Excess production of phage λ delayed early proteins under conditions supporting high *Escherichia coli* growth rates

Magdalena Gabig,¹ Michał Obuchowski,¹ Alicja Węgrzyn,²
Agnieszka Szalewska-Pałasz,¹ Mark S. Thomas³ and Grzegorz Węgrzyn¹

Author for correspondence: Grzegorz Węgrzyn. Tel: +48 58 346 3014. Fax: +48 58 301 0072.
e-mail: wegrzyn@biotech.univ.gda.pl

¹ Laboratory of Molecular Genetics, Department of Molecular Biology, University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland

² Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Laboratory of Molecular Biology affiliated to the University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland

³ Division of Molecular and Genetic Medicine, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

Bacteriophage λ is unable to lysogenize *Escherichia coli* hosts harbouring the *rpoA341* mutation due to a drastic reduction in transcription from CII-activated lysogenic promoters (p_E , p_I and p_{aQ}). In addition, the level of early transcripts involved in the lytic pathway of λ development is also decreased in this genetic background due to impaired N-dependent antitermination. Here, it is demonstrated that despite the reduced level of early lytic p_L - and p_R -derived transcripts, lytic growth of bacteriophage λ is not affected in rich media. The level of the late lytic, p_R -derived transcripts also remains unaffected by the *rpoA341* mutation under these conditions. However, it was found that whilst there is no significant difference in the phage burst size in *rpoA*⁺ and *rpoA341* hosts growing in rich media, phage λ is not able to produce progeny in the *rpoA341* mutant growing in minimal medium, in contrast to otherwise isogenic *rpoA*⁺ bacteria. Provision of an excess of the phage replication proteins O and P *in trans* or overproduction of the antitermination protein N restore the ability of phage λ to produce progeny in the *rpoA341* mutant under the latter conditions. These results suggest that in rich media phage λ produces some early proteins in excess of that needed for its effective propagation and indicate that replication proteins may be limiting factors for phage lytic growth in poor media.

Keywords: bacteriophage λ development, *Escherichia coli* *rpoA341* mutation, phage λ gene expression, λ replication proteins, bacterial growth rate

INTRODUCTION

As a temperate phage, λ is able to enter either of two alternative modes of development upon infection of *Escherichia coli*: the lytic and lysogenic pathways (for reviews see Echols, 1986; Ptashne, 1992). However, the physiological conditions under which the lytic pathway is entered may vary considerably. For example, although lysogenic development is preferred in bacteria infected by many phages simultaneously and growing in poor media, there is only a small possibility that lysogenization will occur when the host cell is infected by a single phage irrespective of the growth conditions (for reviews see Herskowitz & Hagen, 1980; Herskowitz, 1985; Taylor & Węgrzyn, 1998). Therefore, λ must be able to maximize its yield of progeny under these different conditions. Although bacteriophages have played a crucial role in the development of molecular biology, most experiments on phage genetics and

biochemistry have been performed under laboratory conditions supporting near maximum growth rates of the host bacterium, a situation which may be far removed from that found in the natural environment of *E. coli*. Therefore, the full extent to which growth conditions and the physiological state of the host cell influences phage development, as well as phage strategies which could ensure its effective propagation under different environmental conditions, may have been overlooked (see Hadas *et al.*, 1997).

We demonstrated previously that a mutation, *rpoA341*, in the gene encoding the α subunit of *E. coli* RNA polymerase prevents lysogenization by bacteriophage λ (Węgrzyn *et al.*, 1992a). We proposed, and recently confirmed experimentally, that impaired lysogenization of *rpoA341* hosts results from drastically decreased transcription from the CII-activated promoters p_E , p_I and p_{aQ} , which are necessary for establishment of a λ

prophage (Węgrzyn *et al.*, 1992a; Szalewska-Pałasz *et al.*, 1996; Obuchowski *et al.*, 1997b). In addition, we found that the level of the longer transcripts derived from the early lytic p_L and p_R promoters was also decreased in the phage-infected *rpoA341* mutant due to impaired antitermination at the t_{L1} and t_{R1} terminators (Szalewska-Pałasz *et al.*, 1996; Obuchowski *et al.*, 1997a) (a genetic map of bacteriophage λ is shown in Fig. 1). Thus, it would seem likely that lytic growth of bacteriophage λ should also be compromised in the *rpoA341* mutant. The aim of this work was to examine the effect of the *rpoA341* mutation on λ lytic growth. Contrary to expectation, our results show that there are no phenotypic differences between λ lytic development in *rpoA*⁺ and *rpoA341* hosts growing in rich media, whereas an effect is observed under conditions supporting slow growth of the host. These observations can be rationalized as part of an overall strategy for the effective propagation of the phage in the host cell under a range of nutritional conditions.

METHODS

Bacterial strains, phages and plasmids. Otherwise isogenic [*F*⁻ *araD139* Δ (*argF-lac*)U169 Δ (*his-gnd*) *thi rpsL150 gltS*, *flbB5301 relA1 deoC1 rbsR*] *rpoA341* and *rpoA*⁺ strains of *E. coli* K-12 (WAM105 and WAM106, respectively) have been described previously (Thomas & Glass, 1991). Bacteriophages λ cl857S7 (Goldberg & Howe, 1969), λ c17, λ cl857cll68 (Obuchowski *et al.*, 1997c), λ c17cll68 (from A. B. Oppenheim, The Hebrew University Hadassah Medical School, Jerusalem, Israel) and λ clb2 (from our collection) were used. For providing O and P proteins *in trans* we used plasmid pTC λ 1 (Herman-Antosiewicz *et al.*, 1998), which contains the phage λ O and P genes under control of the *tetA* gene promoter. This promoter is repressed by the pTC λ 1-encoded TetR repressor, and transcription of the O and P genes can be induced by autoclaved chlortetracycline (which does not act as an antibiotic but is still active as an inducer). Overproduction of the phage antiterminator protein, N, was achieved using plasmid pSS125 (Sullivan & Gottesman, 1992; Obuchowski *et al.*, 1997a) harbouring the N gene under control of the p_{lac} promoter and the *lacI*^q allele (IPTG was used as an inducer). Plasmid pGAW9035 was constructed by ligation of a 9035 bp λ DNA fragment, stretching from the *EcoRI* site at 44972 through *cos* to the *BamHI* site at 5505, with the 3315 bp *EcoRI*–*BamHI* fragment of pBR328 (Soberon *et al.*, 1980).

Culture media. The following media were used: Luria–Bertani (LB) broth (Sambrook *et al.*, 1989); MMGluCaa (termed minimal medium 1 by Węgrzyn *et al.*, 1991), composed of salts (for details see Węgrzyn *et al.*, 1991), thiamin (10 μ g ml⁻¹), Casamino acids (1%, w/v) and glucose (1%, w/v); and MMGlu, in which the Casamino acids of MMGluCaa are replaced with L-histidine and L-cysteine (each at a final concentration of 100 μ g ml⁻¹).

DNA techniques. All DNA manipulations (molecular cloning and preparation of DNA templates for construction of labelled probes) were carried out according to Sambrook *et al.* (1989).

Kinetics of bacteriophage λ lytic growth and burst size determination. Lytic development of bacteriophage λ was investigated by one-step growth experiments as described previously (Szalewska *et al.*, 1994). Briefly, bacteria growing exponentially in the appropriate medium were infected by λ

phage in the presence of 3 mM NaN₃ (to prevent unsynchronized phage development) at the indicated m.o.i. and adsorption was carried out for 10 min. Following centrifugation, the bacterial pellet was resuspended in the same medium (with NaN₃) containing anti- λ serum and incubated for 5 min to neutralize unadsorbed phages. The suspension was then diluted 1000-fold with the prewarmed medium (devoid of NaN₃) and aerated in a water-bath shaker. The number of 'infective centres' was estimated by plating of samples taken during the first 10 min after dilution (time 0–10 min). The estimated number of 'infective centres' is in fact the sum of the number of infected bacteria and the number of free, unadsorbed phages. However, as most of the unadsorbed phage particles were neutralized by anti- λ serum the second value was ignored during the calculation of the burst size. The number of intracellular progeny phages was estimated by plating of chloroform-treated samples of the infected culture withdrawn at different times. The burst size was calculated as the ratio of the number of progeny phages to the number of infective centres. In Table 1, the burst size is provided as the only measurement of λ lytic growth and was calculated from samples taken at times when the phage growth curve reached a plateau.

Estimation of the rate of bacteriophage λ O protein synthesis.

Bacteria growing at 30 °C in LB or MMGlu medium were infected with phage λ cl857S7 at an m.o.i. of 10 and samples were taken for pulse labelling 5 min post-infection as O protein synthesis is maximal at this stage (Węgrzyn *et al.*, 1992b). Measurement of the synthesis of bacteriophage λ -O protein was performed by pulse labelling (5 min) with [³⁵S]methionine and immunoprecipitation of cell lysates with anti- λ O serum followed by SDS-PAGE and autoradiography as described previously (Węgrzyn *et al.*, 1992b). Because LB medium contains a relatively high concentration of peptides and amino acids, a 10-fold higher amount of radiolabelled methionine was used in experiments performed in this medium relative to experiments performed in MMGlu medium. Moreover, during autoradiography, the films from different experiments were exposed for various times to achieve the best resolution. Therefore, the results presented in Fig. 2 may be directly compared within one panel but not between panels.

Analysis of phage protein synthesis in λ -infected cells.

Phage protein synthesis in λ -infected cells was measured in pulse-labelling experiments as described previously (Gottesman *et al.*, 1981). The bands on the autoradiogram, identified on the basis of their migration in the gel relative to molecular mass standards, were quantified by densitometry using a UVP Enhanced Analysis System (EASY).

Measurement of the level of phage transcripts.

The level of longer, antiterminated (due to Q protein activity), late transcripts derived from the bacteriophage λ p_R promoter was estimated on the basis of Northern hybridization. Northern blot experiments were performed as described previously (Węgrzyn, A. *et al.*, 1995). Bacteria were infected with phage λ cl857S7 at an m.o.i. of 10 and samples were withdrawn 35 min post-infection, as the amount of late transcripts reaches a maximum around this time (Echols, 1971). The template for labelling of the probe designed for detection of p_R -derived transcripts was the *EcoRI*–*BamHI* λ DNA insert of plasmid pGAW9035.

Analysis of λ E protein abundance.

Detection of the λ E protein in phage-infected cells was performed by Western blotting as described previously (Węgrzyn, A. *et al.*, 1995). Anti- λ E serum was provided by A. Taylor, S. Kędzierska and E. Laskowska (University of Gdańsk, Poland).

RESULTS

Lytic development of bacteriophage λ in the *E. coli* *rpoA341* host

We examined whether impaired N-dependent anti-termination at t_{L1} and t_{R1} compromises lytic growth of phage λ in an *rpoA341* background. Surprisingly, estimation of the efficiency of plating (Szalewska-Pałasz

et al., 1996), burst size measurements with *λcIb2* (Table 1) and kinetics of production of phage progeny (measured in one-step growth experiments, data not shown) revealed no significant differences between λ lytic development in *rpoA⁺* and *rpoA341* strains growing in LB. To eliminate the possibility that these results were due to differences in the phage strains used or conditions employed for measuring transcript abundance on the

Table 1. Effects of the host *rpoA341* mutation, phage *cI7* mutation and overproduction of phage O, P and N proteins on mean phage burst size

Otherwise isogenic *rpoA341* (WAM105) and *rpoA⁺* (WAM106) strains were used as hosts. Infections were performed at an m.o.i. of 0.05 in different media at 30 °C. Mean burst size (in p.f.u. per infective centre) was calculated on the basis of one-step growth experiments performed as described in Methods. The data presented are based on at least three measurements.

Growth medium*	Plasmid in host†	<i>λcIb2</i>		<i>λcI7</i>		<i>λcI7cII68</i>	
		<i>rpoA341</i>	<i>rpoA⁺</i>	<i>rpoA341</i>	<i>rpoA⁺</i>	<i>rpoA341</i>	<i>rpoA⁺</i>
LB	None	109	115	111	96	94	146
MMGluCaa	None	27	32	29	44	31	35
MMGlu	None	<0.01	4	21	32	16	17
MMGlu	pTCλ1	23	35	NT	NT	NT	NT
MMGlu	pSS125	15	85	NT	NT	NT	NT

NT, Not tested.

* Media compositions are described in Methods. Bacterial growth rates in different media were: for the *rpoA341* (WAM105) strain, 1.90, 1.55 and 1.10 doublings h^{-1} in LB, MMGluCaa and MMGlu, respectively; for the *rpoA⁺* (WAM106) strain, 2.00, 1.60 and 1.15 doublings h^{-1} in LB, MMGluCaa and MMGlu, respectively.

† Overexpression of the O and P genes from plasmid pTCλ1 was achieved by addition of autoclaved chlortetracycline to the medium to a final concentration of 10 $\mu g\ ml^{-1}$. Overexpression of the N gene from plasmid pSS125 was achieved by addition of IPTG to the medium to a final concentration of 1 mM. Both inducers were present in the medium overnight and throughout the whole experiment.

Fig. 1. Map of the bacteriophage λ genome. The most important genes and their roles in phage development are indicated. Transcripts specifying lytic functions are marked as thick arrows; transcripts necessary for the establishment of the lysogenic pathway of λ development and maintenance of the prophage are marked as narrow arrows. The main promoters (p) and terminators (t) are also shown. N-dependent antitermination occurs at t_{L1} as well as at t_{R1} and more distal terminators. Q-dependent antitermination occurs at the t_{R1} terminator. The scheme is not drawn to scale.

Fig. 2. Synthesis of the phage O protein in λ cl857S7-infected *rpoA341* (WAM105) and *rpoA*⁺ (WAM106) hosts growing in LB medium (a) and MMGlu (b) between 5 and 10 min post-infection at 30 °C. Note that due to different amounts of [³⁵S]methionine used in the different media and the different times of film exposure during autoradiography, the results presented in this figure (particularly band intensities) should not be directly compared between panels (see Methods for details).

one hand (Szalewska-Pałasz *et al.*, 1996; Obuchowski *et al.*, 1997a) and lytic growth on the other, we repeated measurements of the burst size of λ cl857S7 and λ clb2 phages at different temperatures and m.o.i. values. However, under these alternative conditions we were again unable to detect any significant differences in the lytic development of bacteriophage λ in the two host strains (data not shown).

Synthesis of bacteriophage λ O protein in the *E. coli rpoA341* host

The results presented above suggest that decreased synthesis of certain delayed early proteins can still support normal lytic growth of the phage in rich media. To test this idea we examined the synthesis of O protein, the initiator of λ DNA replication, which is encoded by the N-antiterminated, *p_R*-derived, delayed early lytic transcript (Fig. 1). As expected, we observed a marked reduction in the level of O protein synthesis in λ cl857S7-infected *rpoA341* bacteria relative to the *rpoA*⁺ host (Fig. 2a). These results indicate that in a bacterial host growing in rich media bacteriophage λ produces O protein (and probably other delayed early proteins) in excess of that required to achieve normal lytic growth. One implication of this observation is that effective growth of the phage may be achieved even under conditions which are far from optimal for lytic growth, i.e. conditions which might result in decreased O synthesis or activity. In control experiments we found no significant differences in the synthesis of the immediate early gene product, Cro (the gene for which is located upstream of the *t_{R1}* terminator), in UV-irradiated λ cl857S7-infected *rpoA*⁺ and *rpoA341* hosts (data not shown). In the same experiments, decreased synthesis of the replication proteins O and P was again observed in the *rpoA341* mutant relative to the *rpoA*⁺ strain (data not shown).

Bacteriophage λ lytic development under different growth conditions

One prediction from our observations is that under certain conditions delayed early lytic proteins will become limiting for λ growth in the *rpoA341* host but not in the wild-type host. Therefore, we investigated λ lytic development in *rpoA*⁺ and *rpoA341* hosts under different nutritional conditions. We found that although there was no significant difference between λ clb2 lytic development in *rpoA*⁺ and *rpoA341* hosts growing in relatively rich media (LB or MMGluCaa), the phage could not produce its progeny efficiently in the mutant host in poor medium (MMGlu), in contrast to the situation in the *rpoA*⁺ strain in which the yield of progeny was decreased relative to that in rich media but was still significant (Table 1). This observation cannot be attributed to differences in growth rates between the two hosts as both have similar doubling times (Table 1).

Overexpression of phage replication proteins can restore efficient λ lytic development in the *rpoA341* host growing in minimal medium

We found that, similarly to the situation observed in a rich medium (Fig. 2a), synthesis of O protein was significantly decreased in the *rpoA341* host relative to the *rpoA*⁺ strain growing in a poor medium (MMGlu, Fig. 2b). Therefore, we investigated whether this protein was a limiting factor for λ lytic development under these growth conditions. We found that artificially raising the level of the phage replication proteins (O and P) *in trans* resulted in an increase in the yield of phage λ clb2 progeny in the *rpoA*⁺ host growing in minimal medium to a level similar to that obtained in MMGluCaa in the absence of overproduced O and P (Table 1). Even more striking was the restoration of the ability of λ to develop lytically in the *rpoA341* mutant under these growth conditions, again to a level similar to that observed in MMGluCaa (Table 1). In analogous experiments, when O and P were oversupplied in *rpoA*⁺ and *rpoA341* bacteria growing in LB or MMGluCaa medium, we did not observe any increase in the phage burst size (data not shown). These results indicate that the activity of the replication proteins is likely to be a limiting factor for phage lytic growth under conditions unfavourable for growth of the host, but not under favourable conditions.

The O and P proteins are encoded by genes which are transcribed from the *p_R* promoter but are located downstream of the weak *t_{R1}* terminator (Fig. 1). Thus, for maximal expression the O and P genes require N-dependent transcriptional antitermination at *t_{R1}* (for a review see Friedman & Court, 1995). It was demonstrated that N-mediated antitermination is less efficient in the *rpoA341* mutant and that this defect can be compensated for by overproduction of N protein (Obuchowski *et al.*, 1997a). Accordingly, we found that overexpression of the N gene from plasmid pSS125 results in improved phage lytic development in both *rpoA*⁺ and *rpoA341* hosts growing in MMGlu, though the yield of phage progeny was higher in the *rpoA*⁺

Fig. 3. Western blotting analysis of the abundance of the λ E protein in *rpoA341* (WAM105) and *rpoA*⁺ (WAM106) hosts following infection by λ c1b2 (λ cII⁺) and λ c1857cII68 (λ cII⁻) phages at 30 °C. Samples were withdrawn 10, 20 and 30 min post-infection, cells were lysed and following 12.5% SDS-PAGE of lysates and transfer to a nitrocellulose membrane, the λ E protein was detected by Western blotting using anti- λ E serum. The OmpA protein was detected on the same blot and served as an internal control.

strain (Table 1), presumably because N synthesis from pSS125 is not sufficient to restore maximum anti-termination efficiency in the mutant host (Obuchowski *et al.*, 1997a). In the *rpoA*⁺ host we observed a burst size almost as high as in hosts growing in LB medium (Table 1). This more substantial effect of N overproduction than provision of O and P functions may result from the fact that O complementation is known to be relatively poor *in trans* (Wyatt & Inokuchi, 1974) due to instability of uncomplexed O protein in *E. coli* cells (Lipińska *et al.*, 1980; Gottesman *et al.*, 1981; Węgrzyn *et al.*, 1992b), or it may be due to the provision of increased levels of other N-dependent delayed early lytic proteins which would otherwise be limiting under these conditions.

We also investigated lytic development of λ carrying the *c17* mutation, which allows for effective expression of the O and P genes in the absence of N-dependent antitermination due to the creation of another rightward promoter (Packman & Sly, 1968; Rosenberg *et al.*, 1978). The λ c17cII68 mutant was also used to eliminate any possible effects of the *cII* gene product which is inactive in the *rpoA341* host. As expected, we observed growth-rate-regulated development of the phages in both the *rpoA*⁺ and *rpoA341* strains, but no significant differences were detected between the burst sizes in these two hosts under any of the growth conditions tested (Table 1). These results further support our contention that delayed early proteins become limiting for growth of phage λ in MMGlu and that the effect is more marked in the *rpoA341* host due to the impairment of N-dependent antitermination.

Bacteriophage λ late transcription and expression of the late gene, E, in the *E. coli rpoA341* host

Phage burst size is also dependent on the level of capsid protein synthesis. The head and tail genes are transcribed from the late promoter p_R , but they also require the action of a delayed early lytic protein, the Q

antiterminator, for their expression (Fig. 1). The expression of the Q gene, like O and P, is dependent on N-stimulated transcriptional readthrough of t_{R1} and downstream terminators (Fig. 1) and would therefore be expected to be depressed in the λ -infected *rpoA341* mutant. Any such decrease in Q synthesis may be manifested in an observed reduction in the level of p_R -derived transcripts. However, we found that the level of p_R -derived late transcripts is almost identical in *rpoA*⁺ and *rpoA341* strains following infection with λ c1857S7 (data not shown). As late gene expression in phage λ is down-regulated through the action of CII protein at the p_{aQ} promoter we speculated that the absence of anti-Q RNA synthesis in the *rpoA341* host compensates for this decreased readthrough transcription into Q. To test this idea we measured the abundance of one of the λ late proteins, E, in *rpoA*⁺ and *rpoA341* bacteria infected by λ cII⁺ and λ cII⁻ phages. As expected, we found that expression of the E gene is enhanced in the *rpoA*⁺ host infected by the λ cII⁻ mutant relative to λ cII⁺ phage infection (Fig. 3). However, no such effect was observed in the *rpoA341* host as this strain causes a phenotypic CII⁻ condition (Fig. 3).

DISCUSSION

Although levels of bacteriophage λ transcripts derived from the p_R and p_L promoters are decreased in the *E. coli rpoA341* mutant relative to the *rpoA*⁺ host due to impaired N-dependent transcriptional antitermination (Szalewska-Pałasz *et al.*, 1996; Obuchowski *et al.*, 1997a), we did not observe any significant differences in phage lytic development between both strains growing in LB medium, i.e. under standard laboratory conditions. This is despite the fact that the phage replication proteins O and P are produced less efficiently in the *rpoA341* mutant under these conditions. The most straightforward hypothesis to explain the absence of any obvious impairment of phage lytic development in the presence of depressed delayed early gene expression

is that phage λ produces an excess of certain proteins in rich media. One might expect that synthesis of all phage components should be optimally co-regulated during the infection cycle and that the amount of each component should not exceed that necessary for maximum growth under any given set of conditions. Since this does not appear to be the case, there must be a reason why evolution preferred overproduction of some phage proteins. We propose that under favourable growth conditions (for example, in LB medium with good aeration, etc., i.e. under standard laboratory conditions) phage λ produces at least some proteins in excess of that needed for its effective propagation. Thus, efficient lytic growth of the phage may be achieved even under less favourable conditions. Accordingly, we found that whereas a mutant host which retards expression of early lytic genes is able to support normal lytic growth of phage λ in rich media, it fails to support phage growth in nutritionally poor medium. Thus, we propose that whilst the level of certain phage proteins such as O and P decreases as the nutritional quality of the growth media decreases to the point where they become limiting for phage growth in wild-type bacteria, the level of these proteins in the *rpoA341* mutant is lower still, such that effective phage lytic development becomes impossible.

The inhibition of lytic growth in the N-antitermination-defective *rpoA341* mutant growing in MMGlu suggests that the activity of one or more products of the delayed early lytic transcripts is limiting under these conditions. Looking for potential candidates for the limiting factors in λ lytic development under unfavourable conditions, we found that overproduction of the replication proteins O and P, as well as overexpression of the N gene (encoding an antitermination protein), can restore the ability of λ to produce progeny in the *rpoA341* host growing in minimal medium. Therefore, the replication proteins may be the limiting factors or, alternatively, limited expression of N might result in decreased levels of O and P due to inefficient transcriptional antitermination at t_{R1} . However, overproduction of O and P in MMGluCaa and LB has no effect on lytic growth of the phage. Furthermore, whilst lytic growth of the phage is downregulated in cells growing in MMGluCaa relative to cells growing in LB, the burst size in the mutant host remains the same as in the wild-type. Since the synthesis of O protein in the mutant host growing in MMGluCaa is depressed relative to the wild-type, the level of O cannot be limiting for phage growth under these conditions. By implication, the observed fall in the burst size in this medium probably results from decreased activity of some other phage protein(s) (though not delayed early proteins) or, more likely, the involvement of host factor(s). Why does the activity of O and/or P become limiting under conditions allowing slow growth of the host? One possible reason for this is that the activity of the p_R and/or p_L promoter(s) may be growth-rate-regulated. However, using p_R -*lacZ* and p_L -*lacZ* fusions, we were not able to detect an obvious positive correlation between the bacterial growth rate and the activities of these promoters (data not shown).

The possibility remains that the level of at least some phage components is depressed in poor media as a consequence of a decrease in the translational capacity of the cell at slow growth rates. Such a model has been recently proposed as an explanation of decreased efficiency of bacteriophage T4 development in *E. coli* cells growing in poor media (Hadas *et al.*, 1997).

Our proposal that under favourable growth conditions phage λ produces an excess of certain proteins is supported by some recently published studies on λ O protein. As mentioned above, the O protein is very unstable in the free form (Lipińska *et al.*, 1980; Gottesman *et al.*, 1981) but is stabilized in the replication complex (Węgrzyn *et al.*, 1992b; Pawłowicz *et al.*, 1993; Węgrzyn, A. *et al.*, 1995), a structure which is inherited by one of two daughter DNA copies after a replication round and may function in the next replication event (Kur *et al.*, 1987; Węgrzyn & Taylor, 1992; Szalewska-Pałasz & Węgrzyn, 1994; Szalewska-Pałasz *et al.*, 1994; Taylor & Węgrzyn, 1995; Węgrzyn, 1995; Węgrzyn & Węgrzyn, 1995; Węgrzyn, A. *et al.*, 1996). It was demonstrated that overproduction of ClpXP protease, which specifically degrades the O protein, does not affect the replication of phage λ and plasmids derived from this phage in rich media (Szalewska *et al.*, 1994). This observation can be reconciled with our hypothesis that O protein is produced in excess under these conditions such that the level of the unproteolysed form of this protein is still sufficient to maintain efficient λ phage and plasmid replication. Moreover, *clpP* and *clpX* null mutations had no significant effect on phage λ burst size or λ plasmid copy number in bacteria growing in rich media (Szalewska *et al.*, 1994), again indicating that O protein is probably produced in excess under these growth conditions.

Further support for our proposed hypothesis comes from studies on the role of the host *dnaA* gene function in replication of bacteriophage λ . It was demonstrated that DnaA positively regulates the activity of the phage promoter p_R (Węgrzyn, G. *et al.*, 1995a, 1996; Szalewska-Pałasz *et al.*, 1998). These studies also indicated that both the level of p_R -derived transcripts and the synthesis of O protein are significantly decreased in a temperature-sensitive *dnaA46* mutant at 43 °C. Nevertheless, lytic development of phage λ in this host was found to be apparently normal at the non-permissive temperature in rich medium (Węgrzyn, G. *et al.*, 1995b). The experimental system described above is analogous to that described in this paper, i.e. a host mutant (*dnaA46* or *rpoA341*) that causes a decrease in the level of λ replication proteins is nevertheless able to support normal lytic growth of the phage in rich media. Staying with this analogy, we have subsequently found that phage λ lytic development is impaired in the *dnaA46* mutant growing in minimal medium at 43 °C (data not shown).

We have also demonstrated that in the λ -infected *rpoA341* mutant the level of the longer Q-antiterminated p_R -derived transcripts is not depressed relative to the

rpoA⁺ strain. This might seem unexpected in view of the fact that late gene expression requires the action of the Q antiterminator, the synthesis of which is dependent on N-mediated transcriptional antitermination at *t*_{R1} and more distant terminators (Fig. 1). Since this process is impaired in the *rpoA341* mutant (Obuchowski *et al.*, 1997a), one might consequently expect a lower level of the Q protein and a correspondingly decreased level of longer *p*_R-derived transcripts. However, in addition to the positive effect of N protein on Q gene expression, Q protein synthesis is negatively regulated by antisense RNA appearing as a result of the activity of the CII-dependent *p*_{aQ} promoter (Ho & Rosenberg, 1985; Hoopes & McClure, 1985). Since we have demonstrated that the level of *p*_{aQ}-initiated transcripts is drastically decreased in the λ -infected *rpoA341* mutant (Szalewska-Pałasz *et al.*, 1996), the simplest explanation of our results is that the absence of negative regulation of Q gene translation by *p*_{aQ}-derived antisense RNA compensates for the decrease in N-dependent transcription of Q. In short, although the level of Q transcripts is depressed in the *rpoA341* host, they may be translated more efficiently.

ACKNOWLEDGEMENTS

We thank A. B. Oppenheim for providing phage mutants and A. Taylor, S. Kędzierska and E. Laskowska for providing anti- λ E serum. M.O. is a recipient of the fellowship from the Foundation for Polish Science in 1997. This work was supported by the Polish State Committee for Scientific Research (KBN, project number 6 P04A 059 09).

REFERENCES

- Echols, H. (1971). Regulation of lytic development. In *The Bacteriophage Lambda*, pp. 247–270. Edited by A. D. Hershey. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Echols, H. (1986). Bacteriophage λ development: temporal switches and the choice of lysis or lysogeny. *Trends Genet* 2, 26–30.
- Friedman, D. I. & Court, D. L. (1995). Transcription antitermination: the λ paradigm updated. *Mol Microbiol* 18, 191–200.
- Goldberg, A. R. & Howe, M. (1969). New mutations in the S cistron of bacteriophage lambda affecting host cell lysis. *Virology* 38, 200–202.
- Gottesman, S., Gottesman, M., Shaw, J. E. & Pearson, M. L. (1981). Protein degradation in *E. coli*: the *lon* mutation and bacteriophage lambda N and cII protein stability. *Cell* 24, 225–233.
- Hadas, H., Einav, M., Fishov, I. & Zaritsky, A. (1997). Bacteriophage T4 development depends on the physiology of its host *Escherichia coli*. *Microbiology* 143, 179–185.
- Herman-Antosiewicz, A., Śrutkowska, S., Taylor, K. & Węgrzyn, G. (1998). Replication and maintenance of λ plasmids devoid of the Cro repressor autoregulatory loop in *Escherichia coli*. *Plasmid* (in press).
- Herskowitz, I. (1985). Master regulatory loci in yeast and lambda. *Cold Spring Harbor Symp Quant Biol* 50, 565–574.
- Herskowitz, I. & Hagen, D. (1980). The lysis-lysogeny decision of phage λ : explicit programming and responsiveness. *Annu Rev Genet* 14, 399–445.
- Ho, Y. S. & Rosenberg, M. (1985). Characterization of a third, CII-dependent, coordinately activated promoter on phage λ involved in lysogenic development. *J Biol Chem* 260, 11838–11844.
- Hoopes, B. C. & McClure, W. (1985). A cII-dependent promoter is located within the Q-gene of bacteriophage λ . *Proc Natl Acad Sci USA* 82, 3134–3138.
- Kur, J., Górski, I. & Taylor, K. (1987). *Escherichia coli dnaA* initiation function is required for replication of plasmids derived from coliphage lambda. *J Mol Biol* 198, 203–210.
- Lipińska, B., Podhajski, A. & Taylor, K. (1980). Synthesis and decay of λ DNA replication proteins in minicells. *Biochem Biophys Res Commun* 92, 120–126.
- Obuchowski, M., Węgrzyn, A., Szalewska-Pałasz, A., Thomas, M. S. & Węgrzyn, G. (1997a). An RNA polymerase α subunit mutant impairs N-dependent transcriptional antitermination in *Escherichia coli*. *Mol Microbiol* 23, 211–222.
- Obuchowski, M., Giladi, H., Koby, S., Szalewska-Pałasz, 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., Shotland, Y., Koby, S., Giladi, H., Gabig, M., Węgrzyn, G. & Oppenheim, A. B. (1997c). Stability of CII is a key element in the cold stress response of bacteriophage λ infection. *J Bacteriol* 179, 5987–5991.
- Packman, S. & Sly, S. (1968). Constitutive λ DNA replication by λ c17, a regulatory mutant related to virulence. *Virology* 34, 778–785.
- Pawłowicz, A., Węgrzyn, G. & Taylor, K. (1993). Effect of coliphage λ P gene mutations on the stability of the λ O protein, the initiator of λ DNA replication. *Acta Biochim Pol* 40, 29–31.
- Ptashne, M. (1992). *A Genetic Switch: Phage λ and Higher Organisms*, 2nd edn. Cambridge, MA: Cell Press/Blackwell Scientific Publications.
- Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. (1978). The relation between function and DNA sequence in an intercistronic regulatory region in phage λ . *Nature* 272, 414–423.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Soberon, X., Covarrubias, L. & Bolivar, F. (1980). Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. *Gene* 9, 287–305.
- Sullivan, S. L. & Gottesman, M. E. (1992). Requirement for *E. coli* NusG protein in factor-dependent transcription termination. *Cell* 68, 989–994.
- Szalewska, A., Węgrzyn, G. & Taylor, K. (1994). Neither absence nor excess of λ O initiator-digesting ClpXP protease affects λ plasmid or phage replication in *Escherichia coli*. *Mol Microbiol* 13, 469–474.
- Szalewska-Pałasz, A. & Węgrzyn, G. (1994). An additional role of transcriptional activation of *ori λ* in the regulation of λ plasmid replication in *Escherichia coli*. *Biochem Biophys Res Commun* 205, 802–806.
- Szalewska-Pałasz, A., Węgrzyn, A., Herman, A. & Węgrzyn, G. (1994). The mechanism of the stringent control of λ plasmid DNA replication. *EMBO J* 13, 5779–5785.
- Szalewska-Pałasz, A., Węgrzyn, A., Obuchowski, M., Pawłowski, R., Bielawski, K., Thomas, M. S. & Węgrzyn, G. (1996). Drastically decreased transcription from CII-activated promoters is responsible for impaired lysogenisation of the *Escherichia coli rpoA341* mutant by bacteriophage λ . *FEMS Microbiol Lett* 144, 21–27.

- Szalewska-Pałasz, A., Węgrzyn, A., Błaszczak, A., Taylor, K. & Węgrzyn, G. (1998). DnaA-stimulated transcriptional activation of *oriλ*: *Escherichia coli* RNA polymerase β subunit as a transcriptional activator contact site. *Proc Natl Acad Sci USA* **95**, 4241–4246.
- 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 *Molecular Microbiology*, pp. 81–97. Edited by S. J. W. Busby, C. M. Thomas & N. L. Brown. Berlin & Heidelberg: Springer.
- Thomas, M. S. & Glass, R. E. (1991). *Escherichia coli* *rpoA* mutation which impairs transcription of positively regulated systems. *Mol Microbiol* **5**, 2719–2725.
- Węgrzyn, A. & Węgrzyn, G. (1995). Transcriptional activation of *oriλ* regulates λ plasmid replication in amino acid-starved *Escherichia coli* cells. *Biochem Biophys Res Commun* **214**, 978–984.
- Węgrzyn, A., Węgrzyn, G. & Taylor, K. (1995). Protection of coliphage λ O initiator protein from proteolysis in the assembly of the replication complex *in vivo*. *Virology* **207**, 179–184.
- Węgrzyn, A., Węgrzyn, G., Herman, A. & Taylor, K. (1996). Protein inheritance: λ plasmid replication perpetuated by the heritable replication complex. *Genes Cells* **1**, 953–963.
- Węgrzyn, G. (1995). Amplification of λ plasmids in *Escherichia coli* *relA* mutants. *J Biotechnol* **43**, 139–143.
- Węgrzyn, G. & Taylor, K. (1992). Inheritance of the replication complex by one of two daughter copies during λ plasmid replication in *Escherichia coli*. *J Mol Biol* **226**, 681–688.
- Węgrzyn, G., Neubauer, P., Krueger, S., Hecker, M. & Taylor, K. (1991). Stringent control of replication of plasmids derived from coliphage λ . *Mol Gen Genet* **225**, 94–98.
- Węgrzyn, G., Glass, R. E. & Thomas, M. S. (1992a). 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., Pawłowicz, A. & Taylor, K. (1992b). Stability of coliphage λ DNA replication initiator, the λ O protein. *J Mol Biol* **226**, 675–680.
- Węgrzyn, G., Szalewska-Pałasz, A., Węgrzyn, A., Obuchowski, M. & Taylor, K. (1995a). Transcriptional activation of the origin of coliphage λ DNA replication is regulated by the host DnaA initiator function. *Gene* **154**, 47–50.
- Węgrzyn, G., Węgrzyn, A., Konieczny, I., Bielawski, K., Konopa, G., Obuchowski, M., Helinski, D. R. & Taylor, K. (1995b). Involvement of the host initiator function *dnaA* in the replication of coliphage λ . *Genetics* **139**, 1469–1481.
- Węgrzyn, G., Węgrzyn, A., Pankiewicz, A. & Taylor, K. (1996). Allele specificity of the *Escherichia coli* *dnaA* gene function in the replication of plasmids derived from phage λ . *Mol Gen Genet* **252**, 580–586.
- Wyatt, W. M. & Inokuchi, H. (1974). Stability of lambda O and P replication functions. *Virology* **58**, 313–315.

Received 24 December 1997; revised 27 April 1998; accepted 14 May 1998.