JOURNAL OF VIROLOGY, Nov. 1988, p. 4362–4364
0022-538X/88/114362-03$02.00/0

Vol. 62, No. 11

Evaluation of the Interaction of φX174 Gene Products E and K in E-Mediated Lysis of *Escherichia coli*

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Received 25 January 1988/Accepted 23 July 1988

Gene *K* of bacteriophage φX174 was cloned, and its gene product was localized in the cell envelope of *Escherichia coli*. Compared with the sole expression of the φX174 lysis gene *E*, the simultaneous expression of the *K* and *E* genes had no effect on scheduling of cell lysis. Therefore, a direct interaction of proteins E and K could be excluded. In contrast, φX174 infection of a host carrying a plasmid expressing gene *K* resulted in a delayed lysis and an apparent increase in phage titer.

The φX174 genome codes for 11 genes (13), all of which are essential for in vivo production of mature viruses except genes *E*, required for host cell lysis (8), and *K*, with unknown function (14). Cloning of gene *E* revealed that gene product E (gpE) with an M_r of 10,000 is the only protein of bacteriophage φX174 which is required and sufficient to cause lysis (7, 17). The exclusive localization of gpE in the envelope fraction of *Escherichia coli* (1, 3) suggests a mechanism in which gpE acts as a membrane-attacking protein. Like gene *E*, which is entirely embedded within gene *D*, the *K* reading frame (56 codons) overlaps with the essential genes *A*, *B*, and *C* (13). With the aid of φX174 *D*/*K* and *A*/*K* double amber mutants, the predicted gene product K (gpK) was shown to be produced in infected cells (16). A^+ revertants of A^-/K^- double mutants did not influence phage maturation but showed smaller plaques and a two- to threefold-reduced burst size relative to φX174 wild type in a nonsuppressor host (16). More recently, this reduced plaque phenotype was unambiguously assigned to gene *K* by using site-directed mutagenesis to create an amber codon in gene *K* which did not affect the overlapping gene *A* reading frame (5). These observations suggested a function of gpK in the phage life cycle, and the lack of effect on phage maturation made some role in lysis an attractive possibility. As deduced from the nucleotide sequence, gpE and gpK share homologies in their hydrophobic amino termini (2, 14). Since gpE is known to be a membrane protein (1, 3), a membrane-associated function was also likely for gpK and an interaction of both proteins seemed to be possible. This was supported by the conservation of the amino terminus of gpK in the φX174-related phage G4 (6). To determine the subcellular location of gpK and to define its possible role in φX174 lysis, we cloned gene *K* into different plasmids.

Cloning of gene *K*. Replicative form of φX174 DNA was prepared as described previously (17) and digested with *Hpa*I. Gene *K* was isolated on a 1,264-base-pair (bp) *Hpa*I fragment containing the complete φX174 genes *C*, *D*, *E*, *J*, and *K* and part of gene *F*. The 1,264-bp fragment was subsequently cleaved with *Sfa*NI to obtain a 341-bp fragment containing gene *K* and a part of gene *C*. After the 3′ recessed ends created by *Sfa*NI were filled, the fragment was subcloned into the *Sma*I site of plasmid pSB50 (U. Bläsi, Ph.D. thesis, University of Munich, Munich, Federal Republic of Germany, 1987). This provided suitable ends for insertion as a 376-bp *Eco*RI-*Pst*I fragment into plasmid pLa2311 (12), giving rise to plasmid pSB150 (Fig. 1). In plasmid pSB150, gene *K* is under transcriptional control of the lambda p_L promoter (12).

To construct a plasmid carrying both genes *K* and *E*, we isolated a 1,011-bp *Eco*RI fragment of φX174 DNA comprising gene *E* from plasmid pSB12 (4). This *Eco*RI fragment was inserted into the unique *Eco*RI site of plasmid pLa2311. The resulting plasmid, pSB102 (Fig. 1), carries gene *E* under control of lambda p_L. For additional cloning of gene *K* into pSB102, a 1,128-bp *Mlu*I-*Sma*I fragment containing gene *K* and the lambda p_L promoter-operator region was isolated from plasmid pSB150. Then the *Mlu*I site of the 1,128-bp fragment was filled in, and the fragment was cloned into the unique *Fsp*I site of pSB102. The orientation of gene *K* in the recombinant plasmid pSB152 (Fig. 1) was determined by *Xho*I and *Ava*I digestion. In plasmid pSB152, both genes are transcribed under control of lambda p_L. Gene *K* is transcribed clockwise, whereas gene *E* is transcribed counterclockwise.

Identification of gpK. *E. coli* pop2135 (F^- *endA thi hsdR malT cI857* pR *malPQ*; kindly provided by O. Raibaud, Institute Pasteur, Paris, France) carries a chromosomal copy of the phage lambda thermosensitive repressor gene *cI857*. Strain pop2135(pSB150) was grown in M9 minimal medium (11) at 28°C to a density of 10^8 cells per ml and divided into two portions. One portion was preincubated at 42°C to induce lambda p_L and labeled for 2.5 min with a [^{14}C]amino acid mixture (Amersham Corp., Arlington Heights, Ill.). The other portion was kept at 28°C and labeled in the same manner. After labeling, cells were washed and disrupted by boiling in sodium dodecyl sulfate sample buffer, and the labeled polypeptides were analyzed on a 40-cm 17.5% polyacrylamide gel (9). The gene *K* product was identified as a 6.4-kilodalton peptide in the protein pattern of the induced sample (Fig. 2, lane 2) and was absent in the uninduced sample (Fig. 2, lane 1). Although differing from a previous report (16) in which gpK was identified with an M_r of 8,000, the observed M_r for gpK is consistent with the molecular weight of 6,380 predicted from the gene *K* DNA sequence (13).

To determine the subcellular localization of gpK, we prepared cytoplasmic and envelope fractions of the induced cells. Cells were induced and labeled as described above,

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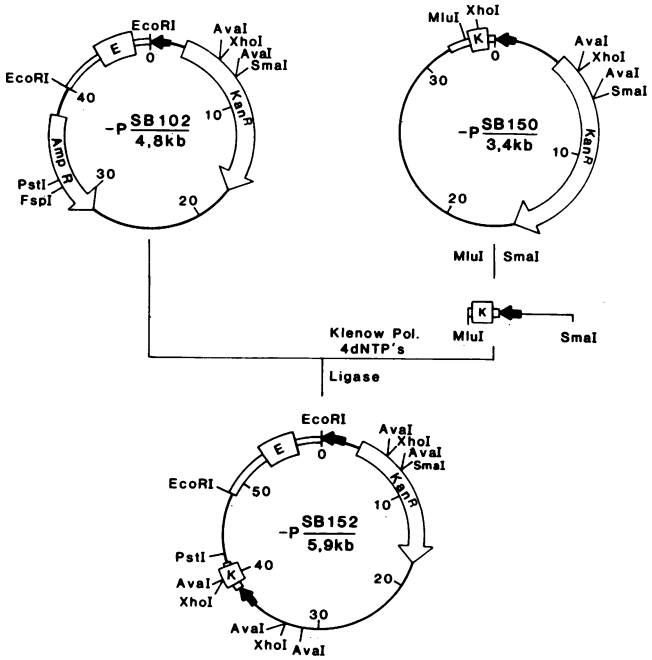

FIG. 1. Basic features of plasmids pSB102 and pSB150 and construction of plasmid pSB152. Directions of transcription are given by arrows. If not indicated otherwise, the open bars signify ϕX174 DNA. Expression of the ϕX174 information is under control of the thermoinducible p_L promoter of phage lambda (short dark arrows). The plasmid maps are subdivided into sections of 1 kilobase (kb) and not drawn to scale. Only relevant restriction sites are shown. Pol., Polymerase; dNTP's, deoxynucleoside triphosphates.

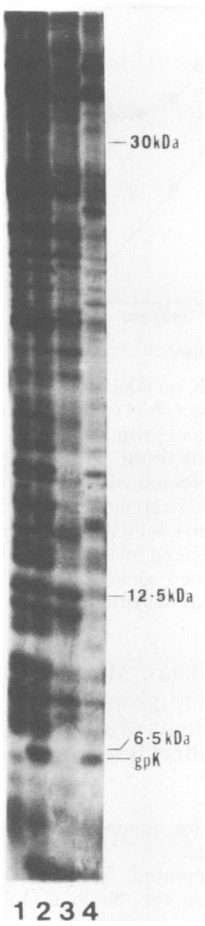

FIG. 2. Identification of gpK in *E. coli*. Experimental conditions were as outlined in the text. Lanes 1 and 2, Polypeptides synthesized in *E. coli* pop2135(pSB150) at 28 and 42°C, respectively. Lanes 3 and 4, Labeled polypeptides in the cytoplasmic fraction and membrane fraction, respectively, of thermally induced *E. coli* pop2135(pSB150). The positions of molecular size markers and of gpK are indicated at the right (kDa, kilodaltons).

suspended in M9 buffer (11), and disrupted in a French pressure cell. Unbroken cells were removed by low-speed centrifugation, and the supernatant was centrifuged at 100,000 × *g* for 2 h to pellet the membrane fraction. The envelope fraction was washed twice with M9 buffer. The supernatant (cytoplasmic fraction) was precipitated by the addition of an equal amount of 10% trichloroacetic acid. Both cytoplasmic and membrane samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described above. The *K* gene product was found exclusively in the membrane fraction (Fig. 2, lanes 3 and 4).

It should be noted that gene *K* is highly expressed in strain pop2135(pSB150), to the extent that after prolonged induction, gpK could be visualized by Coomassie staining (data not shown). However, gpK produced at this high level had no effect on cell growth. *E. coli* pop2135(pSB150) and pop2135(pLa2311), which carried the parental plasmid of pSB150, showed the same growth rate at 42°C.

Effect of gpK on ϕX174- and *E*-directed lysis of *E. coli*. To test the idea that gpK might be involved in disruption of the cell envelope at the time of lysis, we asked whether overproduction of gpK in a host cell would affect the timing of ϕX174 lysis. The lysis profile of cells expressing gene *K* constitutively from plasmid pSB150 was significantly delayed compared with that of control cells carrying the parental plasmid pLa2311. Onset of lysis occurred at 42 min after ϕX174 infection compared with 22 min for the control, and the rate of lysis after its inception was much slower (Fig. 3A). Furthermore, 80 min after infection there was a five-fold-higher titer of progeny phage in the supernatant of PC1363(pSB150) than in the supernatant of PC1363 (pLa2311). Taken together, these data suggested that gpK acts to delay the lytic function of lysis protein E.

To test this notion, we cloned both *E* and *K* genes into the same plasmid. The simultaneous expression of both genes from plasmid pSB152 (Fig. 1) enabled us to evaluate a possible interaction of gpK and gpE by comparing the timing of lysis directed by plasmid pSB152 with the timing of lysis mediated by the sole expression of gene *E* from plasmid pSB102 (Fig. 1). Expression of the lambda p_L-controlled genes in pop2135(pSB152) resulted in a lysis profile (Fig. 3B) indistinguishable from that obtained with pop2135(pSB102). This result is evidence against a direct interaction of gpK and gpE which would affect lysis timing.

To reconcile the apparent difference between gpK effects on phage- and *E*-induced lysis, it is necessary to conclude that other phage functions are involved in lysis of the host cell. Some evidence exists for this. Lysis induced by the phage differs from lysis directed by the action of gpE without the phage context. *E. coli* mutants resistant to gpE can be lysed by ϕX174 (G. Halfmann, unpublished data). Furthermore, ϕX174-induced lysis causes massive degradation of the peptidoglycan (10), whereas only a minor degradation was observed in cells lysed by induction of cloned gene *E* (A.

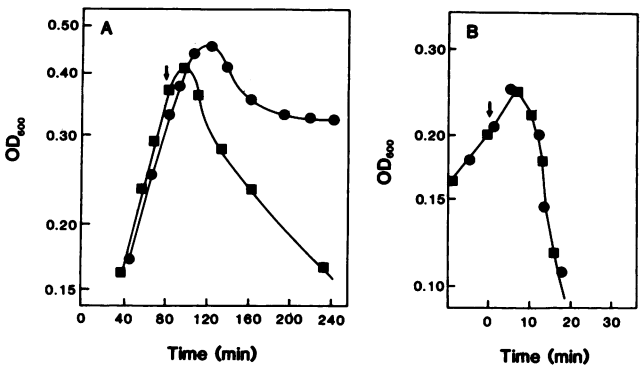

FIG. 3. Effect of gpK on ϕX174 lysis and *E*-mediated lysis of *E. coli*. (A) *E. coli* PC1363 (*pheA*) harboring plasmid pSB150 (●) or plasmid pLa2311 (■) was grown at 37°C in the presence of 50 μg of kanamycin per ml in LB broth (11). At the time indicated by the arrow, the cells were infected with ϕX174 (multiplicity of infection of 5). (B) *E. coli* pop2135 carrying plasmid pSB102 (■) or plasmid pSB152 (●) was grown in LB medium supplemented with antibiotics at 28°C. At the time indicated by the arrow, the cultures were shifted to 42°C to induce cloned gene *E* (pSB102) or genes *E* and *K* (pSB152). OD_{600}, Optical density at 600 nm.

Witte, unpublished data). Moreover, mutations in ϕX174 gene *H* can impair the phage-induced lysis (8, 15). Thus, there may be interactions of gpK with phage proteins other than gpE which can affect lysis. This possibility is now being investigated.

We thank Bill Roof for comments on the manuscript and Lisa Lohman for the graphics.

This work was supported by Public Health Service grant GM27099 to R.Y. from the National Institutes of Health and Deutsche Forschungsgemeinschaft grant Lu 213/4-5 to W.L. U.B. was supported by a postdoctoral fellowship (B1 262/1-1) from the Deutsche Forschungsgemeinschaft.

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