

Isolation of the Bacteriophage Lambda Receptor from *Escherichia coli*

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Received for publication 9 August 1973

A factor which inactivates the phage lambda can be extracted from *Escherichia coli*. This factor is a protein and is located in the outer membrane of the bacterial envelope. It is found in extracts of strains which are sensitive to phage lambda, but not in extracts of strains specifically resistant to this phage. We conclude that this factor is the lambda receptor, responsible for the specific adsorption of the phage lambda to *E. coli* cells. A partial purification of the lambda receptor is described. Inactivation of the phage by purified receptor is shown to be accompanied by the release of deoxyribonucleic acid from the phage.

The cell envelope of *Escherichia coli* is a complex, multilayered structure composed of the cytoplasmic membrane and the cell wall which itself includes the peptidoglycan layer and the outer or L membrane. Little is known about the morphology or the function of the outer membrane. It was considered by some workers as a penetration barrier and its study was approached through the analysis of mutants with altered antibiotic sensitivity (2, 5, 9, 11, 16). Another approach is the investigation of receptor molecules located on the bacterial surface. Receptors for colicin E (12) and colicin K (12, 21) have been identified as molecules containing protein moieties. Receptors for phage T2 and T6 have also been demonstrated to involve a protein moiety, whereas T3, T4, T7, and C21 adsorb to lipopolysaccharide components of the outer membrane (20).

The isolation of the receptor for phage lambda, reported here, is another example of this type of approach. The purification and characterization of this receptor should yield information on the structure and function of the bacterial cell envelope. Also, it will provide a means to study the mechanism of phage adsorption and other early steps in the phage-host interaction.

The genetics of the lambda receptor is well documented. It has been demonstrated that all mutations specifically impairing adsorption of phage lambda inactivate a single cistron, *lam B* (80.5 min on the genetic map of *E. coli*) (17; and M.

Hofnung, Ph.D. thesis, Paris, 1972). This cistron is in one of the operons involved in maltose metabolism. Therefore, *lam B* can be inactivated either directly by mutations inside the gene, or indirectly by mutations in other genes of the maltose system. About 20% of all resistant mutations map inside *lam B* and yield λ *Mal*⁺ phenotype. The remaining 80% of λ mutants are *Mal*⁻. Most of these have a mutation in *mal T* (66 min), the positive regulator of the maltose system. Polar mutations in *mal K*, a maltose permease gene located in the same operon as *lam B*, also yield a λ *Mal*⁻ phenotype. There are no host range mutants of lambda which infect any λ *Mal*⁻ strain. However, host range mutants can be selected which grow on some λ *Mal*⁺ *lam B* mutants. This may be because λ *Mal*⁻ bacteria contain no *lam B* gene product, whereas some λ *Mal*⁺ mutants may have a modified gene product which allows the adsorption of phage which have a mutant host range. The *lam B* gene may code for an enzyme involved in the synthesis of lambda receptors or it may code for a structural component of the receptor itself. The work presented here utilizes λ -resistant mutants to correlate the receptor with a protein which is solubilized from the outer membrane and which inactivates phage lambda. The partial purification and characterization of this protein is discussed.

MATERIALS AND METHODS

Abbreviations and definitions. Abbreviations used are as follows: PFU, plaque-forming units; MOI, multiplicity of infection; λ r and λ s, resistant and

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sensitive to phage λ , respectively; Mal⁺ and Mal⁻, able and unable to use maltose as a carbon source, respectively; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid; Tris buffer, tris(hydroxymethyl)aminomethane (pH was adjusted with HCl). Room temperature ranged from 22 to 24 C.

QAE-Sephadex, an anionic exchange resin, was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Radioactive isotopes were obtained from Commissariat à l'Énergie Atomique, France. Sodium cholate was purchased from Schuchardt München, Germany.

Media and bacterial strains. The minimal medium (M63) used for growth and the complete medium used for plating phage were described elsewhere (15). Strain HfrG6 (15) is the wild-type λ Mal⁺ strain. Pop 1730 is a spontaneous λ rMal⁻ mutant of HfrG6; it carries the deletion MB Δ 17 covering part of the gene *mal K* and probably part of the gene *lam B* (M. Hofnung, D. Hatfield, and M. Schwartz, J. Bacteriol., in press). The λ rMal⁺ strain CR63 (1) is the standard host for host range mutants of λ . Several other strains are used only in the experiments described in Table 2 and Fig. 2; their genotype is given in the legends.

Phage λ V is a virulent mutant of λ^+ (8). The phage λ Vh, a spontaneous host range mutant of λ V, was isolated in this laboratory by plating λ V on strain CR63. In the text λ V is referred to simply as λ and λ Vh as λ h.

The indicator strain used for plating phage was HfrG6.

Assay of receptor activity. Unless otherwise indicated, inactivation of λ h was assayed in the absence of CHCl₃, as described here. One milliliter of a phage stock containing between 3×10^8 and 6×10^8 PFU in 10^{-2} M MgSO₄ was mixed with 1 ml of cholate-EDTA extract (see below) diluted as given for each experiment in 10^{-2} M Tris buffer (pH 7.5). In the control experiments the extract was replaced by Tris buffer. The mixture was incubated at 37 C. At times as indicated, samples of 0.2 ml were taken and added to tubes, kept at 37 C, containing 10^9 indicator bacteria in 0.1 ml 10^{-2} M MgSO₄. After an incubation of 5 min to allow adsorption, 3 ml of soft agar was added to each tube and the contents were plated.

Assay of receptor activity in the presence of CHCl₃. Purified λ h, and to a lesser extent, purified λ , are sensitive to chloroform. Efficient protection against the inactivation of those phages by chloroform is obtained by adding BSA to the phage suspension. Hence the phage stocks used in these experiments were diluted in 10^{-2} M MgSO₄ containing 200 μ g of BSA per ml. One milliliter of such a phage dilution containing between 3×10^8 and 6×10^8 PFU was mixed with 1 ml of a dilution of cholate-EDTA extract. About 10 drops of CHCl₃ were added, and the mixture was shaken vigorously. At the times indicated 0.2-ml portions were transferred into 10 ml of 10^{-2} M MgSO₄, and 0.1 ml of those dilutions were plated with indicator bacteria.

Extraction of receptor. Two liters of *E. coli* HfrG6 cells were grown at 37 C in minimal medium contain-

ing 0.02% histidine and 0.4% maltose. The bacteria were harvested in exponential phase at an optical density of $1 A_{600}/\text{ml}$ (5×10^8 cells/ml), centrifuged, and suspended in 100 ml of 1.0% cholate, 2×10^{-3} M EDTA, 10^{-2} M Tris buffer, pH 7.5. After shaking 30 min at 37 C, the suspension was centrifuged at 20,000 $\times g$ for 40 min, and then dialyzed versus 2% cholate, 10^{-2} M Tris buffer (pH 7.5). Protein concentration was determined by the method of Lowry et al. (10), using BSA as a standard.

Chloroform-methanol treatment. Two volumes of CHCl₃ and 1 volume of CH₃OH were added per volume of extract, and the mixture was stirred at room temperature for 5 min. The phases were separated by low-speed centrifugation. The precipitate at the interface was carefully removed, suspended in 10 ml of 2% cholate- 10^{-2} M Tris buffer (pH 7.5), and dialyzed versus the same buffer to remove remaining CH₃OH and CHCl₃. The dialysis fluid was then changed to 10^{-2} M Tris (pH 7.5).

Column chromatography. Routinely, 15 ml of extract, at a protein concentration of approximately 3 mg/ml, was applied to a QAE-Sephadex column (30 cm by 1.5 cm) equilibrated with 10^{-2} M Tris buffer (pH 7.5). The column was eluted at 6 ml/h with a 300-ml salt gradient from 0 to 0.8 M NaCl in Tris buffer, followed by a detergent-salt wash (2% cholate, 1.5 M NaCl in 10^{-2} M Tris, pH 7.5). Ninety-seven percent of the protein applied, but no receptor activity, was washed off the column by the salt gradient. All detectable receptor activity was found in the cholate wash. Fractions containing the activity were pooled and dialyzed versus 10^{-2} M Tris buffer (pH 7.5) and then applied to a second QAE-Sephadex column (25 cm by 0.8 cm). Elution was with a 100-ml gradient from 0 to 0.6 M NaCl in 2% cholate, 10^{-2} M Tris buffer (pH 7.5). The fractions containing receptor activity were pooled and dialyzed versus 1% cholate, 10^{-2} M Tris buffer (pH 7.5). The purification is summarized in Table 1.

³H- and ¹⁴C-labeled bacteria. Labeled bacteria

TABLE 1. Purification of the lambda receptor^a

Purification step	Sp act	Recovery (%)
Cholate-EDTA extraction	1	100
CHCl ₃ :CH ₃ OH treatment	1	100
1st QAE-Sephadex column	11	39
2nd QAE-Sephadex column	73	34

^a Conditions for each step in purification are given in detail in Materials and Methods. The specific activity is expressed relative to that observed in the crude cholate-EDTA extract. It is calculated as follows: the rate constant K (s^{-1}) is determined for each fraction (see legend to Fig. 1), multiplied by the appropriate factor to account for differences in volume between the various fractions and the crude extract, and divided by the total protein content of the given fraction (in the purification given here protein content was calculated from ³H-leucine present in each fraction).

were grown in a minimal medium containing 0.4% maltose, 0.4% glycerol, 0.02% histidine, 0.02% Casamino Acids, and either 2.5 mCi of ^3H -leucine per liter (1 Ci/mmol) or 0.25 mCi of ^{14}C -leucine per liter (48 mCi/mmol). Uptake of 75% of both labels had occurred by late exponential phase when the bacteria were harvested. Extraction and purification of the receptor from labeled bacteria were as given above.

Gel electrophoresis. Gel electrophoresis in the presence of SDS was carried out by the procedure of Weber and Osborn (19). Samples were heated to 100 C for 5 min before application to 8-cm-long, 10% acrylamide gels. Electrophoresis was carried out at room temperature (22 C) at 8 mA/gel. Gels were stained in 0.05% Coomassie brilliant blue in CH_3OH - CH_3COOH - H_2O (5:1:5) and destained by soaking in repeated changes of 7.5% CH_3COOH -5% CH_3OH .

Gel slicing. Gels to be eluted for activity studies were run as described above and cut in half longitudinally. One-half was fixed and stained, the other half was cut into 1-mm slices and each slice was put into a tube containing 0.5 ml of 0.1% SDS. After 3 h at room temperature samples of the solution were tested for receptor activity. When analyzed for radioactivity the slices were initially placed in scintillation vials, and after incubation with 0.5 ml 0.1% SDS scintillation fluid was added and the samples were counted.

Scintillation counting. Portions from radioactive columns and gels were counted in 10 ml of Bray solution which contained: 60 g of naphthalene, 4 g of 2,5-diphenyloxazole (PPO), 0.2 g of *p*-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene (POPOP), 100 ml of methanol, and 20 ml of ethylene glycol, brought up to 1 liter with dioxane. Counting was carried out in an Intertechnique scintillation counter.

Preparation of outer membrane. The procedure of Wolf-Watz, Normak, and Bloom was followed (manuscript in preparation). One hundred milliliters of *E. coli* was grown in minimal media, harvested in exponential phase, centrifuged, and suspended in 5 ml of 30% sucrose in 5×10^{-2} M Tris buffer (pH 7.8). Ten milligrams of lysozyme and 0.5 ml of 10^{-1} M EDTA were added to the cell suspension. After a 10-min incubation period at room temperature, 0.5 ml of 2×10^{-1} M MgCl_2 was added and the suspension was then centrifuged at $27,000 \times g$ for 45 min. The pH value of the supernatant was adjusted to 5.0 by the addition of HCl. The outer membrane precipitate which formed was collected by centrifugation at $27,000 \times g$ for 20 min and resuspended in 10^{-2} M Tris buffer (pH 7.5).

The receptor was solubilized from the outer membranes by making the suspension 1% cholate and 2×10^{-3} M EDTA. After incubation at 37 C for 30 min, the suspension was centrifuged at $100,000 \times g$ for 1 h. The supernatant fluid containing solubilized receptor was tested for activity.

RESULTS

Assay of the receptor. We shall operationally define the λ receptor as the molecule, or smallest molecular complex, responsible for the adsorption of phage λ to sensitive bacteria. We

developed an assay by assuming that the receptor extracted from the bacterial membrane would retain its specific affinity for the phage, and that a phage which had reacted with a soluble receptor would be unable to form a plaque on a lawn of bacteria because it would be unable to associate with a receptor present on the surface of a bacterium.

Inactivation of the phage is in fact observed when a cholate-EDTA extract of intact bacteria (see Materials and Methods) is incubated with a host range mutant of phage λ (λh). This extract will also inactivate wild-type lambda but only in the presence of chloroform. This difference will be discussed later (see below).

Inactivation of λh by the extract in the absence of CHCl_3 follows pseudo first-order kinetics (Fig. 1a). The rate of inactivation is directly proportional to the concentration of extract added (Fig. 1b). The inactivation displays an absolute requirement for Mg^{2+} as does adsorption of the phage in vivo. In both cases the optimum is at about 5 mM Mg^{2+} (data not shown).

Correlation of the solubilized activity with the lambda receptor. There is an exact correlation between the sensitivity of a strain of bacteria to λ or λh and the ability of an extract from that strain to inactivate that phage. Extracts from wild-type bacterial strains, which are sensitive to both λ and λh inactivate both phage strains. Extracts from strains resistant to λ and λh do not inactivate either phage. As shown in Table 2 there is no inactivation of either phage by extracts from resistant strains carrying *mal T* mutations, polar *mal K* mutations, or some of the *lam B* mutations, whereas extracts of those *lam B* mutants which are resistant to λ but sensitive to λh inactivate the latter, but not the former (Fig. 2). None of the extracts tested inactivate a hybrid phage $\lambda\text{h y80}$ which has the structural proteins and hence the host range of $\phi 80$.

This correlation of the in vivo phage sensitivity with the in vitro phage inactivation indicates that the inactivation is specific and representative of the lambda receptors synthesized under the direction of the gene *lam B*.

Extraction of receptor from bacteria. The receptor can be released from *E. coli* cells by a variety of treatments. When the bacteria are sonically treated, greater than 90% of the receptor activity remains associated with the membrane fragments sedimenting at high speed ($100,000 \times g$ for 1 h). The addition of detergent is necessary to solubilize the activity. Treatment of whole bacteria with sodium deoxycholate (0.05%) saturated with toluene, or Triton

X-100 (0.5%) plus EDTA (2×10^{-3} M) efficiently releases the receptor activity in a soluble form. The cholate-EDTA extraction method is preferred to those mentioned above because cholate is easier to remove from samples than

is Triton X-100, and it does not precipitate in the presence of salts as does deoxycholate.

Stoichiometric phage receptor interaction.

At sufficiently high phage concentration a 30-min incubation period with receptor fails to

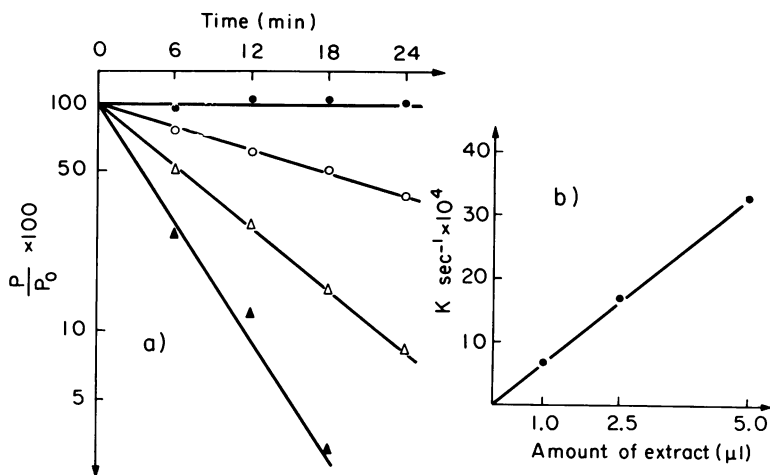


FIG. 1. Inactivation of phage λ_h by an extract of *Escherichia coli*. a, The technique used is described in Materials and Methods. A 1-ml amount of a λ_h dilution in 10^{-2} M $MgSO_4$, containing 3.8×10^8 PFU was incubated at 37 C with 1 ml of a cholate-EDTA extract from HfrG6 diluted 200-fold (\blacktriangle), 400-fold (\triangle) or 1,000-fold (\circ) in 10^{-2} M Tris (pH 7.5). In the control experiment (\bullet) the extract was replaced by Tris buffer. The number of plaques, P , obtained at time, t , is plotted as percent of that (P_0) obtained at time $t = 0$. b, The data in Fig. 1a show that the inactivation follows the equation $P = P_0 e^{-Kt}$. Here, K , calculated from the data in Fig. 1a, is plotted versus the amount of extract in the inactivation incubation (expressed as microliters of the original, undiluted extract present in the final incubation mixture).

TABLE 2. Inactivation of λ and λ_h by extracts of various *E. coli* strains

Strain	Nature of mutation	Phenotype	Protein concn in extract (mg/ml)	In vitro inactivation of phage: ^a	
				λ	λ_h
HfrG6	Wild type	λ_s Mal ⁺	0.84	+	+(0.95)
Pop 1048	Wild type	λ_s Mal ⁺	0.54	+	+(0.89)
Pop 1749 ^b	<i>mal J2</i>	λ_s Mal ⁻	0.70	+	+(0.72)
Pop 1753 ^b	<i>mal J1</i>	λ_s Mal ⁻	0.80	+	+(2.10)
Pop 1760 ^b	<i>mal K</i>	λ_s Mal ⁻	0.88	+	+(1.40)
Pop 1761 ^b	<i>mal K</i> (amber)	λ_s Mal ⁻	0.57	+	+(0.32)
Pop 1729 ^b	Polar deletion in <i>mal K</i>	λ_r Mal ⁻	0.69	-	-(<10 ⁻³)
Pop 1730 ^b	Deletion in <i>mal K</i> and <i>lam B</i>	λ_r Mal ⁻	0.70	-	-(<10 ⁻³)
Pop 1071 ^c	<i>lam B</i>	λ_r Mal ⁺	0.72	-	-(<10 ⁻³)
Pop 1073 ^c	<i>lam B</i>	λ_r Mal ⁺	0.80	-	-(<10 ⁻³)
HfrG6MA Δ 102	Deletion in <i>mal T</i>	λ_r Mal ⁻	0.82	-	-(<10 ⁻³)

^a The (+) indicates inactivation of phage in an in vitro assay done in the presence of $CHCl_3$. The (-) indicates no inactivation. The numbers in parentheses are the rate constants for λ_h inactivation in the absence of $CHCl_3$. They were measured as described in Fig. 1 using dilutions of the extract and multiplying by the dilution factor to obtain the value for K in the undiluted extracts.

^b Single-step mutants of HfrG6, to be described by Hofnung, Hatfield, and Schwartz (J. Bacteriol., in press).

^c Single-step mutants of pop 1048 which is itself a *his⁺ met A lac rif* derivative of HfrG6; they will be described elsewhere (Hofnung, manuscript in preparation). HfrG6MA Δ 102 was obtained by transducing deletion MA Δ 102 (6) into HfrG6.

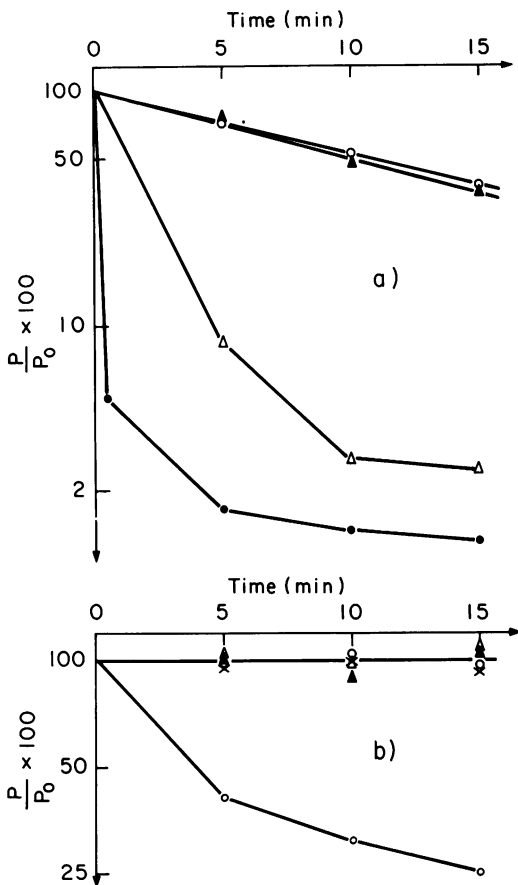


FIG. 2. Inactivation of λ and λ_h by extracts of various strains, in presence of CHCl_3 . The assay for inactivation of λ_h (a) and λ (b) is described in Materials and Methods. Unless otherwise stated, a 100-fold dilution of cholate-EDTA extracts from the following strains was used. Symbols: \bullet , pop 1048 (λ λ_h s wild type); \circ , pop 1071 ($\lambda_r \lambda_h$ lam B mutant of pop 1048); Δ , pop 1068 ($\lambda_r \lambda_h$ s lam B mutant of pop 1048); \times , pop 1068 extract diluted only 10-fold; \blacktriangle , control (buffer instead of extract). In a similar experiment, extracts of CR63, the reference $\lambda_r \lambda_h$ s strain, behaved like extracts of pop 1068. All of the $\lambda_r \lambda_h$ strains listed in Table 2 behaved like pop 1071. Inactivation of λ_h by extracts of pop 1068 or CR63 is also observed in absence of CHCl_3 but it is less complete.

inactivate all the input phage (Fig. 3a). Under these conditions longer incubation time does not increase the extent of inactivation (data not shown). The phage concentration at which the receptor begins to show incomplete inactivation of the phage is proportional to the amount of receptor present in the incubation. These results strongly suggest that the reaction is stoichiometric, rather than catalytic. Therefore, it

is possible to titer the number of available receptor sites in the extract. An experiment similar to that of Fig. 3a can be plotted so that extrapolation to zero phage survival gives a "titration point" representing 5×10^{12} receptor sites per ml (Fig. 3b). This titration may give an underestimate of the concentration of available receptor since the phage stock may contain particles which are unable to form plaques but which retain the ability to interact with the receptor. The titer of the phage stock used, estimated by ultraviolet (UV) absorption, is 7.5 times greater than that estimated by plating; thus the upper estimate of the receptor concentration is 3.7×10^{13} receptors per ml. Assuming no receptor sites are masked due to aggregation, it can be calculated that the extraction proce-

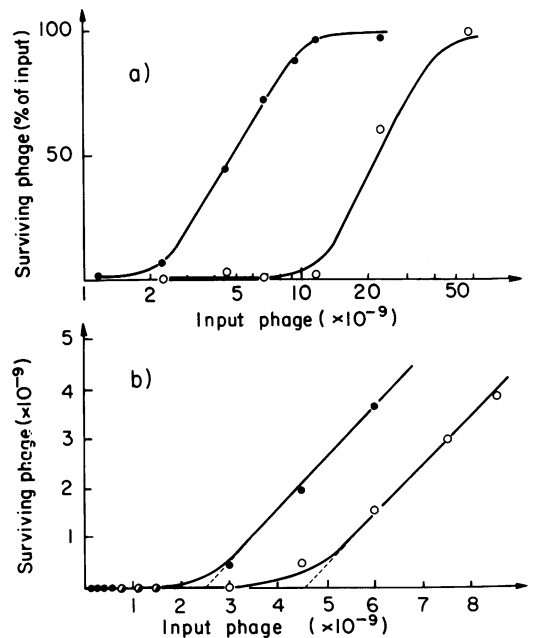


FIG. 3. Titration of λ receptor in extracts of *Escherichia coli*. a, λ_h suspensions (1 ml) containing the number of PFU indicated on the abscissa were incubated with 0.1 ml of a cholate-EDTA extract from HfrG6 (same extract as in Fig. 1) diluted 20-fold (\circ) or 100-fold (\bullet) in 10^{-2} M Tris buffer (pH 7.5). After a 30-min incubation period at 37 C, 0.8 ml of 10^{-2} M MgSO_4 was added and serial dilutions were plated on indicator bacteria. The number of PFU obtained was plotted as percentage of the input PFU. b, Same experiment as above, with a different stock of λ_h and a different cholate-EDTA extract of HfrG6. The extract was diluted 100-fold (\circ) or 200-fold (\bullet). The actual number of surviving PFU is plotted, so that a titration point can be determined by extrapolation to zero phage survival.

dures releases between 500 and 3,700 receptors per bacterium.

Titration of receptor sites in vivo. An attempt was made to estimate the number of receptor sites on the bacterial surface by studying the competition for receptor sites between two strains of phage. As shown in Fig. 4, the rate of adsorption of one phage decreased about eightfold in the presence of the competing phage at an MOI of 10^8 . This result could be taken as evidence that the number of receptor sites is of the order of 10^8 per bacterium. However, as shown in Fig. 5, at a phage MOI of 10^8 , the surface of the cell appears to be completely covered with phage heads. Hence, even if there were more than 10^8 sites on the bacterial surface, the remaining free sites could not be reached by superinfecting phage particles once 10^8 phage had adsorbed. Thus we can conclude that the minimum number of receptor sites per bacterium is 10^8 , a value of the same order of magnitude as the amount of receptor extracted from a single bacterium.

Localization of the receptor in the outer membrane. The use of two independent techniques demonstrates that the receptor is located in the outer membrane. Cytoplasmic membrane proteins are selectively solubilized from cell

envelopes by Triton X-100 in the presence of Mg^{2+} . Re-extraction of the insoluble cell wall fraction with Triton X-100 plus EDTA releases about one-half of the cell wall protein (4, 13, 14). Table 3 shows the distribution of receptor activity after successive extraction of cell envelopes by Triton X-100 plus Mg^{2+} and Triton X-100 plus EDTA. The Triton X-100- Mg^{2+} soluble fraction which should contain the solubilized cytoplasmic membrane has a very low level of receptor activity. Greater than 90% of the detectable receptor activity is found in the cell wall fraction, solubilized by Triton X-100-EDTA. Wolf-Watz et al. (personal communication) have developed a method for isolating outer membrane free of cytoplasmic membrane. *E. coli* cells are plasmolyzed and the outer membrane is released by a lysozyme-EDTA treatment (Materials and Methods). Receptor activity can be solubilized with cholate-EDTA from outer membrane prepared by this method.

When the cell envelope of a lambda-resistant strain is fractionated by either the Triton X-100 or lysozyme-EDTA method, no receptor activity is detected in any of the fractions.

Partial purification. Gel electrophoresis in the presence of SDS of the most purified receptor fraction (see Materials and Methods) reveals three bands. Receptor activity can be eluted only from the slowest migrating band, but attempts to separate the three proteins have been unsuccessful. If this protein band corresponds to the receptor, it should be absent in fractions purified from a λ -resistant mutant. A double-label experiment was conducted to confirm this. Lambda-sensitive bacteria (HfrG6) grown on 3H -Leu were mixed with λ -resistant bacteria (pop 1730) grown on ^{14}C -Leu and the mixture was extracted by the routine cholate-EDTA treatment. The double-labeled extract was purified as given in Materials and Methods. Figure 6 shows the distribution of 3H and ^{14}C eluted from the second QAE-Sephadex column. The fractions containing the receptor activity are enriched for 3H -Leu. Again, gel electrophoresis in SDS of the fraction containing the receptor activity reveals three bands (Fig. 7). The slowest migrating band, the only band from which receptor activity can be eluted, contains only 3H -Leu. Thus it corresponds to a protein which is synthesized by the wild-type strain and not by the λ -resistant mutant. The fastest migrating band, which contains about 50% of the 3H label, also contains ^{14}C and therefore represents a contaminant. The middle band, quite unexpectedly, contains only 3H . The λ -resistant strain (pop 1730) used in this experiment carries a deletion

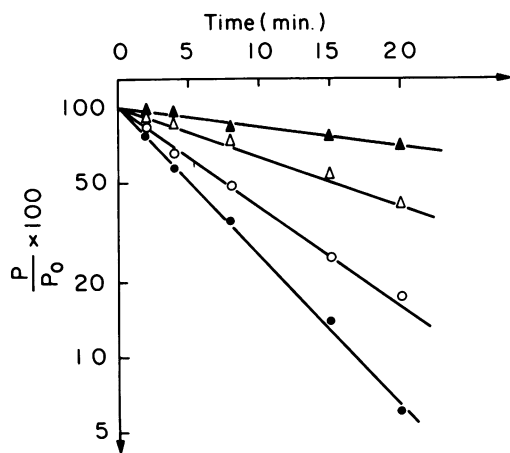


FIG. 4. Competition between two strains of phage λ for adsorption on *E. coli*. Sensitive bacteria (HfrG6) (2.5×10^7) were preincubated for 5 min at 37°C in a volume of 0.5 ml λCI_{57} at a MOI of 0 (●), 320 (○), 640 (△) or 960 (▲); 1.5 ml of a λV suspension was then added at an MOI of 1. At the indicated time intervals, 0.1-ml samples were diluted into 10 ml of 10^{-2} M $MgSO_4$ saturated with $CHCl_3$. Further dilutions were in 10^{-2} M $MgSO_4$. The diluted phage mixture was plated with an indicator bacterial strain lysogenic for λ [C600(λ)] (λV forms plaques on this strain and λCI_{57} does not). The percentage of unadsorbed λV is plotted against the time of incubation.

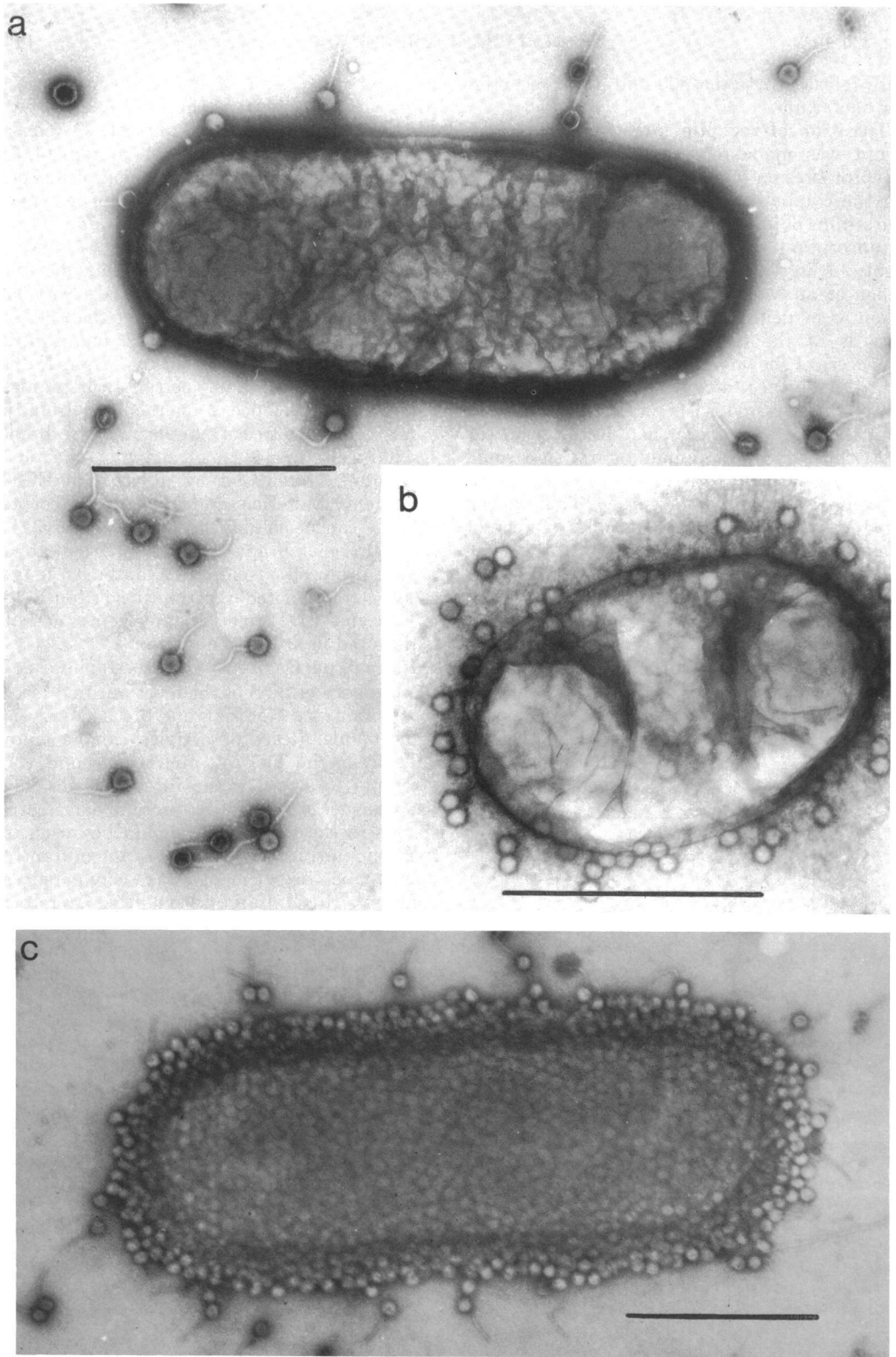


FIG. 5. Adsorption of phage λ to *E. coli* cells. Phage λ at the indicated MOI and bacteria (2×10^8 cells/ml) were incubated for 5 min at 37 C in 100 μ liters of 10^{-2} M $MgSO_4$. The mixtures were then transferred to 4 C and immediately prepared for negative staining and observed under the electron microscope. a, Pop 1730 (λ resistant) cells infected at MOI of 1,000. b, HfrG6 (wild-type) cells infected at MOI of 100. c, HfrG6 cells infected at MOI of 1,000. The scale markers represent 1 μ m.

which extends into *mal K*. Therefore, this ^3H -labeled protein may correspond to the *mal K* gene product. Alternatively, it may be an inactive form of the lambda receptor.

Receptor protein moiety. Incubation of the partially purified receptor with trypsin results in a loss of the receptor activity (Fig. 8). A similar loss of activity is obtained by treatment with either papain or Pronase, thus indicating that a protein moiety is essential for the λ receptor activity. We have not yet determined if lipid or carbohydrate moieties are involved. The receptor activity in the crude extract is completely resistant to inactivation by trypsin. In addition, the crude extract loses no receptor activity after boiling in SDS for 5 min, but the purified receptor is sensitive to the same treatment.

Mechanism of phage inactivation in vitro. The receptor inactivates the phage by caus-

ing it to release its DNA. Ultracentrifugation studies (Fig. 9) demonstrate that in the case of λh the DNA is released when the phage is incubated with the receptor, whereas the release of DNA from λ requires the addition of chloroform and the receptor simultaneously. Figure 9a demonstrates the effect of incubating λh with purified receptor. In addition to some unidentified nonsedimenting material, two UV-absorbing species are detected. One, which exhibits the fast sedimentation of intact phage particles, disappears after incubation with purified receptor. The other species, which sediments more slowly, is increased after incubation with receptor. This second component is converted to nonsedimenting material by treatment with DNase (Fig. 9b). Thus, it represents DNA released from the phage by treatment with the receptor. Presence of some free DNA in untreated λh stocks was always observed.

As mentioned previously, incubation of wild-type λ with receptor does not inactivate the phage unless chloroform is added simultaneously. This observation is confirmed by the results in Fig. 9c and d which demonstrate that the receptor fails to trigger the release of DNA from λ unless chloroform is added. Addition of chloroform alone causes some DNA to be released (Fig. 9d). This is consistent with the observation that chloroform partially inactivates purified λ phage unless BSA is added to protect the phage (Materials and Methods).

DISCUSSION

A factor which specifically inactivates phage λ can be extracted from the cell envelope of

TABLE 3. Localization of the lambda receptor^a

Fraction	Receptor activity	
	HfrG6 (λs)	Pop 1730 (λr)
Triton X-100-Mg ²⁺ soluble	0.1	<10 ⁻⁴
Triton X-100-EDTA soluble	9.4	<10 ⁻⁴
Outer membrane preparation	1.1	<10 ⁻⁴

^a The Triton X-100 fractions were prepared by the method of Schnaitman (13, 14). Fractions were diluted in 10⁻² M Tris buffer (pH 7.5) and assayed for inactivation of λh as described in Materials and Methods. Receptor activity is expressed as K (s⁻¹) in undiluted extracts (see Fig. 1 for method of calculation).

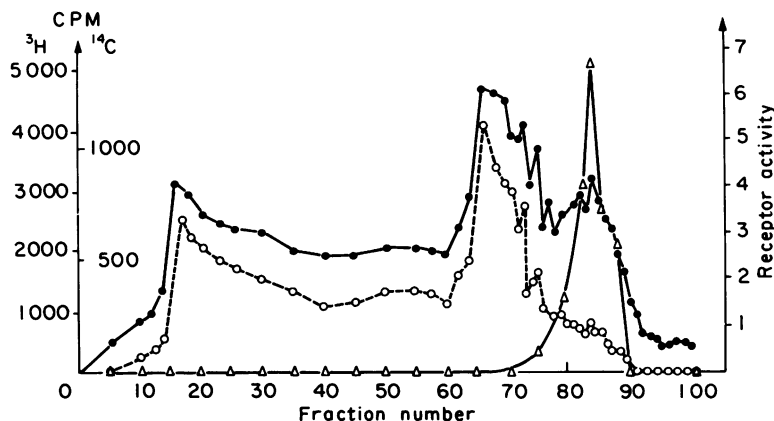


FIG. 6. Distribution of ^3H - and ^{14}C -labeled protein eluted from the second QAE-Sephadex. The column chromatography is described in Materials and Methods. The gradient applied was 100 ml from 0 to 0.6 M NaCl in 2% cholate, 10⁻² M Tris buffer (pH 7.5). Fraction volume was 1 ml. Symbols: ●, counts of ^3H -leucine per min, HfrG6 (wild type, λ sensitive); ○, counts of ^{14}C -leucine per min, pop 1730 (λ -resistant mutant); Δ, receptor activity as assayed by inactivation of λh . Activity is expressed in units of K (s⁻¹) and was determined for each fraction as given in legend of Fig. 1.

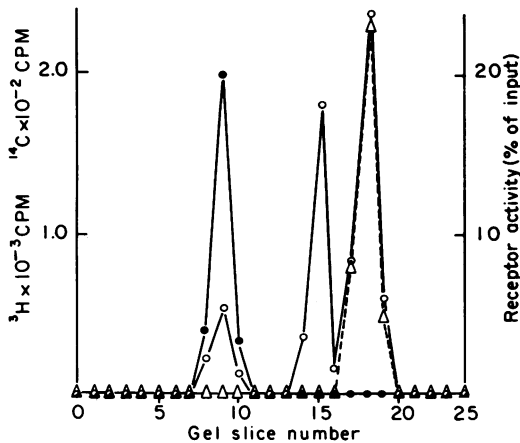


Fig. 7. Gel electrophoresis of purified radioactive λ receptor. Fractions containing receptor activity from QAE-Sephadex column shown in Fig. 6 (fractions 78 to 85) were pooled, and the distribution of radioactive protein was examined by gel electrophoresis (Materials and Methods). Only the first 25 of 80 slices are shown here, no radioactivity or receptor activity was detectable in slices 26 to 80. Receptor activity was determined by inactivation of phage λh and is expressed as percentage of recovery of total activity applied to the gel. Symbols: O, counts of ^3H -leucine per min, HfrG6 (wild type, λ sensitive); Δ , counts of ^{14}C -leucine per min, pop 1730 (λ -resistant mutant); \bullet , receptor activity.

λ -sensitive strains of *E. coli*. This factor is absent in extracts of λ -resistant bacteria which have an inactive *lam B* gene. We conclude that this factor is the receptor for the phage λ . The partially purified receptor contains a protein moiety essential for its activity. The receptor must be purified to homogeneity before we can determine if lipid or carbohydrate moieties are involved, but at this point we shall refer to the receptor as a "protein." The interaction of the receptor protein and the phage is stoichiometric.

The lambda receptor is located in the outer membrane and exhibits hydrophobic properties which facilitate its purification. Soluble proteins can be washed off an ion exchange column with salt, but detergent is required to elute the receptor protein. Other unusual characteristics of the receptor protein may be related to its hydrophobic nature. For example, its resistance in crude extract both to boiling in SDS and to digestion by proteases may be due to aggregation or to association with lipids.

Mutants of *E. coli* have been isolated which are simultaneously resistant to several phages, including lambda. Since these mutations map

outside gene *lam B* (3, 7, 16), it is possible that they alter components in the membrane which interact with the receptor protein. Tamaki et al. (16) have shown that one multiple-resistant mutant is deficient in lipopolysaccharide. Investigation of the relationship of lipopolysaccharide and the receptor protein is underway.

After incubation in vitro with the most purified preparation of receptor, the phage lambda loses its ability to infect *E. coli* and loses its DNA. Since the preparation used in this experiment was not purified to homogeneity, caution must be exercised in positively attributing both effects to a single polypeptide species. If we assume that the in vitro inactivation of the phage is the result of the loss of the DNA, we can conclude that only one protein, the receptor, is involved since we know that the protein eluted from one band on an SDS gel of the purified preparation inactivates the phage. However, DNA-release experiments have not yet been done using this single protein species, and therefore we cannot be certain that the receptor alone causes the in vitro release of DNA from the phage.

Further investigation is necessary to determine if the DNA release observed in vitro reflects an in vivo phenomenon. The requirement for chloroform as well as the receptor to trigger the release of DNA from wild-type lambda may indicate that membrane components in addition to the receptor are involved when the phage ejects its DNA after adsorption on the bacterium. The in vitro release of DNA

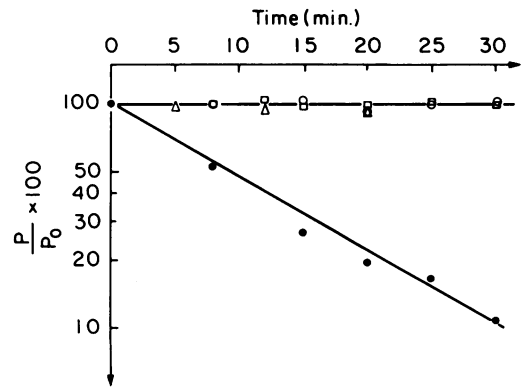


Fig. 8. Treatment of receptor with proteases. The partially purified receptor was incubated at 37 C for 30 min in 10^{-2} M Tris buffer (pH 7.5) in the presence of: 50 μg of trypsin per ml (O); 5 μg of trypsin per ml (Δ); 100 μg of papain per ml (\square); buffer only (\bullet). The mixtures were then diluted 2×10^8 -fold in 10^{-2} M Tris buffer (pH 7.5) and assayed for inactivation of λh as described in Materials and Methods.

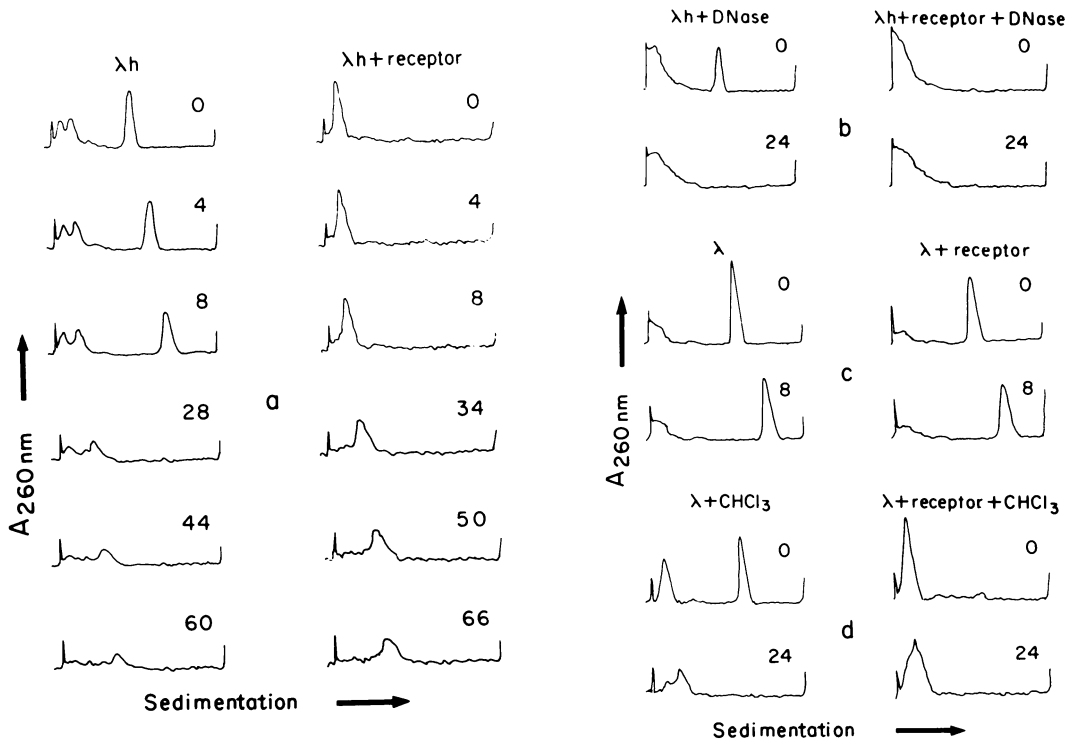


FIG. 9. Ultracentrifugation analysis of receptor-treated phage. Band centrifugation (18) was done at room temperature in a model E Beckman ultracentrifuge at 18,000 rpm. During the rotor acceleration, a 20- μ liter sample was layered at the surface of a CsCl solution ($\rho = 1.3$). UV-absorbing material was followed by scanning at 260 nm. The first tracing shown for each centrifugation was recorded about 15 min after the rotor had reached its maximum velocity. The number above each tracing indicates time (in min) after the initial tracing. The λh stock used had an A_{260} of 2.0 and contained 2×10^{11} PFU/ml. The λ stock had an A_{260} of 7.2 and contained 1.2×10^{12} PFU per ml. The purified receptor was that from double label preparation described in the text. The samples (20 μ liter) used were taken after incubation at 37 C for 15 min in mixtures of the following composition. a, Left: 50 μ liters of λh , 10 μ liters of 10^{-2} M $MgSO_4$, 10^{-2} M Tris buffer (pH 7.5) (TM buffer). Right: 50 μ liters of λh , 10 μ liters of receptor (in TM buffer). The PFU survival was 3%. b, Left: 50 μ liters of λh , 10 μ liters of TM buffer, 2 μ liters of deoxyribonuclease (DNase) (1 mg/ml). Right: 50 μ liters of λh , 10 μ liters of receptor, 2 μ liters of DNase (1 mg/ml). c, Left: 15 μ liters of λ , 45 μ liters of TM buffer; Right: 15 μ liters of λ , 35 μ liters of TM buffer, 10 μ liters of receptor. The PFU survival was 80%. d, Left: 15 μ liters of λ , 45 μ liters of TM buffer, 10 μ liters of $CHCl_3$. The PFU survival was 60%. Right: 15 μ liters of λ , 35 μ liters of TM buffer, 10 μ liters of receptor, 10 μ liters of $CHCl_3$. The PFU survival was 7%.

from λh by the receptor alone could be explained if the host range mutation were to make the phage less stable and thus unable to retain its DNA upon interaction with the receptor protein. In fact, purified λh stocks have been observed to spontaneously lose plaque-forming units more rapidly than do purified λ stocks.

ACKNOWLEDGMENTS

We thank Alex Fritsch for his guidance in the ultracentrifugation experiments, Maurice Hofnung for some of the strains and useful suggestions, Madeleine Jolit and Celestine Derval for their skillful technical assistance, and Antoinette Ryter for the electron microscopy.

This investigation was supported by grants from the National Institutes for Health, the Centre National de la Recherche Scientifique, the Délégation Générale à la Re-

cherche Scientifique et Technique, and the Commissariat à l'Énergie Atomique. L. R. H. thanks the "Fondation pour la Recherche Médicale Française" and "Fondation Philippe" for granting her a fellowship during the course of this work.

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