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Biochemistry

Heat shock regulatory gene *htpR* influences rates of protein degradation and expression of the *lon* gene in *Escherichia coli*

(abnormal proteins/protease La/cellular stress/bacterial proteases/ATP-dependent proteases)

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ABSTRACT **Upon a shift to high temperature, *Escherichia coli* increase their rate of protein degradation and also the expression of a set of "heat shock" genes. Nonsense mutants of *htpR* (also called *hin*), suppressed by a temperature-sensitive suppressor, show lower expression of heat shock genes at 30°C and fail to respond to a shift to 42°C. These mutants were found to have a lower capacity to degrade abnormal or incomplete proteins than that of wild-type cells. This reduction in proteolysis equals or exceeds that in *lon* mutants, which encode a defective ATP-dependent protease, protease La, and is particularly large in *htpR lon* double mutants. The activity of protease La was higher in wild-type cells than in *htpR* mutants grown at 30°C and increased upon shift to 42°C only in the wild type. To determine whether *htpR* influences transcription of the *lon* gene, a *lon-lacZ* operon fusion was utilized. Introduction of the *htpR* mutation reduced transcription from the *lon* promoter at 30°C and 37°C. This defect was corrected by a plasmid (pFN97) carrying the wild-type *htpR* allele. Induction of the heat shock response with ethanol had little or no effect in *htpR* mutants but stimulated *lon* transcription 2–3 fold in wild-type cells and *htpR* cells carrying pFN97. Thus, *lon* appears to be a heat shock gene, and increased synthesis of protease La under stressful conditions may help to prevent the accumulation of damaged cellular protein.**

In *Escherichia coli*, as in other cells, proteins with abnormal structures are rapidly degraded (1–3). Such polypeptides may arise as a result of biosynthetic errors, mutations, or postsynthetic damage. Upon exposure of cells to high temperatures, a number of proteins may become unfolded or partially denatured. In *E. coli* above 40°C, the breakdown of normally stable proteins rises significantly (4, 5), and many temperature-sensitive proteins are rapidly degraded (6). Under these conditions, protein breakdown may assume a particularly important role in preventing the accumulation of denatured polypeptides.

A variety of evidence suggests that an initial step in intracellular protein breakdown (6, 7) is catalyzed by the ATP-stimulated protease, protease La. This enzyme is encoded by the *lon* gene (8–10), and the *lon* mutants studied thus far contain a partially active protease (8, 11, 12) and have a 50–80% reduction in their ability to degrade abnormal proteins (6–8, 13). On the other hand, in strains containing a multicopy *lon* plasmid, the level of protease La is increased 2- to 4-fold, and the degradation of abnormal proteins is 3- to 5-fold faster (unpublished data). Protease La also may play a crucial role in the degradation of certain short-lived normal proteins (14, 15), and stabilization of such proteins in *lon* cells may account for the various phenotypes characteristic of these strains, such as their greater sensitivity to UV irradiation (15) and excessive production of capsular mucopolysaccharides (16).

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Since rates of protein breakdown rise with increased temperature (4, 5), we have tested for a relationship between intracellular rates of protein breakdown, the levels of protease La, and the ability of cells to undergo the "heat shock response." Upon shifts to high temperature, *E. coli* (17–19), like eukaryotic cells (19), transiently increase the synthesis of a limited number of constitutive polypeptides, generally referred to as "heat shock proteins." Strains that carry the amber mutation *htpR* (also called *hin*) and a temperature-sensitive suppressor fail to show this response (18, 20, 21) but will undergo a heat shock response if transformed with a plasmid carrying the wild-type *htpR* allele (22). The product of the *htpR* locus is postulated to act as a positive regulator of the heat shock genes (21–24). At 30°C, where the *htpR* mutation is partially suppressed, these cells show reduced basal levels of the heat shock proteins (21, 23). Increased expression of the heat shock genes occurs not only during a shift to high temperatures but also in various adverse conditions, such as exposure to ethanol or oxidants (24–27). This response probably serves an important protective role, since *htpR* mutants lose viability during such stresses (20, 23, 26).

The present studies have attempted to define systematically the influence of the *htpR* locus on the cells' ability to degrade abnormal proteins, on their content of protease La, and on the rates of expression of the *lon* gene under various conditions. To examine whether the *htpR* gene product influences transcription of the *lon* gene, we have utilized an operon fusion between the *lon* and *lacZ* genes. Independently, Baker *et al.* (28) have found in *htpR* cells a marked decrease in the degradation of a nonsense mutant of β-galactosidase (X-90) and of a missense mutant of the σ subunit of RNA polymerase (RPO D800). In addition, Phillips *et al.* (29) have shown that one of the proteins induced during the heat-shock response corresponds to the *lon* gene product. The present studies demonstrate that the *htpR* mutation decreases the cells' capacity to degrade various abnormal proteins, apparently by reducing transcription of the *lon* gene.

MATERIALS AND METHODS

E. coli strains GW1000 (30) [*recA441*, *sulA11*, Δ(*lac*)*U169*, *thr-1*, *leu-6*, *his-4*, *argE3*, *ilv*(ts), *galK2*, *rpsL31*] and its *htpR* derivative, GW4701 [*htpR*(am), *malPQ*::Tn*5*], and SC122 [F⁻ *lac*(am), *trp*(am), *pho*(am), *mal*(am), *rpsL*, *supC*(ts)], and its derivative, K165-37Rb (21) [*htpR*(am)], were kindly provided by Graham Walker (Massachusetts Institute of Technology). GW4701 was derived from GW1000 by P1 transduction of *htpR*(am) from K165-37Rb (21). Our unpublished studies indicate that strains GW1000 and GW4701 also carry an unidentified amber suppressor. Plasmid pFN97 carrying the wild-type *htpR* allele (22) was kindly provided by Frederick Niedhardt (University of Michigan). Bacteria were grown at 30°C with shaking in glucose minimal media, either M9 (31) or potassium 4-morpholinepropane sulfonate (Mops) (32). Because of frequent reversion from the temperature-sensitive phenotype, these cultures were routinely monitored for their inability to plate at 42°C.

Measurement of Protein Breakdown. The degradation of abnormal proteins was assayed as described (8, 33) in cells growing on M9 medium containing glucose and the essential amino acids (60 μg/ml). To induce the synthesis of incomplete polypeptides, these cells were exposed to puromycin (300 μg/ml) for 15 min during exponential growth, and [^{3}H]phenylalanine (1 μCi/ml; 20 Ci/mm, ICN; 1 Ci = 37 GBq) was added for 5 min. To follow the breakdown of complete polypeptides containing amino acid analogs, we centrifuged the cells and suspended them in media containing the arginine analog canavanine (125 μg/ml) for 15 min prior to the addition of [^{3}H]phenylalanine for 5 min. The cells then were washed twice and resuspended in a medium containing a large excess of nonradioactive phenylalanine (500 μg/ml). The degradation of the radioactive canavanine-containing or puromycin-containing proteins was estimated by following the appearance of free [^{3}H]phenylalanine. The extent of protein breakdown is expressed as the percentage of ^{3}H-labeled protein present at time zero that was converted to a form soluble in trichloroacetic acid (33).

Construction of *tsx*::Tn*10* and Linked *lon* Mutants. In order to transduce wild-type and mutant *lon* alleles with a drug-resistant phenotype, Tn*10* was transposed into the adjacent *tsx* locus (34), which determines resistance to infection by phage T6. This was accomplished by using a defective λ bacteriophage (λNK55, *cI857 b221 029*am cIII::Tn*10*), which requires nonsense suppression to replicate and which harbors the transposon Tn*10* in the *c*III gene. Tetracycline-resistant cells were selected after infection with this phage to insure that transposition of Tn*10* had taken place, and the colonies were further selected for resistance to infection with T6 phage (*tsx*). The resultant isolates were tested for linkage between *lon* and Tn*10* by cotransduction. To construct strains with *lon* mutations (*lon100* and *CapR9*) linked to a selectible phenotype, we used this *tsx*::Tn*10* for P1-mediated transduction of the tetracycline resistance and screened for crossovers between the various *lon* mutants and *tsx*.

Construction of *lon-lac* Operon Fusion. An operon fusion between the *lon* and *lacZ* genes was constructed *in vitro* by standard recombinant DNA techniques. The *lon*-containing plasmid, pJMC40 (35), and an operon fusion vector, pMLB1022 (36), were isolated and digested with the restriction enzymes *Eco*RI and *Bam*HI (New England Biolabs). The activity of these enzymes was then destroyed by heating to 70°C for 4 min and by chloroform extraction, and the DNA fragments were precipitated with ethanol. At low concentrations, these fragments were ligated by using T4 DNA ligase (New England Biolabs). Calcium-treated cells with a *lac* deletion were transformed with the ligated material, and the cells were plated on broth plates containing ampicillin (25 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (100 μg/ml). Blue (*lac*$^+$) colonies were chosen and used for plasmid isolation. Several *lon-lac* operon fusion plasmids were isolated from the *Lac*$^+$ transformants, and the structure of the fusion was verified by restriction enzyme cleavage followed by 1% agarose gel electrophoresis. This plasmid was designated pSG1012.

To integrate this fusion into the host chromosome, it was placed on the bacteriophage λNF1955 (37). Phage λ DNA was isolated and digested with *Eco*RI. This digest was ligated to pSG1012 DNA cut with *Eco*RI, and the ligated mixture was packaged into λ particles by an *in vitro* packaging system (38). Phage λ-sensitive cells with *lac* deletions were infected and plated on rich plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. Blue plaques were isolated, and the presence of the *lon-lac* operon fusion was verified by restriction analysis of the purified λ DNA. In order to form stable lysogens, the fusion-carrying phage was crossed with wild-type phage λ, and the resulting blue plaques were screened for turbidity on a *lac* host that did not carry an amber suppressor. This fusion-containing phage is designated λSG101. SC122 and K165 were transduced to *mal*$^+$ in order to lysogenize the strains with λSG101.

Enzyme Assays. The amount of the ATP-dependent protease, protease La, was assayed as described by Waxman and Goldberg (39). Isogenic *htpR* and wild-type strains were grown at 30°C. After sonication, equal amounts of cell protein were applied to phosphocellulose columns at 4°C, and the bound material was fractionated by a salt gradient. The enzyme was dialyzed against assay buffer (10 mM Tris, pH 7.5/0.1 mM NaEDTA/1 mM 2-mercaptoethanol/20% glycerol). Protease La was assayed with the specific fluorometric substrate, glutaryl-L-alanyl-L-alanyl-L-phenylalanine-H-methoxynapthylamine (Enzyme Systems Products, Livermore, CA) in the presence or absence of ATP (1 mM). The levels of β-galactosidase in strains carrying *lon-lacZ* or *cps-lacZ* operon fusions were determined by cleavage of *o*-nitrophenyl-β-D-galactopyranoside (Sigma) (31).

RESULTS

Protein Degradation in *htpR* Cells. Incomplete proteins containing puromycin are degraded rapidly in the wild-type cells (1, 2, 33), and this process was found to occur more slowly by a factor of 3–5 in *htpR* strains at 30°C (Fig. 1). At this temperature the nonsense mutation in *htpR* is partially suppressed (24). We focused on rates of protein breakdown and *lon* expression at 30°C because, at high temperatures (e.g., at 42°C), the viability of the *htpR* mutants decreases with time and interpretation of results becomes complicated. Since a lower rate of proteolysis was observed in *htpR* mutants at 30°C, where *htpR* and wild-type strains grow at similar rates, this defect cannot be a secondary consequence of their lower viability or failure to undergo the heat shock response. Instead, this defect appears to result from the decrease in functional *htpR* gene product.

The *htpR* mutants also degraded complete proteins containing the arginine analog canavanine more slowly by a factor of 3–4 than did the parental strains (Fig. 2) at 30°C. Thus, these bacteria appear to have a general reduction in their capacity to degrade abnormal proteins. This process requires ATP (2, 3, 33) and is reduced in *lon* mutants (8, 13). The defect in degradation of puromycyl fragments (Fig. 1) and canavanine-containing proteins (Fig. 2) in the *htpR* bacteria was roughly equivalent to or greater than that observed in strains carrying *lon* alleles *R9* or *100*, both of which produce a defective ATP-dependent protease (8, 11, 12). Therefore, we constructed strains carrying both *htpR* and these *lon* mutations to determine if these defects in proteolysis were additive. As shown in Fig. 2, the double mutants degraded analog-containing proteins much more slowly than did the wild-type strain and more slowly by a factor of 2–3

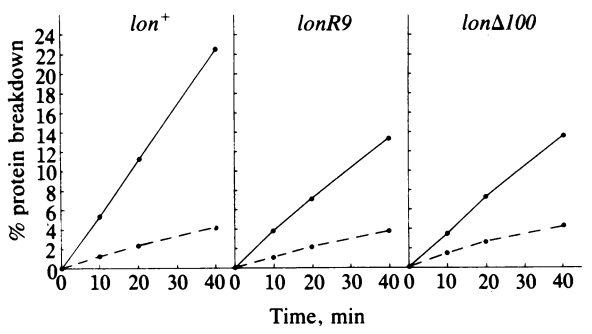

FIG. 1. The effect of *htpR* and *lon* mutations on the degradation of puromycyl polypeptides in *E. coli* SC122 (*htpR*$^+$) (—) and K165 (*htpR*) (---). During exponential growth, the cells were exposed at OD_{600} = 0.4 to puromycin to induce the production of incomplete polypeptides, and protein degradation was measured as described. Similar data were obtained in three different experiments.

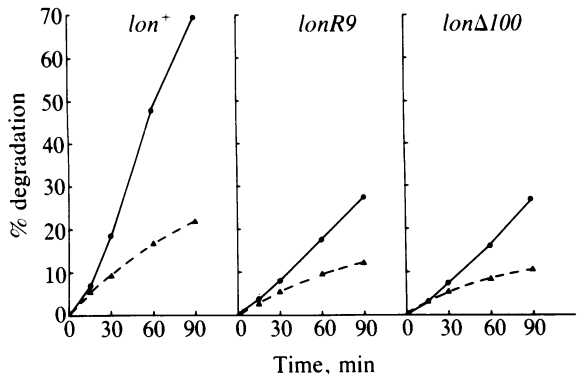

FIG. 2. The effects of *htpR* and *lon* mutations on the degradation of canavanine-containing proteins at 30°C in *E. coli* GW1000 (*htpR*$^+$) (—) and GW4701 (*htpR*$^-$) (---). During exponential growth, these arginine auxotrophs were resuspended in medium containing canavanine for 20 min to cause the production of abnormal canavanine-containing polypeptides. In parallel studies, no clear difference was observed in the ability of these strains to degrade normal cell proteins (containing arginine), which were hydrolyzed at ≈1–2% per hr. Similar data were obtained in three different experiments.

than the bacteria carrying either mutation alone. Similar effects were obtained when *htpR* was combined with the *lon* allele *R9* or *100*. The magnitude of these effects varied with the protein substrate and the genetic background. For example, in strain SC122, the degradation of puromycyl fragments is reduced by 80–90% by the *htpR* mutation, and introduction of the *lon* mutation did not significantly decrease rates of proteolysis further, unlike the findings with analog-containing proteins.

Influence of *htpR* on Levels of Protease La. This ATP-dependent protease, the *lon* gene product, can be rapidly isolated by using phosphocellulose chromatography, and Waxman and Goldberg (39) have recently described a specific assay using the fluorogenic substrate glutaryl-L-alanyl-L-alanyl-L-phenylalanine-4-methylnaphthylamine in the presence of ATP. As shown in Table 1, protease La was present in extracts of *htpR* but at levels that were 40–50% lower than in wild-type cells grown in parallel at 30°C. A similar reduction in protease content was observed repeatedly in these strains, with no apparent alteration in the ATP stimulation or temperature stability of the enzyme. By contrast, the protease La in *lon* strains carrying the *lonR9* or *lon100* alleles is completely inactivated by this isolation method (8, 9, 11).

Upon shift to 42°C, wild-type cells express the heat shock proteins at high rates for a short period and then continue to express them at an intermediate rate (17). Upon incubation of wild-type cells for 1 hr at 42°C, there was a 60–125% increase in the total content of protease La in the wild type. This increase in protease content was prevented by the addition of chloramphenicol to block protein synthesis (data not shown). In the *htpR* mutants, protease levels did not increase significantly after a shift to 42°C. Thus, the *htpR* mutation seemed to decrease the level of protease La at 30°C and to prevent its increase in response to heat shock.

Influence of *htpR* on Transcription of the *lon* Gene. Additional experiments used strains carrying a *lon-lacZ* operon fusion to test whether this reduction in protease La content (Table 2) is a consequence of decreased transcription of the *lon* gene. As shown in Table 3, the *htpR* cells expressed this gene less (i.e., showed less β-galactosidase activity) than did the parent during growth at 30°C. Similar effects of *htpR* were observed in wild type and *lon* backgrounds. When plasmid pFN97, which contains a wild-type *htpR* allele, was introduced into the *htpR* strain K165, the transcription of the *lon-lacZ* operon fusion was restored to wild-type levels (data not shown). At 30°C the *htpR* amber mutation is partially suppressed in strain K165; at 37°C, this suppressor, *supC*(ts), is less effective (24). Accordingly, in *htpR* mutants, unlike in the wild type, expression of the *lon-lacZ* fusion was less at 37°C than at 30°C.

We also attempted to show by this approach that the *lon* gene is induced by shifts to 42°C, but the enzyme encoded by the *lon-lacZ* operon fusion is unstable at this temperature (data not shown); therefore, the experimental data are uninterpretable. In *E. coli*, as in eukaryotes, the heat shock response can be induced by other noxious stimuli, such as exposure to ethanol (4%). When cells containing the *lon-lacZ* operon fusion were exposed to ethanol during growth at 30°C (Fig. 3), the amount of β-galactosidase activity increased 2-fold in the wild-type cells, but little or no increase was observed in the *htpR* strain. In *htpR* mutants carrying the wild-type allele on the plasmid pFN97, exposure to ethanol stimulated transcription of *lon* as in the wild-type cells (data not shown). Furthermore, exposure of cells to ethanol for 1 hr increased the total cellular content of protease La in a similar fashion as did exposure to a temperature shift (Table 1). Together, these various observations provide further evidence

Table 1. Influence of *htpR* mutation on intracellular levels of protease La during growth (30°C) and after a shift to 42°C or exposure to ethanol

Strains	Protease La, units		
	30°C	42°C	% increase
K165 (*htpR*$^-$)	1.2	1.4	—
SC122 (*htpR*$^+$)	2.1	3.9	87
SC122 (*htpR*$^+$)	1.9	4.1	122
+ Ethanol (4%) at 30°C	3.3	—	73

Wild type and *htpR* strains were grown on Luria broth at 30°C to an OD_{600} of 0.5. One-half was incubated at 30°C, and the other at 42°C for 1 hr (before cell lysis was evident). Protease La was partially purified from equal amounts of cell protein by phosphocellulose chromatography (36). The amount of protease La was determined by its ability to cleave a fluorometric substrate as discribed in the presence of 1 mM ATP. In the absence of added ATP, hydrolysis of the fluorometric substrate was 10–15% of the values shown. Each unit of protease La corresponds to the hydrolysis of 30 nM of the substrate at 37°C in 1 hr. These data are averages of results obtained in three different experiments, each of which gave similar results. The response to ethanol was determined after a 1-hr exposure of the cells to 4% EtOH. These data are the averages of two independent experiments, each of which gave similar results.

Table 2. Transcription from the *lon* promoter evaluated with a *lon::lacZ* operon fusion during growth at 30°C or 37°C

Strain	Genotype	β-Galactosidase units	
		30°C	37°C
GW1000	Wild type	845 (n = 3)	—
GW4701	*htpR*	498 (n = 3)	—
SC122	Wild type	540 (n = 13)	602 (n = 4)
K165	*htpR*	276 (n = 13)	172 (n = 4)

Isogenic wild-type and *htpR* strains were lysogenized with bacterial phage λ containing a *lon-lac* operon fusion, constructed as described. These strains were grown to an OD_{600} of ≈0.3 in Mops minimal medium at 30°C or 37°C. The level of β-galactosidase produced by the operon fusion was assayed by the method of Miller (31). At 37°C, suppression of the *htpR* mutation is less effective than at 30°C (23). Each value is the average of n determinations on individual cultures grown from a single colony. The difference in *htpR* and wild type at 30°C is highly significant ($P < 0.01$) as is the decrease seen in strain K165 between 30°C and 37°C ($P < 0.01$), as evaluated by the paired Student t test. When plasmid pFN97, which contains a wild-type *htpR* allele, was introduced into K165, the level of β-galactosidase was similar to that in SC122 (data not shown).

Table 3. Comparison of the phenotypic effects of *hptR* and *lon* mutations

Mutations	Phenotypic effects	
	Wild type	*htpR*(am)
	Mucoidy	
Wild type	−	±
lonR9	+ + + +	+
lon100	+ + + +	+
	UV sensitivity, % survival	
Wild type	62	88
lon100	22	18

Wild type, *lon*, *htpR*, and double mutants were plated on minimal and rich agar medium at 30°C and scored visually for the presence of a mucoid phenotype. Nonmucoid control cells were scored −, while highly mucoid *lon* cells were scored + + + +. Sensitivity to ultraviolet irradiation was measured after aerobic growth of the cells to an OD_{600} of 0.3 in Mops minimal medium at 30°C. Cells were exposed to UV for 30 sec at a distance of 45 cm and plated after serial dilution. The ability to form colonies relative to unexposed cells was used as a measure of cell survival. In two experiments, *htpR* mutants did not exhibit greater sensitivity to UV than did the wild type, unlike *lon* or *htpR lon* cells.

that *lon* is one of the heat shock genes (29), whose transcription is under positive control by the *htpR* gene product (21, 23, 24).

Comparison of Phenotypes of *htpR* and *lon* Mutants. Although *htpR* mutants resemble *lon* strains in their decreased capacity for protein breakdown (Figs. 1 and 2), they do not exhibit the mucoid phenotype (16) that is obvious upon visual inspection of *lon* colonies (Table 1). The *htpR* colonies by contrast resemble those of wild type. Surprisingly the *htpR lon* double mutants showed less mucoidy than does the *lon* parent. In *lon* strains (40), transcription of *cps* (capsular polysaccharide synthesis) genes rises many fold, and this effect appears responsible for the enhanced capsular polysaccharide production (16). To explore this unexpected ability of the *htpR* mutation to reduce mucoidy, we have utilized *lon* strains that contain a *cps-lacZ* operon fusion, kindly provided by Stephen Garrett (Frederick Cancer Research Laboratories). In the *lon* strains, expression of the *cps-lacZ* fusion is markedly increased,* but introduction of the *htpR* mutation reversed this effect of *lon*. Although the mechanism of this unexpected *htpR* effect is unclear, this decrease in *cps* expression can account for the ability of *htpR* to prevent mucoidy in *lon* strains.

Another characteristic of *lon* bacteria is their greater sensitivity to UV irradiation and DNA damaging agents (15, 26, 40). This effect has been explained by the reduced capacity of *lon* strains to degrade an inhibitor of septation (15), the *sulA* gene product, which is induced in the SOS response (40). Unlike *lon* strains, the *htpR* strain did not appear more sensitive to UV irradiation than did the wild type (Table 3). The UV sensitivity of double mutants resembles that of *lon* strains. Thus, the *htpR* and *lon* phenotypes are quite distinct, although both show reduced capacity for proteolysis (Figs. 1 and 2).

DISCUSSION

The present findings (Figs. 1 and 2) and the observations of Baker *et al.* (28) demonstrate a marked defect in the ability of *htpR* cells to degrade incomplete or unfolded polypeptides. This reduced capacity to eliminate abnormal proteins is evident even at the permissive temperature of 30°C and, therefore, is not simply a secondary consequence of a failure of *htpR* mutants to undergo a heat shock response. The most likely explanation of this defect is the lower content of protease La in the *htpR* strains (Table 1), although it is possible that, in addition, the content of other proteases also may be reduced.

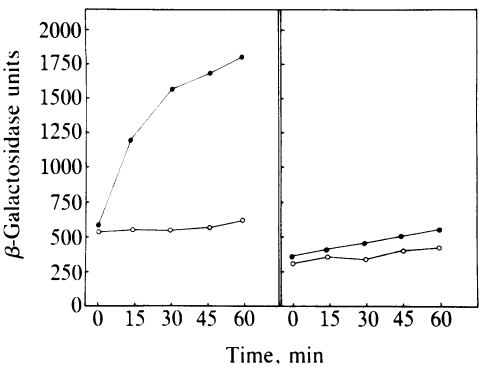

FIG. 3. Induction of the *lon-lacZ* operon fusion by ethanol. Wild-type (SC122) (*Left*) and *htpR* (K165) (*Right*) cells containing the operon fusion were grown at 30°C in Mops minimal medium to an OD_{600} of 0.3. Ethanol (4%) was added to one half of the culture (●), while the other served as a control (○). Similar data were obtained in four different experiments. When plasmid pFN97, which contains a wild-type *htpR* allele was introduced into strain K165, these cells responded to ethanol treatment in a similar fashion to the wild-type SC122 (data not shown).

In vivo protease La appears to catalyze an initial ATP-requiring step in the degradation of abnormal proteins (7, 9, 11), and a variety of recent evidence indicates that small changes in its content can have a marked effect on the ability of the cells to degrade abnormal proteins. For example, in *lonR9* and *lon100*, this activity is present in approximately half the normal level (8), and the degradation of abnormal polypeptides is reduced by a factor of 2–4 (refs. 6, 8, and 13 and Figs. 1 and 2). Similarly, at 30°C the *htpR* mutation lowered the cells' content of protease La (Table 1) and the transcription of the *lon* gene (Table 2) by about half, and caused a 60–80% reduction in the capacity of the cell to hydrolyze abnormal proteins. On the other hand, in *lon* plasmid-containing strains in which protease La activity is 2–4 times normal levels, the breakdown of such proteins is increased 3- to 5-fold (unpublished data).

Since the *htpR* gene product appears necessary for both basal and increased transcription of the heat shock genes (18, 21), the simplest explanation of the present findings is that this protein serves as a positive regulator of the transcription of the *lon* gene and that protease La is therefore one of the heat shock proteins (7, 18, 29). The molecular weight of its subunit, 94,000 (8–10), in fact corresponds to one of the *htpR*-dependent polypeptides, and, using two-dimensional gel electrophoresis, Phillips *et al.* (29) have observed induction of this polypeptide after a temperature shift from 28°C to 42°C. In accord with this conclusion, the cellular content of protease La rises with increased temperatures but not in *htpR* strains (Table 1). Furthermore, induction of the heat shock response at 30°C by exposure to ethanol promoted transcription from the *lon* promoter in wild-type or *htpR* cells with pFN97, but not in *htpR* strains (Fig. 3). Thus, by all criteria examined, this protease is a heat shock protein.

The *htpR* mutants show a defect in degradation of abnormal polypeptides that is similar to or greater than that seen in *lon* mutants. The protease La isolated from all *lon* strains examined thus far shows reduced activity and is quite labile. For example, the enzymes from *lonR9* (8, 9, 11) and *lon100* (unpublished results) are completely inactivated by chroma-

*Torres-Cabassa, A. S. & Gottesman, S., Annual Meeting of the American Society for Microbiology, March 8, 1984, St. Louis, MO, H91, p. 106 (abstr.).

tography on phosphocellulose. By contrast, the ATP-dependent protease in the *htpR* strains remains active after this treatment (Table 1), as would be expected for a regulatory mutation that affects protease content and not protease structure. Presumably, the additive effect of *lon* and *htpR* seen with analog-containing proteins results from the presence of a labile enzyme synthesized in low amounts.

Despite their marked defect in proteolysis, the *htpR* mutants are neither mucoid nor UV sensitive, unlike *lon* strains. The greater sensitivity of the *lon* mutants to DNA damage seems to result from their inability to degrade rapidly an inhibitor of septation induced during the SOS response (14, 15). Therefore, the lack of UV sensitivity of *htpR* strains would appear surprising. (Possibly the low amount of protease remaining in *htpR* strains can degrade the inhibitor of septation more effectively than can the labile protease in *lon* strains.) It is also noteworthy that *htpR* mutants, unlike *lon* strains, do not show excessive production of capsular polysaccharides or increased transcription of the *cps* genes. On the contrary, *htpR* blocks the appearance of mucoidy in *lon* strains (Table 3). It is difficult to explain such observations by simple models involving regulation of the *cps* genes by proteolysis. Finally, the *lon htpR* mutants grow almost as well as wild-type strains, despite their very low capacity for breakdown of abnormal proteins. Therefore, the *htpR* strains and *htpR lon* double mutants clearly should prove of appreciable value for the cloning of various rapidly degraded polypeptides.

Induction of protease La as part of the heat shock response would appear to be highly advantageous to the organism by helping to prevent a potentially dangerous accumulation of aberrant polypeptides. Rates of protein degradation rise in bacteria at high temperatures (4, 5), and under such conditions, a number of cellular proteins may be partially unfolded or even denatured. In eukaryotes (19, 41) and in *E. coli* (24–26), the heat shock response also can be elicited by various treatments that either cause the synthesis of abnormal proteins such as exposure to amino acid analogs, high temperature, and ethanol (25) or cause protein denaturation, such as increased temperature, oxidants (27), or ethanol. Under such adverse environmental conditions, increasing the cell content of protease La and thereby enhancing its capacity to eliminate abnormal polypeptides could serve an important protective function for the organism.

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1. Pine, M. J. (1967) *J. Bacteriol.* **93,** 1527–1533.
2. Goldberg, A. L. & St. John, A. C. (1976) *Annu. Rev. Biochem.* **45,** 747–803.
3. Hershko, A. & Ciechanover, A. (1982) *Annu. Rev. Biochem.* **51,** 335–364.
4. St. John, A. C. & Goldberg, A. L. (1978) *J. Biol. Chem.* **253,** 2705–2716.
5. Lin, S. & Zabin, I. (1972) *J. Biol. Chem.* **247,** 2205–2211.
6. Gottesman, S. & Zipser, D. (1978) *J. Bacteriol.* **133,** 844–851.
7. Kowit, J. D. & Goldberg, A. L. (1977) *J. Biol. Chem.* **252,** 8350–8357.
8. Chung, C. H. & Goldberg, A. L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4931–4935.
9. Charette, M. F., Henderson, G. W. & Markovitz, A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4728–4732.
10. Waxman, L. & Goldberg, A. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4883–4887.
11. Chung, C. H., Waxman, L. & Goldberg, A. L. (1983) *J. Biol. Chem.* **258,** 215–221.
12. Charette, M. F., Henderson, G. W., Kezdy, F. J. & Markovitz, A. (1982) *J. Mol. Biol.* **162,** 503–510.
13. Bukhari, A. I. & Zipser, D. (1973) *Nature (London) New Biol.* **243,** 238–241.
14. Gottesman, S., Gottesman, M., Shaws, J. E. & Pearson, M. L. (1982) *Cell* **24,** 565–573.
15. Mizusawa, S. & Gottesman, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 353–362.
16. Markovitz, A. (1979) in *Surface Carbohydrates of the Prokaryotic Cell*, ed. Sutherland, I. (Academic, New York), pp. 415–462.
17. Lemaux, P. G., Herendeen, S. L., Bloch, P. L. & Neidhardt, F. C. (1978) *Cell* **13,** 427–434.
18. Yamamori, T. & Yura, T. (1980) *J. Bacteriol.* **143,** 843–851.
19. Schlessinger, M. J., Ashburner, M. & Tissiers, A., eds. (1982) *Heat Shock: From Bacteria to Man* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Cooper, S. & Ruettinger, T. (1975) *Mol. Gen. Genet.* **139,** 167–176.
21. Neidhardt, F. C. & VanBogelen, R. A. (1981) *Biochem. Biophys. Res. Commun.* **100,** 894–900.
22. Neidhardt, F. C., VanBogelen, R. A. & Lau, E. T. (1983) *J. Bacteriol.* **153,** 597–603.
23. Yamamori, T. & Yura, T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 860–864.
24. Yamamori, T., Osawa, T., Tobe, T., Ito, K. & Yura, T. (1982) in *Heat Shock: From Bacteria to Man*, eds. Schlessinger, M. J., Ashburner, M. & Tissiers, A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 131–137.
25. Travers, A. A. & Mace, H. A. F. (1982) in *Heat Shock: From Bacteria to Man*, eds. Schlessinger, M. J., Ashburner, M. & Tissiers, A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 127–130.
26. Gottesman, S. & Neidhardt, F. C. (1983) in *Gene Function in Prokaryotes*, eds. Beckwith, J., Davies, J. & Gallant, J. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 163–183.
27. Lee, P. C., Bochner, B. R. & Ames, B. N. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7496–7500.
28. Baker, T. A., Grossman, A. D. & Gross, C. A. (1984) *Proc. Natl. Acad. Sci. USA*, in press.
29. Phillips, T. A., VanBogelen, R. A. & Neidhardt, F. C. (1984) *J. Bacteriol.* **159,** 283–287.
30. Kenyon, C. J. & Walker, G. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2819–2823.
31. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 431.
32. Neidhardt, F. C., Block, P. L. & Smith, D. F. (1974) *J. Bacteriol.* **119,** 736–747.
33. Goldberg, A. L. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 422–426.
34. Bachmann, B. J. (1983) *Microbiol. Rev.* **47,** 180–230.
35. Zehnbauer, B. A. & Markovitz, A. (1980) *J. Bacteriol.* **143,** 852–863.
36. Silhavy, T. J., Berman, M. L. & Enquist, L. W. (1984) *Experiments with Gene Fusions* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
37. Hui, I., Maltman, K., Little, R., Hastrup, S., Johnsen, M., Fiil, N. & Dennis, P. (1982) *J. Bacteriol.* **152,** 1022–1032.
38. Hohn, B. & Hohn, T. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2372–2376.
39. Waxman, L. & Goldberg, A. L. (1985) *J. Biol. Chem.*, in press.
40. Walker, G. (1984) *Microbiol. Rev.* **48,** 60–93.
41. Ashburner, M. & Bonner, J. J. (1979) *Cell* **17,** 241–254.