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The product of the *lon* (*capR*) gene in *Escherichia coli* is the ATP-dependent protease, protease La

(proteolytic enzymes/energy requirement/intracellular protein degradation)

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ABSTRACT In *Escherichia coli*, degradation of abnormal proteins is an energy-requiring process; it is decreased in mutants in the *lon* (*capR* or *deg*) gene. We find that the protein encoded by the *lon* gene is an ATP-dependent protease and is identical to protease La, recently described in *E. coli*. Both proteins are serine proteases that hydrolyze casein and globin, but not insulin, in the presence of ATP and Mg^{2+}. Both respond to ATP, less well to other nucleoside triphosphates, and not to nonhydrolyzable ATP analogs. The purified *lon* protein has an apparent M_r of 450,000 and appears to be composed of four identical subunits. Its size, chromatographic behavior, and sensitivity to various inhibitors and heat are indistinguishable from those of protease La. Moreover, in a strain that carries additional copies of the lon^+ allele on a plasmid, the content of protease La, but not of other proteases, is 2- to 10-fold greater than in the lon^+ parent strain. Strains carrying the nonsense mutations *capR9* and $capR^-$ also contain this ATP-dependent proteolytic activity, but it is present in substantially lower amounts and is inactivated by phosphocellulose chromatography, unlike the wild-type enzyme. Degradation of abnormal proteins in these lon^- strains, which is slower than in the wild type, still requires ATP. Alterations in the ATP-dependent protease in the lon^- mutants can account for the defect in intracellular proteolysis and perhaps also for the other phenotypic effects of this pleiotropic gene.

Like eukaryotic cells, *Escherichia coli* has an efficient system for selectively degrading abnormal polypeptides (1, 2), such as may arise by nonsense (3–5) or missense mutations (6) or by incorporation of puromycin or amino acid analogs (7). One of the intriguing features of the breakdown of abnormal proteins (and also of normal proteins) in bacterial and mammalian cells is that this process requires metabolic energy (1, 8–11). Studies with metabolic inhibitors have demonstrated that ATP is required for an initial endoproteolytic step in the degradation of abnormal proteins in *E. coli* (5); in extracts, degradation of globin and of nonsense fragments of β-galactosidase can be stimulated 2- to 4-fold by ATP (12, 13). Swamy and Goldberg (10, 11, 14, 15) have demonstrated that *E. coli* contains eight distinct proteases, and one of these, named "protease La," is completely dependent on ATP and Mg^{2+} for activity. Recently, Larimore *et al.* (16) have shown that proteolysis by this enzyme requires concomitant hydrolysis of the ATP. Various observations further suggest that, *in vivo*, protease La catalyzes the rate-limiting, ATP-dependent steps for protein breakdown (14, 16).

Mutants with decreased ability to hydrolyze abnormal proteins have been isolated by Bukhari and Zipser (4) and were initially called *deg*. These mutations were located at 11.4 min on the *E. coli* genetic map and were shown to coincide with *lon* (also called *capR*) mutations (4, 16). All such mutations result in many other phenotypic alterations, including overproduction of capsular polysaccharides due to derepression of biosynthetic enzymes, defective cell division leading to filament formation, and increased sensitivity to UV light and to radiomimetic agents (18–22). It is not clear which of these effects is primary and how the decreased capacity for proteolysis is related to the other phenotypic effects of the *lon* mutation.

In vivo, the lon^- mutation was shown to cause a decrease in the same initial cleavages of nonsense polypeptides that are prevented by ATP depletion (5). The identification of a single ATP-dependent protease in *E. coli* (10, 11, 14, 15) raised the possibility that this enzyme might be the product of the *lon* gene or might be regulated by it. One argument against this possibility is that extracts of lon^- strains showed some ATP-stimulated proteolysis (12). Recently, the *lon* gene product was purified as a DNA-binding protein by Zehnbauer *et al.* (23) and shown to be a 94,000-dalton polypeptide by $NaDodSO_4$/polyacrylamide gel electrophoresis.

The present studies were therefore undertaken to test whether the purified *lon* gene product might be a protease, to clarify its possible relationship to protease La, and to examine the effects of lon^- mutations on this activity.

MATERIALS AND METHODS

The following *E. coli* K-12 strains were provided for these investigations by A. Markovitz: RGC121 which carries the wild-type lon^+ ($capR^+$) allele, RGC121/pJMC40 which carries the lon^+ ($capR^+$) allele both on the chromosome and on plasmid pSC101, RGC123 which is isogeneic with RGC121 but contains the lon^- (*capR9*) allele, and another lon^- ($capR^-$) strain (24). The *capR9* and $capR^-$ are both nonsense mutations and have identical lon^- phenotypes, but the *capR9* allele (unlike the $capR^-$) is dominant to the wild type when present on a plasmid (23).

Bacteria were grown to stationary phase in Luria broth at 37°C. For the strain carrying plasmid pJMC40, the medium was supplemented with tetracycline at 5 μg/ml (24) to prevent loss of the plasmid. For phosphocellulose chromatography (23), one-half of the harvested cells were washed, resuspended, and lysed in buffer P [0.1 M K phosphate, pH 6.5/10 mM 2-mercaptoethanol, 1 mM EDTA, 20% (vol/vol) glycerol]. Chromatography on phosphocellulose was carried out by the method of Zehnbauer *et al.* (23). For analysis on DEAE-cellulose columns the other half was suspended in buffer D (10 mM Tris·HCl, pH 7.8/5 mM $MgCl_2$). Preparation of cell-free extracts and chromatography on DEAE-cellulose were performed as described (14).

The purified lon^+ ($capR^+$) and lon^- (*capR9*) gene products were provided for these studies by M. F. Charette and A. Markovitz. These polypeptides had been purified as DNA-binding proteins and identified as 94,000-dalton polypeptides (23), which we confirmed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Partially purified protease La was kindly provided by F. S. Larimore. It was prepared as described by Larimore *et al.* (16) from frozen *E. coli* K-12 (Grain Processing, Muscatine, IA).

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α,β-Methylene-ATP, β,γ-methylene-ATP, and adenosine 5′-(*O*-3′-thiotriphosphate) were obtained from PL Biochemicals; DEAE-cellulose (DE-52) and phosphocellulose (P-11) were purchased from Whatman. ^{125}I-Labeled insulin (^{125}I-insulin) was obtained from Cambridge Nuclear Corp. [^{3}H]Methylcasein and [^{14}C]methylapohemoglobin were prepared as described (25).

Proteolytic activity against these substrates was assayed as described by Swamy and Goldberg (14). All assays were carried out in 25 mM Tris·HCl, pH 7.8/5 mM $MgCl_2$ in the presence and absence of 1 mM ATP. The incubations were for 0.5–1 hr at 37°C with either the purified *lon* gene product or partially purified protease La (16) in a final volume of 0.5 ml. The reaction was stopped by the addition of 60 μl of 100% (wt/vol) trichloroacetic acid and 40 μl of bovine serum albumin (30 mg/ml) as a carrier. The assay tubes were kept on ice for 30 min and, after centrifugation, the amount of acid-soluble radioactivity in 0.4 ml of the supernatant was assayed in 4 ml of Liquiscint (National Diagnostics, Parsippany, NJ). The acid-soluble products of ^{125}I-insulin hydrolysis were assayed in a Beckman gamma spectrometer.

The degradation of puromycin-containing polypeptides *in vivo* was measured as described by Goldberg (7). The various strains were grown at 37°C in M9 medium supplemented with 0.2% glucose, thiamine at 5 μg/ml, and leucine, tryptophan, proline, and adenine at 50 μg/ml.

RESULTS

In the presence of ATP and Mg^{2+} the *lon* gene product that had been purified as a DNA-binding protein hydrolyzed [^{3}H]casein and [^{14}C]globin to acid-soluble material but did not digest ^{125}I-insulin (Table 1, Exp. A). In a typical experiment using casein at 30 μg/ml and 5 mM $MgCl_2$, half-maximal proteolytic activity (K_m) was observed with 7.8 μM ATP. By contrast, no activity was evident with ATP or Mg^{2+} alone (data not shown). Because casein was hydrolyzed more rapidly, all further experiments were performed with this substrate. The pattern of substrates cleaved (Table 1, Exp. A) and the requirement for ATP and Mg^{2+} resemble earlier observations with the partially purified protease La (14, 16). The preparation of the *lon* protein used in these studies was approximately 90% pure 94,000-dalton polypeptide as analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (data not shown). Identical proteolytic activity was observed when the *lon* gene product was purified to homogeneity by an adaptation (15) of the procedure of Zehnbauer *et al.* (23) (unpublished observations).

Table 1. Proteolytic activity of the purified *lon* (*capR*) gene products

Substrate	μg substrate hydrolyzed/hr/mg enzyme: No ATP	With ATP
Exp. A: With *capR*$^{+}$ protein		
[^{3}H]Casein	2.2	712.0
[^{14}C]Globin	0.8	73.9
^{125}I-Insulin	0.9	1.1
Exp. B: With *capR9* protein		
[^{3}H]Casein	0.1	0.0
[^{14}C]Globin	0.0	0.0

The *capR*$^{+}$ protein, which was purified to near homogeneity, and the *capR9* gene product, which was partially purified on phosphocellulose, were both provided by M. F. Charette. Both proteins appeared to be 94,000-dalton polypeptides.

Table 2. Effects of different nucleotides and ATP analogs on casein hydrolysis by the *lon* gene product

Nucleotide or analog (1 mM)	Activity, %
ATP	100
ADP	3
AMP	0
GTP	14
UTP	65
CTP	74
Adenosine 5′-(*O*-3′-thiotriphosphate)	29
α,β-Methylene-ATP	18
β,γ-Methylene-ATP	0

Similar data were previously obtained with protease La by Larimore *et al.* (16).

Comparisons of Protease La and *lon* Gene Product. Larimore *et al.* (16) have recently shown that protease La requires cleavage of ATP for its proteolytic activity. Proteolysis was inhibited by sodium vanadate, a potent ATPase inhibitor (26), and the effect of ATP could not be reproduced with the nonmetabolizable ATP analog β,γ-methylene ATP or with the poorly metabolizable analogs α,β-methylene-ATP and adenosine 5′-(*O*-3′-thiotriphosphate). CTP and UTP stimulated protease La activity (16) but less effectively than ATP, and other nucleotides tested had little or no effect. Nearly identical results with these nucleotides and analogs were obtained for the *lon* gene product (Table 2), which thus also seems to require ATP cleavage for proteolytic activity.

Because of the similarities between the two ATP-dependent proteolytic activities, they were further compared with respect to sensitivity to various inhibitors, stability against heat, and molecular weight. Like protease La (16), the *lon* gene product was sensitive to diisopropyl fluorophosphate and therefore probably is a serine protease. N-Ethylmaleimide and antipain also inhibited the two activities to a similar extent (Table 3).

Larimore *et al.* found that protease La is rapidly inactivated at 42°C but that ATP could prevent this thermal inactivation (16). In the absence of ATP the activity of the *lon* gene protease decreased by about 80% upon preincubation at 42°C for 1 hr but, when ATP was added, no loss in activity occurred (Table 3).

Upon glycerol density gradient centrifugation, the *lon* gene

Table 3. Effects of inhibitors and temperature on the ATP-dependent proteolytic activity of the *lon* gene product and protease La

Additions	Activity, %: Protease La	*lon* protein
Exp. A		
Control	100	100
Diisopropyl fluorophosphate:		
1 mM	65	70
10 mM	5	9
N-Ethylmaleimide (5 mM)	35	37
Antipain (30 μg/ml)	66	70
Sodium vanadate (0.1 mM)	25	11
Exp. B		
Preincubation for 1 hr at 42°C:		
No ATP	21	14
With ATP	100	100

These data compare the purified *lon*$^{+}$(*capR*$^{+}$) gene product (23) and partially purified protease La (16). Neither preparation showed any proteolytic activity in the absence of ATP. The data on heat stability of protease La were taken from Larimore *et al.* (16).

product and protease La showed identical molecular weights of 450,000 (Fig. 1). This value was confirmed by gel filtration on a Sephacryl S-300 column from which both activities were eluted as sharp peaks (data not shown; see Table 4). Zehnbauer *et al.* (23) found that the *lon* gene product, purified as a DNA-binding protein, migrated as a 94,000-dalton protein on $NaDodSO_4$/polyacrylamide gel electrophoresis. Thus, the native *lon* gene product probably is composed of at least four, or at most five, identical 94,000-dalton subunits. Furthermore, our most purified preparations (15) of protease La contain a major band of 94,000 daltons as well as several contaminating polypeptides (data not shown). These similarities in size further argue that the *lon* gene product and protease La are identical.

Protease La Activity of lon^+ and lon^- Strains. To obtain clearer evidence on whether or not the *lon* gene encoded protease La, the levels of this enzyme were compared in a wild-type strain, a wild-type strain carrying additional copies of the lon^+ allele on a plasmid, and lon^- (*capR9* and $capR^-$) mutants. Cell-free extracts from these strains were prepared and fractionated by chromatography on DEAE-cellulose to separate protease La from the ATP-independent proteases (14, 15). The activity of the protease La peak was 2- to 10-fold greater (depending on the experiment) in the strain carrying additional copies of the lon^+ allele than in the wild-type parent (Fig. 2). Conversely, both lon^- mutants showed some ATP-dependent proteolytic activity eluting where protease La is found but in significantly lower amounts (approximately 50%) than in the wild-type strain (Fig. 2; Table 4). Similar results were found in three additional experiments. In contrast, the levels of the various ATP-independent proteolytic activities against casein were indistinguishable in all four strains (Fig. 2). Thus, by all criteria tested, the *lon* gene product is identical to protease La.

To confirm that the ATP-dependent proteolytic activity remaining in these mutants corresponds to protease La, the ATP-stimulated proteins obtained from the DEAE-cellulose column (Fig. 2) were pooled and compared with the wild-type enzyme (Table 4). On gel filtration with Sephacryl S-300, the activity from *capR9* and $capR^-$ showed the same size (450,000 daltons) as the wild-type protein. In addition, the enzymes from these two mutants lost most of their activity when incubated at 42°C for 1 hr but both could be completely stabilized by addition of ATP, as had been observed with wild-type enzyme (15) and purified *lon* gene product (Table 3).

In our initial studies, we observed that the *capR9* gene product purified on phosphocellulose did not show any ATP-dependent proteolytic activity (Table 1, Exp. B), in contrast to the results obtained with DEAE-cellulose (Fig. 2). A possible explanation for this apparent contradiction is that purification by phosphocellulose chromatography may selectively inactivate the mutant protease. Therefore, cell-free extracts were prepared and fractionated on phosphocellulose as described by Zehnbauer *et al.* (23). The wild-type protease could be isolated from this column (15) with greatly increased specific activity (Fig. 3). However, no ATP-dependent proteolytic activity was recovered after phosphocellulose chromatography of the *capR9* and $capR^-$ strains. Zehnbauer *et al.* (24) have shown that the 94,000-dalton *capR9* polypeptide could be eluted from a phosphocellulose column in a similar fashion as the $capR^+$ gene product. Therefore, the ATP-dependent proteolytic activity from the mutants appears to be more labile than that from the

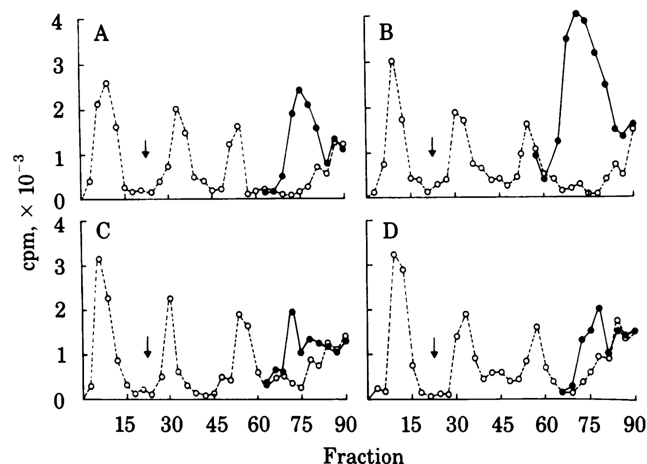

FIG. 2. Proteolytic activities in *E. coli* strains carrying different *lon* alleles fractionated by DEAE-cellulose chromatography. Cell-free extracts were prepared in buffer D from an *E. coli* strain carrying the lon^+ allele (*A*), a derivative carrying additional copies of the lon^+ allele on a plasmid (*B*), a derivative carrying a mutant lon^- allele, *capR9* (*C*), and another lon^- strain, $capR^-$ (*D*). After dialysis against the same buffer, the extracts (each containing 342 mg of protein) were adsorbed to DEAE-cellulose columns (1.5 × 18 cm) which were washed with two bed volumes of buffer D. The proteins were eluted with linear 0–0.25 M gradients of NaCl in buffer D in a total volume of 500 ml. Fractions (4 ml) were collected at a flow rate of 35 ml/hr and assayed against [^{3}H]casein in the presence (●) and absence (○) of ATP (1 mM). Arrows indicate the beginning of gradient. The peak of the proteolytic activity observed only in the presence of ATP eluted at the position of protease La (14–16). Proteolytic activity elsewhere in the gradient was not stimulated by ATP (14).

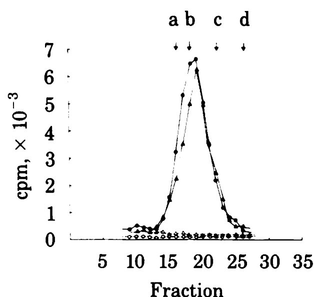

FIG. 1. Comparison of protease La and the *lon* gene product in glycerol density gradient centrifugation. The *lon* gene product (●, ○) and protease La (▲, △) preparations were applied onto 5 ml of 10–30% glycerol density gradients. These preparations contained 24 and 325 μg of protein, respectively. Centrifugation was then performed at 200,000 × *g* for 6 hr. Fractions (7 drops; 150 μl) were collected from the bottom and 50 μl of each fraction was assayed in the presence (●, ▲) and absence (○, △) of ATP. Arrows, peaks of marker proteins: a, thyroglobulin, 669,000; b, β-galactosidase, 467,000; c, catalase, 240,000; d, alkaline phosphatase, 82,000.

Table 4. Protease La activities in lon^+ and lon^- strains

	$capR^+$	*capR9*	$capR^-$
Total activity:			
Recovered from DEAE-cellulose, %*	100	47	51
Size on Sephacryl S-300, daltons	450,000	450,000	450,000
Activity after 1 hr at 42°C:†			
No ATP, %	27	39	31
With ATP, %	100	100	100

The data on enzyme recoveries were obtained from DEAE-cellulose chromatography as in Fig. 2. These results are the averages of four independent experiments that gave similar results. The measurements on size and heat stability were performed twice on material from the DEAE-cellulose columns. In each case, a single symmetric peak of ATP-dependent proteolytic activity was observed. The column was 1.0 × 27 cm and was eluted with buffer D containing 10% glycerol.

* Relative to wild type.

† Relative to activity present initially.

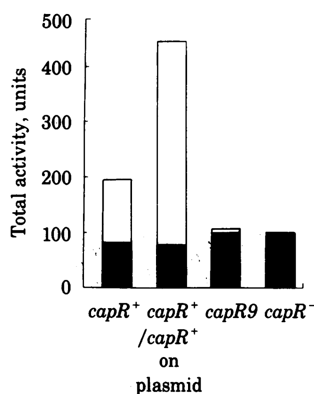

FIG. 3. Proteolytic activities in *E. coli* strains carrying different *lon* alleles, eluted from phosphocellulose columns. Cell-free extracts were prepared in buffer P from the same *E. coli* K-12 strains studied in Fig. 2. After dialysis against this buffer, the extracts (each containing 410 mg of protein) were adsorbed to phosphocellulose columns (1.5 × 18 cm) which were washed with two bed volumes of buffer P. The adsorbed proteins were eluted with buffer P containing 0.35 M phosphate. The nonadsorbed fractions did not contain ATP-stimulated proteolytic activity, but contained 20 times more ATP-independent activity (data not shown). The eluted proteins were assayed against [^{3}H]casein in the presence (open bar) and absence (solid bar) of ATP. Units are defined as μg of casein hydrolyzed per hour.

wild type. In related unpublished studies, we have found that the inactive *capR9* protein eluted from phosphocellulose is capable of inhibiting the wild-type protease, and this property may account for its phenotypic dominance (23, 24).

Energy Requirement for Protein Degradation in *lon*$^+$ and *lon*$^-$ Cells. These observations would predict that the *lon*$^-$ mutants *in vivo* still degrade proteins by an ATP-dependent process, but do so more slowly than the wild-type cells. We therefore compared the rates of degradation of puromycin-containing polypeptides in the *capR*$^+$, *capR9*, and *capR*$^-$ cells under normal conditions and when energy metabolism was inhibited. Both *capR9* and *capR*$^-$ mutations resulted in a 50% reduction in degradation of puromycyl polypeptides (Fig. 4) as previously observed with other *lon*$^-$ strain (27, 28). In both mutant and wild type cells, removal of glucose from the medium

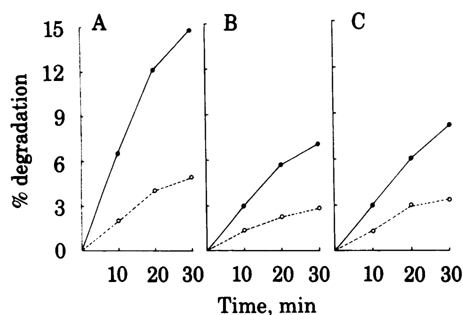

FIG. 4. Effects of energy depletion on the degradation of puromycin-containing polypeptides in *capR*$^+$ (*A*), *capR9* (*B*), and *capR*$^-$ (*C*) strains. To induce production of abnormal proteins (7), growing cultures of the *lon*$^+$, *lon*$^-$ (*capR9*), and *lon*$^-$ (*capR*$^-$) strains were incubated with puromycin at 100 μg/ml for 15 min. [^{14}C]Phenylalanine was then added for 5 min to label incomplete proteins synthesized under these conditions. These cultures were washed to remove unincorporated phenylalanine and the puromycin. One-half of the cells were resuspended in the growth medium (●) and the other half, in medium containing 0.1 mM 2,4-dinitrophenol and lacking glucose (○). The washes and incubation medium contained a large excess (300 μg/ml) of nonradioactive phenylalanine to prevent reincorporation of [^{14}C]phenylalanine. Aliquots were withdrawn at each time point, and acid-soluble radioactivity was measured.

and addition of the uncoupling agent 2,4-dinitrophenol decreased the degradation of abnormal protein by approximately 70%. Thus, in accord with the cell-free data (Fig. 2), an ATP-dependent proteolytic system functions in the strains carrying the *capR9* or *capR*$^-$ mutation, but its capacity to degrade abnormal proteins is decreased.

DISCUSSION

The *lon* gene product, purified as a DNA-binding protein, has been demonstrated to be an ATP-dependent protease; by all criteria tested, it is identical to protease La. The purified *lon* gene product and partially purified protease La are of similar size (Fig. 1), have similar specificities (i.e., hydrolyze casein and globin but not insulin) (Table 1; refs. 14–16), are eluted similarly from DEAE-cellulose columns (Fig. 1; ref. 23), are serine proteases as shown by their sensitivity to diisopropyl fluorophosphate, are equally sensitive to *N*-ethylmaleimide and vanadate (Table 3), and are rapidly inactivated at 42°C but stabilized by ATP (Table 3). Both proteins show proteolytic activity only in the presence of ATP and Mg^{2+} (probably an ATP^{2-}–Mg^{2+} complex) and they show similar responses with various nucleotides and with nucleotide analogs (Table 2; ref. 16). Finally, the strains carrying additional copies of the *lon*$^+$ gene on a plasmid show an increased level of protease La activity but not of the other *E. coli* proteases; nonsense mutations in this locus decrease this activity specifically (Fig. 2). We therefore refer to protease La and the *lon* gene product interchangeably.

It is of particular interest that cleavage of ATP appears to be essential for proteolytic activity. Casein hydrolysis is not stimulated by nonmetabolizable ATP analogs (Table 2; ref. 16) and is sensitive to vanadate, a potent inhibitor of various ATPases (Table 3; ref. 26). The enzymological mechanisms linking ATP cleavage and proteolytic activity are not clear. Related studies have demonstrated that this unique protease has ATPase activity which is stimulated by protein substrates (ref. 29; unpublished data). In addition, Larimore *et al.* (16) have shown that protease La does not have protein kinase, protein adenylase, or ubiquitin-conjugating activity (30, 31). The direct activation of protease La by ATP is not consistent with the model suggested by Hershko, Rose, and colleagues (30, 31) to account for the ATP requirement for intracellular proteolysis in reticulocytes. According to their model, ATP is required by a multiple-component system that conjugates a small polypeptide, ubiquitin, to protein substrates to enhance their susceptibility to ATP-independent proteases (30, 31).

The requirement for ATP hydrolysis also differentiates the *lon* gene product from the other known proteolytic enzymes, including those activated by ATP. During induction of prophage λ, in the presence of ATP the *recA* gene product catalyzes the endoproteolytic cleavage of the phage repressor (32). However, that reaction does not seem to require ATP hydrolysis because it occurs severalfold more effectively with adenosine 5′-(*O*-3′-thiotriphosphate) than with ATP, unlike that with the *lon* gene product. Proteases from liver or reticulocyte cytoplasm have been described that they are stimulated 2- to 3-fold by ATP, but they also do not require ATP hydrolysis (33, 34).

The ATP requirement of protease La can account for the energy requirement for degradation of normal and abnormal proteins *in vivo* (1, 8–11). Earlier studies showed that in the degradation of nonsense fragments of β-galactosidase the *lon* mutation affects the same endoproteolytic step that is decreased by inhibitors of energy metabolism (5). Furthermore, both the *capR9* and *capR*$^-$ mutations caused a similar decrease, of about 50%, in protease La activity (Fig. 2) and in the capacity of the cells to degrade puromycyl polypeptides (Fig. 4). In addition, the relatively high affinity of this enzyme for ATP can account for the finding that intracellular ATP levels must be decreased

to <10% of normal levels before protein degradation decreases (2, 9). These various observations support the earlier suggestion (14, 15) that protease La is responsible for the rate-limiting steps (5) in the degradation of abnormal proteins *in vivo*.

We have previously demonstrated ATP stimulation of proteolysis in crude extracts of lon^- as well as lon^+ strains (12). In the present studies, protease La was demonstrated by DEAE-cellulose chromatography to be present in lon^- extracts, but in reduced amounts (Fig. 2). The mutant enzyme was also shown to resemble the wild type in multimeric size and heat stability (Table 4). Furthermore, although decreased in rate, the degradation of abnormal proteins in these mutants cells still required energy (Fig. 4). In additional experiments, extracts from a strain (N5115) carrying a deletion within the *lon* gene (kindly provided by Susan Gottesman) were found to contain protease La activity in lower amounts than in the wild-type parent (N5116), in accord with the results of Fig. 2. In such cells, the degradation of abnormal proteins also occurred at a decreased rate and still required energy (unpublished data). The presence of the ATP-dependent protease even in the nonsense and the deletion mutants and our failure to find strains truly lacking this activity suggest that this enzyme may be essential for the survival of the cells.

Although phosphocellulose chromatography is particularly useful in purifying the ATP-dependent protease from the extracts of wild-type cells (15), this step completely destroyed the activity in the *capR9* and $capR^-$ extracts. This inactivation explains our initial failure (Table 1) to find the residual ATP-dependent proteolytic activity in lon^- mutants as well as the conclusion of Charette *et al.* (29) that these mutants completely lack this activity.

Both $capR^-$ and *capR9* are nonsense mutations. The 50% reduction observed in the total activity of protease La in these mutants (Fig. 2) may underestimate the effect of the mutation on enzyme function because the *capR9* strain has been reported to overproduce this polypeptide (23, 24). The *capR9* mutation must be located close to the carboxyl terminus because its polypeptide, purified as a DNA-binding protein, is indistinguishable from that of the wild-type on $NaDodSO_4$/polyacrylamide gel electrophoresis (24) and on gel filtration on a Sephacryl S-300 column (Table 4). It is noteworthy that, after phosphocellulose chromatography has inactivated the *capR9* enzyme, the protein retains DNA-binding activity (23) and it can inhibit the wild-type protease La (unpublished data). The finding of an ATP-dependent proteolytic activity in the $capR^-$ extract is particularly interesting because Zehnbauer *et al.* (23) were unable to find the 94,000-dalton polypeptides in such extracts. However, we did observe a normal-size multimeric enzyme in this strain (Table 4).

The demonstration that the *lon* gene product is a protease provides a possible explanation for the ability of *lon* mutations to affect a remarkable variety of physiological processes. Among these are capsular polysaccharide synthesis, cell division, filamentation, transcription of the *gal* operon, and sensitivity to UV light and radiomimetic agents (18–22). Possibly these various processes involve short-lived proteins that are normally degraded by the ATP-dependent proteolytic enzyme. For example, lon^- cells are defective in lysogenization of phage λ, and one of the short-lived regulatory proteins involved in this process, the *N* gene product, is stabilized by the lon^- mutation (35). A similar decrease in the proteolytic inactivation of various cellular regulatory proteins or enzymes may account for many other phenotypic consequences of the *lon* mutation.

This work was initiated in this laboratory as a collaboration with Mr. M. F. Charette and Dr. A. Markovitz who preferred to publish independently (29). We are very grateful to Professors Howard Green and Bernard D. Davis for their helpful advice. Our colleagues Drs. Fred S. Larimore, Sreedhara Swamy, Lloyd Waxman, and Lian Yeh have made many helpful suggestions and provided materials used in this study. We also thank Ms. Maureen Rush for help in preparation of this manuscript. Our studies have been supported through grants from the National Institute of Neurological and Communicative Diseases and Stroke, the Juvenile Diabetes Foundation, and the Kroc Foundation.

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