Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5492–5496, July 1989
Genetics

UV induction of coliphage 186: Prophage induction as an SOS function

(LexA/SOS response/noninducible coliphages/antirepressor)

IAIN LAMONT*, ANTHONY M. BRUMBY, AND J. BARRY EGAN†

Department of Biochemistry, University of Adelaide, Adelaide S.A. 5001, Australia

Communicated by Ira Herskowitz, April 3, 1989 (received for review August 10, 1987)

ABSTRACT **Our results show that UV induction of the 186 prophage depends upon the phage function Tum, with the mutant phenotype of *tu*rbid plaques on *m*itomycin plates and the expression of which is controlled by the host LexA protein. Tum function, encoded near the right-hand end of the coliphage 186 chromosome, is under the control of promoter *p*95. This promoter is overlapped by a sequence closely related to the consensus sequence of the LexA-binding site. It is proposed that inactivation of LexA after UV irradiation (or by genetic means) leads to prophage induction by permitting expression of Tum which, by unknown means, induces prophage. This mechanism is basically different from that seen with the UV-inducible lambdoid coliphages, which are not regulated by LexA.**

The temperate coliphages have been traditionally classified as being either inducible or noninducible, with λ and P2, respectively, the classical archetypes. Coliphage 186 is not related to λ but is very closely related to P2 (1–3). It was, therefore, with some surprise that we found coliphage 186 to be UV-inducible (4).

UV induction of the λ prophage reflects the fact that the prophage repressor protein undergoes proteolytic cleavage involving activated RecA protein in UV-irradiated cells (for review, see ref. 5). We began the present experiments after Kalionis *et al.* (6) observed that the coliphage 186 repressor protein does not display the characteristic sequence seen in the carboxyl domain of four proteins cleaved by RecA-activated proteolysis. We therefore isolated a series of noninducible mutants of coliphage 186 expecting that their amino acid changes, as deduced from their DNA sequences, would indicate the region of the phage 186 CI repressor protein important for protease recognition. However, the loci of the noninducible mutants were mapped some distance outside the *c*I gene and were not therefore mutants of the CI repressor. Our results indicate that the function so identified, Tum (with the mutant phenotype of *tu*rbid plaques on *m*itomycin plates), is necessary for induction and that Tum synthesis is regulated by the host LexA protein.

MATERIALS AND METHODS

Strains. The *lexA3* inducible-deficient (Ind$^-$) bacterial strain, in an *Escherichia coli* strain C600 (7) background, was constructed in our laboratory by cotransduction with *malB*::Tn*10* from *E. coli* strain JC13067 (A. J. Clark, University of California, Berkeley). The *lexA41* temperature-sensitive (Ts) (KP84) and *lexA51* defective (Def) (DM1420) bacterial strains and their Δ (*rec*A–*srl*R)306 derivatives were in an *E. coli* AB1157 background from the laboratory of D. Mount (University of Arizona, Tucson). The phage strains used were from our laboratory.

General Methods. General bacteriological media and methods were as described by Woods and Egan (4). General recombinant DNA technology used is described by Maniatis *et al.* (8).

RESULTS

Isolation of Non-UV-Inducible Mutants. Woods and Egan (4) had originally described four non-UV-inducible mutants of coliphage 186 *cItsp* that they isolated as *tu*rbid plaques on *m*itomycin plates and called Tum mutants (Tum1–4). We used the same method of mutagenesis and detection to isolate Tum mutants in either a *cItsp* (Tum8 and Tum9) or a *c*I$^+$ (Tum13, -14, -16, and -17) background. All the Tum mutants tested showed a reduced frequency of UV-induced and spontaneous induction (Table 1). The attempted UV-induction pattern of a Tum$^-$ prophage (Tum16) is recorded in Fig. 1; no detectable induction occurred. The Tum mutations were not concerned with excision, as Tum$^-$ prophage carrying a *cItsp* allele were readily induced by temperature elevation (data not shown). Nor was the pattern a reflection of the inability of a Tum mutant to propagate in a UV-irradiated cell because Tum$^-$ mutants gave a normal burst size upon UV irradiation followed by temperature induction (data not shown), although the burst was delayed, as expected (9). We concluded that the Tum phenotype was directly concerned with UV induction of the prophage.

Locating the Tum Mutations on the Coliphage 186 Chromosome. A map of the coliphage 186 chromosome is shown in Fig. 2. Restriction enzymes *Xho* I and *Bgl* II cut the chromosome uniquely at 68% and 79% of its length, respectively, such that double digestion of coliphage 186 DNA with these enzymes gives a small 3.5-kb fragment, which includes the *c*I gene, and a large 26.5-kb fragment joined at the cohesive ends. We expected the Tum mutations to lie within the *c*I gene of coliphage 186. To test this hypothesis, the small *Xho* I–*Bgl* II fragments from each of four Tum mutants (Tum2, -9, -14, and -16) were purified and recombined *in vitro* with the purified large *Xho* I–*Bgl* II fragment from wild-type coliphage 186 and *vice versa*. The recombinant plaques were tested for their Tum phenotypes by infecting lawns of C600 indicator bacteria on mitomycin plates. Unexpectedly, the Tum phenotype in each case was determined by the large *Xho* I–*Bgl* II fragment, so that the Tum mutations had to be outside the *c*I gene.

We next exploited the unique *Not* I site at 89% of the chromosomal length and recombined *in vitro* the *Not* I–*cos* (89–100%) fragment from each Tum mutant with the large *cos*–*Not* I (0–89%) fragment from coliphage 186 mutant *Aam*24. The *Aam*24 mutation resides in the small *Not* I–*cos*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: Tum mutants, non-UV-inducible mutants of coliphage 186 isolated as *tu*rbid plaques on *m*itomycin plates.

*Present address: Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand.
†To whom reprint requests should be addressed.

Table 1. Induction of Tum mutants

Mutant	Titer of unirradiated culture	UV induction: Titer of irradiated culture	UV induction: Phage titer after irradiation, -fold increase	UV induction: Titer relative to irradiated Tum$^+$ culture
Tum$^+$	1.6×10^7	1.9×10^9	119	1
Tum2	1.6×10^6	1.3×10^7	8	6.8×10^{-3}
Tum8	8.5×10^5	8.4×10^6	10	4.4×10^{-3}
Tum9	7.2×10^3	2.1×10^4	3	1.1×10^{-5}
Tum13	3.7×10^2	4.7×10^3	13	2.4×10^{-6}
Tum14	7.7×10^5	9.1×10^6	12	4.8×10^{-3}
Tum16	9.0×10^1	2.6×10^2	3	1.4×10^{-7}
Tum17	1.7×10^2	2.4×10^3	14	1.3×10^{-6}

All cultures were grown at 30°C and centrifuged; the bacteria were then resuspended in 10 mM $MgSO_4$. Portions of each culture were UV irradiated (see Fig. 1 legend), and the unirradiated and irradiated bacteria were diluted into L-broth and then incubated with aeration at 30°C. After 4 hr aliquots were chloroformed and assayed for free phage.

region, and its use allowed selection against uncut parental phage after transfection of a nonsuppressing strain, as the large *Not* I–*cos* fragment (27 kb) could not be fully separated from intact phage DNA (30 kb) by electrophoresis. In each instance the Tum phenotype was associated with the small *Not* I–*cos* fragment, and we therefore concluded that the Tum mutation was located in the 89–100% region designated in Fig. 2.

The DNA sequence from the 89–96% region was known (10). This sequence contained a promoter, *p*95, known to be active *in vitro* (12), but, more significantly, the sequence indicated the existence of a potential LexA-binding site overlapping its −10 region (see Fig. 4). Because LexA is central to the SOS regulon and therefore intimately associated with the response of the cell to UV irradiation (5), we investigated the role of LexA in UV induction of the 186 prophage.

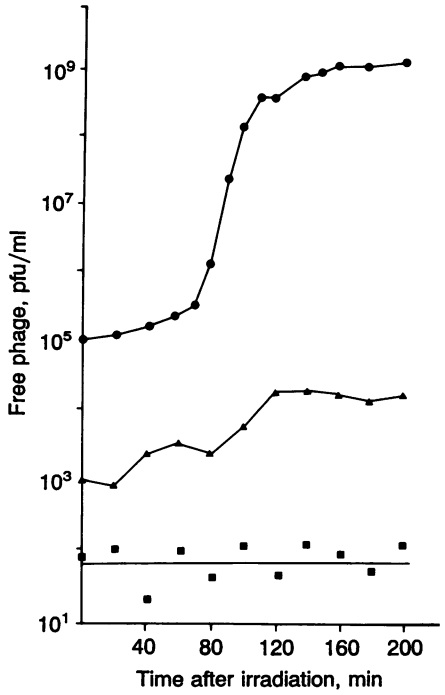

FIG. 1. Phage production after UV-irradiation of lysogens. Cultures were grown with aeration at 37°C to 3×10^8 colony-forming units per ml. The cultures were centrifuged, and the bacteria were resuspended in 10 mM $MgSO_4$. Portions were transferred to glass Petri dishes to a depth of 1 mm and UV-irradiated at 15 J/m^2 (254 nm) with a 15 W General Electric germicidal lamp. Bacteria were then diluted into L-broth and incubated with aeration at 37°C. Samples were taken at intervals, treated with chloroform, and assayed for free phage. ●, C600 *lexA*$^+$(186 Tum$^+$); ▲, C600 *lexA3*(Ind$^-$)(186 Tum$^+$); ■, C600 *lexA*$^+$(186 Tum16).

Role of the *lexA* Gene in UV Induction of 186 Prophage. We carried out the following series of experiments to investigate the role of LexA in UV induction of the 186 prophage.

Phage 186 forms clear plaques on a lexA*(Def) bacterial strain.* Coliphage 186 forms turbid plaques on wild-type indicator bacteria, characteristic of a temperate phage. However, on the *lexA*(Def) indicator bacteria, which do not form active LexA protein, coliphage 186 formed clear plaques. In contrast, coliphage λ formed turbid plaques on both *lexA*$^+$ and *lexA*(Def) bacterial strains. These results suggested a function antagonistic to lysogeny that was under LexA control.

186 Tum$^-$ phage forms turbid plaques on a lexA*(Def) strain.* On bacterial lawns of *lexA*(Def) indicator Tum9, Tum16, and Tum17 formed turbid plaques, whereas the Tum2 and Tum14 plaques were slightly turbid. These results confirmed that the lysogeny antagonist was encoded by the presumptive phage gene *tum* and was not simply a property of the mutant host.

186 lysogens of a lexA(*Ind$^-$*) *host are not UV inducible.* The *lexA*(Ind$^-$) host, *lexA3*, encodes a mutant LexA protein that is not degraded after activation of RecA (13). When the 186 lysogen *lexA3*(186) was UV-irradiated there was no sharp increase in plaque-forming units as seen after UV irradiation of the *lexA*$^+$ lysogen, but rather a very gradual increase, accompanied by an overall yield 10^5-fold lower than that of the wild-type control (Fig. 1). In contrast, phage infection of UV-irradiated *lexA3* bacteria gave a phage burst similar to that obtained with *lexA*$^+$ bacteria (data not shown). This confirmed that the blockage to phage production in the UV-irradiated lysogen *lexA*3(186) was at the level of prophage induction and not within the replication cycle after induction of prophage 186.

lexA *(Ts)(186) lysogens are temperature-inducible.* The *E. coli tsl* mutant was thought to encode a temperature-sensitive LexA protein (14), but it has recently been shown that the temperature-sensitive phenotype of *tsl* strains is largely due to increased degradation of the mutant LexA protein at high temperatures (15). The prophage in a 186 lysogen of the *tsl* strain KP84 was induced by a temperature-shift, whereas a Tum$^-$ prophage was not (Fig. 3). However, the *tsl* strain could host the temperature induction of the Tum$^-$ prophage when it carried the *cIts* allele.

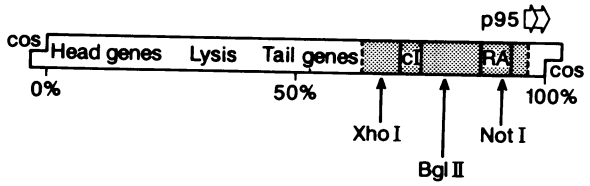

FIG. 2. Chromosomal map of bacteriophage 186. Only relevant genes and transcripts are shown; the portion of the chromosome that has been sequenced is stippled (6, 10, 11). One percent is equivalent to ≈300 base pairs (bp). The *cI* gene encodes coliphage 186 immunity function that prevents expression of lytic genes from lytic promoter P_R in the lysogenic state. The RA gene is essential for bacteriophage replication and contains the *Aam*24 mutation (10). Promoter *p*95 generates two *in vitro* transcripts of 0.59 and 1.54 kilobases (kb) in length with identical 5′ ends (12). Relevant restriction sites are shown below the map.

From these results we concluded that the expression of a presumptive gene *tum*, whose product is necessary for UV induction of the 186 prophage, was under LexA control.

Role of the *p*95 "Operon" in UV Induction of 186 Prophage. As mentioned earlier, a potential LexA binding site exists, overlapping the known promoter *p*95. It was therefore considered possible that the Tum function was encoded on a transcript from the *p*95 promoter under LexA control, and we sought next to confirm this expectation.

There is a *Pst* I site at 94.0% some 140 bases upstream of the −35 region of the *p*95 promoter (10), a *Hgi*AI site 45 bases downstream from its +1 position (10), and a *Sph* I site at 98.4% (unpublished result). We isolated the 1.3-kb *Pst* I–*Sph* I fragment from wild-type 186 and cloned the fragment into pBR322 to give pEC300 (Fig. 4). Our intended experiment was to see whether this clone would complement the Tum defect, and if it did, to determine whether the complementation was under LexA control. As a control we used the clone pEC301 that carried the equivalent *Pst* I–*Sph* I fragment from phage 186 Tum16. To focus on the *p*95 promoter we also studied the *p*95-less clone pEC302, which carried the 1.1-kb *Hgi*AI–*Sph* I fragment from wild-type coliphage 186. The two phenotypes used in the complementation study were UV induction of prophage and plaque phenotype on a *lexA*-(Def) indicator.

The coliphage 186 Tum16 lysogen was not inducible by UV light (Fig. 1). However, in the presence of pEC300, but not pEC301 or pEC302, the prophage was UV inducible (Fig. 5), indicating that the wild-type Tum function was encoded on the *Pst* I–*Sph* I fragment and that its expression was under the control of a signal located between the *Pst* I and *Hgi*AI sites. Furthermore, this expression was under LexA control, as pEC300 could not potentiate UV induction of the 186 Tum16 prophage in a *lexA*(Ind$^-$) background (Fig. 5).

Confirmatory results were seen with plaque phenotypes. As earlier indicated, the Tum mutant gave a turbid plaque on the *lexA*(Def) strain, whereas the wild type gave a clear plaque. However, in the presence of pEC300, coliphage 186 Tum16 gave a clear plaque, reflecting the supply of the Tum function from pEC300. This conclusion was supported by the fact that the Tum mutant continued to give a turbid plaque in the presence of the Tum$^-$ plasmid pEC301. The inability of pEC300 in a *lexA*$^+$ background to provide a clear plaque phenotype to coliphage 186 Tum16 was consistent with LexA control of gene expression from the clone. The probability that *p*95 was the promoter involved was enhanced by the fact that in its absence, as with the *Hgi*AI–*Sph* I clone pEC302, complementation of the Tum defect was lost. Thus, phage 186 Tum16 gave a turbid plaque on *lexA*(Def) carrying pEC302.

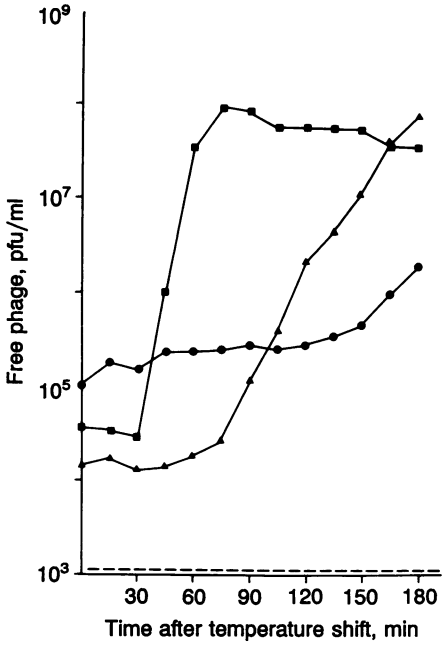

FIG. 3. Phage production from lysogens after temperature-shift. Cultures were grown with aeration at 30°C to 2 × 10^8 colony-forming units per ml. Cultures were then diluted into L-broth prewarmed to 41°C, and incubated was continued with aeration at this temperature. Samples were taken at intervals, chloroformed, and assayed for free phage. ●, AB1157 *lexA*$^+$(186*c*I$^+$ Tum$^+$); ■, AB1157 *lexA41*(Ts) (186 *cItsp* Tum16); ▲, AB1157 *lexA41*(Ts) (186 *c*I$^+$ Tum$^+$); ---, AB1157 *lexA41*(Ts) (186 *c*I$^+$ Tum16). The number of plaque-forming units per ml for AB1157 *lexA41*(Ts) (186 *c*I$^+$ Tum16) was $<10^3$.

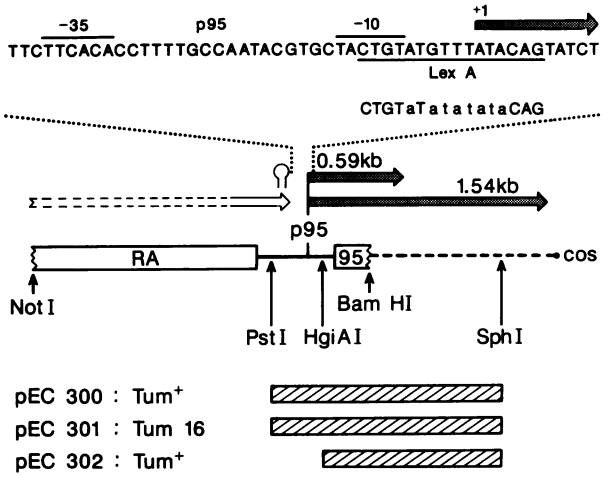

FIG. 4. Genetic map of the far right of the bacteriophage 186 chromosome from the *Not* I site at 89% to the cohesive end. That portion of the chromosome yet to be sequenced is shown as a dashed line. The DNA sequence of *p*95 is given above the figure and locates the predicted LexA-binding site overlapping the −10 region of the promoter (10, 12). The consensus LexA-binding site sequence is given beneath the proposed site (16). Known *in vitro* transcripts from *p*95 (12) are represented by stippled arrows, and the presumed *in vivo* transcript from the lytic promoter P_R is shown as a dashed arrow. Predicted open reading frames based on sequence data are shown as open boxes (10), RA being the replication gene and 95 the computer-predicted protein CP95. Plasmids pEC300 and pEC301 contain the 1.3-kb *Pst* I (94.0%)–*Sph* I (98.4%) fragment from 186 Tum$^+$ and 186 Tum16, respectively, ligated into the *Eco*RI–*Bam*HI backbone of pBR322. The plasmid pEC302 was constructed identically, with the same insert orientation, by using the 1.1-kb *Hgi*AI (94.8%)–*Sph* I (98.4%) fragment from 186 Tum$^+$. Before ligation the overhanging 5′ or 3′ end generated by a restriction enzyme digestion was removed by either the 5′→3′ polymerase activity, or the 3′→5′ exonuclease activity, respectively, of DNA polymerase I (Klenow fragment).

From these studies we concluded that the Tum function was encoded on the *Pst* I–*Sph* I fragment and that its expression, from a site between the *Pst* I and *Hgi*AI sites, was under LexA control.

Further Properties of the Tum Function. *On the role of RecA in the antilysogenic activity of Tum.* Phage 186 formed clear plaques on a *recA lexA*(Def) strain (DM 2571), indicating that the RecA product was not needed for the clear plaque phenotype of 186 on the *lexA*(Def) strain and suggesting that the RecA product was not essential for the antilysogenic activity of the Tum product. Furthermore the *recA lexA* (Ts)(186) lysogen was temperature inducible (data not shown), indicating that the RecA protein was not necessary for the induction of the prophage upon temperature-induced degradation of the mutant LexA protein.

The Tum activity has no effect on P2 or λ lysogeny. We used the *lexA*(Def) strain carrying pEC300, in which we expect the Tum function to be constitutively expressed, to

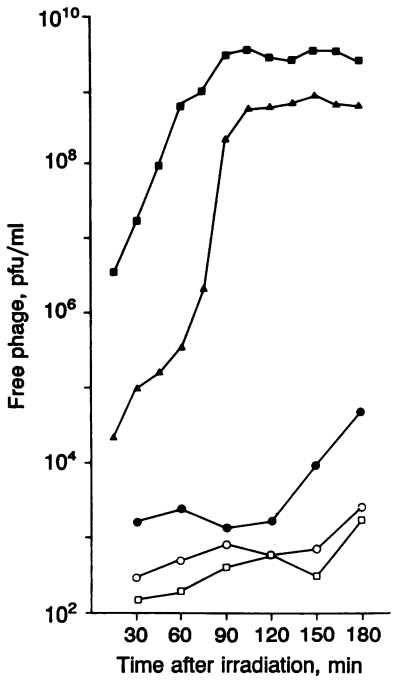

FIG. 5. Phage production after UV irradiation of plasmid-containing lysogens. The procedure followed is described in the Fig. 1 legend. ■, C600 *lexA*$^+$(186 Tum16), pEC300; ▲, C600 *lexA*$^+$(186 Tum$^+$); ●, C600 *lexA*3(Ind$^-$)(186 Tum16), pEC300; ○, C600 *lexA*$^+$ (186 Tum16), pEC302; □, C600 *lexA*$^+$(186 Tum16), pEC301.

test the specificity of the antilysogenic activity of Tum. We found, in contrast to coliphage 186, that both P2 and λ gave turbid plaques, suggesting that Tum was coliphage 186-specific.

DISCUSSION

Our results show that coliphage 186 encodes a trans-acting factor, which we term the Tum factor and assume to be a protein that causes and is necessary for prophage induction. Expression of the gene involved (the *tum* gene) is controlled by the host SOS system, so that induction of the SOS system allows expression of the gene, causing prophage induction (Fig. 6). Blocking this process, either with a noncleavable LexA protein or with a mutation in Tum, prevents UV-induction of the prophage; constitutive expression of the gene in a *lexA*(Def) host prevents lysogeny, even in the absence of UV-damaged DNA, presumably due to "constitutive induction" of the prophage. The Tum factor is also involved in spontaneous induction of the prophage, as shown by the low frequency of spontaneous induction of Tum$^-$ mutants (Table 1). Our results indicate that RecA is not directly involved in inactivation of CI repressor protein during prophage induction and thus explain the apparent absence of a RecA–protease cleavage site in the coliphage 186 CI repressor protein noted previously (6).

The Tum16 mutation is encoded on the 1.3-kb *Pst* I–*Sph* I fragment (94.0–97.8%), shown by the fact that a clone of this fragment (pEC300) complements the mutation. Furthermore, that the 1.1-kb *Hgi*AI–*Sph* I fragment (94.6–97.8%) does not complement the mutation indicates either (*i*) that the mutation resides on the 200-bp *Pst* I–*Hgi*AI fragment, with marker rescue rather than complementation operative, or more probably (*ii*) that complementation was indeed operative, with the Tum factor encoded entirely on the *Pst* I–*Sph* I fragment and transcribed from the established promoter *p*95 that is situated at 94.4% (12). This complementation capacity from the *Pst* I–*Sph* I fragment was shown to be under LexA control and,

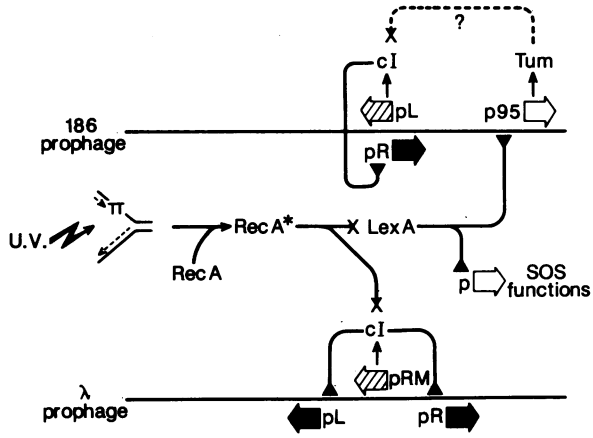

FIG. 6. Comparison of the postulated mechanism for UV induction of the coliphage 186 prophage with the mechanism of induction for the lambdoid-like prophage. The common step in the two systems is the activation of the RecA* proteolytic function in the cell in response to UV irradiation. This proteolytic activity is essential for normal cellular survival after UV irradiation because it is responsible for irreversibly inactivating the cellular repressor LexA and, hence, relieving the transcriptional repression of a battery of LexA-controlled genes, the SOS genes, which function to increase cellular survival (5). In a λ lysogen the RecA* proteolytic activity is directly responsible for also inactivating the lysogenic maintenance protein, CI. This allows expression from the CI-repressed lytic promoters P_L and P_R, and hence induction of the λ prophage (17). However, in a 186 lysogen, the lysogenic maintenance protein CI, which represses the early lytic promoter P_R, appears to be unaffected by RecA* activity. Instead, upon relief of LexA repression mediated by RecA*, a phage-encoded SOS function, Tum, is expressed from the promoter *p*95 and then presumably acts in some as yet to be defined manner to activate lytic development.

indeed, overlapping the −10 region of *p*95 is the sequence CTGTATGTTTATACAG. This represents a 14/16 match with the consensus LexA binding site CTGTaTatatataCAG, which is a better match than many sites known to bind LexA protein (16). The candidate gene for the *tum* gene is the computer-predicted protein CP95, which starts at 94.6% and is presumably encoded on the 0.59-kb transcript that initiates from *p*95 and terminates ≈96.5% (12).

The possibility that the LexA-controlled *tum* gene is simply encoding a function concerned with prophage excision was considered but disregarded because the Tum mutant carrying the *cItsp* allele forms a prophage that gives a normal one-step growth curve after prophage induction by a temperature shift. We favor the view that the Tum factor acts as an antagonist of the prophage 186 repressor protein (i.e., an antirepressor), thereby allowing excision and lytic development of the prophage. Another possibility is that the Tum factor may cause activation of a promoter that is not under control of a repressor and that causes expression of the replication and lytic genes of coliphage 186. Whatever the actual mechanisms of Tum action, the overall system of UV induction of prophage 186 clearly differs from that of the lambdoid phages. With such phages, as exemplified by λ, activation of the RecA protein after UV induction is directly involved in the proteolysis of the phage (lysogenic) repressor, whereas with coliphage 186 activation of the RecA protein leads to the derepression of a host-controlled, phage-encoded inducing factor (Fig. 6).

An antirepressor function Ant has been described for the lambdoid phage P22 (for review, see ref. 18). This protein reversibly inhibits various lambdoid (lysogenic) repressors, apparently by inhibiting repressor oligomerization (18). The antirepressor has no role in either spontaneous or UV induction of P22 prophage. In this way it differs from the Tum system of coliphage 186, which is pivotal to induction. A

further difference is the relative specificity of the coliphage 186 Tum system, which appears to have no action against the (lysogenic) repressor of the closely related phage P2; nor is it active against the CI repressor of λ. Antirepressor systems have been described for coliphage P1 (19) and P4 (20), but these have not been characterized. We have initiated studies to determine the mechanism of action of Tum. We are interested to compare ultimately the nucleotide sequence of the far-right region of coliphage 186 that encodes Tum with that of the analogous region of the closely related phage P2, as this phage is non-UV inducible (and therefore, naturally, Tum^-) and the region constitutively expresses the anti-λ function known as the Old function (for review, see ref. 20).

In conclusion we note that the LexA control of coliphage 186 gene expression is a rare example of a host repressor controlling phage gene expression. The possibility does exist, however, of LexA control of gene expression in λ. In 1983, Sprizhitsky and Kopylov (21) noted the presence of a sequence homologous to the LexA-binding site in the promoter of the *oop* transcript. As *oop*-encoded RNA inhibits expression of CII protein (22), the proteolysis of LexA after UV irradiation and subsequent derepression of the *oop* promoter would be expected to favor the lytic response. If so, then a lysogen of the noncleavable mutant *lexA*(Ind^-) might show poor UV inducibility. Indeed, Quillardet *et al.* (23) found a low efficiency of prophage induction, measured by infectious centers, with *lexA*(Ind^-) (λ), even in the presence of elevated levels of RecA protein, and Ian Dodd (personal communication) has confirmed the finding, showing a depressed burst size after UV induction of *lexA*(Ind^-) (λ).

In summary, we have shown that UV induction of coliphage 186 prophage is under LexA control; the properties of this "SOS function" of *E. coli* require further characterization.

We are grateful to Don Ennis and Belva Fisher for sending us strains, and to Bill Kalionis for pointing out the probable LexA binding site over *p*95. We also thank Jon Anargyros for skilled technical assistance. We acknowledge Program Grant Support from the Australian Research Grants Scheme (J.B.E.), a National Research Fellowship (I.L.), and a Commonwealth Postgraduate Award (A.M.B.).

1. Bradley, C., Ong, P. L. & Egan, J. B. (1975) *Mol. Gen. Genet.* **140,** 123–135.
2. Younghusband, H. B., Egan, J. B. & Inman, R. B. (1975) *Mol. Gen. Genet.* **140,** 101–110.
3. Skalka, A. & Hanson, P. (1972) *J. Virol.* **9,** 583–593.
4. Woods, W. H. & Egan, J. B. (1974) *J. Virol.* **14,** 1349–1356.
5. Walker, G. C. (1984) *Microbiol. Rev.* **48,** 60–93.
6. Kalionis, B., Dodd, I. B. & Egan, J. B. (1986) *J. Mol. Biol.* **191,** 199–209.
7. Appleyard, R. K. (1954) *Genetics* **39,** 440–452.
8. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
9. Hooper, I. & Egan, J. B. (1981) *J. Virol.* **40,** 341–349.
10. Sivaprasad, A. V. (1984) Ph.D. thesis (University of Adelaide, Adelaide, Australia).
11. Richardson, H. & Egan, J. B. (1989) *J. Mol. Biol.* **206,** 251–255.
12. Pritchard, M. & Egan, J. B. (1985) *EMBO J.* **4,** 3599–3604.
13. Little, J. W., Edmiston, S. H., Pacelli, L. Z. & Mount, D. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3225–3229.
14. Mount, D. W., Low, K. B. & Edmiston, S. (1972) *J. Bacteriol.* **122,** 886–893.
15. Peterson, K. R. & Mount, D. W. (1987) *J. Mol. Biol.* **193,** 27–40.
16. Wertman, K. F. & Mount, D. W. (1985) *J. Bacteriol.* **163,** 376–384.
17. Roberts, J. W., Roberts, C. W. & Craig, N. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4714–4718.
18. Susskind, M. M. & Youderian, P. (1983) in *Lambda II*, eds. Hendrix, R., Roberts, J., Stahl, F. & Weisberg, R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 347–363.
19. Sternberg, N. & Hoess, R. (1983) *Annu. Rev. Genet.* **17,** 123–154.
20. Bertani, L. E. & Six, E. W. (1988) in *The Bacteriophages*, ed. Calendar, R. (Plenum, New York), Vol. 2, pp. 73–143.
21. Sprizhitsky, Y. A. & Kopylov, V. M. (1983) *FEBS Lett.* **160,** 7–10.
22. Krinke, L. & Wulff, D. L. (1987) *Genes Dev.* **1,** 1005–1013.
23. Quillardet, P., Moreau, P. L., Ginsburg, H., Mount, D. W. & Devoret, R. (1982) *Mol. Gen. Genet.* **188,** 37–43.