ISOLATION OF PLAQUE FORMING λ PHAGES WHICH CARRY BACTERIAL GENES INVOLVED IN THE FUNCTIONAL ORGANIZATION OF THE CELL MEMBRANE

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Mutants of the bacteriophage lambda have been isolated which can form plaques as well as transduce a series of bacterial loci. Included amongst the loci carried by the phages are the chlA genes which control membrane components involved in the nitrate respiration complex of E. coli. By using the appropriate chlA deletion mutants and λpchlA phage, it was possible to transduce control of formic dehydrogenase activity with the chlA genetic locus.

We have chosen to study in some detail the "nitrate respiration" complex of E. coli which contains a number of membrane-bound enzymes (1,2,3,4). This system provides several experimental advantages for an analysis of both membrane organization and biogenesis. The nitrate respiratory complex is an association of nitrate reductase, cytochrome b_1, formate dehydrogenase (FDI) and formate hydrogenlyase. Chlorate reduction, which is effected by the nitrate reductase enzyme of the complex is a reaction lethal to the cell. Mutants which are resistant to chlorate and lack nitrate reductase activity have been isolated. In

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many cases, these mutants exhibit a pleiotrophic phenotype in that they also show the concomitant loss of the formate hydrogenlyase system which consists of a formate dehydrogenase activity (FDII) and a hydrogenase activity (2,5,6,7).

Genetic analysis of chlorate-resistant mutants has shown that at least five genetic loci (chlA, B, C, D and E) can affect nitrate reductase activity (5,6,7,8,9,10). Recently it was shown by gel electrophoresis that the chlA locus controls several membrane components that can be detected in both aerobically and anaerobically grown cells (11,12). A series of chlD, chlA and chlE deletion mutants were isolated, characterized, and genetically reconstructed by the addition of specific λ transducing phages (12,13). This paper describes the isolation and the characteristics of a series of plaque-forming λ transducing phages (λpchlA) which carry bacterial genes that are involved in the functional organization of the cell membrane of E. coli.

MATERIALS AND METHODS

The bacterial strains KA56 (11), AT2687 C25 (a point mutant in the chlA locus) (5), KB70 (a mutant with a deletion through both the chlA and chlE loci) and KB70 (λdchlA) (12) have been described elsewhere. The phage P2 lysogens AT2687 C25(P2) [hereafter referred to as C25(P2)] and KB70(P2) were prepared for this investigation. The phage λ lysogens HNB4, HNB5, HNB6, HB11 and HB14 are independent heterogenotes in strain C25 and are (λ^+) (λdbio$^+$uvrB$^+$chlA$^+$) double lysogens (5). The heterogenotes were initially isolated by selecting for chlA$^+$ transductants (N), bio$^+$chlA$^+$ (NB) and bio$^+$ (B) colonies. The relevant bacterial genes and the characteristics of several of the strains used in this study are shown in Figure 1.

The bacteriophage P2 was kindly provided by Dr. B. Molholt. The

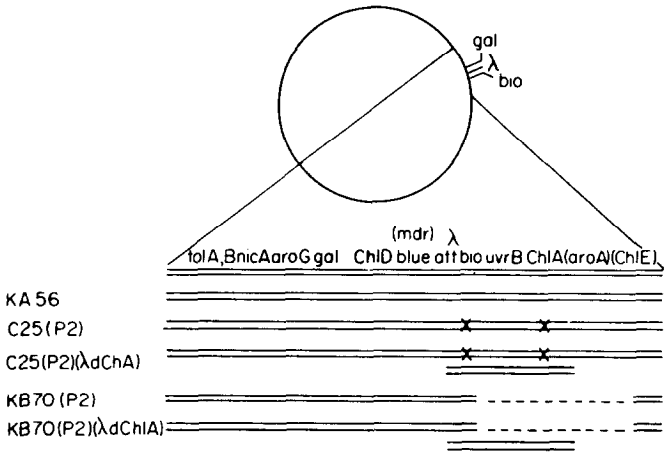

Figure 1. Relevant bacterial genes on the E. coli chromosome. The cross represents point mutations in the bio and chlA genes while the dashed line represents the deleted genes. The precise order of genes (aroA) and (chlE) has not been determined.

phage λ strain λdbiouvrBchlA (hereafter called λdchlA) has been described previously (5,12). Phage λles and the E. coli-Salmonella hybrid strain it plates on, were kindly provided by Dr. G. Kayajanian (14). This phage contains a number of mutations which make it independent of the control of the N cistron of phage λ (a λNIN phage) (14; Dr. W. Szybalski, personal communication).

The following rationale was used to isolate plaque-forming transducing phages of phage λ. Wild type λ^+ phage plates on a host which is lysogenic for phage P2 with an efficiency between 10^{-6} and 10^{-7} because the prophage interferes with λ phage replication. Mutant λ phages which can plate with an efficiency of 1.0 can be isolated and frequently shown to be plaque-forming λ biotin transducing phages (16). Moreover, these λ phage mutants are unable to form plaques on recombination-deficient bacteria of the $recA^-$ class, but they can grow on $recA^-recB^-$ double mutants.

Recently, it was shown that mutant phages of λ described as λNIN phages (cistron N independent) could be crossed with λdbio phages

(defective λ transducing phages) to give rise to plaque-forming λ transducing phages (14). In addition, these infectious transducing phages could plate on hybrid strains of E. coli-Salmonella (14) and on P2 lysogens but not on recA$^-$ strains (Rolfe, unpublished). Independent λ mutants which can form plaques on E. coli-Salmonella hybrids have been isolated and characterized (14). These mutants, called λles (14), contain several mutations which enable the phage to complete an infectious cycle independently of the phage N cistron. Since the λdchlA$^+$ phages are defective because they are deleted of the phage N cistron, the construction of plaque-forming λpchlA$^+$ phages was attempted by crossing the λles phage mutations conferring N cistron independence into these defective phages and selecting for ability to form plaques on phage P2 lysogens.

The phage cross and isolation of the plaque-forming transducing phages was performed as follows. The different λ phage lysogens (HN, HNB and HB series) were grown overnight, diluted into prewarmed nutrient broth, and regrown into exponential phase. The cell suspensions were centrifuged, resuspended in 0.02 M $MgSO_4$ and irradiated with an inducing dose of UV light. Phage λles at a final m.o.i. of 3 to 5 was added to 4 ml samples of the UV-irradiated cells. This suspension of phage-infected cells was kept at 4°C in an ice bucket for 5 minutes before the total contents of each sample tube were added to 16 mls of prewarmed broth containing 0.02 M $MgSO_4$. This procedure of phage adsorption in the cold followed by dilution into warmed broth optimizes the synchrony of phage adsorption and injection (15; Dr. W. Sly, personal communication). These cell suspensions were then shaken at 37°C until cell lysis occurred (usually after 120 minutes). The lysed cultures were chloroformed, centrifuged and the phage lysates assayed on various indicator strains. Care was taken throughout this procedure to minimize photoreactivation.

The various enzyme assays used in this investigation have been described in detail elsewhere (12). The results were calculated as a percent of the specific activity of the parent strain and indicated as present (+) (90-100% activity) or absent (-) (no activity detected).

RESULTS AND DISCUSSION

Phage crosses were performed between phage λles and several induced λdchlA$^+$ lysogens as described in the MATERIALS AND METHODS. The different lysates were assayed first on strains C25 and C25(P2) and in each case the frequency of plaque formation on strain C25(P2) was about 10^{-7} relative to strain C25. Individual single plaques arising on C25(P2) were picked, resuspended in $MgSO_4$ buffer and reassayed on a group of indicator strains (TABLE 1). Clearly the properties of the phage isolates are those of λ phage mutants which have lost their sensitivity to phage P2 interference, are sensitive to the phage λ

TABLE 1. Plating Efficiency of Single Plaque Isolates.

Single Plaque Isolates	Indicator Strains. C25	C25(P2)	C25(λ)	CS hybrid	recA$^-$	recA$^-$ recB$^-$
λ.HNB5	1.0	1.0	0	0.6	0	1.0
λ.HNB6	1.0	1.0	0	0.5	0	1.0
λ.HB11	1.0	1.0	0	0.4	0	1.0
λ.HB14	1.0	1.0	0	0.2	0	1.0

Single plaques formed on C25(P2) were picked, resuspended in 0.02 $MgSO_4$- buffer and treated with chloroform. Dilutions were plated with the indicator strains and scored for plaque-forming units (PFU) after overnight incubation at 30°C. The plating efficiency for isolates derived from the corresponding λdchlA$^+$ lysogen is the number of plaques on a particular indicator strain compared with the PFU on strain C25. The single plaque isolates were designated λ.HNB5, λ.HNB6, λ.HB11 or λ.HB14 to indicate their origin (induced heterogenote x λles). At least 10 independently isolated plaques were tested from each group. All the plaques tested had transduction activity. Indicator strain CS hybrid is the E. coli-Salmonella hybrid strain of Kayajanian (14).

repressor, can plate effectively on hybrids of E. coli-Salmonella and fail to form plaques on recA$^-$ bacterial mutants although they can plate on recA$^-$recB$^-$ double mutants. These phage isolates were then tested for their transduction capacity. The results summarized in TABLE 2 show that phage isolates from each prepared lysate can transduce the bacterial markers bio$^+$, uvrB$^+$ and chlA$^+$ originally carried by the parental λdchlA phages.

Although the λpchlA phages can transduce the chlA$^-$ point mutant

TABLE 2. Transduction Capacity of Phage Isolates.

Single Plaque Isolates	Bacterial Marker Transduction bio$^+$	uvrB$^+$	chlA$^+$
λ.HNB5	+	+	+
λ.HNB6	+	+	+
λ.HB11	+	+	+
λ.HB14	+	+	+

Transduction of the bacterial marker bio$^+$ was after the procedure of Becker and Dunbar (unpublished). Strain AT2687 (bio$^-$, met$^-$, str^r) was grown overnight in nutrient broth, washed extensively and the washed cells diluted one in twenty. An aliquot of cells (containing about 2 x 10^7 cells/ml) plus a sample from a phage dilution were poured over the surface of a supplemented minimal agar plate. These plates were supplemented with methionine and contained 200 μg/ml streptomycin which minimized growth of any contaminant cells that might cause biotin cross-feeding. The assay plates were left at 30°C for 2 days. Plaques were observed after the first day and as the plaques enlarged bio$^+$ colonies appeared within the centre of the plaque. The use of washed diluted cell suspensions allows the optimum development of plaque formation and clarity of bio$^+$ transduction. The plaque-forming units of a phage preparation were found to be approximately 10 times higher than the bio$^+$ transduction frequency.

The transduction of uvrB$^+$ was carried out with nutrient agar plates spread with strain KB70 or KB70(P2) (deleted of the uvrB marker) and spotted with samples from different phage isolates. These plates were irradiated with a dose of UV light sufficient to give about a 0.1% survival of the spread KB70 cells. Transduction of chlA$^+$ was assayed on plates containing lactate nitrate medium by the method of Venables and Guest (5) using strains C25 and C25(P2) as recipient strains.

C25 to chlA$^+$ this result does not reveal whether they carry the complete chlA region or only a portion of the genes which may be present at this locus. Clearly, a set of λpchlA phages, some which carry all the chlA genes and some which carry only a portion, would provide a powerful genetic tool for deletion mapping of the locus as well as an aid in the biochemical investigation of the membrane function of the chlA locus. Some indication of the variation between the different λpchlA phages was obtained by examining a number of membrane-bound functions of the nitrate respiration system. The results summarized in TABLE 3 show that while all the isolates can supply the missing components of strain C25(P2) they do not all carry the complete chlA locus, as seen from the results obtained with strain KB70(P2). This strain has lost both the chlA and chlE loci by deletion and has no nitrate reductase or formic hydrogenlyase activity. KB70(P2) cells transduced by phage λ.HNB5 and λ.HNB6 have about the same specific activity for the formic dehydrogenases (FDI and FDII) as the parent strain, although they still lack the nitrate reductase and the complete formic hydrogenlyase activities. By contrast, KB70(P2) cells transduced with λ.HB11 or λ.HB14 phage still lack the two formic dehydrogenase activities (i.e., no observed FDI or FDII functions). Moreover, none of the tested λpchlA phages carried both chlA$^+$ and chlE$^+$ loci together.

Mutants of chlA and chlE loci are normally pleiotrophic, lacking both the nitrate reductase and the formic hydrogenlyase activities (2, 5,9). Transduction of the chlA,E deletion strain, KB70(P2), by some λpchlA phages restores one component of the formic hydrogenlyase complex (FDII activity but not gas production). This suggests that the chlE locus is preferentially concerned with the hydrogenase component rather than the FDII component of the complex. In contrast, on the basis of other work (12), the loci chlA and chlD appear to control more directly the functional organization of the FDII activity of the complex.

TABLE 3. Extent and Enzyme Characteristics of the chlA Locus Carried by the λpchlA Phages.

Bacterial Strains	Formate Hydrogenlyase		Nitrate Reductase Complex	
	Acid and Gas Production	FDII	FDI	Nitrate Reductase
KA56	AG	+	+	+
$C25^{+}$	AG	+	+	+
C25(P2)	A	-	-	-
C25(P2) (λ.HNB5, 6, 11 or 14)	AG	+	+	+
KB70(P2)	A	-	-	-
KB70(P2) (λ.HNB5 or 6)	A	+	+	-
KB70(P2) (λ.HB11 or 14)	A	-	-	-

Acid and gas production tests for the complete presence of the hydrogenlyase system and is observed in tubes containing nutrient broth plus glucose, indicator dye and small inverted Durham tubes. The cells of the λ lysogens of C25(P2) and KB70(P2) used in these experiments were always checked to make sure of the continued presence of the λ prophage.

Putting a bacterial gene on to a plaque-forming phage can increase by at least a 100-fold the number of gene copies present in the cell (17). With the availability of a large number of chlA gene products *in vivo*, it will now be possible to investigate more precisely the regulation and integration of these gene products into the *E. coli* membrane.

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