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Genetics

Heat shock regulatory gene (*htpR*) of *Escherichia coli* is required for growth at high temperature but is dispensable at low temperature

(heat shock protein/nonsense mutation/nucleotide sequence/transcriptional control/σ factor)

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ABSTRACT **Nonsense mutations affecting the positive regulatory gene (*htpR*) of heat shock response have been obtained in a strain of *Escherichia coli* carrying no suppressor. The mutants can grow only at temperatures below 34°C–35°C. Heat, ethanol, and coumermycin induce major heat shock proteins in the wild-type but not in the *htpR* mutants. In contrast, the level of heat shock proteins synthesized at low temperature is unaffected. The *htpR* gene product is thus required for induction of heat shock proteins by heat or other stresses but not for their "basal-level" synthesis. Nucleotide sequence has been determined for the wild-type and the mutant alleles of *htpR*. The coding region appears to consist of 852 nucleotide pairs that correspond to 284 amino acids. Sequences commonly considered as signals for transcriptional initiation and termination were found flanking the coding region. Within this region, six amber, one opal, and two missense mutations were identified; the nonsense mutations are scattered along the gene, some being very close to the presumed amino terminus. These results indicate that the absence of *htpR* gene product is directly responsible for the failure to respond to heat shock or other stresses and for the inability to grow at high temperature. We propose that *htpR* represents a new class of genes that are essential for growth only at high temperatures (>35°C). Implications of the sequence homologies found among htpR, rpoD, and nusA proteins are discussed.**

A specific set of proteins called heat shock proteins (HSP) is induced when organisms or cells are exposed to high temperature or other environmental stresses. Such induction of HSP seems to represent part of homeostatic response, at the cellular level, to environmental changes in both eukaryotes and prokaryotes. Extensive structural homology between some of the HSP genes (1) and proteins (2) from a variety of distantly related organisms suggests that the response to heat shock or stress is universal and well conserved during evolution. In spite of the recent progress in this field, little is known about the mechanisms involved in regulating heat-shock response.

Induction of HSP in *Escherichia coli* occurs primarily at the transcriptional level (3, 4). Analysis of a mutant defective in heat shock induction led us to identify a gene (*htpR* or *hin*) whose product is apparently required for the enhanced transcription of heat shock operons upon exposure to high temperature (4, 5). Furthermore, heat shock induction controlled by the *htpR* gene appears to play a critical role in bacterial growth at high temperature and in acquired thermotolerance under certain conditions (4). The importance of *htpR* in heat shock induction and growth at high temperature was further substantiated by isolation and characterization of additional *htpR* mutants (6). The *htpR* gene has been cloned into multicopy plasmids, and its product has been identified as a protein with an apparent molecular weight of 33,000–36,000 (6, 7). The recombinant plasmids carrying *htpR* (or part thereof) have also been used to localize various mutations within the gene (6).

In the course of these studies, we found that some of the temperature-sensitive *htpR* mutants isolated from a strain carrying no suppressor actually carry a nonsense rather than a missense mutation. The experiments reported here lead us to propose that the *htpR* gene is uniquely required for growth at high temperature. The present results of complete nucleotide sequence of wild-type and mutant *htpR* alleles establish the essential role of *htpR* in heat shock response in *E. coli*.

MATERIALS AND METHODS

Bacterial Strains, Phages, and Plasmids. Temperature-sensitive *htpR* mutants carrying a *htpR* mutation and a transposon closely linked to *htpR* (*zhf50*::Tn*10*) have been obtained in a strain carrying no suppressor (MC4100: F⁻ *araD* Δ*lac rpsL*) (6). Derivatives of strain CA274 (HfrC *lacZam trpam*; see ref. 8) to which the amber *htpR* mutations were transduced by selecting for tetracycline resistance were also used. Stock cultures of *htpR* mutants were stored in 15% (vol/vol) glycerol at −80°C. Strain KY1426 (*htpR165 zhf50*::Tn*10*) was used to transduce *htpR165* into other strains. Bacteriophages carrying an amber suppressor (λ*supD* and φ*80supF*) were kindly supplied by H. Ozeki. Plasmids pKV3 and pKV6 that carry the *htpR*⁺ or the *htpR165* allele, respectively, have been described (6).

Media and Chemicals. Minimal medium used was medium E (9) with 0.5% glucose, thiamine (2 μg/ml), and other supplements. Polypeptone broth and L broth have been described (6). L-[4,5-^{3}H]leucine (60 Ci/mmol) and deoxyadenosine 5′-[α-^{35}S]thio triphospate (410 Ci/mmol) were obtained from Amersham (1 Ci = 37 GBq). M13 vectors, dideoxynucleoside triphosphates, and primer for DNA sequencing were obtained from P-L Biochemicals, and DNA polymerase Klenow fragment was from Boehringer Mannheim.

Cloning and Sequencing of *htpR*. Segments of the *htpR* gene were subcloned into M13 vectors from multicopy plasmid pKV3 carrying the intact *htpR*⁺ gene or from pKV6 carrying *htpR165* (6). For cloning part of the *htpR* region from the rest of mutants, bacterial DNA was digested with restriction enzymes, electrophoresed through agarose, and fragments of expected mobility were recovered. The fragments were ligated with M13 vectors (mp10 and mp11) and were used to transfect JM103 bacteria, essentially as described (10). White plaques containing DNA inserts were picked, propagated, and culture fluids (phage DNA) were screened by hybridizing them with a tester phage that contains the complementary DNA strand of the known *htpR* segment. DNA sequence was determined by the dideoxy chain termination method of Sanger *et al.* (11), using 17-base-long primer and deoxyadenosine [^{35}S]thio triphosphate.

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Abbreviations: HSP, heat shock protein(s); kb, kilobase(s).

RESULTS

Suppression of Temperature-Sensitive *htpR* Mutations by Amber Suppressors. We have recently isolated and characterized several *E. coli* mutants that are defective in induction of HSP and able to grow at 30°C but not at 42°C (6). These mutants were thought to carry a missense and not a nonsense *htpR* mutation, because the gene and its product have been believed to be essential for cell growth (4, 5, 12), and the parental strain used (MC4100) does not contain any nonsense suppressors. Contrary to such an expectation, transduction of amber suppressor *supF* into some of these mutants by phage P1 was found to render the cell able to grow at 42°C: among the nine mutants tested, five (*htpR5*, *-6*, *-16*, *-18*, and *-22*) became temperature resistant, whereas the rest (*htpR1*, *-11*, *-15*, and *-23*) did not. Lysogenization with a specialized transducing phage carrying an amber suppressor (*supF* or *supD*) conferred on the former but not the latter mutants the temperature resistance. These results suggested that an amber *htpR* mutation by itself (without suppressor) was responsible for the inability of the former mutants to grow at high temperature.

Transduction of *htpR165* into Suppressor-Free Strains. The *htpR165* (amber) mutation was originally isolated in a strain carrying a temperature-sensitive suppressor (*supCts*), which is active at low (30°C) but not at high (42°C) temperature (12). In view of the above finding with our new *htpR* mutants, however, we asked whether *htpR165* could be transduced into strains containing no suppressor. Strains MC4100 and CA274 were used as recipient, and strain KY1426 carrying Tn*10* near *htpR165* was used as donor. Tetracycline-resistant transductants were selected at 25°C and examined for their ability to grow at 42°C. The majority (70%–80%) of transductants obtained with either recipient was unable to grow at 42°C and they became temperature resistant when lysogenized with *φ80supF* or transformed with a plasmid containing the *htpR*$^+$ gene (pKV3). These results demonstrate that *htpR165*, like other amber *htpR* mutations, permits cell growth at low temperature in the absence of suppressor.

Growth Characteristics of *htpR* Amber Mutants. All *htpR* amber mutants containing no suppressor grow normally at 30°C in glucose minimal medium with a generation time of 80–90 min. When a mutant culture grown at 30°C was transferred to 34°C, growth continued almost normally; when transferred to 36°C, optical density increased for 3–4 hr and then stopped (Fig. 1). At still higher temperatures (39°C or 42°C), growth was arrested more abruptly. The number of viable cells (colony formers) hardly increased at 42°C, and it decreased gradually after 2–3 hr (Fig. 1). Similar results were obtained with polypeptone broth or L broth, although the maximum temperature that permits growth was slightly lower (by ≈1°C). Microscopic observation revealed that cells were slightly elongated and granular structures became apparent after exposure to 42°C for several hours. All amber mutants exhibit similar growth characteristics, provided that special care is taken to avoid secondary mutations, such as suppressor mutations that may occur in stock cultures. These results suggest that the *htpR* gene product may be required for cell growth only at high temperatures (above 34°C or 35°C). It should be noted that the "high temperature" as used here includes 37°C, the optimal temperature for growth of normal *E. coli* cells.

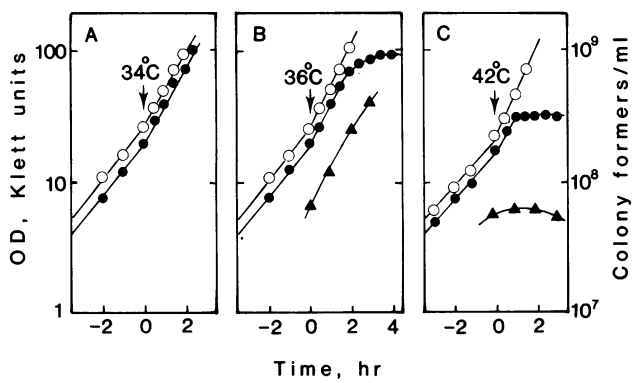

FIG. 1. Growth of *htpR* amber mutant after upshift of temperature. Isogeneic wild-type (*htpR*$^+$) and mutant (*htpR6*) cultures (CA274 and its derivative) were grown in minimal medium supplemented with L-tryptophan (20 μg/ml) at 30°C for several generations and were shifted to 34°C (*A*), 36°C (*B*), or 42°C (*C*) at time 0. Circles represent optical density (Klett units with a no. 54 filter) for wild type (○) and mutant (●). ▲, Viability (colony formers) for mutant culture as determined by plating appropriate dilutions on L agar and incubating the plates at 30°C.

HSP Synthesis in *htpR* Amber Mutants. To determine the rate of synthesis of HSP in *htpR* amber mutants, cells of the representative mutants (*htpR6* and *htpR165*) carrying no suppressor were pulse-labeled with [^{3}H]leucine at 30°C and after transfer to 42°C. The labeled groE protein (a major HSP) was precipitated with the specific antiserum and quantitated after separation by NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 2). The differential synthesis rate of groE protein at 30°C was found to be similar to that of the wild type (0.2% of total protein synthesized). Upon transfer to 42°C, synthesis of groE protein was markedly induced in the wild type, whereas no significant induction occurred in the mutant, although the rate of synthesis appeared to increase gradually. Similar results have been obtained with the *htpR165 supCts* strain (4, 6). Thus, heat induction but not the "basal-level" synthesis of groE protein is affected by the *htpR* amber mutations.

Since coumermycin A1 (inhibitor of DNA gyrase) and ethanol mimic heat shock in inducing some of the HSP in *E. coli* (14), we examined the response of *htpR* amber mutants to these agents. Synthesis of at least two major HSP (groE and dnaK proteins) was induced in the wild type, although the extents of induction were less marked than with the temperature shift (data not shown). In contrast, little or no induction occurred in the *htpR* amber mutants, suggesting that the *htpR* gene is also involved in induction of HSP by these

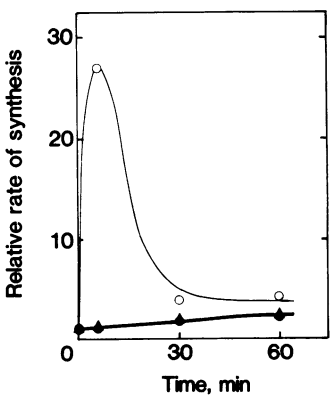

FIG. 2. Synthesis of groE protein in *htpR* mutants. Isogeneic *htpR*$^+$ (MC4100) and *htpR* amber mutants were grown in minimal medium supplemented with L amino acids (except leucine, isoleucine, and valine) at 30°C, and shifted to 42°C at time 0. Samples were taken at the times indicated, pulse-labeled with [^{3}H]leucine for 3 min and chased for 2 min. Immunoprecipitation of groE protein and NaDodSO$_4$ gel electrophoresis were carried out as described (4, 13). The rate of groE protein synthesis was determined from the ^{3}H/^{14}C ratio of the protein band, and was normalized to the 0 time value for *htpR*$^+$. The 0 time values presented are averages of two determinations. ○, *htpR*$^+$; ●, *htpR6*; ▲, *htpR165*.

chemical agents. Thus the *htpR* gene product appears to be responsive not only to heat shock but also to other stresses. Similar observations were recently reported using UV light and nalidixic acid as inducing agents (15).

Determination of *htpR* Nucleotide Sequence. To substantiate the above conclusion about the role of *htpR* in stress response as well as to gain further insight into the structure and function of the gene, the nucleotide sequence of *htpR* was determined. The strategy of sequencing is shown in Fig. 3, and the results are presented in Fig. 4. The coding region of *htpR* appears to consist of 852 nucleotide pairs that correspond to 284 amino acids. This inference is supported by the facts that (*i*) it represents the only reading frame consistent with the expected size of the product protein, (*ii*) typical initiation signals for transcription (Pribnow box) and translation (Shine–Dalgarno sequence) are found at the expected locations, (*iii*) a typical termination signal for transcription is found downstream of the termination codon, and (*iv*) the locations of all nonsense mutations (amber and opal) are consistent with the above reading frame (see below).

Fig. 4 also shows the amino acid sequence of the putative *htpR* gene product as deduced from the nucleotide sequence. The molecular weight is calculated to be 32,435. Although direct evidence on the structure of isolated htpR protein is lacking, certain properties seem apparent. When compared to "average protein" (16), it has a high content of charged amino acids (32%) and methionine (3.9%), and low contents of proline (1.8%), glycine (4.2%), and cysteine (0%). Judging from the codon usage (17), it represents a moderately abundant (≈1000 mol per genome) protein.

Altered Nucleotide Sequence in *htpR* Mutants. Based on the previous results of intragenic location of *htpR* mutations (6), either the *Hin*dIII/*Pst* I fragment [0.7 kilobase (kb)] or the *Pst* I/*Pvu* II fragment (0.2 kb) was cloned directly from chromosomal DNA of each mutant into M13 vectors and used for identification of sequence alterations (pKV6 was the source of DNA in cloning the *htpR165* allele). As shown in Fig. 5, single nucleotide changes were detected for all the mutations examined. Altogether six amber, one opal, and two missense mutations were identified by the sequence study within the coding region of *htpR*. It may be noted that the nonsense mutations are well scattered along the coding region of *htpR* and that some (*htpR11* and *htpR16*) affect an amino acid (at the 26th residue) very close to the amino terminus. The fact that a couple of missense mutations were identified in the *htpR* gene rules out the possibility that the observed phenotypes of *htpR* mutants are due to the decreased expression of some unknown gene(s) located downstream of *htpR* as a result of the polar effect of *htpR* nonsense mutations.

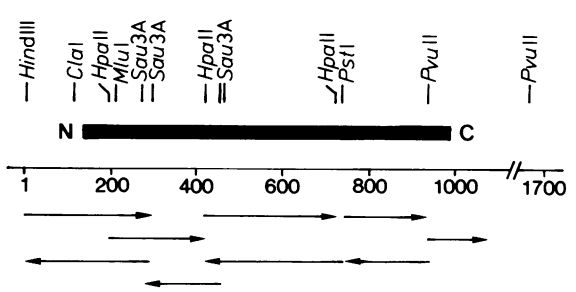

FIG. 3. Strategy of DNA sequencing. The *Hin*dIII/*Pst* I (0.7 kb), *Pst* I/*Pvu* II (0.2 kb), and *Pvu* II/*Pvu* II (0.7 kb) fragments were obtained from pKV3 and cloned into M13 vectors. The *Hin*dIII/*Pst* I fragment was further digested with *Hpa* II or *Sau*3A to clone subfragments. Upper bar shows position of *htpR* coding region; N and C indicate amino and carboxyl termini, respectively, of the protein. Arrows indicate direction and extent of sequencing for each M13 recombinant clone used.

Sequence Homology with Known Regulatory Proteins. To find possible structural similarity to other regulatory proteins, the nucleotide sequence and the predicted amino acid sequence of *htpR* were compared with the known sequences of several regulatory genes, including *crp* (cAMP receptor protein), *rpoD* (RNA polymerase σ factor), and *nusA*. Extensive homology was found between *htpR* and *rpoD* in two separate regions; one includes nucleotides 276–461 (amino acids 49–110) containing a cluster of 14 identical amino acids, and the other includes nucleotides 864–968 (amino acids 245–279) (Fig. 6). A segment of the nusA protein, also known to bind to core RNA polymerase (21), appears to be homologous to htpR and rpoD proteins with respect to the latter region. In addition, a significant homology is noted between another region of htpR protein and crp protein (Fig. 6).

DISCUSSION

A specific gene of bacteria or other haploid organisms is usually considered essential for growth if temperature-sensitive or other conditionally lethal mutations affecting its function or synthesis can be isolated. Nonsense mutants for a number of essential genes have also been isolated as conditionally lethal mutants from parental strains carrying a nonsense suppressor, which is active only under certain conditions. In the case of *htpR*, the *htpR165* amber mutation was isolated in a strain carrying a temperature-sensitive suppressor (*supCts*) (12). While such an observation alone suggests that the gene is essential for growth at the restrictive condition (e.g., high temperature), it does not exclude the possibility that the gene might be dispensable under other conditions. It is shown in this paper that a *htpR* nonsense mutation itself causes temperature-sensitive growth without involvement of a temperature-sensitive suppressor.

The proposal that the *htpR* gene is dispensable at low temperature depends on the fact that the *htpR* nonsense mutants we have isolated or constructed do not contain functionally active suppressors. No significant amounts of β-galactosidase have been detected in derivatives of strain CA274 (*lacZam*) carrying each of the *htpR* amber mutations, indicating that the level of suppressor activity, if any, is <0.1% of *supF*. Another important point is whether incomplete polypeptides (fragments) produced by the nonsense mutants in the absence of suppressor can support growth at low temperature. This also seems quite unlikely, because some of the mutations (e.g., *htpR11* and *htpR16*) were found to occur very close to the amino terminus (at the 26th residue) of the putative htpR protein (Fig. 4). Taken together, the present results lend strong support to the idea that *htpR* represents a new class of genes that are required for growth at high temperature (>35°C) but are dispensable at lower temperatures. Since the basal-level expression of *groE* (and presumably other HSP genes) occurs normally in the absence of htpR protein, the htpR protein appears to be specifically required to cope with the cellular demands for increased expression of HSP genes upon exposure to high temperature or other stress. The fact that the *E. coli* cell requires *htpR* even at 37°C might indicate that the cell is under some stress at the optimal temperature. Although the molecular mechanism underlying these observations remains obscure at present, it seems significant that the maximum temperature that allows growth of *htpR* nonsense mutants (34°C–35°C) coincides well with the temperature (≈34°C) at which the *E. coli* cell begins to respond to heat by inducing the HSP synthesis (3).

The complete nucleotide sequence presented here is based on the results obtained with both wild-type and a number of mutant *htpR* alleles examined. The single nucleotide changes have been unequivocally identified for all nine mutants tested. The finding that *htpR11* and *htpR15* represent, respec-

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AAGCTTGCAT TGAACTTGTG GATAAAATCA CGGTCTGATA AAACAGTGAA TGATAACCTC GTTGCT    1-66

CTTAA GCTCTGGCAC AGTTGTTGCT ACCACTGAAG CGCCAGAAGA TATCGATTGA GAGGATTTGA   67-131

ATG ACT GAC AAA ATG CAA AGT TTA GCT TTA GCC CCA GTT GGC AAC CTG GAT TCC  132-185
Met Thr Asp Lys Met Gln Ser Leu Ala Leu Ala Pro Val Gly Asn Leu Asp Ser    1-18

TAC ATC CGG GCA GCT AAC GCG TGG CCG ATG TTG TCG GCT GAC GAG GAG CGG GCG  186-239
Tyr Ile Arg Ala Ala Asn Ala Trp Pro Met Leu Ser Ala Asp Glu Glu Arg Ala   19-36

CTG GCT GAA AAG CTG CAT TAC CAT GGC GAT CTG GAA GCA GCT AAA ACG CTG ATC  240-293
Leu Ala Glu Lys Leu His Tyr His Gly Asp Leu Glu Ala Ala Lys Thr Leu Ile   37-54

CTG TCT CAC CTG CGG TTT GTT GTT CAT ATT GCT CGT AAT TAT GCG GGC TAT GGC  294-347
Leu Ser His Leu Arg Phe Val Val His Ile Ala Arg Asn Tyr Ala Gly Tyr Gly   55-72

CTG CCA CAG GCG GAT TTG ATT CAG GAA GGT AAC ATC GGC CTG ATG AAA GCA GTG  348-401
Leu Pro Gln Ala Asp Leu Ile Gln Glu Gly Asn Ile Gly Leu Met Lys Ala Val   73-90

CGC CGT TTC AAC CCG GAA GTG GGT GTG CGC CTG GTC TCC TTC GCC GTT CAC TGG  402-455
Arg Arg Phe Asn Pro Glu Val Gly Val Arg Leu Val Ser Phe Ala Val His Trp   91-108

ATC AAA GCA GAG ATC CAC GAA TAC GTT CTG CGT AAC TGG CGT ATC GTC AAA GTT  456-509
Ile Lys Ala Glu Ile His Glu Tyr Val Leu Arg Asn Trp Arg Ile Val Lys Val  109-126

GCG ACC ACC AAA GCG CAG CGC AAA CTG TTC TTC AAC CTG CGT AAA ACC AAG CAG  510-563
Ala Thr Thr Lys Ala Gln Arg Lys Leu Phe Phe Asn Leu Arg Lys Thr Lys Gln  127-144

CGT CTG GGC TGG TTT AAC CAG GAT GAA GTC GAA ATG GTG GCC CGT GAA CTG GGC  564-617
Arg Leu Gly Trp Phe Asn Gln Asp Glu Val Glu Met Val Ala Arg Glu Leu Gly  145-162

GTA ACC AGC AAA GAC GTA CGT GAG ATG GAA TCA CGT ATG GCG GCA CAG GAC ATG  618-671
Val Thr Ser Lys Asp Val Arg Glu Met Glu Ser Arg Met Ala Ala Gln Asp Met  163-180

ACC TTT GAC CTT GCT TCC GAC GAC GAT TCC GAC AGC CAC GCG ATG GCT CCG GTG  672-725
Thr Phe Asp Leu Ala Ser Asp Asp Asp Ser Asp Ser His Ala Met Ala Pro Val  181-198

CTC TAT CTG CAG GAT AAA TCA TCT AAC TTT GCC GAC GGC ATT GAA GAT GAT AAC  726-779
Leu Tyr Leu Gln Asp Lys Ser Ser Asn Phe Ala Asp Gly Ile Glu Asp Asp Asn  199-216

TGG GAA GAG CAG GCG GCA AAC CGT CTG ACC GAC GCG ATG CAG GGT CTG GAC GAA  780-833
Trp Glu Glu Gln Ala Ala Asn Arg Leu Thr Asp Ala Met Gln Gly Leu Asp Glu  217-234

CGC AGC CAG GAC ATC ATC CGT GCG CGC TGG CTG GAC GAA GAC AAC AAG TCC ACG  834-887
Arg Ser Gln Asp Ile Ile Arg Ala Arg Trp Leu Asp Glu Asp Asn Lys Ser Thr  235-252

TTG CAG GAA CTG GCT GAC CGT TAC GGC GTT TCC GCT GAG CGT GTA CGC CAG CTG  888-941
Leu Gln Glu Leu Ala Asp Arg Tyr Gly Val Ser Ala Glu Arg Val Arg Gln Leu  253-270

GAA AAG AAC GCG ATG AAA AAA TTG CGT GCT GCC ATT GAA GCG TAA TTTCCGCTAT  942-996
Glu Lys Asn Ala Met Lys Lys Leu Arg Ala Ala Ile Glu Ala  *              271-284

TAAGCAGAGA ACCCTGGATG AGAGTCCGGG GTTTTTGTTT TTTGGGCCTC TGTAATAATC AATT  997-1057
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FIG. 4. Nucleotide sequence of the *E. coli* K-12 *htpR* gene. Sequence of the coding strand of DNA is shown from 5′ to 3′, starting from the end of the *Hin*dIII site. Deduced amino acid sequence is shown below the DNA sesquence. Upstream to the coding region are sequences possibly involved in initiation of transcription (boldface) and of translation (underlined), whereas downstream is a region of dyad symmetry presumably involved in transcription termination (boldface with underlining).

tively, opal and missense mutations is consistent with the previous observation that they are slightly or moderately leaky mutants, respectively (6). In the case of *htpR165*, the possibility of an additional alteration that may explain the observed anomaly in reversion study (5, 12) has not been rigorously excluded.

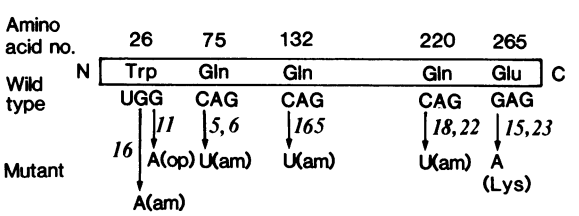

FIG. 5. Altered nucleotide sequences of *htpR* mutant alleles. Upper portion shows nucleotides and amino acids for wild type. Arrows indicate mutational changes observed (the mutant allele numbers are indicated with each arrow). (am), Amber; (op), opal.

The striking sequence homology found between segments of htpR and rpoD proteins (and nusA protein) suggests that there may be a common structural domain or domains that are involved in specific functions, such as binding to RNA polymerase core enzyme or to DNA. Interestingly, the carboxyl-terminal region of homology in both σ (*rpoD*) and nusA proteins have been shown to be dispensable for complementing the respective mutations when cloned into multicopy plasmids (22, 23). It is possible that this region determines the affinity of the proteins to core RNA polymerase. The occurrence of missense mutations (*htpR15*, *htpR23*) within this region implies that the hypothetical domain structure of htpR protein may be essential for heat shock induc-

A

htpR	**Ala** Ala **Lys** Thr Leu Ile Leu Ser His **Leu** **Arg** Phe **Val** Val His **Ile** **Ala** Arg Asn **Tyr** Ala	49-69
rpoD	**Ala** Lys **Lys** Glu Met Val Glu Ala Asn **Leu** **Arg** Leu **Val** Ile Ser **Ile** **Ala** Lys Lys **Tyr** Thr	375-395
htpR	Gly Tyr **Gly** **Leu** Pro Gln Ala **Asp** **Leu** **Ile** **Gln** **Glu** **Gly** **Asn** **Ile** **Gly** **Leu** **Met** **Lys** **Ala** **Val**	70-90
rpoD	Asn Arg **Gly** **Leu** Gln Phe Leu **Asp** **Leu** **Ile** **Gln** **Glu** **Gly** **Asn** **Ile** **Gly** **Leu** **Met** **Lys** **Ala** **Val**	396-416
htpR	Arg Arg **Phe** Asn Pro Glu Val **Gly** Val Arg Leu Val Ser Phe **Ala** Val His **Trp** **Ile** Lys	91-110
rpoD	Asp Lys **Phe** Glu Tyr Arg Arg **Gly** Tyr Lys Phe Ser Thr Tyr **Ala** Thr Trp **Trp** **Ile** Arg	417-436
htpR	Val **Ala** **Arg** Glu **Leu** Gly **Val** **Thr** **Ser** Lys Asp **Val** Arg Glu Met	157-171
crp	Met **Ala** **Arg** Arg **Leu** Gln **Val** **Thr** **Ser** Glu Lys **Val** Gly Asn Leu	120-134
htpR	**Leu** **Asp** **Glu** Asp Asn Lys **Ser** **Thr** **Leu** Gln **Glu** **Leu** **Ala** Asp Arg Tyr Gly **Val** Ser Ala **Glu**	245-265
rpoD	Ile **Asp** Met Asn Thr Asp Tyr **Thr** **Leu** Glu **Glu** Val Gly Lys Gln Phe Asp **Val** Asp Arg **Glu**	565-585
nusA	**Leu** Val **Glu** Glu Gly Phe **Ser** **Thr** **Leu** Glu **Glu** **Leu** **Ala** Tyr Val Pro Met Lys Glu Leu Leu	372-392
htpR	**Arg** Val **Arg** **Gln** **Leu** **Glu** Lys Asn **Ala** Met Lys **Lys** **Leu** **Arg**	266-279
rpoD	**Arg** Ile **Arg** **Gln** Ile **Glu** Ala Lys **Ala** Leu Arg **Lys** **Leu** **Arg**	586-599
nusA	Glu Ile Glu Gly **Leu** Asp Glu Pro Thr Val Glu Ala **Leu** **Arg**	393-406

B

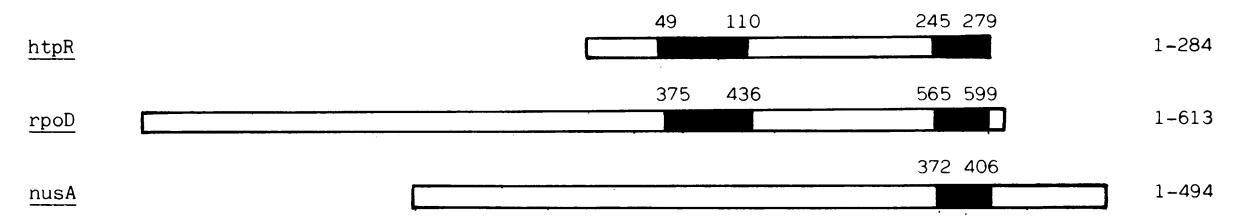

FIG. 6. Amino acid sequence homology between putative htpR protein and some regulatory proteins. Homologous segments are shown in *A*, and regions of homology among htpR, rpoD, and nusA proteins are indicated relative to the intact proteins in *B*. Sequence data for σ protein (*rpoD*), NusA protein (*nusA*), and cAMP receptor protein (*crp*) were taken from refs. 18, 19, and 20, respectively.

tion and high-temperature growth. Also relevant to this discussion may be our previous finding that the level of expression of HSP genes is inversely correlated with the cellular level of σ factor or RNA polymerase holoenzyme (24, 25). More direct experiments are required to assess implications of these various findings. The present results on the nonsense and missense mutants of *htpR* and the nucleotide sequence data should provide a basis for further elucidation of the mechanisms of heat shock response in bacteria, and in particular, the role of *htpR* in transcriptional regulation of the HSP genes.

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