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# Mutations Allowing Growth on Maltose of *Escherichia coli* K12 Strains with a Deleted *mal*T Gene

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*Summary.* Previous work suggests that the *mal*T gene exerts a positive control on two operons. One operon (*mal*B *lam*B) codes for maltose permease and a protein involved in the biosynthesis of cell wall receptors for phage lambda. The other operon (*mal*P *mal*Q) codes for two enzymes of maltose metabolism.

*Mal*T mutants are shown in this paper to recover the ability to grow on maltose if they acquire two additionnal mutations. One mutation (*bym*A) allows the bacteria to take up maltose even when the *mal*B-*lam*B operon is inactive. The other (*mal*$I^c_A$) is a *cis* dominant constitutive mutation allowing some expression (10 to 30% of induced wild type) of the *mal*P *mal*Q operon in absence of *mal*T product.

Some of the *mal*$I^c_A$ mutants retain the ability to be induced with maltose, when a *mal*$T^+$ allele is present. On the other hand, in the absence of inducer, and contrary to what is observed in the L-arabinose system of *E. coli* B, there is no repressive effect of the *mal*$T^+$ allele on the constitutive expression of the *mal*$I^c_A$ mutations.

The results presented here support the hypothesis that the *mal*T product activates transcription or translation of the *mal*P *mal*Q operon.

## Introduction

Two regions of the chromosome of *Escherichia coli* K12 are involved in maltose utilization (Figs. 1 and 2) (Schwartz, 1966). The *mal*A region contains—a) an operon composed of the structural genes (*mal*P and *mal*Q) for two enzymes specifically involved in maltose metabolism.—b) a gene (*mal*T) believed to be the positive regulator of the system (Hatfield, Hofnung and Schwartz, 1969; Hofnung, Hatfield and Schwartz, 1971). The *mal*B region contains one or several genes involved in maltose permeation (Schwartz, 1967a, c; Hofnung, Schwartz, and Hatfield, in preparation). Very close to *mal*B is located a gene, *lam*B, involved in the synthesis of the cell surface receptors specific for phage lambda (Schwartz, 1967b; Thirion, Hofnung and Schwartz, in preparation). There is evidence that *mal*B and *lam*B constitute an operon (Schwartz, 1967b, c; Hofnung, Schwartz and Hatfield, in preparation).

In wild type strains maltose induces the synthesis of amylomaltase (product of *mal*Q) and of maltodextrin phosphorylase (product of *mal*P) (Monod and Toriani, 1950; Schwartz and Hofnung, 1967). It also induces the synthesis of maltose permease (a product of *mal*B) and of the lambda receptors (whose formation requires the product *lam*B) (Wiesmeyer and Cohn, 1960; Schwartz, 1967b). In *mal*T mutants the level of expression of the *mal*P-*mal*Q operon is reduced to 1 to 2% of what it is in the induced wild type strain, and is insensitive to the

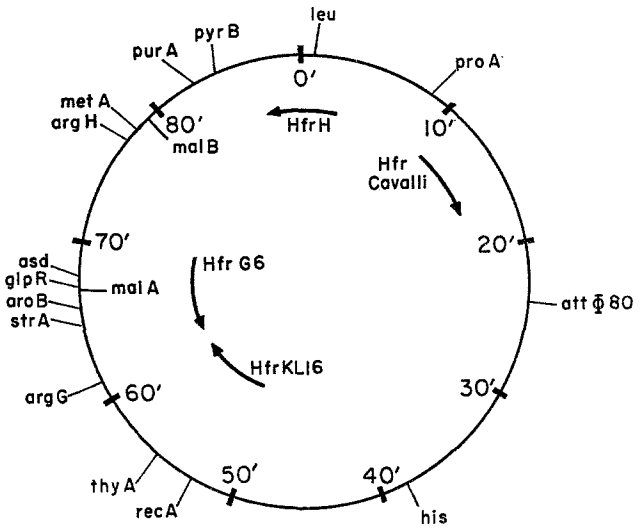


Fig. 1. Location of the *mal*A and *mal*B regions on the genetic map of *E. coli* K12. The drawing is a simplified version of the genetic map of *E. coli* K12 (after Taylor, 1970) graduated in 10 min units and giving the locations of the mutations referred to in this paper. Origins of transfer of Hfr strains are indicated by arrows

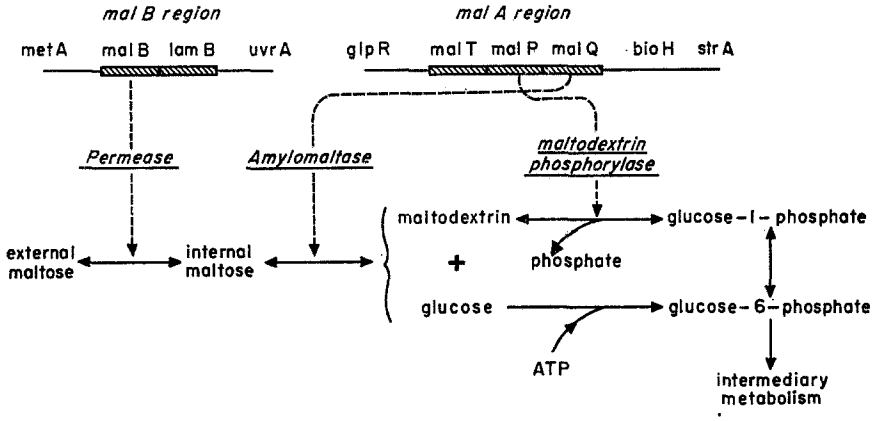


Fig. 2. The maltose system in *E. coli* K12. The structure of the *mal*A region is discussed in this paper. The structure of the *mal*B region was discussed previously (Schwartz, 1967b): gene *mal*B is probably the gene for maltose permease gene *lam*B previously called λ *rec*B (Schwartz, 1967b) is involved in the synthesis of bacterial receptors for phage λ

addition of maltose. The *mal*T mutants are also devoid of maltose permease activity and are resistant to phage lambda. Therefore, the *mal*T mutants have the phenotype which would be expected from a simultaneous inactivation of the *mal*P-*mal*Q operon and of the *mal*B-*lam*B operon. Consequently it is assumed that the *mal*T gene product exerts a positive regulation on these two operons.

Table 1. *Descriptions of strains used*

| Strain | Mating type | Relevant genotype | Observation |
|---|---|---|---|
| pop 338 | F⁻ | *arg*G *met*A *thy*A *his asd str*$^r$ | formely called PA505MS33 (Hofnung *et al.*, 1971) |
| pop 496 | F⁻ | *arg*G *met*A *thy*A *his str*$^r$ | *asd*$^+$ transductant of pop 338 |
| pop 430<br>pop 440<br>pop 441 | F⁻ | *arg*G *met*A *thy*A *his str*$^r$ del (*glp*R *mal*A) | strains carrying respectively the following *glp*R *mal*A deletions: Δ105, Δ3, Δ5. They were obtained by transduction of pop 338 with $P_1$ grown on PA505 MAΔ105, Δ3, or Δ5 (Hatfield *et al.*, 1969) |
| pop 301<br>pop 302<br>pop 303<br>pop 304 | F⁻ | *arg*G *met*A *thy*A *his str*$^r$ del Δ3 (*glp*R *mal*A) *bym*A | independant Mal$^+$ revertants of pop 440 (see text). They respectively, carry *bym*A mutations numbered R1, R2, R3 and R4 |
| pop 305 | F⁻ | *arg*G *met*A *thy*A *his str*$^r$ del Δ5 (*glp*R *mal*A) *bym*A R5 | Mal$^+$ revertant of pop 441 (see text) |
| pop 306 | F⁻ | *arg*G *met*A *thy*A *his leu str*$^r$ del Δ5 (*glp*R *mal*A) *bym*A R6 | Mal$^+$ revertant of pop 441. The *leu* mutation appeared at the same time as the *bym*A R6 mutations (see text) |
| pop 11 | F⁻ | *arg*G *met*A *thy*A *str*$^r$ del Δ3 (*glp*R *mal*A) *bym*A R3 | obtained by transduction of pop 303 to *his*$^+$ with $P_1$ grown in Hfr H |
| pop 12 | F⁻ | *aro*B *met*A *thy*A *str*$^r$ *bym*A R3 | recombinant of a cross between Hfr G61 and pop 11 |
| pop 14<br>pop 15<br>pop 16<br>pop 19<br>pop 20 | F⁻ | *met*A *thy*A *bym*A R3 *str*$^r$ del (*glp*R *mal*A) | strains respectively carrying the following *glp*R *mal*A deletion Δ102, Δ104, Δ105, Δ113, Δ133. They were obtained by transduction of pop 12 with $P_1$ grown on PA 505 MAΔ102, Δ104 etc. (Hatfield *et al.*, 1969) |
| pop 40N | F⁻ | *met*A *thy*A *bym*A R3 *str*$^r$ | obtained by transduction of pop 12 to *aro*B$^+$ with $P_1$ grown on Hfr H |
| Hfr H | Hfr (Hayes type) | *Str*$^s$ | |
| Hfr G61 | Hfr (G6 type) | *Str*$^s$ *his aro*B | |
| Hfr G6 MAΔ105 | Hfr (G6 type) | *Str*$^s$ *his* del Δ105 (*glp*R *mal*A) | obtained by transduction of Hfr G61 with $P_1$ grown on PA 505 MAΔ105 |

Table 1 (Continued)

| Strain | Mating Type | Relevant genotype | Observation |
|---|---|---|---|
| Hfr G6 MAΔ133 | Hfr (G6 type) | *Str*$^s$ *his* del Δ133 (*glp*R *mal*A) | transduction of HfrG 61 with $P_1$ grown on PA505 MAΔ133 |
| Hfr G6 MBΔ101 | Hfr (G6 type) | *Str*$^s$ *his mal*B MBΔ101 | The *mal*B mutation is deletion MBΔ101. It results in a Ma$^-$λR phenotype. It inactivates all known genes in *mal*B (Schwartz, 1967c). |
| S 903 | Hfr | *Str*$^s$ del Δ3 (*glp*R *mal*A) | (Schwartz, 1967c) |
| pop 487 | F′ | chromosome: *str*$^r$ *rec*A *pro arg*H *mal*B (MBΔ101) episome KLF12: *arg*H$^+$ *mal*B$^+$ | episome KLF12 was obtained from Dr. Brooks Low. It extends approximatively from min. 77 to min. 84 |

The symbols delΔ3 (*glp*R *mal*A), delΔ5 (*glp*R *mal*A) etc... refer to deletions like delΔ3, Δ5 etc... described in Fig. 3 and known to extend from *glp*R into somewhere in the *mal*A region (Hatfield *et al.*, 1969).

All the evidence obtained so far favours this hypothesis. It does not conclusively show, however, that the *mal*T gene product *directly* activates transcription or translation of the maltose operons. For instance it would not eliminate models where the *mal*T product would play a role in the inactivation of a hypothetical repressor of the maltose operons. In this paper we describe mutations allowing *mal*T mutants to grow on maltose. The study of these mutations will give strong support to the original hypothesis that *mal*T is a true positive regulator gene, i.e. that the *mal*T product interacts directly with the transcription (or translation) system to allow expression of the maltose operons.

## Material and Methods

### *Abbreviations*

Gene nomenclature is as in Taylor (1970) except for:

*lam*B, which designates a gene characterized by mutations to a λR Mal$^+$ phenotype (Thirion *et al.*, in preparation).

*bym*A, which stands for "bypass of maltose permease", a notation introduced in this paper.

*mal*$I_A$, which designates the initiator of the *mal*P-*mal*Q operon (which is in the *mal*A region). The wild type allele is designated *mal*$I_A^+$ and the constitutive alleles, *mal*$I_A^c$. Deletions in *mal*A will be noted Δ followed by a number (see Fig. 1).

### *Strains*

For bacteria see Table 1.

λV was provided by F. Jacob. The $P_1$ phage used for all transduction is a partially virulent strain given to us by S. Brenner. φ80 *dmal*$_1$ and φ80 *dmal*$_2$ were given to us by D. Schwartz and J. Beckwith. They both carry bacterial genes of the *mal*A region. φ80 *dmal*$_1$ carries *mal*T and *mal*P while φ80 *dmal*$_2$ carries *mal*T *mal*P *mal*Q and *bio*H (Hofnung *et al.*, 1971).

### *Media and Techniques*

All media except TTY were described previously (Hatfield *et al.*, 1969). TTY is M6 3 agar supplemented with 0.2% bactotryptone (Difco) and 0.1% yeast extract (Difco).

*Sexual crosses and enzyme measurements*, were as described in Hatfield *et al.* (1969). Some of the enzyme assays have been performed only once: relative errors can therefore reach, in this paper, about 30% of the values figuring in the tables.

*Transductions:* phage $P_1$ was used for generalised transduction in the following way. Recipient strains were grown in complete medium at 37° C to approximatively $10^9$ cells/ml. $CaCl_2$ was added at a final concentration of $5 \times 10^{-3}$ M and incubation was continued for 20 min. 0.1 ml samples of the culture were then distributed in small tubes and the $P_1$ lysate was added to give multiplicities of about 10, 1, 0.1, 0.01 and 0 (control). Adsorption was allowed to proceed at room temperature for 20 min. 0.2 ml of a 20% sodium citrate was then added and the content of each tube was plated in soft agar on the appropriate selective medium. The plates were incubated at 37° C for 24 to 96 hours and the transductants purified and tested. (Sodium citrate complexes $Ca^{++}$ and thus reduces infection of the transductants by active phage.)

*Technique for detecting amylomaltase activity on plates.* The bacteria are plated on TTY glass Petri dishes. (Glycerol minimal medium can also be used.) The colonies are treated with toluene for 1 hour. The toluene is removed and the plates are dried. A tube containing 3 ml of 0.3% agar in minimal medium, 0.1 ml 20% maltose, 1 ml glucostat, 3 ml chromogen is poured onto the plates. (Glucostat and the corresponding chromogen are from Worthington.) When the colonies contain amylomaltase they will stain orange within 5 to 30 min depending on the amount of enzyme they have. Since the technique is based on the liberation of glucose from maltose it is important not to use a growth medium containing either glucose or a sugar from which the bacteria will produce glucose during growth.

## Results

### *Search for Mal$^+$ Revertants from Strains Carrying a Deletion in the malA Region*

A set of deletions extending from the *glp*R gene into the *mal*A region were previously described (Hatfield *et al.*, 1969; Hofnung *et al.*, 1971). Some of them are shown on Fig. 3. They all lead to a loss of maltose permease and lambda receptors, but they vary in their effect on the *mal*P-*mal*Q operon. The long deletions (Δ113 and Δ133)—but not the short ones (Δ102, Δ104, Δ105)—have a polar effect (*cis* dominant) on the expression of the *mal*Q gene. This was taken as evidence that all of the controlling elements for the operon are located between *mal*T and *mal*P. Deletions Δ3 and Δ5 probably end inside the controlling elements of the operon. Strains carrying these deletions are partially constitutive for the expression of genes *mal*P and *mal*Q, located in *cis* position. Haploïd strains carrying Δ3 or Δ5 are not induced by maltose. When a *mal*T$^+$ allele was introduced on an episome, the operon adjacent to Δ3 was fully induced when maltose was added. The same was not true for Δ5. To explain the properties of Δ3 and Δ5 it has been suggested that both deletions fuse the *mal*P-*mal*Q operon with another unknown operon (Hatfield *et al.*, 1969) and that Δ3 preserves at least partially the controlling elements of the *mal*P-*mal*Q operon, while Δ5 does not (Hofnung *et al.*, 1971) (see Fig. 3).

Mal$^+$ revertants were sought from strains carrying the deletions shown in Fig. 3. In most cases no revertants could be found. The only exceptions were for strains carrying Δ3 or Δ5. In this case, after EMS mutagenesis, revertants could be found, at a frequency of about $10^{-6}$. The results in Table 2 show that

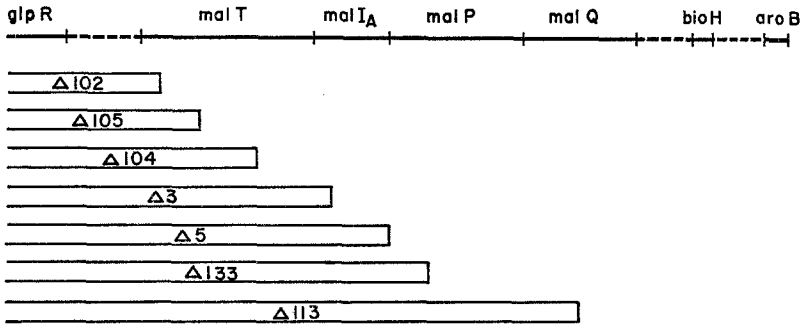


Fig. 3. The *mal*A region of *E. coli*. The map is a simplified version of that published in Hatfield *et al.* (1969). *mal*$I_A$ is defined in this paper as the initiator of the *mal*P-*mal*Q operon. The other genes are as in Taylor (1970). The extent of the deletions used in this paper is shown below the map. Deletion Δ5 is drawn longer than Δ3 because of complementation results (Hofnung *et al.*, 1971) showing that Δ3, but not Δ5, has retained sensitivity to positive regulation by the *mal*T product

these revertants can take up $^{14}C$ maltose, much more than the original strain but still less than the wild type strain. Independent mutants with similar properties have been isolated by D. Hatfield (personal communication).

### *The Mal$^+$ Revertants Carry a Mutation (bym) Leading to a Bypass of the mal*B *Gene Product(s)*

The recovery of the ability to take up maltose exhibited by the revertants described in Table 2 could result from either of two mutational events.

1) a mutation allowing the normal maltose permease to be synthesized in the absence of *mal*T product.

2) a mutation unmasking an independent permeation system with some affinity for maltose.

Two lines of evidence favour the second hypothesis. First, all the revertants are still resistant to phage lambda. If the recovery of the ability to take up maltose was due to an escape of the *mal*B gene(s) from *mal*T control, one would expect *lam*B to escape also from this control, and the revertants to be sensitive to lambda. A stronger argument is that the mutations (thereafter called *bym*, standing for "bypass maltose permease") acquired by the revertants allow growth on maltose of strains which carry a deletion of the *mal*B region.

This was shown as follows: two strains (pop 305 and pop 306) carrying the Δ5 deletion in the *mal*A region, and a *bym* mutation (respectively *bym*A R5, and *bym*A R6) were first lysogenized with $\phi 80\ dmal_2$, and thus rendered sensitive to phage λ. These lysogens were then transduced to Met$^+$ with a $P_1$ stock grown on Hfr G6 MBΔ101, a strain with a deleted *mal*B region. As expected from the cotransducibility of *met*A and *mal*B about 5% of the Met$^+$ transductants were λR because they had acquired the MBΔ101 deletion. All of them were Mal$^+$. Several sexual crosses involving strains with other *bym* mutation also demonstrated that *bym* mutations suppress the Mal$^+$ phenotype normally resulting from the MBΔ101 deletion.

Table 2. *Enzymic characterization of strains carrying the Δ3 or Δ5 deletions in the* mal*A region and a* bym *mutation*

Pop 301 to pop 304 are independent EMS induced Mal$^+$ revertants of pop 440. Pop 305 and pop 306 are revertants from pop 441.

All enzyme activities are expressed as percent of those obtained in the induced wild type strain. The induced wild type strain (pop 496) contains 190 u/mg amylomaltase 160 u/mg phosphorylase, and accumulates 280 nanomoles of maltose per mg protein and per hour. It must be recalled that the $^{14}C$ maltose accumulation—which is measured over 30 min, a period during which it is linear—does not reflect solely the "permease" activity but a combination of permease activity and further metabolism of the sugar.

| Strain | Maltose genotype | Amylomaltase | | Phosphorylase | | Permease | |
|---|---|---|---|---|---|---|---|
| | | un-induced | induced | un-induced | induced | un-induced | induced |
| pop 496 | wild type | 5 | 100 | 4 | 100 | 14 | 100 |
| pop 430 | Δ105 | <1 | <1 | <1 | <1 | 1 | 2 |
| pop 440 | Δ3 bymA$^+$ | 22 | 24 | 23 | — | 0,5 | 9 |
| pop 301 | Δ3 bymAR$_1$ | 21 | 18 | 18 | — | 16 | 16 |
| pop 302 | Δ3 bymAR$_2$ | 24 | 26 | 19 | — | 55 | 57 |
| pop 303 | Δ3 bymAR$_3$ | 25 | 27 | 21 | — | 43 | 41 |
| pop 304 | Δ3 bymAR$_4$ | 21 | 29 | 22 | — | 14 | 14 |
| pop 441 | Δ5 bymA$^+$ | 21 | 18 | 24 | — | 1 | 4 |
| pop 305 | Δ5 bymAR$_5$ | 22 | 27 | 26 | — | 39 | 36 |
| pop 306 | Δ5 bymAR$_6$ | 29 | 28 | 25 | — | 48 | 57 |

### *Some Properties of the bym Mutations*

The six *bym* mutations are linked with *met*A. In crosses between each of the six *bym* strains described in Table 2 with a Cavalli type Hfr strain (S 903), about 70% of the Met$^+$ Str-r recombinants are Mal$^-$, i.e. have lost the *bym* mutation

*bym* is carried by episome KLF12. The *bym* allele is dominant over the corresponding wild type allele. KLF12 was introduced into the six *bym* strains of Table 2. The merodiploids selected as Met$^+$, remained Mal$^+$ like the haploid. In every case, however, Mal$^-$ homogenotes spontaneously appeared in the merodiploid cultures, a result indicating that the *bym*$^+$ allele is carried by KLF12, but that *bym* is dominant over *bym*$^+$.

As all the six *bym* mutations studied have similar locations and phenotypic expressions, we suggest that they define a single locus which will be called *bym*A in the rest of this paper.

The *bym*A locus is located between *mal*B and *leu*. A *mal*B derivative of Hfr G6 was crossed with a *met*A *bym*AR6 *leu* F$^-$ strain. Out of 40 recombinants which acquired both the *mal*B and *leu*$^+$ alleles of the male, 32 also acquired its *bym*$^+$ allele (32 Mal$^-$ among the 40 λR Leu$^+$) while among the 54 recombinants which acquired the *mal*B allele of the male, but not its *leu*$^+$ allele (λR Leu$^-$) only 11 acquired its *bym*$^+$ allele (i.e. are Mal$^-$). This last figure, as well as the gradient of transmission between *met*A, *mal*B, *bym*A, and *leu* (out of 96 *met*A$^+$

Table 3. *Enzymic characterization of strains with a deleted* mal*T gene, and a* mal$I_A^c$ *mutation*

The wild type strain is pop 40N. Pop 22 to pop 33 are independant Mal$^+$ revertants of pop 14; pop 34 to pop 42 are revertants of pop 15; and pop 43 to pop 52 are revertants of pop 16. All enzyme activities are expressed as percent of those found in the induced wild type strain, which contains 150 u/mg amylomaltase and 165 u/mg phosphorylase.

| Strain | Maltose genotype | Amylomaltase | | Phoshorylase | |
|---|---|---|---|---|---|
| | | un-induced | induced | un-induced | induced |
| pop 22 | $I^c_{22}$ Δ102 | 29 | — | 27 | 23 |
| pop 24 | $I^c_{24}$ Δ102 | 19 | 22 | 21 | 23 |
| pop 25 | $I^c_{25}$ Δ102 | 24 | 12 | 12 | 13 |
| pop 26 | $I^c_{26}$ Δ102 | 19 | 19 | 14 | 28 |
| pop 28 | $I^c_{28}$ Δ102 | — | 14 | 14 | 16 |
| pop 29 | $I^c_{29}$ Δ102 | 16 | 16 | 16 | 18 |
| pop 30 | $I^c_{30}$ Δ102 | 13 | 15 | 19 | 11 |
| pop 31 | $I^c_{31}$ Δ102 | — | — | 12 | 11 |
| pop 32 | $I^c_{32}$ Δ102 | 19 | 20 | 19 | 22 |
| pop 33 | $I^c_{33}$ Δ102 | — | — | 25 | 24 |
| pop 34 | $I^c_{34}$ Δ104 | 22 | 18 | 15 | — |
| pop 35 | $I^c_{35}$ Δ104 | — | — | 24 | 26 |
| pop 36 | $I^c_{36}$ Δ104 | 21 | 28 | 18 | 22 |
| pop 37 | $I^c_{37}$ Δ104 | — | 15 | 14 | 20 |
| pop 38 | $I^c_{38}$ Δ104 | — | 16 | 20 | — |
| pop 39 | $I^c_{39}$ Δ104 | — | — | 26 | 32 |
| pop 40 | $I^c_{40}$ Δ104 | — | — | 14 | 17 |
| pop 41 | $I^c_{41}$ Δ104 | — | 15 | 11 | 17 |
| pop 42 | $I^c_{42}$ Δ104 | 12 | 25 | — | — |
| pop 43 | $I^c_{43}$ Δ105 | 11 | — | 15 | 13 |
| pop 44 | $I^c_{44}$ Δ105 | 6 | 16 | — | 19 |
| pop 45 | $I^c_{45}$ Δ105 | 18 | 19 | 21 | 22 |
| pop 46 | $I^c_{46}$ Δ105 | 17 | 10 | 8 | 11 |
| pop 47 | $I^c_{47}$ Δ105 | — | 10 | 11 | 20 |
| pop 48 | $I^c_{48}$ Δ105 | — | — | 14 | 14 |
| pop 49 | $I^c_{49}$ Δ105 | — | — | — | — |
| pop 50 | $I^c_{50}$ Δ105 | — | 18 | — | 10 |
| pop 51 | $I^c$ Δ105 | 24 | — | — | — |
| pop 52 | $I^{51}_{52}$ Δ105 | 17 | 13 | — | 21 |
| pop 14 | I$^+$ Δ102 | <1 | <1 | 1 | 1,5 |
| wild type | I$^+$T$^+$ | 1,3 | 100 | 3,9 | 100 |

recombinants in the same cross, 61 had received *mal*B—i.e. were λR; among those λR recombinants 18 had received *bym*A$^+$ and 7 *leu*$^+$) show that the distance between *mal*B and *bym*A is of several minutes.

Mutations allowing a *mal*B mutant to grow on maltose were previously described (Ricard, Hirota and Jacob, 1970). These mutations, also allowed the bacteria, which were *lac*Y, to grow on lactose and, furthermore, increased their sensitivity to desoxycholate. The *bym*A mutations described herein do not have the latter properties, and are therefore presumably different from the mutations described by Ricard, Hirota and Jacob.

Table 4. *Cotransducibility of the* mal$I_A^c$ *mutations with* aro*B and* mal*T*.

$P_1$ stocks were grown on the *mal*T *mal*$I_A^c$ strains listed in the first column and described in table 3. These stocks were used to transduce pop 12, a *bym aro*B strain with a wild type *mal*A region. Sensitivity to phage λ and ability to synthetize amylomaltase constitutively were tested as described in Material and Methods.

| Donor | Number of Aro$^+$ transductants analyzed | λ sensitive amylomaltase non constitutive | λ resistant amylomaltase constitutive | λ sensitive amylomaltase constitutive | λ resistant amylomaltase non constitutive |
|---|---|---|---|---|---|
| pop 22 | 112 | 60 | 52 | 0 | 0 |
| pop 24 | 47 | 32 | 14 | 1 | 0 |
| pop 25 | 96 | 48 | 45 | 3 | 0 |
| pop 26 | 92 | 51 | 40 | 1 | 1 |
| pop 28 | 95 | 50 | 45 | 0 | 0 |
| pop 29 | 122 | 76 | 45 | 1 | 0 |
| pop 30 | 47 | 20 | 24 | 3 | 0 |
| pop 31 | 47 | 36 | 10 | 0 | 1 |
| pop 32 | 87 | 48 | 34 | 3 | 2 |
| pop 33 | 82 | 43 | 39 | 0 | 0 |
| pop 34 | 24 | 14 | 8 | 1 | 1 |
| pop 35 | 21 | 11 | 10 | 0 | 1 |
| pop 36 | 48 | 25 | 22 | 1 | 0 |
| pop 37 | 75 | 38 | 37 | 0 | 0 |
| pop 38 | 46 | 27 | 18 | 1 | 0 |
| pop 39 | 94 | 47 | 47 | 0 | 0 |
| pop 40 | 92 | 52 | 40 | 0 | 0 |
| pop 41 | 92 | 48 | 44 | 0 | 0 |
| pop 42 | 40 | 23 | 16 | 1 | 0 |
| pop 43 | 20 | 10 | 9 | 1 | 0 |
| pop 44 | 24 | 15 | 7 | 1 | 1 |
| pop 45 | 93 | 54 | 38 | 1 | 0 |
| pop 46 | 94 | 44 | 48 | 2 | 0 |
| pop 47 | 16 | 9 | 7 | 0 | 0 |
| pop 48 | 19 | 14 | 5 | 0 | 0 |
| pop 49 | 66 | 41 | 24 | 0 | 1 |
| pop 50 | 90 | 48 | 41 | 0 | 1 |
| pop 51 | 94 | 51 | 43 | 0 | 0 |
| pop 52 | 48 | 22 | 24 | 2 | 0 |

### *Search for Mal$^+$ Revertants of Strains Carrying a Deletion in the mal*A *Region and a bym*A *Mutation*

The results obtained so far can be summarized as follows. If one starts with a deletion like Δ3 or Δ5 which happens to render the *mal*P-*mal*Q operon constitutive, one can find Mal$^+$ revertants. These revertants carry a mutation (*bym*A) which leads to the appearance of a new permeation system bypassing the normal maltose permease. If one starts with a shorter deletion like Δ102, Δ104 or Δ105, which still inactivates *mal*T, but does not render the *mal*P-*mal*Q operon constitutive, one does not find Mal$^+$ revertants.

Reversion to Mal$^+$ of strains carrying Δ102, Δ104 or Δ105 can be obtained however, when the strains already carry a *bym*A mutation. This is shown in the

following experiment. Strain pop 12 carries a *bym*A mutation and an *aro*B marker. This strain was transduced with $P_1$ lysates obtained on strains carrying the deletions shown in Fig. 3, and Aro$^+$ $\lambda$R recombinants were selected. As expected the transductants which received $\Delta 3$ or $\Delta 5$ were Mal$^+$—a confirmation that the *bym*A mutation is indeed present in the strain—while the transductants which received the other deletions were Mal$^-$. These Mal$^-$ strains were plated on synthetic maltose medium. Spontaneous Mal$^+$ derivatives were obtained (frequency $10^{-7}$ to $10^{-8}$) from the strains carrying the short deletions $\Delta 102$, $\Delta 104$ and $\Delta 105$, but not (frequency less than $10^{-9}$) from the strains carrying $\Delta 133$ or $\Delta 113$. These Mal$^+$ revertants are quite different from wild type. They grow only poorly on maltose, and are still resistant to phage lambda. Furthermore, as shown in Table 3, they make constitutively from 10 to 25% as much amylomaltase and phosphorylase as does the induced wild type and this level is not increased by addition of maltose to the cultures. For reasons that will become clear below, the mutations responsible for the constitutive phenotype will be called *mal*$I^c_A$.

Table 5. *Recombination of some* mal$I^c_A$ *mutations with two deletions*

The donors are Hfr G6 MA$\Delta$105 and Hfr MA$\Delta$133 (see Table 1 and Fig. 3). The acceptors are Mal$^-$ (*mal*Q or polar *mal*P) derivatives of five *mal*T$^+$ *mal*$I^c_A$ strains. The ability of recombinants to synthetize amylomaltase constitutively was determined as described in Materials and Methods.

| Genotype of donor | Genotype of acceptor | Number of {Met$^+$ His$^+$ Mal$^+$} recombinants analyzed | Percentage of amylomaltase constitutive recombinants ($I^c$) |
|---|---|---|---|
| $\Delta 105$ | $I^c_{30}\,Q_{13}$ | 88 | 40 |
| $\Delta 105$ | $I^c_{36}\,Q_{18}$ | 80 | 41 |
| $\Delta 105$ | $I^c_{42}\,P_{20}$ | 68 | 7.4 |
| $\Delta 105$ | $I^c_{44}\,Q_{19}$ | 99 | 93 |
| $\Delta 105$ | $I^c_{46}\,Q_{14}$ | 17 | 30 |
| $\Delta 133$ | $I^c_{30}\,Q_{13}$ | 178 | 100 |
| $\Delta 133$ | $I^c_{36}\,Q_{18}$ | 200 | 100 |
| $\Delta 133$ | $I^c_{42}\,P_{20}$ | 150 | 100 |
| $\Delta 133$ | $I^c_{44}\,Q_{19}$ | 60 | 100 |
| $\Delta 133$ | $I^c_{46}\,Q_{14}$ | 50 | 100 |

### *Genetic Location of the mal*$I^c_A$ *Mutations*

$P_1$ stocks were grown on the 29 independantly isolated *mal*$I^c_A$ mutants listed in Table 3, and used to transduce an *aro*B *bym*A strain (pop 12). As shown in Table 4, the great majority of the Aro$^+$ transductants which are $\lambda$R, *i.e.* which received the *mal*T deletion of the donor, are also Mal$^+$ and amylomaltase constitutive, i.e. also received the *mal*$I^c_A$ mutation of the donor. Conversely the great majority of the Aro$^+$ transductants which inherited the constitutive phenotype of the donor, also inherited its $\lambda$R character. The 29 *mal*$I^c_A$ mutations are thus in the *mal*A region, tightly linked to the *mal*T gene. For about half of

Table 6. *Enzymic characterization of* mal$T^+$ mal$I^c_A$ *strains*

The strains used in this study are some of the "$\lambda$ sensitive amylomaltase constitutive" transductants obtained in the experiment summarized in Table 4. Enzyme activities are expressed in percent of those obtained in the induced wild type strain (pop 40N. See under Table 3).

| Relevant genotype | Amylomaltase | | Phosphorylase | |
|---|---|---|---|---|
| | uninduced | induced | uninduced | induced |
| $I^c_{24}$ | 17 | 27 | 19 | 11 |
| $I^c_{26}$ | 13 | 8 | 20 | 15 |
| $I^c_{29}$ | 16 | 60 | 18 | 58 |
| $I^c_{30}$ | 18 | 75 | 11 | 67 |
| $I^c_{32}$ | 16 | 25 | 15 | 13 |
| $I^c_{34}$ | 13 | 15 | 15 | 10 |
| $I^c_{36}$ | 10 | 32 | 16 | 17 |
| $I^c_{38}$ | 18 | 16 | 16 | 13 |
| $I^c_{42}$ | 13 | 11 | 15 | 9 |
| $I^c_{43}$ | 12 | 10 | 18 | 25 |
| $I^c_{44}$ | 19 | 7 | 21 | 25 |
| $I^c_{45}$ | 18 | 98 | 19 | 120 |
| $I^c_{46}$ | 19 | 65 | 22 | 85 |
| $I^c_{52}$ | 14 | 9 | 15 | 9 |
| $I^+$ | 1.3 | 100 | 3.9 | 100 |

the strains, rare constitutive transductants were obtained which were $\lambda$S and therefore did not inherit the *mal*T deletion from the donor. Some of these *mal*$T^+$ *mal*$I^c_A$ strains, the phenotype of which will be described later, were used to further map *mal*$I^c_A$ mutations.

Mal$^-$ mutants were derived from the *mal*$T^+$ *mal*$I^c_A$ strains after EMS mutagenesis. These mutants were all *mal*Q or polar *mal*P mutants since they could be complemented to Mal$^+$ by a *mal*$Q^+$, but not by a *mal*Q, $\phi$80 *dmal*A bacteriophage. They were crossed with derivatives of Hfr G6 carrying either $\Delta$105 or $\Delta$133 (Table 5). The Mal$^+$ recombinants obtained in such crosses result from crossing over events between the right end of the deletion carried by the Hfr strain and the *mal*$^-$ mutation newly introduced in the F$^-$ strain. When the deletion was $\Delta$105 both constitutive and wild type recombinants were obtained. When the deletion was $\Delta$133 all Mal$^+$ recombinants were constitutive. The *mal*$I^c_A$ mutations used in these crosses are therefore located between the right end point of $\Delta$105 and that of $\Delta$133.

### *Effects of a mal*$T^+$ *Allele on the mal*$I^c_A$ *Mutations*

The *mal*$I^c_A$ mutations allow the *mal*P-*mal*Q operon to be expressed to a certain extent in the absence of maltose and *mal*T product. Two questions can be asked:

—1 Would the *mal*T product affect, in absence of maltose, the constitutive expression of the *mal*$I^c_A$ mutations.

—2 Would the *mal*T product allow induction by maltose of the *mal*$I^c_A$ mutants.

Table 7. *Effect of a* mal$T^+$ *allele on the expression of* trans *located* mal$I^c_A$ *mutation*

The haploid strains are Aro$^+$ Mal$^-$ transductants of Hfr G 61, obtained by infecting it with $P_1$ phages grown on the *Mal*T *mal*$I^c_A$ strains described in Table 3. The merodiploids were prepared by lysogenizing the above transductants with Φ80 *dmal*$_1$. Amylomaltase activity is expressed as % of that found in induced Hfr G 6, which contains 170 u/mg amylomaltase.

| Relevant genotype on the chromosome | Presence of Φ*dmal*$_1$ (T$^+$P$^+$Q$^-$) | Amylomaltase | |
|---|---|---|---|
| | | uninduced | induced |
| I$^+$ T$^+$ | — | 3 | 100 |
| $I^c_{22}$ Δ102 | — | 15 | 20 |
| $I^c_{24}$ Δ102 | — | 8 | 8 |
| $I^c_{29}$ Δ102 | — | 6 | 6 |
| $I^c_{33}$ Δ102 | — | 32 | 28 |
| I$^+$ Δ102 | + | 2 | 86 |
| $I^c_{22}$ Δ102 | + | 20 | 20 |
| $I^c_{24}$ Δ102 | + | 10 | 10 |
| $I^c_{25}$ Δ102 | + | 16 | 18 |
| $I^c_{26}$ Δ102 | + | 10 | 16 |
| $I^c_{28}$ Δ102 | + | 9 | 15 |
| $I^c_{29}$ Δ102 | + | 8 | 40 |
| $I^c_{30}$ Δ102 | + | 14 | 68 |
| $I^c_{31}$ Δ102 | + | 11 | 18 |
| $I^c_{32}$ Δ102 | + | 14 | 19 |
| $I^c_{33}$ Δ102 | + | 36 | 80 |
| $I^c_{34}$ Δ104 | + | 15 | 10 |
| $I^c_{35}$ Δ104 | + | 26 | 28 |
| $I^c_{36}$ Δ104 | + | 27 | 28 |
| $I^c_{37}$ Δ104 | + | 23 | 17 |
| $I^c_{38}$ Δ104 | + | 31 | 23 |
| $I^c_{39}$ Δ104 | + | 32 | 33 |
| $I^c_{40}$ Δ104 | + | 30 | 25 |
| $I^c_{41}$ Δ104 | + | 35 | 26 |
| $I^c_{42}$ Δ104 | + | 16 | 18 |
| $I^c_{43}$ Δ105 | + | 18 | 18 |
| $I^c_{44}$ Δ105 | + | 14 | 11 |
| $I^c_{45}$ Δ105 | + | 24 | 80 |
| $I^c_{47}$ Δ105 | + | 30 | 27 |
| $I^c_{48}$ Δ105 | + | 11 | 7 |
| $I^c_{49}$ Δ105 | + | 12 | 8 |
| $I^c_{52}$ Δ105 | + | 10 | 8 |

As reported above, about half the *mal*$I^c_A$ mutations could be easily separated from the *mal*T deletion with which they were originally associated. The phenotype of the resulting *mal*T$^+$ *mal*$I^c_A$ recombinants is given in Table 6. It can be seen that, in the absence of maltose, the enzyme levels are the same as when the *mal*T gene was deleted. Thus in these 14 strains the *mal*T gene product does not affect, in the absence of maltose, the constitutive synthesis resulting from the *mal*$I^c_A$ mutations. In 4 of the strains, addition of maltose induces the *mal*P-*mal*Q operon, but it has no effect in the remaining strains.

Table 8. *Cis dominance of the* mal$I^c_A$ *mutations*

The haploid strains are Mal⁻ derivatives of some of the *mal*T⁺ *mal*$I^c_A$ strains of Table 6. One of them is a polar *mal*P mutant (the $I^c_{42}$ P20 strain) and the others are *mal*Q mutants. The merodiploids are Φ80 $dmal_2$ lysogens of the above strains. Enzyme activities are expressed as percent of those found in induced pop 40N (see under Table 3).

| Relevant genotype on the chromosome | Presence of a Φ80 $dmal_2$ ($T^+P^+Q^+$) | Amylomaltase | | Phosphorylase | |
|---|---|---|---|---|---|
| | | un-induced | induced | un-induced | induced |
| $I^c_{30}\,Q_{13}$ | — | < 0.6 | < 0.6 | 13 | 14 |
| $I^c_{30}\,Q_{13}$ | + | 2.5 | 84 | 15 | 72 |
| $I^c_{36}\,Q_{18}$ | — | < 0.6 | < 0.6 | 19 | 25 |
| $I^c_{36}\,Q_{18}$ | + | 2.2 | 75 | 15 | 45 |
| $I^c_{42}\,P_{20}$ | — | < 0.6 | < 0.6 | < 0.6 | < 0.6 |
| $I^c_{42}\,P_{20}$ | + | 2.5 | 52 | 2.6 | 24 |
| $I^c_{44}\,Q_{19}$ | — | < 0.6 | < 0.6 | 15 | 16 |
| $I^c_{44}\,Q_{19}$ | + | 5.5 | 84 | 10 | 40 |
| $I^c_{46}\,Q_{14}$ | — | < 0.6 | < 0.6 | 21 | 62 |
| $I^c_{46}\,Q_{14}$ | + | 1 | 72 | 13 | 84 |
| $I^+\,Q^+$ | — | 3 | 100 | 2.1 | 100 |

It could be argued that the 14 *mal*$I^c_A$ mutants studied here are not representative of the whole series of *mal*$I^c_A$ because they were selected for their property of retaining constitutivity in presence of a *mal*T⁺ allele. It was therefore necessary to introduce a *mal*T⁺ allele into all the *mal*$I^c_A$ mutants. This was done as follows. The 30 *mal*$I^c_A$ mutations were first introduced biy $P_1$ transduction, together with the *mal*T deletion with which they were origtnally associated, into Hfr G61. Since this strain does not carry a *bym*A muta ion, the transductants are Mal⁻. Expression of the *mal*$I^c_A$ mutations is the same in this strain as in the original *mal*$I^c_A$ mutants (compare Table 3 and first lines of Table 7). The transductants were then lysogenized with $\phi$ 80 $dmal_1$. The phenotypes of the resulting $\frac{T^+I^+P^+Q^-}{T^-I^cP^+Q^+}$ merodiploids are shown in Table 7. It is obvious that in all cases the constitutive phenotype is retained in the merodiploids and that, therefore, a *mal*T⁺ allele does not repress the constitutive expression of the *mal*P-*mal*Q operon .It is also apparent that one more *mal*$I^c_A$ mutant ($I^c_{33}$), where the constitutive mutation had not been previously separated from Δ102, is inducible when a *mal*T⁺ allele is provided in transposition. This makes a total of 5 (out of 29) *mal*$I^c_A$ mutants where some action of the *mal*T gene product on *mal*P-*mal*Q expression can still be demonstrated.

### *Dominance Properties of the* mal$I^c_A$ *Mutations*

In merodiploids of $\frac{T^+I^+P^+Q^+}{T^+I^cP^+Q^-}$ structure, phosphorylase, but not amylomaltase, is synthesized constitutively (Table 8). The *mal*$I^c_A$ mutations therefore only render constitutive a *mal*P-*mal*Q operon located in *cis*-position.

## Discussion

All known *mal*T mutants of *E. coli* are phenotypically Mal⁻. Experiments reported here show that the ability of these mutants to grow on maltose can be restored by introducing two additional mutations. One of the mutations, called *bym*A, unmasks a new permeation system, independant of the *mal*B product(s). The other mutation, called *mal*$I^c_A$ is a *cis*-dominant constitutive mutation allowing expression of the *mal*P-*mal*Q operon independent of *mal*T.

The *mal*$I^c_A$ mutations described here are similar in many respects to the *ara*$I^c$ mutations obtained in the L-arabinose system of *E. coli B* (Englesberg, Sheppard, Squires and Meronk, 1969). Hence the same notation, standing for "Initiator constitutive". The initiator was defined as the target for the positive regulation. $I^c$ mutations are considered as mutations modifying the initiator so that the operon can be expressed in the absence of the positive regulator.

The fact that all the Ara⁺ revertants from an *ara*C deletion were of the *ara*$I^c$ type was taken as evidence that *ara*C was a positive regulator gene. The same reasoning applies here. If, as often suggested, the *mal*T gene were involved in the inactivation of a hypothetical repressor of the *mal*P-*mal*Q operon, one would expect to get mutants lacking this repressor as Mal⁺ revertants of *mal*T strains (unless these mutants would not be viable).

Some differences between the *ara*$I^c$ and *mal*$I^c_A$ mutations are worth mentioning:

—1 The degree of constitutivity resulting from *mal*$I^c_A$ mutations is generally higher (10–30% of induced wild type) than that resulting from *ara*$I^c$ mutations (1.5 to 8.7%).

—2 Only some *mal*$I^c_A$ mutants are still subject to induction by maltose, while all the *ara*$I^c$ mutants are inducible by L-arabinose. When the control by maltose is lost, the *mal*$I^c_A$ mutation could be due to the insertion of a new promotor into the normal controlling elements.

—3 In no case is the constitutive phenotype of a *mal*$I^c_A$ mutation repressed by a *mal*T⁺ allele. This is contrary to what is observed in the L-arabinose system where, in the absence of L-arabinose, the *ara*C⁺ allele represses the constitutive enzyme synthesis resulting from the *ara*$I^c$ mutations (Englesberg, Squires, Meronk, 1969). The result in the *ara* system is taken as evidence that the *ara*C gene product is a repressor in the absence of L-arabinose and an activator in the presence of L-arabinose. Regulation in the *ara* system is therefore considered to be partly negative and partly positive. Our results suggest that the *mal*T product is an activator in the presence of maltose, but is not a repressor in the absence of maltose. Regulation in the *mal* system is apparently purely positive. To retain the alternative hypothesis that *mal*T is a repressor in absence of maltose, one would have to suppose that all of the 30 independently isolated *mal*$I^c_A$ mutations happened to inactivate the hypothetical operator, target of the repression by *mal*T product.

In the *ara* system, the *ara*$I^c$ mutations restore the ability of any *ara*C mutant to grow on L-arabinose. This is possible because, presumably, *ara*C mutations do not entirely abolish the ability of the cell to take up L-arabinose. The situation is evidently different in the maltose system where *mal*T mutants are totally

unable to take up maltose. Consequently, suppression of the Mal⁻ phenotype of a *mal*T mutant requires, in addition to a *mal*$I_A^c$ mutation, another mutation restoring the ability to take up maltose. Since maltose permease is coded by a gene (genes ?) located in *mal*B, and since *mal*B is apparently controlled by *mal*T, mutations—which would be called *mal*$I_B^c$—allowing *mal*T independent expression of *mal*B, would restore the ability to take up maltose. What is found, instead of *mal*$I_B^c$ mutants, and at a rather low frequency, are mutants with an alternate pathway for maltose permeation, a pathway which involves neither the gene(s) in *mal*B, nor gene *mal*T. This result is still consistent with the hypothesis that the *mal*T product regulates *mal*B. The failure to find *mal*$I_B^c$ mutants may suggest, however, that the *mal*B region is more complex than believed until now, and may contain more than one operon controlled by *mal*T.

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*Note Added on Proofs.* A more accurate localization of the *bym*AR6 mutation has been made recently using the marker "*glucuronate*" which is located at min. 84 (Raymond Portalier, personal communication). The gradient of transmission between *leu*, "*glucuronate*", *bym*A and *met*A shows that *bym*A is located at min. 82 (plus or minus one minute). Out of 200 leu⁺ *strr* recombinants between pop306 and an Hfr (P4x type) carrying a mutation preventing growth on glucuronate, 98 had received the "*glucuronate*" marker, 79 had received the *bym*A⁺ allele and 47 had received the *met*A⁺ allele.

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