Alp, a Suppressor of Lon Protease Mutants in Escherichia coli

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Escherichia coli lon mutants lack a major ATP-dependent protease, are sensitive to UV light and methylmethane sulfonate (MMS), and overproduce capsular polysaccharide. Evidence is presented that an activity (Alp), cloned on a multicopy plasmid, can suppress the phenotypes of lon mutants. The sensitivity to UV and MMS is a reflection of the stabilization of the cell division inhibitor SulA, while the capsule overproduction arises through the stabilization of a transcriptional activator of capsule biosynthetic genes, RcsA. Multicopy alp (pAlp) suppressed capsule formation in Δlon cells, and Δlon cells containing the pAlp plasmid were resistant to MMS treatment. The MMS resistance of Δlon pAlp+ cells correlates with an increase in the degradation of SulA to that found in lon+ cells. Lon-directed degradation of SulA was energy dependent, as was the increase in degradation of SulA in Δlon pAlp+ cells. alp maps close to pheA, at 57 min on the E. coli chromosome. Although pAlp can substitute for Lon, cells lacking alp activity did not have the phenotype on a lon mutant. This study demonstrates that at least one activity, when overproduced in the cell, can substitute for Lon protease.

Protein turnover serves as a regulatory strategy for cells during periods of changing conditions or environmental emergencies. When a crisis is encountered, the cell may require the rapid synthesis or elimination of specific gene products to permit survival. The cell can respond quite rapidly by regulating gene transcription through a variety of interesting mechanisms, but once a gene product is made it is generally long lived (10, 24). After the cell has adjusted to the emergency and resumes normal growth, the emergency-activated gene products can be gradually removed by dilution, provided that the accumulation is not harmful to the normal physiology of the cell. However, if these products must be rapidly cleared for normal growth, then some sort of inactivation of the gene products must occur along with the termination of their synthesis.

The most thoroughly studied proteolytic process in Escherichia coli is that which involves the ATP-dependent protease Lon. Mutations in lon give rise to a pleiotropic phenotype (for a review, see reference 10). Two of these phenotypes, sensitivity to DNA damage and overproduction of capsular polysaccharide, can be explained as a direct result of the unavailability of Lon protein, resulting in the stabilization of two normally unstable proteins (10, 26, 29).

When cells experience DNA damage (e.g., through exposure to UV light or methylmethane sulfonate [MMS]), the SOS repair response in E. coli is activated, giving rise to the expression of specifically repressed genes (19, 31). One expressed gene product is SulA, a cell division inhibitor which inhibits septation by interacting with FtsZ, a component of the cell division apparatus (13–15). In wild-type cells, SulA is very unstable (with a half-life of 1.2 min). This normal instability of SulA permits the cell to resume cell division once the environmental stress has been alleviated. However, in lon mutant cells, the SulA half-life is 20 to 30 min (21, 26). The stabilization of SulA in lon mutants results in irreversible filamentation and subsequent cell death (14).

As is the case with sensitivity to DNA-damaging agents, overproduction of capsular polysaccharide is dependent on an unstable protein, RcsA (29). The regulation of the capsular polysaccharide genes (cps) occurs at the transcriptional level (30). lon+ cells with a lac fusion to one of the cps genes (cpsB10::lac) are Lac−, whereas lon mutant cells with the cpsB10::lac fusion are Lac+. The difference in cps::lac expression correlates with differences in the stability of the cps positive transcriptional regulator, RcsA; the half-life of RcsA is 5 min in lon+ cells, and in lon mutant cells the half-life is increased to 20 min (29). RcsA is apparently limiting for cps expression in lon+ cells; its stabilization in lon mutant cells allows high-level cps expression. The normal instability of RcsA may permit fine tuning of cps gene expression in response to changing environmental conditions. As seen with the stabilization of SulA protein, the stabilization of RcsA protein in lon mutant cells gives rise to a phenotype (overproduction of capsule) that is a disadvantage to the cell for normal growth.

SulA and RcsA proteins represent two examples of normal short-lived proteins whose regulation by Lon-dependent proteolysis may help the cell recover from emergency conditions. We have exploited the behavior of these Lon substrates to probe the cell for other, similar protease functions. Maurizi et al. (21) have demonstrated residual degradation of SulA protein in Δlon cells. Similarly, RcsA turnover is still significant in cells devoid of lon (29). On the basis of these observations, other protease activities that are capable of degrading SulA and RcsA must exist in E. coli. Increasing the activity of such proteases should lead to suppression of the Lon− phenotypes. We have screened fragments of the E. coli chromosome for those which, when present on multicopy plasmids, are capable of suppressing Lon− phenotypes. We have identified an activity capable of suppressing both sensitivity to DNA-damaging agents and capsule overproduction. Therefore, this activity, when present in excess in the cell, can mimic Lon.

MATERIALS AND METHODS

Bacteria and phage strains. The relevant bacterial and phage strains used are listed in Table 1. Hfr strains and additional recipient strains with appropriate markers used for mapping were obtained from B. Bachmann (E. coli Genetic Stock Center, Yale University, New Haven, CT).
TABLE 1. Bacterial and phage strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>JTS100*</td>
<td>Δlon-510 Δgal-165</td>
<td>SG2050 derivative</td>
</tr>
<tr>
<td>SG20780*</td>
<td>Δlon-510 cpxB10::lac-Mu-imaM</td>
<td>3</td>
</tr>
<tr>
<td>SG20781*</td>
<td>lon* cpxB10::lac-Mu-imaM</td>
<td>3</td>
</tr>
<tr>
<td>SG20520*</td>
<td>lon* cpxS</td>
<td>11</td>
</tr>
<tr>
<td>C600</td>
<td>F- thi-1 thr-1 leuB6 lacY1 tonA21 supE44</td>
<td>National Institutes of Health strain collection</td>
</tr>
<tr>
<td>JM101</td>
<td>Δ(lac-proAB) supE thi(r') traD36 proAB lacPZAM15</td>
<td>23</td>
</tr>
<tr>
<td>SG1611</td>
<td>Δlon-510 Δgal-165 Δ(lac-proAB) supE thi(r') traD36 proAB lacPZAM15</td>
<td>JM101 derivative</td>
</tr>
<tr>
<td>DB1255</td>
<td>recBC sbcB15 hasD supF8</td>
<td>33</td>
</tr>
<tr>
<td>λNK1105</td>
<td>e1857 b221 Pam80 nin5 ΔKan plac-transposase</td>
<td>32</td>
</tr>
</tbody>
</table>

* MC4100 derivative; contains ΔlacU169 (4).

Conn.). The procedures for Hfr mating and P1 transduction have been described elsewhere (25).

Construction of library and selection of lon-complementing plasmids. Chromosomal DNA was isolated (28) from cells carrying a Δlon-510 mutation (JTS100), partially digested with Sau3A (28), and cloned into the BamHI site of pBR322. This multi-copy plasmid library was transformed into Δlon cells containing the cpxB10::lac fusion (SG20780). Ampicillin-resistant colonies were replica plated onto either Luria broth plates containing 0.05% methylmethane sulfonate (MMS) (12) or plated directly onto lactose-MacConkey agar plates containing 50 μg of ampicillin per ml. Plasmids that conferred MMS resistance also conferred UV resistance.

Insertional mutagenesis of plasmid DNA. Transposon mutagenesis of plasmid-cloned DNA was performed as described previously (32) by using the phage λNK1105, which was provided by N. Kleckner. The mini-transposon carried on this phage (ΔKan) confers resistance to kanamycin and contains the outer ends of Tn10 (32). Wild-type cells (SG20520) containing pAlp were infected with λNK1105 and Kan' colonies were selected. Plasmid DNA was isolated from the Kan' cells and retransformed into wild-type cells (C600). Transformants were selected for Kan' and screened for Amp'. Plasmids containing the ΔKan inserts were isolated, and restriction analysis was performed (20) to determine the location and orientation of the ΔKan. alp-210::ΔKan is a ΔKan transposon that eliminates alp activity from the plasmid, and alp-ΔZ27::ΔKan is a ΔKan transposon that does not affect alp activity but is located in the chromosomal DNA carried by the plasmid.

Both ΔKan insertions in the chromosomal insert were transferred to the chromosome by linearizing the plasmid with the restriction endonuclease Nhe1 and transforming DB1255 (33) as previously described (3, 16). The ΔKan was mapped by Hfr matings and P1 transductions (25).

In vivo SulA turnover. lon' (JM101) cells were transformed with pBR322, and lon mutant (JM101 derivative, SG1611) cells were transformed with either pBR322, pAlp, or alp-210::ΔKan plasmid. Ampicillin-resistant transformants were transformed with a pBR322-compatible plasmid (conferring chloramphenicol resistance) containing the sulA locus under control of an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible lac promoter. Ampicillin-resistant, chloramphenicol-resistant cells were grown at 32°C in M56 minimal medium (9) supplemented with 0.4% glycerol, essential amino acids (50 μg/ml) excluding methionine and cysteine, 50 μg of ampicillin per ml, and 25 μg of chloramphenicol per ml. To induce transcription of SulA, IPTG (final concentration, 5 mM) was added to exponentially growing cells (optical density at 600 nm [OD600], 0.6) and incubation was continued at 32°C for an additional 45 min. Cells were pulse-labeled for 1 min with [35S]methionine (10 μCi/ml, Amersham Corp.) and chased with a 10-fold excess of unlabeled methionine. Samples were removed at various times after the pulse-chase and precipitated with 10% (vol/vol, final concentration) cold trichloroacetic acid. Acetonitrile-washed pellets were suspended in Tris-buffered saline (10 mM Tris, pH 7.8, 150 mM NaCl) containing 0.1% sodium dodecyl sulfate and 1% Nonidet P-40. Suspended pellets were boiled, and aprotinin (0.1 U; Sigma Chemical Co.), phenylmethylsulfonyl fluoride (1 μM; Sigma), Nα-tosyl-L-lysine chloromethyl ketone (100 μM; Sigma), and Nα-tosyl-L-phenylalanine chloromethyl ketone (100 μM; Sigma) were added to inhibit proteolytic activity. Anti-SulA antibody, negatively absorbed against total protein from a sulA E. coli strain, and Staphylococcus A protein (IgG; Sigma, Enzyme Center) were used to immunoprecipitate labeled SulA. Immunoprecipitates were analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (10% to 20% linear gradient gels). Fluorograms (using T.S. McLean Research Products) followed by autoradiography was used to visualize the labeled protein bands. To determine whether alp activity required energy, the same procedure was followed except that at the time unlabeled methionine was added (chase), 10 mM potassium cyanide (KCN) was included to inhibit ATP generation.

RESULTS

Identification of lon-suppressing activity from a multicopy plasmid library. We have used the two characteristic phenotypes of cells carrying a lon mutation, sensitivity to DNA-damaging agents and high-level expression of genes for capsular polysaccharide synthesis, to test the hypothesis that other protease activities in the cell can substitute for Lon protease if present in excess. A multicopy plasmid library consisting of chromosomal DNA from Δlon cells (JTS100) was transformed into Δlon cells containing the cpxB10::lac fusion (SG20780 Lac'; MMS sensitive). This strain permitted us to examine the fate of two lon substrates, SulA (through sensitivity to MMS) and RcsA (through β-galactosidase levels). Twenty thousand colonies were screened on MacConkey agar-lactose plates containing ampicillin or on Luria broth plates containing MMS. The three classes of lon-suppressing plasmids identified in the screen are listed in Table 2. lon' cells are MMS resistant and Lac', whereas Δlon cells are MMS sensitive and Lac'. Seven plasmids were identified (class 1) that suppressed the Lac' phenotype of Δlon cells but did not affect MMS sensitivity. One plasmid was identified (class 2) that conferred MMS resistance on Δlon cells but did not affect the Lac phenotype. Class 1 and 2 plasmid activities might represent a protease or other proteins that are specific to the SOS or capsule pathways. Restriction map analysis of these plasmid inserts (data not shown) suggested that the activities are not known components of the SOS or capsule pathways. Two plasmids identified in the screen (class 3) suppressed both phenotypes of a lon deletion. The two lon-suppressing plasmids of class 3 were identified in separate screens. One plasmid was identified by its ability to confer resistance to MMS and then screened for the Lac phenotype. The other plasmid was
selected for its ability to reverse the Lac\(^+\) phenotype of \(\Delta lon\) cells to Lac\(^-\) and then screened for the MMS phenotype. Restriction analysis revealed that the two \(lon\) suppressing plasmids of class 3 contained an identical 4.5-kilobase DNA insert. We chose to focus our analysis on the plasmid that suppressed both \(lon\) phenotypes (class 3). We reasoned that the ability to suppress two independent \(lon\) phenotypes was most easily explained as an increased protease activity in cells carrying the multicopy plasmid. We have named this plasmid pAlp and the putative gene \(alp\). We confirmed that the ability to suppress the \(lon\) phenotypes resided entirely in the plasmid by isolating pAlp and retransforming it into \(\Delta lon\) cells (SG20780). Once again, both MMS sensitivity and high \(cps:\lac\) expression were suppressed.

\(\Delta Kan\) transposon insertions were isolated in pAlp to physically map the activity responsible for suppressing the \(lon\) phenotypes. The Amp\(^+\) Kan\(^-\) plasmids were isolated, transformed into \(\Delta lon\) \(cps:\lac\) cells (SG20780), screened for the MMS and Lac phenotypes, and analyzed by restriction analysis. Two types of \(\Delta Kan\) insertions in the plasmid were identified: (i) insertions that did not affect the MMS or Lac phenotypes and (ii) insertions in pAlp that abolished suppression of both \(\Delta lon\) phenotypes (Fig. 1). On the basis of the phenotype and DNA restriction analysis, we determined that \(alp\) activity resided in a DNA fragment of 750 bases. Neither of the \(\Delta Kan\) insertions identified affected just one of the phenotypes. This suggests that a single gene locus, in multiple copies, mimics the effects exerted by Lon in the cell. We have named this locus \(alp\) (for alternative activity to Lon protease).

To quantitate the effect of pAlp on \(cps:\lac\) expression, isogenic \(lon^+\) (SG20781) and \(\Delta lon\) (SG20780) cells containing a \(cps:\lac\) protein fusion were assayed for \(\beta\)-galactosidase in the presence of the wild-type plasmid and \(\Delta Kan\) insertion plasmids. In \(lon^+\) cells, the \(cps:\lac\) fusion was expressed at low levels (Table 3, line 1). In \(\Delta lon\) cells, \(cps:\lac\) expression was approximately 100-fold higher than in \(lon^+\) cells (Table 3, line 2). This difference has been interpreted as a reflection of the effect of Lon protease on the stability of the regulator of \(cps\) operons, RcsA (29). \(\Delta lon\) cells carrying pAlp had \(\beta\)-galactosidase levels similar to those seen in \(lon^+\) cells (Table 3, line 3). When pAlp contained a \(\Delta Kan\) that inactivates its ability to suppress \(lon\) (\(alp-210:\Delta Kan\)), \(cps:\lac\) expression returned to high levels in \(\Delta lon\) cells (Table 3, line 4). However, the plasmid suppressed \(cps:\lac\) expression when the \(\Delta Kan\) was inserted elsewhere in the plasmid (\(alp^+\) \(zfh-27:\Delta Kan\)) (Table 3, line 5). Additionally, \(lon^+\) cells containing any of the three plasmids expressed \(cps:\lac\) at the same levels as those without the plasmid (Table 3, lines 6 to 8).

\(\text{In vivo proteolysis of SulA protein: a biochemical examination of pAlp effects.}\) The decrease of \(\beta\)-galactosidase levels in

\begin{table}[h]
\centering
\caption{Effect of multicopy \(alp\) on the expression of the \(cpsB10-lac\) fusion}
\begin{tabular}{|l|l|l|l|}
\hline
Host genotype & Plasmid & Phenotype & \(\beta\)-Galactosidase activity
\hline
\(\text{inacti- morphotype} & (\text{strain}) & (\text{gene}} & (\text{activity}) ~
\hline
\(lon^+\) (SG20781) & Lac\(^-\) & 8
\(\Delta lon\) (SG20780) & Lac\(^+\) & 760
\(\Delta lon\) & pAlp & Lac\(^-\) & 9
\(\Delta lon\) & \(alp-210:\Delta Kan\) & Lac\(^+\) & 604
\(\Delta lon\) & \(alp^+\) \(zfh-27:\Delta Kan\) & Lac\(^-\) & 18
\(lon^+\) & pAlp & Lac\(^-\) & 7
\(lon^+\) & \(alp-210:\Delta Kan\) & Lac\(^-\) & 8
\(lon^+\) & \(alp^+\) \(zfh-27:\Delta Kan\) & Lac\(^-\) & 8
\hline
\end{tabular}
\end{table}

\(\text{Notes:}\)
\(a\) Lac phenotype was determined on lactose-MacConkey indicator agar at 32°C.
\(b\) Cells were grown in glucose minimal medium at 32°C and assayed for \(\beta\)-galactosidase specific activity (expressed as Miller units [25]). Plasmid-containing strains were grown in the presence of ampicillin (50 \(\mu\)g/ml).

\(\text{Restriction enzymes used: EcoRI, EcoRV; H, HindIII; P, PstI.}\)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{restriction_map.png}
\caption{Restriction map of cloned insert in pAlp plasmid. Symbols: 210, \(alp-210:\Delta Kan\) insertion which inactivates \(alp\) activity; 27, \(alp^+\) \(zfh-27:\Delta Kan\) insertion which does not inactivate \(alp\). Restriction enzyme sites are abbreviated as follows: R, EcoRI; V, EcoRV; H, HindIII; P, PstI. The following restriction enzymes did not cleave this fragment: BamHI, Clal, KpnI, Nhel, NruI, and PvuII.}
\end{figure}
Δlon cells carrying pAlp is consistent with an increase in turnover of RcsA. Resistance to MMS in cells carrying the pAlp plasmid is consistent with an increase in degradation of the cell division inhibitor, SulA. The effect of pAlp on the stability of SulA protein was directly tested in lon+ and Δlon strains (JM101 and SG1611, respectively) containing a pBR322-compatible plasmid with the sulA locus under the control of an IPTG-inducible lac promoter. The IPTG-induced cells were pulse-labeled with [35S]methionine and chased with an excess of unlabeled methionine, and immunoprecipitated samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography. An example of this experiment is illustrated in Fig. 2. SulA is very unstable in lon+ cells (with a half-life of less than 5 min) (Fig. 2A). The instability of SulA is a function of Lon (Fig. 2B) in that SulA is stabilized in Δlon cells (with a half-life of greater than 20 min). Introducing the pAlp plasmid into Δlon cells (Fig. 2C) decreased SulA half-life to that which was observed in lon+ cells (less than 5 min). Δlon cells carrying the alp-210::ΔKan mutation on the plasmid (Fig. 2D) showed a pattern of SulA decay similar to that found in the Δlon parent, indicating the elimination of the multicopy alp effect by the ΔKan insertion. A similar effect of pAlp on the stability of SulA was seen when SulA was synthesized from a transducing phage in UV-irradiated cells (data not shown). In both cases, pAlp increased the degradation of SulA in lon mutant cells to that found in lon+ cells.

Proteolytic activity by Lon requires energy in the form of ATP in vitro (5, 6). We wanted to determine whether the increase in degradation of SulA by pAlp required energy. The same procedure was followed as illustrated in Fig. 2A to D, except that potassium cyanide was added at the time of the chase to quench energy-generating systems. Inhibition of ATP generation by cyanide stabilized SulA in lon+ cells (Fig. 2E). This result was expected, given the in vitro ATP dependence of Lon, and had been previously observed by D. Canceill and O. Huisman (personal communication). The activity from pAlp that decreased the stabilization of SulA in Δlon was also reversed by the addition of cyanide (Fig. 2F). This result indicates that, like Lon, the pAlp mechanism requires energy to affect the stability of SulA.

These results, taken together, suggest that we have identified a gene which, when present in multiple copies, acts directly or indirectly to impart a Lon+ phenotype to the cell. The only component that the SOS system and the capsule system are known to have in common are unstable substrates whose stabilities are affected by Lon. Since multicopy alp suppresses the lon phenotype for both of these systems, alp may code for a protease which, when overproduced, is capable of degrading SulA and RcsA. It is also possible that the alp product may be a regulatory protein that increases synthesis of a protease or a site that titrates a negative repressor from protease genes.

Chromosomal alp mutants. The ΔKan inserts in pAlp were transferred to the chromosome by linearizing the alp-210::ΔKan plasmid with a single restriction cut and transforming the linear plasmid DNA into a nuclease-deficient strain (3, 16). PICMclrl00 was grown on the resulting KanR AmpR transformants that arose through homologous recombination, and the lysate was used to transduce the ΔKan into the Hfr strains used for mapping by interrupted mating. The ΔKan was transferred early in interrupted matings with Hfr KL16 (point of origin, 60 min) and PK191 (point of origin, 44 min), consistent with alp mapping in the 44- to 60-min region. The transductions of the ΔKan into appropriate recipient strains with markers in this area demonstrated that alp cotransduces with pheA (57 min [1]) at a frequency of 50%.

FIG. 2. SulA stability in lon+ cells, Δlon cells, and Δlon cells plus pAlp or alp-210::ΔKan plasmid. IPTG-induced cells carrying a pBR322-compatible plasmid containing the sulA locus under the control of an IPTG-inducible lac promoter were pulse-labeled for 1 min with [35S]methionine (10 μCi/ml) and chased with 104-fold-excess unlabeled methionine. Samples were immunoprecipitated and analyzed by electrophoresis as described in Materials and Methods. Labeled protein bands were visualized by fluorography. The position to which SulA (18 kilodaltons) migrated in this gel system is indicated on the figure. (A) lon+ (JM101) + pBR322 control vector. (B) Δlon (SG1611) + pBR322 control vector. (C) Δlon (SG1611) + pAlp. (D) Δlon (SG1611) + alp-210::ΔKan plasmid. (E) lon+ (JM101) + pBR322 control vector. (F) Δlon (SG1611) + pAlp. For panels E and F, cells were incubated with potassium cyanide as described in Materials and Methods. Cells were labeled for 1 min (lane 1) and chased for 1 (lane 2), 2 (lane 3), 3 (lane 4), 5 (lane 5), 7 (lane 6), 12 (lane 7), and 20 min (lane 8).
Comparison of the pAlp restriction map (Fig. 1) with that of the published restriction map of the E. coli chromosome (18) permitted us to accurately locate alp at kilobase pair 2765. Saito et al. have reported that grpE, a gene encoding a heat shock-induced cellular substrate of pheA and a fragmental protein of 61% (27). grpE is carried on clones EBF2 and 22D7 of Kohara et al. (18); these phage overlap in a region at least 10 kilobase pairs from alp (S. Gottesman and C. Georgopoulos, unpublished observations).

The ability to transfer the alp::AKan insertion from the plasmid to the chromosome suggested that alp is not essential for normal cell growth. To confirm that secondary mutations or rearrangements were not occurring, we carried out P1 transductions from the original alp::AKan isolates into pheA::Tn10 recipients, selected either kanamycin resistance or prototrophy for phenylalanine and screened for the nonselected marker. Control transductions were carried out using the closely linked alp+ zfr-27::AKan insertion, which was also transferred to the chromosome by linear transformation. Both alp-210::AKan and alp+ zfr-27::AKan showed essentially identical linkage to pheA regardless of the original selection. Therefore, if secondary mutations are present in cells carrying alp-210::AKan, they must be tightly linked to the kanamycin resistance marker. Duplications in the alp region would be expected to be unstable and might show unusual transduction behavior; we have not encountered problems associated with these characteristics. alp-210::AKan was also introduced into Δlon-510 hosts by P1 transduction, once again by selecting pheA+ and screening for the nonselected kanamycin resistance marker. The pheA::AKan linkage was identical in lon+ and Δlon strains. Apparently, the alp activity, as defined by the plasmid insertion mutations, is not essential for cell growth in the presence or absence of Lon. Finally, lon+ cells containing the alp-210::AKan mutation did not show any lon phenotypes (sensitivity to MMS or capsule overproduction). Thus, the pAlp suppression of lon mutations was not due to any direct and necessary role of alp in degrading Lon substrates.

**DISCUSSION**

Proteolysis, combined with controls on gene expression, can serve as an important mechanism for the fine tuning of protein availability and plays a necessary role in the rapid response to emergency conditions and recovery from these emergencies (10). In E. coli, Lon has been identified as a major, ATP-dependent protease. lon mutants directly or indirectly affect the stability of at least two proteins in different global response networks, SulA and RcsA. In the absence of Lon, the stability of SulA interferes drastically with the ability of the cell to recover from even mild DNA-damaging treatments. Capsule synthesis in the absence of Lon is carried out at a rate too high for rapid cell growth. The residual turnover of SulA, RcsA, and many abnormal proteins in cells devoid of lon, as well as the continued rapid turnover of unstable lambda proteins unaffected by lon mutations (10, 21), suggests that proteases other than Lon must be present in E. coli. Identification of the natural cellular substrates of these proteases will be an important step in understanding the full possibilities for regulation by proteolysis. In addition, some foreign proteins cloned into E. coli are unstable; while lon mutations have some stabilizing effect on this degradation, residual degradation is frequently significant (for a review, see reference 24). We reasoned that we could identify other activities which may overlap with Lon in substrate specificity by overproducing these activities in the cell. We have identified a gene present on a multicopy plasmid that can suppress the lon phenotypes of MMS sensitivity and overproduction of capsule. This activity is a newly identified locus of E. coli which we have named pAlp.

When alp is present on a multicopy plasmid, it functions similarly to Lon, as demonstrated in this study, by suppression of two Lon phenotypes. We have demonstrated directly that suppression of MMS sensitivity is correlated with an increase in SulA degradation in Δlon cells carrying alp on a multicopy plasmid and that energy is required for pAlp activity. We noted other similarities between cells carrying alp on a multicopy plasmid and those carrying lon on plasmids. Goff and Goldberg reported that lon cloned on high-copy-number plasmids acquires insertion elements that inactivate the lon gene (8). Similarly, the pAlp plasmid rapidly acquires mutations which significantly reduce its ability to suppress lon mutations, although these mutations do not appear to be due to insertion elements (J. Trempy, unpublished observations). Presumably, increased levels of Lon or Alp lead to degradation of essential cell components. Additionally, we have observed that cells carrying the lon+ or alp+ plasmid are temperature sensitive and are unable to grow on minimal media even at lower temperatures (unpublished observations), suggesting once again that overproduction of either lon or alp is similarly unfavorable for normal cell growth.

A function coded for by the Alp plasmid and inactivated by insertions at one site within the plasmid can increase degradation of the SulA protein. The easiest way to explain this effect is that a limiting component of a protease, normally with only limited ability to degrade SulA or RcsA, is coded for by the alp gene. If so, it would seem that this protease is not normally essential for cell growth, since transfer of the alp-210::AKan mutation into the chromosome was easily accomplished. Similarly, if a positive activator for a protease or proteases were present on the plasmid, mutations that inactivate it might be protective for the protease when present on the chromosome but might still give significant levels of activity. However, we have not yet identified an alp product, although numerous attempts have been made by using maxicell analysis and in vitro transcription-translation systems. It is possible that a site rather than an open reading frame for a protein is on the plasmid and is inactivated by the alp-210::AKan insertion. If a site for a negative regulator were on the plasmid such that a protease repressor were being titrated, it might not be surprising to find that inactivation of the single site on the chromosome had no phenotype. Since five ΔKan insertions which inactivate alp appear to be at the same point in the plasmid, as determined by restriction mapping (possibly reflecting a transposon hot spot), we cannot rule out a site. The absence of lon phenotypes in lon+ cells carrying the alp-210::AKan insertion in the chromosome demonstrated that alp is not necessary for lon synthesis or activity and that lon does not act indirectly by activating or increasing synthesis of alp.

What might alp do for the cell? No known proteases or suspected proteases of E. coli for which genes have been identified correspond to alp (10, 17, 24). If we assume that alp is a component of a new protease, the absence of dramatic phenotypes may suggest that its natural substrates are not detected under ordinary growth conditions. SulA, for instance, is a lethal Lon substrate, but the Lon effect on SulA is not visible until induction of synthesis by DNA damage. If alp substrates are the members of other emergency-response systems, the detection of a phenotype may
require the appropriate induction protocol. A better understanding of the nature of the alp locus and the conditions under which it normally functions would help us to identify putative substrates for Alp.

The use of an overproduction phenotype provided by the presence of a locus on a multicopy plasmid is simple and has been successfully used in other systems to either suppress mutant functions or inhibit wild-type activities (2, 7, 22). In our case, we did not have information about the natural substrates of the putative protease, which might have allowed development of a selection for inactivation of the protease. In addition, we were anxious not to eliminate from our screening procedure genes essential for E. coli growth. Selection for increased activity of a given function and screening for crossover activities of the plasmid-borne function, as described here, serve as useful alternatives to identification of genes by inactivation.

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LITERATURE CITED