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Deg Phenotype of *Escherichia coli lon* Mutants

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Deg, one of the *Escherichia coli* systems for degrading abnormal polypeptides (e.g., nonsense fragments), is also involved in the degradation of some classes of missense proteins. Both missense proteins of β-galactosidase and temperature-sensitive phage products appear to be degraded by the Deg system. Mutations in the Deg system are indistinguishable from mutations classically called *lon* or *capR;* all map near *proC*, all are mucoid, defective in protein degradation, sensitive to radiomimetic agents, and defective in P1 lysogenization. All are able to propagate temperature-sensitive phage better than *lon*$^+$ parental strains. Mutations that suppress the radiation sensitivity of these strains (*sul*) also suppress the P1 lysogenization defect, but do not affect mucoidy or the degradation defect.

Escherichia coli appears to have two general mechanisms for degradation of its own protein. One of these systems is activated when cells are starved for amino acids, nitrogen, or a carbon source. Upon starvation, a three- to fourfold increase in proteolysis is observed. This activity does not require metabolic energy. A separate system of protein degradation is responsible for hydrolysis of abnormal proteins. Proteins containing amino acid analogs, such as canavanine or *p*-fluorophenylalanine, and some proteins containing missense mutations and nonsense fragments turn over much more rapidly than bulk cellular protein. This degradation is inhibited by energy poisons such as cyanide or azide. (For review, see references 6 and 7.) Very little is known about the role, if any, of short-lived native proteins in *E. coli.* Some subclasses of proteins appear to turn over rapidly, but whether protein degradation has a role in modulating protein function in *E. coli* is unknown.

One approach to understanding the role of protein degradation is to study mutants defective in degradation. Mutations of this kind have been selected, using strains that would not grow unless β-galactosidase nonsense fragments were stabilized to allow complementation (1, 2). These mutants, originally called *deg,* turned out to have a number of other phenotypes as well, including mucoidy and UV sensitivity. Some were mapped at the site of the previously described *lon* mutations, which are also mucoid and UV sensitive (19). On the other hand, some *lon* mutations were shown to stabilize nonsense fragments against degradation, a feature they had not previously been known to possess (19).

lon mutants were first isolated in 1964 as a class of UV-sensitive mutants (9), which make extremely mucoid colonies. The mucoidy is the result of overproduction of the normal mucopolysaccharides made in *E. coli. lon* cells are also derepressed for the *gal* operon and other enzymes in the pathway to mucopolysaccharide synthesis (10, 13). In addition, phage λ lysogenizes *lon* cells poorly (23) apparently because they produce less λ repressor after infection than wild-type strains (21). Lysogens, once formed, are stable. Phage P1, which has a very different mode of lysogeny, also does not lysogenize well in *lon* strains (20). To find the nature of the genetic lesion in *lon* cells and to clarify the relation between the Deg phenotype and the other aspects of the Lon phenotype, we have begun an analysis of the genetics of *lon* mutations. This paper demonstrates that the physiology and genetics of *deg* and *lon* mutations are indistinguishable. We also describe some new results on the relationships among various phenotypes expressed by these mutants. In addition, some aspects of the physiology of degradation are described. We do not yet know whether any of the defects in the *lon* mutants are direct or indirect effects.

Since work on *lon* and *deg* has been largely carried out independently and with quite different conceptual emphases, and since the phenotypes involved are so remarkably pleiotropic, a complex and redundant nomenclature has evolved. We now have a situation in which one genetic locus has three names, *deg, lon,* and

capR. We will refer to the genetic locus under discussion as *lon* and the term Deg will refer to the phenotypic property of slowing protein degradation characteristic of *lon* mutations. Information about mutant origin will be preserved in the description of strains given in Table 1. In Table 1 we have also assigned new *lon* allele numbers to the mutations originally called *degT* and *degR*. These allele numbers will be used throughout the rest of the paper.

MATERIALS AND METHODS

Bacterial and phage strains. All strains used are listed in Table 1. The *lon* allele in SA1500 is a putative deletion. It was isolated as a temperature-resistant derivative of a strain containing λcI857 in *tsx*. This lysogen, isolated by Shimada et al. (18) was not fully T6 resistant when tested by us. The temperature-resistant derivative (isolated by Sankar Adhya), which simultaneously lost all phage genes and became mucoid and UV sensitive, is *tsx*$^+$. We suspect that the

TABLE 1. *Bacterial strains used*

Strains	Relevant genotype	Source of reference
Bacterial (all *E. coli* K-12)		
SA1500	F$^-$ *his lon*Δ *str*	S. Adhya; found as temperature-resistant survivor of λcI857 lysogen in *tsx* (18)
SA1384	F$^-$ *his capR6 str*	S. Adhya; *capR6* isolated by Markovitz and Rosenbaum (14)
DT521	F'42*lacZ521/lacZ521 deg-2*	Reference 2; in this paper *deg*-2 will be referred to as *lon*-t2
HR521	F'42*lacZ521/lacZ521 degR*	Reference 1; in this paper, this *degR* allele will be referred to as *lon*-r1
RC277	F$^-$ *lacY proC tsx/lon*$^+$ *thr leu tonA str*	Roy Curtiss via C. Pratt
W3747	F' 13	Hirota (8) via S. Adhya
N998	F 8-1 *gal*$^+$*/gal sup*$^+$ *str*	National Institutes of Health bacterial collection; from D. Korn, F' is temperature sensitive for replication
SG4007	F$^-$ *sup*$^+$ *gal*$^+$ *lac proC str*	594 (*gal lac proC*$^+$) × CA7087 (Hfr *proC* Sm^s from J. Beckwith)
SG4008	F$^-$ *pro*$^+$ *lon*-r1 *lac str*	P1 (HR521), to SG4007, select pro$^+$
SG4009	F$^-$ *lon*-t2 *lac str*	P1 (DT521), to SG4007, select pro$^+$
SG4010	F$^-$ *lon lac str*	P1 (SA1500), to SG4007, select pro$^+$
SG4011	F$^-$ *capR6 lac str*	P1 (SA1384), to SG4007, select pro$^+$
SG12002	RC277 *pro*$^+$ *tsx*$^+$ *capR6*	P1 (SA1384) to RC277
SG12008	RC277 *pro*$^+$ *tsx*$^+$ *lon*-t2	P1 (DT521) to RC277
SG12007	RC277 *pro*$^+$ *tsx*$^+$ *lon*Δ	P1 (SA1500) to RC277
SG12009	RC277 *pro*$^+$ *tsx*$^+$ *lon*-r1	P1 (HR521) to RC277
SG4030	F$^-$ *proC* Δ(*gal-att-bio*) *sup*$^+$ *str*	Deletion found as temperature-resistant survivor of SG4007 (λcI857)
SG4033	*pro*$^+$ *lon*Δ	P1 (SA1500) to SG4030
SG4034	*pro*$^+$ *lon*-r1	P1 (HR521) to SG4030
SG4035	*pro*$^+$ *lon*-t2	P1 (DT521) to SG4030
SG4036	*pro*$^+$ *capR6*	P1 (SA1384) to SG4030
SG4405	F' 13 *lon*$^+$*/proC lac str*	W3747 × SG4007; select *lac*$^+$ Sm^r
SG4406	F' 13 *lac*$^+$ *lon*$^+$*/lac lon*-r1	W3747 × SG4008; select *lac*$^+$ Sm^r
SG4407	F' 13 *lac*$^+$ *lon*$^+$*/lac lon*-t2	W3747 × SG4009; select *lac*$^+$ Sm^r
SG4408	F' 13 *lac*$^+$ *lon*$^+$*/lac lon*Δ	W3747 × SG4010; select *lac*$^+$ Sm^r
SG4409	F' 13 *lac*$^+$ *lon*$^+$*/lac capR6*	W3747 × SG4011; select *lac*$^+$ Sm^r
Phage		
λcI857		From collection of D. Botstein
λcI857*Ots*		Obtained from Nancy Kleckner
λ*Pts*41		Obtained from Nancy Kleckner
λcI60*Kts*12		Obtained from Phil Youdarian
T5$^+$		D. Botstein
T5*ts*126		These phage are temperature-sensitive mutants isolated at M.I.T. as part of a bacterial genetics laboratory course; they are in the collection of D. Botstein
T5*ts*345		
P1Cm*clr*100		Lee Rosner (17)

original insertion was not within the *tsx*$^+$ gene but was in a controlling element or was polar on *tsx*. The putative *lon* deletion presumably restores transcription and translation, possibly by attaching the *tsx* gene to a new promoter. Although not conclusive, the following facts lead us to believe this strain carries a deletion of *lon:* (i) mutations created by abnormal excisions of lambda are frequently deletions (18); (ii) in reversion tests, no precise revertants have been detected under conditions where other *lon* mutations give revertants; (iii) the cotransduction of *proC* and *lon* is increased for this mutation over other *lon* alleles tested (Table 2).

Media and buffers. Bacteria were grown in liquid in either TBMM solution (10 g of tryptone, 5 g of NaCl per liter, 0.2% maltose, 0.01 M $MgSO_4$, and 10 μg of vitamin B_1 per ml) or in LB solution (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl per liter of water). Tryptone-B_1 plates (10 g of tryptone, 5 g of NaCl, 10 g of agar per liter and 10 μg of vitamin B_1 per ml) and LB plates (LB with 15 g of agar per liter) were used for titrating phage and assessing bacterial growth. LB-CM plates contained 12.5 μg of chloramphenicol per ml in addition to the LB ingredients. MMS plates contained 0.04% methylmethanesulfonate added to LB agar. Bacteria and phage were diluted in TMG buffer (0.01 M tris(hydroxymethyl)-aminomethane, pH 7.4, 0.01 M $MgSO_4$, 0.1 mg of gelatin per ml) and plated in TB top agar (tryptone broth + 6.5 g of agar/liter). Minimal plates contained 0.2% carbon source, M56 or M9 salts, B_1, 1.5% agar, and appropriate amino acid additions.

P1 transductions and lysates. P1Cm*clr*100 (17) lysogens were formed in strains, and lysates were made by induction, as described by Miller (15). Transductions were also done according to Miller. Pro$^+$ transductants were selected on glucose minimal plates, on which mucoid colonies can easily be distinguished from nonmucoid colonies. In experiments where the efficiency of P1 lysogenization was being measured, mid-log-phase cultures grown in LB broth at 37°C were made 0.005 M in $CaCl_2$. A 0.1-ml portion of a P1Cm*clr*100 lysate was incubated with 0.1 ml of cells for 30 min at 30°C. Appropriate dilutions of this adsorption mix were spread on LB agar and LB-CM agar and incubated at 30°C overnight. Efficiency of lysogenization was calculated as follows: no. of colonies on LB CM plates/no. of colonies on LB plates.

TABLE 2. *Cotransduction of lon and proC*

Donor genotype			*proC*$^+$ transductants[a]		
proC	*tsx*	*lon*	T6 sensitive (%)	Mucoid (%)	Mucoid *tsx*$^+$ (%)
+	+	+	35	<0.5	—
+	+	*lon*-r1	35	9	28
+	+	*lon*-t2	30	8	16
+	+	*capR6*	NT	7	NT
+	+	*lon*Δ	23	23	70

[a] Percentage mucoid determined on initial selection plates. Recipient genotype: *lac proC tsx lon*$^+$; NT, not tested; —, no mucoids observed.

Lambda burst sizes. The burst size of temperature-sensitive phage in the *lon* strains was determined as follows: SG4007 (*lon*$^+$), SG4008 (*lon-r*1), SG4009 (*lon-t*2), and SG4010 (*lon*Δ) were grown at 37°C in TBMM to an optical density at 600 nm of 0.5. Cultures were heated to 40°C, and phage was added at a multiplicity of infection of 0.05 to 0.1. After adsorption at 40°C for 10 min, the cultures were incubated with shaking at 40°C for 90 min; chloroform was added; and the resulting lysate was titered at 30°C on a wild-type permissive strain. Cells to be infected with T5 were grown in LB. Burst size was calculated as phage out/phage in; adsorption in all cases was ≥90%.

Although the *lon* strains are mucoid even on TB plates at 30°C, most phage could be titrated on these strains. Titers on the Lon$^-$ and Lon$^+$ strains at 30°C were identical. Strains carrying *gal*$^+$ *capR6* adsorbed lambda poorly, and plaques could not be seen at 30°C.

MMS sensitivity. The bacteria were grown in LB broth at 37°C to stationary phase, and appropriate dilutions were spread on LB plates and LB-MMS plates. The plates were incubated overnight at 37°C. MMS-resistant colonies were purified and tested on glucose minimal plates for degree of mucoidy. Purified colonies were also tested on MMS plates to confirm their phenotype; all colonies purified from MMS plates were as MMS resistant as the parent Lon$^+$ strain. Mucoid and nonmucoid colonies were saved and further analyzed, as discussed below.

Strain construction. The *lon* strains used (except those referred to in Table 2 as parent strains) were made by transduction into *proC lon*$^+$ backgrounds, selecting *pro*$^+$. Mucoid transductants were purified three times and tested for their other phenotypes (MMS sensitivity, λ*Ots* plating). Construction of *gal lon* strains was done by constructing an F$^-$ *proC gal*/F′(Ts) *gal*$^+$. This strain was used as a recipient for P1 transduction at 30°C, and, after purification of mucoid transductants, Gal$^-$ segregants were isolated by streaking at 40°C. The presence of the *lon* allele was confirmed in the purified segregants by streaking on a lawn of F′ *gal* or λp*gal8*, to restore mucoidy.

Measurements of degradation of nonsense and missense proteins. Nonsense fragment degradation was measured as described in Miller and Zipser (16).

Degradation of missense proteins was measured as described in Zipser and Bhavsar (24); detailed kinetics of missense degradation were carried out by the same techniques used for the long nonsense fragment of *lacZX90* as described in Miller and Zipser (16).

RESULTS

Mutations at *lon* locus have Deg phenotype. Shineberg and Zipser (19) have shown that at least one mutation with the Deg phenotype cotransduces with *proC,* as *lon* mutations do, and that Deg, UV sensitivity, and mucoidy are all transferred together. We have extended these findings with two mutations isolated for their Deg phenotype and a *lon* deletion strain. These three strains and a *capR* mutant (14)

were used as donors in transductions to a *proC tsx* host, selecting Pro$^+$ recombinants. The results of this transduction are given in Table 2. All four strains show cotransduction of mucoidy with *proC*. We have used efficiency of colony formation on agar plates containing MMS as a simple test for UV sensitivity (11). When mucoid colonies are purified and characterized, they are also all MMS sensitive. In addition, cells receiving *proC*$^+$ and mucoidy from phage T6-sensitive strains (*tsx*$^+$) also received the *tsx*$^+$ allele. These data are consistent with the map order previously reported for *lon* (9, 19).

Other properties of the *lon* strains are cotransduced with mucoidy. Each of the parental *lon* strains and their transductants in two different genetic backgrounds are sensitive to MMS; resistant colonies arise on plates at frequencies of 10^{-3} to 10^{-5} of total colony formers. The ability of these strains to be lysogenized by P1 can be measured by adsorbing P1Cm to the strains and plating for chloramphenicol (Cm)-resistant survivors. As has been previously reported by Takano (20), under conditions where *lon*$^+$ strains give 27% Cm$_r$ colonies among survivors, the *lon* strains gave 0.2% to 1% Cm$_r$ colonies. The overall frequency of surviving bacteria was not significantly different for *lon*$^+$ and *lon* strains.

To confirm that our transductants, which seem to have received the *lon* alleles, are in fact still Deg$^-$ as originally defined (2), we have compared half-times for decay of a β-galactosidase nonsense fragment in the original strains and the transductants. F′ *lac* episomes containing *lac* nonsense mutations, which produce unstable β-galactosidase fragments, were introduced into the set of transduced Deg$^-$ and *lon* deletion strains. In these merodiploids the decay of β-galactosidase fragments was followed as a function of time (Table 3). All *lon* strains have a greater persistence of the *lac* nonsense fragment, although quantitative differences appear between the different *lon* alleles and between the same allele in different genetic backgrounds. These quantitative differences are difficult to reproduce, and so not much significance can be attributed to them at this time. From these results we conclude that mucoidy, slowing of nonsense fragment degradation, defective P1 lysogenization, and UV sensitivity are all phenotypes brought about by genetic lesions at the *lon* locus.

Suppression of missense mutations in Deg$^-$ strains. Deg mutations were originally defined by their ability to stabilize the rapidly degraded protein fragments formed in the presence of puromycin (6). If the Deg pathway is the major route for ridding the cell of unusable protein, it may also be responsible for degradation of missense proteins. This hypothesis has been tested in two ways.

Zipser and Bhavsar (24) have examined a large number of *lac* missense mutations to determine the in vivo half-lives of β-galactosidase in wild-type cells. They found that a minority of such missense mutations produced β-galactosidase, which is rapidly degraded. We have examined some of these mutations in *lon* strains for stability of the β-galactosidase protein. Table 4 gives the half-life estimates for these β-galactosidase proteins in *lon*$^+$ and *lon* cells. It is apparent that the *lon* mutation stabilizes missense proteins as well as nonsense fragments.

If Deg is the primary system for degradation of missense proteins and if many missense proteins are turned over rapidly, we might expect that some classes of leaky mutations would be phenotypically suppressed by *lon* mutations. This expectation depends on two assumptions: that more of a slightly defective protein will restore function, and that degradation of defective proteins is a major pathway for lowering the activity of these proteins in the cell. To

TABLE 3. *Decay of β-galactosidase nonsense fragments in Lon$^+$ and Lon$^-$ strains*

Strain background	*lon* genotype	Half-time for decay (min)	Steady-state auto-α[a]
Parental	+	11.9	=1
	lon-t2	19.1	3.2
	lon-r1	60.0	19.1
Transductants to SG4007	+	9.2	2.9
	lon-t2	41.5	19.4
	lon-r1	14.6	3.7
	*lon*Δ	28.5	14.8

[a] The steady state level of auto-α is indicative of its rate of degradation: the more auto-α, the slower its decay.

TABLE 4. *Effect of lon-t2 and lon-r1 on degradation of missense proteins*[a]

lac genotype	*deg* genotype	Avg half-life (min)	Standard deviation	No. of expt
lacZ279	+	13.9	3.2	5
lacZ279	*lon*-r1	79	64	3
lacZ279	*lon*-t2	30	16	3
lacZ237	+	12.2	0.4	2
lacZ237	*lon*-r1	94	—	1
lacZ237	*lon*-t2	54	36	2

[a] Lac missense mutations and measurements of degradation rates are described in Zipser and Bhavsar (24).

investigate this possibility, we chose to test temperature-sensitive phage mutants. A selection of lambda and T5 temperature-sensitive mutants were spotted at 42°C on several *lon* isogenic strains. Some phage carrying temperature-sensitive mutations grew significantly better on the *lon* strains than on the parent strain, and these phage were examined further.

The most dramatic effect is seen with λ*Ots;* this phage forms plaques at an efficiency of less than 10^{-3} on a *lon*$^+$ strain, and with an efficiency of 0.1 to 0.7 on our *lon* strains. This improvement in plating efficiency cotransduces with the other parts of the Lon phenotype. Therefore, the suppression of λTs mutants can be added to the list of these phenotypes. To quantitate this effect, the burst sizes of a number of lambda and T5 temperature-sensitive mutants were determined, under the nonpermissive conditions, in *lon* strains. The results, shown in Table 5, indicate that all the Ts mutations tested are partially suppressed by growth in a *lon* strain. Some, such as the λ*Pts*, show a very small improvement in their growth relative to their growth in the wild-type strain. For others, such as the λ*Ots*, the increase in burst size is enough to allow plaque formation at the nonpermissive temperature. The results strongly suggest that degradation of many temperature-sensitive mutant proteins is carried out by the Deg system and that this destruction of protein adds to the observed defects in growth of such mutant phage.

Outside suppressors of *lon*. One way of dissecting complex phenotypes is to select revertants for one characteristic and determine what happens to the others.

lon revertants that suppress the UV-sensitive phenotype have been isolated and mapped at two sites (4, 5, 11). These are variously called *sfiA* and *sfiB* or *sulA* and *sulB*. The *sulA* and *sfiA* site cotransduce with *pyrD;* the *sulB* and *sfiB* sites cotransduce with *leu* and are unlinked to *lon*. These outside *lon* suppressors eliminate UV sensitivity, filament formation (11), and the lysogenization defect for phage λ (4). They are still mucoid and carry the original *lon* mutation.

We have isolated our own outside suppressors of *lon* and examined them for a variety of phenotypes. *lon* strains were plated on MMS plates, and resistant colonies were picked and purified. Two classes were found for each strain: mucoid and nonmucoid. On purification, both classes were totally MMS resistant. Table 6 gives the results of testing of a *lon* strain and both classes of revertants for plating of temperature-sensitive phage, lysogenization by P1, MMS sensitivity, and mucoidy. It is apparent that the nonmucoid class (class 1) of revertants acts as if it had reverted for all the measurable phenotypes of *lon*. It no longer supports growth of phage carrying temperature-sensitive mutations, and it is now lysogenized by P1 at high efficiency. The mucoid class (class 2), however, still allows growth of temperature-sensitive phage; presumably this class represents *lon sul* bacteria. The P1 lysogenization defect is suppressed by the *sul* mutation. The postulated genotypes of these strains were confirmed by P1 transduction; Table 7 shows the frequency of mucoid recombinants in a transduction to a *proC* recipient and of MMS^r among *pyrD*$^+$ recombinants after transduction to a *lon pyrD* recipient. As expected, class 1 no longer contains a *lon*-type mutation, or, if there, it is not easily separable from its suppressing mutation. Class 2, however, contains both a *lon* mutation (column 1) and a second mutation, closely linked to *pyr*D, which suppresses the MMS sensitivity of *lon* strains.

One can suppress the mucoidy of *lon* strains by blocking the pathway to synthesis of mucopolysaccharide. Mutations at a number of loci, for instance *galE*, will render *lon* cells nonmu-

TABLE 6. *Phenotypes of MMS^r Lon mutants*

Phenotype	Efficiency of plating on MMS plates	Mucoidy	Efficiency of plating λ*Ots* (40° C)	Efficiency of lysogenization P1Cm*clr* 100
+	1.0	−	5×10^{-4}	0.5
Lon-t2	10^{-4}	+	0.9	3×10^{-3}
MMS^r class 1	1.0	−	1×10^{-2}	0.75
MMS^r class 2	1.0	+	1.0	1.0

TABLE 5. *Growth of temperature-sensitive phage mutants in Deg$^-$ strains at 40° C*[a]

Genotype	Burst size (burst size as % of parental phage burst)						
	λcI857	λ*Ots*	λ*Pts*	λ*Kts*	T5$^+$	T5*ts*126	T5*ts*345
+	460	0.34 (0.07)	4.4 (0.95)	0.0037 (0.0008)	31	0.29 (0.9)	0.26 (0.8)
lon-r1	45	1.8 (4)	6.0 (13.3)	0.041 (0.09)	4.1	0.85 (2.0)	0.61 (14.8)
lon-t2	65	1.2 (1.8)	1.2 (1.8)	0.0058 (0.009)	7.2	0.50 (6.9)	0.30 (4.1)
*lon*Δ	160	1.4 (0.9)	2.0 (1.2)	0.28 (0.17)	—	—	—

[a] Burst size was calculated as phage out/phage in; adsorption in all cases was 90% or better. The data in parentheses were obtained by normalizing the burst sizes of the temperature-sensitive phage to the burst size of λcI857 or T5$^+$ in the same strains at 40° C. Further details can be found in the text.

coid. Such a mutation, of course, is not blocked in derepression of the *gal* operon, but it does allow us to test whether derepression of *gal* and the resulting mucoidy has an effect on the plating of bacteriophage, for instance, or on sensitivity to UV or MMS. Therefore, an isogenic set of strains was constructed, containing the same *lon* alleles as used above, but containing, in addition, a deletion of the *gal-bio* region of the bacterial chromosome. Such strains are nonmucoid; when an F′ *gal*$^+$ episome is introduced into the strains, these strains become mucoid. Table 8 shows the results of testing these strains for some of the *lon* phenotypes. As would be expected, the strains are still capable of supporting growth of phage with temperature-sensitive mutations at high efficiency; in fact, *capR6*, which is normally extremely mucoid, seems to allow better growth of λ*Ots* in Gal$^-$ strains than in Gal$^+$ strains. The MMS sensitivity is identical to that of the *lon gal*$^+$ parent. The P1 lysogenization defect persists but is not as marked as in *gal*$^+$ *lon* strains. The isolation of *sul* mutations in these *gal lon* strains increases P1 lysogenization frequencies at least 10-fold (data not shown). We conclude that the mucopolysaccharide overproduction does not play a significant role in determining the other phenotypes of *lon* cells.

TABLE 7. *Transduction analysis of MMS^r derivatives of Lon$^-$ strains*

Recipient genotype:	*proC lon*$^+$	*pyrD lon*	*leu lon*	Deduced genotype of donor
Selected marker:	*pro*$^+$	*pyrD*$^+$	*leu*$^+$	
Phenotype scored[a]:	% Mucoid	% MMS^r	% MMS^r	
Donor				
Wild type	0.1	5	6	*lon*$^+$ *sul*$^+$
lon-t2	9	5	6	*lon sul*$^+$
MMS^r class 1	0.3	5	6	*lon*$^+$ *sul*$^+$
MMS^r class 2	10	65	6	*lon sulA*

[a] Mucoidy was determined by appearance of colonies on the transduction plates; sensitivity to MMS was determined by restreaking *pyrD*$^+$ colonies to MMS and LB plates. All *proC*$^+$ mucoid transductants tested were also MMS sensitive.

TABLE 8. *Characteristics of gal lon strains*[a]

Lon allele	Efficiency of plating of λcI857*Ots*	Efficiency of lysogenization by P1Cm*clr*100	Efficiency of plating on MMS plates
+	<0.001	0.23	0.4
*lon*Δ	0.45	0.07	1.8×10^{-4}
capR6	0.84	0.06	1.1×10^{-5}
lon-t2	0.55	0.02	4.0×10^{-4}
lon-r1	0.85	NT	1.0×10^{-4}

[a] The *lon*$^+$ parent for these strains is SG4030. *lon* derivatives were constructed by P1 transduction. NT, Not tested.

Temperature dependence of missense protein decay. All the information that has been gathered about the Deg system indicates that this is the system responsible for decay of nonsense and missense proteins in the cell. It had been observed previously that decay of amber fragments was extremely temperature dependent and proceeds much more rapidly at high temperatures than at low (12); Table 9 demonstrates that this temperature dependence of decay also holds for missense proteins. Since we have already demonstrated (Table 4) that this degradation is dependent upon the *lon* gene, we can predict that other *lon*-dependent degradations will also be temperature dependent. It is not at all clear whether this reflects a high temperature coefficient for the protease involved in degradation or an increase in the unfolding of nonfunctional proteins at high temperature and, therefore, increased susceptibility to degradative proteases.

***lon*$^+$/*lon* partial diploids.** It has been reported that some *lon* mutations are recessive in merodiploids, whereas others, presumed to be nonsense mutations, seem to be dominant under some conditions. In particular, Markovitz and Rosenbaum (14) have found that a *lon* mutation (*capR9*) is dominant for mucoidy when it is on an episome but not when on the chromosome. This mutation was selected in a partial diploid for the *lon* region.

An F′ 13 episome was introduced into an isogenic set of *lac lon str* strains by selecting for Lac$^+$ Sm^r. The colonies growing upon minimal lactose plates were purified and tested for the presence of *lac* and *lon*. The same culture was plated on MMS plates, lactose minimal plates, and used as lawns to titer λ*Ots* stocks at 40°C (Table 10). It is clear that the *lon* alleles on the chromosome are recessive for mucoidy, since less than 5% of colonies are mucoid under conditions where greater than 90% of the haploid parent strains are mucoid. MMS sensitivity also appears to be recessive, as has been found pre-

TABLE 9. *Temperature effects on missense degradation*[a]

Expt	Temp	Half-life (min)
1	32	22.3
2	32	35.4
1	37	14.3
2	37	14.7
1	42	5.1
2	42	5.7

[a] A strain carrying *lacZ279* missense mutation was used.

TABLE 10. *Expression of Lon phenotypes in Lon⁺/⁻ diploids*[a]

Relevant genotypes	Efficiency of plating on MMS plates	Efficiency of λ*Ots* plating	% Mucoid
F_{13}' *lac⁺ lon⁺/proC lac lon⁺*	0.84	10^{-3}	0.1
F_{13}' *lac⁺ lon⁺/lac lon-r1*	0.23	10^{-3}	5
F_{13}' *lac⁺ lon⁺/lac lon-t2*	0.46	10^{-2}	3
F_{13}' *lac⁺ lon⁺/lac lonΔ*	0.13	NT	2
F_{13}' *lac⁺ lon⁺/lac capR6*	0.23	10^{-2}	1

[a] Mucoid, Lac⁻ segregants could be isolated from 50% of the non-mucoid colonies growing on *lac* minimal plates. Colonies isolated from MMS plates do not segregate mucoid colonies, although many still segregate Lac⁻ colonies. This is apparently due to the rapid segregation of the episome on MMS plates combined with the strong selection against *lon* cells on such plates. NT, Not tested.

viously for the UV sensitivity defect of *lon* strains (22).

DISCUSSION

A dividing *E. coli* must necessarily correlate chromosome and plasmid replication and distribution to daughter cells, cell wall synthesis, septa formation, the deposition of the mucopolysaccharide layer, and certainly many more processes. *lon* mutants appear to have a major disturbance in some of these processes. Mucopolysaccharide is strikingly overproduced; septa cease forming after UV irradiation, leading to long, snakelike filaments and eventually to cell death. What is the mechanism of this disturbance? In the experiments described above we have begun to investigate the interrelationship of the *lon* phenotypes. One can reach the following conclusions: *deg* mutants and *lon* mutants are not distinguishable by any of their phenotypes or mapping characteristics. All the *lon* mutants tested except *capR9* (selected in a diploid) are recessive to wild type. Revertants can be found that revert all phenotypes simultaneously. Therefore, at the least, we conclude that these are single mutations, presumably in the same operon and possibly in the same gene.

In addition, we have been able to extend the range of proteins on which the Deg system is known to act. Not only does it degrade grossly abnormal proteins, such as puromycin fragments or amber fragments, but, in addition, it degrades some classes of missense proteins. Since *lon* strains now allow the growth of some temperature-sensitive phages, we postulate that degradation contributes to the defect found in some missense mutations. This effect of *lon* strains on some missense proteins has been independently observed by J. Schrenk and D. Morse (personal communication). This raises the possibility that some effects of *lon* in apparently suppressing mutations in a variety of phage and bacterial genes—for instance, in *cro* (23)—is not due to involvement of the *lon* product in *cro* regulation but simply represents "saving" of the defective *cro* protein so that its residual activity can be expressed.

Every T5 and λ*ts* mutant whose burst size was measured showed an improvement in the *lon* strains. A large number of T4 mutations with temperature-sensitive defects in late genes showed no such improvement (data not shown). This may be the result of the inactivation of the Deg system after T4 infection, since T4 nonsense fragments do not degrade (Larry Gold, personal communication). The observation that *lon* simultaneously reduces protein degradation and labilizes the cell to division anomalies perhaps adds some support to the theory that short-lived proteins play a role in the division cycle.

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