DIVERGENT OPERONS AND THE GENETIC STRUCTURE OF THE MALTOSE B REGION IN *ESCHERICHIA COLI* K12

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ABSTRACT

Complementation and polarity suppression data are interpreted in terms of the genetic structure of the maltose B region. It is proposed that this region comprises two divergent operons. One operon includes *mal*K, a cistron involved in maltose permeation, and *lam*B the only known cistron specifically involved in λ receptor synthesis. The other operon includes $malJ_1$ and $malJ_2$ which are most probably two different cistrons, both involved in maltose permeation*. It is further assumed that expression of the two operons is controlled by *mal*T, the positive regulatory gene of the maltose system, located in the *mal*A region. The target(s) for the action of the *mal*T product is (are) most likely to be located between $malJ_1$ and *mal*K. There is an indication that the two operons might overlap in the region of their promoters. The structure of such an overlap as well as the possible function of the products of the different cistrons in *mal*B are briefly discussed.

THE genetic system for maltose utilization by *Escherichia coli* K12 comprises two regions of the chromosome, *mal*A and *mal*B (Figure 1) (SCHWARTZ 1966). The activator protein, product of the *mal*T gene located in the *mal*A region, is believed to play a positive regulatory role in the expression and in the induction by maltose of all other activities encoded in these two regions (Figure 2) (SCHWARTZ 1967 a and b; HOFNUNG, SCHWARTZ and HATFIELD 1971). The *mal*A region contains, besides gene *mal*T, an operon composed of *mal*P and *mal*Q the structural genes for maltodextrine phosphorylase (SCHWARTZ and HOFNUNG 1967) and amylomaltase (HATFIELD, HOFNUNG and SCHWARTZ 1969). The promoter and initiator of this operon are located between *malT* and *mal*P (HATFIELD, HOFNUNG and SCHWARTZ 1969; HOFNUNG, SCHWARTZ and HATFIELD 1971; HOFNUNG and SCHWARTZ 1972). The *mal*B region, the genetic structure of which is discussed in this paper, contains all the known genes involved specifically in maltose permeation (SCHWARTZ 1967a; HOFNUNG, HATFIELD and SCHWARTZ 1973) and phage λ adsorption (THIRION and HOFNUNG 1972). An analysis of this region is thus not only relevant to positive regulation but also to two functions of the cell envelope and to possible interactions between them.

Phenotypic characterization and mapping of *mal*B mutations led to the proposal that the *mal*B region consisted of three genes *mal*J *mal*K and *lam*B (HOFNUNG, HATFIELD and SCHWARTZ 1973). *malJ* and *malK* are both involved in

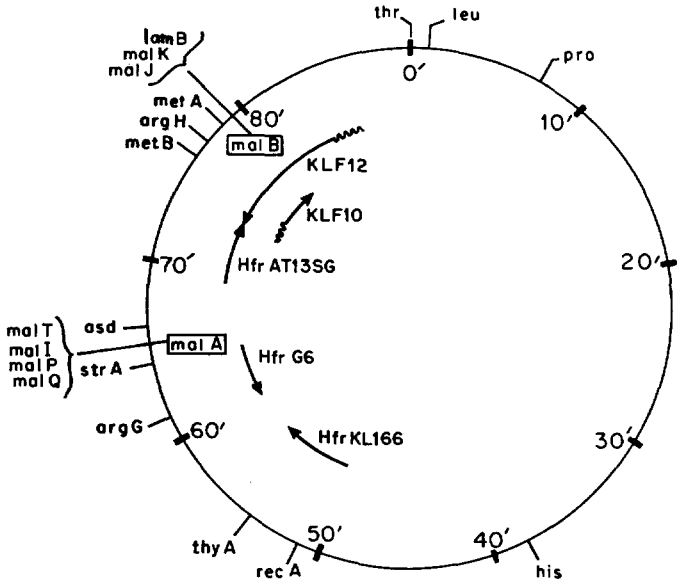


FIGURE 1.—A simplified genetic map of *Escherichia coli* K12 (TAYLOR and TROTTER 1972). Relevant markers, origins of Hfr strains, and extensions of episomes are indicated.

maltose permeation but their inactivation affects the *mal*P *mal*Q operon in different ways: *mal*J mutations result in a silent *mal*P *mal*Q operon, while *mal*K mutations yield, at least in some strains, a partially constitutive expression of this operon* (SCHWARTZ 1967a; THIRION and HOFNUNG 1972). *lam*B was found

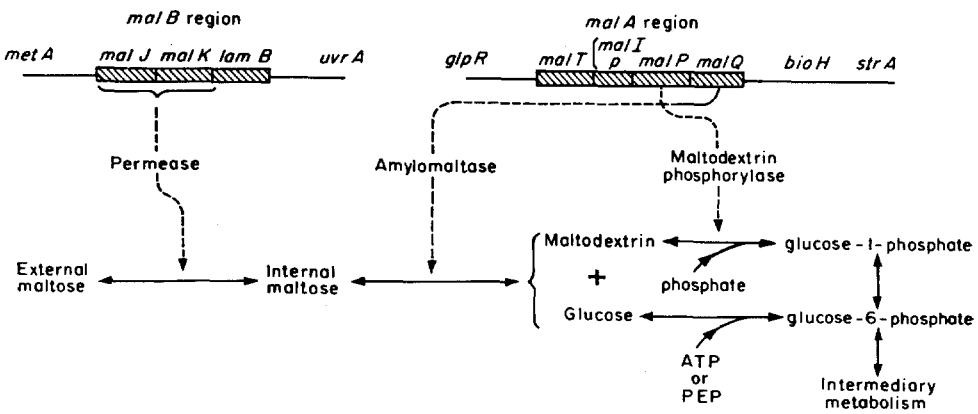


FIGURE 2.—The maltose system in *Escherichia coli* K12. The structure of the *mal*A region was presented previously (HOFNUNG, SCHWARTZ and HATFIELD 1971) and is redescribed briefly in the introduction. The promoter (binding site for RNA polymerase) is denoted p and the initiator (target for positive regulation) is denoted *mal*I. The order of these sites is unknown. The structure of the *mal*B region is discussed in this paper and in HOFNUNG, HATFIELD and SCHWARTZ (1973).

* One possible explanation for such a constitutivity is that *mal*K mutants accumulate an internal inducer of the maltose system. (See DISCUSSION).

to be the only cistron specifically involved in the synthesis of the bacterial receptor sites for phage λ (THIRION and HOFNUNG 1972). It has been suggested that *mal*K and *lam*B constitute an operon of polarity *mal*K→*lam*B (HOFNUNG, HATFIELD and SCHWARTZ 1973). Since the λ receptors are inducible by maltose, it can be assumed that expression of this operon is controlled by the *mal*T product. This assumption also accounts for the inducibility of maltose permease. Previous data do not allow one to say whether *malJ* expression is or is not inducible by maltose.

Studies of complementation between Mal⁻ mutations reported here lead me to propose that gene *mal*J consists of two cistrons *mal*J_1 and *mal*J_2. Maltose permeation would thus involve three cistrons of the *mal*B region, *mal*J_1, *mal*J_2 and *mal*K. The results obtained allow me also to present a model for the genetic structure of the *mal*B region and to suggest a location for the target of positive regulation in this region.

MATERIALS AND METHODS

Abbreviations, media and techniques for genetic transfers have been described already (HOFNUNG and SCHWARTZ 1972; HOFNUNG, HATFIELD and SCHWARTZ 1973). Bacterial strains are listed in Table 1.

Construction of mal*B strains for complementation tests:*

Since the episomes used recombine at high frequency with the chromosome in Rec+ strains, all complementation tests were performed in *rec*A strains.

a) *Construction of* mal*B* rec*A strains*

Most of the *mal*B mutations (Table 2; Figure 3) were introduced into strain pop 356 by exchange with the *met*A marker. This was done either by conjugation or by P_1 transduction. In the first case one *mal*B *arg*H *his thy*A recombinant was kept. In the second case one *mal*B *arg*H *his thy*A *asd* transductant was kept and the *asd* marker was crossed out with Hfr AT13SG. Then each of the *mal*B *arg*H *his thy*A strain populations 600 to 645 in Table 1 was crossed with Hfr KL166. One *rec*A *mal*B *arg*H *his* recombinant (pop 550 to 595) was used as recipient in complementation studies and one *rec*A+ *mal*B *arg*H *his* recombinant (pop 500 to 545) was used as recipient in recombination studies.

b) *Transfer of a chromosomal* mal*B mutation to the episome F′ KLF12*

The *mal*B *arg*H *his thy*A strains (pop 600 to pop 645) were crossed with Hfr AT13SG and one *mal*B *arg*H *str*+ derivative (pop 650 to 695) was kept in each case. The F′ KLF12 episome was introduced from strain pop 422 (KLF12) into each of the *mal*B *arg*H *str* strains by conjugation. Selection was done on glu B_1 agar plates. Two hundred colonies of recipient bacteria harboring the F′ (F ductants) from each cross were then, without reisolation, streaked in small square patches on the same medium (plates called "A"). After 36 hours of incubation at 37° the plates "A" were replicated onto EMB maltose agar plates. The patches on EMB maltose were incubated 8 hours at 37° and checked by replica for (a) transfer of the *arg*H+ marker into strain pop 424 (glu B_1 thy str agar plates seeded with pop 424); (b) growth on maltose (EM mal agar plates); (c) sensitivity to streptomycin (MLstr agar plates). On plates "A" about 25% of the patches were Mal⁻ and around 10% of those contained an autonomous F′ *arg*H+ which was considered as a candidate *mal*B derivative of F′ KLF12. Among the Mal+ patches 65% contained an autonomous F′ *arg*H+. The proportions of the different types of patches were about the same for the different *mal*B mutations studied.

The putative *mal*B derivatives of F′ KLF12 were picked on the glu B_1 thy str plates and reisolated at least twice on the same medium. They were kept in *arg*H+ F ductants of strain pop 424. All the candidates were found to have the characteristics expected from *mal*B derivatives of F′ KLF12. In particular, it was checked in each case that the *mal*B mutation carried by the episome did not recombine with the corresponding chromosomal mutation it came from, but

TABLE 1

Bacterial strains

Strain	Mating type	Relevant markers (except maltose)	Maltose genotype
Hfr G_6	Hfr	*his*	Mal^+
AT13SG *	Hfr	*thr leu*	Mal^+
KL166	Hfr	*recA* rif^r	Mal^+
pop422(KLF12) †	F'	chromosome : *argH pro recA str*	*malB*Δ101
		episome : $argH^+$	$malB^+$
JC1553(KLF10) **	F'	chromosome : *argG metB leu his recA str*	*malA*
		episome : $metB^+$	$malB^+$
pop459 to 470 ††	F'	chromosome : *argH thyA recA str*	*malB*Δ101
		episome : $argH^+$	one of the *malB* alleles studied
pop356	F^-	*asd argH metA his thyA* rif^r *str*	
pop 424	F^-	*argH thyA recA str*	*malB*Δ101
pop650 to 695	F^-	*argH*	one of the *malB* mutations listed in Table 2
pop600 to 645	F^-	*argH thyA his str*	"
pop550 to 595	F^-	*argH his recA str*	"
pop500 to 545	F^-	*argH his str*	"
DM 33 ***	F^-	*trp* E9851 *thyA*	Mal^+
DM 1013 ***	F^-	*suA* Δ (lac pro)	*malA*
pop804	F^-	*argH metA trp*E9851 *his thyA ilv str*	Mal^+
pop815	F^-	*argH metA trp*E9851 *his thyA str*	Mal^+
pop817	F^-	*argH metA trp*E9851 *his thyA suA str*	Mal^+
pop900 to 912	F^-	*argH trp*E9851 *his thy str*	one of the *malB* mutations listed in Table 2
pop913 to 925	F^-	*argH trp*E9851 *his thy suA str*	"
pop931 to 950	F^-	*argH trp*E9851 *str*	"
pop951 to 970	F^-	*argH trp*E9851 *recA str*	"
pop931 bis to 950 bis	F^-	*argH trp*E9851 *suA str*	"
pop951 bis to 970 bis	F^-	*argH trp*E9851 *recA suA str*	"

All strains require vitamin B_1. Details about their construction are given in MATERIALS AND METHODS.

* *str*+ (λ−) derivative of Hfr AT13.

† Episome from Dr. B. Low.

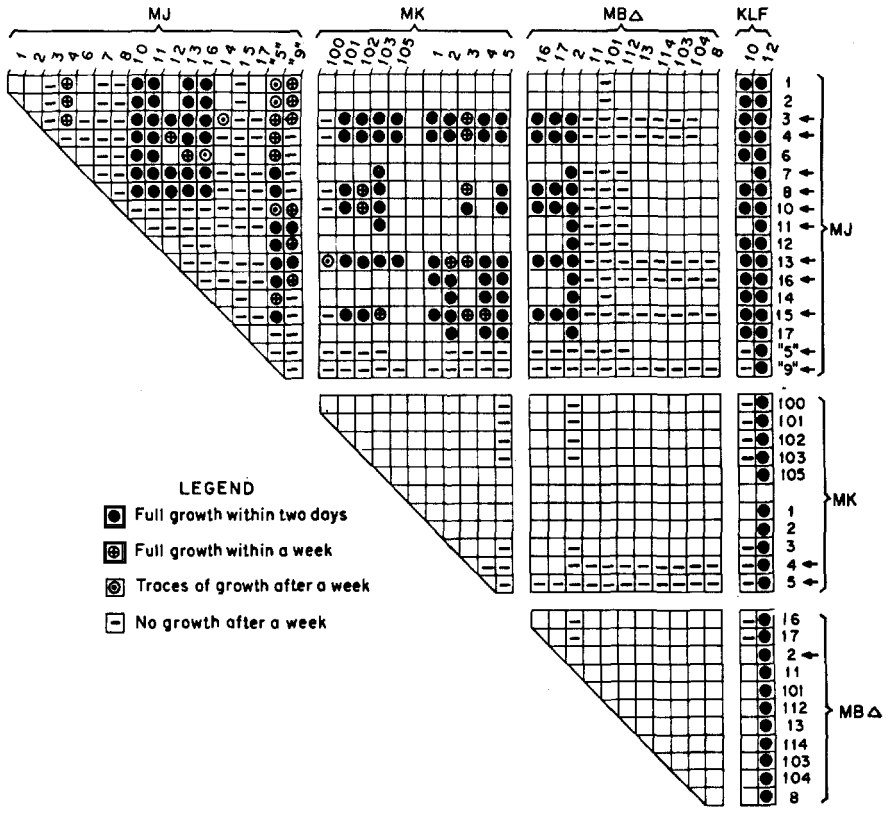
** From Dr. B. Low.

†† The *malB* mutations which carried an F' episome are marked by an arrow on Table 2 (pop468 carries wild type KLF12).

*** Obtained from Dr. Sunshine.

TABLE 2

Complementation between Mal⁻ mutations of the malB *region in an* su*A+ background*



The mutations have been separated in three classes *mal*J *mal*K *mal*BΔ (noted here as MJ,MK and MBΔ). KLF10 and KLF12 are the two parental episomes used. KLF10 carries the Mal-λr mutations *mal*B15;KLF12 has a wild-type *mal*B region. *mal*"J5" and *mal*"J9" are probably double mutations. Mutations which have been transferred on F'KLF12 have been marked by an arrow (right column). The complementation pattern of all the mutations tested was qualitatively the same whether the mutations were carried by the chromosome or by an episome. Full growth within a week was considered as indicative of complementation. In the cases when only traces of growth were detected after one week (here 6 cases out of 406 tested) the results were considered to be doubtful.

recombined with most of the other *mal*B mutations. When the original mutation was of the nonsense type, it was shown that the mutation of the episome was also of the nonsense type.

c) *Mutations used in complementation tests*

The F⁻ strains carried *mal*B mutations yielding either a λ sensitive (λs) or a λ resistant (λr) phenotype (Table 2). Among the λs *mal*B mutations used, fifteen mapped in *mal*J (*mal*J mutations), five in *mal*K (mutations *mal*K1 to *mal*K5) and two have been tentatively considered as double mutations affecting *mal*J and *mal*K (mutations *mal*"J5" and *mal*"J9"). Among the λr *mal*B mutations five were revertable and mapped in *mal*K (mutations *mal*K100 to *mal*K103, and *mal*K105) and eleven were deletions inactivating *mal*K and for most of them cutting in *mal*J or *lam*B or both (*mal*BΔ mutations). The episomes used included fourteen derivatives of F'KLF12 carrying one of the above mutations (marked by an arrow on Table 2) and F'KLF10 which

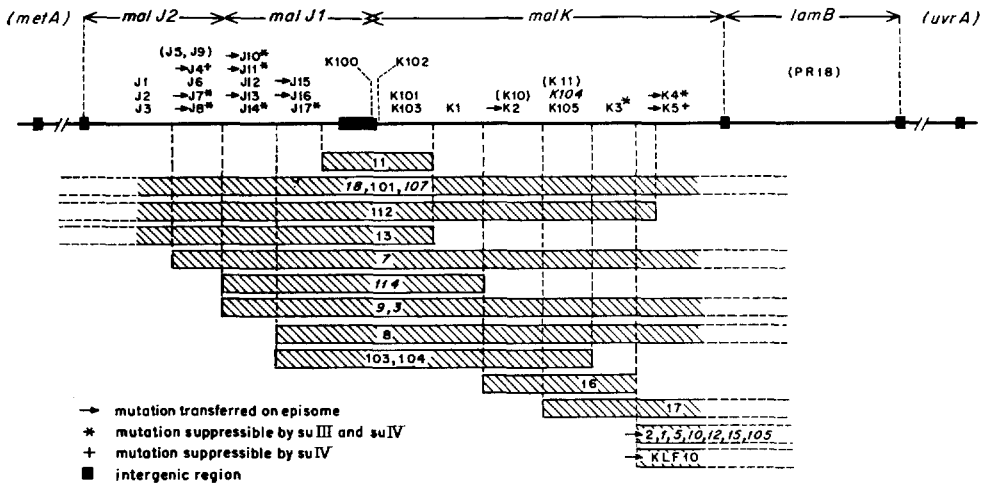


FIGURE 3.—A map of the *maltose* B region. The map takes into account complementation data presented in this paper. In particular these were used to order the markers *mal*K100 *mal*K102 and *mal*K101. Mutations *mal*"J5" and *mal*"J9" were tentatively classified as double mutations affecting at the same time *mal*J and *mal*K (HOFNUNG, HATFIELD and SCHWARTZ 1973). Their complementation patterns agree with this idea they have been renamed *mal*J5, *mal*K10 and *mal*J9, *mal*K11, respectively. *mal*J and *mal*K mutations have been noted J and K, respectively. Hatched bars figure *mal*BΔ deletions. The deletions have been represented by their numbers. KLF10 indicates the *mal*B15 mutation carried by F'KLF10. Italicized numbers stand for mutations which were not studied by complementation. An asterisk indicates that a mutation is suppressible by *su*IV. A + indicates that a mutation is suppressible by *su*IV but not by *su*III.

TABLE 3

Restoration of lam*B activity by* su*A in* λ*r* mal*B mutants*

*mal*B allele	*su*A$^+$ background	*su*A background
*mal*J8 (λs control)	1	1
*mal*BΔ11	$< 10^{-10}$	$< 10^{-10}$
*mal*K100	$< 10^{-10}$	10^{-3}
*mal*K101	$< 10^{-10}$	10^{-3}
*mal*K102	$< 10^{-10}$	1
*mal*K103	$< 10^{-10}$	1
*mal*BΔ16	$< 10^{-10}$	10^{-4}

Dilutions of a stock of a virulent mutant of λ(λv) were spotted on lawns of the different mutants seeded on ML agar plates by means of soft top agar. The figures indicate the approximate efficiency of plating of λv on the mutants determined according to the highest dilution which gave rise to lysis. Efficiency of plating on λs control (*mal*J8) was taken as one. Phage λvh80 plates with the same efficiency on all those strains.

carries the *mal*B mutation *mal*B 15 (ref. in HOFNUNG, HATFIELD and SCHWARTZ 1973). F'KLF12, which complements to Mal+ all the *mal*B mutations studied, was always used as a control.

d) *Construction of quasi-isogenic* su*A*+ *and su*A mal*B strains*

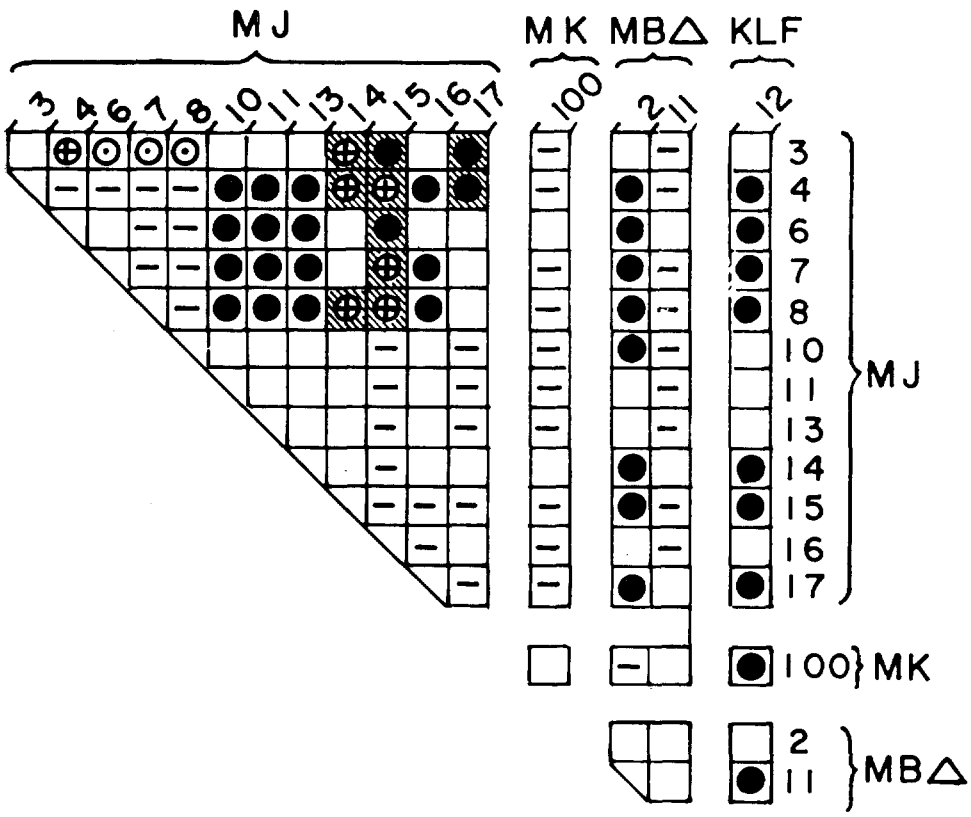
The polarity resulting from the ochre mutation *trp*E9851 renders the strains which harbor it sensitive to inhibition by high concentration of anthranilate (100 μg/ml) unless they carry the *su*A suppressor (MORSE and PRIMAKOFF 1970). All the strains used for polarity suppression studies carried mutation *trp*E9851 so that resistance to growth inhibition by anthranilate was a test for the *su*A genotype.

The mutations (listed in Table 4) which played a critical role for the interpretation of the data were transferred into a different genetic background of two quasi-isogenic strains, one of which carried the polarity suppressor *su*A. The relief of polarity for *lam*B was tested directly by looking at the ability of the *su*A strain to support phage λ growth (Table 3). The relief of polarity for other cistrons was looked at by complementation. In the two *su*A+ strains the same results were found (Table 2). The results obtained in presence of *su*A were different (Table 4) and constitute a critical test for the model proposed.

The two quasi-isogenic Mal+ λs strains, one *su*A+ (pop 815) and one *su*A (pop 817) were constructed by transducing strain pop 804 to Ilv+ with a P_1 stock grown on strain DM1013. pop

TABLE 4

Complementation between Mal⁻ mutations of the mal*B region in a* su*A background*



The crosshatching indicates results which show a clear effect of *su*A.

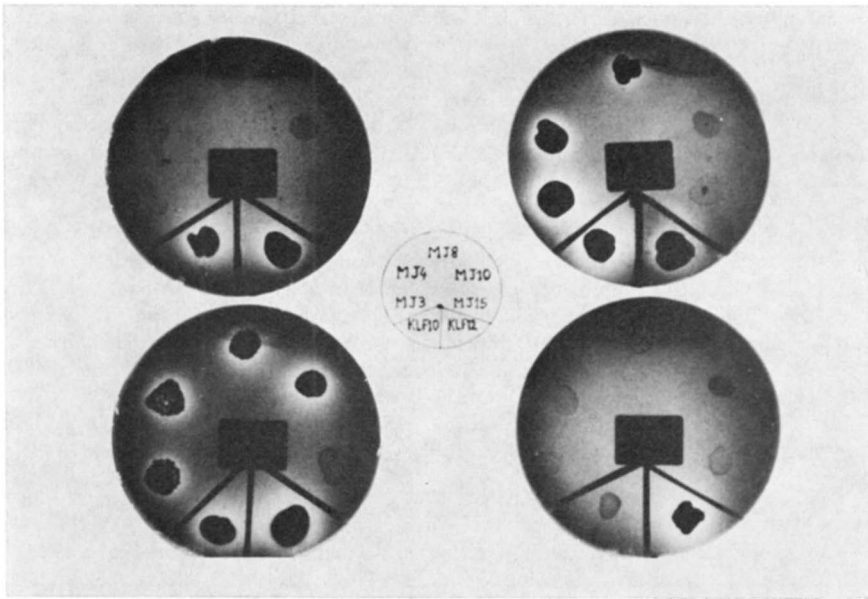


FIGURE 4.—Complementation and recombination in the *mal*B region determined by spot tests. EM MalB$_1$ trp agar plates were seeded, respectively, with F⁻*mal*J15 *su*A+ *rec*A (top left), *mal*J15 *su*A *rec*A (top right) *mal* J15 *su*A+ *rec*A+ (bottom left), *mal*K100 *su*A *rec*A (bottom right). One drop of each donor strain was spotted as described in MATERIAL AND METHODS. The seven donor strains were harboring F'KLF12, F'KLF10 or derivatives of F'KLF12 carrying the *mal*B mutations *mal*J3, *mal*J4, *mal*J8, *mal*J10, *mal*J15, respectively. *mal*J15 recombines with all other mutations used here. *mal*J15 complements mutations of the *mal*J$_2$ cistron only in the presence of *su*A. *mal*K100 does not complement any of the mutations tested.

815 and pop 817 were transduced to *met*A+ *mal*B with P$_1$ phage grown on different *mal*B mutants. The *mal*B *su*A+ (pop 931 to 950) and *mal*B *su*A (pop 931 bis to 950 bis) derivatives were then made *rec*A+ *thy*A+ *his*+ (pop 951 to 970) and *rec*A *thy*A+ *his*+ (pop 951 bis to 970 bis) by crossing with Hfr KL166 for the purpose of recombination and complementation studies.

Spot test for complementation

Complementation tests were performed by spot test (Figure 4). The F⁻ strain was grown overnight to saturation in ML medium. The F′ strain was grown overnight to saturation in minimal glucose medium supplemented with thymine and vitamin B1. Such a medium ensures selection for the presence of the episome since the chromosome carries the *arg*H marker while the episome carries the *arg*H+ allele. In the morning the culture of F′ strain was diluted tenfold in ML medium, and grown at 37° until it reached a density of about 5×10^8 bacteria/ml. It was centrifuged and resuspended in the same volume of M63 medium. One drop was then spotted on each of two plates seeded with 0.3 ml of the F⁻ strain culture. The first plate contained minimal glucose agar medium supplemented with histidine or tryptophan and vitamin B1; it indicated the efficiency of episome transfer. The second plate contained a minimal EM maltose agar medium supplemented with histidine or tryptophan and vitamin B1; it indicated if complementation to Mal+ occurred. In the cases when complementation to Mal+ occurred, two Mal+ F ductants were isolated from the spots and were shown to bear the expected genetic characteristics. Isolation of colonies from the spots proved possible only when full growth occurred. Traces of growth in the spots were not interpreted as clearcut proof for or against complementation (Table 4; Table 5).

Remarks on the interpretation of complementation and polarity suppression data:

A summary of the different cases encountered in the analysis of complementation data is given below. It is meant to facilitate the reading of interpretations presented in this paper.

1) *Complementation groups and the number of cistrons*

The *cis-trans* test (BENZER 1957) was designed to reveal units of functions. In the system originally studied, each cistron corresponded to a single complementation group and to a single polypeptide chain. This classical situation is at the origin of the aphorism "one cistron—one polypeptide chain". However, such a phrase must be used carefully: the number of complementation groups does not always reflect so simply the number of "classical cistrons."

On the one hand two mutations may affect the genetic determinant for two different polypeptide chains—i.e., two different classical cistrons—without complementation being found in the *trans* test. This can happen if the unit of function is an enzymatic complex comprising the two polypeptide chains and if one of the mutations is dominant (negative complementation): then one expects to find no complementation in the *cis* test either. It can also happen if the unit of function is a unit of expression—an operon—comprising the two genetic determinants involved and if one of the mutations is polar. Then, one expects the polar mutation to be more proximal to the promoter of the operon than any mutation of the other classical cistron. Moreover, such a polar mutation should at least in some cases complement mutations in the other classical cistron in the presence of a polarity suppressor.

On the other hand, two mutations may complement although they both affect the determinant for a single polypeptide chain (CRICK and ORGEL 1964; GILLIE 1966). This intracistronic complementation is generally less efficient than intercistronic complementation. Moreover, it should generally not occur if the two mutations are of the nonsense types (or if one of the two mutations is an extensive deletion covering the site of the other mutation) even in the presence of a polarity suppressor.

The term cistron is used throughout the present paper to designate the genetic determinant for a single polypeptide chain—a "classical cistron."

2) *Polarity suppression*

The polarity suppressor *su*A (BECKWITH 1963; MORSE and PRIMAKOFF 1970; MORSE and GUERTIN 1972) restores the activities corresponding to genes distal to most polar mutations without suppressing the mutation itself. The efficiency of restoration varies with the system but for polar nonsense mutations it does not depend on the type of nonsense codon nor on its map position in the mutated gene (CARTER and NEWTON 1971).

RESULTS

Complementation tests were performed by introducing *mal*B episomes into F⁻ *mal*B *rec*A strains and by looking at the ability of the resulting merogenotes to use maltose as a sole carbon source (MATERIALS AND METHODS; Figure 4). Some of the tests were also performed in the presence of the polarity suppressor *su*A (MORSE and PRIMAKOFF 1970) in order to detect a possible polarity effect of the mutations. Whether *su*A is present or not, the complementation data (Tables 2 and 4) can be organized into linear complementations maps (Figures 5 and 6) which are colinear with the genetic map. The complementation maps are interpreted below in terms of the structure of the *mal*B region.

The malK lam*B operon*

a) *Complementation*

In almost all the cases tested (the only exception is *mal*K100; see below) *mal*J and *malK* revertable mutations complement each other. Also, deletions cutting

into *mal*K but not into *mal*J complement all *mal*J revertable mutations tested. This brings further support to the idea that *mal*J and *mal*K are different genes. Since mutations affecting *mal*K were never found to complement each other, *mal*K is likely to be a single cistron.

b) *Polarity suppression*

Four λr *mal*K revertable mutations as well as a deletion whose site is included in *mal*K(*mal*BΔ16) were tested for suppression by *su*A. The *su*A allele does not restore (at least to a detectable amount) *mal*K activity since the *su*A *mal*K strains are still Mal⁻. However with all five mutations the *su*A allele restores *lam*B activity, although to different degrees (Table 3). This shows that *mal*K and *lam*B are indeed two different cistrons, as already suggested, and that they belong to an operon of polarity *mal*K→*lam*B.

The fact that the smallest known deletion (*mal*BΔ11) cutting out the region between *mal*J and *lam*K is insensitive to *su*A for *lam*B expression (Table 3) brings further support to the idea that the promoter of the *mal*K *lam*B operon is located there.

The malJ *"gene"*

The mutations mapped within *mal*J build up five complementation groups (Figure 5), the properties of which provide information about the mode of expression of the *mal*J gene (see remarks in MATERIALS AND METHODS).

a) *Number of cistrons*

Group (3) and (4) complement with good efficiency, although they both

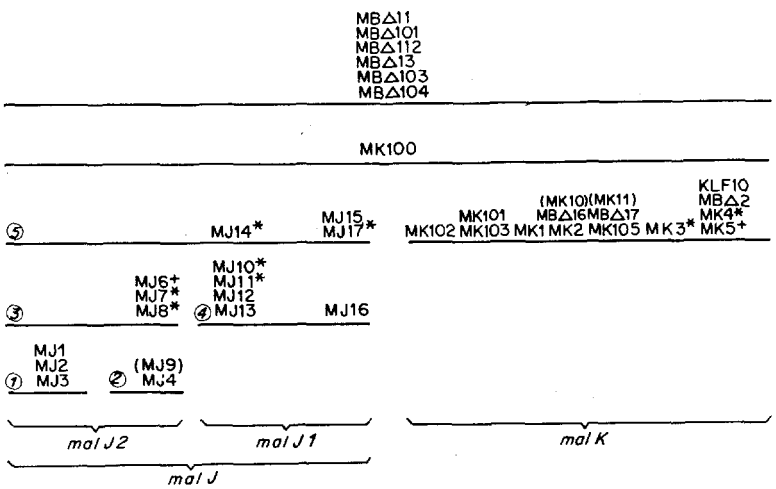


FIGURE 5.—Complementation map of the *mal*B region in an *su*A+ background. This map is based on the data of Table 2. Mutations have been noted from left to right in the order of the genetic map. The name of the deletions which enter into both *mal*J and *mal*K have been written in the intergenic region. The first straight line drawn under the name of a mutation covers the names of all other mutations which do not complement with it. Identification of the mutations indicated between brackets is not complete. Legend as in Figure 3, except that *mal*J, mal*K* and *mal*BΔ mutations are denoted here as MJ, MK and MBΔ, respectively.

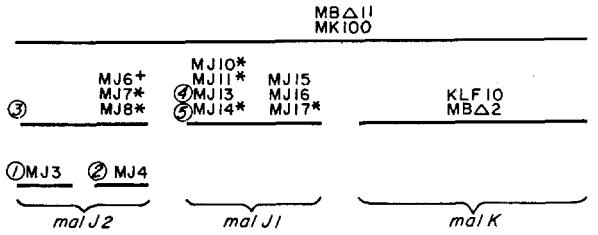


FIGURE 6.—Complementation map of the *mal*B region in an *su*A background. This map is based on the data of Table 4. Notations as in Figure 5. Complementation groups (5) and (4) are now indistinguishable. Complementation groups (3) and (1) are still represented as different, although a very weak growth was detected when complementation between mutation *mal*J3 and mutation of group (3) was tested in an *su*A strain (see text).

contain nonsense mutations. I propose that they correspond to two different cistrons which I call *mal*J_1 (for group (4)) and *mal*J_2 (for group (3)). Mutations of group (5) should then be *mal*J_1 mutations exerting a polar effect on *mal*J_2 expression. Polarity suppression data confirm this prediction, since in presence of *suA*, mutations of group (5) behave like mutations of group (4) and complement all mutations of groups (1), (2) and (3) (Table 4; Figure 6).

Groups (1) and (2) complement with poor efficiency. While no mutation of these two groups is suppressible by suIII or suIV, the mutations of group (3), which map at the same locus as the mutations of group (2) and do not complement with group (1) mutations, are all of the nonsense type. This leads me to suggest that the complementation between groups (1) and (2) is of intracistronic nature. This hypothesis is compatible with the fact that, in presence of *su*A, only traces of growth are detected in the test between group (1) and (3) mutations (Table 4; Figure 6). These traces are interpreted as a proof of intracistronic, as opposed to intercistronic, complementation.

b) *Polarity*

Polar effects are always exerted from right to left, as represented in Figure 5: group (5) mutations exert polarity on groups (3) and (4) mutations and groups (3) mutations exert polarity on groups (1) and (2) mutations. This suggests strongly that transcription of *mal*J_1 and *mal*J_2 proceeds in the order *mal*J_1–*mal*J_2. This direction corresponds to a counter-clockwise orientation on the *E. coli* genetic map. Since transcription of the *malK* gene was found to progress in the opposite direction, the promoter for *mal*J is likely to be located between *mal*J and *mal*K.

The deletions affecting at the same time *mal*J and *mal*K do not complement any *mal*J revertable mutation (Figure 5). In particular, the shortest of the deletions *mal*BΔ11 does not complement any mutation of the groups (1), (2) and (3): since group (4) includes nonsense mutations recombining with *mal*BΔ11, this gives another argument to say that the polarity of transcription for *mal*J is from right to left (Figure 5) and that deletion *mal*BΔ11 cuts off the promoter for *mal*J. As expected in this hypothesis the complementation pattern of deletion *mal*BΔ11 is not affected by *su*A.

It can therefore be proposed that *malJ* is an operon comprising two cistrons

malJ_1 and *mal*J_2 and that the promoter for this operon is located between *mal*J_1 and *mal*K. Since some intracistronic complementation is found in *mal*J_2, the active product of this gene should be an oligomeric protein (CRICK and ORGEL 1964).

c) *Alternative hypotheses*

Definitive proof of the above proposal requires that the products of *mal*J_1 and *mal*J_2 be purified and shown to be independent polypeptide chains. Meanwhile other possible, although less likely, hypotheses are not eliminated. First, concerning groups (3) and (4) one could, for example, say that between the regions corresponding to those two groups there is an initiation point, but no stop point, for translation. However, in this case, one is still led to postulate that the polypeptide chain corresponding to group (3) can have an existence independent from that of the polypeptide chain corresponding to group (4): the products of *mal*J would be one polypeptide chain corresponding to groups (3) *and* (4) and one chain corresponding to group (3). Second, regarding groups (1) and (3), one could say that the traces of growth detected in complementation tests between these groups (Table 4) could be due, for instance, to an intracistronic initiation point for translation which would be detectable, in strains harboring a mutation of group (3), only in an *su*A background. This initiation point could be preceded by a stop point so that although it seems very unlikely to me, the possibility is not completely ruled out that groups (1) and (2) correspond to two different cistrons (i.e., *mal*J_2 would then consist of two cistrons).

Mutation mal*K100 and the interoperonic region*

Mutation *mal*K100 is a spontaneous and revertable mutation which is therefore probably a point mutation, an insertion, or a small inversion. It inactivates at the same time the *mal*J_1 *mal*J_2 and the *mal*K *lam*B operons. Since none of the other revertable *mal*K mutations abolishes at the same time *mal*J_1 *mal*J_2 expression and since the effect of *mal*K100 is purely *cis* dominant, it appears likely that this mutation is located closer to the *mal*J_1 *mal*J_2 operon than mutations *mal*K101, *mal*K102 and *mal*K103. This remark allows me to suggest for those mutations a more precise order than that obtained from recombination data alone (Figure 3).

The existence of a mutation like *mal*K100 might indicate the existence of an overlap between the *mal*J_1 *mal*J_2 and the *mal*K *lam*B operons. Polarity suppression restores some *lam*B activity in a strain carrying *mal*K100 (but not deletion *mal*BΔ11). It can be tentatively suggested that *mal*K100 acts at least partially at the translational level for blocking the *mal*K *lam*B operon. The fact that no *mal*J_2 activity is found under the same conditions is an indication—but the evidence is negative since a weak restoration of *mal*J_2 activity might not be detected—that *mal*K100 could block transcription or translation initiation for the *mal*J_1 *mal*J_2 operon. The possible structure of the overlap region will be further examined in the DISCUSSION.

DISCUSSION

The genetic structure of the mal*B region*

According to the model proposed, the *mal*B region consists of two operons. One,

*mal*K *lamB*, is oriented clockwise; the other one, *mal*J_1 *mal*J_2, is oriented counterclockwise on the genetic map of *E. coli* K12. The existence of the *mal*K *lam*B operon had already been suggested (HOFNUNG, HATFIELD and SCHWARTZ 1973). It is further supported by the present data, which show that *mal*K and *lam*B are indeed different genes and that *mal*K is probably monocistronic. The other operon —formerly called gene *mal*J—comprises two genes, *mal*J_1 and *mal*J_2, which are also likely to be monocistronic. Recent biochemical evidence is in agreement with the idea that *mal*J_1 and *mal*J_2 are indeed different genes: *mal*J_1 mutants, but not *mal*J_2 mutants, are devoid of an inducible periplasmic maltose binding protein (KELLERMAN 1972). The occurrence of complementation between some *mal*J_2 mutations was taken as an argument to say that the active product of this gene is an oligomeric protein; however, the hypothesis that *mal*J_2 includes more than one cistron has not been completely eliminated.

Expression of the two divergent operons can be abolished by the spontaneous and revertable mutation *mal*K100. This was taken as suggestive of the existence of an overlap between the promoter proximal parts of the operons. Although little experimental data are yet available concerning the interoperonic region one may try to speculate about its structure. Since the λ receptor sites (absent in *lam*B mutants) and the maltose binding protein (absent in *mal*J_1 mutants) are inducible by maltose and are lacking in *mal*T mutants, it can be assumed that both operons are under the positive control of the activator protein, product of the *mal*T gene. This assumption accounts for the fact that no mutant expressing all of the *malB* region functions in absence of *malT* product have been found, although a powerful selection technique was available (HOFNUNG and SCHWARTZ 1972): if they exist, single mutations resulting in a *mal*T-independent expression of both *mal*B operons should be rare.

Three other systems of divergent operons are known. One is found in phage λ (TAYLOR, HRADECNA and SZYBALSKI 1967; SZYBALSKI *et al.* 1969) in which one operon (promoter *pr*) is under negative control of the immunity repressor and the other operon (promoter *prm*) containing the structural gene for the repressor, under positive control of the repressor (EISEN, PEREIRA DA SILVA and JACOB 1968; ECHOLS and GREEN 1971; REICHARDT and KAISER 1971). The two other systems, the *arg* ECBH cluster (ELSEVIERS *et al.* 1972; JACOBY 1972) and the *bio* ABEFGCD cluster (CLEARY, CAMPBELL and CHANG 1972; GUHA, SATURNEN and SZYBALSKI 1971) in *E. coli*, are under negative control. However in the three cases all the data are compatible with the existence of an overlap between the targets for the regulation of each operon. Moreover, in the *arg* ECBH cluster, such an overlap seems very likely since 0^c mutants selected for the constitutive expression of the *arg*C gene are also constitutive for the expression of the *arg*E gene, which is transcribed in a direction opposite to that of the *arg* CBH operon (JACOBY 1972).

Following this remark one may suggest that at least part of the initiators of each operon in the *mal*B region overlap (Figure 7). Other structures for the interoperonic region are possible, especially since the order of promoters and initiators is not known. However, any of them should account for two facts: (1) no recombination was found between mutations *mal*K100 and *mal*K102, which

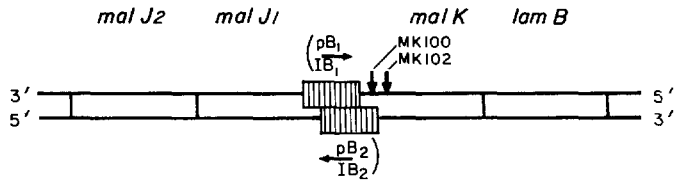


FIGURE 7.—The structure of the *mal*B region. The hatched region represents the promoter initiator complexes for the two divergent operons: pB_1, IB_1 for the *mal*K *lam*B operon and pB_2, IB_2 for the *mal*J_1 *mal*J_2 operon. It is suggested that the two complexes overlap at least partially by their initiator parts. However, other possibilities are not excluded since the order of promoters and initiators is not known.

should therefore be very close to one another; (2) in the presence of *suA* some expression of the *lam*B gene can be detected in a *mal*K100 mutant; mutation *mal*K100 should therefore not abolish totally the activity of the promoter for the *mal*K *lam*B operon. One can also note that the type of structure tentatively proposed would allow two operons to be submitted to a common regulation and at the same time to have different pattrens of expression. For example, the promoters can have different affinities for RNA polymerase, so that the range of induction of the operons could be different, or one of the two operons can be submitted to other regulations. In that respect it appears likely that both the *mal*B operons are sensitive to catabolite repression: λ receptor synthesis (PEARSON 1972; YOKOTA and KASUGA 1972) as well as the periplasmic maltose-binding protein synthesis (SZMELCMAN, unpublished results 1973) are sensitive to catabolite repression.

Function of the malB *region products*

The genes of the *mal*B region are all involved in cell envelope functions: *mal*J_1 *mal*J_2 and *mal*K are involved in maltose entry while *lam*B is implicated in λ receptor synthesis. Despite the limited amount of data available, it is tempting to draw a tentative picture which could serve as a working hypothesis in the study of the functions encoded by the *mal*B region.

The fact that, at least in some strains, *mal*K mutants express the *mal*P *mal*Q operon in a partially constitutive way can be interpreted by saying that in such mutants there is accumulation of an internal inducer which in the wild type can leak out of the cell thanks to the *mal*K product (the K protein). It can therefore be suggested that the K protein is responsible for facilitated diffusion of maltose through the cytoplasmic membrane. The *mal*J_1 product (J_1 protein) is probably a periplasmic maltose-binding protein (KELLERMAN 1972). By analogy with what was found in the transport system for histidine in Salmonella (AMES and LEVER 1970; LEVER 1972), one may suggest a sequential action for the J_1 and K proteins. The *mal*J_2 product (J_2 protein) could then play a role in the energetic coupling for the active transport of matose.

The reason—if any—for the genetic association between gene *lam*B and the maltose permease genes is still obscure. It might reflect a functional association between the products of the *mal*B region: the *lam*B product is presumably the structural protein for the λ receptor (RANDALL-HAZELBAUER and SCHWARTZ

1973; HOFNUNG, unpublished results) which may interact in some way with the permease. It might also reflect a metabolic relation: the *lam*B product could have an enzymatic activity (yet undiscovered) on a precursor or a derivative of maltose. This genetic association might be due to both of these reasons or to some other hidden evolutionary cause.

Further genetic analysis of the region, as well as the *in vitro* characterization of its products, might help answer these questions and elucidate the functions and mode of expression of the products of the *mal*B region.

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Note added in proof: To be more consistent with the recommended genetic nomenclature, the two cistrons *malJ*$_1$ and *malJ*$_2$ will be called *mal*E and *mal*F respectively in subsequent publications.

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