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Identification of a Temperature-Sensitive Mutation in the *htpR* (*rpoH*) Gene of *Escherichia coli* K-12

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A new mutation in the *htpR* (*rpoH*) gene of *Escherichia coli* K-12 was identified. The mutation resulted in a temperature-sensitive phenotype in terms of cell growth and bacteriophage λ development. As in the case of the classical *htpR tsn-165* mutation, synthesis of heat shock polypeptides was not induced in strains carrying the mutation described here.

Shifting *Escherichia coli* cultures to high temperature results in a pronounced elevation of synthesis of several polypeptides (heat shock proteins). Neidhardt et al. (15) have proposed that the high-temperature-production regulon of *E. coli* consists of a set of operons that are coordinately controlled by a single regulator gene, designated as *hin* or *htpR* and more recently as *rpoH* (7). Although a number of *rpoH* mutants have been described (8, 19), information on the role of the regulator gene has been based primarily on work with a single amber mutation, *tsn-165* (*hin-165*). The mutation results in a temperature-sensitive phenotype in strains carrying a temperature-sensitive suppressor tRNA (4) and in little or no induction of the synthesis of heat shock polypeptides (14, 21). We describe here cloning and other studies on another mutant isolate. Our findings show that a mutation in the *htpR* gene is responsible for the temperature-sensitive characteristics of cell growth and the inability of the cells to support growth of phage λ at high temperatures.

TABLE 1. *E. coli* K-12 strains

Strain	Derivation	Characteristics	Source or reference
AB2531		F⁻ *ilv arg thy deo his gal*	6
TC28-2	From AB2531 by nitrosoguanidine mutagenesis	F⁻ *ilv arg thy deo his gal*(Ts)	6
TC28-2 *str*	From TC28-2 by spontaneous mutation	Like TC28-2 and *str*	This laboratory
TC28-2 *recA str*	KL16-99 × TC28-2 *str* mating	F⁻ *liv arg deo his gal recA str*(Ts)	This laboratory
F140/TC28-2 *recA str*	MAF1/JC1553 × TC28-2 *recA str* mating		This laboratory
F141/TC28-2 *recA str*	KLF41/JC1553 × TC28-2 *recA str* mating		This laboratory
KL16-99		Hfr *recA*	D. Hoar; 11
MAF1/JC1553		*arg*⁺/*arg his leu met recA*	B. J. Bachmann; 1, 12
KLF41/JC1553		*arg*⁺/*arg his leu met recA*	B. J. Bachmann; 1, 12
159		*gal uvrA*	P. Ray; 17
159*supF*		*gal str uvrA supF*	18
WC5041*supE*		*str uvrA leu supE*	18
C600/T6		*thr leu lac tsx*	9
W3350		*gal lac*	3
KOT226		*trp tyr hin* [80 *supF*(Ts)]	C. Georgopoulos; 21
NF1514		*recA srb*::Tn*10 trk*	H. Murialdo
W3350ts	TC28-2 × W3350 *malT* (P1 transduction)	Temperature sensitive	This laboratory
C600/T6ts	W3350ts × C600/T6 *malT* (P1 transduction)	Temperature sensitive	This laboratory
W3350ts *recA*	NF1514 × W3350ts (P1 transduction)	*recA*(Ts)	This laboratory

E. coli strains used were as described in Table 1. Isolation and initial characterization of TC28-2, the original mutant isolate, have been described previously (6). The temperature-sensitive site in TC28-2 was mapped to 76.0 min by a series of P1 transduction tests which resulted in a value of 3.4% cotransduction of the temperature-sensitive site with *malT*. This was considerably higher than the frequency of cotransduction of temperature sensitivity with *aroB*, imply-

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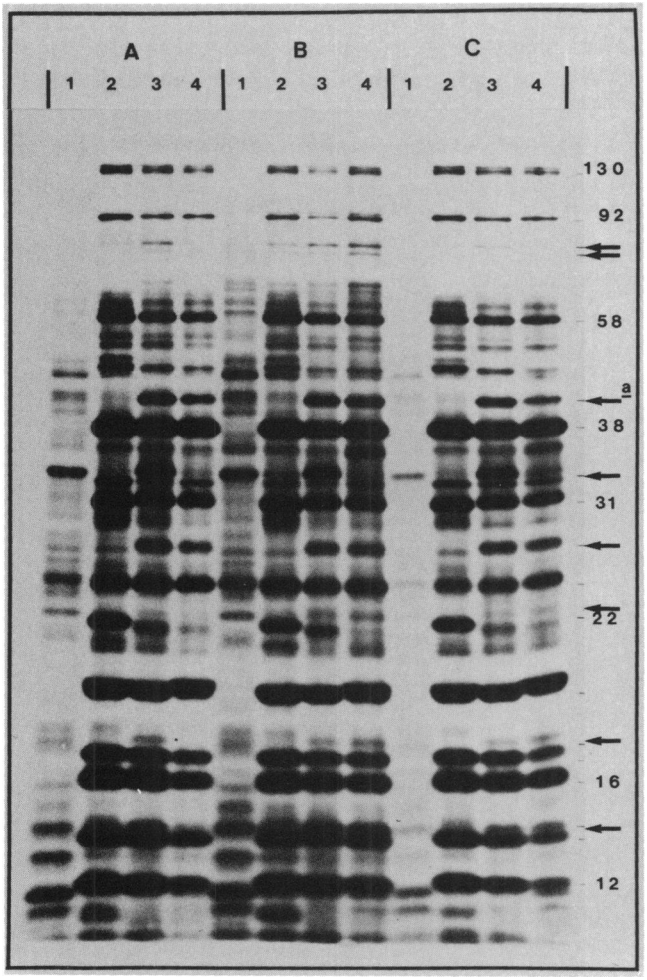

FIG. 1. Proteins synthesized by λts⁺, λts⁺am31, and λ *c*I ts857 *S*am7 control phage. Phage proteins were labeled with [^{35}S]methionine after infection of UV-irradiated cells incubated at 40.5°C. (A) 159, (B) 159*supF*, and (C) WC5041*supE* uninfected (lane 1) or infected with λ *c*I ts857 *S*am7 (lane 2), λts⁺ (lane 3), or λts⁺am31 (lane 4). Molecular weights of several lambda proteins are indicated in the right margin. Arrows indicate polypeptides that are synthesized by the λts⁺ transducing phage but not by λ *c*I ts857 *S*am7. The arrow marked with a superscript a denotes a band composed of two polypeptides that are clearly resolved in additional autoradiographs.

ing that the chromosomal order of the markers is *aroB-malT*(Ts). All transductants which were temperature-sensitive for bacterial growth were also temperature-sensitive for phage λ growth.

Merodiploids of TC28-2 *recA* were also tested for growth capability and phage λ production at high temperature. Isolates carrying F-prime factor F140 or F141 (12) were capable of colony formation on nutrient agar at 42°C, and their doubling time in liquid culture at 34 and 40.5°C was essentially the same. Thus, it could be inferred that these F-prime factors carry the wild-type allele of the temperature-sensitive site and that the wild-type allele (ts⁺) is dominant. At least partial dominance of ts⁺ over temperature sensitivity was also evident from measurements of λ phage production by phage-infected merodiploid cultures or lysogens.

The wild-type allele of the gene coding for temperature sensitivity was cloned on a 15-kilobase (kb) *Hin*dIII fragment from C600/T6 into the λ *Hin*dIII replacement vector NM762 (13). The procedure involved packaging of DNA in vitro (2) and selection of plaque formers on W3350ts at 42°C. Dilysogens of temperature-sensitive strains carrying the ts⁺

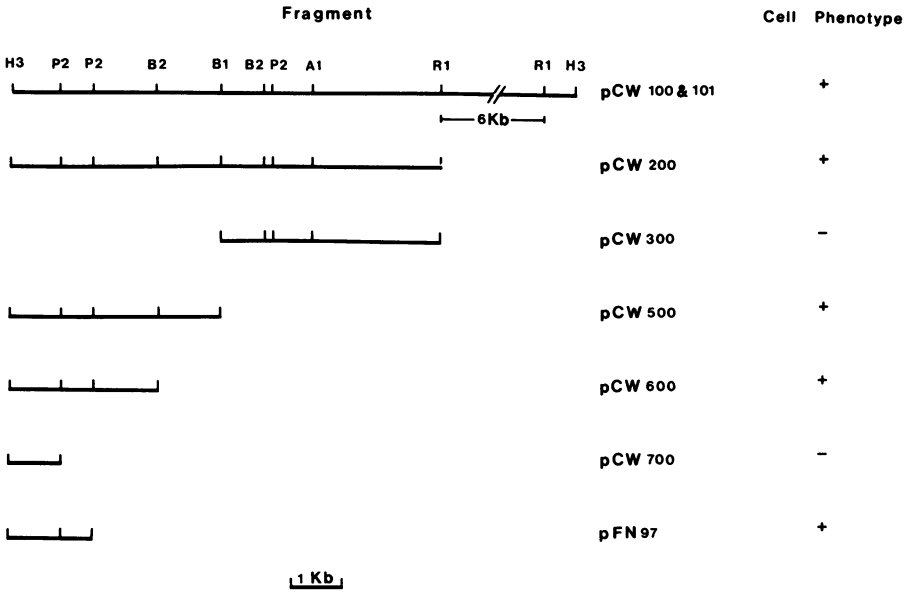

FIG. 2. Localization of the wild-type, temperature-sensitive gene on cloned DNA fragments. Restriction maps of cloned *E. coli* fragments and the phenotype of transformants are shown. Restriction sites are indicated by A1 (*Ava*I), B1 (*Bam*HI), B2 (*Bgl*II), H3 (*Hin*dIII), P2 (*Pvu*II), and R1 (*Eco*RI). Ampicillin-resistant transformants of W3350ts *recA* were tested for growth at 42°C; (+) and (−) entries indicate growth and no growth, respectively. With plasmid pCW700, recombinants capable of growth at 43°C were detected in tests with *recA*⁺ cells. Plasmid pFN97, provided by R. VanBogelen, was derived from a larger plasmid, pOX1 (16). The restriction sites mapped here are in agreement with the map of the overlapping region of pOX1.

transducing phage, λts$^+$, and λ *E*am4 *c*I$^+$, a helper phage required for lysogenization, grow well at high temperature, indicating that the cloned gene is expressed and able to compensate for the mutant chromosomal temperature-sensitive gene.

In UV-irradiated cells infected with λts$^+$, at least nine polypeptides were synthesized that are not produced in cells infected with normal λ particles (Fig. 1). Infection of minicells resulted in the synthesis of the same polypeptides, indicating that they were indeed coded by the infecting phage rather than the host cell (data not shown). Pulse-chase experiments failed to provide evidence of a precursor-product relationship between any of the polypeptides uniquely associated with λts$^+$ infection.

Two amber mutants of λts$^+$ were isolated on the basis of their inability to grow in nonsuppressing temperature-sensitive host cells at high temperature. Unlike the λts$^+$ parent phage, which produced normal numbers of progeny particles in temperature-sensitive host strains at 40.5°C, the burst size of the amber mutants in a nonsuppressing temperature-sensitive host was reduced about 100-fold at this temperature. It is apparent from Fig. 1 that one of the amber mutants, λts$^+$am31, does not produce six of the nine polypeptides made by λts$^+$. Like λts$^+$am31, the other amber mutant, λts$^+$Δ4am17, also does not produce these six polypeptides. The inability of the amber mutants to produce most of the polypeptides made by λts$^+$ is most easily accounted for by assuming that the mutations have occurred in a regulator gene, but whether that gene controls expression of an operon within which the ts$^+$ gene is located or the ts$^+$ gene itself regulates an operon on the cloned fragment was not established by the results obtained.

The 15-kb *Hin*dIII fragment in λts$^+$ was cloned into the *Hin*dIII site of pBR322 (Fig. 2). Plasmids pCW100 and pCW101 carry the fragment in opposite orientations. Subclones of these plasmids were constructed by restriction enzyme digestion of pCW100 with *Eco*RI (pCW200), pCW101 with *Bam*HI (pCW500), pCW200 with *Bam*HI (pCW300), and pCW500 with *Bam*HI and *Bgl*II (pCW600) or *Pvu*II (pCW700) and religation with T4 DNA ligase. W3350ts *recA* cells transformed to ampicillin resistance with pCW plasmid subclones were used to localize the ts$^+$ activity of the 15-kb *Hin*dIII fragment in λts$^+$ to the 2.8-kb *E. coli* DNA fragment carried by pCW600 (Fig. 2). This fragment also conferred ts$^+$ growth characteristics on KOT226, which carries the *htpR tsn-165* mutation. The plasmid pFN97, which is known to carry the *htpR* gene on a 1.69-kb fragment (15), was also shown to confer ts$^+$ growth characteristics on cells carrying the temperature-sensitive mutation. W3350ts *recA* transformants carrying plasmid pCW700 remained temperature-sensitive for growth, but ts$^+$ cells in transformant cultures of a *rec*$^+$ strain occurred approximately 100 times more frequently than did ts$^+$ revertants in cultures of untransformed cells. The increased frequency of cells able to grow at high temperature (43°C) is attributed to recombination between the plasmid and host cell DNA. Production of ts$^+$ cells by recombination implies that the 0.95-kb fragment in pCW700 carried part of the gene in which the temperature-sensitive mutation was located. Neidhardt et al. (15) have localized the *tsn-165* mutation to the same DNA restriction fragment. The nucleotide sequence of this region reveals a single 852-nucleotide open reading frame bounded by transcriptional and translational signals (10). In vivo and in vitro, the cloned segment produces a single protein that migrates in gels with the cellular protein (F33.4) implicated as the *htpR* product (15; unpublished results). Hence, we conclude that the temperature-sensitive mutation under investigation is a mutation in the *htpR* gene.

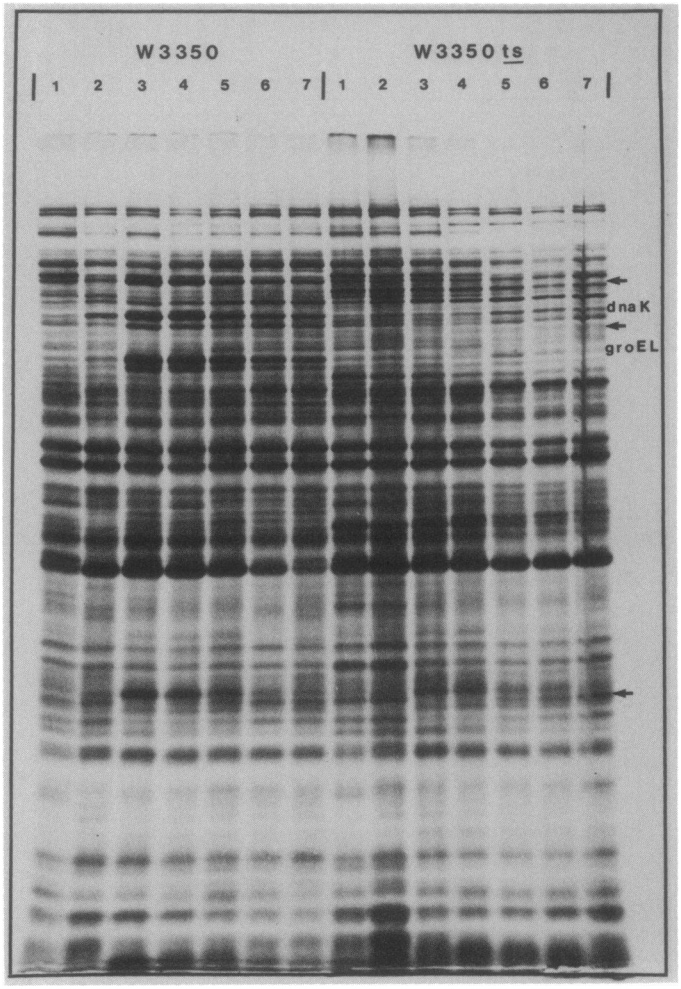

FIG. 3. Heat shock response in W3350 and W3350ts strains. Cells were labeled for 3 min with [^{35}S]methionine and chased for 2 min with unlabeled amino acid at 30°C (lane 1) or after shift to 42°C and incubation for 0 (lane 2), 5 (lane 3), 10 (lane 4), 20 (lane 5), 30 (lane 6), or 60 (lane 7) min. Arrows indicate polypeptide bands which are more intense after temperature shift of the ts$^+$ strain. Included are two bands that correspond in molecular weight to the *dnaK* and *groEL* gene products. Results similar to those shown for W3350ts were obtained with 159ts and C600/T6ts cultures. The viable cell number of these temperature-sensitive strains remains essentially constant during prolonged incubation at 42°C.

Figure 3 shows the pattern of protein synthesis by ts$^+$ strains at 30°C and after transfer to 42°C. Induction of several *E. coli* proteins (indicated by arrows) at high temperature can be seen in W3350. Included are gpdnaK and gpgroE, two of the proteins known to be among the heat shock proteins produced by wild-type cells (14). In W3350ts cells, however, there is no obvious induction of any of these proteins over a period of up to 1 h. Two-dimensional analysis of proteins synthesized at 30°C or from 5 to 10 min after shift to 42°C has also indicated that induced synthesis of specific heat shock proteins characteristic of ts$^+$ cells does not occur in the temperature-sensitive mutant cells (data not shown).

Our findings confirm the results obtained previously with the *tsn-165* mutation. Since we have found that the temperature-sensitive mutation in *htpR* described here confers

temperature-sensitive characteristics on phage λ growth, phage development must also require induction of host heat shock proteins. It is interesting that, of the few heat shock proteins genetically identified, three are known λ host factors. The *dnaK* gene product is involved in λ DNA replication, and the products of the *groE* genes (gpgroEL and gpgroES) are required for proper head assembly (5). As reported elsewhere, we have obtained evidence of abnormalities in phage head assembly in *htpR*(Ts) host cells at high temperature, but there is also a defect in tail fiber formation (20).

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