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QUARTERLY

This paper is dedicated to the memory of Professor Karol Taylor who introduced us to the field of lambdology several years ago

Biochemical and genetic analysis of λ^W, the newly isolated lambdoid phage[o]

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Otherwise isogenic *Escherichia coli* CP78 (*relA*$^+$) and CP79 (*relA*$^-$) strains are commonly used in studies on the stringent control, the bacterial response to amino acid starvation. We found that these strains are lysogenic for a phage which is spontaneously induced with a low frequency, producing virions able to infect other *E. coli* strains. Genetic studies, restriction analysis of the phage DNA genome, and electron microscopy revealed that this phage is very similar to, but not identical with, bacteriophage λ. We called the newly isolated phage λ^W, and found that most of CP78/CP79 ancestor strains are lysogenic for this phage.

Stringent control is the bacterial response to amino acid starvation. This response has been found to exist in many bacterial species but it is best characterised in *Escherichia coli* (for a recent review see [1]). The first event of the stringent response is production of high amounts of a specific alarmone, guanosine-5′-diphosphate-3′-diphosphate (ppGpp). This nucleotide interacts with RNA polymerase [2] which results in inhibition of transcription of many genes, especially those coding for rRNAs and tRNAs, and activation of some promoters. Although there are two proteins capable of synthesizing ppGpp, ppGpp synthetase I (the *relA* gene product) and ppGpp synthetase II (the *spoT* gene product) [3–6], only the first enzyme is active during amino acid starvation. Therefore, mutants in the *relA* gene do not produce ppGpp in amino acid-starved cells. This leads to a decrease in

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ppGpp level, and such a phenotype is called the relaxed response [1].

One of the most widely used, otherwise isogenic, pairs of the *E. coli* $relA^+$ and $relA^-$ variants are in the background of strains CP78 and CP79 [7]. These strains are derivatives of *E. coli* K-12. Although the originally isolated K-12 strain was reported to be lysogenic for bacteriophage λ [8], its derivatives were treated many times with UV light and other mutagens which led to the loss of the prophage.

Bacteriophage λ belongs to the family of lambdoid phages [9]. All members of this family are temperate phages, produce similar virions and posses several other common features, such as similar genome organization and dependence of their lytic development on several host heat shock proteins.

In the course of our study on the stringent response we found that strains CP78 and CP79 are lysogenic for a phage able to spontaneous induction. The aim of this work was to characterize this phage. We also investigated all available ancestors of CP78 and CP79 to find that most of them are lysogenic for this phage.

MATERIALS AND METHODS

Bacterial strains, phages and plasmids. *Escherichia coli* K-12 strains are listed in Table 1. Bacteriophages λpapa, *λcIb2* (from our collection), *λimm21* (from W. Szybalski), *λcI857S7* [10] and λ^W (isolated in this work) were used. Plasmid pKB2 [11] is a plasmid derived from bacteriophage λ which contains a replication region of the λ genome (from the p_R promoter to the *ren* gene) and a kanamycin resistance gene. Plasmid pAS3 [12] was constructed by replacement of the p_R promoter with p_{lac} promoter in plasmid pKB2.

Propagation and purification of phages. Phages were propagated from induced lysogens, by the plate lysate technique, or by infection of cells growing in a liquid medium as earlier described [13, 14]. When the plate lysate technique was used, phages were recovered from soft agar according to Obuchowski & Stopa [15]. The virions were purified by ultracentrifugation in a cesium chloride gradient as described by Sambrook *et al.* [14].

DNA manipulations. Isolation and purification of phage and plasmid DNA as well as DNA restriction analysis were performed as described by Sambrook *et al.* [14].

Efficiency of plating. Efficiency of plating of phages on *E. coli* host strains was measured according to Arber *et al.* [13].

Efficiency of transformation. Efficiency of transformation of *E. coli* cells with plasmid DNA was estimated as described previously [16].

Electron microscopy. Phage samples were prepared for electron microscopy analysis by negative staining with 4% phosphotungstic acid, neutralized with KOH, according to Wischnitzer [17] and Bozzola & Russell [18].

RESULTS

We found that supernatants of centrifuged liquid cultures of *E. coli* CP78 and CP79 strains, contrary to many other commonly used laboratory *E. coli* strains, contain low concentrations of phages (from 10^{-6} to 10^{-5} plaque forming units per one cell in the culture) which form plaques on wild-type *E. coli* strains, for example MG1655. The plaques resemble those formed by bacteriophage λ (not shown). The presence of phages in a non-infected bacterial culture indicates that a strain is lysogenic and phage particles appear due to spontaneous induction of prophages, which is a relatively rare but rather usual phenomenon.

The phage was propagated to obtain a high titer lysate and purified by ultracentrifugation in a cesium chloride gradient. When the centrifugation was performed to the equilibrium state, we found that the buoyant density of virions of this phage was exactly the same

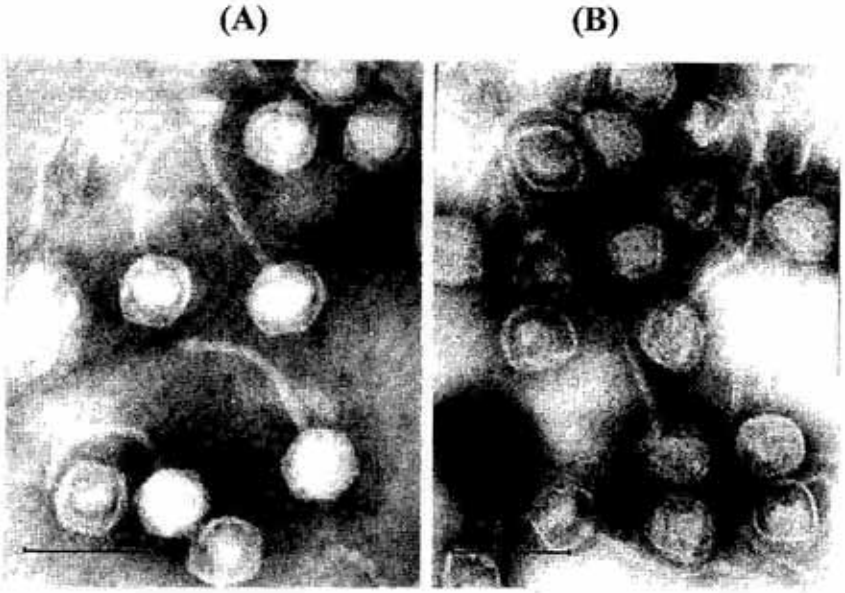

Figure 1. Electron micrographs of virions of phages *λcI857S7* (A) and λ^W (B) negatively stained with phosphotungstic acid.

Bars represent 100 nm.

as that of bacteriophage λ virions (on the basis of measurement of the refractive index of fractions containing phage particles). Electron microscopy indicated that the size and morphology of the virions are very similar to those of bacteriophage λ (Fig. 1). Assuming that the investigated phage may belong to the family of lambdoid phages we called it λ^W.

We isolated DNA from λ^W virions and analysed it using restriction endonucleases. We found the restriction pattern of λ^W DNA to be indistinguishable (at least using the method of agarose gel electrophoresis for separation of restriction fragments) from that of λ DNA for *Bam*HI, *Bst*XI, *Dra*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Sal*I, *Sma*I, *Nhy*I, *Not*I and *Xho*I endonucleases (not shown). These results confirm that λ^W is closely related to λ and perhaps belongs to the family of lambdoid phages.

Replication of bacteriophage λ DNA is dependent on functions of host genes coding for DnaB helicase and DnaK, DnaJ and GrpE heat shock proteins which act also as molecular chaperones (for reviews see [19, 20]). Mutants in these genes are not permissive hosts for development of lambdoid phages. We also found that phage λ^W was unable to form plaques on such mutants (Table 2).

The *malT* gene product of *E. coli* is a membrane receptor for bacteriophage λ. We found that phage λ^W did not form plaques on the *malT1* mutant (the PR100 strain), whereas it did on the otherwise isogenic *malT*$^+$ host (not shown). Therefore, it seems that λ and λ^W use the same receptor on the surface of *E. coli* cells.

All the results presented above might suggest that the phage isolated from the cultures of CP78 and CP79 strains, called λ^W, is identical with bacteriophage λ. There are, however, differences between these two phages. Lambdoid prophages are inducible with UV light, thus lysogenic bacteria are sensitive to low dosage of UV irradiation due to prophage excision and subsequent lytic development leading to production of mature virions and cell lysis. We found that while *E. coli* strains lysogenic for λ or *λimm21* are sensitive to UV dosage as low as 5 J/m^2, strains CP78 and CP79 can grow normally even after UV irradiation at 20 J/m^2 (not shown). Moreover, UV irradiation of CP78 and CP79 cultures resulted in an increase in the concentration of λ^W plaque forming units in the supernatants after culture centrifugation only by about one order of magnitude, while similar irradiation of cultures of bacteria bearing other lambdoid prophages gave by several orders of magnitude more (not shown).

E. coli strains lysogenic for a lambdoid phage are immune to superinfection by the same phage, but are sensitive to other lamb-

Table 1. *Escherichia coli* strains

Strain	Known genotype	Source/reference/construction
MG1655	*rph*	[22]
BM857	As MG1655 but lysogenic for *λcI857S7*	This work, by lysogenization
BM21	As MG1655 but lysogenic for *λimm21*	This work, by lysogenization
C600	*supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21*	[23]
BM29	As C600 but *dnaB15* (*groPA15*)	[24]
BM237	As C600 but *grpE280 zfh*::Tn*10*	[25]
BM238	As C600 but *dnaK756 zaa*::Tn*10*	[25]
BM239	As C600 but *dnaJ259 thr*::Tn*10*	[25]
PR100	*thr-1 leuB6 lacY1 rpsL132 xylA7 mtlA2 thi-1 malT1*	From *E. coli* Genetic Stock Center (GSC)
BM100	As PR100 but *malT*$^+$	This work, by P1 transduction from MG1655
WG1	Wild-type K-12 / F1-1	[8], from GSC
679	As WG1 but *thr-1* / F1-1	[8], from GSC
679-680	As 679 but *leuB6 rfbD1* F$^-$	[8], from GSC
Y10	As 679-680 but *supE44 thi-1*	[8], from GSC
Y53	As Y10 but *lacY1*	[8], from GSC
W1	As Y53 but *malT1*	[8], from GSC
W480	As W1 but *fhuA2*	[8], from GSC
W583	As W480 but *galT1 xyl-7 ara-13*	[8], from GSC
W595	As W583 but *mtlA2 osmZ1*	[8], from GSC
W660	As W595 but *gal*$^+$	[8], from GSC
W677	As W660 but *gal-3 fic-1*	[8], from GSC
CP78	As W677 but *his-65 argH46 relX*	[7]
CP79	As CP78 but *relA2*	[7]

doid phages. This is due to the fact that all lambdoid phages bear different *immunity* region, with the *cI* gene coding for the repressor which is the main protein responsible for blocking the activity of early promoters of both the prophage (which leads to its maintenance) and the infecting phage (which inhibits its development). The CI repressor, which is extensively produced by the prophage, recognizes the respective operator sequences derived from the same, but not from other phages. We found that λ^W was able to grow on the *λimm21* lysogen but not on λ lysogen (Table 3), which might suggest that it contains an *immunity* region identical to that of λ. However, subsequent experiments revealed that this is not true. Since CP78 and CP79 strains bear the *malT1* mutation, we were not able to investigate their sensitivity to λ. Nevertheless, similar test could be performed using plasmids derived from bacteriophage λ. Standard λ plasmids bear the replication region of bacteriophage λ genome which contains all genes and regulatory sequences necessary for initiation of DNA replication. The replication genes, *O* and *P*, are under control of the p_R promoter. Transcription initiated from this promoter is necessary not only for production

Table 2. Efficiency of plating of bacteriophages *λcIb2* and λ^W on different *E. coli* host mutants

Host strain	Relevant genotype	Efficiency of plating	
		λcIb2	λ^W
C600	"Wild type" (control)	1[a]	1[b]
BM29	*dnaB15*	$< 10^{-6}$	$< 10^{-6}$
BM237	*grpE280*	$< 10^{-6}$	$< 10^{-6}$
BM238	*dnaK756*	$< 10^{-6}$	$< 10^{-6}$
BM239	*dnaJ259*	$< 10^{-6}$	$< 10^{-6}$

[a]Efficiency of plating value of 1 corresponds to 1×10^{10} plaque forming units per ml; [b]Efficiency of plating value of 1 corresponds to 2×10^{10} plaque forming units per ml.

of mRNA required for synthesis of O and P proteins, but also serves in the so called transcriptional activation of *oriλ*, which seems to be the main process regulating the frequency of replication initiation [21]. Since p_R promoter is strongly repressed by the CI protein, phage λ promoters. It is clear, therefore, that the *immunity* regions of λ and λ^W, although very similar, are not identical. Moreover, λ^W cannot be identical with any other known lambdoid phage as all of them are able to grow lytically on λ lysogens.

Table 3. Efficiency of plating of bacteriophages *λcIb2*, *λimm21* and λ^W on *E. coli* hosts lysogenic for *λcI857S7* (*λimmλ*) and *λimm21* phages

Host strain	Prophage	Efficiency of plating		
		λcIb2	*λimm21*	λ^W
MG1655	None (control)	1[a,c]	1[b]	1[c]
BM857	*λcI857S7*	$< 10^{-6}$	0.9	$< 10^{-6}$
BM21	*λimm21*	0.9	$< 10^{-6}$	0.8

[a,c]Efficiency of plating value of 1 corresponds to 1×10^{10} plaque forming units per ml for (a) and (b), and 2×10^{10} plaque forming units per ml for (c).

wild-type λ plasmids (as pKB2) cannot replicate in strains lysogenic for λ (Table 4). In control experiments we used a derivative of wild-type λ plasmid (pAS3) which contains the p_{lac} promoter instead of p_R. Since p_{lac} is not repressed by the CI protein, bacteria lysogenic for λ could be easily transformed with this plasmid (Table 4). However, strains CP78 and CP79 (lysogenic for λ^W) could be transformed efficiently with both pKB2 and pAS3 (Table 4). These results suggest that the CI repressor produced by λ can inhibit the activity of early promoters of both λ and λ^W, but an analogous protein produced by λ^W is not able to repress

We tried to check whether CP78 and CP79 are the only strains bearing the λ^W prophage or is it present also in the ancestor strain(s). Knowing the pedigree of CP78 and CP79 ([7, 8], Mary Berlyn, personal communication) we collected all the ancestor strains available from the *E. coli* Genetic Stock Center (Yale University). Using all the tests presented in preceding paragraphs, we have found that most of the strains from the lineage leading to CP78 and CP79 are lysogenic for λ^W (Table 5). The prophage appeared in the lineage relatively early, with the strain named 679-680 being the first lysogenic for λ^W.

Table 4. Efficiency of transformation of *E. coli* hosts lysogenic for *λcI857S7* (*λimmλ*) and λ^W phages with λ plasmids

Host strain	Prophage	Efficiency of transformation[a]	
		pKB2[b]	pAS3[c]
MG1655	None (control)	7.3×10^4	5.0×10^4
BM857	*λcI857S7*	$< 10^1$	2.4×10^4
CP78	λ^W	3.5×10^4	5.0×10^4
CP79	λ^W	3.4×10^4	5.1×10^4

[a]Efficiency of transformation is calculated in transformants per 1 μg of plasmid DNA; [b]pKB2 is a wild-type λ plasmid; [c]pAS3 is a derivative of pKB2 in which the λ p_R promoter was replaced with the p_{lac} promoter.

DISCUSSION

We described a lambdoid phage, called λ^W, isolated after spontaneous induction of appropriate prophage from the *E. coli* strains CP78 and CP79, commonly used in studies on the stringent response. This phage is very similar to λ (for example: the same size and morphology of virions, the restriction pattern of the phage DNA indistinguishable from that of λ DNA after digestion with several enzymes), but reveals two important differences: the UV-mediated λ^W prophage induction is of a very low efficiency relative to other lambdoid phages, and although λ^W cannot grow on λ lysogens, λ plasmid replication (and thus transcription from at least λ p_R promoter) is allowed in λ^W lysogens. It is perhaps difficult to

Table 5. The pedigree of *E. coli* strains CP78 and CP79 (on the basis of ref. [8] and Mary Berlyn, personal communication), and the presence or absence of the λ^W prophage in the ancestor strains

Strain[a]	Treatment[b]	Availability in GSC[c]	Presence of λ^W prophage[d]
WG1	X-ray	Yes	No
679	X-ray	Yes	No
679-680	X-ray	Yes	Yes
Y10	UV	Yes	Yes
Y53	N-mustard	Yes	Yes
W1	T1 selection	Yes	Yes
W480	UV	Yes	Yes
W566	UV	No	Not determined
W582	UV	No	Not determined
W583	UV	Yes	Yes
W595	None	Yes	Yes
W660	UV	Yes	Yes
W677	UV	Yes	Yes
CP76	UV	No	Not determined
CP77	Cross with Hfr Cavalli	No	Not determined
CP78	UV	Yes	Yes
CP79	None	Yes	Yes

[a]Each strain is a derivative of its ancestor presented in the preceding line; [b]Indicated treatment of each strain resulted in the appearance of its derivative presented in the next line; [c]Availability of strains in the *E. coli* Genetic Stock Center (GCS) is indicated; [d]Strains were checked for the presence of λ^W prophage as described in the text.

decide whether λ^W should be considered a newly discovered lambdoid phage or a mutant of bacteriophage λ. In fact, mutants of λ producing prophages resistant to UV induction are already known (so called ind$^-$ phenotype). On the other hand, our results suggest that CI repressor produced by λ can inhibit the activity of early promoters of both λ and λ^W, but an analogous protein produced by λ^W is not able to repress phage λ promoters. Such a phenotype is unique to λ^W suggesting that it may be considered a separate phage (related much closely to λ than other lambdoid phages) rather than a mutant of λ.

The λ^W prophage could appear in the 679-680 strain (the first strain in the lineage being lysogenic for λ^W; see Table 5) due to infection and lysogenization. This scenario should be preferred if λ^W is indeed a newly discovered phage, which separated from λ relatively late during evolution. Alternatively, one may suspect that early mutagenesis of the wild-type *E. coli* K-12 strain (which was lysogenic for λ) resulted in the appearance of a mutation leading to the ind$^-$ phenotype in the prophage (still observed in λ^W), and subsequent mutagenesis events produced changes in the *immunity* region (including the *cI* gene) which are now characteristic of λ^W. It is worth noting, however, that although the CI protein produced by λ^W is not able to repress bacteriophage λ promoter(s) (at least the p_R promoter and perhaps also p_L), it must be fully functional in repressing λ^W early promoters as the λ^W prophage is stably maintained and its spontaneous induction is relatively rare. Moreover, if the hypothesis that λ^W is a mutant of the λ prophage present in the original K-12 strain were true, two results would be hard to explain. First, we did not find the prophage in ancestors of the strain 679-680 (its lack in the strain 679 is the most intriguing). Second, if the changes in the *immunity* region of λ^W accumulated due to mutations in a λ prophage at later steps of strains' construction, one might expect that at least some strains in the linneage should not be permissive for λ plasmid replication due to production of the unchanged λ CI repressor. Contrary to this prediction, we found that all tested strains of the lineage (including 679 and 679-680) can be efficiently transformed by wild-type λ plasmid, pKB2 (not shown). These results may again suggest that λ^W can be considered a newly discovered lambdoid phage rather than a mutant of bacteriophage λ. On the other hand, it would be tempting to speculate that a kind of microevolution (from λ to λ^W), accelerated by many mutagenesis events, could be provoked and observed during construction of *E. coli* K-12 derivative strains.

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