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The General Stress Sigma Factor σ^S of *Escherichia coli* Is Induced during Diauxic Shift from Glucose to Lactose

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The general stress sigma factor σ^S (RpoS) of *Escherichia coli* is strongly induced in response to glucose starvation. This increase in the cellular σ^S level is due to stabilization of σ^S, which under non-stress conditions is subject to rapid proteolysis. In the present study, it is demonstrated that σ^S is also induced during the diauxic shift from glucose to lactose, i.e., under conditions of glucose exhaustion in the presence of another, less-preferred carbon source that eventually gets utilized. This σ^S induction, which is due to stabilization, is transient and precedes the induction of β-galactosidase. In parallel, σ^S-dependent genes are transiently activated, as was shown here for *osmY*. Although σ^S can mediate transcription of *lacZ* in vitro, σ^S does not contribute to the induction of β-galactosidase during the diauxic lag phase. Rather, the induction of σ^S and the general stress response during the diauxic shift plays the role of a rapidly activated emergency system, which is shut off again as soon as the cells are able to cope with the stress situation by utilizing a more specific and more economical system.

The σ^S subunit of RNA polymerase (or RpoS) is the master regulator of the general stress response in *Escherichia coli*. While σ^S levels are low in rapidly growing cells not exposed to any particular stress, σ^S is induced in response to a variety of rather diverse environmental stress conditions that include starvation for various nutrients, stationary phase in general, high osmolarity, and high or low temperature (for recent reviews, see references 3 and 4). These stresses differentially affect *rpoS* transcription and translation as well as the rate of proteolysis of σ^S, which under non-stress conditions is a highly unstable protein (11). Glucose starvation in particular is one of the conditions that interferes with σ^S turnover (11, 24). σ^S induction is then followed by the activation of numerous σ^S-dependent genes, which results in rather dramatic changes in physiology, including the expression of a strong general stress resistance, and even in cellular morphology (4, 7, 13).

The reaction to glucose exhaustion has been studied extensively under conditions where the medium contains a second utilizable, even though less-preferred, carbon source such as lactose. During growth on glucose, uptake and utilization of lactose are inhibited due to inducer exclusion and low-level expression of the *lac* operon mediated by enzyme IIA^{Glc}, a component of the PTS^{Glc} uptake and signal transduction system (14, 20–22). Here, glucose exhaustion results in a transient growth arrest termed the diauxic lag phase, during which lactose permease and β-galactosidase are induced, which then allow the cells to resume growth on lactose as the carbon source (2, 16).

The regulation of the *lac* operon has provided the basis for paradigms of specific gene regulation in bacteria, but the diauxic shift has not been looked at as a bacterial stress response. Therefore, we asked whether entry into the diauxic lag phase, i.e., starvation for glucose in the presence of another carbon source that eventually gets utilized, provokes a stress response similar to that observed upon glucose starvation in a medium that does not contain other carbon sources, i.e., during entry into stationary phase. Under the latter conditions, induction of σ^S results in major physiological and morphological changes. However, when another carbon source could replace the missing glucose, the induction of such a complex response would not be necessary. *E. coli* might thus be able to discriminate between the two situations.

However, we observed σ^S induction during the diauxic lag phase, which precedes the induction of the *lac* operon and lactose utilization. As cells resume growth on lactose, σ^S levels are reduced again. Thus, σ^S induction, which is due to inhibition of σ^S proteolysis, has the characteristics of an immediate emergency system that the cells transiently resort to, even if in the somewhat longer run they can afford not to make use of it.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* W3110 [F^- IN(*rrnD-rrnE*)*1 thyA36 deoC2*] (1, 19) was used in the present study. The strain was kindly provided by the *E. coli* Stock Center (New Haven, Conn.). As it was recently observed that strain W3110 exists in a number of variants with respect to σ^S levels and even σ^S molecular weight (6), we initially tested σ^S levels and stress inducibility and found that these were very similar in our W3110 strain and in strain MC4100, in which σ^S has been well characterized previously (11). The *rpoS* mutant derivative of W3110 was obtained by P1 transduction (15) with strain RH90 (MC4100 *rpoS359*::Tn*10* [10]) as a donor.

Standard batch cultures were grown at 37°C under aeration in a rotary shaker in minimal medium M9 (15) supplemented with 0.03% glucose and 0.1% lactose for diauxie experiments. In single-carbon-source experiments, glucose and lactose were used at concentrations of 0.2%. Growth was monitored by measuring the optical density at 578 nm (OD_{578}).

SDS-PAGE and immunoblot analysis. Sample preparations for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8) and immunoblot analysis were performed as described previously (11). Twenty micrograms of total cellular protein per lane was used on SDS gels. For detection of σ^S and β-galactosidase, polyclonal sera produced in rabbits were used. Bands were visualized by using a goat anti-rabbit immunoglobulin G alkaline phosphatase conjugate (Sigma) and the chromogenic substrates BCIP (5-bromo-4-chloro-3-indolylphosphate)–nitroblue tetrazolium (Boehringer Mannheim). Relative σ^S and β-galactosidase levels determined were normalized for the levels obtained during exponential growth on glucose.

Pulse labelling of cells and immunoprecipitation. Pulse labelling of cells with L-[^{35}S]methionine and immunoprecipitation of σ^S were previously described (11). The OD_{578} of the samples was adjusted with supernatant from the same cultures obtained by centrifugation immediately before taking the samples for

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pulse labelling. The pulse time was 1.0 min. For the determination of relative rates of σ^S synthesis, the chase time was 0.25 min, whereas for the determination of σ^S half-life, chase times varied between 0.25 and 10 min. For immunoprecipitation, a polyclonal serum against σ^S (11) was used. Protein bands on autoradiographs were quantitated densitometrically. The intensity of bands representing σ^S was calculated relative to the intensity of bands representing stable proteins that weakly cross-reacted with the antisera used.

Preparation of mRNA and primer extension. For the quantitation of *osmY* mRNA, strain W3110 transformed with pNH5, a pBR322 derivative carrying the *osmY* gene on a 1.4-kb *Pst*I insert (4a), was used (W3110 carrying just the single copy of *osmY* on the chromosome does not produce enough *osmY* mRNA for reliable detection and quantification; *osmY* exhibits similar regulation when present in single or multiple copies [9], consistent with all its known regulators [9] being abundant proteins). Total RNA was prepared by hot phenol extraction of cells sampled during the different phases of the diauxie experiment. For primer extension reactions, a 5′ digoxigenin-labelled oligonucleotide with the sequence 5′-CAGTCTTGTCATAGTCATGG-3′, which is complementary to a region near the 5′ end of *osmY*, was used. The reactions were performed according to standard procedures (23) with 5 μg of total RNA and 12.5 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) as previously described (9, 12). As a reference, double-strand sequencing reactions were performed with the same primer as that used in the primer extension experiments.

β-Galactosidase assay. β-Galactosidase activity was assayed by use of ONPG (*o*-nitrophenyl-β-D-galactopyranoside) as a substrate and is reported as micromoles of *o*-nitrophenol per minute per milligram of cellular protein, as described by Miller (15).

RESULTS AND DISCUSSION

Cellular σ^S levels rapidly and transiently increase due to stabilization of σ^S during the diauxic lag phase. Classical diauxie experiments (16) were performed with *E. coli* W3110 by using a combination of 0.03% glucose and 0.1% lactose as carbon sources in a minimal medium batch culture. Relative cellular levels of σ^S and β-galactosidase were determined by immunoblot analysis (Fig. 1). As expected, a diauxic lag phase of approximately 40 min was observed (Fig. 1A). Immediately after the onset of this lag phase, we found an approximately threefold induction of σ^S (Fig. 1B and C). Increased σ^S levels were maintained throughout the lag phase but started to gradually decline again as soon as the cells resumed growth. By contrast, a more than 20-fold induction of β-galactosidase occurred with slower kinetics and reached a permanent maximum after the cells had already started to grow on lactose. Similar results (fivefold induction of σ^S) were obtained when the diauxic shift experiment was performed under batch fermentation conditions, where much higher cellular densities are reached (25).

When cells are grown on glucose alone as a carbon source, glucose exhaustion results in a rapid stabilization of σ^S (11, 24). The rapid kinetics of σ^S induction at the onset of the diauxic lag phase (Fig. 1) also suggested an inhibition of proteolysis rather than regulation at the genetic level. Therefore, we tested σ^S degradation in pulse-chase experiments during the different phases of the diauxie experiment (Fig. 2). We determined a σ^S half-life of approximately 2 min during the "glucose phase" (Fig. 2A), which is very similar to previously observed half-lives during growth on glucose alone (11, 17, 18, 24). In the diauxic lag phase, however, σ^S proteolysis was completely inhibited (Fig. 2B). σ^S turnover remained relatively inefficient in the "lactose phase" (as determined approximately 45 min after cells had started to grow on lactose), with the σ^S half-life being considerably higher (more than 20 min) than during the initial growth phase on glucose (Fig. 2C).

After the diauxic lag phase, when cells resume growth on lactose, the cellular σ^S content gradually declined to levels similar to those observed during initial growth on glucose (Fig. 1). Yet σ^S stability was clearly higher during the lactose phase than during the glucose phase (Fig. 2), indicating that the rate of σ^S synthesis might be low after cells start to grow on lactose. The 30% reduction in the relative rate of σ^S synthesis in

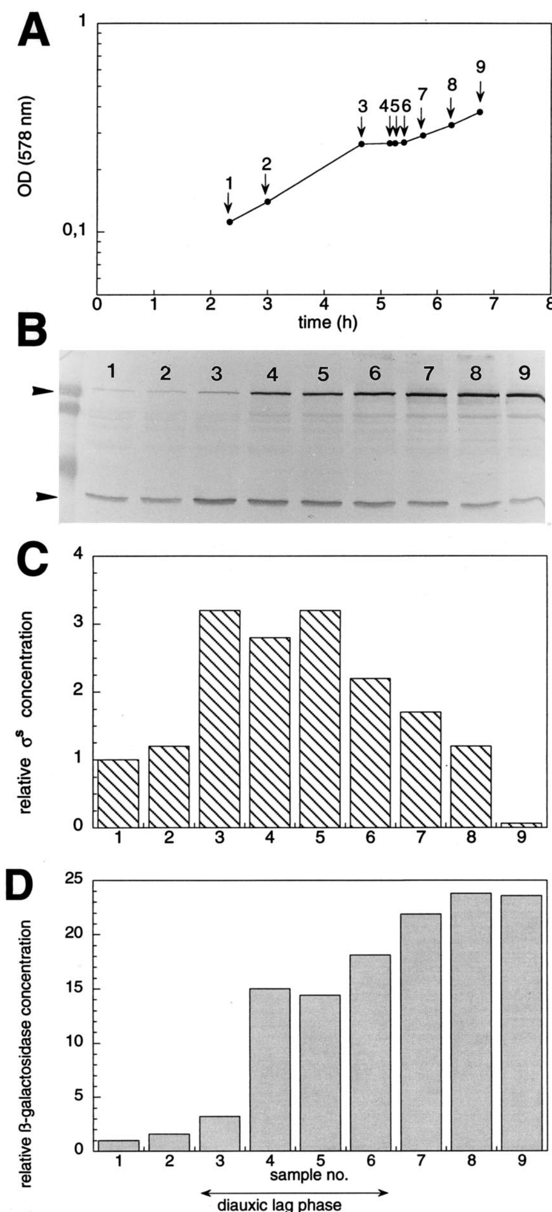

FIG. 1. Induction of σ^S and β-galactosidase during the diauxic shift from glucose to lactose. Strain W3110 was grown in M9 minimal medium supplemented with 0.03% glucose and 0.1% lactose. Samples are designated with the same numbers throughout the entire figure. OD_{578}s were measured (A) and relative levels of σ^S and β-galactosidase were determined by immunoblot analysis (B) (with size standard proteins of 106, 80, and 49 kDa shown at the left side of the figure). Bands representing σ^S (lower arrowhead) and β-galactosidase (upper arrowhead) were quantitated densitometrically (C and D, respectively), and the data obtained were normalized for the values determined during exponential growth on glucose (sample 1).

W3110 cells growing on lactose compared to that observed in cells growing on glucose (data not shown) did not seem sufficient to account for the observed decrease in σ^S levels during the lactose phase. Whether and how various carbon sources or specific growth conditions differentially modulate rates of σ^S synthesis and degradation, even though the resulting σ^S levels might be similar, remains to be determined.

We conclude that glucose starvation, no matter whether another eventually utilizable carbon source is present or not,

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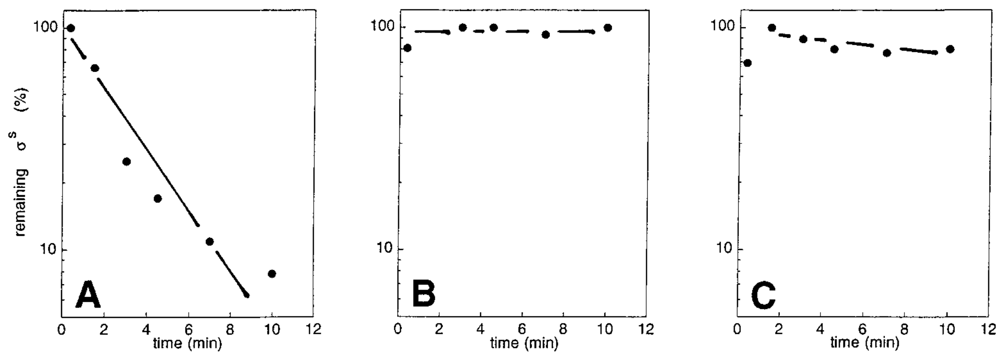

FIG. 2. In vivo turnover of σ^S during the different phases of the diauxic shift experiment. Strain W3110 was grown in M9 medium supplemented with 0.03% glucose and 0.1% lactose. Samples were taken during initial growth on glucose (OD_{578} = 0.16) (A), approximately 10 min after the onset of the diauxic lag phase (OD_{578} = 0.26) (B), and during growth on lactose (OD_{578} = 0.35) (C). The samples were pulse labelled with [^{35}S]methionine as described in Materials and Methods. σ^S was immunoprecipitated from cell extracts made of pulse-labelled cells and was visualized by SDS-PAGE and autoradiography. A densitometric quantitation of the autoradiographic data is shown.

initially represents a stress condition the cells respond to by inhibiting σ^S proteolysis and thus rapidly increasing their σ^S content. As soon as the cells start to utilize lactose as the alternative carbon source, σ^S levels are reduced again. Interestingly, this decrease in σ^S content is not due to rapid proteolysis; rather, σ^S synthesis seems to be reduced.

The increase in the cellular σ^S level during the diauxic lag phase is accompanied by the induction of σ^S-dependent genes. The general stress response is dependent on many genes that require σ^S for expression, but their expression profiles do not necessarily follow that of σ^S, since often additional regulatory proteins are involved in their control. Many σ^S-dependent genes (e.g., *osmY* [9]) are under the negative control of cyclic AMP (cAMP) receptor protein-cAMP complex (CRP-cAMP) (4). As cAMP levels rise during diauxic shift (5), we wondered whether those genes would really be activated in parallel to σ^S induction.

osmY mRNA was quantitated by primer extension before, during, and after the diauxic lag phase. We found that *osmY* mRNA levels increased in parallel to the σ^S content of the cells during diauxic growth arrest. As soon as cells resumed growth on lactose, *osmY* mRNA rapidly decreased again to hardly detectable levels (Fig. 3).

We conclude that not only σ^S but also *osmY* (and probably other similarly regulated σ^S-dependent genes) are induced during the diauxic lag phase, even though *osmY* is negatively controlled by CRP-cAMP (9). During the diauxic lag phase, cAMP levels increase in parallel to β-galactosidase levels (5, 19a), i.e., they lag behind the induction of σ^S and *osmY*. This may explain why CRP-cAMP does not interfere with *osmY* activation. Rather, CRP-cAMP could contribute to the rapid and apparently complete shutoff of *osmY* and perhaps other σ^S-dependent genes, i.e., to the termination of the general stress response around the end of the diauxic lag phase (before cAMP levels decrease again during the early phase of growth on lactose [5]).

Does the increase in the cellular σ^S level during the diauxic lag phase play a role in the induction of the *lac* operon? In in vitro transcription assays, the *lac* promoter can be recognized by RNA polymerase holoenzyme containing either σ^{70} or σ^S (25). Therefore, it seemed possible that increased σ^S levels during the diauxic lag phase could contribute to the expression of the *lac* operon. This would also be consistent with the kinetics of induction of σ^S and β-galactosidase. However, induction of β-galactosidase during the diauxic lag phase is very similar in wild-type and otherwise isogenic *rpoS* mutant cells

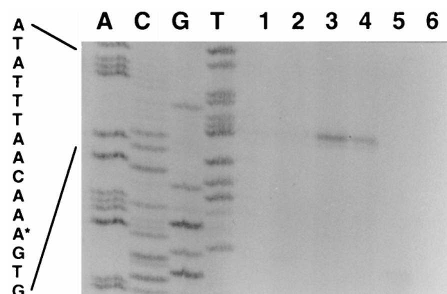

FIG. 3. Analysis of *osmY* mRNA during the different phases of the diauxic shift experiment. Strain W3110 carrying pNH5 was grown in M9 medium supplemented with 0.03% glucose and 0.1% lactose. RNA was prepared during growth on glucose (lane 1, OD_{578} = 0.15; lane 2, OD_{578} = 0.20), during the beginning (lane 3, OD_{578} = 0.27) and the end (lane 4, OD_{578} = 0.29) of the diauxic lag phase, and during growth on lactose (lane 5, OD_{578} = 0.31; lane 6, OD_{578} = 0.35). The reverse *osmY* transcript (for details, see Materials and Methods) is indicated by an arrowhead. As a reference, sequencing reactions were performed with pNH5 and the same primer as that used for primer extension. The sequence covering the *osmY* transcriptional start site is given, with the start site marked by an asterisk.

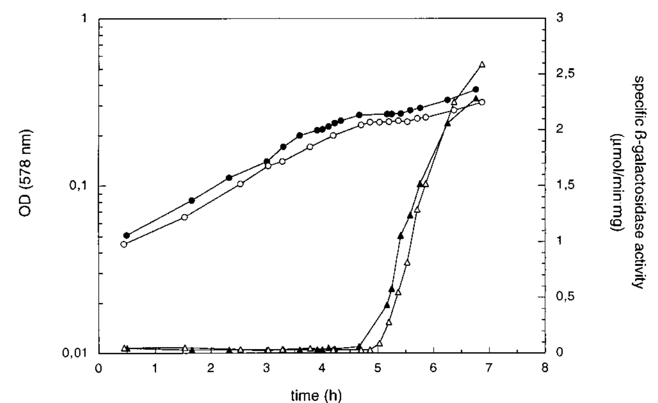

FIG. 4. Induction of β-galactosidase during the diauxic lag phase is not affected by a mutation in *rpoS*. Strain W3110 (closed symbols) and its *rpoS*::*Tn10* derivative (open symbols) were grown in M9 minimal medium supplemented with 0.03% glucose and 0.1% lactose. Optical densities (circles) and specific β-galactosidase activities (triangles) were determined.

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(Fig. 4), which demonstrates that σ^S does not play a role in the expression of the *lac* operon in vivo.

Conclusions. During the diauxic lag phase, *E. coli* cells transiently induce σ^S and σ^S-dependent genes; i.e., they activate the general stress response, even though in the somewhat longer run they do not need this complex response. What is the function of increased levels of σ^S and σ^S-dependent stress-protective proteins during the diauxic lag phase?

With its rapid kinetics and transient nature, this induction of σ^S and of the general stress response has the characteristics of a rapid emergency response. It may be that this response is initiated whenever the nutritional situation deteriorates but is reversed as soon as the cells induce another, more economical system that allows them to cope with the situation. Thus, the general stress and starvation response in *E. coli* is a flexible, very rapidly inducible, and probably at any time reversible response. Nevertheless, if not reversed, the physiological and morphological consequences and the protective potential of this σ^S-mediated stress response are considerable and, in principle, comparable to the consequences of sporulation. With these properties, the general stress response is a key to the remarkable flexibility of enteric bacteria in surviving frequent, rapid, and extreme changes in their natural environments.

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