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Adsorption of Bacteriophage Lambda on the LamB Protein of *Escherichia coli* K-12: Point Mutations in Gene *J* of Lambda Responsible for Extended Host Range

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LamB is the cell surface receptor for bacteriophage lambda. LamB missense mutations yielding resistance to lambda group in two classes. Class I mutants block the growth of lambda with the wild-type host range (λh$^+$) but support the growth of one-step host range mutants (λh). Class II mutants block λh but support the growth of two-step host range mutant (λhh*) phages. To identify amino acid residues in the J protein (the tail fiber of phage λ) responsible for the extended host range phenotype of mutants of phage (λh$^+$), we selected a series of one-step (λh) and two-step (λhh*) host range mutants and analyzed their corresponding *J* genes. Three different class I LamB missense mutants (mutations at sites 247, 245, and 148) were used to select 11 independent, new, one-step host range mutants (λh phages). DNA sequence analysis revealed a single-amino-acid change in each case. The 11 alterations affected only three residues in the distal part of J, corresponding to a Val→Ala change at site 1077 in five cases, a Thr→Met change at site 1040 in three cases, and a Leu→Pro change at site 1127 in three cases. Recombination experiments confirmed that in the cases tested, the mutations identified were indeed responsible for the extended host range phenotype. The class II LamB mutant (Gly→Asp at site 151) was used to select two-step extended host range mutants (λhh* phages) from three new λh phages, corresponding to different amino acid modifications in the J protein (at sites 1040, 1077, and 1127). The new λhh* phages analyzed corresponded to either double or triple point mutations located at the distal end of the J protein. In all, seven residues involved in the extended host range properties of λ mutants were identified in the distal part of the J protein, suggesting that the last C-terminal portion of the J protein participates directly in the adsorption of the phage onto LamB. In agreement with the fact that the λh mutants (and the λhh* mutants) could grow on all of the *lamB* class I mutations tested, we found that the nature of the J mutations did not depend on the LamB class I mutant used to select them. This is interpreted as meaning that the mutated residues in the J protein and in the LamB mutants are not involved in allele-specific protein-protein interactions. Rather, the LamB mutations would block a step in phage adsorption, and this block would be overcome by the mutations in the J protein.

Bacteriophage λ adsorbs to its *Escherichia coli* K-12 host by interacting with a protein encoded by gene *lamB* and located in the outer membrane of the host. This protein is named the λ receptor or the LamB protein (26, 32, 35). LamB also serves as a specific receptor for several other bacteriophages (7, 19, 27).

The active form of the LamB protein is a homotrimer (22, 24, 25) that contains nonspecific channels that allow passive diffusion of hydrophilic molecules with molecular weights of less than 600 across the outer membrane. In addition, LamB facilitates diffusion of maltose and maltodextrins across the outer membrane; hence, its other name, maltoporin (15, 34). The mature LamB protein contains 421 amino acids (aa) (10). Two-dimensional models of LamB folding have been elaborated from the analysis of its primary sequence and refined by genetic, immunological, and biochemical data (reviewed in reference 8). In our current model (6, 8), LamB spans the membrane 16 times, with loops protruding on either side of the lipid bilayer. The high-resolution three-dimensional structure of LamB has not been solved, but crystallographic data are available for three other porins, including the porin of *Rhodobacter capsulatus* and the OmpF and PhoE porins of *E. coli* (12, 36). These porins are all organized as homotrimers. Each subunit consists of a 16-stranded antiparallel β-barrel building up a pore. Although the amino acid sequence of LamB is not homologous to those of these porins, it is likely that it shares this overall structural organization (20, 31).

In early studies, a series of missense mutations in the *lamB* gene, leading to tight resistance to phage λ with the wild-type host range (λh$^+$), were isolated and characterized (17). One-step extended host range mutants of λ, named λh, able to infect these class I LamB mutants were selected. *E. coli* strains with new missense mutations in *lamB*, called class II mutants, conferring tight resistance to λh phages were then selected. Finally, two-step extended host range λ phages, named λhh*, able to infect class II LamB mutants were selected from the λh phages (1, 17). In that study, all of the λh phages were able to infect not only the class I LamB mutant used for their selection but also all other class I LamB mutants. In other words, the mutations in λh were not specific of the LamB class I allele on which they had been isolated. The λhh* mutants of λ could infect the class II LamB mutants and all of the class I LamB mutants. As expected from the isolation procedure, all of the λh phages were still able to infect the LamB$^+$ strain (extended host range). Although this was not imposed by the selection, this was also true for the λhh* mutants.

A large number of class I and class II LamB missense

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mutants have been analyzed (5, 6, 11, 16). Such mutations slightly affected or did not affect the electrophoretic mobilities of the mutant receptors and did not affect the total amount of LamB expressed by the cells (3, 11, 17). Genetic mapping of the mutations showed that class I and II mutations clustered mainly in a few regions of the *lamB* gene (18). This study identified 12 aa residues in the LamB protein essential for λ phage adsorption. Class I mutants corresponded to positions 18, 148, 152, 163, 164, 245, 247, 249, 250, 259, and 382 of the mature LamB polypeptide, and all class II mutants corresponded to position 151 (6).

In contrast, little is known about the J protein of phage lambda, which constitutes the receptor-binding protein of the phage. It has been shown that the J protein was the target of lambda-neutralizing antibodies (4, 13) and was a structural component of the tail fiber (molecular mass, 130 kDa) (21). The number of J polypeptides present per tail is not clear: two to three copies most likely form the tip of the tail fiber (23). The entire nucleotide sequence of the lambda genome has been determined (30). The *J* gene is 3,399 nucleotides long. Most of our knowledge about the sites affected in host range mutants of λ comes from a study carried out 15 years ago (33). In vivo recombination experiments, complemented by comparisons of two-dimensional tryptic maps of J peptides from λh$^+$, λh, or λhh* phages and heteroduplex analyses of DNAs from λh$^+$ and λhh* showed that the host range mutations were located in the last distal (5 to 10%) portion of gene *J*.

To identify the amino acids of the J protein involved in overcoming of the LamB mutations, we selected a series of new one-step and two-step host range mutants of phage λ and analyzed the corresponding *J* genes.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. *E. coli* K-12 strain P4X8 (Hfr P4X8 *metA strS rifS metD proA*) carries the wild-type *lamB* gene on its chromosome. It was used to propagate all of the λ phages. P4X8 *lamB500* is a spontaneous maltose$^+$, dextrin$^-$, λ-resistant mutant of P4X8 (null mutant) that we selected for resistance to λ with the wild-type host range (λh$^+$). No LamB protein was detectable in this strain (data not shown). C600 (F$^-$ *thr leu tonA lacY1 supE*) was used as a LamB$^+$ strain in the selection of new λh host range mutants.

Several class I LamB mutants in an Hfr G6 background (Hfr *trpE galE galZ rpoB*) were used to select or control the λ host range phenotypes (17), i.e., pop7086 (*lamB108*; mutation, Gly-245→Arg), pop7079 (*lamB101*; mutation, Ser-247→Leu), pop1111 (*lamB63*; mutation, Gly-249→Asp), and pop7090 (*lamB112*; mutation, Glu-148→Lys). Strain pop1091 carries a *lamB* gene with the class II mutation affecting aa 151 (change, Gly→Asp) in an Hfr G6 background (Hfr *metA trpE galE galY rpoB*).

Transformations with pUC18-derived plasmids were performed by standard procedures (9). Strain JM501 was the recipient for all of the recombinant plasmids that were sequenced. JM501 is a derivative of strain JM101 carrying the deletion Δ*malB12* (which removes the distal part of *malK* and the entire *lamB* gene). JM501 also contains *malG*::Tn*10* allele *malG100*. This strain was constructed by P. Quillardet (Institut Pasteur, Paris, France).

The virulent λ derivative of wild-type λ phage, λ $b_2$vir (abbreviated as λh$^+$) (18), and its one-step extended host range mutant λ$b_2$vh° (abbreviated as λh°) were previously described (reviewed in reference 3). Sensitivity to phages was assayed by spot tests on lawns of the different strains plated on Luria-Bertani (LB) agar.

The media, chemicals, and growth conditions used were described previously (17).

Selection of host range mutants of λ. (i) Isolation of one-step host range mutants (λh). One-step host range mutants of λ, λh, were isolated by using a previously described procedure (17). The number attributed to each λh mutant corresponds to the mutated amino acid position of the LamB mutant used for its selection. For example, phages λh247-1 to λh247-7 were isolated on a LamB mutant with a Ser-247→Leu mutation. Briefly, after an overnight culture of LamB$^+$ strain C600 and the class I LamB mutant strain in complete medium, bacteria were collected by centrifugation and washed in 10 mM $MgSO_4$. A 100-μl volume of the LamB$^+$ strain was then mixed with 100 μl of the class I LamB mutant, incubated with 500 PFU of λh$^+$ for 15 min at 37°C, and finally overlaid on tryptone solid medium and incubated overnight at 37°C. λh mutant phages were isolated from clear sectors which appeared at the periphery of turbid plaques (λh$^+$, which is able to lyse only the LamB$^+$ strain, made turbid plaques, while the λh mutants, able to lyse both strains, made clear plaques). This procedure thus necessarily leads to the selection of extended host range mutants of λ able to use the wild-type and mutant receptors.

(ii) Isolation of two-step host range mutants (λhh*). Two-step host range mutants of λ were obtained directly by incubating (for 15 min at 37°C) 100 μl of a concentrated lysate of λh (with a titer between 10^9 and 10^{12} PFU/ml) with 100 μl of an overnight culture of class II mutant strain pop1091 washed in 10 mM $MgSO_4$. The mixture was then overlaid on tryptone solid medium and incubated overnight at 37°C. Plaques were isolated and purified twice. They will be referred to as λhh* mutants. The number assigned to each λhh* mutant corresponds to the mutated amino acid position of the λh mutant phage from which it was selected. For example, phages λhh*1040-1 to λhh*1040-4 were isolated from λh245-2 carrying the mutation Thr-1040→Met (see Results).

Each host range mutant was purified twice on the corresponding class I or II LamB mutant. Large liquid phage stocks were prepared on strain P4X8 as previously described (2).

The efficiencies of plating of all of the λh phages on the different LamB mutant strains were checked as previously described (3) and compared with that on LamB$^+$ strain P4X8. Although this was not required by the selection procedure, all of the λhh* mutants were able to grow on the LamB$^+$ strain.

Cloning and DNA sequencing. DNAs from λh phages were prepared by using the standard procedure described in reference 2. Phage DNAs were digested to completion with restriction enzyme *Mlu*I. A 2.2-kb *Mlu*I DNA fragment encompassing the last third of gene *J* was purified from agarose gel and subcloned into vector pUC18 linearized by *Sma*I restriction enzyme digestion (Fig. 1) in accordance with standard procedures (27). DNA sequencing was performed with dideoxy-chain terminators, a Sequenase Version 2 Kit (U.S. Biochemicals, Cleveland, Ohio) with the universal and reverse M13 primers, and lambda primers corresponding to the sequence of the wild-type *J* gene, i.e., primers 2413 (5′ TCGGCGAGCGATG ATGCGGAAGGTTACCT 3′), 2717 (5′ ACCCGGCAAA CGGGAATGAAACGCCGATGTTT 3′), 2878 (5′ GCGG ATATCAGTGGCAGTGTGAATGCGAAC 3′), and 3094 (5′ CGCCAGATAGTGGTGCTTCCGCTGACGTTT 3′) on the coding strand of gene *J* and primers 3399 (5′ TCAGACC ACGCTGATGCCCAGCGCCTGTTT 3′), 3197 (5′ GCACC GTTCATCAGTTTCAGA 3′), and *lom* (5′ CGGGATCC CAGCAACGGCAATACACACATTACGCATCG 3′), corresponding to the N terminus of gene *lom*, on the noncoding

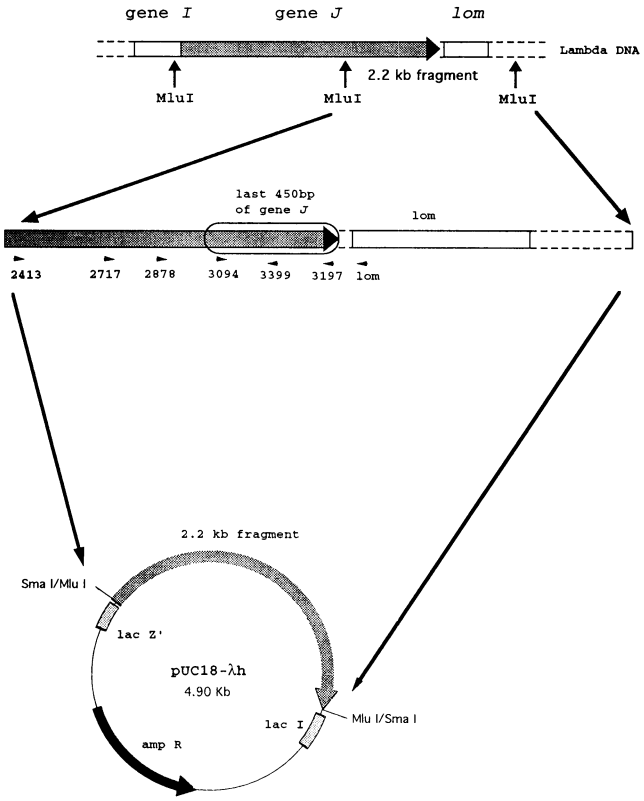

FIG. 1. Positions of the *Mlu*I sites on the lambda DNA region comprising gene *J* are shown at the top. Localization of the primers used for amplification and sequencing in the 2.2-kb *Mlu*I fragment is shown in the middle. The region circled corresponds to the approximate size of the distal part of gene *J* comprising all of the mutations found. A schematic map of the recombinant pUC-2.2kb-λ plasmids constructed by subcloning of the 2.2-kb *Mlu*I fragment into the *Sma*I site of pUC18 is shown at the bottom.

strand. Numbering corresponds to the first 5′ P nucleotide of the primer relative to the *J* gene sequence. All of the λ primers were synthesized with a Milligen/Bioresearch Cyclone Plus DNA synthesizer (Millipore).

For sequence analysis of the λhh* phages and λh recombinants (see the next section), the distal part of gene *J* was amplified by PCR from a single plaque treated with proteinase K (2), with either primer 2878 or primer 3094 and the *lom* primer as the primer pair (29). PCR products were purified with Centricon 100 filtration units. Thermal cycle sequencing was then performed, either by end labelling of the primer with [γ-^{33}P]ATP or by direct incorporation of [α-^{35}S]ATP, with a ds DNA Cycle Sequencing System kit (GIBCO-BRL) (Fig. 1).

Recombination experiments. Class I LamB mutant strain pop7079 was transformed with a recombinant pUC plasmid containing the 2.2-kb *Mlu*I DNA fragment from the λh mutant tested, encompassing the distal part of *J* (Fig. 1). Two λh phages were tested in recombination experiments: λh245-2 and λh247-6. The resulting strains were called pop7079 (pUC-λh245-2) and pop7079 (pUC-λh247-6), respectively.

A 100-ng sample of purified wild-type λh$^+$ DNA was introduced into these recombinant strains by transfection (29). After transfection and spreading onto LB solid medium, individual plaques were isolated. These plaques corresponded to recombinant λh-like phages, since the parental wild-type λh$^+$ phage could not infect class I LamB mutant pop7079.

The recombinant phage plaques were then purified twice on the same strain, and their phenotype was checked. DNA sequencing was performed directly from plaques as described above.

RESULTS

Selection and DNA sequence analysis of one-step host range mutants of λ. Three different class I LamB mutants (strains pop7079, pop7086, and pop7090), corresponding, respectively, to mutations at residues 247, 245, and 148 in the LamB protein, were used to select 11 spontaneous, independent, new, one-step extended host range mutants of lambda (λh phages) (see Materials and Methods).

Seven phages were isolated on strain pop7079; they were named λh247-1 to λh247-7. Three phages were isolated on strain pop7086 (λh245-1 to λh245-3), and one was isolated on strain pop7090 (λh148-1). The phenotypes of the λh host range phages were checked by spot testing on lawns of different *E. coli* strains. All of the λh phages selected grew on P4X8 (LamB$^+$), on the class I LamB mutant used for the selection, and on all of the other class I strains tested, with an efficiency of plating of 1. They did not form plaques on class II LamB strain pop1091 or on LamB null strain P4X8 *lamB500*. Thus, the extended host range phenotype of all of the new λh phages was not allele specific, in agreement with what had been observed for the previously described λh host range mutants (17).

Phage DNAs were purified and restricted with *Mlu*I. The last third of gene *J* comprised within a 2.2-kb DNA fragment was subcloned into a pUC plasmid vector and subjected to DNA sequencing (see Materials and Methods). The cloned fragment of gene *J*, comprising the last 1,100 bp of the gene, was completely sequenced for phages λh247-2, λh245-1, and λh245-2. For all of the other phages, only the last 450 bp of gene *J* were sequenced.

For each of the 11 new λh phages, DNA sequence analysis revealed a single-amino-acid change (Table 1). This observation is in agreement with the high (10^{-5} to 10^{-6}) frequencies of spontaneous mutation previously observed upon direct selection of λh mutants (17). All of the mutations were clustered in the distal 10% of the J protein (the J polypeptide is 1,133 residues long, and the most proximal change found was at aa 1040). The alterations in the 11 new λh phages affected only three residues within J. Phages λh247-1, λh247-3, λh247-4, λh247-5, and λh247-6 corresponded to a Val→Ala change at aa 1077, phages λh247-2, λh245-1, and λh245-2 corresponded to a Thr→Met change at aa 1040, and phages λh247-7, λh245-3, and λh148-1 corresponded to a Leu→Pro change at aa 1127.

Several λh phages isolated from different class I LamB mutant strains corresponded to the same amino acid change in the J protein. For example, strains pop7079 (LamB mutant, Ser-247→Leu), pop7086 (LamB mutant; Gly-245→Arg), and pop7090 (LamB mutant; Glu-148→Lys) led to the independent selection of three λh phages with the same change (Leu→Pro) at aa 1127 (phages λh247-7, λh245-3, and λh148-1) (Table 1). Conversely, phages corresponding to the three different mutations in J (Thr-1040→Met, Val-1077→Ala, and Leu-1127→Pro) were isolated from the same LamB mutant (pop7079 at aa 247 of LamB). This is in agreement with and accounts for the absence of allele specificity of λh mutants with respect to *lamB* class I mutations.

Selection and DNA sequence analysis of two-step host range mutants of λ. For selection of two-step extended host range mutants of lambda (λhh* phages), we chose three different λh

TABLE 1. DNA and amino acid sequence of one-step host range mutants

E. coli strain[a]	Alteration in LamB	Selected λh phage	DNA alteration in gene *J*[b]	Alteration in protein J[c]
pop7079	Ser-247→Leu	λh247-1	3230; GTA→GCA	Val-1077→Ala
		λh247-2	3119; ACG→ATG	Thr-1040→Met
		λh247-3	3230; GTA→GCA	Val-1077→Ala
		λh247-4	3230; GTA→GCA	Val-1077→Ala
		λh247-5	3230; GTA→GCA	Val-1077→Ala
		λh247-6	3230; GTA→GCA	Val-1077→Ala
		λh247-7	3380; CTG→CCG	Leu-1127→Pro
pop7086	Gly-245→Arg	λh245-1	3119; ACG→ATG	Thr-1040→Met
		λh245-2	3119; ACG→ATG	Thr-1040→Met
		λh245-3	3380; CTG→CCG	Leu-1127→Pro
pop7090	Glu-148→Lys	λh148-1	3380; CTG→CCG	Leu-1127→Pro

[a] *E. coli* strains corresponding to class I *lamB* missense mutations.
[b] Each number corresponds to the position of the first base of the triplet in the nucleotide sequence of gene *J*.
[c] Each number indicates the position of the amino acid in the protein J sequence.

phages, each one corresponding to a different amino acid modification in the distal part of protein J, i.e., λh245-2 (Thr-1040→Met), λh148-1 (Leu-1127→Pro), and λh247-6 (Val-1077→Ala) (Table 2). The new two-step host range mutants were selected on class II LamB mutant strain pop1091 (Gly-→Asp at aa 151 of LamB). They were called λhh*1040, λhh*1127, and λhh*1077, respectively. From each selection, four different, but not necessarily independent, λhh* plaques were purified and further analyzed.

DNA sequence analysis revealed that, in each selection, all four of the λhh* phages isolated carried identical mutations. The λhh* phages selected contained either two amino acid changes (for the four λhh*1040 and the four λhh*1127 phages) or three amino acid changes (for the four λhh*1077 phages) (Table 2).

Each newly selected λhh* phage had kept the parental (λh) alteration (Fig. 2). The four λhh*1040 phages (derived from phage λh245-2) corresponded to Thr-1040→Met and Ser-1011→Gly. The four λhh*1127 phages (derived from phage λh148-1) corresponded to Leu-1127→Pro and Gln-1078→Arg, and the four λhh*1077 phages (derived from phage λh247-6) corresponded to Val-1077→Ala, Ala-1076→Ser, and Glu-1075→Val amino acid changes.

In agreement with the sequence data, the λhh* mutants corresponding to the acquisition of only one additional amino acid change, like λhh*1040 and λhh*1127, were obtained at a frequency of about 10^{-6}. In contrast, the λhh* mutants corresponding to the acquisition of two additional amino acid changes, like λhh*1077, were obtained at a frequency of 10^{-11} to 10^{-12}. This correlation is also an indication that there were no mutations other than those found that were responsible for the λhh* host range phenotype.

The alterations found in the λhh* phages correspond to seven residues, all located in the distal part of protein J. The most proximal change found was Ser-1011→Gly.

Recombination experiments. To ensure that the alterations found in the distal parts of the *J* genes were responsible for the host range phenotypes of the mutants analyzed in this study, recombination experiments were conducted with two of the λh mutants, λh245-2 (Thr-1040→Met) and λh247-6 (Val-1077→Ala) (see Materials and Methods for details).

The principle of the assay was to obtain a double recombination event between the plasmid DNA and the phage DNA, leading to transfer of the mutation from the plasmid to the phage DNA. After encapsidation and cell lysis, the recombinant phages carrying the host range mutation gain the ability to grow on the class I LamB mutant strain and thus to form plaques (λh host range phenotype).

The distal part of each λh mutant tested was subcloned into a pUC plasmid vector, and the recombinant plasmids were used to transform class I LamB mutant strain pop7079 (see Materials and Methods for details). Recombinant strains pop7079 (pUC-λh245-2) and pop7079 (pUC-λh247-6) were then transfected with wild-type $λh^+$ DNA. For the λh247-6 mutation, four individual plaques were purified and further analyzed, and for the λh245-2 mutation, two plaques were analyzed. The recombinant phages were purified twice on the same strain, and their phenotype was checked by the spot test procedure on lawns of different *E. coli* strains (see Materials and Methods).

TABLE 2. DNA and amino acid sequence of two-step host range mutants

Phage λh used for λhh* selection	Amino acid altered in protein J	λhh* phages selected	Alterations in gene *J*[a]	Amino acid altered in protein J[b]
λh245-2	Thr-1040→Met	λhh*1040-1–λhh*1040-4	3319; ACG→ATG	Thr-1040→Met
			3031; AGC→GGC	Ser-1011→Gly
λh148-1	Leu-1127→Pro	λhh*1127-1–λhh*1127-4	3380; CTG→CCG	Leu-1127→Pro
			3233; CAG→CGG	Gln-1078→Arg
λh247-6	Val-1077→Ala	λhh*1077-1–λhh*1077-4	3230; GTA→GCA	Val-1077→Ala
			3226; GCG→TCG	Ala-1076→Ser
			3224; GAG→GTG	Glu-1075→Val

[a] Each number is the position of the first base of the triplet in the gene *J* nucleotide sequence.
[b] Each number is the position of the amino acid in the protein J sequence.

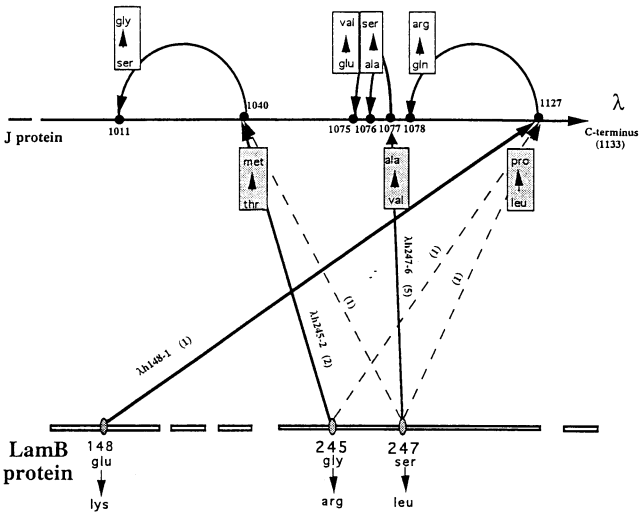

FIG. 2. Genealogy of extended host range lambda mutants. Amino acid positions and changes in the LamB mutants used for selection of λh one-step host range mutants are shown in the bottom diagram. The bold and dotted arrows indicate the LamB mutant from which the different types of one-step λh mutations were selected. From LamB site 245, two different types of λh mutations were obtained, at sites 1040 (two phages) and 1127 (one phage) of the J protein. From LamB site 247, three different types of λh mutations were obtained, at sites 1040 (one phage), 1077 (five phages), and 1127 (one phage). The bold arrows indicate the λh mutants further used for selection of two-step λhh* mutants. Amino acid changes identified in the one-step and two-step λh and λhh* mutants are shown in the diagram at the top. The position and nature of each amino acid change are indicated. The grey boxes correspond to the amino acid changes found in the λh mutants, and the white boxes correspond to the changes found only in λhh* derivatives. In all cases, the λhh* derivatives corresponded to the presence of one or two additional mutations located upstream the parental (λh) mutation. This is represented by the arrows which link the parental λh mutation and the new λhh* mutation for each λhh* derivative. Two λhh* mutants (λhh*1127 and λhh*1040) were double mutants, and one (λhh*1077) was a triple mutant.

With both types of recombinants, all of the recombinant phages kept the ability to form plaques on the wild-type LamB$^+$ strain and acquired the ability to grow on all of the class I LamB mutants tested, like the λh phages they derived from. Moreover, direct DNA sequencing of the plaques confirmed (i) that in all cases the corresponding recombinant phages acquired the very mutation that was present on the initial pUC recombinant plasmid and (ii) that no other mutation in the distal part of gene *J* was present (in the case of λh245-2, we determined the sequence of the entire gene *J* part of the 2.2-kb *Mlu*I fragment without finding any mutation other than the Thr-1040→Met change). These results confirmed that the single-amino-acid changes Thr-1040→Met in phage λh245-2 and Val-1077→Ala in phage λh247-6 were responsible for their one-step extended host range phenotype.

DISCUSSION

We have identified seven residues at the distal part of the J protein which are involved in the extended host range properties of mutant λ phages. The data confirm that the amino acids affected by the mutations in LamB, and the amino acid changes in the J proteins of the extended host range phage mutants, are not involved in allele-specific protein-protein interactions. They strongly suggest, however, that the last C-terminal residues of the J protein participate directly in the adsorption of λ onto LamB.

Eleven new one-step λh phages were isolated and analyzed. In each case, a single mutation was found in the distal part of the *J* gene (Table 1). We found no effect of the initial LamB mutation on the changes observed in the λh host range phages (Table 1). These observations can be correlated with the ability of each λh phage to grow on all of the class I LamB mutants (as well as on the wild-type LamB$^+$ strain) with an efficiency of plating identical to that of the parental wild-type phage. This can be interpreted as meaning that all *lamB* class I mutations block a common step in phage adsorption and that this block is relieved by the λh mutations.

We then selected two-step host range λhh* phages on the class II LamB mutant (a Gly→Asp change at site 151 of LamB) from three λh phages corresponding to three different missense mutations in J. DNA sequencing showed that for each selection, the four λhh* mutants analyzed were identical and that all of the new λhh* phages had kept the parental λh mutations. The λhh* mutants corresponded to double- or triple-point mutations, all located in the distal part of the *J* gene (Fig. 2). In this case too, the class II *lamB* mutation can be interpreted as blockage of a step in phage λ adsorption which is relieved by the λhh* mutations. At this stage, it is not possible to say whether the class I and II LamB mutations affect the same step or two distinct steps of λ adsorption.

One may recall here that the selection procedure used to isolate λh mutants (plating on a mixture of the wild-type strain and a LamB class I mutant) can only yield mutants with an extended host range. This may be a reason for the lack of allelic effects. However, it was shown that selection of λh mutants is also possible by plating a fresh stock of λh$^+$ on a LamB class I mutant (17). Thus, it will be possible to examine whether such mutants also lack allele specificity. At any rate, the procedure used to select λhh* mutants (plating on a selective strain) does not require extension of the host range, so that there is no reason to invoke a selection bias in favor of the absence of allele specificity.

Early studies on the sites affected in λh or λhh* host range mutants of λh$^+$ (33) indicated that the mutations in λh and λhh* had a multisite character. In the present work, we found that this was true for the λhh* but not for the λh mutations which corresponded to single-amino-acid changes. We thus decided to perform DNA sequence analysis of the distal part of gene *J* from a previously isolated one-step host range mutant of λ from the laboratory collection, called λh° (17, 33). In this case, two distinct point mutations were identified at the distal end of the *J* gene, corresponding to a Ser→Gly change at aa 1011 and a Thr→Ala change at aa 1040. We do not know whether the two mutations were present in the original isolate or whether a secondary mutation appeared later.

Two main approaches were previously used to study the interactions between LamB and λ. (i) In vitro studies on solubilized LamB and λ led to the proposal of a kinetic model of interaction in which adsorption of the phage on the receptor occurs in several steps: a first step leading to the formation of reversible phage-receptor complexes, followed by a second step of formation of irreversible phage-receptor complexes, and finally ejection of the phage DNA (32). However, it was not demonstrated that the two types of complexes observed in vitro indeed occur in vivo. (ii) Electron microscopic analyses of complexes between λ and LamB allowed the identification of two types of complexes. In type I complexes, the tip of the tail fiber constituted by protein J was visible, while in type II complexes, the tip was no longer visible and the distal end of the tail tube was directly attached to the receptor (28).

It is not possible to draw any obvious relationships between these sets of observations and our data which could lead to a general view of the mechanism of phage λ adsorption and infection.

However, one may speculate, for example, that the phage λ distal tail fiber constituted by the J protein has to pass through an "accessibility gate" located at the surface of the LamB receptor to be able to interact tightly with the binding site of the receptor protein and to allow further phage DNA injection. The effects of class I or II mutations in LamB would be to block the entry of the tail fiber by narrowing the accessibility gate, thus preventing access to the tight binding site. Extended host range mutations in λ would affect the structure of the distal tail fiber in such a way that access to the binding site through the mutated accessibility gate would be restored. The hypothesis of global reduction of the diameter of the tip of the tail fiber from λh^+ to λh and λhh* is in agreement with the ability of the mutant phages to grow on the wild-type LamB strain (and on all class I LamB for λhh* phage mutants).

A study on host range mutants of T-even type bacteriophage Ox2 using the OmpA protein as a receptor has been previously reported (14). Phage Ox2 possesses six long tail fibers with a distal adhesin protein responsible for the first, reversible, step of the interaction—i.e., the recognition of the OmpA receptor protein—and one basal short tail fiber involved in a later, irreversible, step of binding to lipopolysaccharide. Multistep host range mutants that could grow on an altered OmpA protein, then on OmpC, and finally on lipopolysaccharide were isolated. Their analysis showed that single mutations in adhesin protein 38 were responsible for the host range phenotypes. The major difference between these findings and the data presented in our study is that the Ox2 host range mutants were able to bind to new surface receptors and did not systematically keep the ability to grow on the parental receptors. These differences suggest that the molecular mechanisms underlying the phage-receptor interaction are quite different in the two systems.

Further genetic, biochemical, and biophysical studies on the J protein are required to explain how this protein is folded and interacts with its receptor. Determination of the three-dimensional structure of LamB should also help to clarify the way the J protein interacts with LamB. The present work identified the first set of J residues involved in the interactions between LamB and the J protein. This system constitutes a very attractive model for the study of protein-protein interactions and may also produce additional information about the LamB protein itself.

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