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Osmotic Regulation of *rpoS*-Dependent Genes in *Escherichia coli*

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The *rpoS* gene, which encodes a putative alternative sigma factor (σ^S), is essential for the expression of a variety of stationary-phase-induced genes as well as for stationary-phase-specific multiple-stress resistance. As previously shown for the *otsA* and *otsB* genes (R. Hengge-Aronis, W. Klein, R. Lange, M. Rimmele, and W. Boos, J. Bacteriol. 173:7918–7924, 1991), we demonstrate here that additional *rpoS*-controlled genes (*bolA*, *csi-5*) as well as at least 18 proteins on two-dimensional O'Farrell gels could be induced in growing cells by osmotic upshift via an *rpoS*-dependent mechanism. Also, *rpoS*-dependent thermotolerance and resistance against hydrogen peroxide could be osmotically stimulated. In contrast, the expression of *glgS*, while exhibiting strong stationary-phase induction, was only weakly increased by elevated osmolarity, and several *rpoS*-dependent proteins previously identified on two-dimensional gels were not osmotically induced. During osmotic induction of *rpoS*-dependent genes, *rpoS* transcription and the level of σ^S remained unchanged. We conclude that osmotically regulated genes represent a subfamily within the *rpoS* regulon that requires differential regulation in addition to that provided by σ^S.

rpoS is a regulatory gene of central importance for early stationary-phase-induced genes in *Escherichia coli* (14). Since the *rpoS* regulon appears to be fairly large, it is not surprising that *rpoS* was discovered several times independently and in different contexts. Thus, *rpoS* is identical to *katF*, which was identified as a regulatory gene for catalase HPII (*katE*) (22) and for exonuclease III (*xthA*) (31), as well as to *appR*, which is required for the expression of acid phosphatase (*appA*) (34). *rpoS* was also identified as a gene locus (*nur*) conferring near-UV resistance (32, 35). Furthermore, a systematic search for stationary-phase-induced chromosomal *lacZ* fusions yielded a fusion in *rpoS* (*csi-2*::*lacZ*) that is induced fivefold during the transition into stationary phase and confers a highly pleiotropic phenotype, suggesting a regulatory function (21). Thus, *rpoS* mutants are starvation sensitive, do not develop characteristic stationary-phase-specific stress resistance against high temperature, hydrogen peroxide (21), high osmolarity (27), and ethanol (14a), and do not accumulate glycogen (21) and trehalose (16). In contrast to wild-type *Escherichia coli* strains, *rpoS* mutants do not form small spherical cells in the stationary phase (20). Besides the genes mentioned above, the following genes have been shown to be under the control of *rpoS*: *bolA* (20), *otsBA* and *treA* (16), *osmB* (16), *glgS* (15), *appCBA* (also called the *cyxAB-appA* operon) (6), *appY* (3a), *mcc* (8), and *csi-5* (*osmY*) (unpublished results). However, an analysis on two-dimensional O'Farrell gels of total cellular protein indicates that a majority of *rpoS*-regulated genes are still unknown (21, 27). The striking sequence homology of the *rpoS* gene product to the vegetative sigma factor σ^{70} (23, 29) as well as the lack of typical σ^{70} promoters in front of *rpoS*-controlled genes (14) strongly indicates that *rpoS* encodes an alternative sigma factor (σ^S). In vitro experiments confirming this function have recently been been successful (16b).

The large number of genes within the *rpoS* regulon as well as their very diverse functions already may suggest the existence of gene subfamilies that are subject to some differential control. Preliminary evidence for such a subfamily was provided by study of a group of *rpoS*-dependent genes (16) that were originally identified as being osmotically regulated. These include *otsA*, *otsB*, and *treA*, which are involved in trehalose synthesis and metabolism, thermotolerance, and osmoprotection (4, 10, 16), and *osmB*, which encodes a lipoprotein of unknown function (12, 19). For *otsA* and *otsB*, it was shown that both osmotically regulated expression and growth-phase-dependent expression require *rpoS*, a result indicating that the same promoter(s) is used in both situations (16).

These findings raise several interesting questions. Does *rpoS* have a role in osmotic regulation in addition to its role in early stationary-phase gene regulation, and is *rpoS* itself induced by osmotic upshift? Are all *rpoS*-dependent genes osmotically regulated, or do osmotically induced genes indeed represent a subset of *rpoS*-regulated genes that require some sort of differential regulation in addition to that provided by σ^S? In this report, we present evidence for this latter possibility by demonstrating that some, but not all, *rpoS*-dependent genes or proteins can be significantly induced by osmotic upshift, that for a given gene the extents of osmotic induction and of stationary-phase induction do not necessarily correlate, and that the expression of *rpoS* itself is not increased by a shift to high-osmolarity conditions.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are listed in Table 1. P1 transductions performed as described by Miller (28) were used for strain constructions. In *bolAp*$_1$::*lacZ*, the *bolAp*$_1$ promoter is fused to *lacZYA* followed by the kanamycin resistance gene (2), and λMAV103, which carries this construct, is integrated at *att* in MC4100. *csi-5*::*lacZ* is a λp*lac*Mu55 transcriptional fusion in the chromosomal copy of *csi-5* and is one of a series of carbon starvation- and stationary-phase-induced fusions previously isolated (21). The *lacZ* fusion in *glgS* encodes a β-galactosidase hybrid protein and was

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TABLE 1. Bacterial strains

Strain	Relevant genotype[a]	Reference or source
MC4100	F⁻ Δ(*arg-lac*) *U169 araD139 rpsL150 ptsF25 flbB5301 rpsR deoC relA1*	33
RH90	MC4100 *rpoS359*::Tn*10*	21
RH100	MC4100 Δ*rpoS360 zfi3251*::Tn*10*	R. Hengge-Aronis
RO22	MC4100 *otsA1*::Tn*10* Φ(*otsB*::*lacZ*)*8* λ*plac*Mu55)	16
RO35	MC4100 [λ*RL1-25* Φ(*rpoS*::*lacZ*) (hybr)]	This study
RH95	MC4100 [λMAV103 Φ(*bolAp*$_1$::*lacZYA*) Km^r]	20
RH96	RH95 *rpoS359*::Tn*10*	20
RO151-a	MC4100 Φ(*csi-5*::*lacZ*) (λ*plac*Mu55)	21
RH99	RO151-a *rpoS359*::Tn*10*	This study
RH105	MC4100 [λRH704 Φ(*glgS*::*lacZ*) (hybr)]	15
RH106	RH105 *rpoS359*::Tn*10*	15

[a] Δ*rpoS360* is a chromosomal deletion between the two *Kpn*I restriction sites flanking the *rpoS* open reading frame. hybr, translational fusion.

constructed on a plasmid (pRH704) and then crossed into phage λRZ5, which was then lysogenized into MC4100 (15). The translational *rpoS*::*lacZ* fusion in RO35 was constructed as follows. Tn*lac* (25) insertions in *rpoS* on plasmid pRH320 (21) were isolated and stabilized by deleting the Tn*lac* region downstream of *lac* as well as the pRH320 insert downstream of the *rpoS*::*lacZ* fusion joint by use of *Hin*dIII restriction sites in Tn*lac* and in the pBR322 portion of pRH320. The fusion used in this study was crossed into λRZ5 (30), and the resulting phage (λRL1-25) was then lysogenized into MC4100 at *att*. The *rpoS*::*lacZ* fusion joint was determined (19a) as being at bp 399 downstream of the *Nru*I restriction site (see the *rpoS* sequence in reference 29).

Cultures were grown at 37°C under aeration in M9 minimal medium (28) with 0.4% glycerol as a carbon source. Growth was monitored by measuring the optical density at 578 nm (OD_{578}). After at least three generations of exponential growth, the cultures were divided when they reached an OD_{578} of approximately 0.3. A final concentration of 0.3 M NaCl or 0.464 M sucrose was added to increase the osmolarity of culture aliquots, whereas control aliquots did not receive any supplement, and incubation was continued under the same conditions.

β-Galactosidase assay. β-Galactosidase activity was assayed by use of *o*-nitrophenyl-β-D-galactopyranoside as a substrate and is reported as micromoles per minute per milligram of protein (28).

Stress resistance assays. Thermotolerance or H_2O_2 resistance was tested by determining CFU for heat-shocked or H_2O_2-exposed cell suspensions. At 90 min after division of the cultures into aliquots and osmotic shift (see above), the cells were diluted in 0.9% NaCl to approximately 6×10^3 cells per ml (for thermotolerance assays) or 6×10^7 cells per ml (for H_2O_2 resistance assays). One-milliliter samples were immediately exposed to the respective stress conditions (51.5 to 52°C; 15 mM H_2O_2 at 37°C). Aliquots (100 μl) were withdrawn at various times and plated onto Luria-Bertani medium (LB) plates without further dilution (thermotolerance assays) or after a 10^{-4} or 10^{-3} dilution (H_2O_2 resistance assays). Initial 100% survival was determined immediately before exposure to stress conditions. All stress resistance assays were performed at least in duplicate.

Western blot (immunoblot) analysis. Cultures for Western blot analysis were grown as described above. Samples were taken from cultures grown in the presence or absence of 0.3 M NaCl and adjusted to an OD_{578} of 0.3 by use of the same media as those used for the growth of the respective cultures. Samples (1.5 ml) were precipitated with trichloroacetic acid (10%), washed in acetone, and resuspended in sodium dodecyl sulfate electrophoresis sample buffer. Aliquots of these samples were run on sodium dodecyl sulfate-polyacrylamide (12%) gels. Gels were blotted onto Immobilon polyvinylidene difluoride membranes (Millipore). For gel electrophoresis and blotting, the modules of a Bio-Rad Mini-Protean device were used in accordance with the instructions of the manufacturer. For immunostaining, a polyclonal antibody against σ^S (KatF) (serum raised in a rabbit), a secondary antibody conjugated to alkaline phosphatase (Sigma), and 4-nitroblue tetrazolium chloride and 5′-bromo-4′-chloro-3′-indolyl-phosphate (both from Boehringer Mannheim) were used.

Two-dimensional O'Farrell gel electrophoresis. Cultures were grown as described above. At 20 min after the cultures were divided into aliquots and 0.3 M NaCl was added to one culture aliquot, samples (1 ml; adjusted to an OD_{578} of 0.15 by use of the same media as those used for the growth of the respective cultures) were labeled with 10 μCi of L-[^{35}S]methionine (>1,000 Ci · mmol^{-1}; Amersham) and prepared for electrophoresis as described previously (21). Two-dimensional gel electrophoresis conditions were exactly as described previously (21). The pH gradient in the first dimension ranged from approximately 7 to 4.8 (left and right sides of the gel, respectively, in Fig. 2).

RESULTS

Osmotic induction of *rpoS*-dependent genes. We previously showed that *otsA* and *otsB*, which encode the trehalose synthesis enzymes, require *rpoS* for growth-phase-dependent expression as well as for osmotic induction (16). Since osmotic regulation may be a general feature of *rpoS*-regulated genes, we tested several other such genes for which *lacZ* fusions were available to us. The *bolA* gene encodes a secondary regulator that is involved in the morphological changes that occur during entry into the stationary phase, and its expression is stimulated 5- to 10-fold in an *rpoS*-dependent manner when the cells enter the stationary phase (1, 3, 20). The *csi-5* gene was first identified as an *rpoS*-dependent stationary-phase-induced *lacZ* fusion (21), and the sequence of the *csi-5* gene (16a) turned out to be identical to that of the recently described hyperosmotically inducible *osmY* gene, which encodes a periplasmic protein of unknown function (36). The expression of the chromosomal transcriptional *csi-5*::*lacZ* fusion used here is induced approximately 25-fold during entry into the stationary phase in LB medium (to a final activity of about 0.5 μmol · min^{-1} · mg^{-1}) (unpublished results). *glgS* plays a role in glycogen synthesis, presumably in glycogen priming.

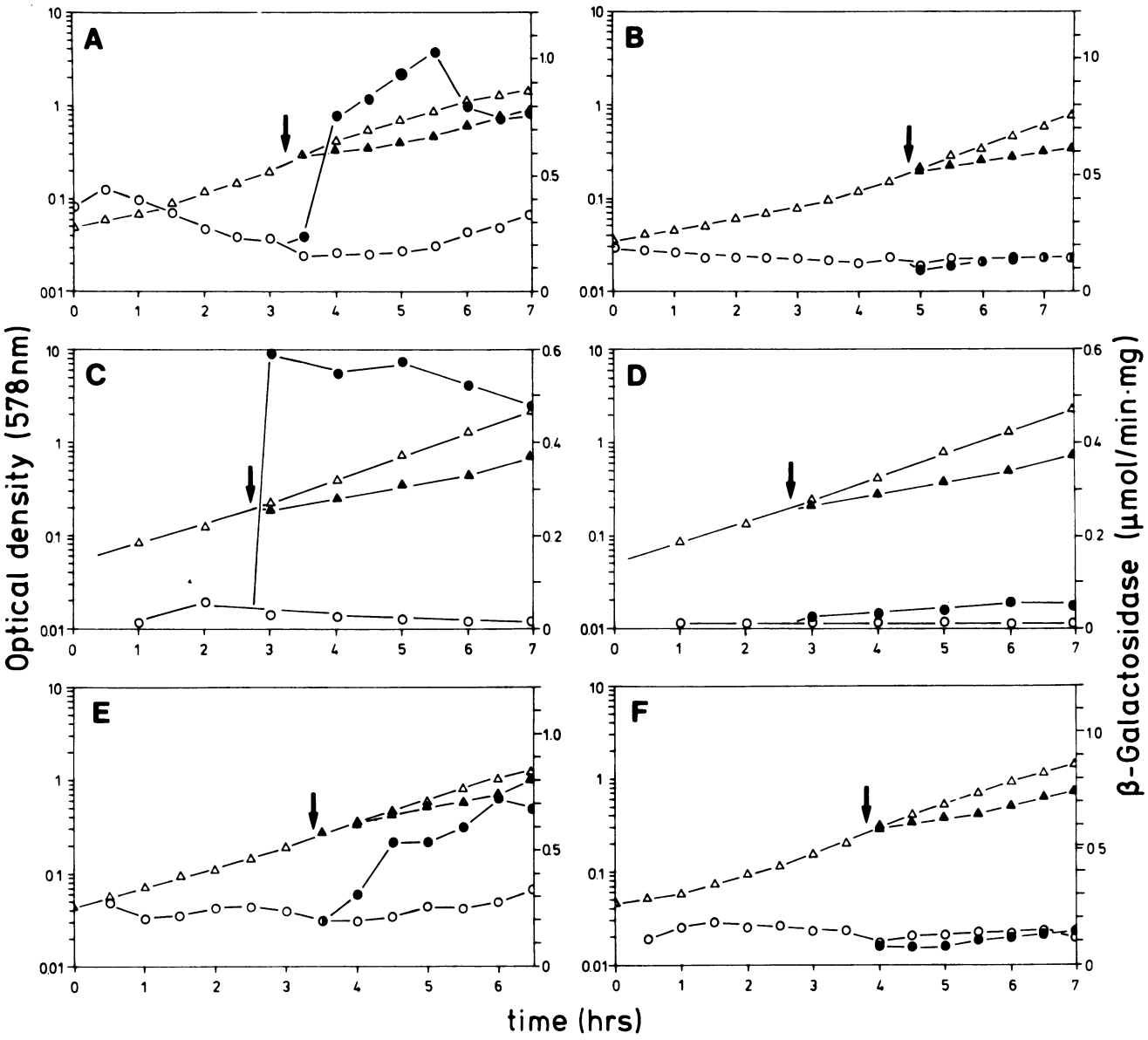

FIG. 1. Osmotic induction of *rpoS*-dependent genes. MC4100 carrying *bolAp*$_1$::*lacZ* (A), *csi-5*::*lacZ* (C), and *glgS*::*lacZ* (E) as well as the isogenic *rpoS*::Tn*10* derivatives (B, D, and F, respectively) were grown in M9 minimal medium with 0.4% glycerol. At the times indicated by the arrows, the cultures were divided into aliquots and 0.3 M NaCl was added to one of the two aliquots in each case. The OD_{578} (triangles) and β-galactosidase specific activities (circles) were determined in cultures grown in the presence (closed symbols) or absence (open symbols) of NaCl.

The expression of *glgS*::*lacZ* is induced 30- to 50-fold in the early stationary phase, and *glgS* is probably one of the most strongly expressed stationary-phase-specific genes (the activity of a chromosomal translational *glgS*::*lacZ* fusion reaches approximately 7.0 μmol · min^{-1} · mg^{-1}). About half of this stationary-phase induction is due to the presence of a σ^S-requiring promoter (*glgSp*$_2$), whereas *glgSp*$_1$ is dependent on cyclic AMP-cyclic AMP receptor protein (15).

lacZ fusions to *bolA*, *csi-5*, and *glgS* were tested for osmotic induction. The fusion strains were grown in minimal medium with glycerol as a carbon source to avoid potential interference with osmotic induction of the various osmoprotectants present in LB medium. Figure 1 shows that expression could be stimulated by an osmotic upshift in all three cases and that this induction required an intact *rpoS* allele. For *bolA*::*lacZ* and *csi-5*::*lacZ*, the factor of induction and the final level of β-galactosidase activity corresponded more or less to the values found during the transition into stationary phase. *glgS*::*lacZ*, however, was only very weakly osmotically induced (approximately twofold), a result in pronounced contrast to its strong growth-phase-related induction.

Using two-dimensional gel electrophoresis, it was shown that at least 16 (21) or up to 32 proteins (27) were absent or produced at a reduced rate in *rpoS* mutants. Figure 2 shows that numerous proteins on two-dimensional gels were induced in strain MC4100 by the addition of NaCl to the growth medium. *rpoS* was required for the osmotic induction

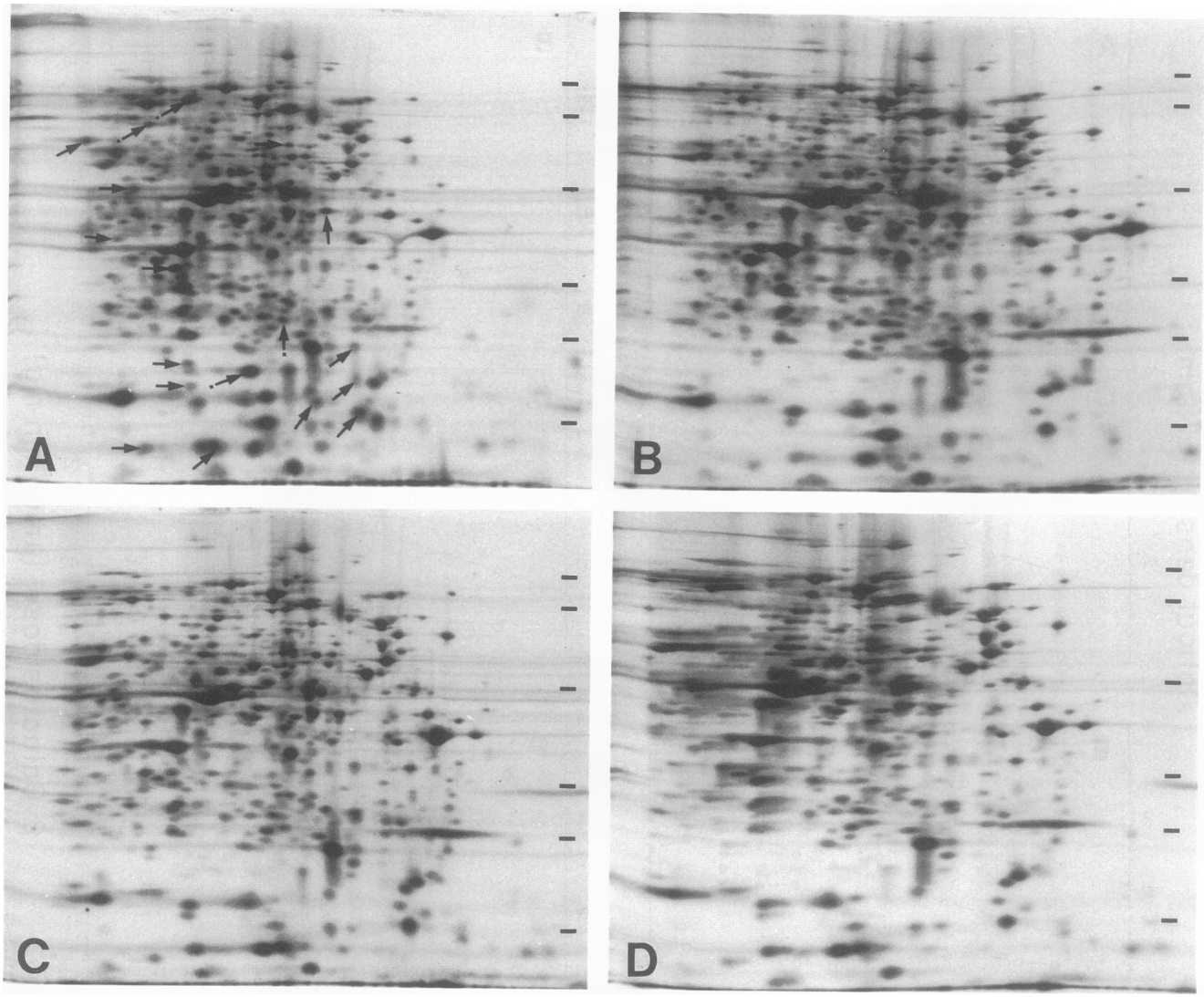

FIG. 2. *rpoS*-dependent osmotic induction of proteins visualized by two-dimensional O'Farrell gel electrophoresis. [^{35}S]methionine-labeled total cellular proteins of strain MC4100 (A and C) and of the Δ*rpoS* mutant RH100 (B and D) grown in the presence (A and B) or absence (C and D) of 0.3 M NaCl were separated by two-dimensional O'Farrell gel electrophoresis. Protein spots that were induced by growth in high-osmolarity medium in MC4100 (compare A and C) but not in the Δ*rpoS* mutant (compare with B) are labeled by arrows in A. Among these, the proteins previously identified as being *rpoS* dependent (21) are marked by an additional dot. The positions of molecular mass standards are indicated at the right side of the gels (from top to bottom: 106, 80, 49.5, 32.5, 27.5, and 18.5 kDa).

of 18 of these proteins (labeled by arrows in Fig. 2A; compare with Fig. 2B). However, only four of these protein spots corresponded unequivocally to *rpoS*-dependent protein spots previously identified (in a Δ*cya* mutant background in which *rpoS* expression was increased) (21). Thus, many of the osmotically inducible proteins shown in Fig. 2A have been newly identified as being under *rpoS* control. On the other hand, the expression of several *rpoS*-dependent proteins previously found was not affected by increased osmolarity. These findings indicate differential control of different genes within the large *rpoS* regulon.

Osmotic induction of *rpoS*-dependent stress resistance. Stationary-phase cells exhibit strong resistance against high-temperature heat shock (>50°C) (17). *rpoS* mutants fail to develop this thermotolerance (21). In contrast, adaptive thermotolerance in growing cells that can be induced by a nonlethal temperature upshift is not under the control of *rpoS* (16) but may require σ^E (9). Figure 3 shows that thermotolerance in growing cells could also be induced by an increase in medium osmolarity (caused by the addition of 0.3 M NaCl or 0.464 M sucrose) and that this type of thermotolerance required an intact *rpoS* allele. Whereas the addition of NaCl significantly reduced the rate of growth of the culture (from a doubling time of 110 min to that of 170 min), an iso-osmolar concentration of sucrose only weakly affected the growth rate (doubling time, 120 min). Therefore, thermotolerance was not induced as a consequence of a reduced growth rate.

Thermotolerance is a complex phenotype that apparently involves the gene products of several unknown structural genes. As one of these factors, trehalose synthesis was implicated in *rpoS*-dependent stationary-phase thermotolerance. The *ots* genes, which are required for trehalose synthesis (10), have been shown to be under *rpoS* control, and

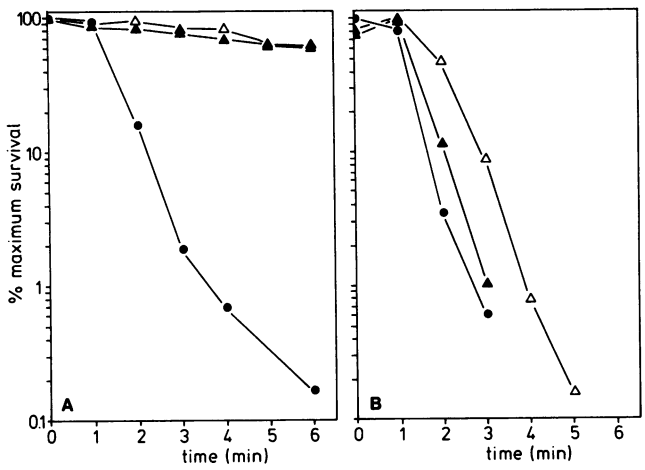

FIG. 3. Osmotic induction of thermotolerance. Exponentially growing cells of strains MC4100 (A) and RH90 (*rpoS*::Tn*10*) (B) were exposed to 52°C after 90 min of incubation in the presence of 0.3 M NaCl (▲), 0.464 M sucrose (△), or no osmotically active supplement (●). Viable cell numbers were determined as CFU by plating aliquots on LB plates.

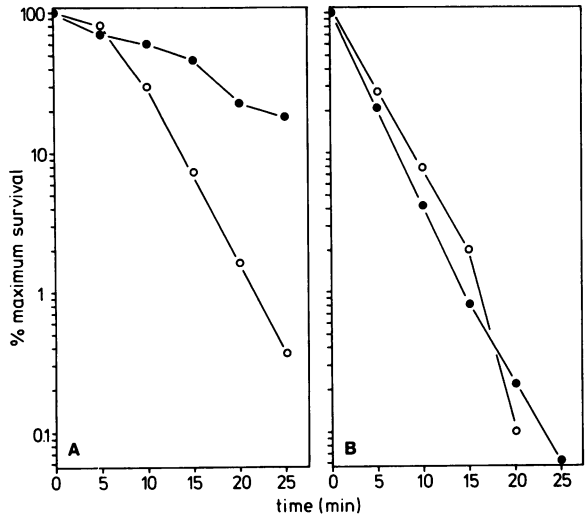

FIG. 4. Osmotic induction of H_2O_2 resistance. Strains MC4100 (A) and RH90 (B), grown for 90 min in the presence (●) or absence (○) of 0.3 M NaCl, were exposed to 15 mM H_2O_2. Viable cell numbers were determined as CFU by plating appropriately diluted aliquots on LB plates.

an *otsA otsB* mutant is significantly more thermosensitive than wild-type strains when grown in minimal medium (16). Trehalose is massively accumulated in minimal medium of high osmolarity, in which it acts as an osmoprotectant (10). However, it is not essential for osmotically induced thermotolerance, which was similar in the trehalose-free *otsA otsB* mutant and in an isogenic trehalose-synthesizing strain (data not shown). This result suggests that an *rpoS*-dependent thermoprotective mechanism apart from trehalose synthesis is present in osmotically stressed but not stationary-phase cells.

Starved cells develop resistance against hydrogen peroxide (17) that is dependent on *xthA* (7) and *katE* (27), both of which require *rpoS* for expression. H_2O_2 resistance was also stimulated by an increase in osmolarity (Fig. 4). Again, this osmotic induction was dependent on an intact *rpoS* allele.

Osmotic induction of *rpoS*. For understanding the osmotic regulation of *rpoS*-dependent genes, it is of crucial importance to know whether *rpoS* itself is induced in response to an osmotic upshift. The osmotic induction of *rpoS*-dependent genes is a rapid process. This fact is illustrated by the analysis of *lacZ* fusions to *otsBA* (16), *bolA*, and *csi-5* (Fig. 1) and also by the 18 *rpoS*-dependent osmotically induced protein spots in Fig. 2 (for which proteins were labeled 20 min after the addition of NaCl). Figure 5 shows the expression of a chromosomal single-copy *rpoS*::*lacZ* fusion in response to the addition of 0.3 M NaCl. For at least 90 min, β-galactosidase activities remained the same in the presence or absence of NaCl. After this time, an approximately twofold increase in *rpoS*::*lacZ* expression could be observed in NaCl-treated cells, a result most likely due to the reduced growth rate, since the expression of *rpoS* is correlated with the doubling time of a culture (21a). This slight induction of *rpoS* expression, however, was too late to be involved in the osmotic induction of the genes mentioned above. The *rpoS* fusion used for this experiment is translational and is carried by a lysogenic lambda phage, but a similar experiment with a chromosomal *rpoS* transcriptional fusion (21) yielded identical results (data not shown). Therefore, the pattern of expression of the *rpoS*::*lacZ* fusion shown in Fig. 5 reflects *rpoS* transcription. Western blot analysis indicated that at least for 90 min, the level of the σ^S protein was identical in osmotically stressed and control cells as well (Fig. 6). We conclude that a potential stabilization of σ^S after an osmotic upshift, which might result in a higher cellular σ^S concentration even in the absence of transcriptional induction, can also be ruled out.

DISCUSSION

In this report, we show that some of the known *rpoS*-dependent genes and phenotypes are not only induced during the transition into the stationary phase but are also osmotically induced. Moreover, the analysis of the total protein synthesis pattern on two-dimensional O'Farrell gels demonstrated that there is a large group of proteins that

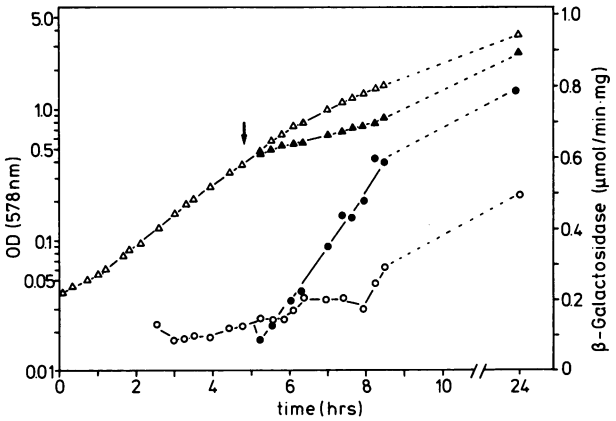

FIG. 5. Expression of *rpoS*::*lacZ* after an osmotic upshift. The OD_{578} (triangles) and β-galactosidase activities (circles) were determined for the *rpoS*::*lacZ*-carrying strain RO35 in the presence (closed symbols) or absence (open symbols) of 0.3 M NaCl. Growth and osmotic shift conditions were as described in the legend to Fig. 1.

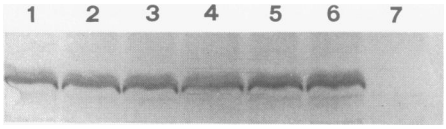

FIG. 6. Cellular levels of the σ^S protein after an osmotic upshift. Strain MC4100 was grown and osmotically shifted as described in Materials and Methods. Samples were prepared by trichloroacetic acid precipitation from cultures grown in the absence (lanes 1, 2, and 3) or in the presence (lanes 4, 5, and 6) of 0.3 M NaCl. Samples were withdrawn 15 min (lanes 1 and 4), 30 min (lanes 2 and 5), and 90 min (lanes 3 and 6) after the culture was divided into aliquots and NaCl was added to one of the two aliquots in each case. As a control, a similar sample from the Δ*rpoS* mutant RH100 (grown without NaCl) was used (lane 7). The amount of protein in each lane corresponds to 0.75 ml of a culture with an OD_{578} of 0.3. After the gel was blotted, the σ^S bands were visualized by immunostaining with a serum raised against purified σ^S.

exhibit *rpoS*-dependent osmotic induction (18 proteins). Although medium osmolarity was generally increased by the addition of NaCl, the addition of an iso-osmolar concentration of sucrose induced thermotolerance (Fig. 3) and *csi-5*::*lacZ* expression (data not shown) equally well, indicating an osmotic rather than a salt effect. Whereas NaCl clearly reduced the rate of growth of the cultures, sucrose did not do so. Therefore, we can exclude the possibility that the observed effects were simply caused by a change in the growth rate. Moreover, we also found that the level of thermotolerance was not inversely correlated with the growth rate, since MC4100 cells growing in LB or minimal glucose medium were more thermoresistant than cells growing in minimal glycerol or acetate medium (data not shown). We conclude that the induction of the genes and resistance phenotypes described here is a true osmotic response. In all cases, this osmotic response was abolished in an *rpoS* mutant background. Thus, the osmotically regulated promoters are the directly or indirectly σ^S-dependent ones, and additional promoters that are subject to a different type of control (e.g., the cyclic AMP-dependent $glgSp_1$ promoter) are not involved.

Our results appear to assign *rpoS* a novel role as an osmoregulator. Although many osmotically regulated genes or operons, such as *proU* (5, 26) or the *osm* genes (13), have been found and in some instances upstream regulatory regions have been characterized in detail (18, 24), no general osmoregulatory protein has been identified so far. However, it is also possible that the presence of the *rpoS* gene product is the basic requirement for expression of the genes under study here and that the actual level of expression after an osmotic upshift or in the stationary phase is determined by additional factors. Our results are consistent with this latter hypothesis, for the following reasons.

(i) Whereas the expression of *csi-5* and *bolA* (this study) or *otsBA* (16) immediately increased after an osmotic upshift, no changes in *rpoS* transcription and translation or in σ^S levels could be detected for at least 90 min. After this period, we found a twofold increase in *rpoS*::*lacZ* expression. This weak induction might have been caused by the reduced growth rate rather than by the increased osmolarity and was clearly too late to be of relevance for the osmotic induction of the genes mentioned above.

(ii) For a given *rpoS*-dependent gene or protein, the level and factor of osmotic induction did not necessarily correspond to the extent of induction during entry into the stationary phase. Whereas osmotic induction and stationary-phase induction of *otsBA*, $bolAp_1$, and *csi-5* were similar, stationary-phase induction drastically exceeded osmotic induction for *glgS* (even when only the activity of the *rpoS*-controlled $glgSp_2$ promoter was taken into account). Also, for *osmB*, stationary-phase induction was stronger than osmotic induction (18). In addition, not all of the *rpoS*-dependent proteins previously identified on two-dimensional gels were found to be osmotically induced. On the other hand, there appears to be osmotic induction of an *rpoS*-dependent mechanism that is not expressed in starved cells: the *otsA otsB* mutant, which is unable to synthesize trehalose, is significantly impaired in stationary-phase-induced thermotolerance (16) but exhibits osmotically induced thermotolerance, indicating the existence of an *rpoS*-controlled thermoprotective mechanism that is specifically induced by increased osmolarity. In addition, several osmotically induced *rpoS*-dependent proteins on the two-dimensional gels have been newly identified under high-osmolarity conditions, and it is possible that at least some of these are predominantly expressed after osmotic stimulation.

We conclude that within the *rpoS* regulon there is differential expression of genes induced either by nutrient limitation or by an osmotic upshift or both. This differential expression cannot be explained by changes in the level of total σ^S or of active σ^S alone (even if there were significant such changes after an osmotic shift). Rather, besides an essential direct or indirect requirement for σ^S, the promoters involved respond differentially to these environmental changes. This strongly suggests the existence of additional factors that modulate the activities of these promoters. The expression or activities of these factors should be controlled by sensor mechanisms that respond to the respective conditions. The molecular details of the regulatory mechanisms involved are presently being studied.

The role of σ^S in gene regulation appears to be more complex than that of several other, alternative σ^{70}-like sigma factors. The expression of the heat shock genes, for instance, is a direct function of the cellular concentration of active σ^{32}, and no evidence for additional modulation has been reported (11). In contrast, osmotic induction of *rpoS*-dependent genes takes place in the absence of changes in the level of σ^S. σ^S may function more like σ^{70}, which in many cases uses additional regulatory proteins for efficient transcription initiation. The concentrations and activities of these regulators determine the levels of expression of subsets of genes.

The results presented in this paper also demonstrate that σ^S not only is an early stationary-phase regulator but also plays a role in the expression of genes that are important under various conditions of slow growth, regardless of whether a reduction in the growth rate is caused by nutrient limitation, by changes in osmolarity, or perhaps by some other nonoptimal growth conditions. Therefore, *rpoS* is probably of major importance for switching between different physiological states because of the "feast and famine" existence as well as because of variable stress conditions in the natural environments of *E. coli*.

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