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Increased ATP-Dependent Proteolytic Activity in Lon-Deficient *Escherichia coli* Strains Lacking the DnaK Protein

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Extracts made from *Escherichia coli* null *dnaK* strains contained elevated levels of ATP-dependent proteolytic activity compared with levels in extracts made from *dnaK*$^+$ strains. This ATP-dependent proteolytic activity was not due to Lon, Clp, or Alp-associated protease. Comparison of the levels of ATP-dependent proteolytic activity present in *lon rpoH dnaK* mutants and in *lon rpoH dnaK*$^+$ mutants showed that the level of ATP-dependent proteolytic activity was elevated in the *lon rpoH dnaK* mutant strain. These findings suggest that DnaK negatively regulates a new ATP-dependent proteolytic activity, independently of σ^{32}. Other results indicate that an ATP-dependent proteolytic activity was increased in a *lon alp* strain after heat shock. It is not yet known whether the same protease is associated with the increased ATP-dependent proteolytic activity in the *dnaK* mutants and in the heat-shocked *lon alp* strain.

Several ATP-dependent proteases have been identified in *Escherichia coli*. Lon protease degrades abnormally truncated or misfolded polypeptides (9, 32) as well as certain normal regulatory proteins (24, 37). Strains which lack Lon protease (22) cannot remove certain normally short-lived regulatory proteins from the cell, which results in the *lon* phenotypes of UV sensitivity (12, 21) and excess capsular polysaccharide synthesis (21). These *lon* phenotypes can be suppressed by overproduction of the product of the *alp* gene (38). Alp may be an ATP-dependent protease or a regulator of an ATP-dependent protease; in either case, the *alp*-associated protease seems able to degrade several Lon substrates in vivo (38). No other function has been found for the presumptive Alp protease.

A third ATP-dependent protease, Clp, is composed of an ATPase component, ClpA, and a proteolytic component, ClpP (13, 14). The function of the Clp protease is not known; in contrast to *lon* mutants, *clpA* mutants do not display any obvious phenotypes, and when assayed in vivo, they are not defective in the degradation of misfolded polypeptides (14).

ClpP protein and Lon protease appear to be heat shock proteins (8, 17, 29). Cells exposed to an upshift in growth temperature show a rapid, but brief, increase in the synthesis of a small set of heat shock proteins, after which the level of synthesis decreases to a new steady-state level (26). Initiation of transcription of most heat shock genes is directed by σ^{32}, the product of the *rpoH* gene (10), in association with RNA polymerase. Recently another RNA polymerase cofactor, σ^E, has been described (6). σ^E initiates transcription at P3 heat shock promoters (6). Transcription of the σ^{32}-independent *htrA* gene is under σ^E control (6, 18), as is transcription of the *rpoH* gene at 50°C (6).

Heat shock proteins in addition to Lon and Clp proteases seem to be involved in protein degradation (33). Various observations indicate that the DnaK protein can affect proteolysis. Mutants lacking a functional DnaK protein hyperdegrade the X90 nonsense fragment of β-galactosidase at 30°C; at 42°C the situation is reversed, and the X90 fragment is hypodegraded (33). A temperature-sensitive LacI protein is also rapidly degraded in the absence of DnaK protein (16). Furthermore, misfolded canavanyl proteins and truncated puromycyl polypeptides are degraded at a lower than normal rate in *dnaK* mutant strains (16, 33). DnaK protein appears to regulate the heat shock response by interacting with the σ^{32} protein (11, 36). Since the DnaK protein is a negative regulator of the heat shock response, *dnaK* mutants contain increased levels of heat shock proteins (35). Thus, DnaK-mediated effects on protein degradation may be secondary to changes in the levels of heat shock proteins.

We have found that an ATP-dependent proteolytic activity in *E. coli* cell extracts, not due to the Lon, Clp, or Alp protease, is elevated in extracts of null *dnaK* cells. This increase in proteolytic activity is not σ^{32} dependent.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in these experiments are described in Table 1. All cultures used to make cell extracts for enzyme assays were grown in glucose L broth, which contained (per liter) the following: tryptone, 10.0 g; yeast extract, 5.0 g; NaCl, 5.0 g; and glucose, 2 g.

Preparation of cell extracts for assays of proteolytic activity. Frozen cells were defrosted in an ice bath and diluted three times by weight with lysis buffer (20 mM Tris, pH 8.0, 20% glycerol, and 10 mM β-mercaptoethanol). Cells were lysed by passage through a French pressure cell at 20,000 lb/in^2 and by sonication three times for 15-s intervals. Extracts were centrifuged at 250,000 × *g* for 1.5 h. The supernatants were frozen in volumes of 200 μl. Protein concentration was determined by the dye-binding method of Bradford (2) supplied by Bio-Rad, with bovine serum albumin as the standard.

^{3}H-methylated casein preparation and assays for levels of ATP-dependent proteolytic activity. α-Casein (Sigma Co.) was radioactively labeled with [^{3}H]formaldehyde by the method of Rice and Means (31). The specific activity of the resulting ^{3}H-methylated casein was approximately 5 μCi mg^{-1}.

Extracts were defrosted in ice water and diluted in lysis buffer immediately prior to assay. Duplicate assays were carried out for each sample, which was composed of 17.2 μl

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TABLE 1. *E. coli* strains

Strain	Relevant genotype	Source and reference
AB1157	F⁻ *thr-1 ara-14 leu-6 proA2 lacY1 supE37 galK2 his-4 xyl-5 mtl-1 argE3 tsx-33 thi-1*	G. Walker
CAG9292	*rpoH120*::Kan(λ25 *rpoH*⁺)	C. Gross (39)
E103S	*metB*	Our collection
GW4813	AB1157 Δ*dnaK52*::Cm^r	G. Walker (26)
HK101	JK403 *clpA319*::ΔKan	This work
HK102	JK404 *clpA319*::ΔKan	This work
HK103	JK405 *alp-22*::ΔKan	This work
HK104	JK406 *alp-22*::ΔKan	This work
HK105	JK405 *rpoH120*::Kan	This work
HK106	JK406 *rpoH120*::Kan	This work
HK107	Cold-tolerant isolate of GW4813	Our collection
HK108	JK406 *dnaK*⁺	This work
HK109	HK108 *rpoH120*::Kan	This work
JK402	E103S *galE*	Our collection
JK403	JK402 *lon-146*::ΔTn*10*	Our collection (14)
JK404	JK403 Δ*dnaK52*::Cm^r	Our collection
JK405	AB1157 *lon-146*::ΔTn*10*	Our collection
JK406	HK107 *lon-146*::ΔTn*10*	Our collection
JK407	JK402 Δ*dnaK52*::Cm^r	Our collection
SG12045	*clpA319*::ΔKan	S. Gottesman (14)
SG21127	*alp-22*::ΔKan	S. Gottesman (37)

of ^{3}H-methylated casein (1 mg of casein per ml, diluted in 50 mM Tris HCl [pH 7.8 at 4°C]–10 mM $MgCl_2$ to equal 2.5 μg per assay) and 12.5 μl of cell extract. To one set of tubes, 1.6 μl of 60 mM ATP was added; no further addition was made to the second set of tubes. Reaction mixtures were incubated at 37°C for 1 h. Reactions were stopped by the addition of cold trichloroacetic acid at a final concentration of 12% in the presence of 3 mg of bovine serum albumin per ml as the carrier. After centrifugation for 10 min at 4°C, 25 μl of supernatant was added to 5 ml of Redi Value scintillation fluid (Beckman), and the level of radioactivity was determined with a scintillation counter.

Radioactive labeling of protein. Cultures were grown in M9 medium supplemented with 35.5 μg of threonine ml^{-1}, 39.5 μg of leucine ml^{-1}, 230 μg of proline ml^{-1}, 15.5 μg of histidine ml^{-1}, 126.5 μg of arginine ml^{-1}, 2 μg of biotin ml^{-1}, and 30 μg of thiamine ml^{-1}. Cultures of HK105 and HK106 labeled during logarithmic growth for protein level determinations were grown to stationary phase at 17°C, diluted to 3×10^7 cells ml^{-1}, and labeled for five generations at 17°C with 12.5 μCi of a ^{14}C-amino acid mixture (NEC-445 purchased from Dupont-NEN) per ml.

2-D gel electrophoresis. Radioactively labeled cells were processed and cell proteins were separated by two-dimensional (2-D) gel electrophoresis as described previously (17, 28). The heat shock proteins in our gels were located by comparing the locations of the heat shock proteins found in the published gels of Neidhardt et al. (25) and VanBogelen et al. (39) and the coordinates of protein spots in the reference gels of Phillips et al. (30) with the positions of the proteins in our 2-D gels.

Comparisons of the amounts of proteins. Protein spots to be quantitated were excised from 2-D gels of strains HK105 and HK106, dissolved in 0.5% H_2O_2 at 60°C for 48 h, and counted in a scintillation counter. The amounts of radioactivity in spots from HK106 were divided by the amounts of radioactivity in the comparable spots from the HK105 gels. The values thus obtained are the differential levels of proteins between strain HK105 and strain HK106 during logarithmic growth. The differential levels of the non-heat shock proteins C and S (30) and the heat shock protein GrpE (1) were determined in strains HK105 and HK106.

TABLE 2. Effect of a null *dnaK* mutation on the ATP-dependent degradation of ^{3}H-methylated casein in extracts of *E. coli* cells[a]

Strain	Relevant genotype	Amt (μg) of casein degraded[b]				*P* value[c]
		+ATP	−ATP	Net	SD	
JK402	*lon*⁺ *dnaK*⁺	10.54	8.73	1.81	0.787	0.0017
JK407	*lon*⁺ *dnaK*	11.50	8.86	2.64	0.794	
JK403	*lon dnaK*⁺	12.62	11.00	1.62	0.861	0.0001
JK404	*lon dnaK*	11.44	8.70	2.74	0.616	
HK101	*lon clpA dnaK*⁺	9.76	8.74	1.02	1.067	0.0039
HK102	*lon clpA dnaK*	8.78	6.93	1.85	1.075	
JK405	*lon alp*⁺ *dnaK*⁺	8.04	6.90	1.14	0.299	<0.00001
JK406	*lon alp*⁺ *dnaK*	11.40	6.66	4.74	0.729	
HK103	*lon alp dnaK*⁺	8.14	7.12	1.02	0.614	<0.00001
HK104	*lon alp dnaK*	11.77	6.83	4.94	1.094	

[a] All cultures, except those in *alp*-associated experiments, were grown at 30°C until the cells reached late log phase, at which point they were harvested by centrifugation for 30 min at 10,000 × *g*. Cell pellets were stored at −75°C. Cultures in *alp*-associated experiments were grown at 35°C.

[b] These data are averages of at least 10 results obtained from at least five separate experiments. Each assay, except for those associated with *alp*, was carried out with 94 μg of cell protein and 2.5 μg of ^{3}H-methylated casein in the presence or absence of 3 mM ATP. Assays related to *alp* were carried out with 78 μg of cell protein. Reaction mixtures were incubated at 37°C for 1 h. Values given are the amounts of casein degraded in 1 h by 1 mg of cell protein. The net value is the amount of casein degraded in the presence of ATP minus the amount of casein degraded in the absence of ATP. This protocol, except for the amount of cell protein added, was used for all assays of proteolytic activity described in this paper.

[c] The probability that the observed difference between the two samples occurred by chance.

RESULTS

E. coli cells lacking a functional DnaK protein hyperdegrade certain abnormal proteins in vivo (16, 33). The degradation of at least one such protein, the temperature-sensitive LacI protein, in a null *dnaK* mutant strain is ATP dependent (16). To obtain more information about the effects of DnaK protein on energy-dependent proteolysis, the degradation of methylated casein was studied in extracts of null *dnaK* and *dnaK*⁺ bacteria.

Comparisons of the levels of ATP-dependent degradation of ^{3}H-methylated casein, assayed by the conversion of trichloroacetic acid-precipitable to trichloroacetic acid-nonprecipitable radioactivity, in extracts of *dnaK*⁺ (JK402) and null *dnaK* (JK407) strains produced results shown in Table 2. The *dnaK* (JK407) mutant extracts degraded significantly more (about 1.4-fold; $P = 0.0017$) methylated casein than did the *dnaK*⁺ (JK402) extracts. Thus, certain ATP-dependent proteolytic activities, apparently elevated in *dnaK* cells, are detectable in vitro as well as in vivo.

Since Lon and Clp proteases can degrade methylated casein in the presence of ATP (5, 15, 34), we asked whether proteolytic activities other than Lon or Clp can cause the increased level of proteolysis in the *dnaK* strains. *E. coli* mutants lacking the Lon protease or Lon and the ClpA component of the Clp protease, in addition to their lack of the DnaK protein, were constructed by P1 phage transduc-

tion (23). Extracts were made from the resulting mutant strains, and the amounts of ATP-dependent hydrolysis of methylated casein by these extracts were compared. As shown in Table 2, the extracts of *dnaK* mutants, lacking Lon (JK404) or lacking Lon and ClpA (HK102), degraded significantly more (about 1.6-fold; $P < 0.004$) casein than the otherwise isogenic *dnaK*$^+$ extracts (JK403 and HK101). These results indicate that an ATP-dependent protease other than Lon or Clp is associated with the increased proteolytic activity in *dnaK* mutant extracts.

To determine whether the *alp*-associated protease was responsible for the increased degradation of methylated casein in the *dnaK* cell extracts, a null *alp* allele was transduced into both *lon dnaK* and *lon dnaK*$^+$ strains. These strains (HK104 and HK103, respectively) were grown at 35°C to reduce the level of mucoidy associated with their *lon* mutations. Other *lon* strains contained *galE* mutations to reduce mucoidy. Although HK104 and HK103 are not strictly isogenic in that HK104 contains an unknown mutation that permits it to grow below 20°C, our experiments indicate that this mutation does not affect levels of proteolysis in our assays (unpublished data). Extracts of these mutant strains were assayed for ATP-dependent degradation of casein. The results in Table 2 show that the addition of the *alp* mutation made essentially no difference; the *lon alp dnaK* (HK104) extract degraded fourfold more casein than did the *lon alp dnaK*$^+$ (HK103) extract. This result is similar to that observed with extracts of the *alp*$^+$ strains, JK405 and JK406. Thus, a protease other than Lon and Alp is associated with the increased ATP-dependent proteolytic activity present in *dnaK* mutant extracts.

The results presented above indicate that an energy-dependent proteolytic activity distinct from Lon, Clp, or Alp is elevated in extracts of *dnaK* mutants. Since overexpression of genes in *dnaK* mutants appears to be dependent on σ^{32}, we asked whether the increased level of proteolytic activity in *dnaK* mutant extracts is dependent on σ^{32}.

To eliminate σ^{32}-dependent gene expression, we introduced a null *rpoH* mutation (10) into various strains. Null *rpoH* mutants cannot grow above 20°C (40), and most *dnaK* mutants cannot grow below 20°C (reference 4 and our observations). A *dnaK* isolate (HK107) that we recovered from strain GW4813 (4) was able to grow at 17°C. The *dnaK* mutant phenotypes of temperature sensitivity for growth at high temperature (41°C) (27) and of the inability to support the growth of λ phage (7) were present in HK107 (data not shown). Also, HK107 remained chloramphenicol resistant, indicating that the Cm^r gene, inserted in place of the first 933 bp of the *dnaK* coding sequence (27), had not been lost. A null *lon* mutation was transduced by P1 phage (23) into HK107, as previously described (17), to produce strain JK406.

Strain JK406 was converted to *dnaK*$^+$ by conjugation with an F′ strain, KL723, that contains the episome F104. One of the first markers transferred by F104 is *thr*$^+$, followed by *dnaK*$^+$. Exconjugants were selected for *thr*$^+$, the ability to grow at 40°C (*dnaK*$^+$), and tetracycline resistance (*lon-146*::ΔTn*10*). The selected exconjugants were screened for chloramphenicol sensitivity. HK108, one of the exconjugants thus isolated, was able to support plaque formation by λ phage. Subsequently, strains JK406 and HK108 were both transduced by P1 carrying the *rpoH120*::Kan insertion mutation to produce the isogenic null *rpoH* strains HK106 (*lon rpoH dnaK*) and HK109 (*lon rpoH dnaK*$^+$). The resulting strains, HK106 and HK109, were unable to grow at 28°C.

Extracts of the *lon rpoH dnaK* strain (HK106) and of the *lon rpoH dnaK*$^+$ mutant strain (HK109) were compared to determine their relative levels of ATP-dependent degradation of methylated casein. The levels of casein degraded (Table 3) in extracts of strains lacking σ^{32} were significantly higher (2.2-fold; $P = 0.0002$) in the *dnaK* (HK106) extract than in the *dnaK*$^+$ (HK109) extract. Thus, an ATP-dependent proteolytic activity other than Lon is negatively regulated by DnaK, but expression of this activity seems not to be dependent upon σ^{32}.

TABLE 3. Effect of *rpoH* mutation on the ATP-dependent degradation of ^{3}H-methylated casein in extracts of *dnaK*$^+$ and *dnaK* cells[a]

Strain	Relevant genotype	Amt (μg) of casein degraded[b]				*P* value[c]
		+ATP	−ATP	Net	SD	
HK109	*lon rpoH dnaK*$^+$	5.52	4.59	0.92	0.883	0.0002
HK106	*lon rpoH dnaK*	8.15	6.12	2.03	0.927	

[a] Cultures were grown at 17°C to mid-log phase before being harvested.
[b] Assays were done with 72 μg of cell protein. Each result shown is an average of results from 20 assays from two separate experiments. For additional information, see Table 2, footnote *b*.
[c] The probability that the observed difference between the two samples occurred by chance.

The possibility that ClpP protein, associated with a protein(s) other than ClpA, is involved with the increased proteolytic activity described above had not been eliminated. The level of ClpP protein is increased about 1.5-fold in *dnaK* mutants compared with the level of ClpP in *dnaK*$^+$ strains (17); the level of ClpP is also elevated in cells containing overproduced σ^{32} protein (17). It was of interest, therefore, to determine whether the level of ClpP protein in *dnaK* mutants was elevated in the absence of σ^{32}.

To determine whether the elevation of the ClpP protein in *dnaK* mutants was σ^{32} dependent, we quantitated the amounts of ClpP protein in strain HK106 (*lon rpoH dnaK*) and in strain HK105 (*lon rpoH dnaK*$^+$) by analysis of 2-D gels. Representative areas of the fluorographs of the resulting gels are shown in Fig. 1. The ClpP protein and the heat shock protein GrpE (1) are visible in fluorographs of gels of the *rpoH* strains exposed to film for 13 days (Fig. 1), whereas these proteins are visible in fluorographs of gels of an *rpoH*$^+$ strain after a 6-day exposure (data not shown). Although present in decreased amounts, both ClpP and GrpE were synthesized in *rpoH* strains.

The level of ClpP was not increased in the *lon rpoH dnaK*

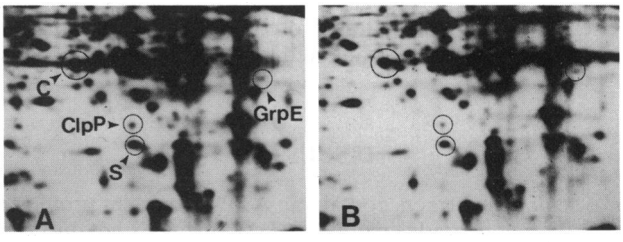

FIG. 1. 2-D gels of proteins synthesized by HK105 (*lon rpoH dnaK*$^+$) (A) and by HK106 (*lon rpoH dnaK*) (B). Cells were labeled with a mixture of ^{14}C-amino acids for five generations at 17°C. The heat shock proteins ClpP (17) and GrpE (1) and non-heat shock proteins C and S (29) are labeled in panel A. Only the regions of the gels corresponding to a pH gradient of 5.0 to 7.0 (in the horizontal dimension) and an M_r of 40,000 to 12,000 (in the vertical dimension) are shown.

TABLE 4. ATP-dependent degradation of ^{3}H-methylated casein in extracts of *lon dnaK*$^+$ *alp* HK103 mutants grown at 28 and 43°C[a]

Growth temp (°C)	Amt (μg) of casein degraded[b]				*P* value[c]
	+ATP	−ATP	Net	SD	
28	9.19	8.71	0.48	0.410	<0.00001
43	12.17	8.20	3.97	1.09	

[a] Cells were grown at 28°C to mid-log phase. Half of the culture was shifted to 43°C and grown for 1 h, and then both cultures were harvested.

[b] Each assay for proteolytic activity was done with 88 μg of cell protein. Each result is the average of results from 20 assays obtained from two separate experiments. See Table 2, footnote *b*, for additional information.

[c] The probability that the observed difference between the two samples occurred by chance.

strain (HK106) compared with that in the *lon rpoH dnaK*$^+$ strain (HK105); dividing the amount of radioactivity in ClpP excised from HK106 gels (average from six gels) by the amount of radioactivity from HK105 gels (average from seven gels) yielded a value of 0.95 (cells were labeled for five generations at 17°C during logarithmic growth). The heat shock protein GrpE and the non-heat shock proteins C and S are also present in similar amounts in strains HK105 and HK106, yielding values of 0.97, 0.83, and 0.99, respectively. It appears that the level of ClpP protein is not negatively regulated by DnaK in the absence of σ^{32} and, therefore, that ClpP is probably not associated with the increase in ATP-dependent proteolytic activity in the *lon rpoH dnaK* cell extracts.

DnaK protein negatively regulates the heat shock response so that *dnaK* mutants contain increased levels of heat shock proteins (35). Lon protease is a known heat shock protein. To determine whether ATP-dependent proteolytic activities other than Lon are affected by an increase in growth temperature, we used an *E. coli* strain that lacked the Lon protease and the *alp*-associated protease to produce cell extracts. It is not known whether the level of the *alp*-associated protease (38) is affected by heat shock. We compared the amount of methylated casein degraded by an extract of *lon alp* (HK103) cells grown at 28°C with the amount of casein degraded by an extract of similar cells that had been shifted from 28 to 43°C and had been grown at the higher temperature for 60 min. The results (Table 4) show that the level of ATP-dependent degradation of methylated casein is about eightfold higher in extracts of the cells that had been upshifted and grown for an hour at 43°C. Therefore, in addition to the DnaK-mediated effects on the level of ATP-dependent proteolytic activity described above, the level of an ATP-dependent proteolytic activity which is not related to Lon or Alp is affected by the heat shock response.

DISCUSSION

E. coli cell extracts contain an ATP-dependent proteolytic activity that degrades methylated casein and is not due to the known ATP-dependent proteases, Lon, Clp, and Alp. This ATP-dependent proteolytic activity is elevated in extracts of null *dnaK* mutants compared with activity in wild-type cell extracts. Furthermore, the ATP-dependent proteolytic activity is elevated in extracts of *lon rpoH dnaK* mutants compared with that in extracts of *lon rpoH dnaK*$^+$ mutants. Thus, a new ATP-dependent proteolytic activity exists in *E. coli*; it appears to be negatively regulated by DnaK, independently of σ^{32}.

Furthermore, an ATP-dependent proteolytic activity is increased in a *lon alp* mutant exposed to heat shock. The results presented do not prove that this ATP-dependent activity is the same as that which is increased in the *dnaK* mutant strains. Since Lon is the only ATP-dependent protease known to increase in association with heat shock, the increased levels of proteolysis after heat shock and in the *dnaK* mutants may, in fact, be associated with the same protease.

Three σ^{32}-independent heat shock proteins have been described: the sigma factor σ^{32} (6), the endopeptidase HtrA (6, 19), and the phage shock protein Psp (3). At 50°C, synthesis of the σ^{32} protein and of the HtrA protein is regulated by σ^E-directed RNA polymerase. The sigma factor involved in transcription of the gene responsible for the Psp protein after heat shock at 50°C has not been determined. HtrA is the only σ^{32}-independent heat shock protein known to have proteolytic activity (20). HtrA is an endopeptidase found in the bacterial cell envelope (18). Although HtrA degrades labeled casein in vitro, the reaction is ATP independent, and therefore, HtrA is probably not responsible for the ATP-dependent proteolytic activity in our casein degradation assays.

The degradation of certain proteins is increased in *dnaK* mutants. Straus et al. (33) showed that the X90 fragment of LacZ is hyperdegraded at 30°C in a *dnaK* mutant. Keller and Simon (16) found that LacI(Ts) is hyperdegraded in a *dnaK* mutant at 30 and 37°C. The increased degradation of LacI (Ts) seen in the *dnaK* mutant is present in cells lacking the Lon protease. Removal of energy from the cell, however, prevents degradation of the LacI(Ts) protein. Thus, an energy-dependent protease other than the Lon protease hyperdegrades the LacI(Ts) protein in the null *lon* null *dnaK* mutant. The proteolytic activity in extracts of *E. coli dnaK* cells hyperdegrades methylated casein, is energy dependent, and is not due to the Lon protease. The protease responsible for the hyperdegradation of LacI(Ts) in vivo, therefore, may also be responsible for the increased degradation of methylated casein observed in *dnaK* mutant extracts in vitro.

The level of the new ATP-dependent proteolytic activity increases in the absence of functional DnaK protein. DnaK negatively regulates the heat shock response, in part by affecting the synthesis (11) and the stability (36) of the σ^{32} protein. Various hypotheses could explain the presence of increased ATP-dependent proteolytic activity in *dnaK* and in *rpoH dnaK* mutant extracts; these hypotheses include the stabilization of the relevant protease, increased synthesis of the protease, or, perhaps less likely, decreased levels of an inhibitor of the protease.

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