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Genetic Analysis of the Maltose A Region in *Escherichia coli*

DOLPH HATFIELD,[1] MAURICE HOFNUNG, AND MAXIME SCHWARTZ[2]
Service de Biochimie Cellulaire, Institut Pasteur, Paris, France

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The genetic map of the maltose A locus of *Escherichia coli* contains at least three closely linked genes, *malT*, *malP*, and *malQ*. The order of these genes is established by deletion mapping. *MalP* and *malQ*, the presumed structural genes for maltodextrin phosphorylase and amylomaltase, belong to the same operon. *MalT* may be a regulator gene involved in the positive control of this operon.

At least three proteins, maltose permease (22), amylomaltase (12, 22), and maltodextrin phosphorylase (18), are involved in maltose metabolism in *Escherichia coli* (Fig. 1). Maltose induces the synthesis of each of these proteins and additionally induces the synthesis of the bacterial receptors for phage λ (17). Mutations impairing maltose metabolism have previously been selected and classified by their phenotypic expression (16). They were found to be located in either of two genetic regions, *malA* or *malB* (15; Fig. 2). Preliminary results dealing with the genetic analysis of these two regions were given elsewhere (M. Schwartz, Ph.D. Thesis, Univ. of Paris, 1967).

The mutations in the *malA* region are known to belong to three distinct classes called MP, MQ, and MT (16). The first is believed to contain mutations in the structural gene for maltodextrin phosphorylase (gene *malP*). Some of these mutations lead to the formation of an enzymatically inactive, but immunologically detectable, phosphorylase protein. The second group consists of mutations in the amylomaltase structural gene (gene *malQ*). This conclusion is thus far based on the fact that bacteria carrying such mutations have lost their amylomaltase activity, but retain complete phosphorylase activity and at least part of their permease activity. Mutations in the third group are highly pleiotropic. They lead to an almost complete lack of permease, amylomaltase, and phosphorylase activities and to the inability to synthesize the receptors for phage λ. It has been suggested (17) that these mutations define a gene (*malT*) whose product is essential for induction of the proteins involved in maltose metabolism and of a protein involved in λ-receptor formation.

[1] Present address: National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

[2] Present address, until October 1969: Biological Laboratories, Harvard University, Cambridge, Massachusetts.

In this study, a more complete genetic analysis of the *malA* region is given. The genetic recombination of mutations belonging to the three classes mentioned above with a set of deletions establishes the order of the *malP*, *malQ*, and *malT* genes, suggests that the *malP* and *malQ* genes belong to the same unit of coordinated genetic expression, and helps to understand the role of the *malT* gene.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used: mal and glp correspond to the ability to use, respectively, maltose and glycerol or L-α-glycerophosphate as a carbon source; arg, met, his, pro and bio correspond to the ability to synthesize L-arginine, L-methionine, L-histidine, L-proline, and biotin; *asd* is the gene for aspartate semi-aldehyde dehydrogenase; *asd* mutants require both meso-α-ε-diaminopimelic acid and L-homoserine for growth; str^r and λ^r correspond to resistance to streptomycin and bacteriophage λ; $glpR^n$ corresponds to an allele of the *glpR* gene leading to noninducibility of the L-α-glycerophosphate system. We have followed the genetic nomenclature proposed by Taylor and Trotter (21). This leads us to modify abbreviations used in previous publications (*argF* becomes *argH*, *dap* + *hom* becomes *asd*). EMS corresponds to ethyl methanesulfonate.

Media. Some of the media (minimal medium M63 and M63 agar, broth, tryptone-agar and soft tryptone-agar, and EMB agar) have been described elsewhere (15). Minimal medium always contains 0.0005% vitamin B_1. Tetrazolium-maltose agar (TTZ maltose; 20) and minimal eosin methylene blue maltose agar (EM-maltose; 5) were also used in this study.

Bacteriophages and bacteria. Bacteriophage P1kc (11) was used for transduction, as previously described (15). A virulent mutant of phage λ (9), λV, was used to select and to score bacterial mutants resistant to phage λ, as previously described (15). The original stocks of P1kc and λV came from the collection of F. Jacob. The bacterial strains used in this study are

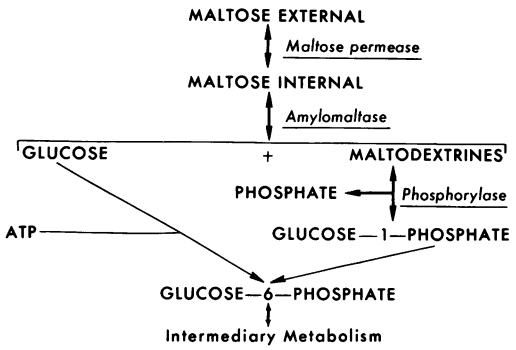

FIG. 1. *Maltose metabolism in E. coli.*

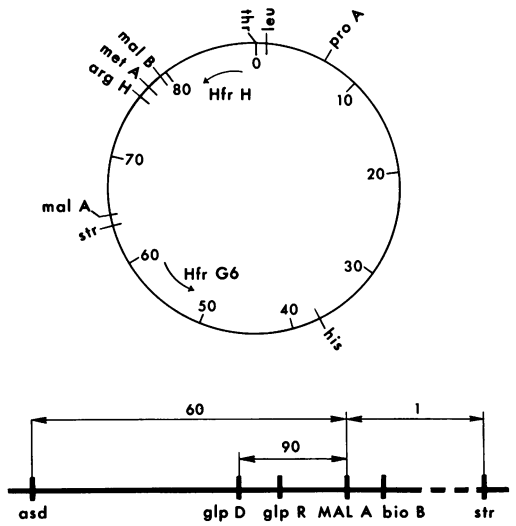

FIG. 2. *"Maltose" loci on the genetic map of E. coli. The top section is a simplified genetic map of E. coli [after that of Taylor and Trotter (21)], giving the location of the mutations carried by the strains listed in Table 1. The map is graduated in 10-min intervals in terms of Hfr × F⁻ conjugation. The origins of transfer of HfrH and HfrG6 are indicated by arrows. The bottom is an enlargement of the malA region. Genetic distances are in cotransduction frequencies by phage P1kc, expressed in per cent.*

listed in Table 1, together with their genotypes and origins.

Mutagenesis. EMS (Eastman Chemical Products, Inc., Kingsport, Tenn.) was used as a mutagen in the following way. Cells of HfrG6 were taken during logarithmic growth (at 2×10^8 cells/ml), concentrated, and resuspended in one-fourth volume of M63. EMS was added to a final concentration of 3%, and the whole was allowed to stand at 37 C for 2 hr. The mutagenized cells were then washed twice in M63 and finally grown in minimal glycerol medium before plating.

Selection of Mal⁻ mutants in HfrG6. MT mutants are resistant to phage λ (*see* introduction). Spontaneous mutants of this class were selected by treating cultures with λV.

EMS-induced Mal⁻ mutants were selected by plating suspensions of mutagenized clones of HfrG6 on EMB or TTZ maltose-agar. This procedure yields MT mutants as well as other types of Mal⁻ mutants. Although MQ mutants were selected from both EMB and TTZ plates, these mutants could be more easily detected on TTZ plates.

MalA mutants were distinguished from *malB* mutants by replica plating onto EM-maltose and glucose agar plates covered with a layer of PA505 MAΔ108, a strain with the entire *malA* region deleted. Mutants which gave prototrophic recombinants on glucose agar plates but which failed to give Mal⁺ prototrophic recombinants on EM-maltose plates were classified as *malA* mutants.

Mutants which lack only phosphorylase grow with maltose as the sole carbon source and therefore could not be detected on TTZ-maltose or EMB-maltose plates. These mutants were detected as amylose-accumulating bacteria by means of an iodine staining procedure, as described previously (16).

The mutants described herein are all independent, since only one mutant of a given phenotype was kept from each original clone of HfrG6.

Selection of deletions in PA505 MS14. F⁻ strains carrying deletions extending into the *malA* region were obtained as Mal⁻ among the glycerol-positive, spontaneous revertants arising from PA505 MS14. [An alternative means of selecting deletions in this region was described previously (15)]. Independent clones of PA505 MS14 were grown in a nonselective medium (broth) and then subcultured in a selective medium (M63 glycerol B_1, arg, met, bio). Biotin was present in the medium to avoid counterselection of deletions covering the "biotin" gene located between *malA* and *str* (15). The cultures were then plated on EMB or TTZ maltose agar for selection of the Mal⁻ clones.

The relative frequency of Mal⁻ among the Glp⁺ revertants was of the order of 10^{-3}, which is in agreement with the data of Cozzarelli et al. (2). Since the frequency of "reversion" of the *glpR*ⁿ mutation is about 10^{-6}, the absolute frequency of the deletions covering at least part of the *glpR* gene and at least part of the *malA* region is about 10^{-9}.

Transduction of MAΔ1, 3, and 5 into PA505 MS11. MAΔ1, MAΔ3, and MAΔ5 are Glp⁺ Mal⁻ mutations obtained in strain 90. Transduction of these mutations into PA505 MS11 was by standard techniques. Asd⁺ Mal⁻ transductants were selected in each case.

Phenotypic study of the mutants. Assay procedures for maltose permease, amylomaltase, and maltodextrin phosphorylase were described previously (16). Analogues of maltose which may be substrates for maltose permease, but would not be further metabolized, are not yet available. Therefore, the "permease" assay measures both the uptake and subsequent metabolism of ^{14}C-maltose.

The substrate used for maltodextrin phosphorylase was acid-hydrolyzed amylose. Amylose (Calbiochem, Los Angeles, Calif.) was suspended in 1 N H_2SO_4 at a concentration of 100 mg/ml, and incubated for 20 min

TABLE 1. *Characteristics of the strains used*

Strain	Sex	Relevant genotype	Origin
HfrH	Hfr	*str*^{s}	W. Hayes, F. Jacob
HfrH U482	Hfr	*asd str*^{s}	F. Jacob, G. N. Cohen
HfrG6	Hfr	*his str*^{s}	T. S. Matney
90	Hfr	*glpR*^{n} *str*^{s}	N. R. Cozzarelli
PA505	F$^-$	*argH metA pro his malA str*^{r}	F. Jacob
PA505 MS11	F$^-$	*argH metA asd str*^{r}	Recombinant HfrH U482 × PA505
PA505 MS13	F$^-$	*argH metA his pro glpR*^{n} *str*^{r}	Recombinant 90 × PA505
PA505 MS14	F$^-$	*argH metA glpR*^{n} *str*^{r}	Recombinant HfrH × PA505 MS13
PA505 MS15	F$^-$	*argH metA str*^{r}	Glp$^+$ revertant of PA505 MS14
PA505 MAΔ108[a]	F$^-$	*argH metA bioB malA str*^{r}	Glp$^+$ Mal$^-$ revertant from PA505 MS14

[a] This strain is one of the Glp$^+$ revertants obtained from PA505 MS14. It carries a deletion of the entire *malA* region and of at least part of *bioB*.

at 100 C. The nonhydrolyzed amylose was then removed by centrifugation, the supernatant fluid was neutralized with $Ba(OH)_2$, and the resulting $BaSO_4$ was removed by centrifugation. The optimal quantity of this material to use as substrate in the maltodextrin phosphorylase assay was determined for each preparation. Use of acid-hydrolyzed amylose gave more consistent results than did the commercial dextrins which were used in previous studies.

Recombination studies. All crosses were between mutants of PA505 MS14 (*argH metA*), carrying deletions in the *malA* region, and *malA* mutants of HfrG6 (*his*). The existence of Mal$^+$ recombinants among the Arg$^+$ Met$^+$ His$^+$ recombinants was studied by two methods: (i) crosses involving mutants lacking amylomaltase activity (i.e., MT, MQ, and extreme polar MP mutants); and (ii) crosses involving MP mutants having amylomaltase activity.

Crosses involving mutants lacking amylomaltase activity: mating on plates. Mutants of HfrG6 were streaked onto tryptone agar (6 to 10 clones per plate) from agar slants. After overnight development, the plates were replica-plated onto a series of EM-maltose plates, each layered with a different deletion-carrying F$^-$ strain. After 7 to 10 days at 37 C, crosses were scored. Control plates contained crosses between the mutants of HfrG6 and PA505 MAΔ108. This procedure permitted a tentative ordering of the mutations.

Mating in liquid medium. Each mutant in HfrG6 was crossed in liquid with all deletions which terminated closest to it on either side, as judged from results of the plate test.

Matings in liquid medium were carried out by standard techniques (10). Both prototrophic and *mal*$^+$ prototrophic recombinants were selected by plating on minimal glucose and minimal maltose, respectively. This technique permits the detection of one *mal*$^+$ recombinant among 10^6 prototrophic recombinants.

Crosses involving MP mutants having amylomaltase activity. Nonpolar MP mutants have amylomaltase activity. Therefore, their growth on maltose minimal agar was sufficient to preclude the study of the recombination of their mutations with the deletions by the above techniques. However, these mutants grow poorly enough in liquid media containing maltose (16) to permit selection of wild-type recombinants arising from mating them with deletion-carrying strains. After mating in liquid medium by standard techniques for about 10^9 F$^-$ cells with about 10^8 Hfr cells, the entire suspension was added to 250 ml of M63 medium supplemented with 0.5% maltose. The whole was subcultured with shaking at 37 C for 55 hr. Subcultures were plated on minimal maltose agar, and the developed plates were stained with iodine (16). The appearance of nonreacting iodine clones (wild type) demonstrated recombination. Control matings between the MP mutants and PA505 MAΔ108 were performed in every case. The resolution of the above selection procedure was determined by mixing MP mutants and the wild type in different proportions. The results demonstrate that this technique permits detection of one wild type among 10^6 MP mutants. This power of resolution is sufficient to establish recombination among the actual number of prototrophic recombinants (approximately 10^6) obtained in each cross. It should be mentioned, however, that no wild-type revertants were found by this technique from MP11, MP12, and MP13, the mutants used in the reconstruction experiments. Such crosses were performed for each MP mutant with all the deletions which do not recombine with any MT mutation.

RESULTS

Results are presented in three sections. The first section describes the phenotypic characteristics of *malA* mutants obtained in HfrG6, an Hfr strain transferring the *malA* region early. In the second, the phenotypic expression of deletions extending into the *malA* region obtained in an F$^-$ strain is given. Lastly, the recombination data are presented.

MalA mutants of HfrG6. Many Mal$^-$ mutants were previously isolated in a derivative of HfrH, and their characteristics have been reported (16). However, this Hfr strain transfers the *malA* region quite late and, therefore, these mutants could not be conveniently used in Hfr and F$^-$ crosses. Thus, a new set of spontaneous and of EMS-induced mutants was isolated from HfrG6

for this study. The phenotypic characteristics of these mutants are given in Table 2.

The MT mutants are resistant to phage λ. Extracts of MT mutants grown on maltose have very little phosphorylase and amylomaltase activity (1 to 4% of the amount found in extracts from induced wild type). Uptake of ^{14}C-maltose by these mutants never exceeds 2% of that found in induced wild type [or noninduced, in this case, since HfrG6 is constitutive for permease (16)], suggesting that the MT mutants have little or no permease activity.

MP mutants all lack phosphorylase activity. HfrG6 MP1 was described previously and shown to synthesize an immunologically detectable, but enzymatically inactive protein (16). MP1, MP11, MP12, and MP13 have amylomaltase activity. They can obtain glucose from maltose by action of amylomaltase (*see* Fig. 1) and, therefore, grow on minimal maltose medium. MP15, however, has little or no detectable amylomaltase activity and, consequently, does not grow on minimal maltose medium. Mutations similar to MP15 have been described previously (16) and interpreted as probable polar mutations in the phosphorylase gene. The mapping data is shown below to be consistent with this hypothesis.

The three MQ mutants described here have properties very similar to those already noted for mutants of this type. In addition to the lack of amylomaltase, the MQ mutants have a lower level of ^{14}C-maltose uptake. This phenomenon most probably reflects the decrease in maltose metabolism in these strains. However, their level of ^{14}C-maltose uptake is much higher than that observed in the MT mutants (*see* Table 2).

Furthermore, the MQ mutants have a noninduced as well as an induced level of phosphor-

TABLE 2. *Phenotypes of malA mutants of HfrG6*

Mutant[a]	Mutagenesis[b]	Resistance to phage λ[c]	Amylomaltase (units/mg of protein)		Phosphorylase (units/mg of protein)		^{14}C-Maltose uptake[d]	
			Uninduced	Induced	Uninduced	Induced	Uninduced	Induced
Wild type		+	19	181	26	220	305	440
MT101	SP	−		<1	3	6		6
MT103	SP	−		<1	6	7		<1
MT104	SP	−		6		2		6
MT105	SP	−		<1		<4		2
MT106	SP	−		2		<4		<1
MT109	SP	−		1		<4		<1
MT110	SP	−		5		2		4
MT151	EMS	−		8		4		2
MT152	EMS	−		6		5		<1
MT153	EMS	−		<1	8	9		6
MT154	EMS	−		<1		<4		<1
MT155	EMS	−		<1	8	7		<1
MT160	EMS	−	<1	<1	8	8		<1
MT162	EMS	−		8		7		<1
MT165	EMS	−	<1	<1	8	9		<1
MT171	EMS	−		4		7		<1
MT173	EMS	−		5		5		8
MP1[e]	UV	+	9	145	2	7		670
MP11	EMS	+	21	168	4	12	380	260
MP12	EMS	+	18	175	3	8	520	240
MP13	EMS	+	20	110	2	5	350	250
MP15	EMS	+	2	12	4	15	45	170
MQ6	EMS	+	6	30	16	288	47	87
MQ7	EMS	+	6	2	56	232	35	74
MQ8	EMS	+	5	<1	76	320	11	67

[a] Complete name of each mutant would be HfrG6, followed by the designation indicated in this column. Data concerning mutants MT9, MT10, MT12, MT13, MT16, and MT17 are very similar to those of the *malT* mutants shown above and are given elsewhere (16).

[b] SP = Spontaneous, EMS = ethyl methanesulfonate, and UV = ultraviolet irradiation.

[c] Symbols: +, sensitive; −, resistant.

[d] Expressed as nanomoles of maltose per milligram of protein per hour.

[e] Data concerning this mutant are taken from Schwartz (16).

ylase which is generally higher than that in wild type. A possible interpretation of this fact is the existence of an increased pool of inducer due to its decreased metabolism.

Deletions in PA505 MS14. The regulator gene for the glycerol system was recently demonstrated to be located between *malA* and *glpD* (2). This gene, *glpR*, is defined by $glpR^c$ and $glpR^n$ mutations, respectively, which lead to a constitutive or a noninducible phenotype. As in the lactose system (23), the noninducible mutants (phenotypically Glp^-) "revert" with a high frequency to constitutive mutants (phenotypically Glp^+). About 10^{-3} of the Glp^+ revertants turn out to be Mal^- and resistant to phage λ. As was suggested by Cozzarelli et al. (2), these Mal^- strains carry deletions of at least part of the *glpR* gene and the *malA* region.

All of the strains listed in Table 3, with the exception of MAΔ1, 3, and 5, were obtained as Glp^+ Mal^- mutants of PA505 MS14, an F^- strain carrying a $glpR^n$ mutation. The phenotype of most of these mutants is similar to that of the MT mutants, i.e., resistance to phage λ and little or no permease, amylomaltase, and phosphorylase activities. MAΔ108, in addition to having these characteristics is also Bio^-, which suggests that this deletion extends beyond the *malA* region into the biotin marker *bioB*, which is located between *malA* and *str* (14, 15). MAΔ149 has a higher constitutive level of amylomaltase than the other deletions, but it has very little or no phosphorylase activity.

A more detailed discussion of the levels of amylomaltase and phosphorylase in these mutants is given after the mapping data.

Deletions MAΔ1, 3, and 5 were obtained in a slightly different manner. A total of 40 Glp^+ Mal^- mutants had previously been isolated in the original strain carrying the $glpR^n$ mutation [strain 90 of Cozzarelli et al. (2)]. Three of them have levels of amylomaltase similar to that observed in MAΔ149. Since strain 90 is Hfr (Cavalli type), the deletions were transferred into an F^- strain for genetic study. As shown in Table 3, MAΔ1 is phenotypically analogous to MAΔ149. Although the two other deletions have an amylomaltase level comparable to MAΔ1 and MAΔ149, they also have a detectable constitutive phosphorylase level.

MAΔ115 and MAΔ131 (Table 3) were not derived from a $glpR^n$ mutant. They were spontaneous λ^r mutations arising in HfrG6 and were transferred to the F^- strain by standard bacterial matings.

Recombination data. The *malA* mutants of HfrG6 were crossed with F^- strains carrying deletions into *malA*, as indicated in Materials and Methods. Prototrophic and Mal^+ prototrophic recombinants were independently selected. A deletion is said to recombine with a point mutation whenever the observed frequency of Mal^+ recombinants among the prototrophs is more than 10^{-6}. The results are summarized in Fig. 3.

The data in Fig. 3 demonstrate that some deletions recombining with all the MP and MQ mutations tested do not recombine with any MT mutation (e.g., MAΔ157). Furthermore, it may be seen that deletions recombining with the three MQ mutations do not recombine with any MT or MP mutations (e.g., MAΔ106) and that the two deletions which do not recombine with any MQ mutation do not recombine with any MT or MP mutation. Thus, the phenotypically defined classes of *malA* mutations, MT, MP, and MQ, appear to map in three distinct parts of the *malA* region, in the order indicated in Fig. 3. The fact that MAΔ108 has a biotin requirement and does not cross with any *malA* mutation is consistent with the interpretation that this deletion extends beyond the amylomaltase gene and into *bioB*. MAΔ131 is indicated as a deletion in Fig. 3 because it does not recombine with six independent *malA* mutants.

All deletions that recombine with at least one MT mutation and, therefore, must terminate in the *malT* gene, have a homogeneous phenotypic expression. They give a low, but very characteristic phosphorylase activity which averages 8 units/mg of protein. The amylomaltase activity is also low and does not exceed 5 to 7 units/mg of protein.

Deletions terminating between the MT mutation mapping farthest to the right in Fig. 3 and MQ mutations mapping farthest to the left fall into four different phenotypic classes. (I) Deletions in this class (MAΔ157 and 161) have a phenotypic expression similar to that of deletions known to terminate inside the *malT* gene. They are most likely to terminate inside the *malT* gene. (II) These deletions (MAΔ106, 107, 109, 110, 112, 114, 121, 122, and 133) give a very low activity in the phosphorylase assay (average 0.9 unit/mg of protein). This activity cannot be attributed, of course, to maltodextrin phosphorylase, since all these deletions are known to cut out part of the *malP* gene. These deletions give a low amylomaltase activity which is comparable to, or slightly higher than, deletions terminating in the *malT* gene. (III) These deletions (MAΔ1 and 149) cut out part of the *malP* gene and give a very low level of phosphorylase activity, which is characteristic of deletions in class II. However, these deletions definitely give a higher constitutive amylomaltase activity. The existence of such

TABLE 3. *Phenotypes of F⁻ strains carrying deletions in the malA region*[a]

Mutant[b]	Deletion terminates in	Amylomaltase (units/mg of protein)		Phosphorylase (units/mg of protein)		Induced ^{14}C-maltose uptake[c]
		Uninduced	Induced	Uninduced	Induced	
Wild type[d]		11	182	11	228	550
MAΔ102	*malT*		<1	9	10	4
MAΔ103	*malT*		<1	10	9	7
MAΔ138	*malT*		5		5	18
MAΔ156	*malT*		<1		7	6
MAΔ105	*malT*	1	1	11	11	6
MAΔ118	*malT*		7		7	6
MAΔ129	*malT*		4		7	8
MAΔ142	*malT*		6		8	6
MAΔ155	*malT*		7		8	9
MAΔ145	*malT*		<1		9	17
MAΔ154	*malT*		6		9	10
MAΔ104	*malT*	<1	1	8	10	8
MAΔ111	*malT*	3	5	8	8	22
MAΔ136	*malT*		<1		7	8
MAΔ101	*malT*		<1	10	8	8
MAΔ157	*malT*(?)		5		8	10
MAΔ161	*malT*(?)		<1		8	14
MAΔ112	*malP*	12	13	2	0.9	2
MAΔ122	*malP*		9		0.5	<1
MAΔ133	*malP*		<1		0.8	<1
MAΔ114	*malP*	13	12	0.6	0.8	3
MAΔ149	*malP*	24	25		1	<1
MAΔ110	*malP*	<1	2	2	0.8	<1
MAΔ107	*malP*	5	7	1.2	1.2	3
MAΔ109	*malP*	11	7	0.8	1.2	2
MAΔ106	*malP* or *malQ*	<1	<1	1.6	1.2	<1
MAΔ121	*malP* or *malQ*		5		0.8	<1
MAΔ151	*malP* or *malQ*		9		0.5	2
MAΔ113	*malQ*	<1	<1	0.6	1.6	<1
MAΔ132	*malQ* or beyond		<1		0.4	8
MAΔ108	Beyond *malQ*	<1	<1	0.8	0.5	2
MAΔ1	*malP*	26	26	0.3	0.8	6
MAΔ3	*malT*, *malP*, or between	43	43	13	8	<1
MAΔ5	*malT*, *malP*, or between	40	43	14	10	<1
MAΔ115	*malT*	<1	<1		4	4
MAΔ131	*malT*	1	4	11	4	3

[a] Deletions with respect to MAΔ1, MAΔ3, and MAΔ5 were transduced from Glp⁺ Mal⁻ derivatives of strain 90 into PA505 MS115. Deletions with respect to MAΔ115 and MAΔ131 were transferred by conjugation from Mal⁻ derivatives of HfrG6 into PA505 MS14. All others were obtained directly in PA505 MS14.

[b] Complete name of each mutant would be PA505, followed by the designation indicated in this column.

[c] Expressed as nanomoles per milligram of protein per hour. The wild-type mutant produced 65 nmoles/mg of protein per hr, uninduced.

[d] PA505 MS15.

deletions provides a strong argument that *malP* and *malQ* belong to the same unit of genetic expression, and the polarity of reading is *malP* → *malQ*. The most likely interpretation of these deletions is that they result in the fusion of the distal part of the *malP–malQ* operon to an unidentified operon, which would be on the left of Fig. 3. There is, however, some difficulty in understanding the nature of the "unidentified operon" to which the distal part of *malA* can be fused. All mutants carrying *malA* deletions have an active *glpD* gene, since these deletions have the

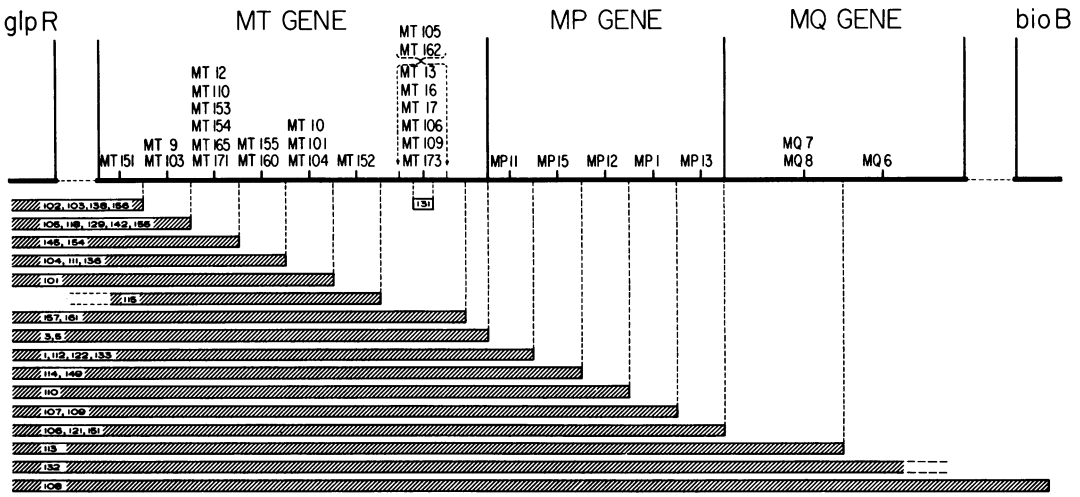

FIG. 3. *Genetic map of the maltose A region. The malT, malP and malQ genes are represented on the line near the top and designated as MT, MP, and MQ genes respectively. The linear arrangement of point mutations in the malT, malP, and malQ genes is also represented on this line. It is not known whether the point mutants, MT 105 and MT 162, are located to the right or left of MAΔ131 in malT, as indicated by the arrows. The hatched bars represent deletions, and each deletion is represented by its number.*

Glp^+ phenotype. The results of Cozzarelli et al. (2) suggest that *glpR* and *glpD* are adjacent. The deletions may thus terminate either in a dispensable part of the *glpD* gene, or inside the *glpR* gene. In the latter case, the level of *glpR* gene product, as reflected by the level of amylomaltase in strains MAΔ1 and MAΔ149, would be about 0.3 to 0.4% of the total proteins in *E. coli*. Such a level is quite high for a repressor molecule (3). However, the $glpR^n$ mutation may not be a classical noninducible mutation (a mutation leading to a structural modification of the repressor), but rather a mutation leading to an overproduction of the repressor (13). In this case, if MAΔ1 and MAΔ149 are fused to *glpR*, then the level of amylomaltase in these deletions would not reflect the level of the *glpR* product. Alternatively, MAΔ1 and MAΔ149 may not be real deletions, but rather transpositions leading to an integration between *malA* and *glpD* of a portion of an operon normally located elsewhere on the chromosome. The occurrence of mutations of this kind was recently demonstrated in the galactose system of *E. coli* (J. Shapiro, J. Mol. Biol., *in press*). Whatever the actual situation, the result remains that deletions covering only part of the *malP* gene can lead to constitutive expression of the *malQ* gene. Comparable situations have been described in other systems (8; D. P. Kessler and E. Englesberg, Bacteriol. Proc., 1967, p. 50). (IV) These deletions (MAΔ3 and 5) terminate between the MT mutation mapping farthest to the right and the MP mutation mapping farthest to the left (Fig. 3). They give a level of amylomaltase activity which is higher than deletions in class III and, additionally, they have phosphorylase activity. This result may be interpreted that *malT* is part of the same unit of genetic expression as *malP* and *malQ*, in which case MAΔ3 and 5 terminate in the *malT* gene, and a part of the *malT* gene is fused to another operon (*see* class III). However, a more likely interpretation is that *malT* does not belong to the *malP–malQ* operon, and the deletions terminate either in a "silent" region of the operon (in a gene which is still unidentified, or in a dispensable part of the *malP* gene) or in a controlling element of this operon [promotor (6) or operator (7)]. The fact that these two deletions terminate further than any of the 23 MT mutations mapped in this study suggests that the first hypothesis—*malT* being part of the *malP–malQ* operon—is less likely than the second.

MP15, a phosphorylase mutant which lies between two well-characterized MP mutations, gives both a low phosphorylase and a low amylomaltase activity. The occurrence of such a mutant in the *malP* gene, which brings about a decreased level of amylomaltase, again suggests that *malP* and *malQ* belong to the same operon and that the polarity of reading is *malP* → *malQ*.

DISCUSSION

On the basis of their phenotypic expression, the *malA* mutations fall into three classes. These

three classes now appear to correspond to three genetically defined regions of the *malA* locus. Therefore, and despite the lack of complementation analysis, it appears very likely that the MT, MP, and MQ mutations define three functionally distinct genes, *malT*, *malP*, and *malQ*, respectively.

MalP and *malQ* are most likely the structural genes for phosphorylase and amylomaltase, whereas the function of *malT* remains to be determined. Further discussion of the function of the *malT* gene is given below. The order of the three genes is unequivocally established in this study. The results of the study also strongly suggest that *malP* and *malQ* belong to the same genetic unit of coordinated expression, or operon. Phosphorylase and amylomaltase are consecutive enzymes in the same pathway; they are coinduced by maltose and appear to be induced in a coordinate manner (although a precise study of coordination has not yet been done); they are simultaneously absent or constitutive in a series of mutants (16; M. Schwartz, Ph.D. Thesis, Univ. of Paris, 1967); and their structural genes are tightly linked (more than 95% cotransducibility). Mutations in the phosphorylase structural gene leading simultaneously to a complete lack of phosphorylase activity and to a decrease in amylomaltase activity, and thus are likely to be polar mutations, have been described both here and elsewhere (16). Lastly, deletions have been found which extend into the phosphorylase gene and give rise to a constitutive amylomaltase synthesis. Such deletions provide strong evidence that *malP* and *malQ* belong to the same operon.

Gene *malT*, defined by mutations leading to a pleiotropic negative phenotype, is tightly linked with the preceding ones and may even be adjacent to *malP*. However, there is thus far no indication that *malT* belongs to the operon defined by *malP* and *malQ*. The fact that none of the deletions terminating before the MT mutation which maps farthest to the right in Fig. 3 leads to a constitutive phosphorylase and amylomaltase would suggest that gene *malT* does not belong to the *malP–malQ* operon.

The exact function of gene *malT* is not yet clear. The present results exclude the possibility that it might be a gene responsible for making a polypeptide common to both phosphorylase and amylomaltase. This would be incompatible with the observation that various levels of amylomaltase activity are observed as the result of deletions which remove the entire *malT* gene. The *malT* product must then in some way take part in the regulation of amylomaltase and phosphorylase synthesis. The fact that deletions of *malT* have a pleiotropic negative phenotype implies that the *malT* product is necessary for the synthesis of the enzymes of maltose metabolism. The hypothesis that the *malT* product is a structural component of the maltose permease, or an enzyme converting maltose into the real inducer (1, 4), cannot be excluded on the basis of the present results. Nevertheless, because of the striking similarity between the gene disposition and function in the maltose system and that in the arabinose system in *E. coli* B (19; E. Englesberg and C. Squires, Proc. 12th Intern. Congr. Genet., Tokyo, 1968, p. 47), we propose that *malT* is a positive regulator gene. Such a gene is defined as having a product which promotes transcription (or translation) of a genetic message. In the presence of maltose, the product of *malT* could promote the transcription (or translation) both of the *malP–malQ* operon and of an operon located in *malB* which contains a gene involved in the synthesis of maltose permease and a gene involved in the synthesis of receptors for phage λ (17).

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