In Vivo and In Vitro Functional Alterations of the Bacteriophage Lambda Receptor in \textit{lamB} Missense Mutants of \textit{Escherichia coli} K-12

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\textit{lamB} is the structural gene for the bacteriophage \(\lambda\) receptor in \textit{Escherichia coli} K-12. In vivo and in vitro studies of the \(\lambda\) receptor from \textit{lamB} missense mutants selected as resistant to phage \(\lambda h^*\) showed the following. (i) Resistance was not due to a change in the amount of \(\lambda\) receptor protein present in the outer membrane but rather to a change in activity. All of the mutants were still sensitive to phage \(\lambda hh^*\), a two-step host range mutant of phage \(\lambda h^*\). Some (10/16) were still sensitive to phage \(\lambda h\), a one-step host range mutant. (ii) Resistance occurred either by a loss of binding ability or by a block in a later irreversible step. Among the 16 mutations, 14 affected binding of \(\lambda h^*\). Two (\textit{lamB}106 and \textit{lamB}110) affected inactivation but not binding; they represented the first genetic evidence for a role of the \(\lambda\) receptor in more than one step of phage inactivation. Similarly, among the six mutations yielding resistance to \(\lambda h\), five affected binding and one (\textit{lamB}109) did not. (iii) The pattern of interactions between the mutated receptors and \(\lambda h^*\) and its host range mutants were very similar, although not identical, in vivo and in vitro. Defects were usually more visible in vitro than in vivo, the only exception being \textit{lamB}109. (iv) The ability to use dextrins as a carbon source was not appreciably affected in the mutants. Possible working models and the relations between phage infection and dextrin transport were briefly discussed.

How the amino acid sequence of a protein determines its structure and therefore its activities is poorly understood. In the case of a membrane protein these questions have gone largely unanswered, because very little is known on the interactions between the protein and the membrane and their relevance to structure and activity. The product of the \textit{lamB} gene (17) is a multifunctional protein located in the outer membrane of \textit{Escherichia coli} K-12 (10), which might be a good tool to study such questions. It is implicated in maltose transport (16) and required for growth on maltodextrins (20; C. Braun-Breton 3rd cycle thesis, University of Paris, Paris, 1977). It also serves as a receptor for phage \(\lambda\) with wild-type host range (\(\lambda h^*\)), its one-step host range mutant (\(\lambda h\)), its two-step host range mutants (\(\lambda hh^*\)) (6, 15), and phages K-10 (11) and TP (19).

Among the \textit{lamB} mutations yielding resistance to phage \(\lambda h^*\), we reported the existence of three classes (6). One class (class III), composed of nonsense mutations and deletions, abolishes all of the known activities of the \(\lambda\) receptor. The \(\lambda h^*\) fragments produced are essentially inactive (3). The other two classes do not abolish at least one of the in vivo activities of the \(\lambda\) receptor: class I allows growth of \(\lambda h\) and \(\lambda hh^*\), and class II allows growth of \(\lambda hh^*\). For this reason and because class I and class II mutations are not affected by nonsense suppressors, it was tentatively concluded that they were of the missense type.

We report here a more detailed analysis of the activities of the \(\lambda\) receptor in vivo and in vitro for class I and class II mutations. Among 16 mutations, none affected the amount of receptor in the outer membrane nor the ability to grow on dextrins. Two mutations allowed efficient reversible adsorption of \(\lambda h^*\) but no inactivation, i.e., they affected a step after reversible binding.

Working models accounting for the interactions seen in vivo and in vitro, as well as possible relations between phage adsorption and dextrin transport, are briefly discussed.

MATERIALS AND METHODS

Media and strains. Media and dextrin preparation were described previously (6, 20).

Phage strains (6) included \(\lambda b2 vir\) with wild-type host range (\(\lambda h^*\)), its one-step host range mutant \(\lambda b2 vir h\) (\(\lambda h^*\)), and its two-step host range mutant \(\lambda b2 vir h h 16\) (\(\lambda hh^*\)). The term \(\lambda\) is used when no specification of host range is needed.

Bacterial strains used are listed in Table 1, and their
construction is explained below. The parental strains (already described in reference 7) were C600 (F' thr leu lacY1 supF tonA21), CR63 (F' supD60 lam63), pop1020 [Hfr G6 metA trpE9780(Am) galE galY], pop1021 [Hfr G6 metA trpE9780(Am) galE galZ], pop1046 [Hfr G6 metA lacZ(Am)] (6). Escherichia coli 6371 was described in reference 14: (i) galY and galZ, previously described (7), are spontaneous mutations located in the gal region which prevent the lethal effect of galactose due to the galE mutation. Except for these mutations, the strains pop1020 and pop1021 are isogenic. (ii) The lamB mutants pop1079 to pop1091 (see Table 1) were isolated as λ^-resistant Mal^- derivatives of pop1020 after ethyl methane sulfonate treatment (6). (iii) pop1076 (Table 1) was constructed by transferring the lamB5 mutation from a metA^- derivative of pop1067 (6) into pop1021 by P1 transduction selecting for metA^- (iv) pop1111 was constructed by transferring the lamB63 mutation of strain CR63 into pop1021 by P1 transduction selecting for metA^- (v) pop1132, pop1134, and pop1136 were constructed as follows: lamB302, 304, and 306 were isolated in strain pop1021 by looking for mutants resistant to λ (λH) after ethyl methane sulfonate mutagenesis. They were then transduced into a non-mutagenized pop1021 background using P1 stocks grown on a metA^- derivative of the mutant clone. In vitro assays for receptor activity. Extraction of λ receptor was performed as described previously (10) in Tris (10^-2 M)–cholate (1%)–EDTA (2 x 10^-3 M) (pH 7.5). The extracts (called R extracts) were kept at 4°C for several days without appreciable loss of activity. (i) Assay for phage inactivation. The ability of an R extract to inactivate phages λ^+, λH, and λH^+ with or without ethanol was examined as described (10; Braun-Breton, 3rd cycle thesis, 1977). The reaction buffer was Tris (10^-2 M)–MgSO_4 (2 x 10^-3 M) (pH 7.5). When ethanol was used, its final concentration was 20%. Bovine serum albumin (2 mg/ml) was added to the reaction buffer to protect the phage against direct inactivation by ethanol. It was checked that the addition of bovine serum albumin does not affect the rate of phage inactivation by the receptor.

**Table 1. In vivo and in vitro properties of wild-type and mutated λ receptors**

<table>
<thead>
<tr>
<th>Strain*</th>
<th>lamB^+</th>
<th>Class</th>
<th>λ Inactivation*</th>
<th>λ Protection*</th>
<th>SDS-PAGE/</th>
</tr>
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<td></td>
<td></td>
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<tr>
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* The bacterial strains are quasi-isogenic. Genetic markers and strain construction are described in the text. pop1080 is used here as a negative control and contains the class III ochre mutation lamB102.

* lamB allele number.

* Initial classification according to in vivo growth of λ^+, λH, and λH^+ (7).

* The results are presented as relative efficiencies of inactivation. The value for wild type is taken arbitrarily as 1; ≤, less than 10^-2; ≤, not tested. The maximum relative error is less than a factor 5. The absolute values for wild type in R extracts containing 1 mg of total protein per ml are as follows (minutes); In vivo: λ^+, 4 x 10^-4 to 5 x 10^-6; λH, 3 x 10^-6 to 6 x 10^-6; λH^+, 1 x 10^-10 to 2 x 10^-10; In vivo: λ^+, 3 x 10^-10 (in the presence of ethanol); λH, 3 x 10^-11; λH^+, 3 x 10^-12.

* The results are presented as relative binding abilities (see text). The value for wild type is taken arbitrarily as 1. Protection was assayed only when no inactivation occurred.

* Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

* The amount of λ receptor protein in each mutant was compared to that in the wild type by sodium dodecyl sulfate polyacrylamide gel electrophoresis (see text). Symbol: I, identical to wild type by visual evaluation of the stained receptor band on the gel. The amount of λ receptor protein in the wild type is about 1% of total proteins (10).

* Two mutations, lamB5 and lamB105, alter the migration of the lamB protein. Symbols: N, normal migration; F, faster migration; S, slower migration.

* Depending on the conditions and genetic background, the efficiency of plating of λH on lamB106 and lamB110 varied between 10^-7 to 10^-5 and 10^-2 to 10^-1, respectively. lamB106 and lamB110 are thus not strictly class I mutations.

* lamB109 allowed plating of λH and λH^+ with reduced efficiency (10^-7 to 10^-5) and is not strictly a class II mutation, but a class I mutation.
All of the results are expressed for an R extract containing 1 mg of protein per ml. We called D50 the final concentration of the R extract which leads to inactivation of 50% of the phage in 30 min at 37°C. The relative activity of an R extract with respect to the activity of the wild-type extract R* was taken as the ratio of D50R*/D50R.

(ii) Assay for reversible phage binding. The ability of an R extract to bind reversibly phages λh*, λh, or λh** could be examined conveniently only when no inactivation occurred. This was done by measuring the protection of the phages by the noninactivating R extract against inactivation by an inactivating R* extract, usually an extract from E. coli 6371 (13, 14). Formation of complexes between phages and λ receptors was allowed by an incubation of 30 min at 37°C in the reaction buffer Tris (10-2 M)–MgSO4 (2 × 10-3 M) (pH 7.5). Inactivation of free phages was then performed by the addition of an excess of R* extract in the same reaction buffer and further incubation for 10 min at 37°C. Surviving phages (associated with λ receptor molecules) were titrated after rapid dissociation of the complexes by dilution in MgSO4 (5 × 10-2 M) in the presence of indicator bacteria. We called P20 the final concentration of the R extract which led to protection of 20% of the phage in the above conditions. All of the results were expressed for R extracts containing 1 mg of protein per ml. The relative binding ability of an R extract with respect to the binding ability of the wild-type extract R* towards phage λh* was taken as the ratio of P20R*/P20R.

In vivo assays for receptor activity. Bacteria growing exponentially at 37°C in maltose minimal medium were harvested at a density of 5 × 10^8 cells per ml.

(i) Assay for phage inactivation. Bacteria were suspended in MgSO4 (2 × 10-3 M) at a density of 5 × 10^7 cells per ml. A mixture of bacteria (2.5 × 10^7 cells per ml) and phages (input multiplicity of 10^3) was incubated in reaction buffer MgSO4 (2 × 10-3 M) at 37°C. Samples were withdrawn at various times and diluted 100-fold in MgSO4 (10-2 M) saturated with chloroform. After 15 min of incubation at room temperature, further dilutions were plated with indicator strain. The initial kinetics of phage inactivation followed the relation \( q = q_0 e^{-Kt} \) (11), where \( q_0 \) is the initial phage concentration, \( q \) is the free phage concentration at time \( t \), \( K \) is the pseudo-first-order rate constant for phage inactivation. We have measured \( K \) for the wild-type and mutant strains. The ratio \( K_{\text{wild type}}/K_{\text{mutant}} \) measures the relative in vivo activity of a lamB mutant to inactivate the phage.

(ii) Assay for reversible phage binding. Bacteria were suspended in Tris (10-2 M; pH 7.5) at a density of 10^9 cells per ml. The procedure was the same as used for in vitro assay of reversible phage binding except that the initial incubation was done with a mixture of phages and dilutions of the bacterial suspension to be tested. The binding ability of a strain was taken as \( Q_0 \) i.e., the concentration of the bacterial suspension which resulted in protection of 20% of the phage in the conditions of the assay. The relative binding ability of a mutant strain with respect to the binding ability of the wild-type extract R* toward phage λh* was taken as the ratio P20R*/Q20.

Examination of the λ receptor protein by electrophoresis in polyacrylamide gels in presence of sodium dodecyl sulfate. Bacteria growing exponentially at 37°C in minimal medium with or without the maltose inducer were harvested at a density of 5 × 10^8 cells per ml and suspended in sterilized water at a density of 10^9 cells per ml. This bacterial suspension was diluted twofold in 0.125 M Tris-hydrochloride (pH 6.8)–20% glycerol–4% sodium dodecyl sulfate–2-mercaptoethanol (0.72 M) and incubated for 5 min at 100°C. Slab gel electrophoresis of such samples was performed using the method of Laemmli (7) as modified by Anderson et al. (1).

RESULTS

Resistance of class I and II mutants to λh+ could be due to the presence of an insufficient amount of λ receptor protein in the cell or modification of the activity of the λ receptor towards the phage or both. One should note that this in vivo activity depends on the specific activity of the λ receptor protein as well as on the position of the λ receptor molecules with respect to the cell surface and on their interaction with the envelope components. Evaluation of the amount of lamB protein by slab gel electrophoresis in the presence of SDS shows that the 16 mutants and the wild-type strain possess comparable amounts of the lamB protein (Fig. 1).

Since the total amount of lamB protein was not appreciably affected by the mutations, we compared the in vivo (in whole cells) and in vitro (in cellular R extracts) activities of the mutated λ receptors towards λh+ and the host range mutants λh and λh**.

Inactivation of phage λ by its receptor is a multistep process (12, 14; Braun-Breton, 3rd cycle thesis, 1977). As a first attempt to compare the mutated receptor proteins with the wild-type λ receptor, we used a simplified view of the inactivation process and assumed the existence of two steps: a reversible binding of λ to its receptor followed by an irreversible binding (inactivation) which leads to λ DNA ejection:

\[
\lambda + R^{\text{(AR)}} \rightarrow \lambda R^{\text{(AR)}} \rightarrow \lambda R^{\text{(AR)}-i} \rightarrow \text{DNA ejection}
\]

where λ is free phage, R is free receptor, (λR) is a reversible complex, (λR)i is an irreversible complex, and \( K_1, K_2, \) and \( K_3 \) are the rate constants.

Inactivation could be assayed conveniently by looking at the disappearance of free phage from a mixture of bacteria (in vivo assay) or cellular R extracts (in vitro) and phages. The initial kinetics of phage inactivation was pseudo first order. The inactivation constant \( K \) could be taken as a measure of the activity of the λ receptor. The relative activity of a mutated λ
which do chloroform assay poplO79 (lamB01l) protein (this figure and unpublished personal communication). wild-type Ah+ (Table 1). Strain pop1079 (lamB101) with maltose; lane 2, strain pop1088 (lamB110) with maltose; lane 3, strain pop1087 (lamB109) with maltose; lane 4, strain pop1084 (lamB106) with maltose; lanes 5 and 6: strain pop1083 (lamB105) with and without maltose, respectively; lanes 7 and 8, strain pop1076 (lamB5) with and without maltose, respectively; lane 9: strain pop1021 (lamB*) with maltose; lane 10: strain pop1080 (lamB102rev) with maltose. lamB = λ receptor; maIQ = amylomaltose; malE = maltose binding protein; ompC and ompF = major outer membrane proteins. None of the 16 lamB mutations reduced significantly the amount of lamB protein (this figure and unpublished data). Two mutations affected the rate of migration of the receptor: lamB5 led to a slower migration and lamB105 led to a faster migration of the lamB protein. In the case of lamB5, the slower migrating band did not appear to be the precursor of the λ receptor or pre-λ receptor. Indeed, the in vitro lamB5 product (9) migrated more slowly than the wild-type pre-λ receptor (D. Perrin, personal communication).

In conclusion, the 16 mutated λ receptors behaved alike in vivo and in vitro towards λh*. Fourteen lost their binding ability towards λh*; two (lamB106 and lamB110) kept their binding ability but lost all capacity to inactivate phage λh*.

Activity towards phage λh. Class I mutants inactivate λh, whereas class II mutants essentially do not. Efficient binding of λh was detected in vivo for one mutant (lamB109).

In vitro, the results were as follows. Seven of the class I λ receptors were able to inactivate λh with approximately the same efficiency as the wild-type λ receptor. One (lamB112) was about 20 times less active even in the presence of ethanol. Two (lamB106 and lamB110) were unable to inactivate λh even in the presence of ethanol but they reversibly bound λh as efficiently as λh*. R extracts of class II mutants did not inactivate λh efficiently. An R extract of mutant lamB109 inactivated λh efficiently.

The pattern of interaction of the mutated λ receptors with λh was thus more complex than with λh*. The 10 class I mutants were able to bind λh in vitro, but 3 were impaired in the receptor with respect to the wild type was defined as the ratio of the constants for the mutant and the wild type (Table 1).

Reversible binding can be assayed only when no inactivation occurs. This is the case in vitro when wild-type λ receptor interacts with phage λh*. In this case inactivation occurs only in the presence of chloroform or ethanol (10, 14). With phages λh (10) and λhh* (Braun-Breton, 3rd cycle thesis, 1977) in vitro inactivation by the wild-type receptor occurs even in the absence of chloroform or ethanol. The reversible binding assay was also used with mutated receptors which do not inactivate the phage. This allowed definition of the relative binding ability of a mutated λ receptor with respect to the binding ability of the wild-type receptor towards phage λh* (Table 1).

Activity towards phage λh*. No inactivation of phage λh* by any of the mutants could be detected in vitro even in the presence of ethanol. Efficient binding in vivo as well as in vitro was detected for two of the class I mutants (lamB106 and lamB110) and none of the class II mutants.

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inactivation process, one slightly (lamB112) and two totally (lamB106 and lamB110). The deficiency of these three mutated receptors was much stronger in vitro and was not cancelled by addition of ethanol. Class II mutants have lost most or all of their affinity for λh. One mutant (lamB109) bound the phage reversibly in vivo without inactivating it efficiently, whereas it inactivated λh efficiently in vitro. In this case release from the membrane allowed the irreversible step to occur.

Activity toward λhh* and growth on maltodextrins. All of the 16 mutated λ receptors were able to inactivate λhh* both in vivo and in vitro. Although the in vivo activities were comparable, the in vitro activities were reduced 3- to 10-fold in four cases (lamB106, 110, 113, and 5). These receptors were partially active or not active at all towards phage λh.

All of the 16 mutants were still able to use maltodextrins as a carbon source (Dex* phenotype). It is interesting to note that, among the mutants isolated as resistant to λh*, all of the missense mutants (class I and class II) were Dex* and sensitive to λhh*, whereas all of the nonsense mutants (class III) were Dex* and resistant to λhh*. The correlation between the pattern of sensitivity to λhh* and the ability to grow on maltodextrins is considered further in the Discussion.

DISCUSSION

Interactions with the phage. We compared the λ receptor of 16 independent class I and II lamB mutants with that of the wild-type strain. The mutations were found not to affect appreciably either the amount of λ receptor protein in the cell or the in vivo rate of inactivation of phage λhh*. This strongly suggests that the effect of the mutations is not due to a modification of the amount of λ receptor protein present in the outer membrane. In fact, reduction in the quantity of receptor results by itself in resistance to λh* only under conditions when the amount of λ receptor present in the outer membrane is well below 1% of the induced wild-type level (B. Colonna and M. Hofnung, manuscript in preparation).

Most of the mutations (14 out of 16) did not affect the electrophoretic mobility of the λ receptor. This is compatible with the idea that they are of the missense type. In two cases the apparent molecular weight was changed. This could be interpreted either as a charge effect due to a missense mutation or as a small deletion (lamB105) or insertion (lamB35). At any rate, elucidation of the exact nature of the mutations requires determination of the DNA sequence change.

For comparison of λ receptor activities, we used two different assays: (i) an inactivation assay which allows evaluation of the relative activity of a mutated receptor for phage inactivation; and (ii) a binding assay which can be used only when no inactivation occurs and which allows evaluation of the relative ability of a mutated receptor to bind the phage. Both assays were performed in vivo as well as in vitro. The results (Table 1) are summarized in the schematic drawing shown in Fig. 2.

Inactivation requires binding. Most of the mutations abolished binding: 14 out of the 16 mutations for λh* and 5 out of the 16 mutations for λh. For these mutations the pattern of interaction was very similar in vitro and in vivo, the defects being usually more apparent in vitro. This is reminiscent of what is found for the interaction between λh* and the wild-type receptor where inactivation in vitro requires ethanol (or chloroform) and is compatible with the idea of a role of the membrane environment in the irreversible step (see equation 1). However, ethanol was never found to relieve the mutational blocks in vitro.

Three mutations affected inactivation without appreciably affecting the binding. One mutation (lamB109) affected inactivation of λh in vivo without appreciably affecting the binding. Inactivation occurs normally in vitro. This could be tentatively interpreted by an inadequate conformation or position of the mutated λ receptor in the membrane. Two class I mutations (lamB106 and lamB110) totally blocked inactivation of λh*. Two class II mutations (lamB106 and lamB110) totally blocked inactivation of λh*.

For λh, inactivation did not occur in vitro and was reduced in vivo. Inactivation of λhh* was slightly affected in vitro. The deficiencies caused by these λ receptor mutations were thus more visible in vitro than in vivo.

The present characterization of mutations lamB106 and 110 revealed a new class of mutations affecting λh* growth at a step later than reversible binding. The other class, composed of the pel mutations, maps outside lamB and allows irreversible adsorption but no DNA injection (12). The block due to lamB106 and lamB110 is thus placed earlier than that due to pel along the pathway for phage infection. The full binding capacities of the lamB106 and 110 receptors show that the simple belief that λh and λhh* mutants would bind weakly to the mutated receptor but would still succeed in infection because they are "fast triggering" does not hold (14).

The pattern of interactions reflects and confirms the in vivo observations that host range mutants of λ are of wider host range rather than
of modified specificity (6; Fig. 2). In fact (i) a mutation affecting the interaction with λhh* always affected the interactions with λh* and λh, (ii) a mutation affecting the interaction with λh always affected the interaction with λh* but not necessarily the interaction with λhh*, (iii) a mutation affecting the interaction with λh* did not affect necessarily the interactions with λh or λhh*.

In the case of gene ompA, there also exists a hierarchical pattern of growth for the host range mutants of phage TuII* (6). It was suggested (5) that this pattern could be explained by differences in the amount of ompA protein. As shown here, such an explanation does not hold for the interactions between the lamB product and phage λ. The hierarchy found between the classes of phage and bacterial mutants must then be accounted for by some kinetic or structural feature or both (see below).

**Working models for phage adsorption.** The binding and inactivating activities (Fig. 2) of a λ receptor are not independent parameters. In the simplified view of phage receptor interaction that we have adopted so far (equation 1), phage binding depended on two constants, $K_1$ and $K_2$, whereas phage inactivation depended on three constants, $K_1$, $K_2$, and $K_3$. The interactions between a λ receptor and phages λh*, λh, λhh* could thus be described with three equations like equation 1. In this case a lamB missense mutation would affect at least some of the rate constants for one or several of the phages.

The properties of the phage and bacterial mutants described, in particular the hierarchy between host range mutants and the properties of the lamB106 and 110 receptors, could be summarized in a working kinetic model (Fig. 3). The receptor would exist in three interconvertible conformations, each of which would be able to bind reversibly one and only one of the classes of phage host range. Transitions between the three types of phage receptor complexes would occur irreversibly along the pathway for inactivation. Evidence for or against this working model could come from more detailed studies of phage-receptor interactions and in particular from a search for different conformations of the receptor and of phage receptor complexes.

Other models could account for the results. For example, the site of interaction between the receptor and the phages could overlap in such a manner that the receptor site for λhh* would be enclosed in the site for λh, itself enclosed in the site for λh*. Such a topological constraint is compatible with the kinetic model presented. If one assumes that the genetic determinants are larger for larger sites, this hypothesis accounts also for the fact that mutants resistant to λh* are more frequent than mutants resistant to λh (6), which are in turn more frequent than mutants resistant to λhh* and insensitive to non-

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**Fig. 2.** Formal representation of the in vivo and in vitro interactions between phages λh*, λh, and λhh*, and the wild-type and mutated receptors. Boxes 1, 2, and 3 represent the binding of phages λh*, λh, and λhh*, respectively, to the λ receptor. The arrow represents the relative rate of inactivation of the phage by the receptor. The size of an arrow depends on this relative rate. For relative rates of less than $10^{-4}$, no arrow is represented. Modification of a symbol always results in modification of the corresponding symbols to its left but not necessarily to its right. First column indicates class and mutation number; second column indicates in vivo interactions; third column indicates in vitro interactions.
sense suppressors (C. Braun-Breton, to be published).

Use of dextrins and phage adsorption. Although growth on dextrins is not appreciably affected by the mutations, the initial rate of transport of maltose at low concentration (3.5 × 10^{-6} M) is impaired to various degrees (16). We have shown here that the effect on transport is not due to the amount of lamB protein made. This agrees with the observation that there is no correlation between transport and the amount of lamB protein in the Ah-resistant derivative of strain CR63 (2). However, in this case the mutations affecting the amount of lamB protein were not characterized and some could map outside lamB.

As mentioned in the introduction, class III mutations are usually nonsense mutations and abolish all of the λ receptor activities in the mutant. In particular, they do not allow use of dextrins nor growth of Ahh*. Conversely, class I and II mutations, which are presumably missense mutations, always allow use of dextrin and growth of Ahh*.

Recently we have isolated reversible lamB mutations which confer resistance to Ahh* and are nonsuppressible by nonsense suppressors (C. Braun-Breton, to be published). These mutations prevent use of dextrins. The existence of such a strict correlation between resistance to Ahh* and inability to use dextrins as a carbon source can fit very well with the idea that the regions of the protein involved in vivo in Ahh* activation would overlap tightly with the part of the λ receptor needed for the transfer of dextrins. However, one should recall that mutations preventing the export of the λ receptor would also affect all of its activities in vivo. It has been proposed that the role of the λ receptor in dextrin transport is firstly to form diffusion channels through the outer membrane with the general properties of porins but, in addition, to bind specifically maltodextrins (4, 8, 18). It is of course an intriguing question whether the lamB nutrient pore has any relation to the route of entry of the λ DNA into the cell. A second role of the λ receptor in the transport of dextrins would involve specific interactions with the periplasmic maltose-binding protein (20). Whether the class III missense mutations lead to a complete loss of λ receptor activities by a drastic effect on its structure or on its proper localization in the outer membrane, or both, is a subject for further studies.

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