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A Mutation That Enhances Synthesis of σ^{32} and Suppresses Temperature-Sensitive Growth of the *rpoH15* Mutant of *Escherichia coli*

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The *rpoH15* mutant cannot grow at or above 34°C, because it produces an altered σ^{32} protein that is largely deficient in the transcription of the heat shock genes. Extragenic suppressor mutations (*suhB*) located at 55 min on the *Escherichia coli* chromosome endowed the mutant cell with the ability to grow at 40°C and the inability to grow at 25°C. One such mutation (*suhB2*), studied in detail, markedly enhanced the rate of σ^{32} synthesis and the *rpoH* mRNA level during steady-state growth at 37 to 40°C but little affected the cellular content of σ^{32} or the induction of heat shock proteins. In the isogenic *rpoH*$^+$ strain, neither σ^{32} synthesis nor the *rpoH* mRNA level was enhanced by the *suhB* suppressor. Furthermore, expression of the *rpoH-lacZ* gene fusion, but not the operon fusion, was much higher in the *suhB* mutant than in the wild type or the *suhB rpoH*$^+$ strain, indicating that *suhB* affects *rpoH* expression primarily at the level of translation. *suhB* probably acts to increase σ^{32} synthesis by affecting the regulatory circuit of *rpoH* expression or by modulating certain parameters in protein synthesis. Consistent with these findings, overproduction of the mutant (*rpoH15*) σ^{32} by multicopy plasmid enabled the *rpoH15* or Δ*rpoH* (deletion) mutant to grow at up to 40°C. Plasmids containing an *E. coli* DNA segment of 1.0 kilobase could complement the cold-sensitive phenotype of the *suhB2* mutant. Nucleotide sequence analysis revealed that the segment contained an open reading frame encoding a protein of 29,128 daltons.

Induction of heat shock proteins in *Escherichia coli* is regulated primarily at the level of transcription (28, 29). A secondary σ factor, σ^{32}, encoded by the *rpoH* (*htpR*) gene, plays a major role in the transcription of the heat shock genes from specific (heat shock) promoters during steady-state growth, as well as upon exposure to a higher temperature (5, 9, 16). At a low temperature (30°C), σ^{32} is produced at a low level and is extremely unstable (10). When shifted to a higher temperature (42°C), both the synthesis rate and the stability of σ^{32} increase markedly though transiently (21). Thus, σ^{32} is actively involved in the regulation of the heat shock response so as to rapidly adjust the cellular content of σ^{32} to cope with the temperature stress (10, 20, 21, 23). The synthesis of σ^{32} can be regulated both at the level of transcription and at the level of translation (6, 7, 21). In the case of the temperature shift from 30 to 42°C, for example, regulation at the translational level appears to play a predominant role (21; H. Nagai, unpublished result).

To study the function and regulation of the heat shock response, we have isolated and characterized temperature-resistant pseudorevertants from various temperature-sensitive *rpoH* mutants. Extragenic suppressors involved in these reversions have been classified into several groups by their map locations on the *E. coli* chromosome. Three classes of suppressors, each obtained from the *rpoH* amber mutants (26), the opal mutant (31), and the deletion mutant (13), have been described previously.

This report deals with still another class of suppressor, designated *suhB*, that can suppress the temperature-sensitive growth of a missense *rpoH* mutation and simultaneously render the cell cold sensitive. It will be shown that a representative *suhB* mutation (*suhB2*) affects a novel gene (located at min 54.6) encoding a 29-kilodalton protein and causes markedly enhanced σ^{32} synthesis during steady-state growth at the permissive temperature (37 to 40°C), though little increase in the σ^{32} level or in the extent of heat shock induction could be demonstrated. The *suhB* mutant (suppressor) may provide a unique opportunity for further analysis of the mechanisms regulating σ^{32} synthesis in relation to the heat shock response in *E. coli*.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and plasmids. All bacterial strains used were derivatives of *E. coli* K-12. Strain MC4100 (3) and isogenic temperature-sensitive heat shock-defective *rpoH* mutants carrying a missense (*rpoH15*), amber (*rpoH6*, *rpoH16*, *rpoH18*, or *rpoH165*), or opal (*rpoH11*) mutation have been described (25, 30, 32). The *rpoH* deletion mutant KY1612 (Δ*rpoH30*::*kan*) of the same genetic background and λ *imm*^{21}-*rpoH*$^+$ phage have also been described (33). The temperature-resistant revertants (*suhB*) were isolated from the *rpoH15* mutant (KY1431) in this study. The *suhB2* mutation was transduced into the *rpoH15* mutant or the wild type (MC4100), and the resulting transductants were used as the *suhB2 rpoH15* strain (KY1450) or the *suhB2 rpoH*$^+$ strain (KY1451), respectively.

A λpF13 derivative carrying an *rpoH-lacZ* operon fusion (H. Nagai, R. Yano, J. W. Erickson, and T. Yura, submitted for publication) contained the entire *rpoH* promoter, the *trpA* Shine-Dalgarno sequence followed by the *trpA-lacZ* fusion gene (designated λOF-0). A λpF13 derivative carrying a gene fusion, on the other hand, contained not only the promoters but also the Shine-Dalgarno sequence and 364 base pairs of the *rpoH* coding region fused in frame to *lacZ* (designated λGF-364; Nagai, unpublished result). Plasmid

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pKV15 carrying the *rpoH15* mutant allele was constructed by inserting *rpoH15* (the *Hpa*I fragment of 2.4 kilobases [kb]) of KY1431 into pBR322 digested with *Pvu*II and transforming the Δ*rpoH* mutant by selecting for ampicillin resistance at 30°C. Plasmids containing the *suhB*$^+$ region (pKV56, pKV60, and pKV61) were obtained by subcloning an *Eco*RV fragment of pLC39-13 (4) into the *Eco*RV site of pBR322 (see Fig. 8).

Media and chemicals. Medium E (27) containing 0.5% glucose, thiamine (2 μg/ml), and amino acids (except methionine and cysteine) was used as minimal medium. L broth has been previously described (25). L-[^{35}S]methionine (1,000 Ci/mmol), [α-^{32}P]dCTP (800 Ci/mmol), and [γ-^{32}P]ATP (3,000 Ci/mmol) were purchased from Amersham International (Amersham, United Kingdom).

P1 transduction. Transduction was carried out with P1*vir* essentially as described previously (19).

Radioactive labeling and gel electrophoresis of proteins. Pulse-labeling and gel electrophoresis of proteins were done essentially as previously described (25). Cells growing exponentially in minimal medium were labeled with [^{35}S]methionine (2 μCi) for 2 min at 37°C (or 30°C) or after shift to 42°C. Labeled cells were treated with 5% trichloroacetic acid and washed with acetone. Whole-cell proteins were dissolved in sodium dodecyl sulfate (SDS)-sample buffer and were loaded onto a SDS–10% polyacrylamide gel for electrophoresis.

Immunoprecipitation of σ^{32} or σ^{32}-β-galactosidase fusion protein. Cells were treated with 5% trichloroacetic acid, washed in acetone, and suspended in 60 μl of 50 mM Tris hydrochloride (pH 8.0) containing 1% SDS and 1 mM EDTA. After heating at 100°C for 3 min, samples (30 μl) were diluted with 1 ml of Triton X-100-containing buffer, centrifuged to remove nonspecific precipitates, treated with antiserum against σ^{32} (gift of D. B. Straus and C. A. Gross) or β-galactosidase (Organon Teknika, Malvern, Pa.) overnight at 4°C, and then treated with *Staphylococcus* protein A conjugates for another hour (11). The immunoprecipitates were collected by centrifugation, washed in Triton X-100-containing buffer and in 10 mM Tris hydrochloride (pH 8.1), and dissolved in SDS-sample buffer. Samples were heated (100°C) for 3 min and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were detected by fluorography and analyzed by a Biomed densitometer SLR-2D/1D (Biomed Instruments, Inc., Chicago, Ill.).

Immunoblotting of the σ^{32} protein. Cells were treated with 5% trichloroacetic acid, washed in acetone, and heated in SDS-sample buffer for 3 min. Proteins (50 μg) were separated by SDS–12.5% polyacrylamide gel electrophoresis and blotted onto an Immobilon transfer membrane (Millipore Corp., Bedford, Mass.). The σ^{32} protein was detected with antiserum against σ^{32}, anti-rabbit immunoglobulin biotinylated antibody, and horseradish peroxidase-streptavidin (Amersham International). Staining was done with a Konica Immunostaining HRP-kit (Konica Co., Tokyo, Japan).

Determination of *rpoH* mRNA by S1 nuclease protection. S1 nuclease protection experiments were carried out essentially as described previously (15). RNA was extracted from cells grown in L broth at the appropriate temperature, hybridized with probe DNA, digested with S1 nuclease, and analyzed by urea-polyacrylamide gel electrophoresis.

Nucleotide sequence determination. DNA fragments containing various portions of the *suhB* region were inserted into M13 vectors, and the nucleotide sequence was determined by the dideoxy-chain termination method with T7 DNA polymerase (U.S. Biochemical Corp., Cleveland, Ohio) (22).

TABLE 1. Growth of cells on L agar plates[a]

Strain and genotype[b]	Growth at (°C)[c]:					
	25	30	34	37	40	42
MC4100 (wild type)	+	+	+	+	+	+
KY1431 (*rpoH15*)	+	+	−	−	−	−
Original *suhB* mutant (*rpoH15 suhB2 X*)	−	−	−	+	+	+
Ts$^+$ td (*rpoH15 suhB2*)	−	−	−	+	+	−
Cs$^+$ td (*rpoH15 suhB$^+$ X*)	+	+	−	−	−	−

[a] Cell suspensions were streaked on L agar plates and incubated for 18 h at the temperatures indicated.

[b] The first three strains are described in Materials and Methods. Temperature-resistant transductants (Ts$^+$ td) and cold-resistant transductants (Cs$^+$ td) were obtained from appropriate crosses (see text), selecting for those that can grow at 40 or 25°C, respectively, on L agar. All of the transductants tested, eight from each cross, exhibited similar growth characteristics, as was reported here. One of the Ts$^+$ transductants was established as KY1450 and was used as the *suhB* mutant in most experiments.

[c] Growth is indicated as present (+) or absent (−).

RESULTS

Isolation of *suhB* suppressor mutations. The *rpoH15* mutant (KY1431) carries a missense mutation affecting residue 264 (glutamic acid to lysine) of the σ^{32} protein (30, 32) and is deficient in the heat shock response and growth at a high temperature (34°C or above). Among the spontaneous temperature-resistant revertants selected at 42°C on L agar, some (1 to 2%) exhibited very slow growth at a low temperature (25°C). Many of these cold-sensitive strains have been shown to carry a suppressor mutation at 55 to 60 min on the *E. coli* chromosome and were tentatively designated *suhB*. A representative mutant of this class, carrying *suhB2*, was examined in this study.

P1 transduction experiments indicated that a single mutation (*suhB2*) was primarily responsible for both suppression of temperature-sensitive growth of the *rpoH15* mutant and cold-sensitive growth. All temperature-resistant transductants obtained from a cross between the *suhB2* mutant (donor) and the *rpoH15* mutant (recipient) turned out to be cold sensitive (Table 1). However, they grew at 40°C but not at 42°C, unlike the original *suhB2* mutant, suggesting that the latter strain had accumulated another mutation (marked as *X* in Table 1) responsible for growth at 42°C. On the other hand, cold-resistant transductants obtained from a cross between the wild type (donor) and the *suhB2* mutant (recipient) were unable to grow at or above 34°C. This indicated that the secondary mutation (*X*) alone had little effect on the growth of the *rpoH15* mutant at a high temperature. Thus, one of the temperature-resistant transductants was established as KY1450 (*rpoH15 suhB2*) and was used as the "*suhB* mutant" for further study.

Specificity of the *suhB2* suppressor. When *suhB2* was transduced into strains with an amber or opal *rpoH* mutation (*rpoH6*, *rpoH11*, *rpoH16*, *rpoH18*, and *rpoH165*), no temperature-resistant transductants were obtained, indicating that the suppression by *suhB2* is specific for *rpoH15*. Consistent with this observation, this class of cold-sensitive strains has been found only among the revertants obtained from missense *rpoH* mutants (*rpoH15* and *rpoH1*) and not from nonsense *rpoH* mutants. Whether *suhB2* suppresses *rpoH1* has not been tested because of the extreme instability of the *rpoH1* allele (25). In addition, when *suhB2* was transduced into an *rpoH*$^+$ strain by selecting for a closely linked transposon (see Fig. 7), the resulting transductants (*rpoH*$^+$ *suhB2*) displayed cold-sensitive growth just as did

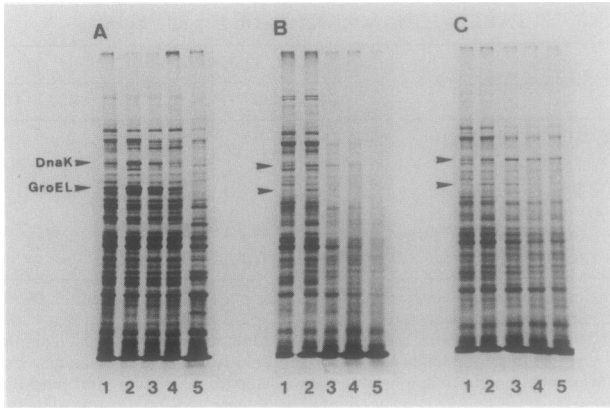

FIG. 1. Gel electrophoresis of proteins synthesized after temperature upshift. Cells of MC4100 (wild type) (A), KY1431 (*rpoH15*) (B), and KY1450 (*suhB2 rpoH15*) (C) were pulse-labeled with [^{35}S]methionine at 37°C (30°C for KY1431) or after shift to 42°C, and proteins (5×10^5 cpm) were analyzed by SDS-polyacrylamide gel electrophoresis, followed by autoradiography. Lanes: 1, cells labeled at 37°C (30°C for KY1431); 2, 5 min at 42°C; 3, 20 min at 42°C; 4, 40 min at 42°C; 5, 60 min at 42°C.

the parental *suhB2* mutant, indicating that *suhB2* by itself is responsible for the cold-sensitive phenotype.

Protein synthesis after temperature upshift. To see whether *suhB2* suppresses the defective induction of heat shock proteins in the *rpoH15* mutant, proteins pulse-labeled with [^{35}S]methionine at various times, after temperature shift from 37 to 42°C, were examined by SDS-polyacrylamide gel electrophoresis. Induction of heat shock proteins in the *suhB* mutant was much less striking than in the wild type but slightly more pronounced than in the parental *rpoH15* mutant observed when shifted from 30 to 42°C (Fig. 1). Thus, the *suhB2* mutation appeared to cause a limited recovery in induction of heat shock proteins, although the growth characteristics of these mutants prevented accurate comparison with the same temperature shift protocol. It should also be noted that the growth rate of the *suhB* mutant at 40°C in minimal medium was much slower (one-fourth) than that of the wild type.

Increased synthesis of σ^{32}. The slight but significant induction of heat shock proteins observed in the *rpoH15* mutant suggested that the altered σ^{32} protein exhibits a low residual activity in transcription from the heat shock promoters. The synthesis of σ^{32} during steady-state growth was examined by pulse-labeling proteins with [^{35}S]methionine, followed by immunoprecipitation with specific antiserum. Electrophoretic analysis of the precipitated proteins revealed that the rate of σ^{32} synthesis in the *suhB* mutant at 40°C was much higher than that in the wild type at 30 or 40°C (Fig. 2A). The rate of σ^{32} synthesis in the *rpoH15* mutant did not differ significantly from that in the wild type at 30°C and increased transiently after shift to 40°C (Fig. 2A and B).

These results suggested that *suhB2* suppresses the temperature-sensitive growth of the *rpoH15* mutant by enhancing the synthesis of σ^{32} at a high temperature. Consistent with this suggestion, when a multicopy plasmid pKV15 that expresses the *rpoH15* allele was transformed into the *rpoH15* or the Δ*rpoH* strain (able to grow only at or below 20°C), the resulting transformants produced large amounts of altered σ^{32} (Fig. 2C) and grew up to 40°C (but not at 42°C); however, the heat shock protein induction was only slightly enhanced, just like in the *suhB* mutant (data not shown). In

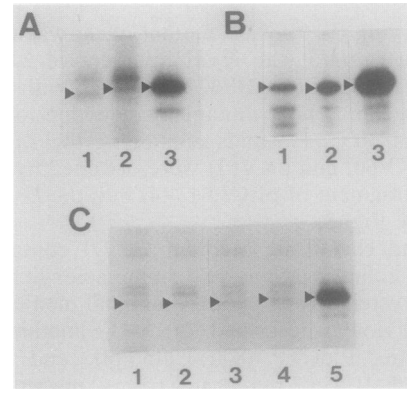

FIG. 2. Synthesis rates of the σ^{32} protein. Portions (0.2 ml) of log-phase cells growing in minimal medium at the temperatures indicated below (2×10^8 cells per ml) were pulse-labeled with 10 μCi of [^{35}S]methionine for 1 min and immediately mixed with an excess of 5% trichloroacetic acid in ice. After centrifugation and washing in acetone, cell lysates were prepared and portions (5×10^6 cpm) were treated with antisera; the immunoprecipitates were analyzed by gel electrophoresis as described in Materials and Methods (12.5% gel was used). Panels A, B, and C represent separate experiments and can not be compared directly. (A) Lanes: 1, MC4100 (wild type) at 40°C; 2, KY1431 (*rpoH15*) at 30°C; 3, KY1450 (*suhB2 rpoH15*) at 40°C. (B) Lanes: 1, KY1431 (*rpoH15*) at 30°C; 2, HY1431 5 min after shift from 30 to 40°C; 3, *suhB2 rpoH15 X* at 40°C. (C) Lanes: 1, MC4100 at 30°C; 2, MC4100 at 40°C; 3, KY1451 (*suhB2 rpoH*$^+$) at 40°C; 4, KY1452 (*suhB2 rpoH15*-λ *imm*^{21}-*rpoH*$^+$) at 40°C; 5, KY1623 (Δ*rpoH30*-pKV15) at 40°C. Arrowheads indicate the σ^{32} protein. The *rpoH15*-encoded σ^{32} showed a mobility slightly slower than that of wild-type σ^{32}. A faster-migrating band(s) may be degradation products of the σ^{32} protein, whereas slower-migrating bands vary among different experiments and may represent artifacts in immunoprecipitation.

contrast to the marked stimulatory effect in the *rpoH15* background, *suhB2* failed to enhance σ^{32} synthesis in the *rpoH*$^+$ strains (Fig. 2C), indicating that the elevated σ^{32} synthesis is somehow prevented by the normal functioning of the σ^{32} protein.

Determination of the σ^{32} level. The cellular contents of the σ^{32} protein during steady-state growth were then compared by immunoblotting. In contrast to the striking increase observed in σ^{32} synthesis, the σ^{32} level in the *suhB* mutant grown at 40°C was not appreciably higher than that in the *rpoH15* mutant grown at 30°C (Fig. 3). It should be noted that the σ^{32} level in the *rpoH15* mutant at 30°C (or after shift to 40°C; data not shown) is severalfold higher than that in the wild type. The σ^{32} level in the *suhB2 rpoH15* mutant lysogenic for λ *imm*^{21}-*rpoH*$^+$ was like that in the wild type rather than that in the *rpoH15* mutant (Fig. 3, lane 5), suggesting that the σ^{32} protein in the *rpoH15* mutant is stabilized because of the reduced proteolytic environment

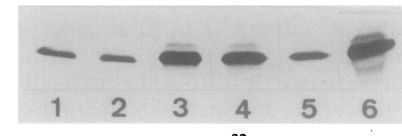

FIG. 3. Immunoblotting of the σ^{32} protein. Proteins (50 μg) from log-phase cells grown at 30 or 40°C were separated by gel electrophoresis and blotted onto a membrane, and the σ^{32} protein was detected by antisera as described in Materials and Methods. Lanes: 1, MC4100 at 30°C; 2, MC4100 at 40°C; 3, KY1431 (*rpoH15*) at 30°C; 4, KY1450 (*suhB2 rpoH15*) at 40°C; 5, KY1452 (*suhB2 rpoH15*-λ *imm*^{21}-*rpoH*$^+$) at 40°C; 6, KY1623 (Δ*rpoH30*-pKV15) at 40°C.

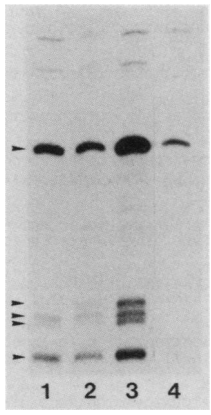

FIG. 4. Quantitation of *rpoH* mRNA. RNA (100 μg) was hybridized with 5'-end-labeled *rpoH* DNA (an *Mlu*I-*Pvu*II segment of 750 base pairs; 31), digested with S1 nuclease at 45°C and subjected to 5% polyacrylamide–8 M urea gel electrophoresis. Lanes: 1, MC4100 at 40°C; 2, KY1431 (*rpoH15*) at 30°C; 3, KY1450 (*suhB2 rpoH15*) at 40°C; 4, KY1451 (*suhB2 rpoH*$^+$) at 40°C. Arrowheads indicate positions of *rpoH* mRNAs transcribed from several known promoters (6, 7; Nagai et al., submitted).

brought about by the *rpoH* mutation (1, 8) and not because of the altered sensitivity of σ^{32} to proteolytic enzyme(s). It has been found that the σ^{32} level in the *rpoH15* (λ *rpoH*$^+$) lysogen is comparable to that in the wild type rather than that in the *rpoH15* mutant (Nagai et al., unpublished result). It may be surmised that the increased synthesis of σ^{32} in the *suhB* mutant was partially cancelled out by increased degradation of σ^{32} and that a small net increase that might be expected in the σ^{32} level was beyond detection by the present experiments.

Increased level of *rpoH* mRNA. The level of *rpoH* mRNA was determined by S1 nuclease protection experiments with a 5'-labeled internal *rpoH* DNA fragment present in excess over the mRNA (Fig. 4). The mRNA level in the *suhB* mutant was found to be three- to fourfold higher than those in the *rpoH15* mutant or in the wild type; transcripts from all known *rpoH* promoters (6, 7; Nagai et al., submitted) were similarly affected. The mRNA level of the wild type was somewhat higher at 40°C than at 30°C (6, 7, 23), whereas that of the *rpoH15* mutant at 30°C or after shift to 40°C was similar to the wild-type value at 40°C. Again, the *suhB2 rpoH*$^+$ cells produced normal wild-type levels of *rpoH* mRNA, in contrast to the *suhB2 rpoH15* mutant.

To see whether the increased mRNA level observed in the *suhB* mutant is correlated with an altered stability of *rpoH* mRNA, RNA was extracted from cells that had been incubated in the presence of rifampin and were similarly examined by the S1 mapping procedure. The results showed a slight increase in mRNA stability in the *suhB* mutant (Fig. 5), which may explain, at least partially, the observed increase in *rpoH* mRNA (Fig. 4). As a control, we determined the levels of *rpsO* mRNA encoding the ribosomal protein S15; the values did not differ significantly among the strains tested (data not shown). All these results together with those for σ^{32} synthesis suggest that the *suhB2* mutation enhances σ^{32} synthesis primarily at the translational level and affects the *rpoH* mRNA level and stability secondarily as the result of increased mRNA translation.

Increased synthesis of σ^{32}–β-galactosidase fusion protein. To further substantiate the above conclusions, we determined expression of the *rpoH-lacZ* operon fusion and the

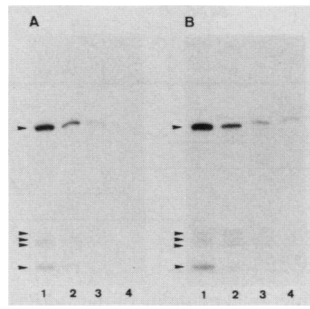

FIG. 5. Stability of *rpoH* mRNA. Rifampin was added to log-phase cultures grown at 40°C, and samples were taken at the indicated intervals. RNA was extracted, hybridized, and analyzed as described in the legend to Fig. 4. (A) MC4100; (B) KY1450 (*suhB2 rpoH15*). Lanes: 1, 0 min; 2, 2 min; 3, 5 min; 4, 8 min. Samples for panel B were diluted threefold so as to give nearly equal signal strengths for panels A and B at time zero.

gene fusion, which should permit us to determine the *rpoH* transcription and translation, respectively. A set of strains lysogenic for λOF-0 or λGF-364 were pulse-labeled with [^{35}S]methionine at 30 or 40°C, extracts were treated with antiserum against β-galactosidase, and the resulting immunoprecipitates were analyzed by SDS-gel electrophoresis. The steady-state synthesis rates of the σ^{32}–β-galactosidase fusion protein from the *rpoH-lacZ* gene fusion were similar to those of σ^{32} and did not differ appreciably between the wild type and the *rpoH15* mutant. However, the synthesis rate in the *suhB* mutant at 40°C was markedly higher (four- to fivefold) than in the wild type, the *suhB2 rpoH*$^+$ strain (at 40°C), or the parental *rpoH15* mutant (at 30°C) (Fig. 6A). In contrast, expression of the *rpoH-lacZ* operon fusion in the same *suhB* mutant was essentially identical to that in the wild type or other strains (Fig. 6B). These results lend strong support to the notion that *suhB2* enhances *rpoH* expression primarily at the level of translation rather than at the level of transcription.

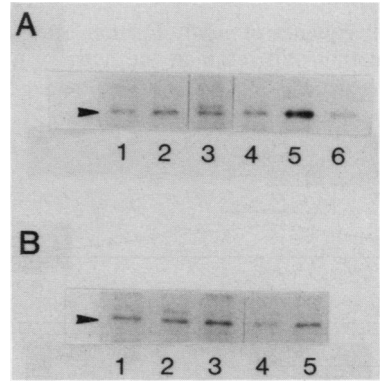

FIG. 6. Synthesis rates of the β-galactosidase fusion protein. Log-phase cells were pulse-labeled with [^{35}S]methionine, cell lysates were treated with antiserum, and immunoprecipitates were analyzed essentially as described in the legend to Fig. 2, except that 1-min pulse-labeling was followed by 3-min chase with unlabeled methionine and antiserum against β-galactosidase was used. A 7% gel was used for electrophoresis. (A) λGF-364 lysogens; (B) λOF-0 lysogens. Lanes: 1, MC4100 (wild type) at 30°C; 2, MC4100 at 40°C; 3, KY1431 (*rpoH15*) at 30°C; 4, KY1451 (*suhB2 rpoH*$^+$) at 40°C; 5, KY1450 (*suhB2 rpoH15*) at 40°C; 6, same as in lane 5 except diluted fivefold. Arrowheads indicate the fusion protein.

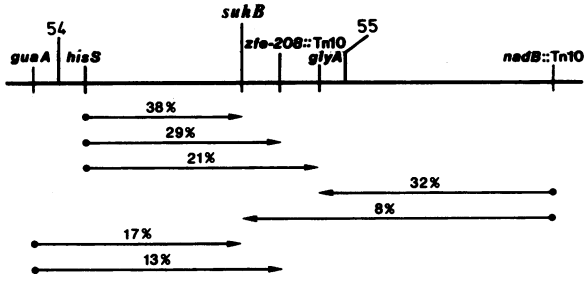

FIG. 7. Transductional mapping of the *suhB* region. The cotransduction frequencies shown are based on several experiments in which transductants were selected for *guaA*$^+$, *hisS*$^+$, or tetracycline resistance (*nadB*::Tn*10*). At least 50 transductants were examined for each cross.

Mapping and cloning of *suhB*. Preliminary Hfr mating experiments suggested that *suhB* is located at 55 to 60 min on the *E. coli* chromosome. P1 phage-mediated transduction experiments with several nearby markers led us to localize *suhB2* more precisely at around 54.6 min (Fig. 7). Several plasmids from the Clarke-Carbon collection (4) were found to complement the cold-sensitive growth of the *suhB2* mutant, suggesting that *suhB* may reside within the overlapped region (ca. 2.1 kb) carried by these plasmids (Fig. 8). The restriction map of this 2.1-kb segment coincided well with that reported recently by Kohara et al. (12). Further subcloning experiments indicated that pKV56 carrying the 2.1-kb segment or pKV61 carrying part thereof (1.0 kb) clearly complements the cold sensitivity of the *suhB2* mutant (Fig. 8). Transformants appeared at 30°C but not at higher temperatures (37 or 40°C). The transformants obtained at 30°C turned out to be unstable at 40°C and formed colonies highly heterogeneous in size on L agar.

A search for the *suhB* gene product with minicells led us to find a unique protein, encoded by pKV56 or pKV61, with a molecular size of ca. 30 kilodaltons, as judged by its mobility in SDS-polyacrylamide gel electrophoresis (data not shown). All these results indicated that the 1.0-kb DNA segment carried by pKV61 contained the intact or nearly intact *suhB*$^+$ gene.

Nucleotide sequence of *suhB*. Further attempts to shorten the fragment that still retained the activity to complement the cold-sensitive growth of the *suhB2* mutant were unsuccessful. The 1.0-kb segment of pKV61 and its various portions were therefore ligated to M13 vectors, and the nucleotide sequence was determined by the standard dideoxy procedure.

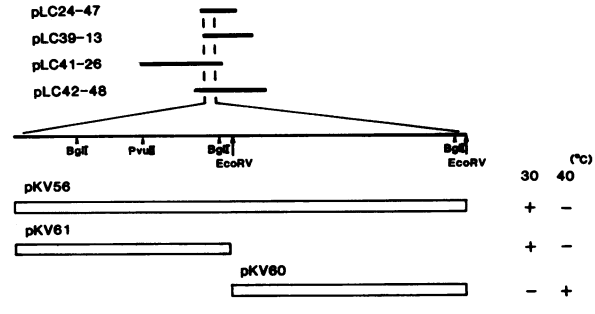

FIG. 8. Complementation of cold-sensitive growth phenotype of the *suhB2* mutant by recombinant plasmids. At the top, four ColE1-derived plasmids (4) that can complement the *suhB2* mutation are shown. The three plasmids shown below the restriction map were obtained by subcloning an *Eco*RV fragment into pBR322 (an *Eco*RV site within the ColE1 vector to the left is not shown). Growth of derivatives of KY1450 (*suhB2 rpoH15*) harboring each plasmid at 30 or 40°C is indicated to the right. Bars indicate chromosomal regions carried by the plasmids.

```
        10         20         30         40         50         60
CATGGCACGGGCAACAGAACCCATATTGCCGGTGTGTGACGTCTCCACCAGCACAATTCG

        70         80         90        100        110        120
AATATTTTGCAGCATTGTCTTTCTTCATCTAAAGATTATTCACGCATCTTATCATAAAAC

       130        140        150        160        170        180
GAAGACAGATGCCGATCTCGCTGCTATACTCTGCGCCGTTTTCCCGTTCTTTAACATCCA

       190        200        210        220        230        240
GTGAGAGAGACCGATGCATCCGATGCTGAACATCGCCGTGCGCGCAGCGCGCAAGGCGGG
            MetHisProMetLeuAsnIleAlaValArgAlaAlaArgLysAlaGly

       250        260        270        280        290        300
TAATTTAATTGCCAAAAACTATGAAACCCCGGACGCTGTAGAAGCGAGCCAGAAAGGCAG
 AsnLeuIleAlaLysAsnTyrGluThrProAspAlaValGluAlaSerGlnLysGlySer

       310        320        330        340        350        360
TAACGATTTCGTGACCAACGTAGATAAAGCTGCCGAAGCGGTGATTATCGACACGATTCG
 AsnAspPheValThrAsnValAspLysAlaAlaGluAlaValIleIleAspThrIleArg

       370        380        390        400        410        420
TAAATCTTACCCACAGCACACCATCATCACCGAAGAAAGCGGTGAACTTGAAGGTACTGA
 LysSerTyrProGlnHisThrIleIleThrGluGluSerGlyGluLeuGluGlyThrAsp

       430        440        450        460        470        480
TCAGGATGTTCAATGGGTTATCGATCCACTGGATGGCACTACCAACTTTATCAAACGTCT
 GlnAspValGlnTrpValIleAspProLeuAspGlyThrThrAsnPheIleLysArgLeu

       490        500        510        520        530        540
GCCGCACTTCGCGGTATCTATCGCTGTTCGTATCAAAGGCCGCACCGAAGTTGCTGTGGT
 ProHisPheAlaValSerIleAlaValArgIleLysGlyArgThrGluValAlaValVal

       550        560        570        580        590        600
ATACGATCCTATGCGTAACGAACTGTTCACCGCCACTCGCGGTCAGGGCGCACAGCTGAA
 TyrAspProMetArgAsnGluLeuPheThrAlaThrArgGlyGlnGlyAlaGlnLeuAsn

       610        620        630        640        650        660
CGGCTACCGACTGCTCGGCAGCACCGCTCGCGATCTCGACGGTACTATTCTGGCGACCGG
 GlyTyrArgLeuLeuGlySerThrAlaArgAspLeuAspGlyThrIleLeuAlaThrGly

       670        680        690        700        710        720
CTTCCCGTTCAAAGCAAAACAGTACGCCACTACCTACATCAACATCGTCGGCAAACTGTT
 PheProPheLysAlaLysGlnTyrAlaThrThrTyrIleAsnIleValGlyLysLeuPhe

       730        740        750        760        770        780
CAACGAATGTGCAGACTTCCGTCGTACCGGTTCTGCGGCGCTGGATCTGGCTTACGTCGC
 AsnGluCysAlaAspPheArgArgThrGlySerAlaAlaLeuAspLeuAlaTyrValAla

       790        800        810        820        830        840
TGCGGGTCGTGTTGACGGTTTCTTTGAAATCGGTCTGCGCCCGTGGGACTTCGCCGCAGG
 AlaGlyArgValAspGlyPhePheGluIleGlyLeuArgProTrpAspPheAlaAlaGly

       850        860        870        880        890        900
CGAGCTGCTGGTTCGTGAAGCGGGCGGCATCGTCAGCGACTTCACCGGTGGTCATAACTA
 GluLeuLeuValArgGluAlaGlyGlyIleValSerAspPheThrGlyGlyHisAsnTyr

       910        920        930        940        950        960
CATGCTGACCGGTAACATCGTTGCTGGTAACCCGCGCGTTGTTAAAGCCATGCTGGCGAA
 MetLeuThrGlyAsnIleValAlaGlyAsnProArgValValLysAlaMetLeuAlaAsn

       970        980        990       1000       1010       1020
CATGCGTGACGAGTTAAGCGACGCTCTGAAGCGTTAATGACTCAGGCGGGTGATATC
 MetArgAspGluLeuSerAspAlaLeuLysArg
```

FIG. 9. Nucleotide sequence of the *suhB* region. The 1.0-kb segment of the *E. coli* chromosome carried by pRY61 was digested with appropriate restriction enzymes, subcloned into M13 vectors, and the nucleotide sequence was determined in both directions. A putative promoter sequence (−35 and −10 regions) and initiation (ATG) and termination (TAA, TGA) codons are underscored.

An open reading frame starting from the ATG codon (base 194) and potentially encoding a protein of 29,128 daltons was obtained, in good agreement with the above results of cloning and product analysis (Fig. 9). In addition, a putative promoter sequence has been located in the nearby upstream region and a putative Shine-Dalgarno sequence was found several nucleotides upstream of the ATG codon (Fig. 9). Although we analyzed these sequence data by using the National Biomedical Research Foundation protein data bank (release 19) and GenBank (release 58), no significant amino acid sequence homology was found between the putative *suhB* product and any of the known proteins.

DISCUSSION

The *rpoH15* missense mutant produces an altered σ^{32} protein largely deficient in transcription from the heat shock

promoters involved in expression of the heat shock genes. It therefore cannot produce heat shock proteins, GroE proteins in particular (13), in amounts sufficient to support cell growth at or above 34°C. The amount of σ^{32} found in the *rpoH15* mutant at 30°C is severalfold higher than that found in the wild type, probably because of reduced proteolytic activities (1, 8; see below). Following temperature shift to 40 or 42°C, synthesis of the mutant σ^{32} is transiently induced as in the wild type (our unpublished result), causing slight induction of heat shock proteins (Fig. 1B).

The *suhB2* mutation suppressed the temperature-sensitive growth of the *rpoH15* mutant and simultaneously caused cold-sensitive growth; the *suhB2* mutant therefore grew only within a narrow temperature range (37 to 40°C; Table 1). The suppression appears to be due to increased synthesis of altered σ^{32} protein; the *suhB* suppression was specific for *rpoH15* and was not effective for the *rpoH* nonsense mutants tested. In addition, overproduction of *rpoH15*-encoded σ^{32} by the multicopy plasmid mimics *suhB* suppression; the plasmid enabled the Δ*rpoH* or *rpoH15* mutant to grow at up to 40°C (but not at 42°C) and exhibited slightly enhanced heat shock induction upon temperature upshift. Furthermore, the increased synthesis of altered σ^{32} seems to occur primarily at the translational level. The *suhB2* suppressor, in combination with *rpoH15*, markedly enhanced the synthesis of σ^{32} to a greater extent than that for *rpoH* mRNA level (Fig. 2 and 4). More importantly, a marked enhancement was observed in the expression of the *rpoH-lacZ* gene fusion but not of the operon fusion (Fig. 6). We believe that the increased level of *rpoH* mRNA observed in the *suhB* mutant is due, at least in part, to stabilization of the mRNA caused secondarily by the increased σ^{32} synthesis. Transient induction of σ^{32} synthesis normally observed after temperature upshift also occurs mostly at the translational level (21; our unpublished result) and secondarily enhances the *rpoH* mRNA level (6, 7).

On the other hand, the steady-state level of the σ^{32} protein and the extent of heat shock induction in the *suhB* mutant were not appreciably higher than those in the parental *rpoH15* mutant (Fig. 1 and 3). This may be explained partially by assuming that degradation as well as synthesis of σ^{32} was elevated in the *suhB* mutant and that increased synthesis was largely cancelled out by increased degradation. The parental *rpoH15* mutant is presumably deficient in proteolysis like other *rpoH* mutants (1, 8) and thus stabilizes σ^{32}. The *suhB* mutation enhances σ^{32} synthesis, thereby increasing the level of heat shock proteins to some extent. This in turn should accelerate degradation of σ^{32}, since some heat shock proteins are known to be involved in negatively regulating the synthesis and stability of σ^{32} (10, 24; D. Straus and C. Gross, personal communication). Such a regulatory circuit would also explain the lack of *suhB* effects on the apparent rate of σ^{32} synthesis in the *rpoH*$^+$ background (Fig. 2), because σ^{32} degradation (even during pulse-labeling) should be much higher; it is also consistent with the specificity of the *suhB* suppressor for *rpoH15*. Thus, *suhB* may directly affect a certain step(s) of the regulatory circuit involved in the *rpoH* translation. Alternatively, *suhB* may enhance σ^{32} synthesis indirectly, for example, by causing accumulation of abnormal proteins. It also remains possible that the increased σ^{32} synthesis observed is not related to the *suhB* suppression; *suhB* might endow the cell to grow at a high temperature by decreasing the cellular requirement for heat shock proteins.

The *suhB* mutation, in both *rpoH15* and *rpoH*$^+$ backgrounds, inhibits cell growth at or below 34°C. The four Clarke-Carbon plasmids known to complement another cold-sensitive mutation, *ssyA3* (18), coincided exactly with those that complemented *suhB2* (Fig. 7). Furthermore, the plasmid carrying only *suhB*$^+$ (pKV61) was shown to complement *ssyA3* (Y. Akiyama and K. Ito, personal communication). *ssyA3* had been isolated as an extragenic suppressor that suppresses the temperature-sensitive mutation (*secY24*) affecting protein secretion and had been shown to cause slower polypeptide chain growth at a low temperature; the chain growth rate is affected directly or indirectly (17). These results indicate that *suhB* and *ssyA* are one and the same gene and that these mutations affect expression of a number of genes at the translational level. GroE proteins have recently been shown to be involved in the secretion of certain proteins in *E. coli* (2, 14). It is therefore conceivable that *suhB* (*ssyA*) affects some step(s) of protein synthesis by facilitating the function of GroE or other heat shock proteins. In any event, further studies, including isolation and characterization of the *suhB* (*ssyA*) gene product, may help to clarify its function and the regulatory mechanisms of σ^{32} synthesis.

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