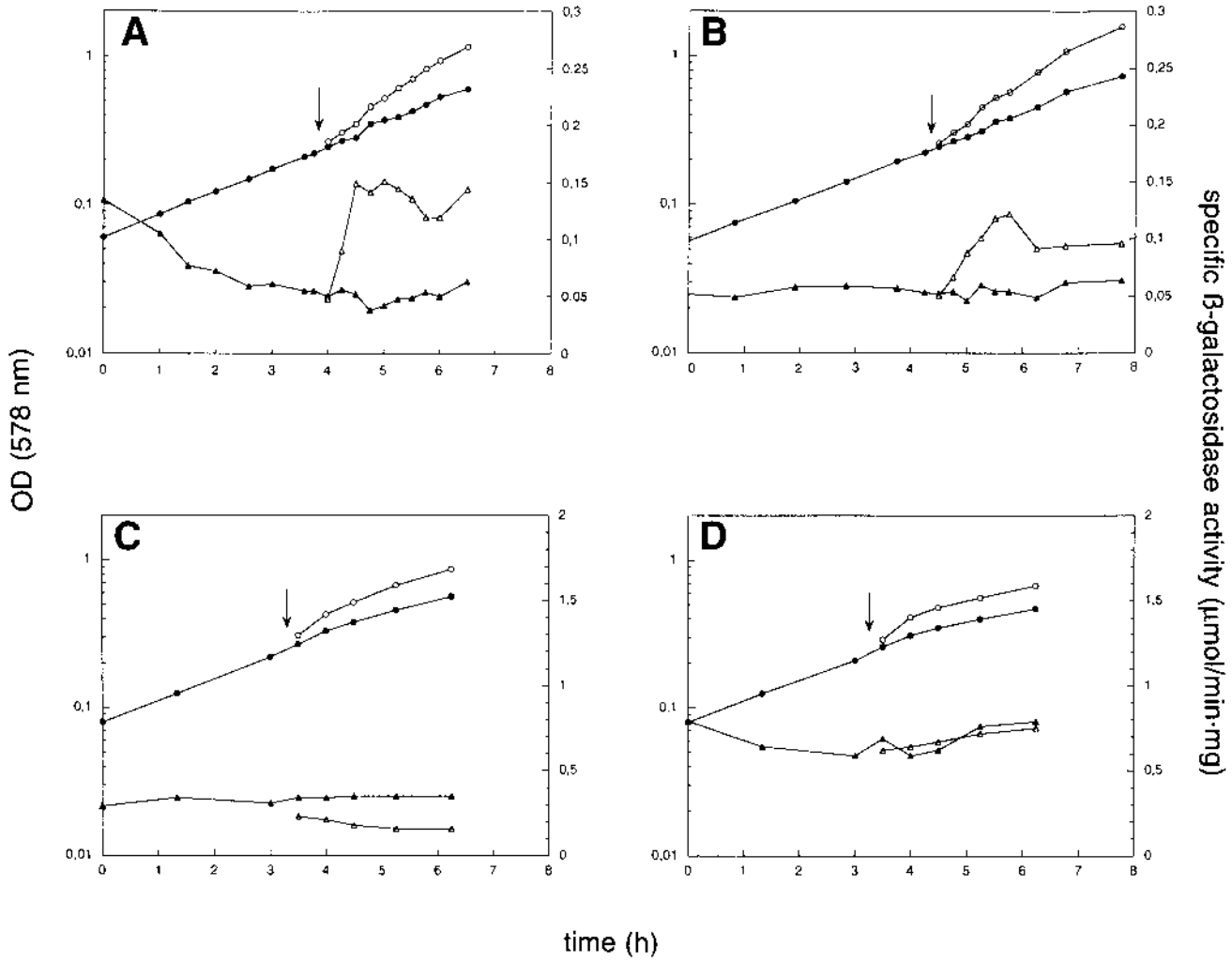

FIG. 4. Differential heat shock induction of σ^S-dependent genes. MC4100 derivatives carrying *lacZ* fusions to *osmY* (A), *otsA* (B), *csiD* (C), and *csiE* (D) were grown and subjected to heat shock (arrows) as described in the legend to Fig. 1. ODs and specific β-galactosidase activities were determined and are denoted by the same symbols as in Fig. 2.

the same rapid heat shock induction and subsequent shutoff of synthesis in otherwise isogenic *rpoS*$^+$ and *rpoS*::Tn*10* strains (data not shown).

While σ^S thus does not seem to be involved in the control of cellular σ^{32} levels, the previously reported observation that *dnaK* mutants have a pleiotropic phenotype mainly associated with stationary phase (28) appeared to point to a role for the heat shock protein DnaK (and thus, indirectly, to a role for σ^{32}) in the control of cellular σ^S levels. Immunoblot analysis indeed demonstrated that σ^S levels are altered in a *dnaK* null mutant (Fig. 5, compare lanes 1 to 3 with lanes 4 to 6). While early-exponential-phase levels of σ^S were somewhat higher in the absence of DnaK, σ^S induction during entry into stationary phase was only twofold in the *dnaK* mutant, compared to a 20-fold induction in the corresponding wild-type strain. This difference resulted in ultimately lower stationary-phase levels of σ^S in the *dnaK* mutant.

DnaK-deficient mutants strongly overproduce σ^{32} and, therefore, heat shock proteins, a phenotype which is suppressed by second-site mutations mapping in *rpoH* that reduce activity or expression of σ^{32} (4). Such a suppressor strain had lower σ^S levels throughout all growth phases than those of the mutant carrying the lesion in *dnaK* alone (Fig. 5, lanes 7 to 9), indicating that the lack of DnaK itself and not σ^{32} and/or heat shock protein overproduction is responsible for the observed reduction in σ^S in stationary phase. The data with the double mutant also indicate that increased levels of σ^S during exponential phase in the *dnaK* mutant are due to overproduction of other heat shock chaperones that can compensate for the absence of DnaK. However, for obtaining high σ^S levels in stationary phase, specifically DnaK seems to be required.

An analysis of *dnaK* effects on various *rpoS*::*lacZ* fusions indicated that DnaK is involved in the posttranscriptional regulation of σ^S. While the transcriptional *rpoS742*::*lacZ* fusion was not significantly affected (Fig. 6A), β-galactosidase activities measured with the translational *rpoS379*::*lacZ* and *rpoS742*::*lacZ* fusions were reduced, with the effect being more pronounced in stationary phase (Fig. 6B and C, respectively). Also, *rpoS742*::*lacZ*, i.e., the fusion which in contrast to *rpoS379*::*lacZ* also reflects σ^S turnover, was more strongly affected, suggesting that *rpoS* translation as well as σ^S stability is reduced in the *dnaK* mutant. While Fig. 6A to C give relative values, Fig. 6D shows absolute specific activities determined with the translational *rpoS742*::*lacZ* fusion, demonstrating reduced station-

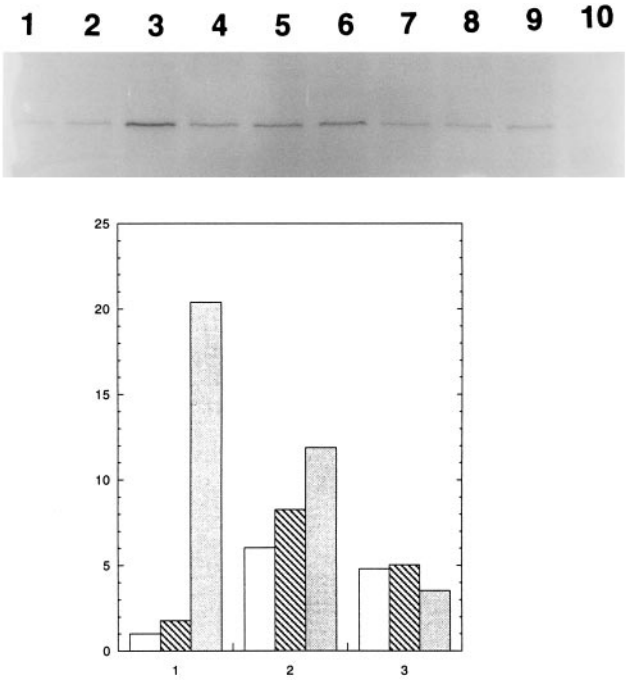

FIG. 5. Altered cellular σ^S levels in *dnaK* mutants. Strains MC4100 (lanes 1 to 3 on the gel and bars 1 in the bar diagram) and its *dnaK52*::*cat* (lanes 4 to 6 on the gel and bars 2 in the bar diagram), *dnaK52*::*cat sidB1* (lanes 7 to 9 on the gel and bars 3 in the bar diagram), and *rpoS359*::Tn*10* (lane 10) derivatives were grown in M9 medium with 0.1% glucose at 30°C. During mid-exponential phase (OD_{578} of approximately 0.3; lanes 1, 4, 7, and 10 on the gel; white bars) and late exponential phase (OD_{578} of approximately 0.6; lanes 2, 5, and 8 on the gel; hatched bars) and 1 h after the onset of starvation (lanes 3, 6, and 9 on the gel; grey bars), samples were taken and subjected to immunoblot analysis with an antiserum against σ^S as described in Materials and Methods. The bar diagram gives quantitative values for the bands shown in lanes 1 to 9 of the gel (normalized to the σ^S level in the wild-type strain in early exponential phase as shown in lane 1).

ary-phase induction in the *dnaK* mutant (which in the wild type under these conditions is due to a combination of increased *rpoS* translation in late exponential phase and a stabilization of σ^S after the onset of carbon starvation [17]).

Even though the decrease in σ^S levels in the *dnaK* mutant may not seem dramatic, it translates into a significant reduction in the expression of σ^S-dependent genes, which is shown here for a *lacZ* fusion to *osmY* (*csi-5*) (Fig. 7). It is therefore likely that the stationary-phase-associated and *rpoS*-like *dnaK* mutant phenotypes previously described (28) are a consequence of lowered cellular σ^S levels.

DISCUSSION

Heat shock regulation of σ^S and σ^S-dependent genes. In the present paper, we demonstrate that the cellular level of the σ^S subunit of RNA polymerase is significantly elevated in response to heat shock. Heat shock is thus one of an ever-increasing number of known environmental stress conditions that result in σ^S induction, which underscores the importance of σ^S as a general stress sigma factor rather than a sigma factor associated only with stationary phase or starvation. In addition, while some other σ^S-inducing stress conditions are associated with reduced growth rates, the classical heat shock, i.e., a shift in growth temperature from 30 to 42°C, accelerates growth. Therefore, a reduction in growth rate is not common to all σ^S-inducing stress situations and thus cannot be a unifying signal for all of these conditions (Fig. 8).

σ^S induction in response to heat shock is accompanied by an induction of σ^S-dependent genes such as *osmY* and *otsA* (Fig. 4). Yet, there are σ^S-dependent genes, e.g., *csiD* and *csiE*, that do not respond to these conditions. Interestingly, expression of these two genes is also not stimulated by other conditions, such as high osmolarity, or in *hns* mutants, that result in increased σ^S levels during exponential phase that are as high as those found in stationary phase (1, 20). It seems that these genes are representatives of a subset of σ^S-dependent genes whose induction is restricted to stationary phase, perhaps by a requirement for some other transcription factor that is present or active only in stationary phase. Possibly, high levels of cyclic AMP (cAMP) such as those observed after glucose starvation (3) are involved, since unlike most other σ^S-dependent genes, *csiD* and *csiE* are positively controlled by cAMP-cAMP receptor protein (20, 38).

What is the mechanism of heat shock induction of σ^S? Different environmental stress conditions have been shown to affect different mechanisms in the posttranscriptional control of the cellular σ^S level (17, 26) (see Fig. 8 for a summary). Here, we present evidence (Fig. 2 and 3) that heat shock interferes exclusively with σ^S degradation, whereas σ^S synthesis is not affected. Two protein factors, the response regulator RssB (2, 24, 27) and the ClpXP protease (29), have been shown to be essential for σ^S degradation. In *rssB* as well as in *clpP-clpX* mutants, σ^S is stable and therefore present in strongly increased amounts. Our observation that σ^S is not heat shock inducible in *rssB* and *clpP* mutants (Fig. 1) is consistent with our finding that heat shock influences only σ^S stability (otherwise, the effects of heat shock and *rssB* or *clpP* mutations should have been more or less additive).

What is the signal transduction pathway for heat shock affecting σ^S turnover? While it is possible that a temperature upshift is sensed via the response regulator RssB, heat shock also results in increased cellular amounts of irreversibly denatured proteins, i.e., substrates for Clp-mediated proteolysis, and, as a consequence, perhaps in a titration of Clp protease (in a manner similar to that suggested for DnaK [32]). Theoretically, this may result in increased stability of Clp protease substrates such as σ^S. However, such a titration effect would be expected to occur rapidly and transiently after heat shock. Yet, the increase in σ^S stability is established with a relatively slow kinetics and seems to be a lasting effect (Fig. 2 and 3). This observation, together with the finding that DnaK plays a positive role in σ^S control by interfering with σ^S degradation (Fig. 6) (see below), may indicate that increased σ^S stability in response to heat shock could be due to the increased steady-state level of the heat shock-induced DnaK chaperone machine which directly or indirectly protects σ^S against degradation.

Role of DnaK in the control of σ^S turnover. Here we have demonstrated that a *dnaK* null mutant contains reduced amounts of σ^S in stationary phase and exhibits reduced expression of σ^S-dependent genes (Fig. 5 and 7). This effect is not due to the abnormally high levels of σ^{32} and other heat shock proteins in *dnaK* mutants, because it can also be observed in a *dnaK* mutant with a secondary mutation in *rpoH* (Fig. 5). These results explain the previously described stationary-phase-associated phenotypes of a *dnaK* mutant which are reminiscent of those of *rpoS* mutants (28).

Our data obtained with *rpoS*::*lacZ* fusions indicate that *rpoS* translation as well as σ^S stability is reduced in the *dnaK* mutant. This is in contrast to the positive effects of *dnaK* mutations on *rpoH* translation and σ^{32} stability, suggesting that DnaK acts on *rpoS*/σ^S in a manner opposite to that in which it

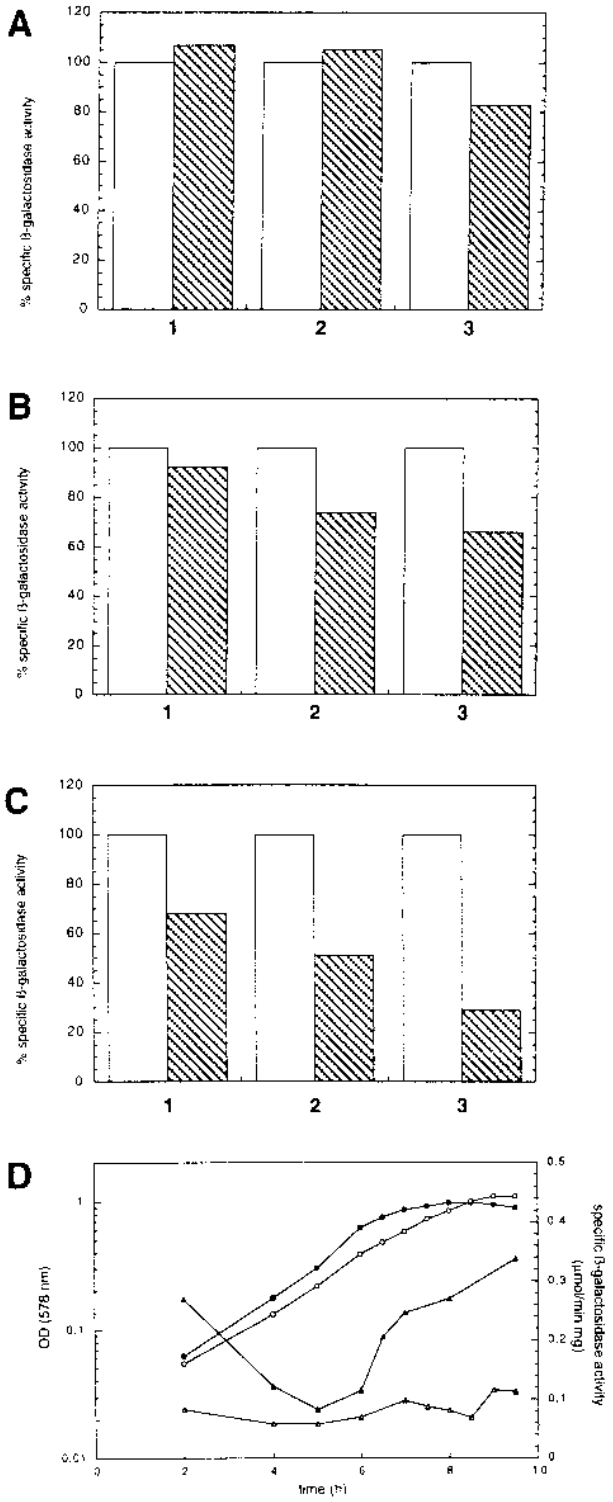

FIG. 6. Effect of a *dnaK* mutation on the expression of transcriptional and translational *rpoS*::*lacZ* fusions. Strains carrying the transcriptional *rpoS742*::*lacZ* fusion (A) or the translational fusions *rpoS379*::*lacZ* (B) and *rpoS742*::*lacZ* (C) in either *dnaK*$^+$ (white bars) or *dnaK52*::*cat* (hatched bars) backgrounds were grown in M9 medium with 0.1% glucose at 30°C. β-Galactosidase activities were determined during mid-exponential phase (OD_{578} between 0.3 and 0.35; bars 1) and late exponential phase (OD_{578} between 0.55 and 0.63; bars 2) and 1 h after the onset of starvation (bars 3). Values for the *dnaK* mutants are given relative to values determined for the *dnaK*$^+$ strains. All values are the average of three measurements with standard deviations between 0.3 and 7%. (D) Absolute values for ODs (circles) and specific β-galactosidase activities (triangles) for strain RO91 which carries the translational *rpoS742*::*lacZ* fusion (closed symbols) and its *dnaK52*::*cat* derivative (open symbols) are shown.

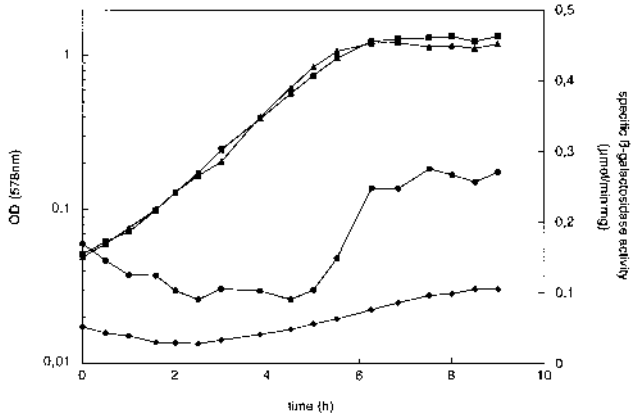

FIG. 7. Reduced expression of the σ^S-dependent gene *osmY* in a *dnaK* mutant. Strains RO151 (carrying a *lacZ* fusion in *osmY*; triangles and circles) and its *dnaK52*::*cat* derivative (squares and diamonds) were grown in M9 medium with 0.1% glucose at 30°C. ODs (triangles and squares) and specific β-galactosidase activities (circles and diamonds) were determined throughout the growth cycle of the cultures.

acts on *rpoH*/σ^{32}. DnaK might have indirect effects by affecting the synthesis, integrity, and/or stability of components involved in *rpoS* translation and/or σ^S stability. Alternatively, a more direct role for DnaK is conceivable, for instance, in protecting σ^S against degradation. The data shown in Fig. 5 indicate that during exponential phase, a lack of DnaK can be compensated for by the strongly increased levels of other heat shock proteins that include GroEL-GroES and other chaperones. During entry into stationary phase, however, DnaK seems to play a more specific and positive role in the control of σ^S that cannot be suppressed by higher levels of other chaperones.

Strikingly, stationary-phase induction of σ^S in the *dnaK* mutant is just twofold, in contrast to the 20-fold induction in the *dnaK*$^+$ strain. Could DnaK play a role in starvation sensing? As outlined above, increased steady-state levels of DnaK some time after the initial temperature upshift may represent the slow heat shock signal in the relatively slow process of stabilization of σ^S which is protected by DnaK (after an initial and

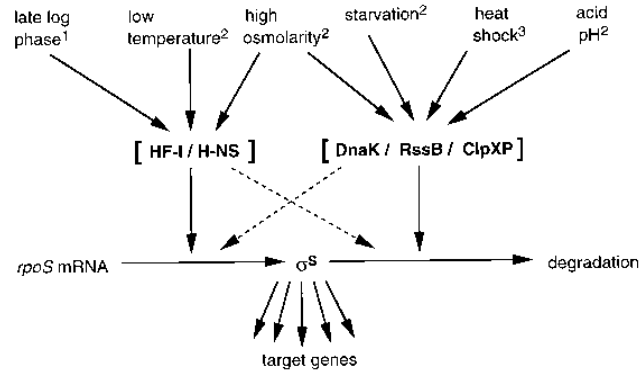

FIG. 8. Various environmental stress signals differentially affect *rpoS* translation and σ^S turnover. Superscript numbers given for stress conditions indicate the effect of the respective stress on the growth rate (1, growth rate unaffected; 2, growth rate reduced; 3, growth rate increased). Specific effects of late-exponential-phase conditions (17), low temperature (31), high osmolarity (17, 26), and starvation (17) as well as the roles of HF-I (25), H-NS (1, 25, 41), RssB (2, 24, 27), and the ClpXP protease (29) in the control of the cellular σ^S level have been described before. Two of the components involved in σ^S regulation, H-NS (1, 41) and DnaK (this study), affect *rpoS* translation as well as σ^S turnover. Effects of these components that are mechanistically unclear are indicated by broken arrows.

transient period of DnaK titration, which results in transient stabilization of σ^{32}, which is destabilized by DnaK). If a similar mechanism is to operate in response to starvation, one has to postulate a functional excess of DnaK after the onset of starvation. Major chaperones such as the DnaK-DnaJ-GrpE and the GroEL-GroES complexes also play an important role in the folding of nascent proteins (5, 8). Since the rate of overall protein biosynthesis rapidly decreases in response to starvation (see examples in reference 17), it is indeed conceivable that starvation may result in a decrease in the cellular amount of chaperone substrates and consequently in an excess of available DnaK which may then protect σ^S against degradation. Experiments to clarify this issue as well as the relationship between DnaK, RssB, and ClpXP are currently in progress.

Functional relationships between the responses mediated by σ^{32} and σ^S. The findings that not only σ^{32} but also σ^S is heat induced and that the heat shock protein DnaK is involved in opposite ways in the control of the two sigma factors suggest a previously unsuspected connection between the stress responses mediated by these two sigma factors.

Are the functions of the stress responses mediated by σ^{32} and σ^S partially overlapping, or do they operate independently from each other? Both regulons include chaperone genes (a σ^S-dependent *dnaJ* homolog, *cbpA*, was recently described [40]), and both contribute to various aspects of thermotolerance (14, 16, 22, 35). Unfortunately, the structural genes essential for thermotolerance have not yet been identified, but it is possible that some of them are under the control of both σ^{32} and σ^S.

The σ^{32}-mediated heat shock response is a rapid emergency response directed specifically against the kind of cellular damage elicited by high temperature. Thermal regulation of σ^S and σ^S-dependent genes suggested that the σ^S regulon may have an auxiliary function in heat adaptation. However, *rpoS* null mutants did not show a reduced growth rate at any temperature tested (our unpublished results) and even normally develop adaptive thermotolerance against a potentially lethal temperature (13). Putative detrimental effects of a σ^S deficiency in heat-shocked cells are also not compensated for by increased levels of σ^{32}, since we have observed completely normal σ^{32} levels before and after heat shock in the *rpoS* mutant (our unpublished results). Taken together, the physiological function of the induction of σ^S and σ^S-dependent genes upon heat shock is not yet apparent. However, it seems unlikely that induction of major parts of a large regulon under conditions where the relative expression of ordinary house-keeping genes is reduced is just a fortuitous event. Most probably, σ^S induction increases cross-protection against other potentially lethal stress conditions that may be relevant under the natural growth conditions of the bacteria but that have not been tested under the usual laboratory conditions.

ACKNOWLEDGMENTS

We thank B. Bukau and S. Gottesman for bacterial strains and B. Bukau for antibodies against σ^{32}. This study was performed in the laboratories of W. Boos, whose interest in our work we greatly appreciate.

Financial support was provided by the Deutsche Forschungsgemeinschaft (Schwerpunktprogramm "Regulatory Networks in Bacteria", He-1556) and by the Fonds der Chemischen Industrie. Andrea Muffler and Christoph Marschall are the recipients of fellowships from the Graduiertenförderung des Landes Baden-Württemberg.

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