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POSITIVE REGULATORY GENE FOR TEMPERATURE-CONTROLLED PROTEINS IN *ESCHERICHIA COLI*

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SUMMARY

A group of nine proteins of *Escherichia coli* K12 vary in steady state level with growth temperature, and are particularly abundant above 40°C. The identities of most of these HTP (high temperature production) proteins are unknown; they are primarily recognizable on two-dimensional polyacrylamide gels by their very high rates of synthesis during the ten-minute period following a shift-up in temperature. This stimulation, as much as 20-fold for some HTP proteins, is abolished by a conditionally lethal nonsense mutation in a chromosomal gene located at 75 minutes. Evidence suggests that this regulatory gene, *htpR*, makes an activator protein that is required for heat induction of HTP proteins.

INTRODUCTION

Recent analyses of the synthesis of proteins in *Escherichia coli* at different temperatures have revealed that several major proteins are derepressed during steady state growth at 42°C or higher (1, 2). These proteins are among those that exhibit very high transient rates of synthesis following even modest shifts-up in growth temperature (3).

One of these proteins is the *groE* (or *mop*) gene product, which is involved in bacteriophage morphogenesis and is essential in an unknown way for the normal growth of *E. coli* (cf., 4-7). The *groE* gene product has been identified as protein B56.5 on the two-dimensional reference gel of *E. coli* proteins (7, 8, and Drahos and Hendrix cited in 1). Lemaux and Cooper (unpublished observations cited in 3) noted that this protein was not made in its normal high quantity at 42°C in a mutant strain of *E. coli* containing a nonsense mutation in an essential gene in the presence of a temperature-sensitive amber suppressor. We now report that this conditionally lethal mutation

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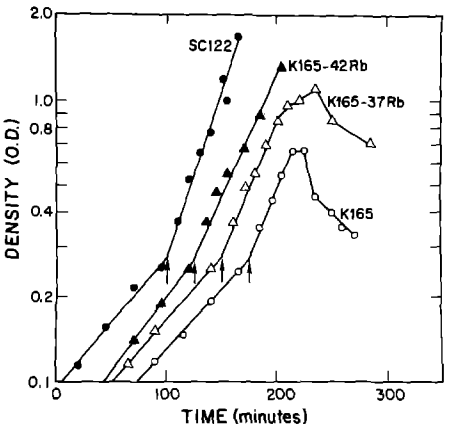

Figure 1. Growth response of mutant strains upon shift from 28°C to 42°C. The four cultures in balanced growth in glucose MOPS rich medium at 28°C were shifted to 42°C at the times indicated by the arrows.

lies not in the groE gene, but elsewhere on the chromosome in a gene that controls the heat-induction of at least nine different proteins.

MATERIALS AND METHODS

Bacterial Strains. Derivatives of E. coli strain K12 were used. SC122 has been described (9); it has the following genotype lac_{amber}, trp_{amber}, pho_{amber}, $supC^{ts}$, str, mal_{amber}. K165 was obtained by mutagenizing SC122 and selecting for temperature sensitive growth (9). K165-37Rb is a spontaneous revertant of K165; K165-42Rb is a spontaneous secondary revertant derived from K165-37Rb. These are described in Table 2.

Media. All media used were defined MOPS medium (10) supplemented with 0.4% glucose (wt/vol), amino acids (0.04 mM methionine), 5 vitamins, and bases (11).

Bacterial Growth. Cells were grown aerobically at selected temperatures as described (1).

Radioactive Labeling of Cultures. Steady state cultures of SC122, K165, K165-37Rb, and K165-42Rb were grown at 28°C from OD_{420nm} 0.05 to 0.30 and then shifted to 42°C. A small portion of each culture was labeled with [^{35}S]methionine (1.25 mCi/mmole; 50 μCi/ml) for a 10-minute period before the shift to 42°C, and a separate portion for the first 10-minute period after the shift.

Analysis by 2-D Electrophoresis. Extracts were made as previously described (12), and proteins were resolved on the O'Farrell equilibrium gel system (13).

RESULTS AND DISCUSSION

Heat-Inducibility of HTP Proteins. A culture of E. coli SC122 growing in glucose rich medium at 28°C was shifted to 42°C (Fig. 1). A small sample

Table 1. The HTP proteins of Escherichia coli SC122

	Protein[a]	Identification[b]	Location on reference gel[c]
1.[d]	B25.3	—	110 x 66
2.	B56.5	groE product	106 x 107
3.	B66.0	dnaK product	108 x 113
4.	C14.7	—	86 x 33
5.	C15.4	—	83 x 34
6.	C62.5	—	92 x 113
7.	D60.5	lysyl-tRNA synthetase	83 x 111
8.	F84.1	—	77 x 119
9.	G93.0	—	50 x 122

[a]The alphanumeric system of nomenclature is described in ref. 14.

[b]Identification of B56.5 as the groE gene product appears in ref. 7; D60.5 as lysyl-tRNA synthetase, form II, unpublished observations and ref. 15; and B66.0 as dnaK gene product (C. Georgopoulos, K. Tilly, D. Drahos and R. Hendrix, personal communication).

[c]The numbers are the x-y coordinates of the protein location in the autoradiogram of Fig. 1B in ref. 8.

[d]These numbers correspond to those in Fig. 2.

of the culture was labeled for 10 minutes with [^{35}S]methionine before the shift, and a second sample labeled for the first 10 minutes after the shift. Nine proteins (B25.3, B56.5, B66.0, C14.7, C15.4, C62.5, D60.5, F84.1 and G93.0), identified in Table 1, were observed to be derepressed at 42°C (Fig. 2, Panels A and B).

These nine proteins exhibit this behavior in all K12 and B strains of E. coli examined so far. As shown next, these proteins share a control system, and we have designated them HTP (high temperature production) proteins.

Mutant Lacking Heat-Inducibility of HTP Proteins. E. coli SC122 makes a tyrosine tRNA (Sup^{ts}) that suppresses amber mutations weakly at 30°C but not at all at 40°C (16). Strain K165 was derived from it by nitrosoquanidine mutagenesis, and is temperature-sensitive for growth as a result of amber mutations in one or more essential genes mapping between approximately 63 and 76 minutes on the recalibrated E. coli genetic map (9). Mutant K165 loses viability and lyses some time after a shift to 42°C.

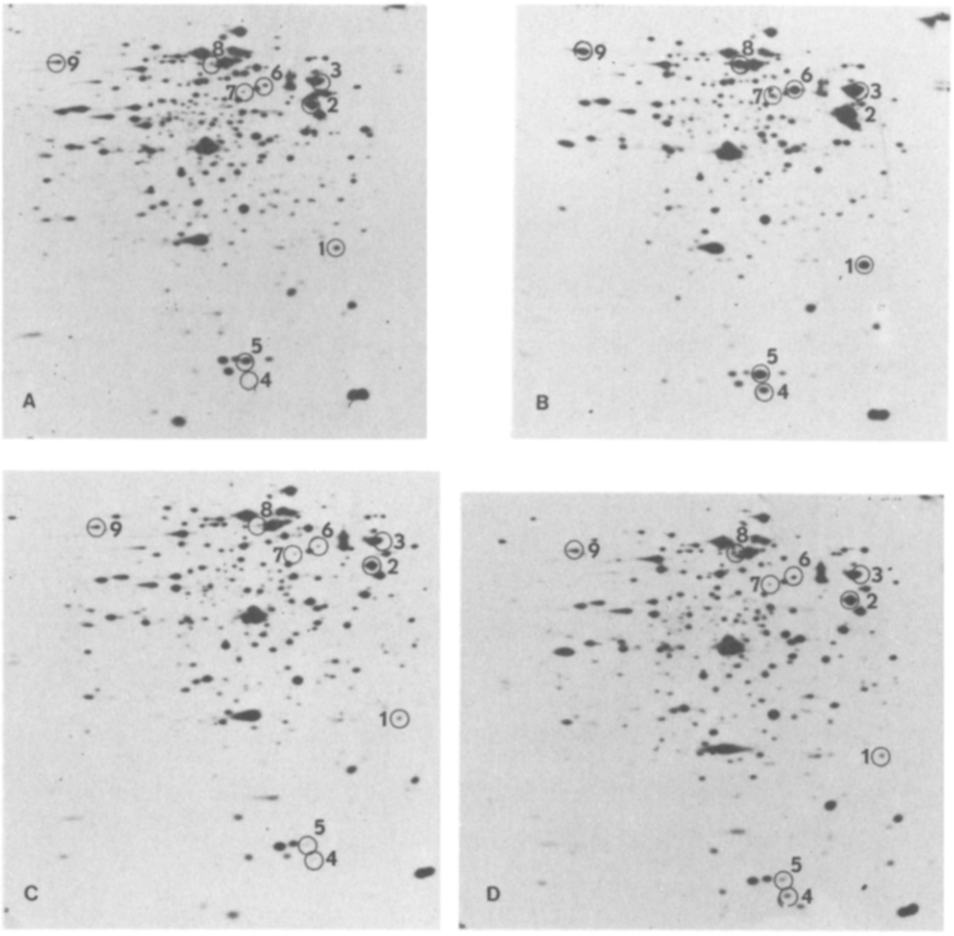

Figure 2. Synthesis of individual proteins in normal and mutant cells at 28°C and 42°C. The autoradiograms were made from two-dimensional gels of total cell extracts of cultures labeled for 10 minutes with [^{35}S]methionine. Strain SC122 (normal HTP control): Panel A, 28°C; Panel B, 42°C. Strain K165 (defect in HTP control) Panel C, 28°C; Panel D, 42°C. Measurement of the radioactivity of selected individual protein spots indicated that the proteins #2, #6 and #8 are derepressed approximately 20-fold in the normal strain at 42°C.

A culture of K165 in balanced growth at 28°C was shifted to 42°C. As expected, there was a normal increase of growth rate for one generation, followed by stasis and eventual lysis of the culture (Fig. 1). Culture samples were labeled as above and examined on two-dimensional gels. All nine HTP proteins continued to be made, but failed to derepress upon the shift to 42°C (Fig. 2, Panels C and D). All other proteins (as far as can

Table 2. Properties of revertants of E. coli K165

Strain plated	Growth temperature selected	Class of revertant (example)[a]	Revertant phenotype: Growth at 42°C	Revertant phenotype: Amber suppressor activity[b]	Revertant phenotype: Heat-inducibility of HTP proteins[c]
K165	42°C	a	+	Sup^+	+
K165	37°C	a	+	Sup^+	+
		b(K165-37Rb)	o	Sup^{ts}	o
K165-37Rb	42°C	a	+	Sup^+	+
		b(K165-42Rb)	+	Sup^{ts}	+

[a] In parenthesis is given the designation of an individual isolate of the indicated class.

[b] Suppressor activity was scored using the lac_{amber} and trp_{amber} markers.

[c] Heat-inducibility of the nine HTP proteins was measured as described in Fig. 2, or by visual inspection of the autoradiograms. ("+" means normal heat-inducibility; "o" means no increase in synthesis rate at 42°C).

be told by visual inspection of the autoradiograms of the gels) retained their normal behavior.

These findings indicated that K165 might have a defect in a regulatory gene, which we tentatively named htpR. To explore this possibility it was necessary to define the mutational lesions in this mutant.

Reversion Analysis of Mutant K165. Table 2 summarizes some of the results of a study of the reversion pattern of mutant K165. Selection for growth of K165 at 42°C yielded only revertants that have acquired high-temperature amber suppressing activity (Sup^+) from the parental temperature-sensitive suppressor (Sup^{ts}), indicating there may be more than one amber mutation in K165 that must be suppressed for growth at 42°C (9). Selection for growth at 37°C yielded some revertants that were still Sup^{ts}. From one of the latter it was easy to select secondary revertants that could grow at 42°C, some of which were still Sup^{ts}. It appears, therefore, that one amber mutation must be reverted (or suppressed) for strain K165 to grow at 37°C, and a second one to grow at 42°C. The Sup^{ts} revertant isolated at 37°C (K165-37Rb) is still unable to derepress the HTP proteins upon a shift to 42°C, while the one derived from it at 42°C (K165-42Rb) has regained vir-

tually normal heat-inducibility for all nine of these proteins (results not shown).

From these results we conclude that the second amber mutation in strain K165 is in a gene (htpR) necessary both for growth at 42°C and for heat-inducility of the nine HTP proteins.

Map Location of htpR. Two plasmids of the Clarke-Carbon recombinant colE1 bank (17), pLC31-16 and pLC31-32, were found to yield transconjugants with K165 and K165-37Rb that grew well at 42°C, were still Sup^{ts}, and had all the other markers of the recipient strains. All nine HTP proteins are heat-inducible in the transconjugants (data not shown). Plasmids pLC31-16 and pLC31-32 had been previously identified as carrying the ftsE gene (cited in 18), loosely mapped at 73-74 minutes. We have found (results not shown) that both plasmids also carry the livJ and livK genes, which map at 75.4 minutes (19). The htpR gene can therefore be assigned a position at approximately 75 minutes. This is consistent with the earlier finding by Cooper and Ruettinger (9) that F'41, covering the region from 63 to 76 minutes, complements the essential gene(s) mutated in K165. The htpR locus is far removed from at least three known structural genes for HTP proteins (groE, 94 min (19); dnaK, 0.5 min (19); and the gene for form II of lysyl-tRNA synthetase carried on pLC4-5 (data not shown) which is non-overlapping with the plasmids carrying htpR).

HTP Control System. Since htpR maps away from the genes it is known to control, and since this function is blocked by an amber mutation, we postulate that htpR codes for a positive regulatory protein that at high temperature stimulates expression of the genes coding for HTP proteins. Shifting from 42°C to 28°C results in immediate repression of at least one HTP protein (3), indicating that the htpR gene product may be a temperature-conformable protein, active as a stimulator at high temperature, inactive at low.

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