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malB Region in *Escherichia coli* K-12: Characterization of New Mutations

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Phenotypic characterization and mapping of more than 50 Mal⁻ mutations located in the *malB* region lead one to divide the site for Mal⁻λs mutations (formerly called gene *malB*) in that region, into two adjacent genetic segments *malJ* and *malK*. *malJ* and *malK* are both involved in maltose permeation. It is suggested that (i) *malK* and *lamB*, the only known gene specifically involved in phage λ adsorption (20), constitute an operon of polarity *malK lamB*. (ii) *malJ* and *malK* correspond to two different genes, and (iii) a promoter for the *malK lamB* operon is located between *malJ* and *malK*. Since λ receptors and maltose permease are inducible by maltose and absent in *malT* mutants, it is likely that the expression of the *malK lamB* operon is controlled by the product of gene *malT*, the positive regulatory gene of the maltose system.

In wild-type *Escherichia coli* K12 maltose induces the three enzymatic activities responsible for its assimilation as well as the bacterial receptor sites for phage λ (17, 21). All the known genes specifically implicated in maltose metabolism and in phage λ receptor synthesis are located in either of two regions of the genetic map: *malA* and *malB* (Fig. 1) (15, 20).

The *malA* region contains an operon composed of *malP* and *malQ* the structural genes for maltodextrin phosphorylase and amylomaltase, and a positive regulatory gene *malT* (3, 4, 16, 18). The activator protein, product of *malT*, allows expression of the *malP malQ* operon in the presence of maltose. The target for this control is the *malI* locus (or initiator) located, like the promoter of the *malP malQ* operon, between genes *malP* and *malT* (Fig. 2) (7, 8).

The structure of the *malB* region is less well known. Mutations affecting this region can be classified according to three phenotypes: Mal⁺λr, Mal⁻λs, Mal⁻λr. It was earlier proposed that Mal⁺λr mutations affect a gene *lamB* involved in λ receptor synthesis but not in maltose metabolism, that Mal⁻λs mutation affect another gene *malB* involved in maltose permeation, and that Mal⁻λr mutations affect both genes. (It is now known [this paper and M. Hofnung, Genetics, submitted for publication] that there is more than one gene of the *malB* region which is involved in maltose permeation. However, we still call here gene *malB* [or simply *malB*] the cluster of genes in the *malB* region defined by Mal⁻λs mutations; the *malB* region includes thus genes *malB* and *lamB*). Some of the Mal⁻λr mutations revert. They were assumed to be polar mutations and their existence was tentatively taken as evidence that the *malB* gene and the *lamB* gene belong to a single operon. In addition, to account for the inducibility of the activities encoded by the *malB* region, as well as for their absence in *malT* mutants, it was assumed that the operon in the *malB* region was like the *malP malQ* operon in the *malA* region-subject to *malT* gene control (17, 20).

A more detailed genetic analysis of the *malB* region appeared to be of interest not only from the point of view of positive regulation but also because it could contribute to the understanding of two functions of the cell envelope, active transport, and phage adsorption, and of possible relations between them. Studies of Mal⁺λr mutations allowed one to propose that *lamB* was a single cistron (20). The characterization of Mal⁻λs and Mal⁻λr mutations reported in this paper suggests that more than one gene of the *malB* region may be involved in the permeation of maltose and that this region is more complex than first thought.

MATERIALS AND METHODS

Strains. Bacterial strains are listed in Table 1. λv is a virulent mutant of phage λ (10). The P1 strain used is a partially virulent mutant of P1kc obtained from S. Brenner. Genetic nomenclature is that of Taylor and Trotter (19). The abbreviations for phenotypes are as follows: λr (λs) means resistant (sensi-

tive) to a virulent mutant of λ. Mal^+(Mal^-) means able (unable) to use maltose as a carbon source.

Media and techniques. Media (3, 4, 15) techniques for transduction (8), conjugation on plates and in liquid (3) ethyl methane sulfonate (EMS) *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) and UV (16) mutagenesis, enzyme assays, detection of amylomaltase activity on plate (8) and screening of Mal^- nonsense mutants (4) were already described. It should be recalled that no analogue of maltose that is a substrate of the permease but is not further metabolized is yet available. Therefore, the permease assay measures both the uptake and subsequent metabolism of ^{14}C-maltose.

Isolation of Mal^-λs mutants with lesions in the malB region. Among 84 independent Mal^- mutants isolated from strain HfrG6 on eosin methylene blue (EMB) or tetrazolium (TTZ) maltose agar after EMS mutagenesis, 65 were shown to have a lesion in the *malA* region (3, 4). The other 19 mutants gave Mal^+ recombinants when crossed with an F^- strain carrying a deletion of the entire *malA* region. They are all λs and, as shown in this investigation, result from defects in the *malB* region. The corresponding mutations have been named *malJ; or malK1, malK3,* and *malK4* according to their levels of *malP malQ* operon expression (Table 2).

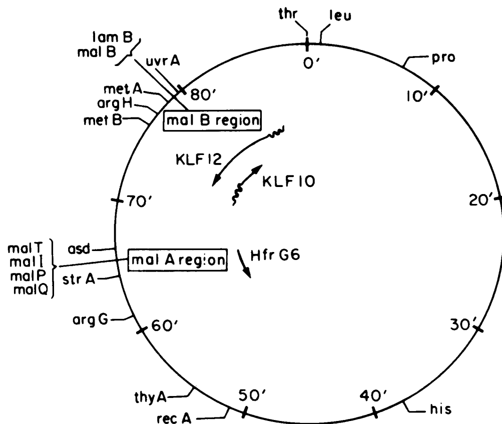

FIG. 1. *A simplified version of the genetic map of E. coli K12 (19). The location of the markers used in this study, the origin of transfer of strain HfrG6 and the portions of the chromosome carried by relevant episomes are shown.*

Three other λs *malB* mutations *malK2, malK5,* and *malJ4*, obtained in a slightly different way have been added to this study. *malK2* and *malK5*, formerly called MB10 and MPE2, were isolated in strain HfrG6 after ultraviolet (UV) mutagenesis (16). Mutation *malJ4*, formerly called MB4, was isolated in strain HfHU482 after NTG treatment (16).

Isolation of Mal^-λr mutants with lesions in the malB region. λr *malB* mutants of strain HfrG6 express the *malP malQ* operon in a constitutive way (M. Schwartz, Ph.D. thesis, Univ. of Paris, 1967; 20). They were isolated as follows. Spontaneous λr mutants were first selected from independent cultures of strain HfrG6 (17) and then plated on M63 B1 his (thiamine-histidine) agar medium containing glucose as a carbon source (8). The developed plates were replicated on M63 B1 his agar medium containing glycerol as a carbon source (8) where the colonies with constitutive amylomaltase activity were detected as already described (8). The corresponding colonies from the master plate were then reisolated. The mutations obtained in this way were designated *malK100* to *malK106* (revertible) or *MalB*Δ (nonrevertible) (Table 3).

Search for spontaneous Mal^+ derivatives of malB mutants. Isolated colonies of the *malB* mutant were inoculated in a flask with 500 ml of ML medium (3) and grown to saturation. A 100-ml amount of the culture was then centrifuged, the pellet was resuspended in 2 ml of M63 medium, and samples were plated on eosine methylene blue minimal (EM) maltose agar medium supplemented with B_1 and histidine. When the *malB* mutant was λr, 10 to 50 Mal^+ derivatives were tested for sensitivity to λ.

Recombination studies. Recombination studies were performed by Hfr$\times$$F^-$ and F'$\times$$F^-$ crosses. It was assumed that the Mal^-λr nonrevertible mutations designated *malB*Δ were deletions. They were introduced into an F^- strain and tested for recombination with all the *malB* mutations (*malJ, malK,* and *malB*Δ). Mutations *malK100* to *malK106* were treated like the *malB*Δ mutations. The *malB* F^- strains used were *argH*$^+$ *metA*$^+$ *malB thr leu his*$^+$ *str*

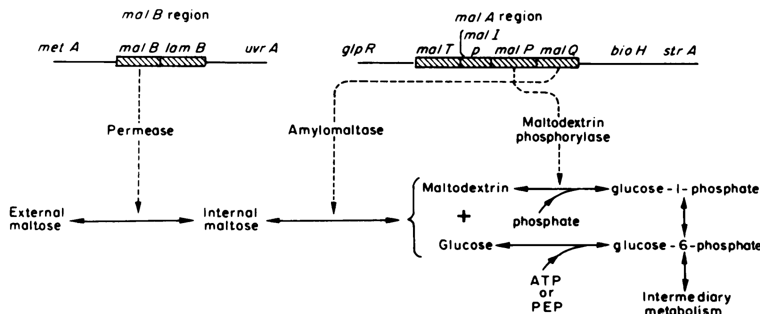

FIG. 2. *The maltose system in E. coli K12. The structure of the malA region was presented previously (7, 8). The structure of the malB region is discussed in this paper and in M. Hofnung, Genetics, submitted for publication.*

TABLE 1. *Bacterial strains*[a]

Name	Mating type	Relevant genetype	Relevant phenotype	Observations
HfrG6	Hfr	*his*	Mal$^+$	From G. Matney
HfrG6 *malJ*	Hfr	*his malB*	Mal$^-$A$^-$λ^s	EMS induced *malB* mutations in HfrG6
HfrG6 *malK*1, *malK*3, *malK*4	Hfr	*his malB*	Mal$^-$A^{c}λs	EMS induced *malB* mutants of HfrG6
HfrG6 *malK*2, *malK*5	Hfr	*his malB*	Mal$^-$A^{c}λs	UV induced malB mutants of HfrG6
HfrG6 *malK*100 to *malK*106	Hfr	*his malB*	Mal$^-$A^{c}λr	Spontaneous *malB* mutants of HfrG6
HfrG6*malB*Δ	Hfr	*his malB*	Mal$^-$A^{c}λr	Spontaneous *malB* mutants of HfrG6; *malB*Δ mutations are deletions
HfrHU482	Hfr (Hayes type)	*asd*	Mal$^+$	
HfrHU482*malJ*4	Hfr	*asd malB*	Mal$^-$A$^-$λs	NTG induced *malB* mutant of HfrHU482
HfrG6*mal* "*J*"	Hfr	*his malB*	Mal$^-$A$^-$λs	EMS induced *malB* mutants of HfrG6 (double mutants?)
pop422 (KLF12)	F′	chromosome: *argH malB*Δ*101 pro recA str*; episome : *argH$^+$ malB$^+$*	Mal$^-$λr (due to chromosome); Mal$^+$λs (due to episome)	Episome from Brooks Low
JC1553 (KLF10)	F′	chromosome: *argG metB leu his malA recA str*; episomeL *metB$^+$ malB*	Mal$^-$λr (due to chromosome; Mal$^-$λr (due to episome	Strain from Brooks Low; resulting phenotype is Mal$^-$λr
pop8	F$^-$	*argH metA thr leu str*	Mal$^+$	
pop8*malB*Δ pop8*malK*	F$^-$	*thr leu str malB*	Mal$^-$A^c	Recombinants between HfrG6 *malB*Δ or Hfr *malK* and pop8
PA505MAΔ108	F$^-$	*arg*H *met*A *bio*H *mal*A (MAΔ108)	Mal$^-$ λR	Reference 3
pop550 to pop587	F$^-$	*arg*H *his mal*B str	Mal$^-$	Strains carrying the various *mal*B mutations and used in F′ × F$^-$ crosses (see reference (9)
PA505MS	F$^-$	*arg*H *met*A *pro str*	Mal$^+$	
PA505 *malK*	F$^-$	*arg*H *met*A *str*	Mal$^-$	Pro$^+$His$^+$ recombinant between HfrG6*malK* and PA505MS

[a] Strains require vitamin B1. When no origin is indicated the strain is from the laboratory collection. A^c, constitutive expression of the *malP malQ* operon. A$^-$, noninducible basal expression of the *malP malQ* operon. All the *malB* mutants of strain Hfr G6 reported here have a collection number comprised between pop 1715 and pop 1768. In the table the name of the strain includes the name of the *malB* mutation carried. The notation is as follows: MJ stands for *malJ*, MK stands for *malK*, and MBΔ stands for *malB*Δ

recombinants obtained by conjugation between the HfrG6 *malB* mutants and strain pop 8 (Table 1). In the mapping experiments, selections for prototrophic (Thr$^+$ Leu$^+$ His$^+$) and prototrophic Mal$^+$ recombinants were done on minimal glucose and minimal EM maltose plates, respectively. Two mutations were said to recombine whenever the observed frequency of Mal$^+$ recombinants among prototrophs was more than 10^{-6}.

F′ × F$^-$ crosses allowed us to check the results of Hfr × F$^-$ crosses and to look for recombination between most of the *malJ* and *malK* mutations. All the mutations marked with an arrow on Fig. 4 were transferred on the KLF12 episome as described in (M. Hofnung, Genetics, submitted for publication). The resulting *malB* episome were then introduced into a series of F$^-$ strains carrying the different *malB* mutations and tested for recombination. The episome KLF10 carrying a Mal$^-$λr mutation named *malB15* (1) was used in a similar way to carry out mapping studies. Episome KLF10 was isolated from HfrJ4 (11) (also called HfrP10 [1]) in which the F′ factor is integrated in the *malB* region (16).

F′ × F$^-$ crosses were performed by a rapid procedure described in reference (M. Hofnung, Genetics, submitted for publication). F$^-$ strains were *argH malB his str*. The F′ factors were carried by a strain harboring on its chromosome the markers *argH malB*Δ*101 recA thyA* (M. Hofnung, Genetics, submitted for publication). In the cases when recombination to Mal$^+$ occurred, two recombinants were isolated and were shown to have the expected genetic characteristics. The F′ × F$^-$ crosses had approximately the same resolving power as the Hfr × F$^-$ crosses and gave identical results which were compatible with the order of markers established by three-point tests (Table 4).

Three mutations, *malK106*, *mal*"*J5*" *and mal* "*J9*", (Tables 2 and 3) have not been represented on the genetic map (Fig. 4). *malK106* had such a high rate of reversion that accurate localization within the

TABLE 2. *Mal$^-$λs mutations in the malB region*[a]

Mutations	Suppression by		Amylomaltase		Phosphorylase	
	suIII	suIV	Noninduced	Induced	Noninduced	Induced
None	−	−	8	100	9	100
*mal*J1	−	−	13	18	7	12
*mal*J2	−	−	20	26	24	28
*mal*J3	−	−	7	6	7	9
*mal*J5	−	−	8	7	8	11
*mal*J6	−	+	20	20	17	23
*mal*J7	+	+	14	17	20	22
*mal*J8	+	+	20	17	20	18
*mal*J9	−	−	22	21	22	20
*mal*J10	+	+	8	6	4	5
*mal*J11	+	+	6	6	9	8
*mal*J12	−	−	12	12	7	16
*mal*J13	−	−	10	18	9	13
*mal*J14	+	+	18	20	19	24
*mal*J15	−	−	11	13	14	15
*mal*J16	−	−	7	12	5	10
*mal*J17	±	±	10	8	10	8
*mal*K1	−	−	45	51	49	42
*mal*K2	−	−	38	44	27	38
*mal*K3	+	+	40	46	32	36
*mal*K4	+	+	27	41	27	40
*mal*K5	−	+	36	45	33	36

[a] The above mutations have been isolated in HfrG6 after EMS or UV treatment. Almost all of them give Mal$^+$ revertants at a frequency higher than 10^{-9}. The two exceptions are *mal*"*J5*" and *mal*"*J9*" which are probably double mutants. Column two indicates whether the mutation is suppressible by suIII or suIV (+ = suppressible ± = doubtful − = not suppressible). Enzyme-specific activities are indicated as the percentage of wild-type induced levels. The permease levels in the mutants were less than 12%. Wild-type induced levels: amylomaltase, 170 Units per milligram of protein; maltodextrin phosphorylase, 180 units per mg of protein; maltose "permease", 201 nmol of maltose per mg of protein per h. Mal$^-$λs mutations can be grouped into two classes according to the level of expression of the *malP malQ* operon they promote. Class I contains mutations *malJ1* to *malJ17*. Class II contains mutations *malK1* to *malK5* (see text).

TABLE 3. *Mal$^-$λr mutations in the malB region*[a]

Mutation	Amylomaltase		Phosphorylase		Permease	
	Noninduced	Induced	Noninduced	Induced	Noninduced	Induced
None	8	100	9	100	50	100
*malB*Δ1	47	54	43	44	5	6
*malB*Δ2	46	55	41	53	5	5
*malB*Δ3	49	61	46	70	7	7
*malB*Δ5	47	68	39	50	6	6
*malB*Δ7	60	46	51	55	10	7
*malB*Δ8	56	60	47	56	6	7
*malB*Δ9	68	59	65	55	7	6
*malB*Δ10	59	53	57	51	6	4
*malB*Δ11	56	61	45	59	6	5
*malB*Δ12	39	59	42	50	6	6
*malB*Δ13	61	51	65	52	5	5
*malB*Δ15	58	64	55	70	5	4
*malB*Δ16	59	75	60	65	4	4
*malB*Δ17	61	70	66	60	6	4
*malB*Δ18	71	66	72	77	4	5
*malB*Δ101	76	59	69	61	5	4
*malB*Δ103	53	×	39	44	4	5
*malB*Δ104	51	46	60	64	9	8
*malB*Δ105	25	25	33	36	4	8
*malB*Δ107	62	62	60	64	9	8
*malB*Δ112	56	62	60	62	7	6
*malB*Δ114	49	44	55	54	10	7
*malK*100	51	70	43	66	6	6
*malK*101	43	48	52	65	9	8
*malK*102	67	57	67	62	5	5
*malK*103	52	39	45	49	5	7
*malK*104	53	54	45	70	5	4
*malK*105	34	41	36	56	4	5
*malK*106	52	56	63	54	5	10

[a] All these mutations are spontaneous and have been isolated in strain HfrG6. None of them is suppressible by suIII or suIV. Mutations *malK100* to *malK106* revert to a Mal$^+$ λs phenotype. The others give rise only to Mal$^+$ derivatives that are still λr: they have been named *malB*Δ followed by a number. Specific activities of enzymes are given as the percentage of wild-type induced levels. Wild-type levels are shown under Table 2.

malB region was not possible. *mal* "*J5*" and *mal* "*J9*" behaved in the crosses as double mutants. Their low reversion rates (Table 4) support this conclusion. This point will be discussed further in the light of complementation data (M. Hofnung, Genetics, submitted for publication).

RESULTS

Mal$^-$λs mutations in the malB region. Twenty-one independent Mal$^-$λs mutations of the *malB* region were obtained in strain HfrG6, as described in Materials and Methods. At least nine of them are of the nonsense type (2) (Table 2). No maltose permease activity is detectable in the mutants and the level of expression of the *malP malQ* operon in the *malA* region is not modified by addition of maltose to the culture medium. Measurement of this level allows one to distinguish two classes. Class I contains the mutants in which the *malP malQ* operon is expressed at a level of about 15% (between 5 and 25%) of that of the fully induced wild type. This rate of synthesis of the *malA* enzymes is approximately the same as that of the uninduced wild type, or slightly higher. Class II consists of five mutants in which the level of expression of the *malP*, *malQ* operon is about 45% (between 35 and 55%) of that of the induced wild type. The distinction between the two classes on the basis of *malP*, *malQ* expression is supported by further analysis of the mutants (this paper and M. Hofnung, Genetics, submitted for publication).

Mal$^-$λr mutations in the malB region. In a haploid strain most Mal$^-$λr mutants have lesions in gene *malT* located in the *malA* region

TABLE 4. *Mal⁺ derivatives of revertable λr malB mutants in strain HfrG6*[a]

λr *mal*B mutation	Frequency of Mal⁺ derivatives (order of magnitude)	Ratio λs/λr colonies among Mal⁺ derivatives
*malK*100	10^{-9} to 10^{-10}	0.2; 1
*malK*101	10^{-9} to 10^{-10}	0.2; 0.3
*malK*102	10^{-7}	>50
*malK*103	10^{-6}	>50
*malK*104	3×10^{-6}	>50
*malK*105	10^{-6}	>50
*malK*106	10^{-5}	>50

[a] Column 2: order of magnitude of the average frequency of Mal⁺ derivatives determined from at least three independant cultures of each mutant. The frequency of Mal⁺ λr derivatives is approximately the same for all the mutants, revertable or not, in which it was tested. This is to be exprected if the Mal⁺λr derivatives are due to a suppressor independant of the original *malB* mutation (see Discussion). This frequency was ranging from 10^{-9} to 10^{-10}. For comparison the frequency of Mal⁺ derivatives of strain HfrG6 *malK1* was about 10^{-8} of strain HfrG6 *malJ11* about 10^{-7}, and strains HfrG6 *mal"J5"* and HfrG6 *mal"J9"* between 10^{-9} and 10^{-10}. Column 3: the values reported for *malK100* and *malK101* are the extreme values found in at least three independant cultures of the mutants.

(17). In strains diploid for the *malA* region most Mal⁻ λr mutants have lesions in the *malB* region (20). All such λr *malB* mutants isolated in a derivative of strain HfrG6 diploid for the *malA* region were shown to express the *malP malQ* operon in a partially constitutive way (20). Mal⁺ *lamB* mutants were never found to express the *malP malQ* operon in a constitutive way (20). The phenotype of λr *malB* mutations in strain HfrG6 is thus that expected if they affect at the same time two activities: the activity corresponding to gene *lamB* (i.e., yield of λr phenotype) and the activity corresponding to the site of class II mutations (i.e., yield of constitutive expression of the *malP malQ* operon). In order to obtain λr *malB* mutations in HfrG6 which is haploid, a selection was devised for λr mutants expressing the *malP malQ* operon constitutively (see Materials and Methods). (The selection technique used here is not general. In some strains λr *malB* mutations do not promote any constitutive synthesis of the *malP malQ* operon. One possible explanation for this difference is that some internal inducer of the maltose system accumulates in λr *malB* mutants of strains like HfrG6, but not in λr *malB* mutants of other strains like pop480 or HfrHU482) (16, 20, M. Schwartz, Ph.D. thesis, Univ. of Paris, 1967.)

Genetic map. Three markers in the *malB* region had previously been mapped with respect to one another and to the neighboring genes *metA* and *uvrA* (Fig. 3) (16). A more detailed map (Fig. 4) of the region has now been constructed by a deletion mapping procedure based on the assumption that the λr *malB* non revertible mutations were deletions (see Materials and Methods). This map has been oriented with respect to the neighbouring genes by ordering the markers *metA*, *malK101*, *malK2*, and *malK105* using three-point tests (Table 5 and Fig. 4).

Examination of the map (Fig. 4) calls for the following observations: (i) the λr *malB* nonrevertible mutations (named *malB*Δ) are indeed deletions. Each of these mutations is unable to recombine with at least two recombining mutations. The Mal⁻λr mutation of episome KLF10 also behaves like a deletion. There is a preferential region for the left end of the deletions, between *malK3* and *malK4* (Fig. 4). The seven deletions (out of 23) ending in this region cover also the site of integration of the F factor of HfrP10. (ii) The *malB* gene defined as the site for Mal⁻ λs mutations in the *malB* region can be divided into two segments according to the level of expression of the *malP malQ* operon promoted by the mutations they contain. Mutations on the *metA* side of the *malB* gene (class I) result in a basal or slightly above basal level of expression of the *malP malQ* operon (Table 2). Mutations on the *uvrA* side (class II) lead to a partially constitutive level of expression of the *malP malQ* operon (Table 2). We call the two segments *malJ* and *malK*, respectively. (iii) The reverible Mal⁻λr mutations (*malK100* to *malK105*) behave as mutations of the *malK* segment: they lie between the last mutations at the right of the *malJ* segment and the last mutations at the right of the *malK* segment (*malK4* and *malK5*) and promote a constitutive expression of the *malP malQ* operon (Table 3). (iv) The λr *malB* revertible mutations as well as the right end points of several λr *malB* dele-

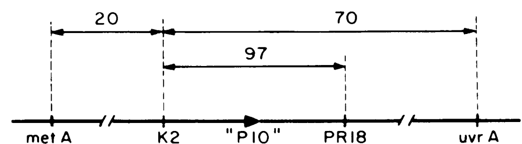

FIG. 3. *The order of three markers in the malB region (after 19). malK2 (noted here K2 and formerly called MB10) is a Mal⁻λs mutation. "P10" (also called malB15) (1) figures the site of the Mal⁻λr mutation resulting from the integration of the F factor in HfrP10. PR18 is a Mal⁺λr mutation. The numbers represent cotransduction frequencies by phage P1.*

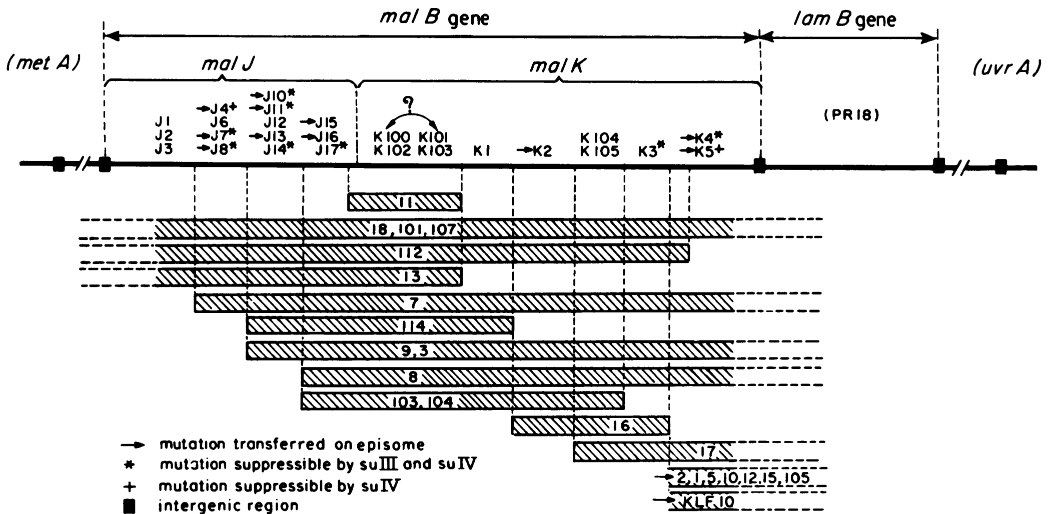

FIG. 4. *A genetic map of the malB region. Gene malB is defined as the locus for Mal$^-$λs mutations in the malB region. The data given in the present work shows that it consists of two genetics segments, designated malJ and malK, which may correspond to separate genes. The relative order of the two clusters of mutations malK100 and malK102 and malK101, malK103 has not been determined. Mutations malJ and malK are noted J and K, respectively. The hatched bars represent the malBΔ deletions. Each deletion is represented by its number. KLF10 designates the malB15 mutation of episome KLF10.*

TABLE 5. *Reciprocal three-point tests*[a]

Donor	Recipient	No. of Mal$^+$ His$^+$ recombinants tested	Met$^+$ Arg$^+$
*malK*2	*malK*101	200	31
*malK*101	*malK*2	199	12
*malK*2	*malK*105	100	9
*malK*105	*malK*2	122	64

[a] Reciprocal three-point tests used to orient the *malB* region with respect to neighbouring markers. Order of markers is: ***argH*, *metA*, *malK101*, *malK2*, *malK105*** (see also Fig. 4). The tests were performed by conjugation. The donor strains were *malB* derivatives of strain HfrG6 (His$^-$). The recipients were F$^-$ strains carrying *malB* mutations and the markers *argH* and *metA* (Table 1).

tions are located inside the *malK* segment. This fact is in agreement with the hypothesis that mutations in the *malB* gene can inactivate the *lamB* activity by polarity.

None of these supposedly polar mutations can be shown to be located entirely within the *malJ* segment. In other words, abolition of the *lamB* activity by such mutations seems to require that the site of the mutation extends into the *malK* segment. This is an argument which, although negative, suggests that there is a barrier for polarity between the *malJ* and *malK* segments. The possible nature of such a barrier will be examined in the discussion.

DISCUSSION

malB region. Phenotypic characterization and mapping of over 50 Mal$^-$ mutations in the *malB* region allows one to say that the *malB* gene, defined as the site for Mal$^-$λs mutations in the *malB* region, comprises two genetic segments: the *malJ* segment and the *malK* segment. Mutations in any of the two segments lead to a lack of maltose permease. Mutations affecting the *malK* segment provoke at least in some strains, a partially constitutive expression of the *malP malQ* operon, whereas mutations affecting the *malJ* segment do not.

About one-half of the Mal$^-$λs mutations in the *malJ* or the *malK* segment is of the nonsense type. The corresponding products are therefore proteins.

Some revertible mutations located in the *malK* segment and several deletions having their right end point inside the *malK* segment lead to a λr phenotype. Such a phenotype was previously shown to result from a purely *cis*-dominant inactivation of gene *lamB* (20). All this agrees with the hypothesis that the *malK* segment and gene *lamB* belong to the same operon with polarity exerted from *malK* to *lamB*. However, it remains to be proved that the *malK* segment and *lamB* are indeed different cistrons.

The order of the markers in the *malB* region is *metA* ... *malJ* segment —*malK* segment —*lamB* ... *uvrA* (Fig. 4). The fact that all the

λr *malB* mutations studied in strain HfrG6 result in a partially constitutive expression of the *malP malQ* operon (20), i.e., inactivate the *malK* segment, confirms the order and suggests that there is no other gene involved in maltose metabolism between the *malK* segment and gene *lamB*.

Nature of the λr malB mutations. It is shown here that the λr *malB* nonrevertible mutations are deletions. They yield Mal^+ derivatives which still have a λr phenotype. The properties of those derivatives are compatible with the idea that they owe their Mal^+ phenotype to a suppressor that allows maltose to enter the cell without expression of the *malB* region. This suppressor does not permit λ receptor synthesis in absence of *lamB* gene activity. Two such suppressors have already been described. One called *bymA* seems to be specific for maltose entry and could act by unmasking a passive transport system for maltose (8). The other suppresses a defect in lactose entry as well as in maltose entry and could operate through a nonspecific change in the permeability of the cell envelope (12).

The revertible λr mutations (*malK100* to *malK105*) all occur in *malK* and inactivate gene *lamB* in *cis* position. They are spontaneous mutations and revert, although at different rates. None of them is suppressible by suIII or suIV. Such mutations could be due to insertions of the type already described (6, 14) which are known to exert a strong polar effect on the expression of all distal genes in the same unit of transcription.

Expression of the malJ and malK segments. One may try to define more accurately to which functional genetic structure the *malJ* and *malK* segments correspond. Do they specify a single protein involved in maltose metabolism and consisting of two regions having different enzymatic activities (9, 13)? Or do they code for two different polypeptide chains both playing a role in maltose entry? In other words, do they correspond to a single gene or to two genes? Two independant arguments encourage us to favor the latter hypothesis. First, if the product of the *malJ* and *malK* segments were a single polypeptide chain, the polarity of transcription of the *malJ* segment should be the same as that of the *malK* segment: i.e., one should have the polarity exerted from the *malJ* segment to the *malK* segment to the *lamB* gene. Then, nonsense mutants in the *malJ* segment would be totally devoid of the activity corresponding to the *malK* segment and would result in a constitutive expression of the *malP malQ* operon as do deletions entering both *malJ* and *malK*. As this is not the case (Table 2) one is led to think that nonsense mutants in the *malJ* segment possess some *malK* segment activity. This is possible only if there is some initiation of translation, transcription or both between the *malJ* and the *malK* segments. Second, it was suggested above, that there is a barrier for polarity between the *malJ* and *malK* segments so that mutations located entirely within *malJ* cannot exert a polar effect on *lamB* expression. An initiation point for translation alone could not account for such a barrier since it should not be able to prevent *all* mutations inside *malJ* from being polar for *lamB* expression. Those two arguments lead one to suggest that there is a promoter for the *malK lamB* operon between the *malJ* and *malK* segments. We therefore propose the hypothesis that the *malJ* and *malK* segments correspond to two genes which we name *malJ* and *malK* and that a promoter for the *malK lamB* operon is located between *malJ* and *malK*. Such proposals are further sustained by complementation data to be published (M. Hofnung, Genetics, submitted for publication). Since the maltose permease and λ receptor sites are inducible by maltose, and absent in *malT* mutants, we suggest that the activator regulates the expression of the *malK lamB* operon. It cannot be said however what the direction of transcription of the *malJ* gene is or whether its expression is inducible or not. Further genetic analysis of the *malB* region and biochemical characterization of its products is necessary to test the hypothesis presented and to describe more precisely the mode of expression of the *malJ* gene.

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LITERATURE CITED

1. Bachmann, B. J. 1972. Pedigrees of some mutant strains of *Escherichia coli* K12. Bacteriol. Rev. **36**:525-557.
2. Garen, A. 1968. Sense and non-sense in the genetic code. Science **160**:149-159.
3. Hatfield, D., M. Hofnung, and M. Schwartz. 1969. Genetic analysis of the *maltose* A region in *Escherichia coli* K12. J. Bacteriol. **90**:559-567.
4. Hatfield, D., M. Hofnung, and M. Schwartz. 1969. Non-sense mutations in the *maltose* A region of the genetic map of *Escherichia coli* K12. J. Bacteriol. **100**:1311-1315.
5. Hirota, Y. 1960. The effect of acridine dyes on mating

type factors in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **46**:57-64.
6. Hirsch, H. J., P. Starlinger, and Ph. Brachet. 1972. Two kinds of insertions in bacterial genes. Mol. Gen. Genet. **119**:191-206.
7. Hofnung, M., M. Schwartz, and D. Hatfield. 1971. Complementation studies in the *maltose* A region of the *Escherichia coli* K12 genetic map. J. Mol. Biol. **61**:681-694.
8. Hofnung, M., and M. Schwartz. 1972. Mutations allowing growth on maltose of *Escherichia coli* K12 strains with a deleted *malT* gene. Mol. Gen. Genet. **112**:117-132.
9. Houston, L. L. 1973. Specialized sub-regions of the bifunctional *his* B gene of Salmonella typhimurium. J. Bacteriol. **113**:82-87.
10. Jacob, F., and E. L. Wollman. 1954. Etude génétique d'un bactériophage tempéré d'*Escherichia coli*. Ann. Inst. Pasteur, Paris **87**:653-673.
11. Low, K. B. 1972. *Escherichia coli* K12 F-prime factors, old and new. Bacteriol. Rev. **36**:587-607.
12. Ricard, M., Y. Hirota, and F. Jacob. 1970. Isolements de mutants de membrane chez *Escherichia coli*. C. R. Acad. Sci., Paris **270**:2591-2593.
13. Setlow, P., D. Brutlag, and A. Kornberg. 1972. Desoxyribonucleic acid polymerase: two distinct enzymes in one polypeptide. J. Biol. Chem. **247**:224-231.
14. Shapiro, J. 1969. Mutations caused by the insertion of genetic material into the galactose operon of *Escherichia coli*. J. Mol. Biol. **40**:93-105.
15. Schwartz, M. 1966. Location of the maltose A and B loci on the genetic map of *Escherichia coli*. J. Bacteriol. **92**:1083-1089.
16. Schwartz, M. 1967. Expression phénotypique et localisation génétique de mutations affectant le métabolisme du maltose chez *Escherichia coli* K12. Ann. Inst. Pasteur, Paris **112**:673-700.
17. Schwartz, M. 1967. Sur l'existence chez *Escherichia coli* K12 d'une régulation commune à la biosynthèse des récepteurs du bactériophage λ et au métabolisme du maltose. Ann. Inst. Pasteur, Paris **113**:685-704.
18. Schwartz, M., and M. Hofnung. 1967. La maltodextrine phosphorylase d'*Escherichia coli* K12. Eur. J. Biochem. **2**:132-145.
19. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K12. Bacteriol. Rev. **36**:504-524.
20. Thirion, J. P., and M. Hofnung. 1972. On some genetic aspect of phage λ resistance in *E. coli* K12. Genetics **71**:207-216.
21. Wiesmeyer, H., and M. Cohn. 1960. The characterization of the pathway of maltose utilization by *Escherichia coli*. Biochim. Biophys. Acta **39**:417-447.