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lon Gene Product of *Escherichia coli* Is a Heat-Shock Protein

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The product of the pleiotropic gene *lon* is a protein with protease activity and has been tentatively identified as protein H94.0 on the reference two-dimensional gel of *Escherichia coli* proteins. Purified Lon protease migrated with the prominent cellular protein H94.0 in *E. coli* K-12 strains. Peptide map patterns of Lon protease and H94.0 were identical. A mutant form of the protease had altered mobility during gel electrophoresis. An *E. coli* B/r strain that is known to be defective in Lon function contained no detectable H94.0 protein under normal growth conditions. Upon a shift to 42°C, however, the Lon protease was induced to high levels in K-12 strains and a small amount of protein became detectable at the H94.0 location in strain B/r. Heat induction of Lon protease was dependent on the normal allele of the regulatory gene, *htpR*, establishing *lon* as a member of the high-temperature-production regulon of *E. coli*.

Mutations in the *lon* gene, first described in 1964 (1, 17), produce several phenotypic changes, including overproduction of mucopolysaccharide (18, 24, 25), increased sensitivity to UV and ionizing radiation (1, 17), defective cell division (1, 17), reduced lysogenization of bacteriophages lambda and P1 (39, 42), and reduced protein degradation (13, 14, 20, 26, 36, 38). The *lon* gene has been cloned (37, 47), and its protein product (here called Lon) has been purified (7, 46) and tentatively identified on the reference two-dimensional gel of *Escherichia coli* proteins (for a preliminary report, see reference 32).

The native Lon protein, a tetramer of 94,000-dalton subunits (6, 7, 46), is an ATP hydrolysis-dependent protease (6, 22) that is also known as protease La (7) and has been shown to hydrolyze α-casein and globin in vitro (6, 7). Although the cellular function of Lon is not completely known, nonsense fragments, missense proteins, and lambda N protein have been shown to be more stable in *lon* mutants than in wild-type cells (13, 14, 20, 38). It is believed that the Lon protein may be the major protease catalyzing the rate-limiting endoproteolytic cleavage of damaged proteins in the cell (7, 22). In addition, Schoemaker et al. (36) have reported that an 11,000-dalton polypeptide is present in extracts of a *lon* mutant and absent in the isogenic *lon*$^+$ strain. The amount of this polypeptide is reduced in *lon* cell extracts treated with Lon protease. Mizusawa and Gottesman (26) have found that the stability of SulA protein, a proposed cell division inhibitor (11, 19), is ca. 15-fold greater in *lon* mutant cells than in wild-type cells. Lon is a DNA-binding protein, and this property has been used in purification procedures (5, 46).

The high-temperature (HTP) regulon of *E. coli* consists of 17 genes that are transiently induced to high rates of expression by a shift up in temperature (31; unpublished observations). These genes are subject to their own individual controls, but their coordinated response to temperature is dependent upon protein F33.4, the product of a regulatory gene, *htpR* (*hin*), located at 76 min on the *E. coli* genetic map (2, 30, 31, 44). Six of the heat-inducible genes have been identified as *dnaJ* (35, 45; C. Georgopoulos, personal communication), *dnaK* (12), *mopA* (*groEL*) (2, 29), *mopB* (*groES*) (2, 40), *lysU* (41), and *rpoD* (15). We report here the identification of *lon* as a seventh HTP gene.

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MATERIALS AND METHODS

Bacterial strains. The *E. coli htpR* mutant strain K165 and its *htpR*$^+$ parent strain, SC122, have been described previously (30). Strain W3110 (from Jonathan Beckwith, Harvard Medical School), was used as a K-12 strain to compare with strain NC3, a B/r strain which has been previously described (27).

Media and culture conditions. Cells were grown aerobically at selected temperatures (23) in defined morpholinopropanesulfonic acid medium (28) containing 0.4% glucose or supplemented with 19 amino acids (minus methionine); five vitamins, thiamine, calcium pantothenate, *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, and 2,3-dihydroxyl benzoic acid; and four bases, adenine, guanine, cytosine, and uracil, as previously described (43). Temperature shifts were accomplished by transferring (small-volume) cultures between water baths.

Radioactive labeling of cultures. For comigration with purified Lon protein, strain SC122 was grown in supplemented medium at 28°C to an optical density (420 nm) of 0.3 and then shifted to 50°C. A 2-ml sample was pulsed for 5 min with [^{35}S]methionine (1,244 Ci/mmol; 50 μCi/ml), starting 3 min after the temperature shift, and chased with 0.167 ml of 0.2 M methionine. For peptide maps, strain SC122 was grown at 28°C in supplemented medium to an optical density (420 nm) of 0.65, shifted to 50°C, and labeled for 10 min with [^{35}S]methionine (1,122 Ci/mmol; 200 μCi/ml). Strain W3110 was labeled in glucose minimal medium with $H_2{}^{35}SO_4$ as previously described (3) for comparison as a reference culture. For comparison of heat-shock proteins in strains NC3 and W3110, 2-ml cultures were grown in supplemented medium at 28°C to an optical density (420 nm) of 0.6 and labeled with [^{35}S]methionine (1,166 Ci/mmol; 50 μCi/ml) for 5 min, starting 3 min after a shift to 45°C. For measurement of relative synthesis rates of heat-shock proteins, 2-ml cultures of strains SC122 and K165 were pulse-labeled with [^{3}H]leucine (69 Ci/mmol; 300 μCi/ml) for 1 min, followed by a chase with a 50× excess of unlabeled leucine at indicated times before and after a temperature shift up. Each sample was harvested (4) and mixed with a reference culture of strain SC122 labeled for several generations at 28°C with [^{14}C]leucine (342 mCi/mmol; 25 μCi/ml).

Two-dimensional gels for visual observations and quantitative measurements. Culture samples to be resolved on O'Farrell two-dimensional gels (33) were processed as previously

described (4). For measuring relative synthesis rates, individual protein spots were cut from the gel and their radioactivity was determined as previously described (23).

Peptide maps. Purified Lon protein (20 μg) was first electrophoresed on two-dimensional gels, which were then stained and dried. The stained protein spots were cut from the gels. Spot H94.0 was cut from dried gels prepared from extracts of strain W3110 labeled with $H_2{}^{35}SO_4$ during exponential growth in glucose minimal medium at 37°C. Putative spot H94.0 was cut from gels prepared from extracts of strain SC122 labeled with [^{35}S]methionine for 10 min after a shift from 28 to 50°C. Peptide mapping of the proteins was carried out by the procedures of Cleveland et al. (8) with *Staphylococcus aureus* V8 protease.

Lon protease. Samples of purified Lon protease were generously supplied by A. Markovitz, University of Chicago, and A. L. Goldberg, Harvard Medical School.

RESULTS

Identification of Lon on two-dimensional gels. Lon (protease La) was identified on the reference two-dimensional gel of *E. coli* K-12 by comigration of the purified protein with $^{35}SO_4$-labeled cellular protein. The purified protein (all samples from both laboratories) migrated on the gels as multiple isoelectric species (usually four to six spots), a finding not uncommon in our experience (34). The two most basic spots migrated with the cellular proteins H94.0 (most basic, major spot) and H94.1 (minor spot). The results (not shown) were similar to those shown in Fig. 1. Purified mutant Lon protein (CapR9 protein from A. Markovitz) also migrated as multiple isoelectric species but was slightly more basic than the protein from the wild-type strain (data not shown).

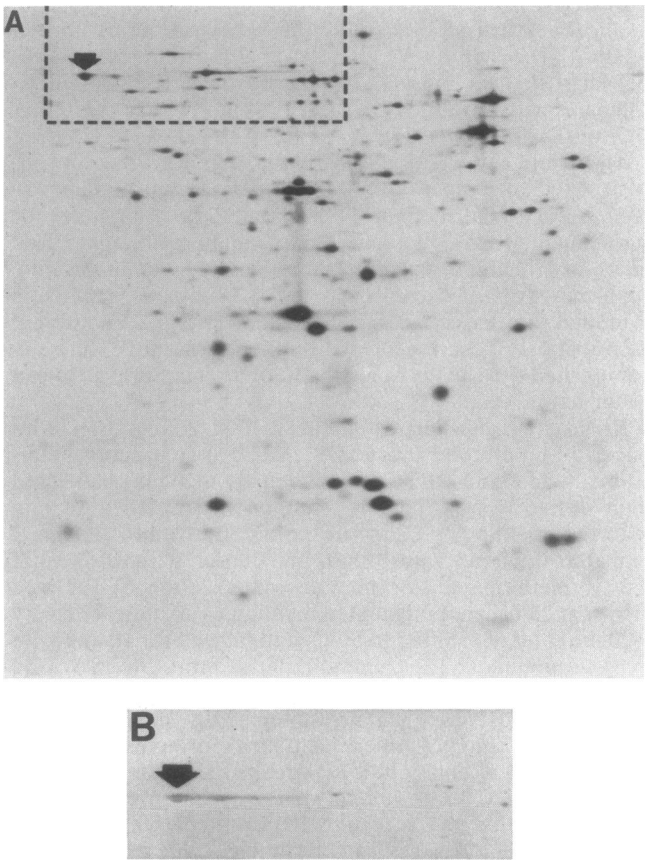

FIG. 1. Comigration of purifed Lon protease and an extract of heat-shocked *E. coli* K-12 strain SC122 on two-dimensional polyacrylamide gels. The first dimension was isoelectric focusing to equilibrium (7,400 V · h), 1.6% pH 5 to 7 and 0.4% pH 3 to 10 ampholine mixture; in the second dimension, an acrylamide concentration of 11.5% and 0.1% sodium dodecyl sulfate were used. (A) Autoradiogram of entire gel showing proteins labeled with [^{35}S]methionine after shift to 50°C. Spot H94.0 is indicated by the solid arrow. (B) Portion of stained gel corresponding to area enclosed by dashed lines in (A). The major stained spot of purified Lon protease (1 μg) coincides exactly with the radiolabeled H94.0 spot indicated in (A).

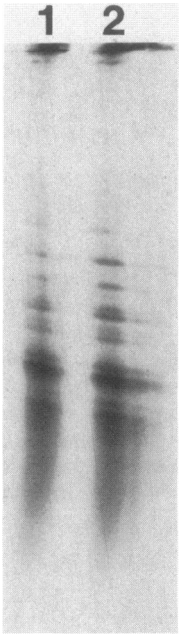

FIG. 2. Peptide band patterns of partial proteolytic digests of protein spot H94.0 from *E. coli* reference strain W3110 (lane 1) and heat-shocked strain SC122 (lane 2).

Behavior of the Lon spot upon shift up in temperature. When proteins from a culture of strain SC122 (*htpR*$^+$) that had been labeled after a shift from 28 to 42°C were resolved on a gel and compared with the proteins of a reference culture (steady-state growth at 37°C), the spot formerly referred to as G93.0 by Neidhardt et al. (31) appeared to be the same as H94.0, the major Lon spot. A check of laboratory records revealed that the original designation had been incorrectly recorded and should have been called H94.0 from its location. Purified Lon protein was therefore mixed with an extract of strain SC122 cells that had been heat shocked, and the mixture was resolved on a two-dimensional gel. An autoradiogram was made and compared with the stained gel (Fig. 1). The Lon protein was recognizable on the stained gel by the abundance of the added marker protein (Fig. 1B). It migrated perfectly with the HTP spot known previously as G93.0 and now more accurately as H94.0. The minor Lon spot, H94.1, was also heat induced. The stained protein spot completely filled the area occupied by the ^{35}S-labeled spot and rendered it slightly diffuse, indicating identity. (Compare the diffuse Lon spot in Fig. 1 with the undiluted spots in Fig. 3.)

Confirmation of Lon spot identity by peptide mapping. Although we have found comigration on two-dimensional gels to be an adequate criterion of protein identity (32), we also compared the peptide maps of purified Lon protein,

spot H94.0 from the reference strain W3110, and the heat-induced spot H94.0 from strain SC122. The radioactive peptide bands produced from the labeled cellular spots (shown on the autoradiogram of Fig. 2) were identical to each other and to the stained peptide bands from the purified protein (not shown because of difficulty in photographing the stained gel).

Absence of protein H94.0 from strain NC3 under normal conditions. Strain NC3 is a B/r strain of *E. coli* and, therefore, like most B and B/r strains, is a *lon* mutant (9, 10). We observed that strain NC3 cells under most growth conditions contain only a barely visible protein spot (not visible on the photograph of the autoradiogram in Fig. 3C) that migrates with purified Lon protein. We took advantage of this fact to confirm that Lon is a heat-shock protein. Strains W3110 (K-12) and NC3 (B/r) were each grown and labeled at 28°C, and then separate cultures were labeled for a brief period shortly after a shift to 45°C. Comparison of autoradiograms of gels at the two temperatures clearly showed the expected induced rate of synthesis of protein H94.0 in the heat-shocked K-12 strain W3110 (Fig. 3A and B). In contrast, B/r strain NC3 contained barely detectable labeled material in the H94.0 spot position at 28°C and only a very small amount after the temperature shift (Fig. 3C and D).

Time course of induction of Lon. Figure 4 shows the time course of induction of the protein products of *lon* and a typical heat shock gene, *dnaK* (B66.0), after shift of a culture of strain SC122 from 28 to 42°C. The induction of *lon* is indistinguishable from that of *dnaK*. Results are also shown for the *htpR* mutant strain K165. It is clear that the heat induction of each protein is dependent on the wild allele of *htpR*, and therefore *lon* must be a member of the HTP regulon.

DISCUSSION

There is reason to believe that the Lon (La) protease is one of the major endoproteolytic enzymes of *E. coli*, and it is presumed to initiate attack on cellular proteins that have been denatured or otherwise damaged or that are unnatural by virtue of being the products of mutant genes or of mistranslation (cf. 14, 22). (At least two properties of Lon, its binding to DNA and the requirement for ATP hydrolysis in its action, are interesting and not fully understood.) Induction of this enzyme by a shift to high temperature could therefore be interpreted as a means to increase the cellular capacity to degrade proteins damaged by heat or by mistranslation at high temperature. This idea has already been suggested for animal cells (16). How important this increase in activity might be for bacterial cell survival has not been evaluated.

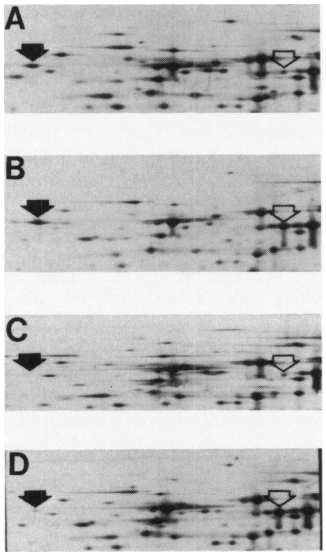

FIG. 3. Autoradiograms of portions of gels of *E. coli* K-12 strain W3110 and B/r strain NC3 labeled at 28°C (A and C) and after shift to 45°C (B and D). Area of gel shown corresponds to the area enclosed in the dashed lines in Fig. 1A. Solid arrows indicate the location of protein H94.0; open arrows indicate the location of another HTP protein, F84.1.

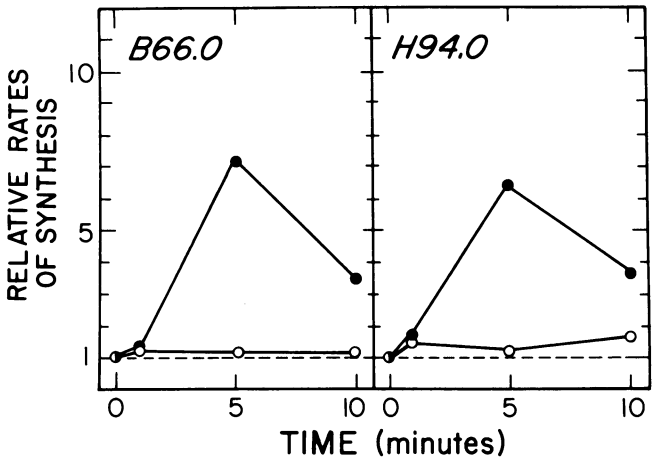

FIG. 4. Time course of synthesis of Lon protease (H94.0) and *dnaK* gene product (B66.0) after shift up in temperature in wild-type and mutant (*htpR*) strains. Symbols: ●, strain SC122 (*htpR*$^+$); ○, strain K165 (*htpR1*).

Identification of *lon* as a member of the HTP regulon has additional significance, for it establishes a potential link between two of the major stress response regulons of *E. coli*. The high levels of SulA protein made as part of the SOS response are believed to be responsible for inhibition of cell division and consequent filamentation of the cells. Recovery from the SOS state requires, among other things, the inactivation of SulA protein by the Lon protease, thus explaining why SOS induction in *lon* cells leads to lethal (irreversible) filamentation (cf. 26). It is not clear, however, why *lon* should be a prominent member of the HTP regulon. We have no evidence that SOS genes, including *sulA*, are induced by high temperature. Is the function of Lon at high temperature to rid the cell of excess SulA? Strain B/r grew well after a shift to high temperature, even though it was deficient in Lon; this fact is not helpful in deducing a function for Lon in heat shock, however, because strain B/r is probably a *sulB* mutant (9). Other *lon* mutants, however, have not been noted as being particularly temperature sensitive (1, 13, 14, 17, 18, 20, 26, 36, 38, 39, 42), whereas one would expect them to experience difficulty if a high level of active Lon is essential at elevated temperature. Therefore, unless these strains have some compensating mutation, it would appear that Lon function in heat shock is redundant whether it is concerned with SulA removal, degradation of damaged proteins, or some other process.

High doses (100 J/m^2) of UV irradiation have been reported to induce at least two of the heat-shock genes (21). If Lon is also induced by this treatment (and this has not been shown), the induction might serve to handle either an excess

of SulA or an increased amount of denatured protein. Nevertheless, a strain that is defective in the heat-shock response (*htpR*$^-$) does not appear to be extra sensitive to radiation (21).

Whatever the physiological significance that *lon* is a member of the HTP regulon, our findings that strain B has a minute amount of Lon and that heat shock increases its level provide an explanation of a long-standing observation about B strains of *E. coli*. It has been known since 1968 that the UV sensitivity of these strains is not as marked at 42°C as at lower temperature (10). Heat-induced synthesis of Lon, even at levels far below that of K-12 strains, could account for the increased radiation resistance.

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