Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6779–6783, November 1984
Genetics

A gene regulating the heat shock response in *Escherichia coli* also affects proteolysis

(*htpR*/*lon*/unstable proteins/RNA polymerase)

TANIA A. BAKER*, ALAN D. GROSSMAN, AND CAROL A. GROSS†

Department of Bacteriology, University of Wisconsin, Madison, WI 53706

Communicated by Bernard B. Davis, June 25, 1984

ABSTRACT **The *htpR* locus in *Escherichia coli* encodes a regulator of the heat shock response. Cells containing the *htpR165* mutation are defective in the induction of synthesis of heat-shock proteins at high temperature. We show that these cells are also defective in degrading two proteins that are normally unstable in *htpR*$^+$ cells. The proteolytic defect is manifest at both 30°C and 42°C. We used a marker rescue technique to map this defect to the *htpR* locus. Although both proteolytic substrates are partially stabilized in *lon*$^-$ strains, we argue that the defect in proteolysis exhibited by the *htpR165* strain does not mimic the *lon*$^-$ state. The *htpR165* strain synthesizes Lon at the normal rate at 30°C and does not show the phenotypes of mucoidy and radiation sensitivity associated with *lon*$^-$ strains.**

When *Escherichia coli* cells are transferred to high temperature, the rate of synthesis of a small number of proteins increases (1, 2). This physiological response to temperature shift, the heat shock response, appears to be universal, as it has been observed in all cell types tested (3, 4). Since many other stimuli, including anoxia, ethanol, and other chemical agents, induce the synthesis of heat shock proteins in various organisms, the response may be part of a general cellular mechanism for adaptation to stress (5, 6). Other agents known to induce heat shock protein synthesis in *E. coli* include UV light, ethanol, amino acid starvation, naladixic acid, and coumermycin (7).

The heat shock response in *E. coli* was initially defined by an analysis of the changes in rates of synthesis of individual proteins after shift to high temperature (1, 2). To date, 17 proteins have been characterized as heat shock proteins (7, 8). Immediately after shift to high temperature, the rates of synthesis of individual heat shock proteins increase 5- to 20-fold, depending on the protein. The increased rate of synthesis peaks at 5–10 min and then declines by 30 min after temperature upshift to a new steady-state rate of synthesis, somewhat greater than that at low temperature (9). Where it has been examined, the increased synthesis of heat shock proteins has been shown to be accompanied by an increase in the rate of synthesis of their mRNAs (refs. 10–12; unpublished results). Thus, initiation of the heat shock response is regulated, at least in part, at the transcriptional level.

The *htpR* gene, mapping at ≈76 min on the *E. coli* chromosome, is involved in regulation of the heat shock response (8, 10, 13, 14). This gene is defined by an amber mutation *htpR165* (*hin165*) carried in a strain that contains a temperature-sensitive suppressor tRNA (10, 13, 15). Cells containing this mutation are temperature sensitive for growth and fail to induce synthesis of heat shock proteins upon temperature upshift. Using a series of suppressors of various efficiencies, all of which insert the same amino acid, Yamamori and Yura (10) have shown that the efficiency of suppression of the *htpR165* mutation is correlated with both the rate of synthesis of heat shock proteins at the peak of the response and the permissive growth temperature. An efficient suppressor functional at all temperatures restores both the heat shock response and high temperature growth (10, 15). Thus, inefficient suppression of the *htpR165* mutation blocks the heat shock response. Cell death ensues at high temperature.

In this report, we show that the *htpR165* mutation confers an additional phenotype; *htpR* mutant cells are defective in proteolysis at both 30°C and 42°C. Both proteolytic substrates tested are also partially stabilized in *lon*$^-$ strains (16–19). We compare the characteristics of *lon*$^-$ cells with *htpR165* cells. In addition, possible relationships between the two phenotypes of the *htpR165* mutant are considered.

MATERIALS AND METHODS

Bacterial Strains and Growth. All strains used are *E. coli* K-12 and are listed in Table 1.

Transductions with P1*vir*, media, plates, and cell growth were as described (16, 20, 21).

Quantitation of Proteins. Double-label two-dimensional gel electrophoresis methods for determining rates of synthesis and degradation of individual proteins were essentially as described (16, 21). Degradation data for the β-galactosidase nonsense fragment were obtained from one-dimensional polyacrylamide gel electrophoresis analysis (22). The amount of radioactivity remaining in β-galactosidase various times after a pulse–chase was determined as a fraction of the amount of radioactivity in the β + β′ subunits of RNA polymerase. β + β′ subunits are stable (data not shown). Alternatively, samples were immunoprecipitated as described (23), using anti-β-galactosidase antibody generously provided by Reid Johnson (24).

RESULTS

***htpR*$^-$ Strains Are Defective in Proteolysis.** The mutant σ subunit of RNA polymerase encoded by the *rpoD800* allele is rapidly degraded at high temperature (16). The rates of degradation of mutant σ subunit in isogeneic *htpR*$^+$ and *htpR165* strains are shown in Fig. 1*A*. At 42°C, mutant σ subunit was rapidly degraded in the *htpR*$^+$ strain ($t_{1/2}$, 6 min) but was quite stable in the *htpR165* strain ($t_{1/2}$, >60 min). The rate of degradation of mutant σ subunit was indistinguishable from that of wild-type σ subunit in the *htpR*$^-$ strain (data not shown).

We examined the degradation of another unstable polypeptide in isogeneic *htpR*$^+$ and *htpR*$^-$ strains. The *lacZX90* mutation is an ochre nonsense mutation located near the 3′ end of the *lacZ* gene (25). The almost full length β-galactosidase polypeptide encoded by *lacZX90* is unstable in wild-type cells (17, 25, 26). However, as was true for σ subunit,

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*Present address: Department of Biochemistry, Stanford University Medical Center, Stanford, CA 94305.
†To whom reprint requests should be addressed.

Table 1. Bacterial strains used

Strain	Genotype
SC122*	*lac*(am) *trp*(am) *pho*(am) *supC*^{ts} *rpsL mal*(am)
K165*	SC122 *htpR165*
CAG456†	SC122 *htpR165*
CAG510‡	SC122 *rpoD800*–Tn*10*
CAG481‡	CAG456 *rpoD800*–Tn*10*
CAG482	CAG481 ϕ80
CAG483	CAG481 ϕ80SuIII (*SupF*)
CAG603§	SC122 *proA/B*::Tn*10*/F'*proAB*$^+$ *lacZX90*
CAG604§	CAG456 *proA/B*::Tn*10*/F'*proAB*$^+$ *lacZX90*
CAG5107	CAG604 pFN92
CAG5110¶	CAG604 pFN92 *htp*$^+$ recombinant

Strains beginning with CAG are from our laboratory collection and were constructed using standard genetic techniques.
*Described in ref. 15.
†CAG456 was made by transducing the *htpR165* mutation linked to *malT*$^+$ into an SC122 derivative that was *malT*$^-$.
‡The Tn*10* is ≈90% linked to *rpoD* (16).
§The F' is from strain CSH21 (20).
¶Temperature-resistant recombinants of CAG5107 were selected at 42°C. The presence of *htpR*$^+$ in the chromosome was verified by recovering the *htpR*$^+$ allele by P1 transduction.

the X90 polypeptide was degraded considerably more slowly ($t_{1/2}$, >60 min) in the *htpR*$^-$ strain than in the *htpR*$^+$ strain ($t_{1/2}$, 7 min) at 42°C (Fig. 1*B*). Thus, the defect in degradation is not specific for mutant σ subunit.

The Degradation Defect Maps to the *htpR* Locus. We mapped the defect in proteolysis by a marker rescue experiment. We were fortunate to obtain from R. van Bogelen, E. Lau, and F. Neidhardt the pFN92 plasmid, which carries a segment of bacterial DNA starting ≈120 base pairs upstream of the NH_2 terminus of the *htpR* structural gene and ending within the *htpR* gene close to its 3' end (7, 27). This plasmid does not complement the *htpR*$^-$ defect; however, *htp*$^+$ recombinants, which are temperature resistant and undergo a normal heat shock response, can be recovered (8). We transformed an *htpR165* strain with pFN92 and then selected *htpR*$^+$ recombinants based on their temperature-resistance phenotype. We compared the ability of these strains (*htpR165*, pFN92, nonrecombinant; *htpR*$^+$, pFN92, recombinant) to undergo the heat shock response (Fig. 2*A*) and to degrade the X90 polypeptide (Fig. 2*B*). The nonrecombinant strain retained the characteristics of the original *htpR*$^-$ strain. It was defective in the heat shock response (Fig. 2*A*, lanes 1 and 2) and in the degradation of X90 (Fig. 2*B*). In contrast, the recombinant strain simultaneously regained the ability to carry out the heat shock response (Fig. 2*A*, lanes 3 and 4) and the ability to degrade X90 (Fig. 2*B*), characteristic of the *htpR*$^+$ strain. *htpR*$^+$ recombinant strains also regain the ability to degrade mutant σ subunit (data not shown). These experiments unambiguously map the defect in proteolysis to a lesion in the *htpR* gene. Both a normal heat shock response and degradation of the X90 and mutant σ subunit polypeptides require the *htpR*$^+$ allele.

The Degradation Defect Is Due to Inefficient Suppression of the *htpR165* Allele. The *supC*^{ts} suppressor present in our standard *htpR165* strain is an inefficient suppressor at the permissive temperature. Thus, the defect in proteolysis observed in *htpR*$^-$ cells at 42°C could be due to a lower amount of the *htpR* product synthesized at 30°C rather than the lack of either the heat shock response or *htpR* synthesis at 42°C. In the former case, the defect in proteolysis exhibited by *htpR*$^-$ cells should occur at 30°C as well as at 42°C. We can compare the rates of σ subunit degradation in isogeneic *htpR*$^+$ and *htpR*$^-$ cells at 30°C, because *rpoD800*-encoded σ subunit is degraded in wild-type cells even at low temperature (16). The mutant σ subunit was degraded more slowly in the *htpR*$^-$ strain than in the *htpR*$^+$ strain at 30°C (Fig. 3*B*). Rapid degradation at 30°C was restored when the more efficient *supF* (SuIII) suppressor was present (Fig. 3*B*).

We asked whether the efficient *supF* (SuIII) suppressor restored the ability of *htpR165* cells to carry out proteolysis at 42°C. We found that ϕ80 SuIII restored the ability of *htpR*$^-$ cells to degrade the unstable σ subunit encoded by

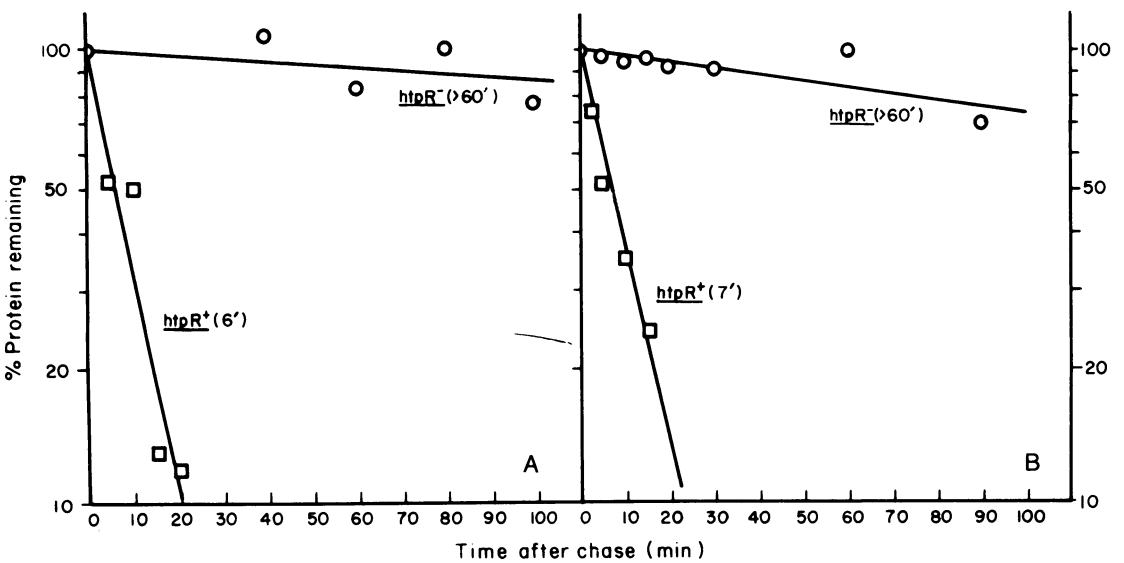

FIG. 1. Degradation of unstable proteins in isogeneic *htpR*$^+$/*htpR*$^-$ strains. (*A*) Degradation of mutant σ subunit (encoded by the *rpoD800* allele). Strains CAG510 (*htpR*$^+$ *rpoD800*) (□) and CAG481 (*htpR*$^-$ *rpoD800*) (○) were labeled for 10 min with [^{3}H]leucine and [^{3}H]lysine during exponential growth at 30°C. After addition of an excess (>200 μg/ml) of nonradioactive leucine and lysine, cultures were shifted to 42°C and sampled periodically. The samples were analyzed for their content of σ subunit on two-dimensional gels as described (16, 21). The initial time point (t = 0) was taken 2 min after addition of chase and is in the range of 2000–4000 dpm in σ subunit. (*B*) Degradation of mutant β-galactosidase (X90). Strains CAG603 (*htpR*$^+$/F'*lacZX90*) (□) and CAG604 (*htpR*$^-$/F'*lacZX90*) (○) growing exponentially at 30°C in M9 glycerol medium were induced with isopropyl β-D-thiogalactopyranoside and shifted to 42°C 10 min after induction. At 20 min after transfer to 42°C, cells were labeled with [^{35}S]methionine for 1.5 min, chased with an excess (>200 μg/ml) of nonradioactive methionine, and sampled periodically. Samples were immunoprecipitated and analyzed for their content of β-galactosidase. The initial time point (t = 0) was taken 2 min after addition of chase and represents 5000 dpm in β-galactosidase.

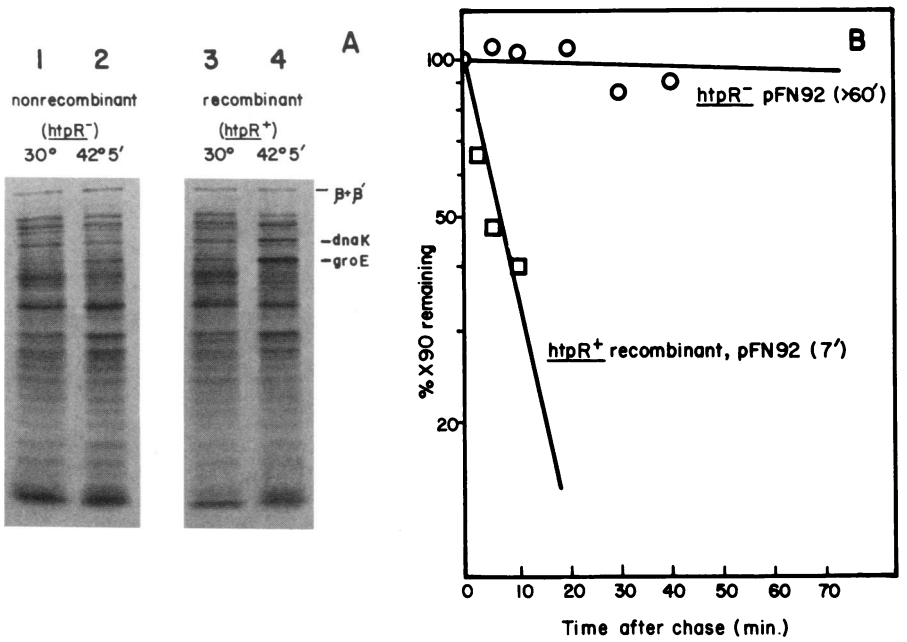

FIG. 2. Heat shock protein synthesis and protein degradation in strains recombinant (*htpR*$^+$) and nonrecombinant (*htpR*$^-$) for the *htpR* allele. The experiments described here were performed on strain CAG5107 (*htpR*$^-$/F′*lacZX90* pFN92) (○) and strain CAG5110 (*hptR*$^+$/F′*lacX90* pFN92) (□). The *htpR*$^+$ strain (CAG5110) was derived from the *htpR*$^-$ strain (CAG5107) by a recombination event between pFN92 and the *htpR*$^-$ allele in the chromosome generating an *htpR*$^+$ recombinant exhibiting ts$^+$ growth at 42°C (See Table 1). (*A*) Restoration of the heat shock response. Strains CAG5107 (lanes 1 and 2) and CAG5110 (lanes 3 and 4) were pulse-labeled at 30°C (lanes 1 and 3) or 5 min after transfer to 42°C (lanes 2 and 4) with [^{35}S]methionine for 2 min and chased with nonradioactive methionine for 1 min. Samples were analyzed for the heat shock response by electrophoresis on $NaDodSO_4$/polyacrylamide gels. The slight heat shock response in the nonrecombinant strain (CAG5107) was indistinguishable from that in the *htpR*$^-$ strain CAG456 and that observed for *htpR*$^-$ strains by other workers (10, 28). The heat shock response in the recombinant strain (CAG5110) was indistinguishable from that in the *htpR*$^+$ strain SC122. (*B*) Degradation of mutant β-galactosidase (X90). Strains CAG5107 (○) and CAG5110 (□) growing exponentially at 30°C in M9 glycerol medium were induced with isopropyl β-D-thiogalactopyranoside and shifted to 42°C 10 min after induction. At 20 min after transfer to 42°C, cells were labeled with [^{35}S]methionine for 1.5 min, chased with an excess of nonradioactive methionine, and sampled periodically. Samples were analyzed on one-dimensional gels for their content of β-galactosidase as a fraction of β + β′ subunits. The initial time point (t = 0) was taken 2 min after addition of chase and represents 5000 dpm in β-galactosidase. In this experiment, the uninduced control represented 20% of the radioactivity in the band for β-galactosidase and was subtracted from each point.

rpoD800 (Fig. 3*A*) along with the ability to carry out synthesis of heat shock proteins (data not shown). Thus, increasing the level of suppression simultaneously restored both a normal heat shock response and proteolysis.

These experiments show that inefficient suppression of the *htpR*$^-$ amber mutation is sufficient to cause the proteolysis defect both at low temperatures under steady-state growth conditions as well as after shift to high temperature.

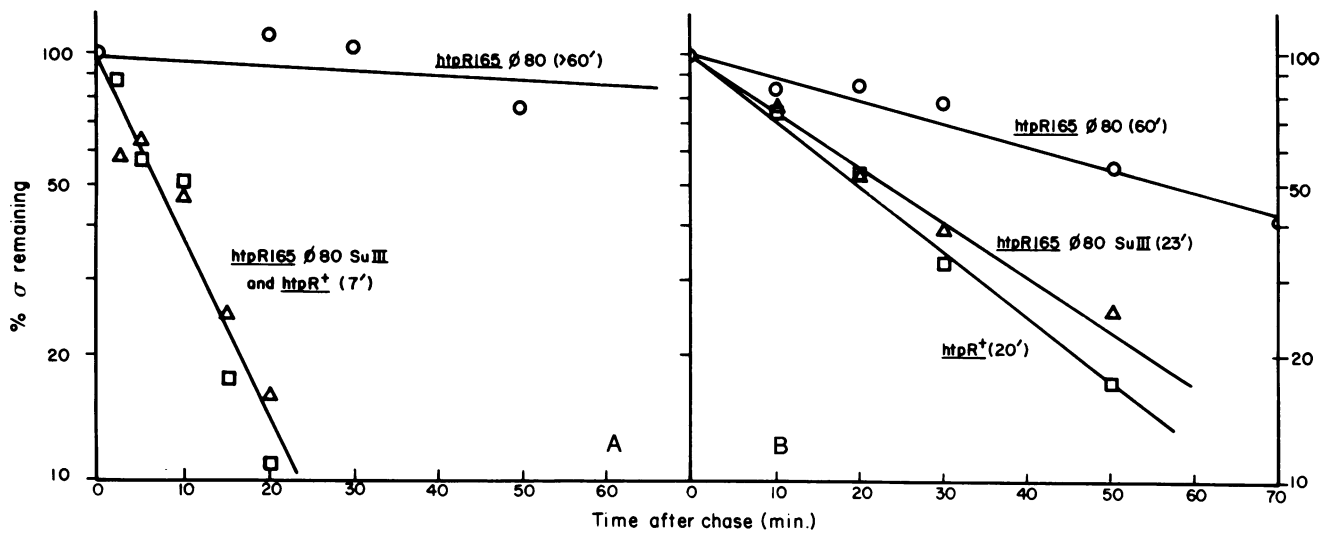

FIG. 3. Degradation of mutant σ subunit (encoded by the *rpoD800* allele) in *htpR*$^-$ strains with and without the *supF* suppressor (SuIII). Degradation of mutant σ subunit was measured in three strains: strain CAG510 (*htpR*$^+$, *rpoD800*) (□), strain CAG483 (*htpR*$^-$, ϕ80pSuIII, *rpoD800*) (△), and strain CAG482 (*htpR*$^-$, ϕ80, *rpoD800*) (○). CAG482 is a control to demonstrate that genes in ϕ80 do not affect the proteolysis phenotype of *htpR*$^-$ cells. Cells growing exponentially at 30°C were labeled with [^{35}S]methionine for 10 min. After addition of an excess (1 mg/ml) of nonradioactive methionine, cultures were shifted to 42°C (*A*) or left at 30°C (*B*) and sampled periodically. The samples were analyzed for their content of σ subunit. The initial time point (t = 0) was taken 2 min after addition of chase and is in the range of 5000–10,000 dpm in σ subunit.

Table 2. Rate of synthesis of selected proteins in *htpR*$^+$ and *htpR*$^-$ strains at 30°C

	rpoD$^+$		*rpoD800*	
Protein	*htpR*$^+$	*htpR*$^-$	*htpR*$^+$	*htpR*$^-$
Lon	0.82	0.74	0.87	0.83
	0.67	0.65	0.81	0.78
F84.1	0.41	0.46	0.44	0.42
	0.44	0.45	0.63	0.45
β-subunit	5.3	5.5	8.7	6.3
	6.1	6.4	7.6	6.4
EF-Ts	1.32	1.18	1.09	1.22
	1.22	1.21	0.97	1.25

Duplicate determinations of the rate of synthesis of two heat shock proteins (Lon and F84.1) and two non-heat shock proteins (β-subunit of RNA polymerase and EF-Ts) in strains SC122, CAG456, CAG481, and CAG510 are presented. The data represent the ratio (^{3}H:^{35}S in protein X)/(^{3}H:^{35}S in total protein).

Increasing the efficiency of suppression of the *htpR165* amber allele restores proteolysis at 30°C and at 42°C. The simplest interpretation of these results is that inefficient suppression by the *supC*^{ts} suppressor leads to a decreased amount of the *htpR* gene product, which results in a cellular defect in proteolysis.

The Degradation Defect Is Not Caused by a *lon*$^-$ Phenotype. Both the *lacZX90* and the *rpoD800* polypeptides are substrates of the *lon* protease system (16–19). Since *lon* is regulated by HtpR (29), the degradation defect in *htpR165* strains could result from decreased synthesis of Lon protein. We quantitated the production of Lon protein using two-dimensional gels. The rate of synthesis of Lon protein in isogeneic *htpR*$^+$ and *htpR165* strains was indistinguishable (Table 2). Thus, the proteolysis defect of *htpR165* cells did not result from decreased expression of the *lon* gene.

Lon may have decreased activity in the *htpR165* strain. We examined two other phenotypes that have been associated with *lon*$^-$ strains. *lon*$^-$ cells are sensitive to radioimetic agents such as nitrofurantoin, and they have decreased plating efficiency in its presence (19). In addition, *lon*$^-$ cells are mucoid (19). A *lon100* derivative of the parental SC122 strain had both of these *lon*$^-$ phenotypes: it was mucoid and plated with an efficiency $\leq 2 \times 10^{-4}$ on LB plates containing 0.5 μg of nitrofurantoin per ml compared to LB plates without nitrofurantoin. In contrast, the *lon*$^+$ *htpR165* derivative had neither phenotype: it was not mucoid and showed equivalent plating efficiency on LB plates alone and on LB plates containing 0.5 μg of nitrofurantoin per ml. Thus, the *htpR165* mutant is not phenotypically *lon*$^-$.

DISCUSSION

The *htpR165* mutation stabilizes the two proteolytic substrates we have examined. Wild-type cells degrade *rpoD800*-encoded σ subunit and the X90 fragment of β-galactosidase with a $t_{1/2}$ of 5–7 min. In contrast, the half-lives of these proteins in the *htpR165* strain are >60 min. This degradation rate is indistinguishable from that measured for the stable wild-type proteins (data not shown). Thus, these mutant proteins are stabilized at least 10- to 15-fold in *htpR*$^-$ cells. *htpR165* cells are not defective in the degradation of all unstable proteins: the λcII gene product is rapidly degraded in wild-type strains (30, 31), and the *htpR165* mutation does not affect its rate of degradation (Y. Ho and M. Rosenberg, personal communication).

The data about *htpR*$^-$ cells that we present raise questions about the relationship of the two *htpR* phenotypes and about the relationship between *htpR* and *lon*. We consider possible relationships below.

The *htpR165* Defect in Proteolysis and in the Heat Shock Response. The fact that *htpR*$^-$ cells are deficient both in the heat shock response and in proteolysis is intriguing and led us to consider whether the two phenotypes could be related. Both phenotypes of *htpR*$^-$ cells could be independent effects of the limiting amount of the *htpR* gene product found in the mutant cells. For example, if HtpR were a positive activator of both heat shock and proteolysis genes, then the low concentration of HtpR could result in inefficient induction of these genes. Alternatively, the *htpR*$^-$ mutation could change cellular characteristics, so that both proteolysis and the ability to sense stress conditions are impaired.

In another class of models, the two phenotypes of *htpR*$^-$ cells are causally related. A defect in expression of heat shock genes could cause the proteolytic defect. Three heat shock proteins—GroEL, GroES, and Lon—are known to be involved in protein processing or degradation (13, 32–34). It is easy to imagine that increased capacity for proteolysis may be advantageous under a variety of stress conditions. Thus, the deficiency of the *htpR*$^-$ mutant in proteolysis may indicate a major function of heat shock proteins. Although this is an attractive notion, we have not detected any effect of the *htpR165* mutation on the rate of synthesis of heat shock proteins (Lon, GroEL, DnaK, F84.1, C62.5, Sigma) at 30°C, while the deficiency in proteolysis is evident at this temperature. However, we cannot rule out the possibility that expression of some less abundant proteins is altered at 30°C in the *htpR* mutant.

Alternatively, the defect in proteolysis could prevent the heat shock response. HtpR itself, or a protein under HtpR control, could be a protease. In response to heat or other forms of stress, this protease would increase transcription of heat shock genes by either cleaving a repressor or processing an activator. Decreased expression of this protease in the *htpR*$^-$ cells would result in the inability of mutant cells to undergo the heat shock response. The SOS response provides a precedent for proteolysis regulating transient changes in gene expression (reviewed in ref. 35). Although Lon is under *htpR* control (29) and involved in proteolysis, there is no indication that Lon is a regulator of the heat shock response. Synthesis of Lon is normal at 30°C in the *htpR165* mutant. In addition, the *lon100* mutation has no apparent effect on the heat shock response (unpublished observations).

Relationships Between *htpR* and *lon*$^-$ Phenotypes. Both of the mutant proteins we describe (*rpoD800*-encoded σ subunit and the X90 fragment of β-galactosidase) are partially stabilized by mutations in *lon* and thus are considered substrates of the Lon proteolytic system (16–19). *lon*$^+$ cells degrade these substrates 2- to 4-fold faster than *lon*$^-$ cells (16–19). A *lon100* derivative of our parental strain likewise partially stabilizes mutant σ subunit ($t_{1/2}$, 25 min vs. 6 min for the wild type; data not shown). Neither substrate was significantly degraded in *htpR*$^-$ cells, although these cells are genotypically *lon*$^+$ and synthesize Lon protein at the normal rate. The mutant proteins are actually more stable in the *htpR165* strain than in the *lon*$^-$ strain. Thus, Lon-dependent proteolysis of these two substrates is HtpR-dependent as well. We consider three explanations:

(*i*) *htpR*$^-$ cells are altered so that Lon protein is inactive. They are phenotypically equivalent to a "null" mutation in *lon*. This explanation is inconsistent with the absence of other *lon*$^-$-associated phenotypes in *htpR*$^-$ cells. *lon*$^-$ derivatives of SC122 are mucoid and radiation sensitive. The isogeneic *lon*$^+$ *htpR*$^-$ strain is neither mucoid nor radiation sensitive (see above and ref. 36). The radiation sensitivity of *lon*$^-$ mutants is thought to be related to their inability to degrade the cell-division inhibitor SulA (37). The fact that *htpR*$^-$ cells are radiation resistant implies that Lon is active in this strain.

(*ii*) There are two pathways for degradation of mutant σ subunit and mutant β-galactosidase, both of which are HtpR dependent and only one of which is Lon dependent. SulA is degraded by a different HtpR-independent pathway.

(*iii*) There is one HtpR-dependent pathway for degradation of mutant σ subunit and mutant β-galactosidase. *lon*$^-$ mutations partially inhibit this pathway. SulA is degraded by a different HtpR-independent pathway.

Our data do not permit us to choose between the latter two alternatives; nor do they imply that HtpR is a direct effector of this process.

The development of *in vitro* degradation systems is a useful approach to study proteolysis. Recently, X90 (38) and SulA (39) degradation have been examined *in vitro*. Neither study found a direct role for Lon. However, these reports did not establish that the previously described *in vitro* proteolytic activities of Lon (40) would be observable under the conditions of the study. Therefore, the question of Lon involvement in initial stages of proteolysis has not yet been definitely addressed. Clearly, further work will be required to untangle the relationships between the various protease systems in *E. coli*.

Note Added in Proof. After this work was completed, the *htpR* (*rpoH*) gene product was purified and shown to be a σ factor (σ^{32}), which promotes transcription initiation from heat shock promoters (41). Based on these findings, we believe that the proteolysis defect in the *htpR165* mutant is due to altered gene expression caused by decreased amounts of σ^{32}.

We thank F. C. Neidhardt, R. Van Bogelen, E. T. Lau, and M. Rosenberg for communicating unpublished information and sharing their plasmids with us. We thank J. Ross, B. Sugden, S. Barclay, E. Craig, M. Rosenberg, C. Mann, J. Flynn, and all the members of our laboratory for their comments on this manuscript.

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