Lysis of *Escherichia coli* by Cloned φX174 Gene E Depends on its Expression

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The lysis gene E of bacteriophage φX174 was cloned under transcriptional control of the lefthanded lambda promoter, giving rise to plasmid pSB12. Plasmid pSB22, identical to pSB12 except for an amber mutation in gene E, was constructed in the same way. Induction of the cloned wild-type gene by heat inactivation of the thermosensitive λ cI857 repressor resulted in lysis of the host bacteria. With plasmid pSB22 only amber suppressor strains of *Escherichia coli* lysed after heat inactivation of λ cI857. Lysis of *E. coli* was shown to depend on the rate of gene E translation and on the growth phase of the bacteria. Stationary cells could not be lysed by the gene E product (gpE), even if present in sufficient amounts to lyse growing cells. By isotopic labelling gpE could be detected among the proteins synthesized in normal *E. coli* as well as in minicells. Determination of gene E expression suggested that gpE synthesis is translationally regulated.

**INTRODUCTION**

The essential role of the gene E product (gpE) of bacteriophage φX174 in the lysis process after infection of *Escherichia coli* was first described by Hutchison & Sinsheimer (1966). More recently, it has been shown that expression of cloned gene E is sufficient to cause lysis of *E. coli* (Henrich *et al.*, 1982a; Young & Young, 1982). From the nucleotide sequence it was determined that gpE consists of 91 amino acids and has a molecular mass of about 10 kDal (Barrell *et al.*, 1976; Pollock *et al.*, 1978). gpE seems to be devoid of enzyme activity (Eigner *et al.*, 1963; Markert & Zillig, 1965; Denhardt & Sinsheimer, 1965) and its lytic function requires a functional autolytic system of the host cell (Lubitz *et al.*, 1984a, b). This system is probably activated by integration of gpE into the cytoplasmic membrane of *E. coli* (Bläsi *et al.*, 1983).

Hitherto, gpE has only been detected by expression of cloned gpE in minicells (Bläsi *et al.*, 1983) or in heavily UV-irradiated φX174-infected cells (Pollock *et al.*, 1978). It has been estimated that roughly 100 to 300 molecules of gpE are made in a single cell after infection with φX174 (Pollock *et al.*, 1978). If such a small number of molecules are sufficient to cause lysis of *E. coli*, then expression of gene E must be highly regulated, taking into account that it is part of all major transcripts of the φX174 genome (Hayashi *et al.*, 1981).

We have recently cloned a 1007 bp AluI fragment of φXRF DNA under the control of the lac promoter (Henrich *et al.*, 1982a). With this system, lysis of *E. coli* occurs within 20 to 120 min after induction of the lac promoter, depending on the amount of inducer used and on the intracellular concentration of lac repressor (Henrich *et al.*, 1983). In this communication we describe the cloning of the same fragment of φX174 DNA under the control of λpL, mRNA initiation at λpL by inactivation of the thermosensitive cl repressor led to lysis of *E. coli* strains which harbour the newly constructed plasmid pSB12 within 10 min after temperature shift.

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Abbreviations: am, amber; gpE, gene E product; λpL, lefthanded λ promoter.
With this system gpE could be detected in normal cells without any reduction in host protein synthesis. Synthesis of gpE, however, is much less efficient than the expression of other proteins which are also encoded by pSB12.

METHODS

Bacteria and plasmids. *E. coli* K12ΔH1Δtrp [rpsL lacZam Δbio-uvrB ΔtrpEA2 (λNam7 Nam53 cIB57 ΔH1)] and plasmids pLC236 and pIC857 were kindly provided by E. Remaut, University of Gent, Belgium. *E. coli* HF4714 (Pro- Arg- Leu- Thi- Thy- Thr- Su+) was from our strain collection. HF4714 (Pro- Arg- Leu- Thi- Thy- Thr- Su+) was from our strain collection.

Ampicillin was added to liquid and solid media to give a final concentration of 200 μg ml⁻¹. Media. Cells were grown in LB medium containing (l⁻¹): 10 g tryptone, 5 g yeast extract and 5 g NaCl. Ampicillin was added to liquid and solid media to give a final concentration of 200 μg ml⁻¹.

Recombinant DNA techniques. Preparation of plasmid DNA, transformation, isolation of DNA fragments and electrophoresis of DNA were done as described previously (Henrich et al., 1982a, b).

SDS-polyacrylamide gel electrophoresis. Polypeptides were separated by electrophoresis for 12 h at 150 V on 14-20% polyacrylamide SDS gels using the discontinuous buffer system of Laemmli (1970). After electrophoresis the gels were processed for fluorography and exposed to Kodak X-ray film (RP-X-Omat) as described by Bonner & Laskey (1974).

Protein labelling in transformed cells. Proteins were labelled with [³⁵S]methionine as described by Reeve (1981). Briefly, *E. coli* K12ΔH1Δtrp harbouring the recombinant plasmid pSB12 or pSB22 was grown at 28 °C in supplemented minimal medium to a density of 2 × 10⁸ cells ml⁻¹ and then shifted to 42 °C. At various times after the temperature upshift 150 μl samples were taken and transferred to tubes containing 10 μCi [³⁵S]methionine (1200 Ci mmol⁻¹; 44-4 TBq mmol⁻¹) and further incubated for 2 min at 42 °C. After labelling, the cells were immediately centrifuged and resuspended in 24 μl sample buffer. The cells were disrupted by boiling for 5 min and analysed directly on a 14-20% polyacrylamide SDS gel.

Preparation of minicells and labelling of proteins. Minicells were prepared from *E. coli* DS410 grown in LB medium at 37 °C. Minicells were separated from nucleate cells by the use of sucrose gradients as described by Reeve (1979, 1981).

Minicells were resuspended in M9 medium (Miller 1972), containing 10⁻³ m-MgSO₄, 0.2% glucose and 20 μg D-cycloserine ml⁻¹, to give a density of 2 × 10¹⁰ cells ml⁻¹. Then 0.5 ml of the minicell suspension was added to 0.15 ml methionine assay medium containing 10 μCi [³⁵S]methionine (1200 Ci mmol⁻¹; 44-4 TBq mmol⁻¹) and further incubated for 45 min at 37 °C. The minicells were collected by centrifugation, resuspended in 50 μl sample buffer and processed as described above.

Enzymes and chemicals. Restriction endonucleases EcoRI and TaqI, and T₄ DNA ligase and DNA polymerase (large fragment) were purchased from Boehringer-Mannheim. Ampicillin and lysozyme were from Sigma. SeaKem ME agarose was from FMC Corporation, Rockland, Maine, USA, [³⁵S]methionine from Amersham, and methionine assay medium from Difco.

RESULTS AND DISCUSSION

Construction of plasmids pSB12 and pSB22

In addition to a series of plasmids carrying the φX174 gene E under the control of the lac promoter-operator region (Henrich et al., 1982a, 1983), we have cloned gene E into plasmid PLC236 (Remaut et al., 1981) under the control of the λpL promoter. For this purpose the EcoRI fragments of plasmids pUH12 and pUH22, carrying the φX174 gene E in its wild-type form (lysis positive) or with the am3 mutation (lysis negative) respectively (Henrich et al., 1982a), were isolated and inserted into the EcoRI site of plasmid PLC236 (Fig. 1). The ligation mixtures were used to transform *E. coli* K12ΔH1Δtrp, which harbours a defective, nonexcisable λ prophage carrying the cI gene that codes for a temperature-sensitive repressor (cI857). Transformants were selected on plates containing ampicillin. After the isolation of plasmid DNA from several colonies, 20% were found to contain plasmids with the expected hybrid size. Digestion of these new plasmids with EcoRI produced two fragments, representing the original cloning vector PLC236 and the 1011 bp φX174 EcoRI fragment from plasmid pUH12 or pUH22. The orientations of the cloned φX174 fragment in the recombinant plasmids pSB12 (gene E wild-type allele) and pSB22 (gene E am3 allele) were determined by TaqI digestion. The restriction patterns obtained showed that both plasmids contain the inserted DNA fragment in the orientation which allows transcriptional control of the gene E sequence by λpL.
however, that the lytic process, once initiated, does not depend on further supply of gpE (Henrich et al., 1982a), seems not to hold in this case. It is more likely that gpE not only activates the autolytic enzyme system at limited and distinct sites (Denhardt & Sinsheimer, 1965; Markert & Zillig, 1965) but also causes a concentration-dependent damage of the cell envelope.

**Influence of the growth phase of E. coli on the lytic action of gpE**

As observed by Denhardt & Sinsheimer (1965) and Lubitz et al. (1984a), \(\phi X174\) only lyses \(E.\ coli\) cells which are actively growing. In accordance with this observation, cultures of strain \(K12\Delta H1\Delta trp(pSB12)\) entering the stationary growth phase cannot be lysed by temperature upshift to 42 °C. The question of whether this is a consequence of a lack of transcription and/or translation of gene \(E\) was investigated by shifting a culture of \(E.\ coli\ K12\Delta H1\Delta trp(pSB12)\) in the stationary growth phase to 42 °C for various periods, followed by reincubation at 28 °C. Lysis was not observed either during prolonged incubation at 42 °C or during the reincubation at 28 °C. However, when such a culture after adjustment of the temperature at 28 °C was diluted with fresh medium to half its density, the bacteria lysed even in the presence of rifampicin and chloramphenicol (Fig. 3). This indicates that neither transcription nor translation of gpE is inhibited in stationary cells. The block which hinders the action of gpE as a lysis protein is coupled with stagnation of growth of the cells, most probably with the regulation of the autolytic system (Lubitz et al., 1984a, b). Stationary cultures of \(E.\ coli\ K12\Delta H1\Delta trp(pSB12)\) which had been kept at 42 °C for up to several hours in the presence of gpE lysed after a signal which is coupled to onset of growth when they were diluted and reincubated at 28 °C.

**Identification of gpE**

The gene \(E\) product of \(\phi X174\) was first identified by Pollock et al. (1978) in heavily UV-irradiated \(E.\ coli\) cells. However, at the position where gpE migrated in the SDS gel used, other host cell proteins were also visible. Using plasmid pSB12, it was possible to detect gpE by separation of total cell proteins (Fig. 4). At a position which corresponds to a molecular mass of 10 kDal, an additional band is visible in the protein pattern of pSB12 transformants which is absent from the pattern of bacteria carrying pSB22. Pulse labelling further revealed that gpE is made throughout the period of induction, between temperature upshift and the onset of lysis (Fig. 4). The latent period between induction and lysis was prolonged by incubating the cells in minimal medium instead of rich medium. Under these conditions of reduced growth, a labelling period of 2 min with \(^{35}\text{S}\)methionine was sufficient to detect gpE. Because other attempts to visualize gpE in untreated bacteria have so far failed, it was of interest to determine the quantities in which gpE is synthesized relative to other proteins encoded by plasmid pSB12.

**Estimation of gpE synthesis in pSB12 transformants**

In order to reduce the amount of labelled host proteins, minicells were used. The amount of gpE formed relative to other plasmid-encoded proteins could be estimated by comparison of the protein patterns of minicells carrying pSB12 or pSB22, respectively.

To prevent expression of the cloned \(\phi X174\) lysis gene at 28 °C, the minicell-producing strain was first transformed with plasmid pcl857 encoding the thermosensitive \(\lambda\) repressor (Remaut et al., 1983). As shown in Fig. 5(a), a distinct band of gpE can be identified among the pSB12 encoded proteins when compared to the protein pattern of pSB22-transformed minicells. The corresponding gpE peak is also clearly visible in a density scan of the autoradiogram (Fig. 5b). The amount of gpE which accumulated during the labelling period of 45 min corresponds, however, only to 2.7% of the total of labelled proteins.

Under conditions where the \(\beta\)-lactamase gene had been cloned under control of \(\lambda\), expression of \(\beta\)-lactamase accounted for 30% of de novo protein synthesis in normal cells (Remaut et al., 1981). However, in such cells, in contrast to minicells, gpE represents only a minor protein (Fig. 4). The relatively low rate of gpE synthesis may thus be due to less efficient translation rather than to ineffective transcription.

As can be deduced from the nucleotide sequence of bacteriophage \(\lambda\) (Sanger et al., 1982) and the cloned fragment of \(\phi X174\) DNA, plasmid pSB12 contains no other genes between \(\lambda\) and...
Fig. 4. Synthesis of gpE in *E. coli* K12ΔH1Δtrp. Proteins synthesized in bacteria harbouring the recombinant plasmids pSB12 (lanes 1–5) or pSB22 (lanes 6–11) were labelled with $^{35}$S methionine and separated by electrophoresis on a 14–20% polyacrylamide SDS gel. The starting times of the labelling periods and the incubation temperatures are given at the top of the figure. The molecular masses of some protein markers, and the position of gpE, are indicated on the left.

gene *E*. The gene *E* ribosome-binding site is the first one on the mRNA which is initiated at $\lambda p_L$. However, translation of gpE seems to be poor. The relatively small amount found even after prolonged time of induction cannot be due to rapid degradation because, as shown above, gpE seems to be quite stable. Therefore other factors should be responsible for its low rate of synthesis. The ribosome-binding site of gene *E* contains only 4 bp which match the Shine–Dalgarno sequence (Barrell *et al.*, 1976). In addition, there is the possibility of some kind of translational attenuation because of the occurrence of 21 leucine residues within the 91 amino acids sequence of gpE, including one stretch of 7 leucines interrupted by one serine (Barrell *et al.*, 1976). More important, however, with respect to gene *E* expressivity, could be the codon usage in gene *E*. Many codons of gene *E*, including almost two-thirds of the leucine codons, correspond to minor tRNA species (Grantham *et al.*, 1980; Gouy & Gautier, 1982) and thus translation efficiency of the lethal gpE seems to be highly modulated. In addition, a perfect hairpin of 7 bp in the C-terminal coding region of gene *E* (Müller & Fitch, 1982) could exert severe influences on the rate of gpE formation.
Fig. 5. Synthesis of gpE in minicells. (a) Polypeptides synthesized in minicells of E. coli DS410 (recA) harbouring pSB12 and pC1857 (lane 1), pSB22 and pC1857 (lane 2), pC1857 (lane 3), and no plasmids (lane 4) were labelled with [35S]methionine and separated on a 14–20% polyacrylamide SDS gel. The molecular masses of protein markers, and the position of gpE, are indicated on the left. (b) Density scan of the autoradiogram shown in (a). | | | | Scan of the DS410(pSB12, pC1857) protein pattern; ...... scan of the DS410(pSB22, pC1857) protein pattern. The position of gpE is indicated by an arrow.

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REFERENCES


