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# Mutations in Bacteriophage λ Repressor That Prevent RecA-Mediated Cleavage

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**In this paper, we report on the isolation and sequence analysis of mutations that confer an induction-deficient phenotype to λ repressor. A total of 16 different mutations, which occur at 13 different sites in the repressor gene, have been characterized. For most of the mutant lysogens, frequencies of spontaneous induction in a *recA*$^+$ strain were reduced dramatically in comparison with those for a wild-type phage, and these mutant lysogens showed little or no prophage induction after UV irradiation. The immunity properties of cells containing the mutant repressors show that all of the mutants but one exhibit operator-binding properties indistinguishable from wild-type repressor.**

When lysogens of temperate phages such as λ, 434, and P22 are treated with UV light or other inducing agents, the prophage repressor is cleaved, and a program of lytic development begins (31). This cleavage of repressor protein does not occur in a *recA* cell, and cleavage under physiological conditions in vitro requires RecA protein (7, 32, 33). Another repressor, the cellular LexA protein, is also subject to RecA-mediated cleavage (18). Cleavage of LexA repressor results in the induction of the cellular SOS response (19).

Jacob and Campbell (12) originally showed that a mutation in λ repressor could prevent UV-mediated prophage induction, and a number of such *ind* mutations have now been mapped to the C-terminal region of the repressor gene (10, 16). Repressor mutations, termed *ind*$^s$, which allow induction under conditions where the wild type is not induced, have also been described (6). In order to better define the structural determinants which are required for RecA-mediated cleavage of λ repressor, we isolated and sequenced a set of *ind* mutations in the λ repressor gene *c*I. Here, we report the sequences of 35 independent *ind* mutations, the inferred DNA-binding properties of these *ind* mutant repressors, and the sequence of the *ind*$^s$*-1* mutation.

## MATERIALS AND METHODS

**Strains and media.** The *Escherichia coli* strains, bacteriophages, and plasmids used in this study are listed in Table 1. Trypticase medium is 1% Trypticase (BBL Microbiology Systems) and 0.5% NaCl. LB broth contains 1% tryptone, 0.5% yeast extract, 0.5% NaCl, and 1 mM NaOH. λ top agar contains 1% tryptone, 0.25% NaCl, and 0.65% agar.

**Enzymes and chemicals.** Materials were obtained from the following sources: restriction enzymes, T4 DNA ligase, and M13 pentadecamer sequencing primer from New England Biolabs, Inc.; the Klenow fragment of *E. coli* DNA polymerase and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside from Boehringer Mannheim Biochemicals; dideoxy and deoxynucleotide triphosphates from P-L Biochemicals, Inc.; [α-$^{32}$P]ATP (400 Ci/mmol) from Amersham Corp.; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine from Aldrich Chemical Co., Inc.; hydroxylamine hydrochloride from Fisher Scientific Co.; and mitomycin C from Sigma Chemical Co.

**Isolation of *ind* mutants.** λ *ind* mutants were identified by the screening procedure described by Mount (26). This method relies on the observation that wild-type λ forms clear plaques on strains DM1187 (*recA441 sulA lexA3-51*), whereas *ind* derivatives form turbid plaques on this strain. The clear-plaque phenotype of wild-type phage is presumed to occur because λ repressor is cleaved constitutively as a consequence of the increased activity and concentration of the *recA441* protein present in strain DM1187.

Phage carrying the *b*2 deletion (λ *b*2 *c*I$^+$) were used for all mutagenesis experiments. Nitrosoguanidine or hydroxylamine mutagenesis was performed by standard procedures (8, 26). For *mutD* mutagenesis, phage stocks were prepared on strain LE30 carrying the *mutD5* allele. UV mutagenesis was performed by suspending phage ($10^8$ PFU/ml) in 0.01 M $MgSO_4$ and irradiating 2 ml of the solution at a dose of ca. 120 J/m$^2$. The irradiated solution (10 μl) was combined with 0.1 ml of log-phase 294(pGW249) cells which had been irradiated with 5 J/m$^2$ of UV light in 0.01 M $MgSO_4$. After absorption for 20 min at room temperature, phage stocks were prepared by growth at 37°C on Trypticase plates for 5 h. For each of the four methods of mutagenesis, the frequency of clear-plaque formers on strain 294 was found to be 0.5 to 2%, and the frequency of turbid-plaque formers on strain DM1187 was found to be 0.1 to 1%. From the DM1187 plating, a single turbid plaque was picked for each independent mutagenesis experiment. Candidates were plaque purified twice on strain DM1187.

**Construction of plasmid pFG541.** Plasmid pKB280/UV44 (3) is a derivative of the tetracycline-resistant plasmid pMB9 that contains the λ *c*I gene cloned under transcriptional control of a *lacUV5* promoter. The *c*IUV44 mutation (M. Hecht, H. C. M. Nelson, and R. T. Sauer, unpublished data) creates a *Cla*I site in the N-terminal coding region of the gene. The 1,776-base-pair *Eco*RI-*Sal*I fragment, containing the repressor gene and *lac* promoter, was excised from pKB280/UV44 DNA, gel purified, and ligated to a preparation of *Eco*RI- and *Sal*I-cut pBR322. After transformation into strain GM48, Tet$^r$ colonies were selected, and plasmid DNA was prepared and screened by restriction analysis for the presence of the 1,766-base-pair *Eco*RI-*Sal*I insert. A plasmid with the correct structure (pFG101) was digested with *Bam*HI and *Bcl*I and was ligated with *Bgl*II- and *Bcl*I-digested λ *3v* DNA. After transformation of strain 294, colonies resistant to ampicillin and phage T4 Δ*r*II were selected. (T4 Δ*r*II resistance selects for transformants which have acquired the *rexA* and *rexB* genes of phage λ.) Plasmid

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DNA was prepared from a single survivor and was cut with *Cla*I. The large backbone fragment was gel purified, religated at low DNA concentration, and transformed into strain 294. Amp$^r$ transformants were selected and screened for sensitivity to T4 Δ*r*II (the deletion of the desired *Cla* fragment removes a portion of the *rexA* gene). A candidate was picked and restriction mapped to confirm the structure shown in Fig. 1. This plasmid is called pFG541.

**Transductional crosses.** Mutations were transferred from the phage *c*I gene to a plasmid *c*I gene by transductional crosses between λ *b*2 *c*I *ind* phage and plasmid pFG541. pFG541 contains λ homology on both sides of the *c*I-*rexA* deletion, and thus an intact *c*I gene can be reconstituted by two homologous recombination events. Since pFG541 contains codons for only the N-terminal 55 (of 236) residues of repressor, recombinant events that generate an intact plasmid *c*I gene must transfer any mutations present in the C-terminal 181 codons of the phage *c*I gene. The structure of such plasmids, as represented by pFG300, is shown in Fig. 2.

Plate stocks of the λ *b*2 *c*I *ind* phage were prepared on strain 294(pFG541) and were used to transduce ampicillin resistance to a naive strain. To do this, ca. $10^8$ log-phase XA102 cells were infected with phage at a multiplicity of infection of approximately one. After absorption for 20 min at room temperature, the cells were grown with aeration for 2 h at 37°C. Cells (0.2 ml) were diluted into 1 ml of LB broth, and aliquots were plated on LB-ampicillin (100 μg/ml) plates, which were seeded with ca. $10^9$ phage each of λ *3v* and λ *3v h*80. The virulent phages select for recombinant plasmids which have acquired an intact *c*I gene and as a consequence are immune. Surviving colonies arose at a frequency of ca. $10^{-4}$ per input phage. Candidates were repurified, and plasmid DNA was prepared and analyzed for the expected structure by restriction mapping.

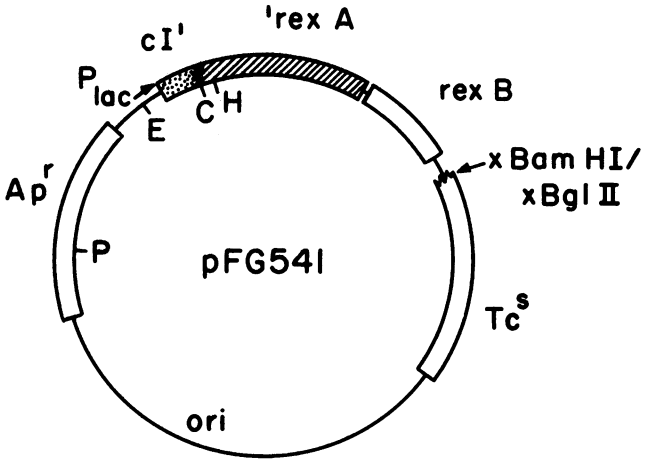


FIG. 1. Physical map of pFG541. The stippled and cross-hatched areas represent a fusion of the N-terminal portion of the *c*I gene and the C-terminal portion of the *rexA* gene. Fusion between these genes occurs at a *Cla*I site. The arrow indicates the direction of transcription from the *lac* promoter. This plasmid contains the beta-lactamase gene (Amp$^r$) but is deleted for part of the tetracycline gene. Its construction is detailed in the text. P, *Pst*I; E, *Eco*RI; C, *Cla*I; H, *Hin*dIII.

TABLE 1. *E. coli* strains, plasmids, and bacteriophages

| Strain, plasmid, or bacteriophage | Genotype | Source or reference |
|---|---|---|
| 294 | *endA hsdR thi pro* | (3) |
| XA102 | *ara thi argE*(Am) Rif$^r$ Nal$^r$ Δ(*lac-pro*) *metB supE* | (25) |
| DM1187 | F$^-$ *his-4 recA441 sulA11 lexA3-51* | (27) |
| LE30 | W3350 *mutD5* Str$^r$ Azi$^r$ *galU95* (*galKTE*$^+$) | L. Enquist |
| GM48 | *thr leu thi lacY galK galT ara tonA tsx dam dcm supE44* | (21) |
| GW5100 | P1 cured derivative of JM103 | G. Walker |
| GW5180 | GW5100 with *cam* insertion in *recA* | G. Walker |
| KL731 | F'116 in KL110 (*leuB6 tonA2 lacY1 supE44 gal-6* λ$^-$ *hisG1 recA1 thyA23 malA1 arg-6 rpsL104 xyl-7 mtl-2 metB1*) | (20) |
| RR1 | F$^-$ derivative of GW5100 | This laboratory |
| RR2 | F$^-$ derivative of GW5180 | This laboratory |
| X90 | F' *lac pro I*$^{q1}$ *ara* Δ(*lac-pro*)*13 nalA argE*(Am) *thi* Rif$^r$ | (1) |
| pKB280 | Tet$^r$ p*lac*-λ *c*I fusion in pMB9 | (3) |
| pGW249 | pKM101 *bla*::Tn*5* | (15) |
| pBR322 | Tet$^r$ Amp$^r$ *ori* ColE1 | (5) |
| pLR1 | *ind*$^s$*-1* version of pKB252 | David Burbee |
| pTP7 | Tet$^r$ p*lac*-P22 *c*II fusion | (30) |
| λKH54 | λ *c*I deletion | (4) |
| λ *c*I *c*17 | λ *c*190 | (39) |
| λ *vir* | *v*2 *v*1 *v*3 | (13) |
| λ *3v h*80 | | This laboratory |
| λ *3v* | *v*2 *v*3c *vs*326 | J. Eliason |
| λ *4v* | *v*2 *v*305 *v*3c *vs*326 | J. Eliason |
| λ *5v* | *v*2 *v*305 *v*3c *vs*326 sv1 | J. Eliason |
| λ *6v* | | J. Eliason |
| λ *b*2 *c*I$^+$ | | This laboratory |
| λ p*lac*5 *imm*$^{21}$ | | This laboratory |
| T4 *r*IIΔ187 | | Fred Winston |
| M13mp8 | | (24) |
| M13mp8 APP1 | Δ*lac* version of M13mp8 | A. Pakula |

**DNA sequence determination.** The positions of potential *ind* mutants were determined by using either the chemical method of Maxam and Gilbert (23) or the chain elongation method of Sanger (34). For dideoxy sequencing, the 581-base *Eco*RI-*Hin*dIII fragment containing the N-terminal two-thirds of *c*I and the 458-base *Hin*dIII-*Pst*I fragment contain-

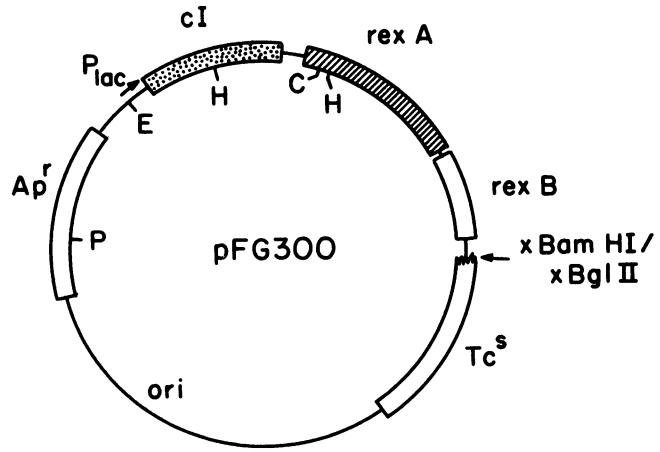


FIG. 2. Physical map of pFG300. The intact *c*I gene is shown as a stippled box, and the *rexA* gene is shown as a cross-hatched box. Plasmids with this structure were produced for each *c*I *ind* gene by the transductional scheme described in the text.

TABLE 2. Sequence changes for 16 different representative *ind* mutants

| Code | Amino acid | Change | Base pair | Codon change | Mutagen[a] | No. of isolates | Plating Phenotype[b] on strain: | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | DM1187 | 294 |
| PL104 | 104 | Pro→Leu | 314 | CCT→CTT | HA | 3 | T | T |
| AT111 | 111 | Ala→Thr | 334 | GCA→ACA | NG | 2 | T | T |
| GR112 | 112 | Gly→Arg | 337 | GGG→AGG | HA, NG | 2 | T | T |
| GE112 | 112 | Gly→Glu | 338 | GGG→GAG | HA, MD, NG | 5 | T | T |
| EK117a | 117 | Glu→Lys | 352, 354 | GAG→AAA | HA | 1 | T | T |
| EK117b | 117 | Glu→Lys | 352 | GAG→AAG | HA, UV | 5 | T | T |
| RK119 | 119 | Arg→Lys | 359 | AGA→AAA | UV | 1 | T | T |
| TI122 | 122 | Thr→Ile | 368 | ACC→ATC | HA | 2 | T | T |
| GD124/DV125 | 124 | Gly→Asp | 374 | GGT→GAT | UV | 1 | T | T |
| | 125 | Asp→Val | 377 | GAT→GTT | | | | |
| DN125 | 125 | Asp→Asn | 376 | GAT→AAT | HA | 1 | T | T |
| DY125 | 125 | Asp→Tyr | 376 | GAT→TAT | HA | 1 | T | T |
| EK127 | 127 | Glu→Lys | 382 | GAG→AAG | HA | 1 | FT | T |
| LF143 | 143 | Leu→Phe | 430 | CTT→TTT | HA | 2 | T | T |
| GD147 | 147 | Gly→Asp | 443 | GGT→GAT | HA, UV | 3 | FT | FT |
| GR185 | 185 | Gly→Arg | 556 | GGG→AGG | UV, HA | 2 | T | T |
| GE185 | 185 | Gly→Glu | 557 | GGG→GAG | HA, UV | 2 | T | T |
| FL189 | 189 | Phe→Leu | 568 | TTT→CTT | UV | 1 | T | T |

[a] HA, hydroxylamine; MD, *mutD*; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.
[b] T indicates that the phage plates turbid on the strain; FT indicates that it plates faintly turbid.

ing the C-terminal one-third of *c*I were cloned into M13appl and M13mp8 respectively.

**Spontaneous and UV induction of *ind* lysogens.** Attachment sites were crossed into the λ *b*2 *c*I *ind* phage to allow formation of stable lysogens. Each λ *b*2 *c*I *ind* parent was crossed with λ $imm^{21}$ p*lac*5, and the progeny were plated on strain 294(pTP7) by using Trypticase plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. Plasmid pTP7 (30) overproduces P22 repressor and prevents plating of the $imm^{21}$ parent or any $imm^{21}$ recombinants. Immunity λ recombinants containing the attachment site were identified as blue plaque formers; the λ *b*2 deletion covers the attachment site and the site of the p*lac*5 substitution, and thus phage that acquire the p*lac*5 substitution also acquire the attachment site.

Spontaneous and UV induction were measured by using a modified version of the procedure of Mount (26). Overnight cultures of both $recA^+$ and *recA* lysogens were harvested by centrifugation, washed with LB broth, reharvested, and then resuspended into LB broth. Portions of these cultures were diluted 100-fold into LB broth and were grown for 4 h at 37°C. These log-phase cultures were then diluted into 0.01 M $MgSO_4$ to give a concentration of $2 \times 10^6$ cells per ml. A portion of the $recA^+$ culture was irradiated with a dose of 20 J/m$^2$. The irradiated and nonirradiated cells were diluted into LB broth and were allowed to grow for 40 min at 37°C. After this time, the number of viable cells was determined by direct plating and the number of PFU was determined by plating on Trypticase tetracycline plates with 294(pBR322) as an indicator strain.

**Inducibility phenotypes of mutant repressors on plasmids.** Cells harboring $ind^+$ and *ind* pFG300 derivatives are immune to superinfection by λ due to the overproduction of λ repressor by the *lac* promoter (3). However, clear-plaque derivatives of λ will superinfect a strain containing an $ind^+$ plasmid if the strain also contains the $lacI^q$ allele, which directs overproduction of *lac* repressor, and if RecA-mediated cleavage of λ repressor is induced by mitomycin C (6). In the case of a strain containing an *ind* plasmid, little or no mitomycin C-induced, RecA-mediated cleavage occurs, and a clear λ phage is unable to superinfect the strain. This difference between strains containing $ind^+$ and *ind* plasmids can be used to distinguish them.

Derivatives of plasmid pFG300, containing mutant or wild-type repressor genes, were transformed into strain X90 containing the $lacI^{q1}$ allele. Log-phase cultures (0.2 ml) were combined with 2.5 ml of λ top agar and were plated onto Trypticase plates or Trypticase plates containing 0.7 μg of mitomycin *C* per ml. Approximately $3 \times 10^8$ clear phage (λKH54) were spotted on the plates, which were incubated overnight at 37°C. In the presence of mitomycin *C*, λKH54 forms a clear spot on X90 strains bearing $ind^+$ repressors and a turbid spot on X90 strains bearing *ind* repressors. In the absence of mitomycin *C*, λKH54 forms turbid spots on strains bearing $ind^+$ or *ind* repressors.

**Immunity properties.** Strains bearing overproducing $ind^+$ and *ind* pFG300 plasmids were cross-streaked against at least $10^6$ clear or virulent derivatives of phage λ on LB-ampicillin (100 μg/ml) plates. The plates were incubated for 5 h at 37°C and were then examined. The immunity properties determined by the cross-streak test indicate the relative level of repressor in a strain. For example, strains bearing lysogen levels of wild-type repressor are resistant to λKH54; strains bearing 14-times-higher concentrations of repressor are resistant to λ *3v*; and 110, 350, and 1,000 times the lysogen levels of repressor are required to mediate immunity to λ *4v*, λ *5v*, and λ *6v* respectively (M. Hecht and R. T. Sauer, manuscript in preparation). Repressor concentrations between 2 and 14 times the lysogen levels are required for immunity to λ *c*I *c*17 and λ *vir*, but precise repressor levels within this range have not been determined.

## RESULTS

**Mutant isolation.** Mount (26) has shown that λ *c*I *ind* phage form turbid plaques on strain DM1187, whereas λ *c*I $ind^+$ phage form clear plaques on this strain (see above). By screening mutagenized λ stocks for turbid plaque formers on strain DM1187, we obtained 39 independent candidates for phage bearing *ind* mutations. Most of the mutant phage were found to form normal turbid plaques on the 294 control strain (Table 2), suggesting that the corresponding phage repressors have nearly wild-type operator DNA-binding activity.

Repressors with increased activity might be expected to cause the formation of deeply turbid plaques on strain 294, whereas those with decreased activity would be expected to result in the formation of lightly turbid plaques on this strain. Three candidates, including GD147, formed lightly turbid plaques on strains DM1187 and 294, and one candidate (EK127) formed a lightly turbid plaque on strain DM1187 but a normal turbid plaque on strain 294.

**Sequences of *ind* and *ind*$^s$ mutations.** Before DNA sequencing, the major portion of the repressor gene (*c*I) from each mutant phage was crossed onto an overproducing plasmid as described above. All *c*I *ind* mutations described to date have been mapped in the C-terminal region of the repressor gene (10, 16), and the crossing procedure was designed to ensure that any mutation in the C-terminal 181 residues of repressor would be crossed from the phage to the plasmid. This region of the plasmid *c*I gene was then sequenced for each mutant candidate. For 35 of the 39 candidates, mutations in the C-terminal coding region of the repressor gene were found, and for 34 of these 35, the mutation resulted in the alteration of a single amino acid. Many of the independent mutations have the same sequence changes. Overall, the collection contains a total of 16 different mutations, affecting 13 residue sites. The sequence changes for representatives of the 16 different mutants are shown in Table 2, and these representative mutants were used for all further studies. For the four candidates for which *c*I mutations were not found by DNA sequencing, it was subsequently shown that the plasmid-encoded repressor did not have an *ind* phenotype (see below). We presume that either the original phage candidates were not *ind* or the *ind* mutations, if present, was not located within the C-terminal coding region of repressor.

To ensure that the sequence changes found in the C-terminal domains of the plasmid-encoded repressors were indeed responsible for the *ind* phenotype of the parental phages, spot tests were performed in the presence and absence of mitomycin C (see above). In the presence of mitomycin, λKH54 produced a clear spot on strains carrying wild-type repressor and on the four plasmid strains for which *ind* mutations were not found. This phage produced a turbid spot on each of the strains carrying repressor plasmids with mutations in the C-terminal domain. In the absence of mitomycin C, λKH54 produced a turbid spot on all of the plasmid strains tested. These results indicate that the sequenced *c*I mutations are responsible for the *ind* phenotypes of the parental phages.

The λ *c*I *ind*$^s$*-1* mutation was also sequenced and was found to encode a missense mutation ($Glu^{233}$→Lys, GAG→AAG) located four residues from the carboxy terminus of the protein. The identity of the *ind*$^s$*-1* sequence change was confirmed by the observation that an *Mbo*II restriction site, present in the wild-type gene, was eliminated by this mutation.

**Activity of the mutant repressors.** The immunity properties of strains bearing *ind* repressor plasmids provide additional information about the operator-binding activities of the mutant repressors. Such results are summarized in Table 3. In the *lacI* deletion strain XA102, the levels of λ repressor expressed from the *lac* promoter are high and, with one exception, the *ind* mutant plasmids, like the wild type, confer immunity to the most virulent phage tested. In strains W3110 *lacI*$^q$ and X90 *lacI*$^{q1}$, λ repressor levels are reduced by the overproduction of *lac* repressor. In strain W3110 *lacI*$^q$, wild-type and 15 of the *ind* mutant plasmids confer immunity to λ *vir* but not to λ 3*v*. In strain X90 *lacI*$^{q1}$, wild-type and 15 of the *ind* mutant plasmids confer immunity to λ *c*I *c*17 but not to λ *vir*. These results suggest that the activities in vivo of all but one of the *ind* mutant repressors are similar to wild-type.

TABLE 3. Immunity properties of the mutant plasmids in different backgrounds

| Allele | Strains and phages[a] | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | XA102 | | | | | | | W3110 *lacI*$^q$ | | | | | | | X90 *lacI*$^{q1}$ | | | | | | | | | | | |
| | A | B | C | D | E | F | G | A | B | C | D | E | F | G | A | B | C | D | E | F | G | | | | | |
| WT | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| PL104 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| AT111 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| GR112 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| GE112 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| EK117a | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| RK119 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| TI122 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| GD124/ DV125 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| DN125 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| DY125 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| EK127 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| LF143 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| GD147 | + | + | + | + | + | | | + | / | | | | | | / | | | | | | | | | | | |
| GR185 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| GE185 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |
| FL189 | + | + | + | + | + | + | + | + | + | + | | | | | + | / | | | | | | | | | | |

[a] Phages: A, λKH54; B, λ *c*I *c*17; C, λ *vir*; D, λ *3v*; E, λ *4v*; F, λ *5v*; G, λ *6v*. +, A strain bearing the repressor gene on an overproducing plasmid survived infection by the indicated phage during the cross-streaking experiment; /, partial survival. The absence of a symbol indicates no survival. WT, Wild type.

In each of the three strains tested, the GD147 plasmid conferred less immunity than wild-type or the other *ind* mutant plasmids. This finding suggests that the GD147 mutant repressor is less active in binding operator DNA. This conclusion is also consistent with the semiclear plating phenotype of the GD147 phage and of two independently isolated *ind* phages which have the same mutation as GD147.

**Spontaneous and UV-mediated prophage induction.** To directly test the inducibility of the λ *c*I *ind* phages, lysogens were constructed in *recA*$^+$ and *recA* hosts, and levels of spontaneous and UV-induced prophage induction were measured. The results for spontaneous induction are listed in Table 4. In the *recA*$^+$ strain, most of the *ind* phage showed levels of spontaneous induction at least three orders of magnitude lower than those of the *ind*$^+$ control. Spontaneous induction of the GD147 and EK127 mutants was similar to the wild type in the *recA*$^+$ strain. In the *recA* strain, the GD147 mutant retained a significant level of spontaneous induction, whereas wild-type and the remaining *ind* phage, including EK127, showed no spontaneous induction (only clear phage were spontaneously released from these lysogens).

UV-mediated prophage induction was also measured for *recA*$^+$ lysogens of each *ind* mutant and for the wild-type *ind*$^+$ control. These data are shown in Table 5. As expected, lysogens of most of the *ind* mutants showed little or no prophage induction after UV irradiation. Mutant GD147 gave about the same number of plaques before and after UV irradiation, and mutant EK127 showed only a small increase in the number of plaques after UV induction.

**Summary of mutant phenotypes.** Of the 16 different *c*I *ind* mutations, 14 affect RecA-mediated inducibility without affecting other repressor functions. Phage bearing the

TABLE 4. Spontaneous induction of lysogens

| Allele | *recA*$^+$ host | | *recA* host | |
|---|---|---|---|---|
| | No. of viable cells per 0.1 ml | No. of turbid plaques per 0.1 ml | No. of viable cells per 0.1 ml | No. of turbid plaques per 0.1 ml |
| Wild type | 32,000 | 550 | 18,000 | 0 |
| PL104 | 8,500 | 2 | 9,200 | 0 |
| AT111 | 37,000 | 1 | 9,900 | 0 |
| GR112 | 54,000 | 0 | 15,000 | 0 |
| GE112 | 42,000 | 0 | 18,000 | 0 |
| EK117a | 21,000 | 0 | 13,000 | 0 |
| RK119 | 35,000 | 8 | 12,000 | 0 |
| TI122 | 40,000 | 5 | 17,000 | 0 |
| GD124/DV125 | 40,000 | 0 | 7,200 | 0 |
| DN125 | 24,000 | 9 | 17,000 | 0 |
| DY125 | 19,000 | 0 | 18,000 | 0 |
| EK127 | 22,000 | 239 | 28,000 | 0 |
| LF143 | 17,000 | 0 | 20,000 | 0 |
| GD147 | 2,300 | 344 | 1,700 | 473 |
| GR185 | 38,000 | 0 | 13,000 | 0 |
| GE185 | 19,000 | 0 | 16,000 | 0 |
| FL189 | 45,000 | 0 | 15,000 | 0 |

Pro$^{104}$→Leu, Ala$^{111}$→Thr, Gly$^{112}$→Arg, Gly$^{112}$→Glu, Glu$^{117}$→Lys, Arg$^{119}$→Lys, Thr$^{122}$→Ile, Gly$^{124}$Asp$^{125}$→AspVal, Asp$^{125}$→Asn, Asp$^{125}$→Tyr, Leu$^{143}$→Phe, Gly$^{185}$→Arg, Gly$^{185}$→Glu, and Phe$^{189}$→Leu amino acid substitutions plate turbid on strain DM1187 and show greatly reduced levels of RecA-mediated spontaneous or UV induction. The mutant repressors bearing these substitutions have activities comparable to that of the wild type when assayed by the plating properties of the mutant phage or the immunity properties conferred by mutant plasmids.

Repressors containing the Gly$^{147}$→Asp substitution (GD147) have reduced DNA-binding activity by the following criteria: (i) phage bearing this mutation form only a lightly turbid plaque on strain 294; (ii) prophage bearing this mutation induce spontaneously at a very high frequency in both *recA*$^+$ and *recA* strains; (iii) the plasmid-mediated immunity of this mutant repressor is significantly less than that of wild-type repressor. The reduced DNA binding of the GD147 mutant repressor makes it somewhat difficult to assess the effect of the mutation on RecA-mediated inducibility. However, UV irradiation does not increase the frequency of induction of GD147 lysogens above spontaneous levels. Moreover, in the presence of mitomycin C, a strain which overproduces this mutant repressor gene does not support the growth of λKH54. Both observations suggest that the GD147 mutant repressor is less sensitive than the wild type to RecA-mediated cleavage.

The Glu$^{127}$→Lys mutation appears to be a leaky *ind* mutation. The levels of RecA-mediated spontaneous induction and UV induction for this mutant are reduced ca. 2-fold and 20-fold, respectively, in comparison with wild-type levels, whereas most of the *ind* mutants show much greater reductions in induction levels. Moreover, phage bearing this mutation form only lightly turbid plaques on strain DM1187 but form normal turbid plaques on strain 294. This latter fact, the plasmid immunity data, and the low level of spontaneous induction observed in *recA* strains suggest that repressors containing the Glu$^{127}$→Lys substitution have normal DNA-binding activity.

## DISCUSSION

The results presented here show that 16 different amino acid substitutions in the λ *c*I repressor result in a noninducible or poorly inducible phenotype. The noninducible mutations occur at 13 residue sites, and 9 of the sites cluster in a 25-residue sequence spanning the central portion of the repressor sequence (Fig. 3). It is within this sequence that the RecA-mediated cleavage of repressor occurs. The data presented here do not prove that the *ind* repressors are resistant to RecA-mediated cleavage. However, preliminary results obtained with six different purified *ind* repressors show this to be the case (F. S. Gimble and R. T. Sauer, manuscript in preparation), and we anticipate that the remaining *ind* mutant repressors will also be resistant to RecA-mediated cleavage.

The site of RecA-mediated cleavage has been identified for four different repressors, including λ repressor, 434 repressor, P22 repressor, and LexA repressor, and in each case, proteolysis occurs at an Ala-Gly dipeptide sequence (11, 37; R. R. Yocum, personal communication). This sequence lies between the N-terminal DNA-binding domain and the C-terminal oligomerization domain in each protein (2, 9, 29). Three of the *c*I *ind* mutations characterized here affect the Ala$^{111}$-Gly$^{112}$ cleavage site of λ repressor. The Ala$^{111}$→Thr, Gly$^{112}$→Glu, and Gly$^{112}$→Arg substitutions result in tight *ind* phenotypes, and we presume that these mutant repressors are not efficiently cleaved in the RecA-mediated reaction. In LexA repressor, substitution for the Ala-Gly cleavage sequence by Ala-Asp is known to result in a noninducible phenotype and greatly reduced RecA-mediated cleavage (18, 22). Taken together, these results show that the RecA-mediated cleavage is strongly sequence specific. It is not clear that Ala-Gly is the only sequence that can be efficiently cleaved. For example, Ala-Ala, Gly-Ala, or Gly-Gly dipeptides might also be susceptible to cleavage. Nevertheless, the λ *c*I *ind* mutations indicate that cleavage depends on the identity of both residues bordering the cleavage site.

TABLE 5. UV induction in a RecA$^+$ strain[a]

| Allele | No. of turbid plaques: | |
|---|---|---|
| | Per 0.1 ml | Relative to wild type[b] |
| Wild type | 13,000 | 1.0 |
| PL104 | 1 | 0.00008 |
| AT111 | 0 | 0 |
| GR112 | 0 | 0 |
| GE112 | 0 | 0 |
| EK117a | 0 | 0 |
| RK119 | 12 | 0.00092 |
| TI122 | 17 | 0.0013 |
| GD124/DV125 | 0 | 0 |
| DN125 | 12 | 0.00092 |
| DY125 | 0 | 0 |
| EK127 | 626 | 0.048 |
| LF143 | 0 | 0 |
| GD147 | 382 | 0.029 |
| GR185 | 0 | 0 |
| GE185 | 0 | 0 |
| FL189 | 0 | 0 |

[a] Since UV induction was measured simultaneously with spontaneous induction, the number of viable cells for the *recA*$^+$ strains is identical to that shown in Table 4.

[b] This number is a ratio of the number of turbid plaques produced by the indicated lysogen relative to the number produced by the wild-type lysogen.

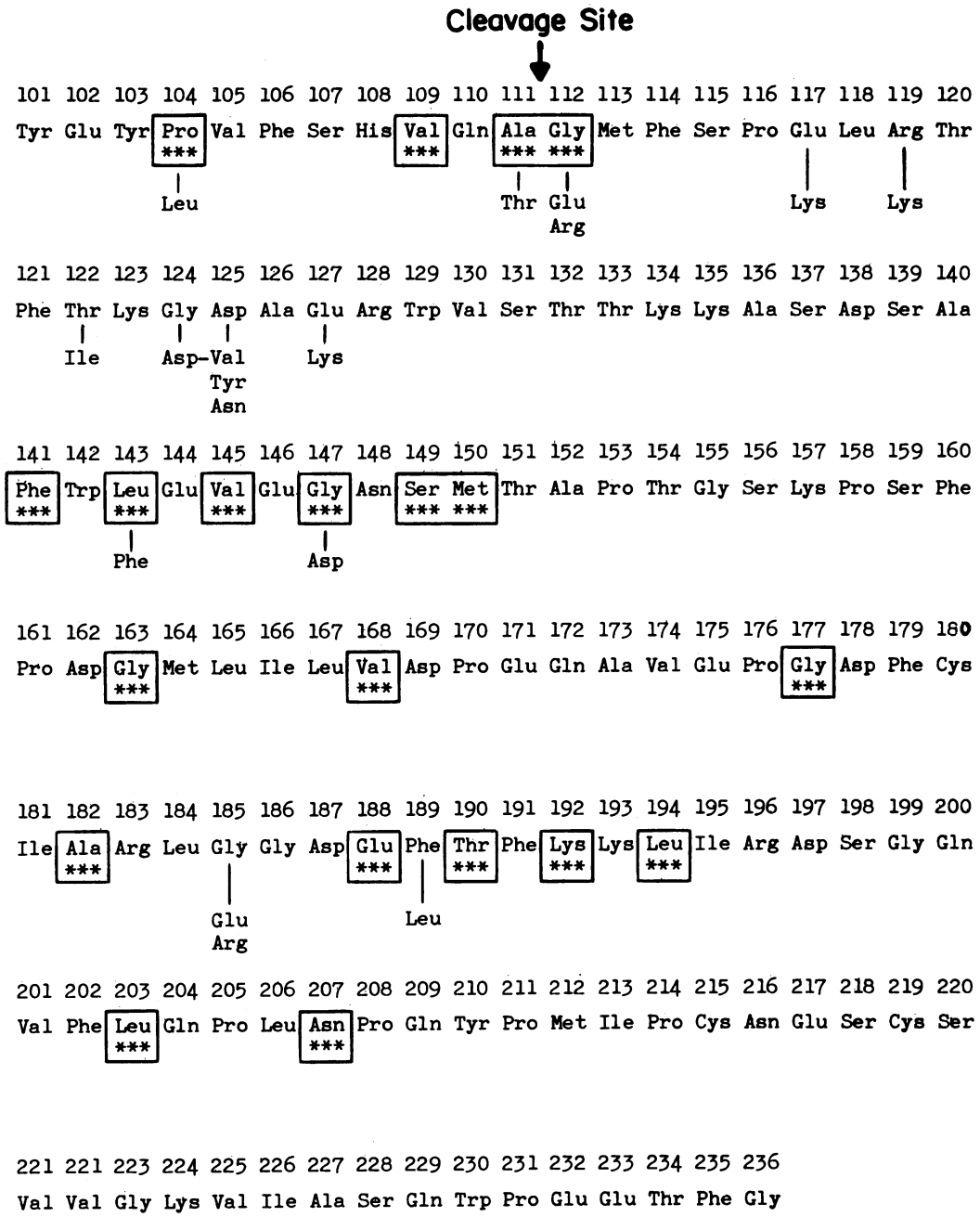


FIG. 3. Partial amino acid sequence of the λ repressor protein and the location of the *ind* mutations. The amino acid sequence of the repressor protein is shown beginning at amino acid 101 and continuing to the carboxy terminus of the protein. The amino acid changes which occur in the *ind* repressors are shown below the wild-type sequence. Residues which are conserved in C-terminal regions of the λ, 434, P22, and LexA repressors are boxed and underlined by asterisks.

How do the remaining *ind* mutations affect RecA-mediated cleavage? Little (17) has presented convincing evidence that cleavage at the Ala-Gly sequence in λ repressor and LexA repressor is actually an autodigestion event, since this cleavage also occurs under alkaline conditions in the absence of RecA protein. Since cleavage in vivo and under physiological conditions in vitro depends on RecA protein (7), it seems likely that RecA protein binds to repressor and stimulates the autodigestion reaction. Moreover, the λ repressor monomer and not the dimer appears to be the substrate for the RecA-mediated cleavage (20). Taken together, these results suggest that *ind* mutations could affect: (i) the Ala-Gly cleavage site, (ii) the repressor side chains that form the RecA protein-binding site, (iii) the repressor side chains required for the autodigestion reaction (17), (iv) the side chains required to couple RecA binding to stimulation of autodigestion, or (v) side chains which could mediate stronger repressor dimerization when mutated. The sites that mediate these functions are likely to be contained entirely within the C-terminal region of λ repressor because a fragment which contains amino acids 93 through 236 is efficiently cleaved in the presence of RecA protein (37).

Moreover, all of the *ind* mutations characterized here and all of the *ind* mutations that have been genetically mapped (10, 16) lie within this region.

The present data do not allow the noncleavage site *ind* mutations to be assigned to particular defects in the RecA-mediated cleavage reaction; this will require biochemical study of the mutant repressors. However, several general points can be made concerning the nature and distribution of the mutations. When the C-terminal sequences of the λ, P22, 434, and LexA repressors are aligned, 20 sequence positions are found to be conserved (38; see Fig. 3). Five of the sites of cI *ind* mutations ($Pro^{104}$→Leu, $Ala^{111}$→Thr, $Gly^{112}$→Arg or Glu, $Leu^{143}$→Phe, and $Gly^{147}$→Asp) affect one of these highly conserved positions. However, over half of the *ind* mutations affect residue positions that show little or no homology among the four repressors. It is clearly not true that only the highly conserved residues affect RecA-mediated induction.

The C-terminal sequence of λ repressor is composed of two regions. Residues 132 through 236 form a compact C-terminal domain that is dimeric in solution (29). Residues 93 through 131 probably form a metastable domain that folds against the C-terminal domain (see reference 36 for discussion). Four of the residue sites changed by *ind* mutations are located beyond residue 132. It seems likely that these *ind* mutations affect a different aspect of the RecA-mediated cleavage reaction than those that cluster in the central region of repressor. Biochemical experiments are consistent with models in which RecA protein binds to the compact C-terminal domain (28, 37), and the *ind* mutations in this domain might affect this binding. There is, however, no direct evidence that bears on this possibility. The *ind* mutations that cluster in the central region of repressor affect six residue sites in addition to the Ala-Gly cleavage site. In four of these cases, a charged residue is altered. The $Glu^{117}$→Lys, $Asp^{125}$→Asn or Tyr, and $Glu^{127}$→Lys mutations result in a more basic local environment, whereas the $Arg^{119}$→Lys mutation replaces one basic residue with another. The $Glu^{117}$→Lys *ind* mutation, which was isolated in six independent experiments here, is identical to the *ind-1* mutation originally isolated by Jacob and Campbell (12; see also reference 35).

Cohen et al. (6) have purified the super-inducible λ $ind^s$-*1* repressor and have shown that it dimerizes poorly but is cleaved more efficiently than the wild type in a RecA-mediated reaction. We find the $ind^s$-*1* mutation to be a $Glu^{233}$→Lys substitution located four residues from the carboxy terminus of repressor. This suggests that the extreme C-terminal portion of the protein plays a role in a repressor dimerization. Phizicky and Roberts (28) originally demonstrated a connection between repressor dimerization and RecA-mediated cleavage by showing that repressor monomers were more efficiently cleaved than were dimers. Although many models are consistent with this data, two simple alternatives are: (i) dimerization buries and therefore blocks the RecA interaction site, and (ii) dimerization buries and makes inaccessible the Ala-Gly cleavage sequence. The cleavage site *ind* mutations described here make this last possibility unlikely. If the wild-type Ala-Gly sequence were buried by dimerization, then mutant dimers containing the *ind* $Ala^{111}$-$Glu^{112}$ or $Ala^{111}$-$Arg^{112}$ sequences should be much less stable than the wild type because of the energetic cost of removing either charged amino acid from an aqueous environment. However, both repressors appear to have near wild-type DNA-binding activity, and since dimerization and DNA binding are coupled equilibria (14), it seems unlikely that these repressors dimerize significantly less well than the wild type. This, in turn, suggests that the wild-type Ala-Gly cleavage sequence is solvent exposed in repressor dimers.

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