Rhizobium meliloti *suhR* Suppresses the Phenotype of an *Escherichia coli* RNA Polymerase σ^{32} Mutant

ANDREW F. BENT† AND ETHAN R. SIGNER*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 1 November 1989/Accepted 10 April 1990

σ^{32} , the product of the *Escherichia coli* *rpoH* locus, is an alternative RNA polymerase sigma factor utilized to express heat shock genes upon a sudden rise in temperature. *E. coli* K165 [*rpoH165*(Am) *supC*(Ts)] is temperature sensitive for growth and does not induce heat shock protein synthesis. We have isolated a locus from *Rhizobium meliloti* called *suhR* that allows *E. coli* K165 to grow at high temperature and induce heat shock protein synthesis. *R. meliloti* *suhR* mutants were viable and symbiotically effective. *suhR* was found to have no DNA or derived amino acid sequence similarity to the genes of previously sequenced sigma factors or other data base entries, although a helix-turn-helix DNA-binding protein motif is present. *suhR* did not restore the phenotypic defects of Δ *rpoH* *E. coli*; suppression of the *E. coli* K165 phenotype is thus likely to involve *E. coli* σ^{32} . Western immunoblots showed that *suhR* caused an approximately twofold elevation of σ^{32} levels in K165; RNA blots indicated that *rpoH* mRNA level and stability were not altered. Stabilization of σ^{32} protein and increased *rpoH* mRNA translation are thus the most probable mechanisms of suppression.

During bacterial growth and differentiation, sets of unlinked but functionally related genes are often coordinately expressed under the control of alternative RNA polymerase sigma factors (13). Processes that utilize alternative sigma factors include sporulation, heat shock, bacteriophage infection, nitrogen assimilation, insecticidal crystal protein production, and chemotaxis (1, 6, 13).

The heat shock response is conserved among many phylogenetic kingdoms (35). In *Escherichia coli*, heat shock leads to increased synthesis of at least 17 heat shock proteins (39). These include the well-characterized La protease (24) as well as proteins involved in secretion and assembly of oligomeric protein structures (GroE), transcription (RpoD or σ^{70}), DNA replication (DnaK, DnaJ, and GrpE), and possibly formation of adenylylated nucleotides (lysyl-tRNA synthetase) (5, 29, 39, 52, 60). In addition, *dnaJ*, *dnaK*, *grpE*, and *groEL* mutants are defective in proteolysis (51). Heat- or toxic chemical-induced transcription of these loci occurs from specific promoters that utilize the alternative RNA polymerase sigma factor σ^{32} , which is encoded by the *rpoH* locus (equivalent to *hspR* and *hin*).

Although the primary sensor(s) of heat shock remains unknown (39), the rate of heat shock gene transcription correlates directly with the level of σ^{32} in the cell (26, 50). During heat shock, the level of σ^{32} increases transiently (21, 50); σ^{70} -dependent transcription of *rpoH* increases only slightly, but the stability of *rpoH* mRNA rises (16, 20). In addition, the half-life of σ^{32} protein, normally on the order of 1 min, increases transiently (50, 54). Within 10 min after temperature shift, expression of heat shock genes decreases to new steady-state levels that vary according to growth temperature, σ^{32} levels decline, and σ^{70} levels increase (39, 52). One of the heat shock proteins, DnaK, is instrumental in this down modulation (26, 53, 54). At very high temperatures, *rpoH* is transcribed from an additional promoter dependent on the recently discovered sigma factor σ^E (15). Additional factors may play a role in regulating σ^{32} -depen-

dent heat shock protein production and certainly are involved in σ^{32} -independent heat shock protein synthesis (59).

σ^{32} is essential for the viability of wild-type *E. coli* at all but very low temperatures (40, 59). Strains carrying the well-studied *rpoH165* amber mutation and a temperature-sensitive amber tRNA suppressor [encoded by *supC*(Ts)] are temperature sensitive for growth and do not induce heat shock protein synthesis (40). *E. coli* strains that carry amber or opal alleles of *rpoH* but no known tRNA suppressor still make small quantities of full-length σ^{32} protein by an unknown mechanism and can grow at intermediate temperatures (56). Suppressors of conditional *rpoH* mutations which map to the *suhA* locus apparently cause both σ^{32} -independent *groE* transcription and stabilization of rare *rpoH* mRNA (56). Strains deleted for *rpoH* (Δ *rpoH*) grow only below 20°C (59) but accumulate spontaneous suppressor mutations that result in overproduction of GroE protein (32). The growth of unsuppressed Δ *rpoH* strains below 20°C may be due to low-level expression of *groE* from non-heat shock promoters (59).

R. meliloti *suhR*, described in this report, is nonessential for growth of *R. meliloti* but allows an *E. coli* mutant deficient in σ^{32} production to grow at high temperature and induce the heat shock response. Suppression by *suhR* requires *E. coli* σ^{32} , and the *suhR* sequence is not similar to that of *E. coli* *rpoH*. *suhR* does not appear to encode either an *R. meliloti* heat shock sigma factor or an amber tRNA suppressor but nevertheless causes elevation of σ^{32} levels in *E. coli* K165. Potential mechanisms of suppression are discussed.

MATERIALS AND METHODS

Bacteriological methods. Information on strains and plasmids is presented in Table 1. All manipulations that used temperature-sensitive strains were done at the permissive temperature of the original strain; nonpermissive temperatures were used only as specifically noted. K165 in particular was maintained at 30°C; 37°C is lethal (although pseudo-revertants appear consistently at a frequency of less than 10^{-5}). Bacteria were generally grown in LB broth or on LB plates. Antibiotic levels used (in micrograms per milliliter)

* Corresponding author.

† Present address: Department of Plant Pathology, University of California-Berkeley, Berkeley, CA 94720.

TABLE 1. Bacterial strains and plasmids used

Bacterial strain or plasmid	Relevant characteristics	Source or reference
Strains		
<i>R. meliloti</i>		
Rm1021	SU47 (wild type) <i>str-21</i>	36
RM9140	Rm1021 <i>suhR::Km^r</i>	This study
RM9145	Rm1021 Δ <i>suhR::Km^r</i>	This study
<i>E. coli</i>		
SC122	<i>trp</i> (Am) <i>lac</i> (Am) <i>mal</i> (Am) <i>pho</i> (Am) <i>rpsL</i> <i>supC</i> (Ts)	9
K165	SC122 <i>rpoH165</i> (Am)	9
DS410	<i>minB</i> <i>ara</i> <i>lacY</i> <i>str</i> <i>malA</i> <i>xyl</i> <i>azi</i> <i>tonA</i> <i>thi</i> <i>mtl</i>	45
MR5	F ⁻ <i>lacZYA524</i> (Oc) Sp ^r	Laboratory collection
MM294A	<i>pro-82</i> <i>thi-1</i> <i>endA1</i> <i>hsdR17</i> <i>supE44</i> Δ (<i>lac-pro</i>)	Laboratory collection
MT607	MM294A <i>recA56</i>	T. Finan
KY1601	F ⁻ Δ <i>rpoH30::kan</i> <i>zhf-50::Tn10</i> <i>araD139</i> Δ (<i>argF-lac</i>)U169 <i>rpsL150</i> <i>relA1</i> <i>flbB5301</i> <i>deoC1</i> <i>ptsF25</i> <i>rbsR</i> (λ pF13-PrpoD _{hs} - <i>lacZ</i>)	32
KY1603	KY1601 <i>suhX401</i>	32
KY1614	KY1603 Tc ^s	T. Yura
CAG9301	MG1655 (wild-type K-12) <i>rpoH120::kan</i> <i>zhg-21::Tn10</i>	59
Plasmids		
pLAFR1	IncP Tc ^r derivative of pRK290 with λ <i>cos</i> site; Tc ^r	19
pKM8	pLAFR1 with ~25-kb <i>R. meliloti</i> genomic fragment carrying <i>suhR</i> ; Tc ^r	This study
pSE153	Derivative of pSC101 with Km ^r from Tn5 and Sm ^r Sp ^r from pDPT427	S. Elledge; 8
pABa2	pSE153 with 1.7-kb <i>Bam</i> HI fragment carrying <i>suhR</i> ; Sp ^r	This study
pABg5	pSE153 with 1.7-kb <i>Bgl</i> II fragment partially carrying <i>suhR</i> ; Sp ^r	This study
pABPE1	pSE153 with 1.2-kb <i>Sph</i> I- <i>Eco</i> RI fragment carrying <i>suhR</i> ; Km ^r	This study
pKP11	pBR322 with <i>E. coli</i> <i>rpoH</i> region	43
pDS2	pKK223-3 with intact <i>E. coli</i> <i>rpoH</i> on 1.3-kb <i>Eco</i> RV fragment from pFN97 in <i>Sma</i> I site; Ap ^r	26
Bluescript	ColE1; Ap ^r	Stratagene
pAB25	Bluescript with 2.5-kb <i>Bgl</i> II- <i>Eco</i> RI fragment of pKM8 (via pABa2 and pABg5); Ap ^r	This study
pAB40	pAB25 <i>suhR::Km^r</i> ; Ap ^r	This study
pAB42	pAB25 ORF-A/B::Km ^r	This study
pAB45	pAB25 Δ <i>suhR::Km^r</i>	This study
pKM40	pKM8 <i>suhR::Km^r</i>	This study
pKM42	pKM8 ORF-A/B::Km ^r	This study
pKM45	pKM8 Δ <i>suhR::Km^r</i>	This study
pRK2013	ColE1 containing transfer region of RK2; <i>npt⁺</i> Km ^r	18
pRK600	pRK2013 <i>npt::Tn9</i> ; Km ^s Cm ^r	T. Finan
pPH1JI	IncP, R751 and R1033 derivative; Cm ^r Gm ^r Sm ^r Sp ^r	30
pHP45 Ω Km	<i>npt⁺</i> from Tn5 in polylinker; Ap ^r Km ^r	17

were as follows: streptomycin, 250; tetracycline, 10 (plates) or 5 (liquid); spectinomycin, 50; kanamycin, 20; ampicillin, 50 or 75; and chloramphenicol, 20. Minimal medium was M9 salts supplemented with glucose or lactose (0.2%), thiamine (2.5 μ g/ml), and where appropriate L-amino acids (20 μ g/ml). Growth rates were determined by monitoring the change in optical density at 675 nm (Δ OD₆₇₅) of duplicate log-phase cultures. Triparental matings were performed by using MM294A(pRK2013) or MT607(pRK600) as helper (12). P1 *vir* transductions were performed as described previously (38); initial selection was for cotransducible antibiotic resistance markers at permissive temperatures unless specifically noted.

Recombinant DNA techniques. Standard procedures were used to construct and analyze plasmids, cosmids, genomic DNA, and RNA (2). DNA sequencing (dideoxy method) was performed from Bluescript plasmids (Stratagene, La Jolla, Calif.), using Sequenase (modified T7 DNA polymerase) and reagents (United States Biochemical Corp., Cleveland, Ohio) and ³⁵S-labeled deoxynucleoside triphosphates. All regions were sequenced at least twice. Southern hybridizations were done by using GeneScreen Plus (Du Pont Co.,

Wilmington, Del.) by standard procedures (instructions of the manufacturer and reference 2).

Marker exchanges and gene replacements. The 2.5-kilobase (kb) *R. meliloti* region shown in Fig. 2 (*Bgl*II-*Eco*RI) was cloned from pABa2 and pABg5 into Bluescript; the resulting construct was named pAB25 (Table 1). The neomycin phosphotransferase locus from Tn5 (via pHP45 Ω Km) was cloned into pAB25 at the *Bgl*II site within open reading frame C (ORF-C) (= *suhR*) or the *Bam*HI site within ORF-A and -B, or at a 0.7-kb deletion bordered by the *Bgl*II and *Bam*HI sites, and exchanged onto pKM8 (the original *R. meliloti* *suhR* genomic clone) by recombination in *E. coli* K165. Gene replacements were isolated by mobilization of the plasmids into *E. coli* MR5, selection with tetracycline, kanamycin, and spectinomycin, and screening for ampicillin sensitivity. These constructs (pAB40, pAB42, and pAB45) were confirmed by restriction analysis to contain only the interrupted version of the relevant *R. meliloti* DNA.

Plasmids pAB40 (*suhR::Km^r*) and pAB45 (Δ *suhR::Km^r*) were mobilized into *R. meliloti* Rm1021 with the pRK2013 derivative pRK600 as helper, and Tc^r Nm^r transconjugants were selected. Plasmid pPH1JI (same incompatibility group

as pAB40 and pAB45) was then mated into these strains, and the gene replacement derivatives Rm9140 and Rm9145 were obtained by selection for Gm^r Sp^r Nm^r and subsequent screening for Tc^s (47).

Heat shock and minicell protein analysis. For heat shock experiments, overnight cultures grown at 30°C in M9 glucose medium (plus antibiotics for plasmid maintenance and amino acids for auxotrophies) were subcultured for at least 2 h at 30°C in the same medium. Mid-log-phase cultures (OD₆₇₅, approximately 0.2) were either labeled with [³⁵S]methionine (5 to 10 µCi/ml) for 5 min or transferred to 37 or 42°C for 5 min and then labeled for 5 min, and they were then chilled on ice or precipitated with 2.5 vol of ice-cold 20% trichloroacetic acid. Minicells were prepared as described previously (45) from *E. coli* DS410 transformed with control and insert-bearing plasmids. Frozen minicells were diluted in M9 medium to an OD₆₇₅ of approximately 0.2, grown at 37°C for 100 min without label and then for 10 min in the presence of [³⁵S]methionine (5 to 10 µCi/ml), and then chilled on ice. Chilled cells or trichloroacetic acid precipitates were pelleted in a microcentrifuge, and the proteins separated on 2.5%/10% stacking sodium dodecyl sulfate (SDS)-polyacrylamide gels (2). Gel molecular weight standards were from Bio-Rad Laboratories (Richmond, Calif.); rainbow pre-stained markers from Amersham Corp. (Arlington Heights, Ill.) were also used. Proteins were visualized by autoradiography of dried gels on SB5 film (Eastman Kodak Co., Rochester, N.Y.).

Computer analysis. University of Wisconsin Genetics Computer Group software as implemented locally at the Whitaker College Computer Facility was used for DNA sequence entry and analysis. In particular, the dot matrix sequence comparison program COMPARE was typically used with a scoring table (MD.cmp) derived from the mutational difference matrix of Dayhoff et al. (10), and IALIGN was used to optimally align consensus sequences with the sequence of ORF-C. CODONPREFERENCE compares codon usage frequency in individual reading frames against a weighted scoring table, distinguishing likely from unlikely codon usage relative to the sequences that make up the scoring table (e_coli.cdf in this analysis).

Plant assays. *Medicago sativa* cv. Iroquois seeds (Agway, Inc.) were surface sterilized in 50% Clorox bleach for 15 min, rinsed with 10 changes of sterile distilled water over >30 min, and germinated in the dark on 1.5% agar plates. Day-old seedlings were transferred to Jensen agar slants (57; two plants per 18-by-150-mm tube, capped with foam rubber stoppers) and grown at 25°C on a 16-h light/8-h dark cycle. On day 3, tubes were inoculated with ~10⁸ CFU of Rm1021, Rm9140, or Rm9145 resuspended from fresh colonies in 250 µl of sterile water or from washed fresh overnight cultures in 250 µl of 10 mM MgSO₄ (five tubes for each treatment). Rate of nodule appearance was observed, and on day 24 whole-plant nitrogen fixation levels were examined by acetylene reduction assay (36).

Western immunoblots. Cells were prepared as for heat shock experiments. Proteins separated by SDS-polyacrylamide gel electrophoresis (PAGE) (2) were blotted onto GeneScreen Plus in transfer buffer (20% methanol, 192 mM glycine, 20 mM Tris), using a Transblot apparatus (Bio-Rad) as instructed by the manufacturer. Blotted filters were blocked for at least 1 h phosphate-buffered saline–0.25% Tween 20–0.25% gelatin and then incubated for 4 h with a 1/500 dilution (in blocking solution) of polyclonal antibody prepared against *E. coli* σ³² (50; kind gift of Carol Gross) that had first been preadsorbed to an extract of sonicated

CAG9301 (*rpoH120::kan*) to reduce background cross-reactivity. Excess antibody was removed from blots by washes in high-salt solution (1 M NaCl, 10 mM EDTA, 0.25% Tween 20, 0.25% gelatin), and bound antibodies were then visualized by using goat anti-rabbit immunoglobulin–gold conjugate as instructed by the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Densitometry was performed by using LKB (Uppsala, Sweden) model 733 transmittance and Hoefer (San Francisco, Calif.) model GS300 transmittance-reflectance scanning densitometers and a Hewlett-Packard model 3390A integrator (Hewlett Packard Co., Beaverton, Ore.). Each lane was scanned in at least four horizontal positions, with sensitivity (signal to noise) sufficient to distinguish bands having one-half the area of the smallest peak. A repeat of the entire experiment, including sample preparation, blotting, and scanning, gave essentially identical results.

RNA analysis. RNA was prepared from logarithmically growing 30°C cultures and from the same cultures 5 min after the shift to 42°C. Rifampin was added to a final concentration of 200 µg/ml at 5.5 min, and additional samples were removed at 8 and 10 min or at 9 min. Culture samples of 50 ml were immediately chilled on an equal volume of crushed ice-M9 salts and centrifuged, and the cell pellet was suspended in 6 ml of 100 mM Tris (pH 7.5)–100 mM MgCl₂–0.5 mg of lysozyme per ml–96 U of DNase (RNase free; Boehringer Mannheim) per ml. Cells were subjected to three rounds of freeze-thaw in dry ice-ethanol and a 37°C bath. Then 0.5 ml of 40 mM acetic acid and 0.6 ml of 10% SDS were added, the suspension was mixed, and three phenol-chloroform-isoamyl alcohol (25:24:1) extractions were performed. Nucleic acids were precipitated from the aqueous phase by addition of 0.11 volume of 2 M sodium acetate (pH 5.2) and 2.5 volumes absolute ethanol, incubation at –20°C, and centrifugation. Slot blot analysis was performed by using GeneScreen Plus (Du Pont) as instructed by the manufacturer and described in reference 2. Equal quantities of total RNA were loaded in each slot of a given set; three dilutions (9×, 3×, and 1×) were blotted. Blots were probed with the 0.7-kb *Clal*-*PvuII* fragment from the *rpoH* locus, isolated from plasmid pDS2. Two independent RNA preparations were each blotted twice; one of these blots is shown in Fig. 8. Densitometry was as above.

RESULTS

Cosmid isolation and phenotypic characterization. *E. coli* K165 [*rpoH165*(Am) *supC*(Ts); 19] is temperature sensitive for production of the heat shock sigma factor. A genomic library from *R. meliloti* Rm1021 in cosmid pLAFR1 (19) was mated into *E. coli* K165, and transconjugants were selected at 30°C. When tested for growth at 37°C, approximately 1 transconjugant in 300 was viable. Ten strains were isolated for further study, and preliminary restriction mapping revealed that five of the isolates carried cosmids with overlapping inserts. Cosmid pKM8 was chosen for further characterization. When pKM8 DNA was transformed into K165 at the permissive temperature (30°C), transformants gained the ability to grow at 37°C.

Heat shock protein synthesis by a set of *E. coli* strains isogenic except for *rpoH* alleles and plasmids is shown in Fig. 1. RpoH⁺ parent SC122 induced the typical heat shock response (39, 40) even at the relatively low temperature of 37°C; note in particular the induced synthesis of major heat shock bands at apparent molecular sizes of ~63 and 70 kilodaltons (kDa), corresponding to the GroEL and DnaK

FIG. 1. Heat shock protein synthesis. Cells grown to mid-log phase in minimal medium at 30°C were pulse labeled with [³⁵S]methionine (5 mCi/ml) for 5 min either at 30°C or 5 min after transfer to 37°C and then chilled on ice. Cells were pelleted in a microfuge and suspended in SDS sample buffer, and the proteins were separated by SDS-PAGE on 2.5%/10% gels as described previously (2). An autoradiograph of the dried gel is presented; arrowheads indicate the migration of protein standards (sizes in kilodaltons). Lanes: A, Rm1021, 30°C; B, Rm1021, 37°C; C, SC122, 30°C; D, SC122, 37°C; E, K165, 30°C; F, K165, 37°C; G, K165(pKM8), 30°C; H, K165(pKM8), 37°C; I, K165(pKP11), 30°C; J, K165(pKP11), 37°C.

proteins, respectively (40). K165 [*rpoH165*(Am) *supC*(Ts)] did not induce these heat shock proteins, but their synthesis was restored by addition of *E. coli rpoH*⁺ on plasmid pKP11. K165 carrying *R. meliloti* clone pKM8 also produced multiple heat shock proteins, suggesting that its growth at 37°C was attributable to the action of a sigma factor.

Figure 1 also gives an indication of the evolutionary divergence of heat shock proteins between *E. coli* and *R. meliloti* (39). *R. meliloti* produced a prominent protein that comigrated (at ~70 kDa) with *E. coli* DnaK.

Characterization of the suppressing *R. meliloti* locus *suhR*. DNA from pKM8 was randomly subcloned in pSE153, a Sp^r Km^r derivative of low-copy-number plasmid pSC101. Plasmid pABa2 (Fig. 2) allowed growth of K165 at 42°C. Plasmid pABg5, which contains an insert partially overlapping that in pABa2, allowed growth at 37°C but only poor growth at 40°C.

The DNA sequence of the *R. meliloti*-derived DNA in pABa2 and pABg5 (Fig. 3) includes three large ORFs, two of which overlap substantially but in opposite orientation (Fig. 2). The third, ORF-C, is carried completely on pABa2 and partially on pABg5. Computer analysis of codon usage frequency with the program CODONPREFERENCE gave marginally significant scores for the majority of ORF-A and ORF-C and nonsignificant scores for ORF-B. Analysis of known *R. meliloti* gene sequences gave scores similar to those from ORF-A and ORF-C (data not shown). In *suhR*, G or C is present at the third position of codons in 201 of 323 cases, or 62.2% of the time (derived from Fig. 3). Whereas Bae et al. (3) observed a strong bias toward G or C at this position for the *R. meliloti* anthranilate synthase gene, the *suhR* usage does not differ significantly from the overall *R. meliloti* G+C content of 62.3 to 62.5% (11).

Plasmid pABPE1 was constructed to contain ORF-C and not ORF-A or -B (Fig. 2). K165 carrying pABPE1 grew at 42°C, implicating ORF-C as the suppressing region. Because our results indicate that ORF-C encodes a suppressor of *E. coli rpoH* rather than an *R. meliloti* sigma factor (see below), we have named this locus *suhR*.

pABPE1 was derived from pAB25 (Materials and Methods). Surprisingly, many constructs such as pAB25 in which ORF-C is carried on high-copy-number vectors did not suppress K165 for growth at high temperature. This phenomenon, which might reflect effects of vector sequences on insert transcription, has not been examined further.

The predicted molecular size of the ORF-C gene product is 35.2 kDa. In *E. coli* minicells (Fig. 4), a protein migrating at an apparent molecular size of ~40 kDa was produced by strains carrying *suhR* on pKM8 or pABa2 but not by those carrying empty vector pLAFR1 or pSE153 (Fig. 4, band A). The protein was synthesized at a low level relative to other plasmid markers such as neomycin phosphotransferase (Fig. 4, band B) and was not observable in maxicells (data not shown).

Mutagenesis of *suhR*. As expected, plasmids pAB40 (*suhR*::Km^r) and pAB45 (Δ *suhR*::Km^r) did not complement

FIG. 2. Map of plasmid inserts, restriction sites, and ORFs from the *R. meliloti* *suhR* region. Plasmid pABPE1 allows growth of *E. coli* K165 at 42°C; ORF-C is therefore designated *suhR*. Arrowheads indicate direction of reading. aa, Amino acid.

```

-175      gcatgcaccactcccgattcgccagccaggtccgctcagacgcgcggcgctgctt -120
-119      cagggcgacctgtctgtctcgattttccgacgtgatcccgacctgacgtgcgaatatc -60
-59      acccctgactgcacgcgggtgtcgggaccgactcggggaaggctgatggccggttagatca 0
1      M S I G G O L T O N A O P G N L S P S T G S g c 60
      atgtcaatcggcggtcagttgcaaaacgcgcaaccgggcaaccttcccttcgacgagc
61      V R T R L L V L O R Q C R N R R R H W I 120
      gttaggacgcgccttctgtgttgcaaaagacaatgccgtaatcgcaggcggcactggatt
121      A A R F E R N L E K K D G A G T R R L E 180
      gccgcacgggttgagcgcgaaccttgaaaagaaagacggcgacggcaccggcgccctggag
181      D D F S S D L H O E T I D G T H S R E I 240
      gatgatcttctcgtcagatctgcacatcaggagacgatcgatggcagcactcccgcgaaata
241      L R S R H E G R H H O R Y R L P E R R S 300
      ctgcgatctcgtcatgaaggcggtcaccagcgggtatcgtctaccggaacgcgcctct
301      G P C A G L P V O E Y R R H V G R S D R 360
      gggccttgcgcgggactaccggttcaagaatatcggcgacgtcggcgaggagcgatcgc
361      S C G L C G C S R R P A E A D E G G D 420
      agctcgcggttgctgcggcggtcgcagtcggagacggcggaagcagatgaaggcgcgat
421      R S T G G A R R F R R P C K G I G E S R 480
      cgctcaaccggagggagcgcgtcgggttctgaaaggccttgcgaaggcactcggcgaaactcgc
481      L A R V H O G P P P A C G R R R P G G I P 540
      ctgcggcggttcatcaaggacctcctccagcctgcggcgcgagggcagggcactccg
541      L A R H T A G N T G V L R K G V A L L G 600
      ctgtctcgtcacactgccggcaacaccggcggttcttctcgaaaggcgttgctgcttcttga
601      A G C T C G T T C G A T C G C C C G T C G A A C G T G T G T G T C G C G G C A C T C G C G G T C T T 660
      agcgtcgttccgcatcgccccgctcgaacgctgctgctgctcgcggcactcgcgggtctt
661      A Y A V G G O T G G M I A A A L P A A I C 720
      gcttatgtcgtcgggggggcaaacaggcagtgatagccgagcttaccgggctgctgctcgc
721      A Y L G G V V F A V L R I A R V L R R C N A A T 780
      gccatctggtgggtgtgttgcgggtactgcgcacgtcgtcgtcctgctgcgcgcgaat
781      L M G L C T G T A P D O P A R R P R M V 840
      ctgatgggctctgtaccggcagggcaccggatcagcccgccggcgccaggaaggtggtc
841      T T G A C C G A T T G C T T C A C G A A C C T G C A G G C A C T G C C G G C A A G G C T T C G G C A G C C C 900
      ttgaccgattggttcacgaaaccctgcaggcactgtccggcaaggcttcggccagccccc
901      T C A C C T C G G C A T C T T T G G C R R S A T P A N R A P T A 960
      tcaccttcggcgatcttggacggcgagcgtaccggcggaaccgggctccgaccgcg
      P L P
961      ccgttaccctgaagatgatcacgactggcatatcgaccaggagccgcgcagcctgccgt 1020
1021      tcgaaagcgcgcttttctggttcggcgcaaggaattc 1068

```

FIG. 3. DNA sequence of *R. meliloti* Rm1021 *suhr* region and derived amino acid sequence of SuhR. Sequence for the 1,237-bp *SphI-EcoRI* fragment (pABPE1 insert) is presented; nucleotides are numbered such that position 1 corresponds to the first base of the *suhr* ORF. The derived amino acid sequence encodes a 35.2-kDa protein.

K165 for growth at high temperature, whereas pKM8 did. pAB42 (ORF-A/B::Km^r) also did not complement K165. Taken together with the information presented above, this result may indicate that *suhr* is transcribed in an operon with ORF-B or that upstream enhancer sites are required for *suhr* expression.

The successful construction of *R. meliloti suhr*::Km^r and $\Delta suhr$::Km^r (Materials and Methods) demonstrates that *suhr* is not essential for the viability of *R. meliloti*. The

FIG. 4. Protein production by *E. coli* minicells carrying *suhr*. Minicells prepared from strain DS410 transformed with *suhr*-carrying or control (no insert) plasmids were pulse-labeled with [³⁵S]methionine, and the proteins were separated by SDS-PAGE (see Materials and Methods). An autoradiograph of the dried gel is presented; migration of protein standards is indicated at the left. Lanes: 1, pLAFR1; 2, pKM8 (25-kb *suhr* region); 3, pSE153; 4, pABa2 (1.7-kb *suhr* region). Arrowhead A marks the putative *suhr* gene product (lanes 2 and 4); arrowhead B marks the neomycin phosphotransferase gene product (lane 3). kD, Kilodaltons.

growth rates of Rm9140 and Rm9145 (Table 1) in LB liquid medium at 30°C were not significantly different from that of the wild type (Rm1021). *M. sativa* inoculated with these three strains were indistinguishable with respect to nodulation and nitrogen fixation.

Absence of similarity to *E. coli rpoH* or other loci. Extensive computer comparisons of both DNA and derived amino acid sequences were made between ORF-C (and also ORF-A and -B) and *E. coli rpoH* (58), *E. coli rpoD* (7), and *R. meliloti ntrA* (46). No significant similarity was observed with alignment and dot matrix comparison algorithms, whether to the entire sigma factor genes or to amino acid sequences of sigma factor consensus regions (25, 37), nor was there any similarity to sequences of the heat shock proteins GroEL and DnaK. Data base searches using 1989 releases of GenBank, EMBL, NAQ, and PSQ libraries returned no sequences significantly similar to ORF-C. The derived amino acid sequence does contain a region with strong similarity to the helix-turn-helix consensus (42; see also references 14 and 37). The sequence, centered on the glycine corresponding to amino acid 190 (see base pair [bp] 568 in Fig. 3, is RHTAGNTGVLRKGVALL.

***suhr* does not encode an amber suppressor or affect *supC*(Ts) activity.** One explanation for suppression of *E. coli* K165 *rpoH165*(Am) is that *R. meliloti suhr* codes for an amber tRNA suppressor. This possibility was examined in K165, which also carries *lac*(Am) and *trp*(Am) alleles. Both pKM8 and a plasmid carrying *E. coli rpoH*⁺ (pKP11) allowed K165 to grow at 37°C. K165 carrying either plasmid remained phenotypically Lac⁺ Trp⁺ at 30°C and Lac⁻ Trp⁻ at 37°C, as determined by colony formation. This result

FIG. 5. Differential rate of β -galactosidase activity in *E. coli* SC122 [*lac*(Am) *supC*(Ts)] carrying pABa2 (*suhR*) or pSE153 (vector, no insert). Isopropyl- β -D-thiogalactopyranoside (IPTG) was added (20 μ g/ml) at time zero to mid-log-phase cultures growing in M9-0.2% glycerol-thiamine-spectinomycin at the indicated temperature. Samples were removed at 10, 30, 50, and 70 min and assayed for β -galactosidase activity (44). Enzyme activity is expressed as differential rate ([change in activity] $\cdot$ [change in culture OD₆₇₅]⁻¹). Data points are derived from individual cultures assayed four times at each time point.

implies that pKM8 does not carry an amber suppressor. Sequence data confirmed that the canonical amber or ochre tRNA structure (5'-PyUA-[10 to 50 bp]-UUC-[12 to 30 bp]-CCA-3') is not encoded by either strand of the insert carried on pABPE1, which is sufficient for suppression (Fig. 2 and 3). Although as little as twofold enhancement (41) of *supC*(Ts) function or increased readthrough of amber codons could explain the elevation of σ^{32} levels observed (see below), no significant effect of *suhR* on β -galactosidase activity was observed in SC122, the isogenic *lacZ*(Am) *supC*(Ts) parent of K165 (Fig. 5).

Suppression versus complementation of *E. coli* *rpoH*. *E. coli* strains deleted for *rpoH* (59) grow only below 20°C, although they also accumulate suppressors that allow growth at higher temperatures (32). Deletion $\Delta rpoH30::kan$ was transduced into K165 carrying plasmid pABa2 (*R. meliloti* insert) or pSE153 (vector only, no insert), with selection at 16°C for a Tn10 insert 70% linked to *rpoH* (56). Of the transductants, approximately 30% [K165(pABa2), 8 of 31; K165(pSE153), 9 of 37] grew at 30°C, and all of these were Km^r, suggesting that for both recipients the remaining 70% were temperature sensitive because they had acquired $\Delta rpoH30::kan$. Similar results were obtained with a wild-type *E. coli* recipient. Thirty K165(pABa2) transductants that were Km^r at 16°C were tested further and found not to grow even at room temperature (21 to 24°C), although some gave a few temperature-resistant colonies, presumably as a result of second-site suppression. Thus, *suhR*, which suppresses *rpoH*165(Am) *supC*(Ts) *E. coli*, does not suppress $\Delta rpoH30::kan$ strains.

The suppressed $\Delta rpoH30::kan$ strain KY1603 grows at temperatures of up to 40°C as a result of overproduction of GroEL (32). The other major heat shock proteins are not elevated upon temperature shift in this strain (Fig. 6; 32), although a few low-molecular-weight proteins are induced. *E. coli* *rpoH*⁺ restores heat shock protein synthesis in KY1603, but *R. meliloti* *suhR*⁺ (in either pABa2 or the original genomic clone pKM8) does not, as expected from its failure to suppress temperature sensitivity. It thus appears that *R. meliloti* *suhR* does not provide a sigma factor that

FIG. 6. Heat shock protein synthesis in *E. coli* KY1603 ($\Delta rpoH30::kan$) with and without *R. meliloti* *suhR* or *E. coli* *rpoH*. Cells grown to mid-log phase in minimal medium at 30°C were pulse-labeled with [³⁵S]methionine (10 mCi/ml) for 5 min either at 30°C or 5 min after transfer to 42°C and then chilled on ice. Cells were pelleted in a microfuge and suspended in SDS sample buffer, and the proteins were separated by SDS-PAGE on 2.5%/10% gels as described previously (2). An autoradiograph of the dried gel is presented; major heat shock proteins (see pKP11, 42°C) are marked at the left. The host strain was KY1603 for all lanes. pKP11 carries *E. coli* *rpoH*⁺; pABa2 and pKM8 carry *R. meliloti* *suhR* (see Table 1).

FIG. 7. (a and b) Western blots of SDS-PAGE protein gels probed with anti-*E. coli* σ^{32} polyclonal antisera. Mid-log-phase cultures were grown at 30°C; for blot b, 30°C samples were removed, cultures were heat shocked at 42°C for 5 min, and a second sample was taken. Sample preparation, SDS-PAGE, blotting, and probing were done by standard methods (see Materials and Methods). Lanes were loaded with equal cell quantities (determined at harvest). Migration of protein standards is indicated at left. *E. coli* σ^{32} migrates at ~32 kDa. (a) Lanes: 1, SC122; 2, K165; 3, K165(pKP11); 4, K165(pKM8); 5, K165(pABa2). (b) Lanes: 1, CAG9301, 42°C; 2, K165, 30°C; 3, K165, 42°C; 4, SC122, 30°C; 5, SC122, 42°C; 6, K165(pKM8), 30°C; 7, K165(pKM8), 42°C; 8, Rm1021, 30°C; 9, Rm1021, 42°C; 10, Rm1021(pKM8), 30°C; 11, Rm1021(pKM8), 42°C. (c) Densitometry tracings of σ^{32} bands on blot b. All traces were done at the same attenuation setting; area integrations (see text) are independent of plotter attenuation setting. Traces: 1, K165, 30°C; 2, K165, 42°C; 3, SC122, 30°C; 4, SC122, 42°C; 5, K165(pKM8), 30°C; 6, K165(pKM8), 42°C. kD, Kilodaltons.

recognizes *E. coli* heat shock promoters but rather suppresses by a mechanism dependent on *E. coli* σ^{32} function.

Intracellular levels of σ^{32} . Densitometry of Western blots probed with polyclonal antisera raised against *E. coli* σ^{32} (Fig. 7a) revealed a twofold (pABa2) to threefold (pKM8) increase in σ^{32} level when *suhR* was added to K165. SC122 (*rpoH*⁺ isogenic parent of K165) and K165(pKP11) (*rpoH*⁺ on pBR322) contained approximately 10-fold more σ^{32} than did K165. We identified the band migrating at 32 kDa as σ^{32} by its mobility and also by the observations that it reacted strongly with antisera, than it was the only band that increased significantly when *rpoH*⁺ (pKP11) was added, and that it was the only band that varied when *suhR*⁺ (pKM8 or pABa2) was added. The relatively high level of this band in SC122 at 30°C is considered in Discussion.

The σ^{32} level increased approximately twofold after heat shock in *E. coli* SC122, K165, and K165(pKM8) (Fig. 7b and c). SC122 (*rpoH*⁺) produced approximately 10-fold more σ^{32} than did K165. More significantly, this experiment confirmed the approximately twofold increase in K165 σ^{32} level in the presence of *suhR* [K165 versus K165(pKM8)]. This was true both at 30°C and after 5 min at 42°C.

For Rm1021, no obvious differences in band intensity were observed (Fig. 7b) before versus after heat shock or between Rm1021 with one versus approximately four to eight copies of *suhR* [Rm1021 versus Rm1021(pKM8)]. Because of the polyclonal nature of the anti-*E. coli* σ^{32} antisera

used, it is premature to conclude that the strongly antigenic ~40-kDa protein is the *R. meliloti* *rpoH* gene product.

***rpoH* mRNA levels and decay.** Analysis of 30 and 42°C heat shock samples from K165(pABa2) and K165(pSE153) cultures revealed no significant (>50%) differences in *rpoH* mRNA levels between strains or temperature treatments (Fig. 8, times 0 min [0'] and 5'). The decay rate of *rpoH* mRNA was similarly unaffected by *suhR*; message levels were down approximately 10-fold 3 min after rifampin addition, but there was no detectable difference in K165(pABa2) versus K165(pSE153) (Fig. 8, times 8' and 10').

DISCUSSION

We have isolated a locus from *R. meliloti* called *suhR* that allows *E. coli* K165 [*rpoH*165(Am) *supC*(Ts)] to grow at high temperature and induce heat shock protein synthesis. Beyond the presence of a helix-turn-helix DNA-binding protein motif, which is thought to be responsible for sigma factor recognition of the -35 region of promoters (22, 48), the derived amino acid sequence of *suhR* bears no similarity to sequences of other RNA polymerase sigma factors. *suhR* does not encode an amber or ochre tRNA suppressor, nor is its DNA or derived amino acid sequence similar to those of genes for thermostabilizing proteins or other data base entries. Southern blotting revealed homology of pKM8, which includes ~25 kb of the *suhR* genomic region, to *E. coli*

FIG. 8. (a) Slot blot of total cellular RNA from K165 with and without *suhR* probed with *E. coli rpoH* DNA. Mid-log-phase cultures were grown at 30°C. A sample was removed at time 0 min (0') (30°C) immediately before a shift to 42°C. A sample was removed at 5 min, rifampin was added to a final concentration of 200 µg/ml at 5.5 min, and then samples were taken at 8 and 10 min. RNA was prepared as described in Materials and Methods; equivalent quantities of total RNA were loaded in all slots. (b) Densitometry tracings of autoradiograph shown panel a; identical attenuation settings for both traces.

genomic DNA (data not shown). Disruption or deletion of *suhR* in *R. meliloti* does not affect viability or symbiotic competence.

Suppression of *E. coli* K165 by *R. meliloti suhR* seems to occur through an RNA polymerase sigma factor, since production of multiple heat shock proteins is induced in suppressed strains. Because sequences for homologous sigma factors from different bacterial species are very similar (cf. GenBank entries for *E. coli* and *B. subtilis rpoD* and for *R. meliloti* and *K. pneumoniae ntrA*), the absence of similarity between *suhR* and *E. coli rpoH* makes it unlikely that we have isolated the *R. meliloti rpoH* locus. Because *suhR* does not thermoprotect or allow heat shock protein synthesis in $\Delta rpoH30::kan$ *E. coli* strains, we conclude that suppression occurs via σ^{32} activity. Preliminary experiments using other *rpoH* point mutations (28, 55) suggest that suppression by *suhR* is allele specific (A. Bent, unpublished data).

The twofold increase in σ^{32} that we observed upon heat shock of wild-type *E. coli* (SC122) is low but within the range of values observed by others using roughly similar systems (2- to 17-fold; 21, 34, 49, 50, 56). This result is most likely due to the high level of σ^{32} in non-heat-shocked SC122 in this study. The dramatic increase in σ^{32} concentration seen upon addition of plasmid pKP11 (Fig. 7a, lanes 2 versus 3) corroborates copy number effects noted by C. Gross and collaborators (personal communication), although the σ^{32} levels that they observe in SC122 are no more than twofold higher than those in K165 and significantly below those of SC122(pKP11).

The rate of heat shock gene transcription correlates directly with the amount of σ^{32} in the cell (26, 50). *E. coli* strains with nonsense alleles of *rpoH* that are nominally Sup⁻ still produce trace amounts of active σ^{32} , perhaps

because of natural leakiness of nonsense alleles or undetected weak suppressors, and this is sufficient for growth at temperatures well above the 20°C limit of $\Delta rpoH$ strains (55, 56, 59). Western blots show that *suhR* causes an approximately twofold elevation of σ^{32} levels in K165 [*rpoH*(Am)], accounting for the ability of *suhR*⁺ K165 to grow at higher temperatures. In principle, this might occur through increased transcription of *rpoH*, message stabilization, increased translation of *rpoH* mRNA, or stabilization of the σ^{32} protein, all of which are known in *E. coli* (16, 20, 50, 54, 56). *suhR* did not alter K165 *rpoH* mRNA levels before or after heat shock, indicating that increased transcription or transcript stabilization is not the mechanism of suppression. A general increase in translation or amber suppression is unlikely, because *suhR* does not increase β -galactosidase activity in SC122 with versus without *suhR*. Specific enhancement of *rpoH* mRNA translation or stabilization of σ^{32} protein is thus the most likely means by which *R. meliloti suhR* allows *E. coli* K165 to grow at elevated temperature and induce the heat shock response.

σ^{32} stabilization and elevated *rpoH* translation are both observed when wild-type *E. coli* is heat shocked (50). A separate example in which gene expression is controlled (in part) by rate of degradation of a regulatory protein is found in the case of λ cII, which is stabilized by cIII and whose degradation is facilitated by HflA (31). In a striking parallel, overproduction of λ cIII induces the heat shock response through stabilization of *E. coli* σ^{32} (4).

No mechanism for SuhR elevation of σ^{32} receives clear support from our data. The two proteins may directly interact, or SuhR may contact *rpoH* mRNA. Alternatively, elevation could reflect an indirect effect on σ^{32} resulting from a general stimulation by SuhR of stress phenomena, which induce the heat shock response (23, 27, 33, 40). DnaK affects proteolysis and modulates the heat shock response (26, 51, 53, 54), and the *suhR* gene product may interact or interfere with DnaK function. The presence of a helix-turn-helix motif suggests that sequence-specific DNA binding may be one aspect of SuhR function, possibly in concert with other DNA-binding protein such as σ^{32} .

ACKNOWLEDGMENTS

We thank the members of our group and the Graham Walker and Carol Gross laboratories for helpful materials and discussion.

This work was supported in part by a National Science Foundation predoctoral fellowship to A.F.B. and by Public Health Service grant GM36370 to E.R.S. from the National Institutes of Health.

LITERATURE CITED

1. Arnosti, D. N., and M. J. Chamberlin. 1989. Secondary sigma factor controls transcription of flagellar and chemotaxis genes in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **86**:830-834.
2. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, and J. A. Smith (ed.). 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
3. Bae, Y. M., E. Holmgren, and I. P. Crawford. 1989. *Rhizobium meliloti* anthranilate synthase gene: cloning, sequence, and expression in *Escherichia coli*. J. Bacteriol. **171**:3471-3478.
4. Bahl, H., H. Echols, D. B. Straus, D. Court, R. Crowl, and C. P. Georgopoulos. 1987. Induction of the heat shock response of *E. coli* through stabilization of σ^{32} by the phage λ cIII protein. Genes Dev. **1**:57-64.
5. Bochkareva, E. S., N. M. Lissin, and A. S. Girshovich. 1988. Transient association of newly synthesized unfolded proteins with the heat-shock GroEL protein. Nature (London) **336**: 254-257.
6. Brown, K. L., and H. R. Whiteley. 1988. Isolation of a *Bacillus thuringiensis* RNA polymerase capable of transcribing crystal

- protein genes. *Proc. Natl. Acad. Sci. USA* **85**:4166-4170.
7. Burton, Z., R. R. Burgess, J. Lin, D. Moore, S. Holder, and C. A. Gross. 1981. The nucleotide sequence of the cloned *rpoD* gene for the RNA polymerase sigma subunit from *E. coli* K12. *Nucleic Acids Res.* **9**:2889-2903.
 8. Cohen, S. N., and A. C. Y. Chang. 1977. Revised interpretation of the origin of the pSC101 plasmid. *J. Bacteriol.* **132**:734.
 9. Cooper, S., and T. Ruettinger. 1975. A temperature sensitive nonsense mutation affecting the synthesis of a major protein of *Escherichia coli* K12. *Mol. Gen. Genet.* **139**:167-176.
 10. Dayhoff, M. O., R. M. Schwartz, and B. C. Orcutt. 1979. A model of evolutionary change in proteins, p. 345-362. In M. O. Dayhoff (ed.), *Atlas of protein sequence and structure*, vol. 5, suppl. 3. National Biomedical Research Foundation, Washington, D.C.
 11. DeLey, J., and A. Russel. 1965. DNA base composition, flagellation and taxonomy of the genus *Rhizobium*. *J. Gen. Microbiol.* **41**:85-91.
 12. De Vos, G. F., G. C. Walker, and E. R. Signer. 1986. Genetic manipulations in *Rhizobium meliloti* utilizing two new transposon Tn5 derivatives. *Mol. Gen. Genet.* **204**:485-491.
 13. Doi, R. H., and L. F. Wang. 1986. Multiple procaryotic ribonucleic acid polymerase sigma factors. *Microbiol. Rev.* **50**:227-243.
 14. Drummond, M., P. Whitty, and J. Wootton. 1986. Sequence and domain relationships of *ntnC* and *nifA* from *Klebsiella pneumoniae*: homologies to other regulatory proteins. *EMBO J.* **5**:441-447.
 15. Erickson, J. W., and C. A. Gross. 1989. Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: a second alternate sigma factor involved in high-temperature gene expression. *Genes Dev.* **3**:1462-1471.
 16. Erickson, J. W., V. Vaughn, W. A. Walter, F. C. Neidhardt, and C. A. Gross. 1987. Regulation of the promoters and transcripts of *rpoH*, the *Escherichia coli* heat shock regulatory gene. *Genes Dev.* **1**:419-432.
 17. Fellay, R., J. Frey, and H. Krisch. 1987. Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for in vitro insertional mutagenesis of Gram-negative bacteria. *Gene* **52**:147-154.
 18. Figurski, D. H., and D. R. Helinski. 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. *Proc. Natl. Acad. Sci. USA* **76**:1648-1652.
 19. Friedman, A. M., S. R. Long, E. S. Brown, W. J. Buikema, and F. M. Ausubel. 1982. Construction of a broad host range cosmid cloning vector and its use in the genetic analysis of *Rhizobium* mutants. *Gene* **18**:289-296.
 20. Fujita, N., and A. Ishihama. 1987. Heat-shock induction of RNA polymerase sigma-32 synthesis in *Escherichia coli*: transcriptional control and a multiple promoter system. *Mol. Gen. Genet.* **210**:10-15.
 21. Fujita, N., A. Ishihama, Y. Nagasawa, and S. Ueda. 1987. RNA polymerase sigma-related proteins in *Escherichia coli*: detection by antibodies against a synthetic peptide. *Mol. Gen. Genet.* **210**:5-9.
 22. Gardella, T., H. Moyle, and M. M. Susskind. 1989. A mutant *Escherichia coli* σ^{70} subunit of RNA polymerase with altered promoter specificity. *J. Mol. Biol.* **206**:579-590.
 23. Goff, S. A., and A. L. Goldberg. 1985. Production of abnormal proteins in *E. coli* stimulates transcription of *lon* and other heat shock genes. *Cell* **41**:587-595.
 24. Goldberg, A. L., K. H. Sreedhara Swamy, C. H. Chung, and F. S. Larimore. 1983. Proteases of *Escherichia coli*. *Methods Enzymol.* **80**:680-702.
 25. Gribskov, M., and R. R. Burgess. 1986. Sigma factors from *E. coli*, *B. subtilis*, phage SPO1, and phage T4 are homologous proteins. *Nucleic Acids Res.* **14**:6745-6763.
 26. Grossman, A. D., D. B. Straus, W. A. Walter, and C. A. Gross. 1987. Sigma-32 synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*. *Genes Dev.* **1**:179-184.
 27. Grossman, A. D., W. E. Taylor, Z. F. Burton, R. R. Burgess, and C. A. Gross. 1985. Stringent response in *Escherichia coli* induces expression of heat shock proteins. *J. Mol. Biol.* **186**:357-365.
 28. Grossman, A. D., Y.-N. Zhou, C. Gross, J. Heilig, G. E. Christie, and R. Calendar. 1985. Mutations in the *rpoH* (*htpR*) gene of *Escherichia coli* K-12 phenotypically suppress a temperature-sensitive mutant defective in the σ^{70} subunit of RNA polymerase. *J. Bacteriol.* **161**:939-943.
 29. Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. W. Hendrix, and R. J. Ellis. 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature (London)* **333**:330-334.
 30. Hirsch, P. R., and J. E. Beringer. 1984. A physical map of pPH1J1 and pJB4J1. *Plasmid* **12**:139-141.
 31. Hoyt, M. A., D. M. Knight, A. Das, H. I. Miller, and H. Echols. 1982. Control of phage λ development by stability and synthesis of cII protein: role of the viral cIII and host *hflA*, *himA*, and *himD* genes. *Cell* **31**:565-573.
 32. Kusakawa, N., and T. Yura. 1988. Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress. *Genes Dev.* **2**:874-882.
 33. Lee, P. C., B. R. Bochner, and B. N. Ames. 1983. AppppA, heat-shock stress, and cell oxidation. *Proc. Natl. Acad. Sci. USA* **80**:7496-7500.
 34. Lesley, S. A., N. E. Thompson, and R. R. Burgess. 1987. Studies of the role of the *Escherichia coli* heat shock regulatory protein σ^{32} by the use of monoclonal antibodies. *J. Biol. Chem.* **262**:5404-5407.
 35. Lindquist, S. 1986. The heat shock response. *Annu. Rev. Biochem.* **55**:1151-1191.
 36. Meade, H. M., S. R. Long, G. B. Ruvkun, S. E. Brown, and F. M. Ausubel. 1982. Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn5 mutagenesis. *J. Bacteriol.* **149**:114-122.
 37. Merrick, M. J., and J. R. Gibbins. 1985. The nucleotide sequence of the nitrogen-regulation gene *ntxA* of *Klebsiella pneumoniae* and comparison with conserved features in bacterial RNA polymerase sigma factors. *Nucleic Acids Res.* **13**:7607-7620.
 38. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 39. Neidhardt, F. C., and R. A. VanBogelen. 1987. Heat shock response, p. 1334-1345. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaecter, and H. E. Umbarger (ed), *Escherichia coli* and *Salmonella typhimurium*; cellular and molecular biology. American Society for Microbiology, Washington, D.C.
 40. Neidhardt, F. C., R. A. VanBogelen, and V. Vaughn. 1984. The genetics and regulation of heat-shock proteins. *Annu. Rev. Genet.* **18**:295-329.
 41. Oeschger, M. P., and G. T. Wiprud. 1980. High efficiency temperature-sensitive amber suppressor strains of *Escherichia coli* K12. *Mol. Gen. Genet.* **178**:293-299.
 42. Pabo, C. O., and R. T. Sauer. 1984. Protein-DNA recognition. *Annu. Rev. Biochem.* **53**:293-321.
 43. Paek, K.-H., and G. C. Walker. 1986. Defect in expression of heat-shock proteins at high temperature in *xthA* mutants. *J. Bacteriol.* **165**:763-770.
 44. Pahel, G., D. M. Rothstein, and B. Magasanik. 1982. Complex *glnA-glnL-glnG* operon of *Escherichia coli*. *J. Bacteriol.* **150**:202-213.
 45. Reeve, J. 1979. Use of minicells for bacteriophage-directed polypeptide synthesis. *Methods Enzymol.* **68**:493-503.
 46. Ronson, C. W., B. T. Nixon, L. M. Albright, and F. M. Ausubel. 1987. *Rhizobium meliloti ntrA* (*rpoN*) gene is required for diverse metabolic functions. *J. Bacteriol.* **169**:2424-2431.
 47. Ruvkun, G. B., and F. M. Ausubel. 1981. A general method for site-directed mutagenesis in prokaryotes. *Nature (London)* **289**:85-89.
 48. Siegle, D. A., J. C. Hu, W. A. Walter, and C. A. Gross. 1989. Altered promoter recognition by mutant forms of the σ^{70} subunit of *Escherichia coli* RNA polymerase. *J. Mol. Biol.* **206**:591-603.
 49. Skelly, S., T. Coleman, C.-F. Fu, N. Brot, and H. Weissbach.

1987. Correlation between the 32-kDa σ factor levels and in vitro expression of *Escherichia coli* heat shock genes. Proc. Natl. Acad. Sci. USA **84**:8365–8369.
50. Straus, D. B., W. A. Walter, and C. A. Gross. 1987. The heat shock response of *Escherichia coli* is regulated by changes in the concentration of sigma-32. Nature (London) **329**:348–351.
51. Straus, D. B., W. A. Walter, and C. A. Gross. 1988. *Escherichia coli* heat shock gene mutants are defective in proteolysis. Genes Dev. **2**:1851–1858.
52. Taylor, W. E., D. E. Straus, A. D. Grossman, Z. F. Burton, C. A. Gross, and R. Burgess. 1984. Transcription from a heat-inducible promoter causes heat shock regulation of the sigma subunit of *E. coli* RNA polymerase. Cell **38**:371–381.
53. Tilly, K., N. McKittrick, M. Zylicz, and C. Georgopoulos. 1983. The *dnaK* protein modulates the heat-shock response of *Escherichia coli*. Cell **34**:641–646.
54. Tilly, K., J. Spence, and C. Georgopoulos. 1989. Modulation of stability of the *Escherichia coli* heat shock regulatory factor σ^{32} . J. Bacteriol. **171**:1585–1589.
55. Tobe, T., K. Ito, and T. Yura. 1984. Isolation and physical mapping of temperature-sensitive mutants defective in heat-shock induction of proteins in *Escherichia coli*. Mol. Gen. Genet. **195**:10–16.
56. Tobe, T., N. Kusukawa, and T. Yura. 1987. Suppression of *rpoH* (*htpR*) mutations of *Escherichia coli*: heat shock response in *suH*A revertants. J. Bacteriol. **169**:4128–4134.
57. Vincent, J. M. 1970. A manual for the practical study of root-nodule bacteria. IBP handbook no. 15. Blackwell Scientific Publications, Oxford, England.
58. Yura, T., T. Tobe, K. Ito, and T. Osawa. 1984. Heat shock regulatory gene (*htpR*) of *Escherichia coli* is required for growth at high temperature but is dispensable at low temperature. Proc. Natl. Acad. Sci. USA **81**:6803–6807.
59. Zhou, Y.-N., N. Kusukawa, J. W. Erickson, C. A. Gross, and T. Yura. 1988. Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor sigma-32. J. Bacteriol. **170**:3640–3649.
60. Zylicz, M., D. Ang, K. Liberek, and C. Georgopoulos. 1989. Initiation of λ DNA replication with purified host- and bacteriophage-encoded proteins: the role of the *dnaK*, *dnaJ* and *grpE* heat shock proteins. EMBO J. **8**:1601–1608.