ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

VIROLOGY

Virology 313 (2003) 622–628

www.elsevier.com/locate/yviro

Toxicity of the bacteriophage λ *cII* gene product to *Escherichia coli* arises from inhibition of host cell DNA replication

Barbara Kędzierska,[a] Monika Glinkowska,[a] Adam Iwanicki,[a] Michał Obuchowski,[a] Piotr Sojka,[a] Mark S. Thomas,[b] and Grzegorz Węgrzyn[a,c,*]

[a] *Department of Molecular Biology, University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland*
[b] *Division of Genomic Medicine, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK*
[c] *Institute of Oceanology, Polish Academy of Sciences, Św. Wojciecha 5, 81-347 Gdynia, Poland*

Received 6 May 2002; returned to author for revision 5 July 2002; accepted 24 April 2003

Abstract

The bacteriophage λ *cII* gene codes for a transcriptional activator protein which is a crucial regulator at the stage of the "lysis-versus-lysogeny" decision during phage development. The CII protein is highly toxic to the host, *Escherichia coli*, when overproduced. However, the molecular mechanism of this toxicity is not known. Here we demonstrate that DNA synthesis, but not total RNA synthesis, is strongly inhibited in *cII*-overexpressing *E. coli* cells. The toxicity was also observed when the transcriptional stimulator activity of CII was abolished either by a point mutation in the *cII* gene or by a point mutation, *rpoA341*, in the gene coding for the RNA polymerase α subunit. Moreover, inhibition of cell growth, caused by both wild-type and mutant CII proteins in either *rpoA*$^+$ or *rpoA341* hosts, could be relieved by overexpression of the *E. coli dnaB* and *dnaC* genes. In vitro replication of an *oriC*-based plasmid DNA was somewhat impaired by the presence of the CII, and several CII-resistant *E. coli* strains contain mutations near *dnaC*. We conclude that the DNA replication machinery may be a target for the toxic activity of CII.

Keywords: Bacteriophage λ; *cII* gene product; Toxic proteins; *Escherichia coli;* DNA replication; *rpoA* mutant

Introduction

The bacteriophage λ CII protein is a crucial regulatory protein involved in the "lysis-versus-lysogeny" decision at an early stage of phage development in *Escherichia coli* cells (for recent reviews, see Taylor and Węgrzyn, 1998; Węgrzyn et al., 2001). This protein is a strong activator of three phage promoters, p_E, p_1, and p_{aQ}, all of them being essential in switching λ development to the lysogenic pathway. At each promoter CII recognises the sequence $TTGC(N)_6TTGC$ overlapping the −35 region. The *cII* gene product is unstable in vivo due to its rapid degradation by the host-encoded HflB/FtsH protease (Herman et al., 1993). HflB is partially inhibited by the phage λ *cIII* gene product, which is also a substrate for this protease (Herman et al., 1997). In addition, CIII plays a more direct role in regulation of CII activity, possibly as a specific molecular chaperone (Latala et al., 2001).

Apart from being a transcriptional activator, the CII protein is highly toxic to host cells (Shimatake and Rosenberg, 1981; Rattay et al., 1984; Obuchowski et al., 1997a; Gabig et al., 1998). One explanation for the mechanism of this toxicity is that CII stimulates or represses expression of certain *E. coli* genes by binding sequences matching the CII DNA recognition sequence, thereby leading to the deregulation of essential cellular processes. This could lead to effective killing of bacterial cells shortly after induction of *cII* gene expression. Indeed, mutations in the gene encoding the β subunit of *E. coli* RNA polymerase (*rpoB*) were isolated, which conferred resistance of the host strain to killing by *cII* overexpression (Hammer et al., 1987). However, these mutants revealed unimpaired activation of CII-dependent λ promoters and normal *cII* gene expression, and

* Corresponding author. Fax: +48-58-301-0072.
E-mail address: wegrzyn@biotech.univ.gda.pl (G. Węgrzyn).

0042-6822/03/$ – see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0042-6822(03)00376-3

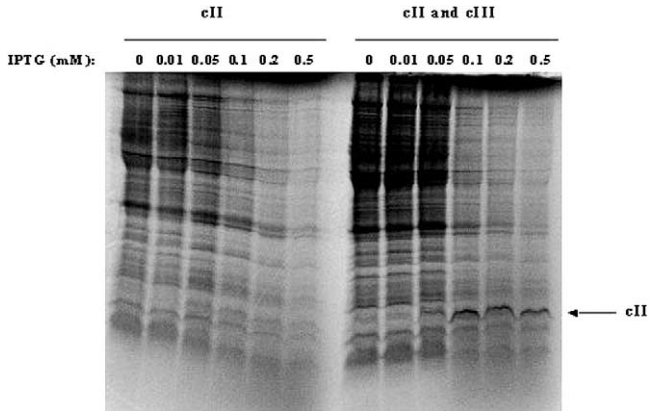

Fig. 1. Synthesis of *E. coli* proteins after induction of *cII* gene expression from plasmid pMO22 and after induction of *cII* and *cIII* expression from pMO23 in strain WAM106 containing plasmid pJMH1. Bacteria were grown in a minimal medium at 30°C to OD_{575} of 0.2. IPTG was then added at the concentrations indicated, and samples of equal cell mass were withdrawn 60 min later. Following pulse-labeling (5 min) with [^{35}S]methionine (2 μCi ml^{-1}), samples were subjected to SDS–PAGE and autoradiography.

most of them were defective in transcription termination and antitermination. Therefore, it was not possible from these results to identify the mechanism of CII toxicity.

In this work we aimed to identify cellular process(es) affected by overproduction of the CII protein, which should help us to understand the mechanism of CII toxicity. On the basis of these experiments, we conclude that the mechanism of CII toxicity is unlikely to be connected to its transcription activation function. In contrast, our results suggest that the toxic effect of CII appears to be specific for DNA replication.

Results

To identify cellular processes affected by overexpression of the *cII* gene, we measured the synthesis of proteins, RNA, and DNA in *E. coli* cells producing CII protein alone or CII together with CIII (in both cases the results were very similar). Bacterial cultures were pulse labeled with [^{35}S]methionine, [^{3}H]uridine, or [^{3}H]thymidine prior to induction of *cII* expression and at different times after induction. We found that protein synthesis was considerably inhibited in cells producing CII, or CII, and CIII, 60 min after induction (Fig. 1). However, this inhibition could be an indirect effect, as such a phenomenon was not observed up to 30 min after induction of *cII* and *cIII* expression, while the products of these genes were abundant as early as 5 min after the induction (data not shown, but see Fig. 3 in Obuchowski et al., 1997b). Synthesis of total RNA was not significantly affected by expression of *cII* even after 60 min of induction (Fig. 2A). However, incorporation of [^{3}H]thymidine into TCA-precipitable material did decrease dramatically relatively shortly (10–15 min) after induction of *cII* expression (Fig. 2B). These results suggest that inhibition of DNA replication may be the primary target of the toxic activity of CII protein.

The CII-mediated inhibition of DNA replication might be an indirect effect of CII- mediated changes in the expression of certain bacterial genes. To investigate the possibility that the activation function of CII is required for this phenomenon, we used previously characterised CII and host RNA polymerase mutants that abolish transcriptional activation by CII protein. It was demonstrated previously that substitution of alanine by threonine at position 30 (A30T) abolishes the CII activation function (Ho et al., 1988). This alanine residue comprises part of the first helix in the helix-turn-helix motif of CII, and it was concluded that this helix is involved in nonspecific interactions of the *cII* gene product with DNA. A plasmid analogous to that expressing the wild-type *cII* gene, pMO23, but harbouring a G-to-A change at nucleotide position 87 of the *cII* gene, leading to substitution of an alanine codon by a threonine codon at position 30, was constructed by site-directed mutagenesis and named pMO23*. Impairment of the transcription activation function by CII protein may also be caused by specific mutations in the *E. coli* chromosome. The *rpoA341* mutation results in a lysine-to-glutamate substitution at position 271 of the RNA polymerase α subunit (Thomas and Glass, 1991). This mutation effectively abolishes transcription activation by CII, most probably due to a defective interaction between the *cII* gene product and the mutant RNA polymerase (Obuchowski et al., 1997b), and also results in impairment of activation of a number of other positively regulated promoters (Giffard and Booth, 1988), causes stimulation of expression of some genes (Gabig et al., 2002), and impairs N-dependent transcriptional antitermination (Obuchowski et al., 1997c).

Induction of expression of the wild-type *cII* gene results in strong inhibition of bacterial growth (Fig. 3). We found

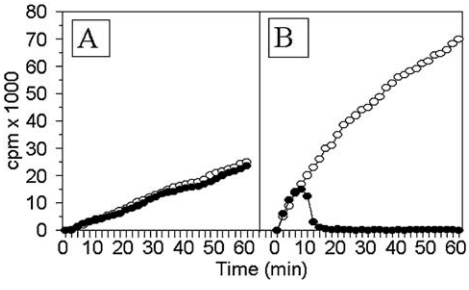

Fig. 2. Synthesis of RNA (A) and DNA (B) in *E. coli* WAM106 containing plasmids pJMH1 and pMO22. Bacteria were grown in a minimal medium at 30°C without (open circles) and with (closed circles) induction of *cII* gene expression from pMO22 by addition of IPTG to final concentration of 1 mM at time 0. At the indicated times, RNA or DNA synthesis was estimated by measurement of incorporation of [^{3}H]uridine or [^{3}H]thymidine, respectively, during 1-min pulses as described under Materials and methods. Similar results were obtained when plasmid pMO23 was used instead of pMO22, or when the *rpoA341* mutant (strain WAM105) was used as a host, and when cultivation was performed at 43°C (data not shown).

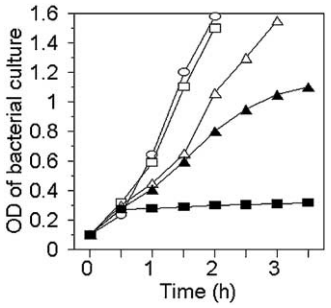

Fig. 3. Growth of the *E. coli* strain WAM106 bearing plasmid pJMH1 (circles) and, in addition, plasmid pMO23 (squares), or plasmids pMO23 and pPS562 (triangles). Bacteria were cultured in LB medium at 43°C. In experiments represented by open symbols, expression of the *cII* gene was not induced, and in experiments represented by filled symbols, *cII* expression from plasmid pMO23 was induced by addition of IPTG to final concentration of 0.1 mM at time 0. Expression of the *dnaB* and *dnaC* genes from plasmid pPS562 was induced at 43°C (a shift of all cultures from 30 to 43°C was performed at time 0). Bacterial growth was monitored by measurement of OD_{575} of cultures at the indicated times.

that the presence of the A30T substitution in overproduced CII had no effect on CII-mediated toxicity (Fig. 4A). Similarly, an *E. coli* host strain harbouring the *rpoA341* mutation is only slightly less susceptible to the toxic effects of CII overproduction (Fig. 4B). These results indicate that CII toxicity is likely to be independent of the transcription activation function of this protein.

Although CII is an activator protein, it can also act as a repressor if the CII DNA binding site is appropriately positioned (Gussin et al., 1986). Thus, CII may repress bacterial genes where DNA sequences corresponding to the CII recognition sequence overlap promoters or are located downstream of promoters where a "roadblock" mechanism could possibly operate (Deuschle et al., 1986; Pavco and Steege, 1991; He and Zalkin, 1992). To investigate this possibility, we performed an in silico analysis of the *E. coli* genome and found that there are 294 sites fully matching the CII-binding sequence, $TTGC(N)_6TTGC$, distributed apparently randomly around the genome (data not shown). In most cases, these sites were located within gene coding sequences, including those for genes whose products are involved in DNA replication (*dnaX*) and other DNA-based processes (*exo, parE, recG, recR, dinD*). Only 11 of the CII recognition sites were identified upstream of coding sequences, where CII binding may potentially interfere with transcription initiation. None of these sites were located near promoters of essential genes. Therefore, we did not identify an obvious target for CII-mediated inhibition of transcription that would lead to an arrest of DNA replication. In addition, no CII-binding sites were found near *oriC*, the origin of *E. coli* chromosome replication, which could enable CII to directly interfere with DNA replication.

To investigate the mechanism of CII-mediated inhibition of DNA replication, we examined the possibility that overexpression of certain genes encoding components of the DNA replication machinery could suppress the toxic effects of CII. Accordingly, we found that overexpression of the *E. coli dnaB* and *dnaC* genes together significantly suppressed the growth inhibition resulting from CII overproduction (Fig. 3). This suppression did not occur when *dnaB* or *dnaC* was overexpressed separately (data not shown). Overexpression of other genes involved in DNA replication regulation, *dnaA* and *seqA*, also did not alleviate CII toxicity (data not shown). In our in silico analysis, no CII-binding sites were found adjacent to or overlapping the *dnaB* and *dnaC* genes (data not shown). Therefore, it seems unlikely that CII influences transcription of these genes.

We asked whether amino acid substitutions in the CII protein and the RNA polymerase α subunit which cause impairment of CII-mediated transcription stimulation influence the alleviation of CII-dependent inhibition of bacterial growth by overexpression of the *dnaB* and *dnaC* genes. We found that overexpression of *dnaB* and *dnaC* improves bacterial growth in the presence of CII in all experimental systems tested (Fig. 4). Interestingly, this improvement was most pronounced in the *rpoA341* mutant (Fig. 4B).

To test whether CII inhibits DNA replication directly, we examined the effects of this protein on in vitro replication of a minichromosome (*oriC*-based plasmid DNA). For these experiments we used a His-tagged derivative of CII which is functional as a transcription activator in vitro (data not shown). *E. coli* protein extracts (Fraction II) were added to a reaction mixture containing purified CII and pBSoriC plasmid DNA (Shotland et al., 2000). We found some inhibition of *oriC*-initiated DNA replication by CII protein in this in vitro system, though the effect was not dramatic (Fig. 5).

If CII inhibits DNA replication by interacting with the replication machinery, particularly with DnaB and/or DnaC,

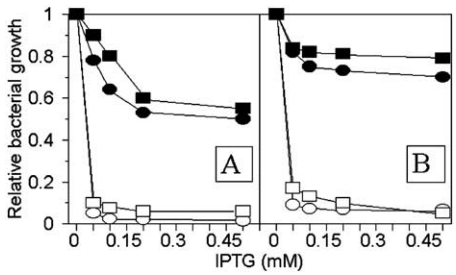

Fig. 4. Relative growth of *E. coli rpoA*$^+$ (WAM106, panel A) and *rpoA341* (WAM105, panel B) hosts bearing plasmids pMO23 (open circles), pMO23* (open squares), pMO23 and pPS562 (filled circles), or pMO23* and pPS562 (filled squares). All strains also harboured plasmid pJMH1. Expression of the wild-type *cII* gene from plasmid pMO23 (circles) or a mutant form encoding the A30T CII mutant from plasmid pMO23* (squares) was induced by addition of IPTG to the concentrations indicated at time 0 (at OD_{575} of bacterial culture = 0.1). Expression of the *dnaB* and *dnaC* genes from plasmid pPS562 was induced at 43°C (a shift of all cultures from 30 to 43°C was performed at time 0). Relative bacterial growth was calculated as the ratio of the increase of the OD_{575} of a given culture in which expression of a *cII* allele was induced, to the increase of the OD_{575} of the control culture, i.e., without induction of *cII* expression, between 60 and 120 min after the induction. A value of 1.0 corresponds to no observed growth inhibition.

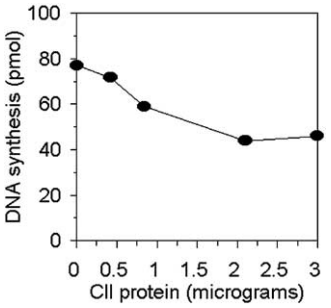

Fig. 5. Effect of CII protein on in vitro replication of an *oriC*-based plasmid, pBSoriC. The reactions were carried out in *E. coli* Fraction II extracts in the presence of the indicated amounts of histidine-tagged CII protein (added to the reaction mixture before addition of Fraction II), as described under Materials and methods.

it should be possible to isolate *E. coli* mutants in *dnaB* and/or *dnaC* gene(s), revealing resistance to CII. Using a series of strains bearing antibiotic-resistance markers located at different positions in the *E. coli* chromosome (Singer et al., 1989), we asked whether it is possible to isolate CII-resistant mutants in *dnaB* and/or *dnaC*. Bacteria bearing a plasmid with *cII* gene under p_{tac} promoter (pMO23) and a plasmid with *lacI*^{Q} gene (pJMH1) were spread on plates containing appropriate antibiotics (to ensure maintenance of pMO23 and pJMH1), 1 mM IPTG (to induce *cII* overexpression), and a mutagen. When NQNO was used as a mutagen at an amount of 10 μg per plate, a few hundred colonies could be obtained. To test if some of these mutants bear changes in the *dnaC* locus, we used the CAG18619 strain, harbouring Tn*10-kan* at 99.5 min of *E. coli* genetic map (*zjj-3188*::Tn*10kan* mutation). This locus is relatively close to *dnaC* (99.1 min). In control experiments, *dnaC1* (ts) mutation was found to be 27% cotransducible with *zjj-3188*::Tn*10kan* (data not shown). After obtaining CII-resistant mutants of CAG18619, P1 transduction experiments were performed, using MG1655/pMO23/pJMH1 strain as a recipient. Randomly selected transductants were tested for CII-resistance by streaking colonies on plates containing 1 mM IPTG (i.e., under conditions of CII overproduction). Seven of nine randomly selected mutants revealed linkage (20–50% cotransduction) of kanamycin resistance and CII resistance. We conclude that these seven mutants are likely to contain mutations in *dnaC*. An analogous selection using strain CAG18609 that bears the *malF3180*::Tn*10kan* mutation, which is close to *dnaB*, did not reveal any linkage of CII resistance to the transposon (data not shown).

Discussion

The bacteriophage λ-encoded CII protein, a transcriptional activator of phage lysogenic promoters, is highly toxic to *E. coli* cells. It might seem likely that the lethal effect of CII arises from overactivation of one or more bacterial genes and subsequent disturbance of cell metabolism. However, as we demonstrate in this article, the toxicity of CII is not connected to its ability to stimulate transcription initiation. Rather, our results suggest that CII toxicity arises from an inhibition of host chromosome replication, as DNA synthesis was strongly inhibited in cells expressing *cII*, and overexpression of the *dnaB* and *dnaC* genes alleviated this inhibition significantly.

The *dnaB* gene codes for DNA helicase, which operates in replication forks, and the *dnaC* gene product is responsible for delivery of the DnaB helicase to the origin of replication (*oriC*) region (for a review see Messer and Weigel, 1996). This, in combination with the observation that (i) overexpression of either *dnaB* or *dnaC* alone did not alleviate CII toxicity and (ii) strong inhibition of DNA replication was observed 10–15 min after induction of *cII* gene expression, while CII protein was found in large amounts in cells as shortly as 5 min after such an induction (Obuchowski et al., 1997b, and data not shown) suggests that the process of initiation of DNA replication may be directly affected by CII.

The inhibition of protein synthesis, observed after induction of *cII* expression, could also result from changes in some postinitiation steps in transcription. A decrease in the activity of β-galactosidase in cells expressing *cII* and harbouring the *lac*Z gene under control of CII-independent promoters has been reported (Obuchowski et al., 1997a). However, the inhibition of protein synthesis was considerably delayed relative to the appearance of large amounts of CII in cells. Moreover, overexpression of only two genes, *dnaB* and *dnaC*, restored bacterial growth in the presence of CII. Therefore, it seems more likely that the general inhibition of protein synthesis in cells overexpressing *cII* may be a secondary effect occurring in response to inhibition of DNA replication.

Given the large number (294) of potential CII binding sites present on the *E. coli* genome, one might argue that the mechanism for the toxicity of overproduced CII most likely involves binding of CII to one or more of these sequences. Moreover, the absence of a requirement for the activation function of CII may suggest that the toxic activity of CII could result from transcription repression. However, the almost immediate effect of CII overproduction on DNA replication may point to a direct interference with the DNA replication process. Transcription near the *oriC* region plays an important regulatory role during initiation of *E. coli* chromosome replication (Messer and Weigel, 1996). Although the exact role of this transcription is not known, the binding of a CII tetramer may interfere with this process and, in turn, with DNA replication initiation. Replication of bacteriophage λ also requires transcription of the origin of λ DNA replication (*ori*λ), which appears to be required for proper positioning of DnaB helicase (Taylor and Węgrzyn, 1995). Interestingly, λ DNA replication is also inhibited under conditions of enhanced expression of the *cII* gene

(Obuchowski et al., 1997a; Gabig et al., 1998). It is not clear whether the mechanism of inhibition of λ DNA replication by CII is related to the mechanism of inhibition of host cell DNA replication. Nevertheless, it is interesting that CII-mediated inhibition of phage replication could be alleviated by overproduction of λ replication proteins, O (an initiator protein) and P (a DnaC functional analog) (Obuchowski et al., 1997a). However, as no CII-binding sites were found near *oriC* in our in silico analysis, one might speculate that CII could inhibit DNA replication by directly contacting the replication machinery. Isolation of CII-resistant strains with mutations localized in the *dnaC* gene region may support this hypothesis. On the other hand, incomplete inhibition of DNA replication by CII in vitro may suggest that the mechanism of CII-mediated toxicity is more complicated in vivo and, as suggested above, it possibly involves interference with transcriptional activation of the *origin* as well as interaction with the replication machinery (note that the requirement for transcriptional activation of the origin is strict in vivo, whereas it may be negligible in vitro).

In summary, we have demonstrated that the replication machinery may be a target for the toxic activity of CII, but more detailed studies are necessary to understand the molecular mechanisms of CII-mediated inhibition of DNA replication.

Materials and methods

Bacterial strains and plasmids

The *E. coli* strain WAM106 (F^-, *araD139*, Δ(*argF-lac*)*U169*, Δ(*his-gnd*), *thi*, *rpsL150*, *gltS*$_0$, *flbB5301*, *relAI*, *deoCI*, *rbsR*) and its *rpoA341* derivative, WAM105, were described previously (Thomas and Glass, 1991). MG1655 wild-type strain (Jensen, 1993) and a series of strains bearing antibiotic-resistance marker at different positions on the chromosome (Singer et al., 1989) were employed. Strain PC1 (*leuB6*, *thyA47*, *deoC3*, *rpsL*, *dnaC1*) was from our collection. Plasmid pPS562, bearing the *dnaB* and *dnaC* genes under control of the p_R and p_L promoters arranged in tandem and conditionally repressed by a temperature-sensitive *cI857* gene product (Elvin et al., 1990), was used. Plasmid pJMH1 (a pSC101-derived replicon bearing the *lac*I^q allele) has already been described (Węgrzyn et al., 1992). Plasmids pMO22 (a p15A-derived replicon bearing the *cII* gene under control of p_{tac}) and pMO23 (a p15A-derived replicon bearing the *cII* and *cIII* genes, each under control of p_{tac}) were described previously (Obuchowski et al., 1997b). Plasmid pMO23* was constructed by introducing a G-to-A substitution at nucleotide position 87 of the *cII* gene, according to the overlap extension method of site-directed mutagenesis described by Ho et al. (1989). Internal primers were P2502 (5′-TGA GAA GAC AAC GGA AGC TGT GGG) and P2503 (5′-CCC ACA GCT TCC GTT GTCTTC TCA), and external primers were PCB2 (5′-TGG CTG ATG GTG CGA TAG TC) and PCB7 (5′-ACG TGC GTC CTC AAG CTG). The integrity of the mutant *cII* gene present in pMO23* was confirmed by DNA sequencing according to Sambrook et al. (1989). Plasmid pBSoriC (Konieczny and Liberek, 2002 and references therein) is a minichromosome (*oriC* plasmid) that was used in this work as a template in in vitro replication assays.

Estimation of bacterial growth rates

Bacterial strains were cultured in LB medium (Sambrook et al., 1989) at the indicated temperatures in water bath shakers. Samples of cultures were withdrawn at time intervals; OD_{575} was measured, and the growth rate was calculated on the basis of growth curve analysis.

Estimation of efficiency of synthesis of proteins, RNA, and DNA

Synthesis of proteins, RNA, and DNA was estimated by measurement of incorporation of [^{35}S]methionine, [^{3}H]uridine, or [^{3}H]thymidine, respectively, during 5-min (labeling with [^{35}S]methionine) or 1-min (labeling with [^{3}H]uridine or [^{3}H]thymidine) pulses according to a previously described method (Węgrzyn et al., 1991). Briefly, bacteria were cultured in a previously described minimal medium (Węgrzyn et al., 1991); samples were withdrawn at the indicated times and [^{35}S]methionine or ^{3}H-labeled nucleosides ([^{3}H]uridine or [^{3}H]thymidine) were added up to 2 or 1 μCi ml^{-1}, respectively, for 1 or 5 min (see above). Then samples were either boiled and subjected to SDS–polyacrylamide gel electrophoresis and autoradiography (labeling with [^{35}S]methionine) according to Sambrook et al. (1989) or placed onto paper filters and transferred immediately to ice-cold 10% TCA, washed in 5% TCA, then 1% TCA and twice in 96% ethanol, and analyzed in a scintillation counter (labeling with [^{3}H]uridine or [^{3}H]thymidine).

Purification of CII protein

Plasmid pET-CII (Shotland et al., 2000) was used for overproduction of N-terminal His-tagged CII protein which was purified as described previously (Shotland et al., 2000). We proved that the purified his-tagged CII protein was active as a transcription stimulator at the λ p_E promoter by carrying out in vitro transcription assays (data not shown).

In vitro DNA replication assay

Replication of an *oriC*-based plasmid (pBSoriC) in vitro, using a crude *E. coli* protein extract (Fraction II), was examined as described by Konieczny and Liberek (2002). Standard reactions contained 300 ng of plasmid pBSoriC template and indicated amounts of the histidine-tagged CII protein in the same volume of reaction buffer. The reactions were carried out at 32°C for 60 min and were stopped by

addition of an excess of trichloroacetic acid. Total radioactive nucleotide incorporation was measured in a scintillation counter following filtration onto Whatman GF/C glass fiber filters, and the amount of synthesized DNA was calculated.

Isolation of CII-resistant mutants

Bacteria bearing plasmids pMO23 and pJMH1 were cultivated in LB medium, and 0.1 ml of a culture was spread onto an LB plate containing appropriate antibiotics, IPTG at final concentration of 1 mM (to induce CII overproduction), and a mutagen (the use of NQNO, 4-nitroquinolone-*N*-oxide, at 10 μg per plate, was the most effective method to obtain CII-resistant mutants). Plates were incubated overnight at 37°C, and colonies were used for mapping of mutations.

Mapping of CII-resistance mutations

To test whether mutations causing CII-resistance are located in *dnaB* or *dnaC* regions, strains bearing a *kan* marker near *dnaB* or *dnaC* were used for isolation of CII-resistant mutants as described above. Then, the obtained mutants were used as donors in P1 transduction experiments (performed according to Silhavy et al., 1984), in which MG1655/pMO23/pJMH1 strain was a recipient. One hundred transductants, selected for kanamycin-resistance, were tested for CII-sensitive phenotype on LB plates containing 1 mM IPTG (thus overexpressing *cII*), and percentage of cotransduction of kanamycin resistance and CII resistance was calculated.

Acknowledgments

We are very grateful to Dr. Igor Konieczny for help in in vitro replication experiments and for discussions. We thank Kirsten Skarstad for providing plasmid pPS562. This work was supported by the Polish State Committee for Scientific Research (project Grant 3 P04A 049 24 to G.W.) and the Wellcome Trust (Grant 050794 to M.S.T.). B.K. and G.W. acknowledge financial support from the Foundation for Polish Science (subsidy 14/2000).

References

Deuschle, U., Gentz, R., Bujard, H., 1986. *lac* repressor blocks transcribing RNA polymerase and terminates transcription. Proc. Natl. Acad. Sci. USA 83, 4134–4137.

Elvin, C.M., Thompson, P.R., Argall, M.E., Hendry, P., Stamford, N.P.J., Lilley, P.E., Dixon, N.E., 1990. Modified bacteriophage lambda promoter vectors for overproduction of proteins in *Escherichia coli*. Gene 87, 123–126.

Gabig, M., Obuchowski, M., Śrutkowska, S., Węgrzyn, G., 1998. Regulation of replication of λ phage and plasmid at low temperature. Mol. Gen. Genet. 258, 494–502.

Gabig, M., Herman-Antosiewicz, A., Kwiatkowska, M., Łoś, M., Thomas, M.S., Węgrzyn, G., 2002. The cell surface protein Ag43 facilitates phage infection of *Escherichia coli* in the presence of bile salts and carbohydrates. Microbiology 148, 1533–1542.

Giffard, P.M., Booth, I.R., 1988. The *rpoA341* allele of *Escherichia coli* specifically impairs the transcription of a group of positively-regulated operons. Mol. Gen. Genet. 214, 148–154.

Gussin, G.N., Temple, E., Brown, S.E., Court, D., 1986. Repression of a mutant derivative of the p_{RE} promoter of bacteriophage lambda by its activator, CII. Gene 46, 171–180.

Hammer, K., Jensen, K.F., Poulsen, P., Oppenheim, A.B., Gottesman, M., 1987. Isolation of *Escherichia coli rpoB* mutants resistant to killing by λ cII protein and altered in *pyrE* gene attenuation. J. Bacteriol. 169, 5289–5297.

He, B., Zalkin, H., 1992. Repression of *Escherichia coli purB* is by a transcriptional roadblock mechanism. J. Bacteriol. 174, 7121–7127.

Herman, C., Ogura, T., Tomoyasu, T., Hiraga, S., Akiyama, Y., Ito, K., Thomas, R., D'Ari, R., Bouloc, P., 1993. Cell growth and λ phage development controlled by the same essential *Escherichia coli* gene, *ftsH/hflB*. Proc. Natl. Acad. Sci. USA 90, 10861–10865.

Herman, C., Thevenet, D., D'Ari, R., Bouloc, P., 1997. The HflB protease of *Escherichia coli* degrades its inhibitor λ cIII. J. Bacteriol. 179, 358–363.

Ho, Y.S., Mahoney, M.E., Wulff, D.L., Rosenberg, M., 1988. Identification of the DNA binding domain of the phage λ *c*II transcriptional activator and the direct correlation of cII protein in stability with its oligomeric forms. Genes Dev. 2, 184–195.

Ho, S.N., Hunt, H.D., Morton, R.M., Pullen, J.K., Pease, L.R., 1989. Site directed mutagenesis by overlap extension using the polymerase chain reaction. Gene 77, 51–59.

Jensen, K.F., 1993. The *Escherichia coli* K-12 "wild types" W3110 and MG1655 have an *rph* frameshift mutation that leads to pyrimidine starvation due to low *pyrE* expression levels. J. Bacteriol. 175, 3401–3407.

Konieczny, I., Liberek, K., 2002. Cooperative action of *Escherichia coli* ClpB protein and DnaK chaperone in the activation of a replication initiation protein. J. Biol. Chem. 277, 18483–18488.

Latała, B., Obuchowski, M., Węgrzyn, G., 2001. Bacteriophage λ *c*III gene product has an additional function apart from inhibition of cII degradation. Virus Genes 22, 127–132.

Messer, W., Weigel, C., 1996. Initiation of chromosome replication, in: Neidhard, F.C., Curtiss III, R., Ingraham, J.L., Lin, E.C.C., Low, K.B., Magasanik, B., Reznikoff, W.S., Riley, M., Schaechter, M., Umbarger, H.E. (Eds.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, American Society for Microbiology, Washington, DC, pp. 1579–1601.

Obuchowski, M., Shotland, Y., Koby, S., Giladi, H., Gabig, M., Węgrzyn, G., Oppenheim, A.B., 1997a. Stability of CII is a key element in the cold stress response of bacteriophage λ infection. J. Bacteriol. 179, 5987–5991.

Obuchowski, M., Giladi, H., Koby, S., Szalewska-Palasz, A., Węgrzyn, A., Oppenheim, A.B., Thomas, M.S., Węgrzyn, G., 1997b. Impaired lysogenisation of the *Escherichia coli rpoA341* mutant by bacteriophage λ is due to the inability of CII to act as a transcriptional activator. Mol. Gen. Genet. 254, 304–311.

Obuchowski, M., Węgrzyn, A., Szalewska-Palasz, A., Thomas, M.S., Węgrzyn, G., 1997c. An RNA polymerase α subunit mutant impairs N-dependent transcriptional antitermination in *Escherichia coli*. Mol. Microbiol. 23, 211–222.

Pavco, P.A., Steege, D.A., 1991. Characterization of elongating T7 and SP6 RNA polymerases and their response to a roadblock generated by a site-specific DNA binding protein. Nucleic Acids Res. 19, 4639–4646.

Rattray, A., Altuvia, S., Mahajna, G., Oppenheim, A.B., Gottesman, M., 1984. Control of bacteriophage lambda *cII* activity by bacteriophage and host functions. J. Bacteriol. 159, 238–242.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shimatake, H., Rosenberg, M., 1981. Purified λ regulatory protein cII positively activates promoters for lysogenic development. Nature 292, 128–132.

Shotland, Y., Shifrin, A., Ziv, T., Teff, D., Koby, S., Kobiler, O., Oppenheim, A.B., 2000. Proteolysis of bacteriophage λ CII by *Escherichia coli* FtsH (HflB). J. Bacteriol. 182, 3111–3116.

Silhavy, T.J., Berman, M.L., Enquist, L.W., 1984. Experiments with Gene Fusions. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Singer, M., Baker, T.A., Schnitzler, G., Deischel, S.M., Goel, M., Dove, W., Jaacks, K.J., Grossman, A.D., Erickson, J.W., Gross, C.A., 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. 53, 1–24.

Taylor, K., Węgrzyn, G., 1995. Replication of coliphage lambda DNA. FEMS Microbiol. Rev. 17, 109–119.

Taylor, K., Węgrzyn, G., 1998. Regulation of bacteriophage λ replication, in: Busby, S.J.W., Thomas, C.M., Brown, N.L. (Eds.), Molecular Microbiology, Springer Verlag, Berlin-Heidelberg, pp. 81–97.

Thomas, M.S., Glass, R.E., 1991. *Escherichia coli rpoA* mutation which impairs transcription of positively regulated systems. Mol. Microbiol. 5, 2719–2725.

Węgrzyn, G., Kwaśnik, E., Taylor, K., 1991. Replication of λ plasmid in amino acid- starved strains of *Escherichia coli*. Acta Biochim. Pol. 38, 181–186.

Węgrzyn, G., Glass, R.E., Thomas, M.S., 1992. Involvement of the *Escherichia coli* RNA polymerase α subunit in transcriptional activation by bacteriophage lambda CI and CII proteins. Gene 122, 1–7.

Węgrzyn, G., Węgrzyn, A., Barańska, S., Czyz, A., 2001. Regulation of bacteriophage lambda development. Recent Res. Dev. Virol. 3, 375–386.