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malB Region in *Escherichia coli* K-12: Specialized Transducing Bacteriophages and First Restriction Map

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By starting from an *Escherichia coli* K-12 strain with a λ phage integrated in the *malB* region, series of transducing phages carrying part or all of the *malB* region have been isolated. Genetic mapping of the transduced *malB* fragments was accomplished by complementation and recombination with known mutations in the region. By using the DNA of these phages, it was found that the *malB* region is cleaved by the restriction enzymes *Bgl*II, *Eco*RI, *Hae*II, *Hinc*II, *Sal*I, and *Sst*I, but not *Bam*HI, *Hind*III, *Kpn*I, *Pst*I, *Xba*I, or *Xho*I. A physical map was constructed and tentatively correlated with the genetic map.

The *malB* region in *Escherichia coli* K-12 has the following three noteworthy properties (5, 7, 8, 15). (i) It codes for the four proteins known to be involved in maltose transport. At least two—and probably all—of these proteins are located in the cell envelope. (ii) Its expression is regulated by a positive control mechanism. (iii) It is composed of two divergent operons.

To know more about the structure of this region, its mode of expression, and the genetic determinants involved in the cellular localization of its products, we have isolated a series of λ transducing phages carrying all or various parts of the *malB* region. The densities of the phages were determined by analytic ultracentrifugation. Characterization of the incorporated *malB* fragments was done by complementation and recombination and by analysis with several restriction enzymes. This allowed construction of a restriction map of the *malB* region. This restriction map should permit the identification, isolation, and study of DNA fragments playing a critical role in the region. It should be possible to fuse such fragments in vitro to other genes and promoters.

MATERIALS AND METHODS

Strains, bacteriological techniques and media. (i) Bacterial strains. Bacterial strains are listed in Table 1. RW597(λa)N was obtained from R. Weisberg. It is a derivative of RW592 in which several copies of λcI857*b*515*b*519 *xisam*6 *Sam*7 (called here λa) are integrated in the *malB* region. RW597(λa)1 is a monolysogen derived from RW597(λa)N by heat shock. The other strains are from the laboratory collection. The construction of the strains for this study is described below.

Strains pop230 and pop260 are *rif metA* transductants of M72su3(P2) and C600 obtained with a P1 stock grown on RCB6. pop240 is a *trp*$^+$ *supF* derivative of pop815 obtained with a P1 stock grown on QD5003. pop251 is a *gal*$^+$ *att*$^+$ *bio*$^+$ transductant of RW597(λa)1 obtained with a P1 stock grown on QD5003. pop252 is a thermoresistant, λ-sensitive derivative of pop251. pop253 is a λa lysogen derivative of pop252.

Three series of *malB* strains were constructed by using P1 stocks grown on pop1743 (*malF1*), pop1754 (*malE12*), pop1758 (*malE16*), pop1760 (*malK1*), and pop1762 (*malK5*) and looking for *metA*$^+$ *malB* transductants. pop231 to pop235 are derivatives of pop230; they were used to isolate *spi* plaque-forming phages carrying *malB* markers. pop241 to pop245 are derivatives of pop240; they were used to isolate plaque-forming phages carrying *malB* markers. pop261 to pop265 are derivatives of strain pop260; they were used for experiments in which no suppression of the *Sam*7 mutations of λa was wanted.

(ii) Phages—strains. λcI857*b*515*b*519 *xisam*6 *Sam*7 (called λa in this study) and λch80 (λ*int att*) were obtained from R. Weisberg. λ*bio*11 was obtained from F. Bregegere. The other strains used, λ*b*2*vh*$^+$, λcI57, λcI857*B*7*N*53*h*80, φ80*VII*, and P1607H, are from the laboratory collection.

(iii) Standard media and methods for bacterial genetics. Standard media and methods for bacterial genetics were described by Miller (8).

MS-mal is a minimal medium containing maltose as well as eosin and methylene blue (5). Mcc-mal is the classical MacConkey medium with maltose instead of lactose. Supplements were added as needed and are not indicated in the text.

Selections for *malB* transducing phages. Low-frequency transducing (LFT) lysates were prepared by heat induction of strain RW597(λa)1. Four different techniques were used to isolate transducing phages from the LFT. The number of phages from each selection retained for further studies is indicated in the four following paragraphs. The names of the phages presented in this paper are indicated in parentheses. In the rest of the paper, these phages are referred to by their full name or by a simplified nomenclature: *malB* followed by a number, 1 to 16.

(i) Selection for *malE16*$^+$ transducing parti-

TABLE 1. *Bacterial strains*

Strain	Relevant genetic characteristic	Origin or reference
Hfr strains		
Type HfrC		
RW592	λ(*gal attλ bio*)	R. Weisberg
RW597(λa)N	λ(*gal attλ bio*) *malF*::(λa)N	R. Weisberg
RW597(λa)1	λ(*gal attλ bio*) *malF*::(λa)1	This paper
pop251	*malF*::(λa)1	This paper
pop252	Δ*malF*	This paper
pop253	(λa) Δ*malF*	This paper
Type HfrG6		
pop1743	*his malF1*	(5)
pop1754	*his malE12*	(5)
pop1758	*his malE16*	(5)
pop1760	*his malK1*	(5)
pop1747	*his malK10*	(5)
pop1761	*his malK3*(Am)	(5)
pop1762	*his malK5*	(5)
pop1728	*his malBΔ15*	(5)
pop1715	*his malBΔ1*	(5)
pop1718	*his malBΔ5*	(5)
pop1721	*his malBΔ8*	(5)
pop1723	*his malBΔ10*	(5)
pop1725	*his malBΔ12*	(5)
F⁻ strains		
QD5003	*pro supF*	Laboratory collection
C600	*lacY1 thr leu supE tonA*	Laboratory collection
pop971	*argH rif gal sup*⁺	C. Braun-Breton[b]
M72*sup*3(P2)	*lac*(Am) *trp*(Am) *lys*(P2) *supF str*	P. Kourilsky
RCB6	*argH metA his rif str malB R*	C. Babinet
pop815	*argH metA his trpE*9851 *sup*⁺*lac gal rif*	(5)
pop230	*supF*(P2) *rif str lac*(Am) *trp*(Am) *metA*	This paper
pop231	*supF*(P2) *rif str lac*(Am) *trp*(Am) *malF1*	Derivative of pop230
pop232	*supF*(P2) *rif str lac*(Am) *trp*(Am) *malE12*	Derivative of pop230
pop233	*supF*(P2) *rif str lac*(Am) *trp*(Am) *malE16*	Derivative of pop230
pop234	*supF*(P2) *rif str lac*(Am) *trp*(Am) *malK1*	Derivative of pop230
pop235	*supF*(P2) *rif str lac*(Am) *trp*(Am) *malK5*	Derivative of pop230
pop240	*supF argH his thy lac gal metA*	This paper
pop241	*supF argH his thy lac gal malF1*	Derivative of pop240
pop242	*supF argH his thy lac gal malE12*	Derivative of pop240
pop243	*supF argH his thy lac gal malE16*	Derivative of pop240
pop244	*supF argH his thy lac gal malK1*	Derivative of pop240
pop245	*supF argH his thy lac gal malK5*	Derivative of pop240
pop260	*supE thr leu lacY1 tonA rif metA*	This paper
pop261	*supE thr leu lacY1 tonA rif malF1*	Derivative of pop260
pop262	*supE thr leu lacY1 tonA rif malE12*	Derivative of pop260
pop263	*supE thr leu lacY1 tonA rif malE16*	Derivative of pop260
pop264	*supE thr leu lacY1 tonA rif malK1*	Derivative of pop260
pop265	*supE thr leu lacY1 tonA rif malK5*	Derivative of pop260

[a] All strains require thiamine. Derivation of the strains constructed for the present study is described in the text.

[b] Braun-Breton, 3rd-cycle thesis, Université Paris, Paris.

cles. Drops of LFT lysates were spotted together with drops of a λa stock on a lawn of pop1758 (*malE16*) and spread on MS-mal plates. Six independent Mal⁺ transductants producing high-frequency transducing (HFT) lysates were studied (λad*malB1*, λad*malB2*, λad*malB3*).

(ii) Selection for *malE16*⁺ and *malF1*⁺ plaque-forming transducers. The LFT lysates were plated with pop243 (*malE16*) and pop241 (*malF1*) on Mcc-mal medium and incubated at 30°C for 48 h. Fifteen lysogens, ten from pop243 (*malE16*) and five from pop241 (*malF1*), producing HFT lysates were isolated from red plaques (λap*malB5*, λap*malB6*, λap*malB7*).

(iii) Selection for *malK1*⁺ plaque-forming transducers of various densities. An LFT lysate was submitted to centrifugation in cesium chloride

(initial density = 1.5) for 60 h at 30,000 × *g* (rotor, SW50L) and 10°C. Fractions were collected and dilutions were plated with strain pop244 (*malK1*) on Mcc-mal at 30°C for 48 h. Nineteen clones of lysogens producing HFT lysates were isolated from white plaques with red papillae (λap*malB8*, λap*malB9*, λap*malB10*) and red plaques (λap*malB4*) (see Fig. 3).

(iv) Selection for *spi* plaque-forming transducers. Plaques from the gradient fractions (see above) were also recovered on strain M72su3 (P2) on tryptone plates. Eleven phages stocks were prepared from the plaques and tested for Mal$^+$ transduction with pop231 (*malF1*) on Mcc-mal plates (λap*malB11*).

All lysogens were tested for thermosensitivity at 42°C, immunity to λ, and presence of the *Sam7* mutation on the phages.

Genetic definition of the *malB* fragments carried by the phages. The portions of *malE*, *malF*, and *malK* carried by the phages were determined by looking for recombination and complementation with known point mutations in those genes. This was done by spot tests with the various lysates on MS-mal (seeded with indicator *malB*λs bacteria).

The portions of *lamB* carried were determined by looking for recombination and complementation with known deletions cutting into *lamB*. This was done in three steps. First, lysates of the transducing phages were prepared by using λ*c*I857*N7N53h80* as a helper; this resulted in phenotypic mixing and production of *h80* transducing particles able to infect bacterial mutants with deletions entering *malK* and *lamB* (Mal$^-$ λr). Mal$^+$ transductants of such strains were then tested for sensitivity and immunity to λ. When all the transductants tested were λs it was considered that an intact *lamB* gene was present on the phage. When no λs transductant was found (out of 10 Mal$^+$ tested), a further step designed to look for recombinants was performed by using the fact that the *lamB* product is needed for dextrin utilization (Dextrin$^+$ phenotype). Cultures of the Mal$^+$λr lysogens were grown in minimal medium with maltose as a carbon source. Then a positive selection for Dextrin$^+$ recombinants was imposed (C. Wandersmann and M. Schwartz, unpublished data). All the Dextrin$^+$ recombinants found were λs. It was estimated that a frequency of recombinants greater than 10^{-4} could be detected by this method.

Selection of recombinant phages carrying an intact *malF* gene. Phages carrying an intact *malF* gene were constructed by genetic crosses between a phage carrying the distal portion of *malF* and various phages carrying the proximal portion of *malF*; the formation of such recombinants involves the *int xis*-mediated recombination pathway of λ (11, 12). Derivatives of pop263 (*malE16*) lysogenic for λad*malB* or λap*malB* phages were infected by phage λap*malB11* (multiplicity of infection ≃ 3). Lysates obtained by thermoinduction of these bacteria lysogenic for two different phages were spotted on pop253 (λa, Δ*malF*). Each parental phage being unable to complement or recombine with pop253 (λa, Δ*malF*), the selection for Mal$^+$ is a selection for an intact *malF* gene. Mal$^+$ colonies were isolated and tested for production of HFT on pop253 (λa, Δ*malF*) (λap*malB12*, λap*malB13*, λap*malB14*, λap*malB15*, λap*malB16*).

Determination of the density of *malB* transducing phages. Lysates of the various phages were obtained by heat induction of lysogenic derivatives of pop263 (*malE16*). Phages were purified by two consecutive cesium chloride gradients (block gradient first and continuous gradient second) (8). Differences in density between the λa helper and the *malB* transducing phages were determined in the analytic ultracentrifuge for 20 h at 34,000 rpm in CsCl (initial density = 1.5) at a temperature of 25°C (4).

Hydrolysis with restriction enzymes. (i) Enzymes. *Hin*cII, *Eco*RI, *Bam*HI, *Hin*dIII, and *Pst*I were purchased from Bethesda Research Laboratories, Inc.; *Hae*II, *Xho*I, *Xba*I, *Sal*I, *Kpn*I, and *Bgl*II were from New England Biolabs. *Sst*I was kindly provided by Alain Rambach and Pierre Tiollais.

(ii) Reactions. All reactions except those with *Pst*I were 10 nM in 2-mercaptoethanol. Reactions containing 2 μg of DNA in 25-μl volumes were incubated at 37°C overnight in microcentrifuge tubes. The buffers used were as follows: *Eco*RI, 0.01 M tris(hydroxymethyl)aminomethane(Tris)-hydrochloride (pH 7.2)–0.05 M NaCl–0.005 M $MgCl_2$; *Bam*HI, 0.002 M Tris-hydrochloride (pH 7.5)–0.007 M $MgCl_2$; *Hae*II, 0.006 M Tris-hydrochloride (pH 7.5)–0.006 M $MgCl_2$; *Hin*cII, 0.01 M Tris-hydrochloride (pH 7.9)–0.006 M $MgCl_2$–0.06 M NaCl; *Hin*dIII, 0.02 M Tris-hydrochloride (pH 7.4)–0.007 M $MgCl_2$–0.06 M NaCl; *Pst*I, 0.02 M Tris-hydrochloride (pH 7.5)–0.01 M $MgCl_2$–0.05 M $(NH_4)_2SO_4$–100 μg of gelatin per ml; *Sal*I, 0.006 M Tris-hydrochloride (pH 7.9)–0.006 M $MgCl_2$–0.1 M NaCl; *Xba*I, 0.006 M Tris-hydrochloride (pH 7.9)–0.006 M $MgCl_2$–0.15 M NaCl; *Xho*I, 0.006 M Tris-hydrochloride (pH 7.4)–0.006 M $MgCl_2$–0.15 M NaCl; *Kpn*I, 0.006 M Tris-hydrochloride (pH 7.5)–0.006 M $MgCl_2$–0.05 M NaCl; *Sst*I, 0.006 M Tris-hydrochloride (pH 7.5)–0.006 M $MgCl_2$–0.1 M NaCl; *Bgl*II, 0.002 M Tris-hydrochloride (pH 7.4)–0.01 M $MgCl_2$–0.006 M KCl.

Agarose vertical slab gel electrophoresis was carried out on agarose gels (Sigma type III; 15 by 14.5 cm by 3 mm) in 0.04 M Tris-acetate (pH 8.0)–0.02 M sodium acetate.

RESULTS

This section is composed of two parts: one presents the isolation and characterization of the *malB* transducing phages, the other one the establishment of the restriction map of the *malB* region.

(i) Isolation and characterization of the *malB* transducing phages. It was first confirmed that in the lysogen from which the transducing phages were isolated [RW597 (λa)1], the phage is indeed integrated in the *malB* region; reversion to Mal$^+$ coincided with the loss of immunity, and complementation to Mal$^+$ could be obtained with an episome carrying the *malB* region, but not with an episome carrying the *malA* region.

***malB* genes carried by the transducing phages.** Four methods were used to isolate transducing phages (see above). The structure

of the different phages can be explained in terms of the excision events which are represented in Fig. 1. The location of the prophage within *malF* and its orientation, namely *malF'-A-J-cI-exo-mal'F-malE-malK-lamB*, are the only ones compatible with those excision events.

The properties of the phages are summarized in Fig. 2. The phages selected by the first method (λad*malB1*, λad*malB2*, λad*malB3*) are defective and carry *malE*, *malK*, *lamB*, and the proximal part of *malF*. The phages selected by the second method (λap*malB5*, λap*malB6*, λap*malB7*) carry *malE*, *malK*, and the proximal parts of *malF* and of *lamB*. With the third method (λap*malB8*, λap*malB9*, λap*malB10*), two peaks could be distinguished in the gradient (Fig. 3). Phages from the peak corresponding to high densities (peak H) carry *malE*, the proximal part of *malK*, and both the proximal and the distal parts of *malF*. Phages from the light peak (peak L) carry the proximal parts of *malF* and *lamB*, *malE*, and *malK*; one of them (λap*malB4*) carries in addition the distal part of *malF*. The fourth method yielded phages carrying the distal part of *malF* (λap*malB11*).

End points within the *malB* genes. The phages which have been chosen from the three first methods of selection carry various lengths of the *malK lamB* operon. The first three phages λad*malB1*, λad*malB2*, and λad*malB3* carry the whole operon and are defective. The end points of λap*malB4* to λap*malB7* within *lamB* were determined by looking for recombination with deletions entering *lamB* (C. Braun-Breton, 3rd-cycle thesis, Université Paris, Paris, France). The end points of λap*malB8* to λap*malB10* within *malK* were determined by looking for recombination with point mutants in *malK* (Fig. 2). For unknown reasons, λap*malB10* which was isolated as *malK1*$^+$ did not give Mal$^+$ recombi-

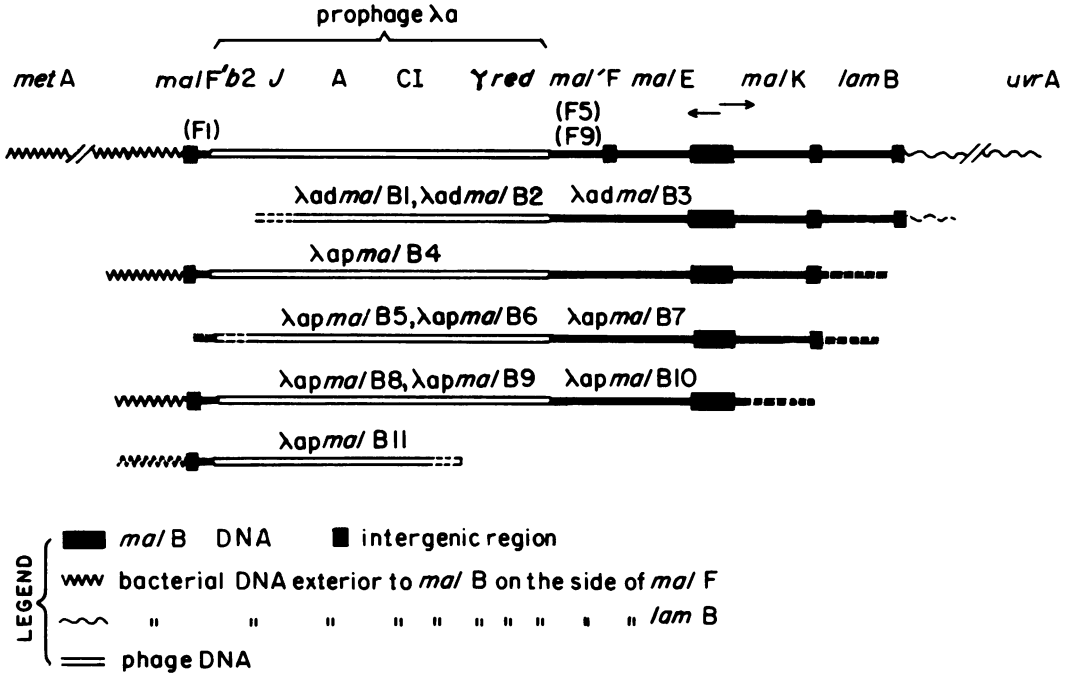

FIG. 1. *Location and orientation of prophage λa in the malB region. The malB region comprises two operons which have opposite polarities and might overlap in their promoters and/or control regions. The expression of both operons is activated in the presence of maltose by the product of gene malT located in the malA region (1, 6, 10). The malE product is a periplasmic maltose binding protein (7). The lamB product is the so called λ receptor, an outer membrane protein (9). The products of the two other genes (malF and malK) are unknown but are most probably envelope proteins (5, 13, 14). The prophage is inserted between the mutations malF1 on one side and malF5 and malF9 on the other side. Complementation data (unpublished) show that the insertion event results in the inactivation of the second cistron but not of the first cistron in malF (5). This has been confirmed by deletion mapping of the location of the inserted prophage (T. Silhavy, personal communication). The types of excision events which were most probably at the origins of the 11 transducing phages presented here have been represented by drawing the regions carried by the various phages. Symbols:* ▬, *malB DNA;* ■, *intergenic region;* ∿∿, *bacterial DNA exterior to malB on the side of malF;* ~~, *bacterial DNA exterior to malB on the side of lamB;* =, *phage DNA.*

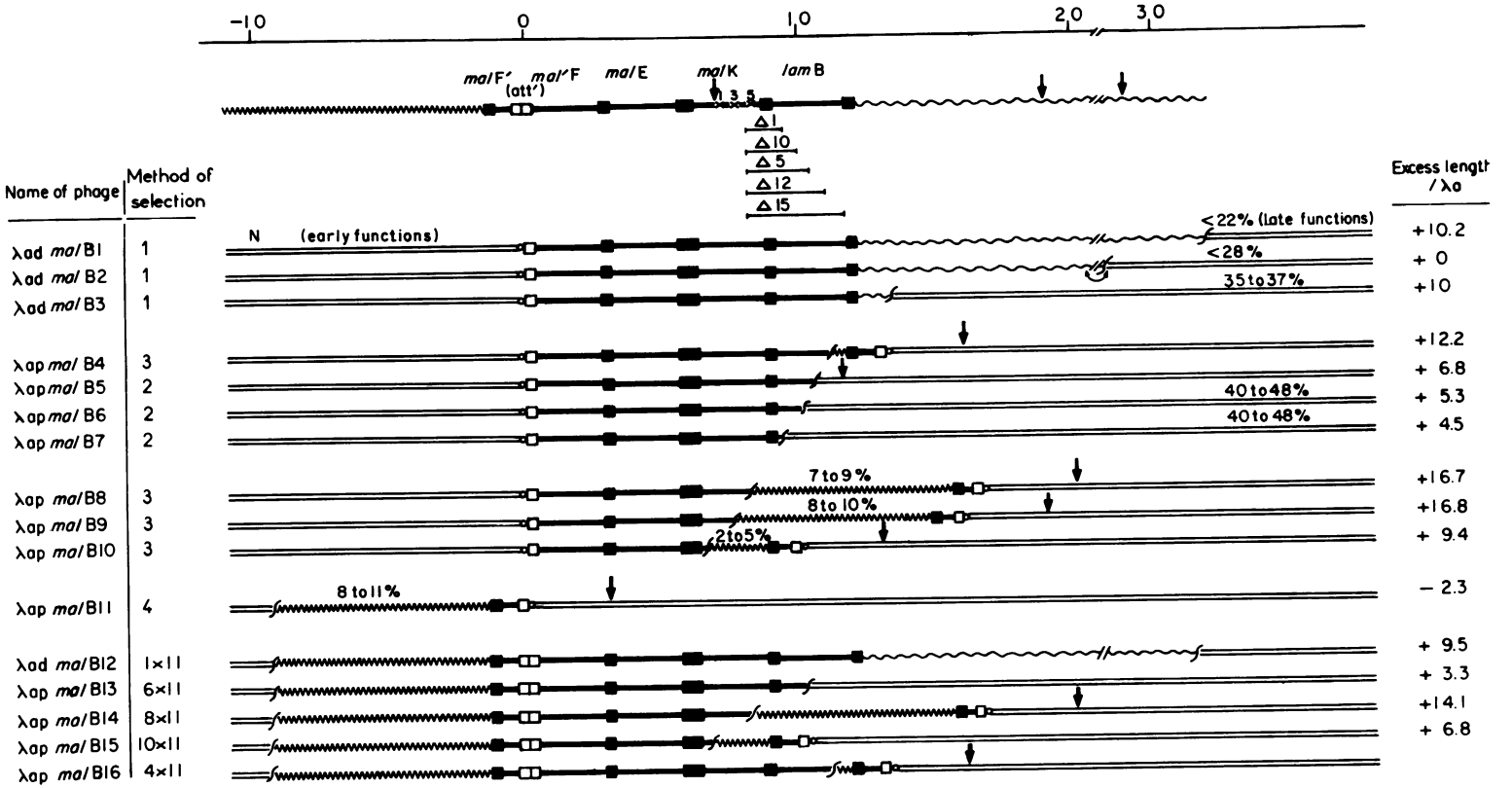

FIG. 2. *Genetic determination of the malB fragments carried by the transducing phages. The transducing phages are compared with a simplified map of the malB region (6; Braun-Breton, 3rd-cycle thesis). The map is drawn at an approximate scale graduated in percentage of λ DNA length (top of figure). For this, it was estimated arbitrarily that the length of each malB gene was 1.5 to 2% of λ. The origin is the site of insertion of bacteriophage λa at the end of malF. The map shows the location of the mutations used to determine the end points of the fragments within malK and lamB. malK1, malK3, malK5 are point mutations, malBΔ1, etc. are deletions. In addition to the mutations, the EcoRI cuts within or near the malB region have been drawn as vertical arrows. Their approximate locations are +7, +19, and +29%. An evaluation of the length of the bacterial DNA exterior to malB carried by the phages has been made starting from the physical map (Fig. 4). It is indicated here in percentage of λ DNA length. Only a portion of the genome of the transducing phages has been represented. The total lengths of the phages (last column on the right) are given as the difference between the transducing phage and λa expressed in percentage of the length of λ. In such units λcI857Sam7 is +11.6%. When present, the EcoRI cut located at 54.3% on the genetic map of λ (17) has been represented by a vertical arrow. When this cut is not present because of a deletion, the length of what remains from late genes DNA has been calculated from the total length of the phage and the physical map of malB (Fig. 4). λadmalB12, λapmalB13, λapmalB14, λapmalB15, and λapmalB16 result from crosses between λapmalB11 and λapmalB1, λapmalB16, λapmalB8 and λapmalB4, respectively. Symbols:* ▬, *malB DNA;* ■, *intergenic region;* □□ *att', bacterial pseudoattachment site for λ in malF;* wwww, *bacterial DNA exterior to malB on the side of malF;* ○, *phage attatchment site;* ~~ , *bacterial DNA exterior to malB on the side of lamB;* ∫ , *illegitimate recombination due to an excision event;* =, *phage DNA.*

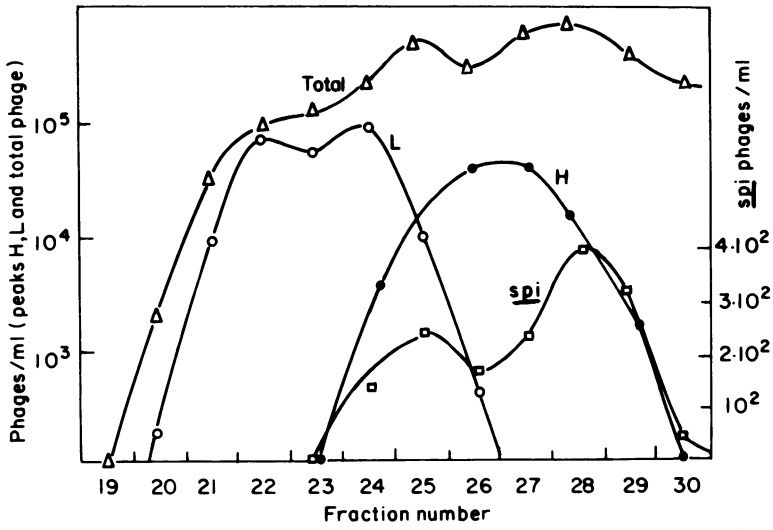

FIG. 3. *Cesium chloride gradient centrifugation of LFT lysate. Centrifugation to equilibrium was performed as described in the text. Peak H corresponds to phages carrying only the proximal part of malK [white plaques with red papillae on Mcc-mal plates seeded with pop244 (malK1), recombination]. Peak L corresponds to phages carrying all of malK [red plaques on Mcc-mal seeded with pop244 (malK1), complementation]. Symbols: for left ordinate (log scale): ●, peak H; ○, peak L; △, total phages; for right ordinate (linear scale): □, peak of spi phages.*

nants with *malK1* in further experiments.

That the phage from the fourth method of selection (λap*malB11*) carries all the distal part of *malF* is shown in the following paragraph.

Phages carrying an intact *malF* gene. Genetic crosses between phages carrying the proximal part of *malF* and phage λap*malB11* (M and MIV) yielded recombinants (λad*malB12*, λap*malB13* to λap*malB16*) which carried all the gene *malF*. In each of the cases tested, and by using its density as a basis to calculate the length of a phage, the length of the recombinant was the algebraic sum of that of its parents. This is to be expected in such site-specific recombination.

(ii) Physical mapping of the *malB* region. A physical map of the *malB* region was constructed with site-specific DNA restriction endonucleases. This map is shown in Fig. 4. The boundaries of the *lamB*, *malK*, *malE*, and *malF* genes are not known with any precision because they are only defined genetically and cannot be exactly placed on the physical map. The location of the end points for the bacterial DNA carried by the phages is known only from the position of the two restriction enzyme cuts between which they fall.

Fragments of DNA resulting from a given digestion were separated by agarose gel electrophoresis and, occasionally, by polyacrylamide gradient gel electrophoresis. The sizes of the fragments were estimated by comparison with *Hin*dIII fragments of simian virus 40 DNA (3, 17).

The λ*malB* DNAs and their parents were digested with *Hin*cII, and the fragments were separated on a 2% agarose gel (Fig. 5A). The principle of the mapping was to use the various λ*malB* phages to establish the order of a large number of *Hin*cII fragments and then to position the cleavage sites of other enzymes within these fragments. Use was also made of arguments based on the estimated lengths of certain fragments. We are able to order all the *Hin*cII fragments within *malB* as follows.

The 1,090-base pair fragment is found in *malB3*, but not in *malB4*. The 720-base pair fragments are both present in *malB6* and missing in *malB8* and so must lie side by side to the left of the 1,090-base pair fragment. The 1,530-base pair fragment is present in *malB8* and missing in *malB10*. The 1,080-base pair fragment is present in the recombinant phage *malB13*, but missing in each of its parent *malB11* and *malB6* and so must contain the site of insertion of the prophage in strain RW597(λa)1. The 4,200-base pair fragment is only present in *malB11* and recombinant phages and so lies to the left of the insertion point. These arguments enable the placement of all but the 1,250- and 640-base pair fragments. We could order these fragments by observing that *Sst*I removes about 70 base pairs from the 1,250-base pair fragment and that this *Sst*I site is 2,400 base pairs to the

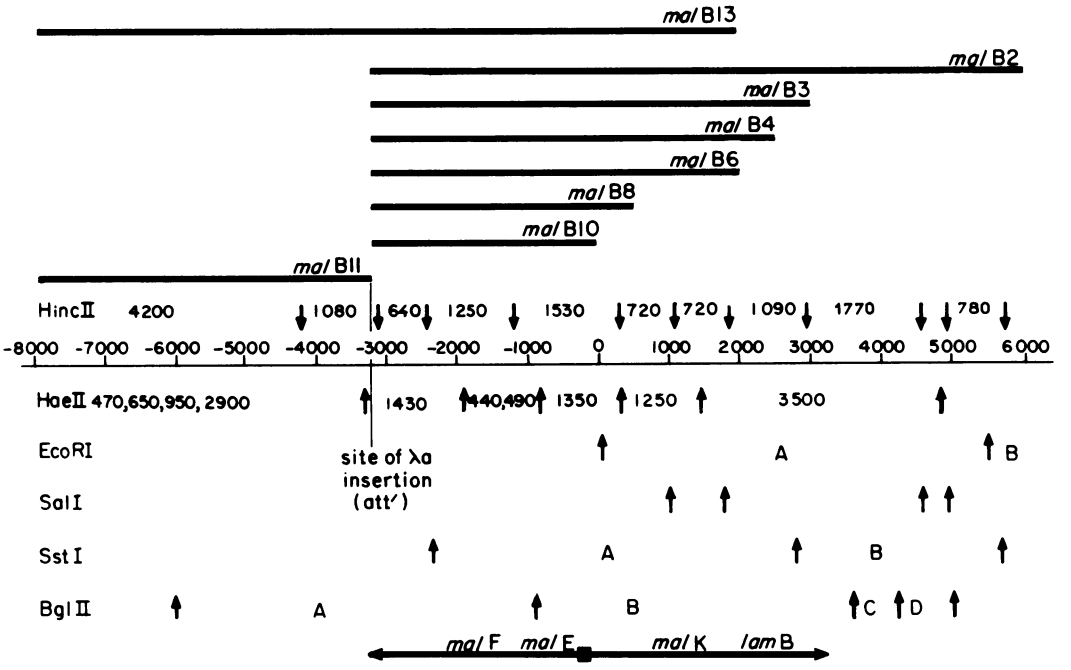

FIG. 4. *Physical map of the malB region. The map is graduated in base pairs (1% of λ length ≃ 470 base pairs). The origin of the scale is the EcoRI cut located within gene malK near the regulatory region of the malB operons. Orientation is positive to the right of the origin and negative to its left. The cuts by the six enzymes HincII, HaeII, EcoRI, SalI, SstI, and BglII are represented by vertical arrows. For HincII and HaeII the length of the fragments is also indicated. At the top of the figure the portions of the malB region carried by several transducing phages have been positioned with respect to the cuts between which they fall. Because the genetic boundaries of these portions are approximately known (Fig. 2), this allows a tentative positioning of the malB genes. It is shown at the bottom of the figure.*

left of the *Eco*RI site chosen as the origin. *Sst*I also splits the 1,090-base pair *Hinc*II fragment into 950- and 140-base pair fragments.

The *Eco*RI site at the origin was accurately positioned within the *Hinc*II map by double digestion. Thus, *Eco*RI divides the 1,530-base pair fragment into 1,180- and 350-base pair fragments. This site is on the right side of the 1,530-base pair fragment because the *Bgl*II site at 950 base pairs is to the left of the *Eco*RI site (as determined by double digestion with *Bgl*II and *Eco*RI) and cleaves the 1,530-base pair fragment into fragments of approximately 1,300 and 200 base pairs.

The cleavage sites of *Hae*II within *malB* were partially determined as above for *Hinc*II by digestion of λ*malB* DNAs (Fig. 5B). The order of the 440- and 490-base pair fragments has not been determined and neither has that of the 950-, 650-, 470-, and 2,900-base pair fragments in λap*malB11*. The 3,500-base pair fragment is placed to the right of the 1,250 fragment because it is the only one between *malB8* and *malB2* which is cleaved by *Bgl*II and it is not cleaved by *Eco*RI. The *Hae*II cleavage sites were positioned with respect to the *Hinc*II sites by observing that the 1,350-base pair *Hae*II fragment is cleaved by *Eco*RI into fragments of 960 and 390 base pairs and by *Bgl*II into fragments of 1,340 and 10 base pairs.

Digestion with *Eco*RI of a variety of *malB* transducing phages, carrying portions of the *malB* region, along with their parent phages λ and λa is shown in Fig. 5C. The second band in the digest of *malB3* is a doublet which can be separated by polyacrylamide gradient gel electrophoresis. It is clear from Fig. 5C that there are two *Eco*RI cleavage sites in *malB1* to the right of *malB3* and, therefore, outside of the *malB* region and that there is a cleavage site in *malB* between *malB10* and *malB8* in gene *malK*.

Digestion of the *malB* DNAs with *Bgl*II revealed that there are three cleavage sites between *malB2* and *malB3*, one site between *malB10* and the insertion site, and one site in *malB11* to the left of the insertion site. These sites were ordered with respect to the *Eco*RI site by double digestion with *Eco*RI and *Bgl*II.

A similar experiment showed that *Sst*I cleaves

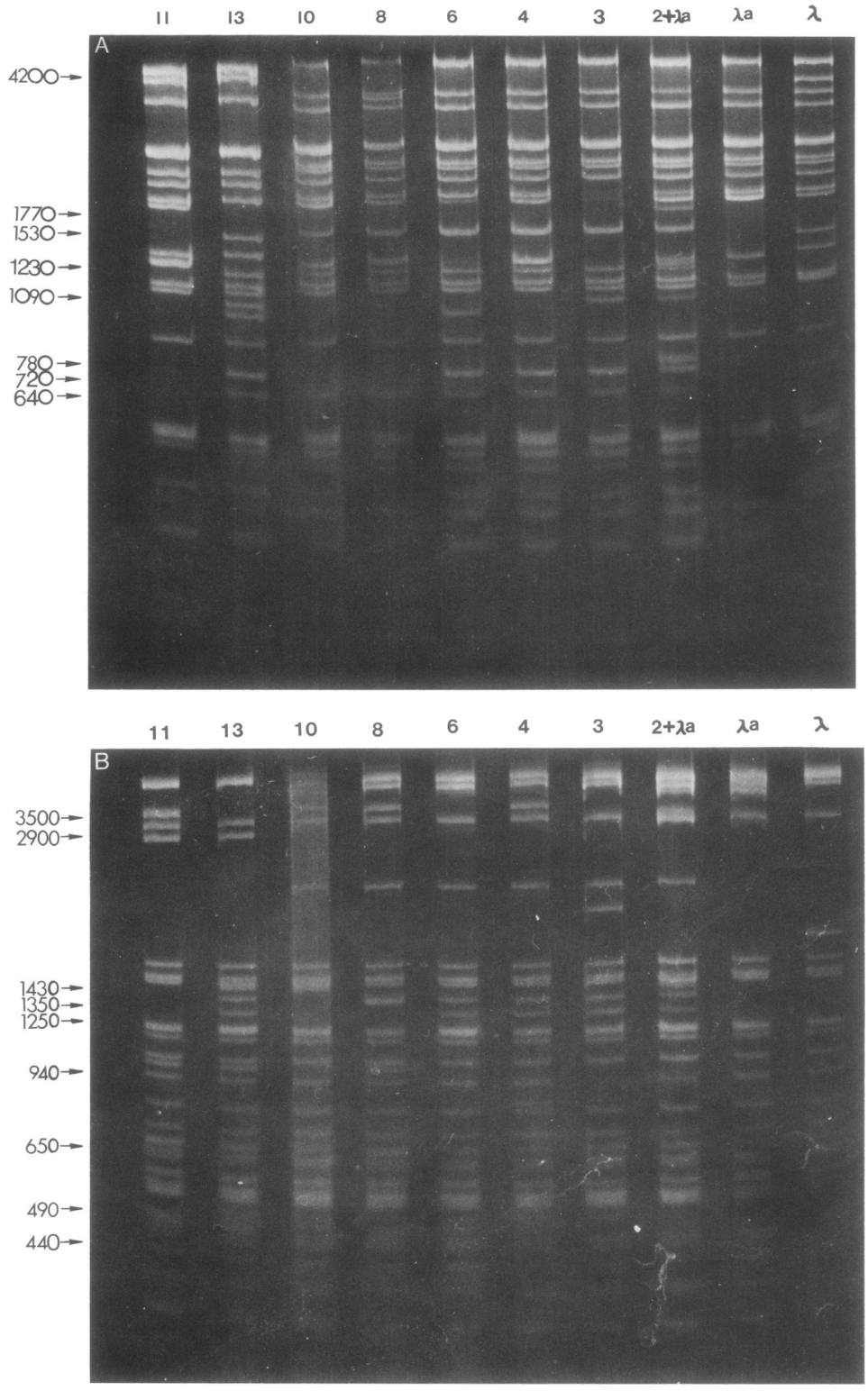
11
13
10
8
6
4
3
2+λa
λa
λ
A
4200
1770
1530
1230
1090
780
720
640
11
13
10
8
6
4
3
2+λa
λa
λ
B
3500
2900
1430
1350
1250
940
650
490
440

FIG. 5A and B

once between *malB2* and *malB3*, once between *malB3* and *malB4*, and once between *malB10* and the insertion site in *malF*. The 2,750- and 2,400-base pair distances of these last two sites from the *Eco*RI site at 0 base pairs were determined by double digestion. The *Sst*I site at +5,650 base pairs is placed to the right of the *Eco*RI site at +5,500 base pairs because the fragment labeled B in the *Sst*I digest of Fig. 4 is shortened by *Eco*RI.

Digestion of λ*malB* DNAs with *Sal*I led to the conclusion that there are two cleavage sites between *malB6* and *malB8* and two sites between *malB2* and *malB3*. The *Sal*I cleavage sites were oriented and positioned with respect to the *Eco*RI and *Bgl*II cleavage sites by double digestions. It was found that the *malB* region is not cleaved by the restriction enzymes *Hin*dIII, *Bam*HI, *Xho*I, *Xba*I, *Pst*I, and *Kpn*I.

DISCUSSION

Formation of the transducing particles. The LFT lysates prepared from strain RW597 (λa)1 had a low titer (10^5 plaque-forming units [PFU] per ml) and contained approximately 10% of the PFU carrying the distal part of *malF* (3% of these PFU were *spi* phages), 10% of the PFU carrying gene *malE* and at least 5% defective particles carrying *malE*, *malK*, and *lamB*.

The characteristics of the transducing particles (Fig. 2) are consistent with the idea that the prophage λa is inserted in gene *malF* by a site-specific recombination event involving attλ (11, 12) and with the orientation indicated in Fig. 1. The genetic structure of the transducing phages agrees with the Campbell model (2) for prophage excision. However, it is striking that an important fraction of the transducing particles (about

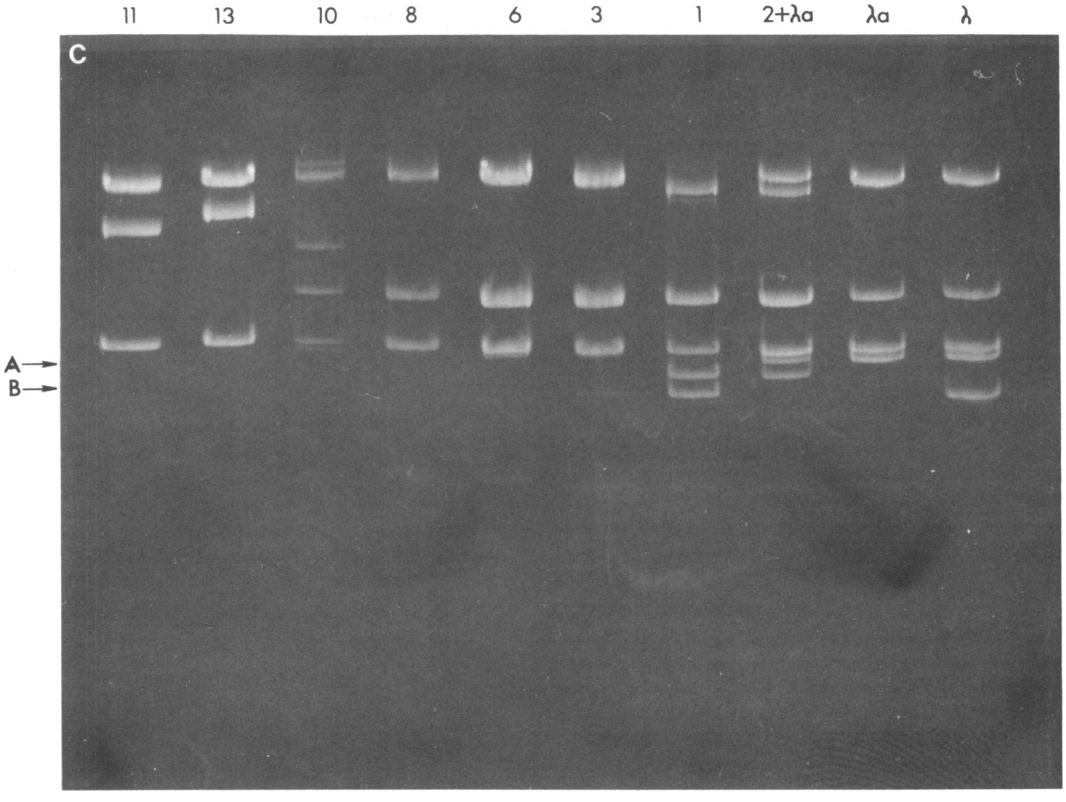

FIG. 5. *Analysis of malB with restriction enzymes. Hydrolysis and gel electrophoresis were performed as described in the text. Arrows on photos indicate the positions of malB fragments provided that both cuts are inside the bacterial DNA carried by the transducing phage. DNA from left to right are: malB11, malB13, malB10, malB8, malB6, malB4, malB3, malB2 with helper λa, λa, and λ. (A) HincII, 2% agarose gel. The numbers next to the arrows indicate the molecular weight of the fragment. Double arrows point to double fragments. (B) HaeII, 2% agarose gel. (C) EcoRI, 0.7% agarose gel. The letters next to the arrows refer to the map of Fig. 2.*

20%) carry both the distal part of *malF* and gene *malE*; their production implies a recombination event between two bacterial DNA regions separated by the prophage. This type of event is not explicit in the original Campbell model. The question may be asked whether such an event would occur in the absence of any prophage and be related to the formation of deletions.

The lengths of the transducing phages varied from about 87.7% of λ to about 106.8% of λ. As calculated from its density, the length of λa is about 90% of the length of λ. It is apparent (Fig. 3) that there is no correlation between the density of the transducing phage and the length of the bacterial DNA carried. This is due to the fact that the length of the phage genome is not the same in the various transducers and is consistent with the multiplicity of possible excision sites.

Restriction map and genetic map. By comparing the restriction fragments with the genetic map of the *malB* deletions carried by the transducing phages, it is possible to approximately locate the restriction enzyme cuts within the *malB* genes. This is shown in Fig. 4. This tentative assignment relies on the following points: (i) λap*malB6*, but not λap*malB8*, carries an intact *malK* gene; (ii) λap*malB3*, but not λap*malB4*, carries an intact *lamB* gene; (iii) λap*malB10* carries the promoter for the *malE malF* operon and does not carry the *Eco*RI cut located in *malK*. Messenger hybridization experiments are consistent with the idea that the 1,570-base pair fragment obtained with *Hin*cII contains the *malB* promoter region (J. M. Clement and J. Greenblatt, unpublished data). (iv) The molecular weight of the *lamB* product is 55,000 and that of the *malE* product is 45,000 (7, 9). This tentative assignment is consistent with the internal deletion analysis of *malB* (O. Raibaud and M. Hofnung, manuscript in preparation).

The *lamB* gene is carried intact by both the *Eco*RI-A fragment (without its promoter) and the *Bgl*II-B fragment (with its promoter) of Fig. 4. It should be possible, for example, to clone the *lamB* gene with or without its promoter in vectors for *Bgl*II and *Eco*RI fragments.

Other restriction enzyme fragments contain part of a particular *malB* gene without containing all of it. For example, the *Hae*II 1,350-base pair fragment should contain the proximal parts of *malE* and *malK*, and the *Hae*II 1,250-base pair fragment should contain the distal part of *malK*. The *Hin*cII 1,080- and 640-base pair fragments contain distal parts of *malF*; the 1,250-base pair fragment contains the distal part of *malE* and the proximal part of *malF*; the 1,350-base pair fragment contains the proximal parts of *malE* and *malK*; the 720-base pair fragments contain the distal parts of *malK* and the proximal part of *lamB*; and the 1,090-base pair fragment contains the distal part of *lamB*.

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