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Isolation and Complementation of Mutants in Galactose Taxis and Transport

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By using a new screening method, we have obtained 43 new *Escherichia coli* mutants defective in ring formation on galactose swarm plates, which score for defects in chemotaxis or transport. They were complemented and compared with mutations previously known to lie in the galactose-binding protein or the β-methylgalactoside (*mgl*) permease, or both. The mutations were all found to lie in three genes, called *mglA*, *mglB*, and *mglC*. *mglB* codes for the gene specifying the binding protein. Based on co-transduction experiments, *mglA*, *mglB*, and *mglC* lie close to each other on the bacterial chromosome.

The motile bacterium *Escherichia coli* carries out chemotaxis: i.e., it accumulates in higher concentrations of certain chemicals, including amino acids, oxygen, and sugars (1). Chemotaxis represents a simple behavioral system in which chemicals are detected, the information is transmitted, and flagella respond appropriately.

The first stage of the process, chemoreception, has been studied for the attractant galactose. It is bound by the galactose-binding protein (2), which has been shown to play roles in galactose taxis (11) and transport (2, 4). Galactose is conveyed into the cell by the β-methylgalactoside (*mgl*) (16) permease (the highest affinity transport system for galactose), of which the galactose-binding protein is part (4). As a result of the binding, appropriate signals are sent to the flagella to cause chemotactic responses.

How interaction of galactose with its binding protein leads to the sending of appropriate signals to the flagella and what these signals are, as well as the mechanism by which galactose is conveyed into the cells, are all unknown. The basic approach we have taken in trying to answer these questions is to isolate and characterize mutants defective in these processes.

Mutants were recognized by their inability to form a normal ring on a galactose swarm plate. It should be pointed out at the outset that this method for isolating mutants depends upon the ability of bacteria to create a gradient of galactose and then to form a ring of cells that pursues the galactose (see ref. 1 and Fig. 1). Mutants with normal taxis but defective transport fail to make such a ring because their utilization of galactose is too slow to form an effective gradient at the low concentration provided. Hence, the method is successful not only for isolating chemotaxis mutants, but also for isolating transport mutants, even transport mutants that are normal in chemotaxis. Mutants defective in galactose metabolism could not grow in galactose minimal medium and hence were discarded.

In the present investigation, we report the isolation and complementation of mutants unable to form a normal ring on a galactose swarm plate and, for reference, complementation of mutants previously known to be defective in galactose taxis or transport, or both. In the accompanying investigation (14) we present quantitative assays of chemotaxis and transport for these newly isolated mutants.

MATERIALS AND METHODS

Unless otherwise indicated, all experiments were done at 35 C, except for centrifugations, which were carried out at room temperature.

Bacterial strains employed are listed in Table 1.

Media. Tryptone broth contained 1% tryptone and 0.5% NaCl. Tryptone agar plates were made by adding 1.5% agar to tryptone broth. For liquid minimal medium, H-1 of Kaiser and Hogness (12) or Vogel-Bonner medium (17) was employed. Thiamine was added at 1 μg/ml. Amino acid supplements were added at 0.3 to 0.5 mM. Minimal agar plates were made by adding 1.5% agar to liquid minimal medium.

Swarm plates containing 0.25% agar were made from 10 mM potassium phosphate, pH 7; 1 mM $MgSO_4$, 1 mM $(NH_4)_2SO_4$, 1 μg of thiamine per ml plus 0.05 mM galactose, 0.1 mM ribose, 0.2 mM fructose, 0.2 mM maltose or 0.2 mM mannitol, plus amino acid supplements each at 0.1 mM. The latter included auxotrophic requirements or, for faster

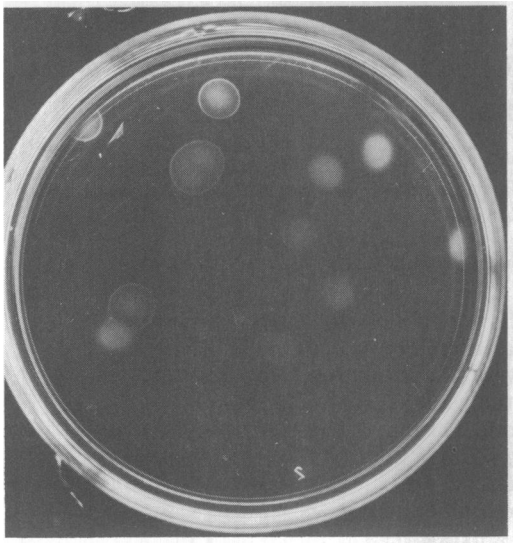

FIG. 1. *Galactose miniswarm plates showing swarms of OW1 (mgl⁺ parent) and OW5 (mglB⁻). The plate included the 11 amino acid supplements. It was incubated for about 24 h at 35 C. The swarms having a ring were OW1, and those lacking a ring (being diffuse at the borders) were OW5.*

growth of bacteria, the following 10 or 11 amino acids: alanine, arginine, glycine, leucine, isoleucine, methionine, proline, phenylalanine, threonine, valine, and sometimes histidine. "Miniswarm" plates (J. S. Parkinson, personal communication) differed from swarm plates in that bacteria were poured into the plate with 10 ml of medium containing 0.325% of agar.

Sugars were of the D configuration, and amino acids were L, except for DL-α-methylaspartate.

Isolation of mutants. (i) Mutagenesis. Strain OW1 was grown in tryptone broth to an optical density at 590 nm (OD_{590}) of 1 to 1.5 (OD_{590} of 1 = 6×10^8 bacteria per ml), centrifuged, washed in 10 mM $MgSO_4$, and resuspended in H-1 at about 2×10^9 cells per ml. The suspension was made 0.3 M in ethyl methane sulfonate, vortexed, and allowed to incubate for 1 h at room temperature. Bacteria were centrifuged, washed twice with H-1, and finally diluted 1:100 or 1:1,000 into Vogel-Bonner medium, supplemented with thiamine, amino acid requirements, and 0.4% glucose. Cultures were grown to stationary phase to allow segregation of new mutants and to dilute out bacteria that had gained new auxotrophic requirements. The method was based on that of Parkinson (personal communication) who determined that it yields mutants 10^3- to 10^4-fold over spontaneous background.

(ii) Screening for mutants on miniswarm plates. This was carried out according to J. S. Parkinson (personal communication). Mutagenized bacteria in stationary phase were diluted 1:50 into Vogel-Bonner medium containing 0.4% glucose, 1 μg of thiamine per ml, and 0.1 mM of the same 11 amino acids used to enrich miniswarm plates (see Media above). When cells had grown to about 2×10^9/ml, they were diluted into miniswarm plates at about 30 bacteria per plate. Plates were incubated about 24 h at 35 C. See Results for description of bacterial swarm morphologies on these plates.

Transduction. (i) Stocks of P1*kc* were obtained by the method of Swanstrom and Adams (15). (ii) P1 transductions were carried out essentially by the method of Lennox (13).

Preparation of recA⁻ derivatives. To introduce the *recA⁻* allele into a strain, a *thyA⁻* mutation was introduced by the method of Dubnau and Maas (9) and then was removed by crossing with an Hfr transferring *thy⁺* early and *recA* 5 min later, as shown below.

To introduce *recA* into a *thy⁻* strain, 0.1 ml of tryptone broth, 0.01 ml of KL16-99 *nalA⁻pro⁺* (for crosses with *str^r* recipient) or KL16-99 *nalA⁻pro⁻* (for crosses with *str^s* recipient), and 0.01 ml of the *thy⁻* recipient—all stationary phase tryptone broth cultures, supplemented with 50 μg of thymine per ml for the *thy⁻* recipient—were mixed and incubated without shaking for 4 to 6 h. Crosses were plated on H-1 glucose plates containing 100 μg of streptomycin per ml or lacking proline to select against the donor and lacking thymine to select against the recipient. Small colonies were streaked on a tryptone plate and subjected to 450 ergs/mm² of ultraviolet light to test for *recA*. *recA* strains were further tested for retention of the *nal⁺* (nalidixic acid sensitivity), *his⁻*, *mgl⁻* (tested on galactose swarm plates), and taxis to ribose (tested on ribose swarm plates) genotype of the recipient parent. The genetic loci for *nalA* and *his* are located about 2 min on either side of *mgl* on the Taylor (16) map (E. N. Kort, personal communication) and were tested to certify that genes close to *mgl* were from the recipient parent. The test for ribose taxis was the control to show that chemotaxis in the recombinants was normal except toward galactose.

Preparation of episomes having mgl mutations. Episomes having *mgl* mutations were prepared by introducing the wild-type episome into *mgl⁻* recipients and obtaining homogenotes. Episome F131, which includes genes required for histidine biosynthesis, converts *mgl⁻* strains to *mgl⁺*: hence, the episome contains wild-type genes for galactose taxis. Partial diploids having *mgl* mutations on the chromosome and wild-type *mgl* genes on the episome were subjected to 320 ergs/mm² of ultraviolet light and were grown in the dark to allow recombination between episome and chromosome. Cells were then poured into galactose-miniswarm plates containing the 10 amino acid supplements (no histidine to assure retention of the episome) and plates were incubated for about 36 h. Swarms lacking rings were cloned on tryptone plates and clones were tested on galactose swarm plates and, as controls, on ribose swarm plates. Episomes were then transferred from partial diploids lacking the ring indicative of galactose taxis into an intermediate host (strain RP263 which is *recA⁻* to prevent recombination) and then back again to the original *mgl⁻* recipient. Episomes that at this step failed to convert the phenotype of the original *mgl⁻* mutant to *mgl⁺* were considered to have appropriate

TABLE 1. *Bacterial strains*[a]

Strain	Source or reference	Relevant properties
AW574	Made by S. Larsen from AW405 (3) crossed with B7 (6)	*thr⁻leu⁻his⁻sup⁰gal⁺str^r* wild type for chemotaxis
OW1	Obtained from crossing AW574 by AB1927 (from K. B. Low) and selecting for ability to show mannitol taxis on swarm plates	
OW1 *mgl⁻* (43 strains)	This work, obtained by mutagenesis from OW1	
AW520 *his⁻*	(11) Made *his⁻* by E. Kort	*mgl⁻his⁻gal⁺str^r*
AW526 *his⁻*	(11) Made *his⁻* by E. Kort	*mgl⁻his⁻gal⁺str^s*
AW543 *his⁻*	(11) Made *his⁻* by E. Kort	*mgl⁻thr⁻leu⁻his⁻met⁻str^r*
AW551 *his⁻*	(11) Made *his⁻* by E. Kort	*mgl⁻thr⁻leu⁻his⁻met⁻str^s*
20SOK⁻ *gal⁺his⁻*	Obtained from crossing 20SOK⁻ (7) by B7 (6), selecting for *gal⁺*; made *his⁻* by penicillin selection (8)	
W3092i *gal⁺his⁻*	Obtained from crossing W3092i (18) by B7 (6), selecting for *gal⁺*; made *his⁻* by penicillin selection (8)	*gal⁺mgl⁻his⁻*
EH3039 *gal⁺his⁻*	Obtained from crossing EH3039 (5) by B7 (6) selecting for *gal⁺*; made *his⁻* by penicillin selection (8)	*gal⁺mgl⁻his⁻*
KL16-99 *nalA⁻*	K. B. Low (via J. S. Parkinson); made *nalA⁻* by mutagenesis	*thy⁺recA⁻str^s nalA⁻*
KL16-99 *nalA⁻pro⁻*	Penicillin selection of above for *pro⁻* (8)	*thy⁺recA⁻pro⁻nalA⁻str^s*
RP263	J. S. Parkinson	*thyA⁻trp⁻his⁻galE⁻* non-motile
KL16-21 *nalA⁻*	(10) Made *nalA⁻* by E. Kort by P1 transduction	*ptsF⁻nalA⁻*
OW1 *nalA⁻ptsF⁻*	Obtained by crossing KL16-21 *nalA⁻* with OW1	*thr⁻leu⁻his⁻mgl⁺ptsF⁻*
F131 (episome)	K. B. Low (via E. Kort)	*mgl⁺his⁺*
F514 (episome)	P. Strigini (via J. S. Parkinson)	*supU⁻* (episome does not contain *mgl*)
F402 (episome)	J. S. Parkinson	*supD⁻his⁺* (episome does not contain *mgl*)

[a] Key to symbols: *mgl* refers to the β-methylgalactoside permease or to galactose taxis, or both; *gal*, to galactose metabolism; *str*, to resistance or sensitivity to streptomycin; *recA*, to defectiveness in recombination; *thr*, *leu*, *his*, *met*, *pro*, *trp*, *thy*, to auxotrophic requirements for threonine, leucine, histidine, methionine, proline, tryptophan, and thymine, respectively; *sup* to presence of suppressor (*sup⁰* means none); *galE*, to defect in galactose metabolism; *ptsF*, to defect in fructose transport; and *nalA⁻*, to resistance to nalidixic acid.

mgl mutations. There was a possibility that deletions of the *mgl* region might have been introduced into the episome during these manipulations but the data of Table 2 suggest that such abnormal events did not occur.

Complementation. In a typical complementation test, strain RP263 having a *mgl⁻his⁺* episome was crossed with a *mgl⁻his⁻recA⁻* recipient by cross-streaking on selective plates (H-1 glucose, thiamine plates which contained only threonine and leucine as supplements). After growth of F-ductants for 2 days, bacteria were spotted on galactose swarm plates enriched with the 10 amino acids (no histidine; see above) by using a toothpick to inoculate from the mating plates, and the swarm plates were incubated for about 24 h. Distance of migration of the ring, if present, was compared to the distance for the same recipient having the wild-type episome.

Tests for amber mutants. F402 was introduced into *mgl* mutants by selecting for *his⁺* clones. F514 was introduced into *mgl* mutants by selecting for *thr⁺* since the *thr* mutation in these *mgl⁻* strains is an amber mutation.

RESULTS

Screening technique for mutants in galactose ring formation. Mutagenized cells were poured into semisolid agar containing galactose. Incubation of these miniswarm plates resulted in growth of clones, each having a ring at its outside border. These rings contained cells carrying out galactose taxis and increased in radius with time. Ahead of the rings was the initial concentration of galactose; behind them were undetectably low concentrations (1). The bacteria in the rings were located in the concentration gradient between these two regions (1). Some clones, however, lacked a ring and hence

TABLE 2. *Complementation of new mutants*[a]

Strain	Mutation on chromosome gene and allele no.	Mutation on episome										
		B4	B5	B6	A 18	A 20	A 22	C 31	C 33	AC 41	AC 43	AC 44	ABC 12
OW2	B-2	−	−	−	++	++	++	+	++	++	++	++	−
OW3	B-3	−	−	−	+	++	+	++	++	++	++	++	−
OW4	B-4	−	−	−	++	++	++	++	++	++	++	++	−
OW5	B-5	−	−	−	++	++	++	++	++	++	++	++	−
OW6	B-6	−	−	−	+	+	++	++	++	++	++	++	−
OW7	B-7	−	−	−	++	++	++	++	++	++	++	++	−
OW8	B-8	−	−	−	++	++	++	+	+	++	+	+	−
OW9	B-9	−	−	−	+	++	+	+	+	+	+	+	−
OW10	B-10	−	−	−	++	+	+	++	++	++	++	++	−
OW11	B-11	−	−	−	++	++	++	++	++	++	++	++	±
OW12	ABC-12	−	−	−	−	−	−	−	−	−	−	−	±
OW13	ABC-13	−	−	−	−	−	−	−	−	−	−	−	±
OW14[b]	A-14	++	++	++	−	−	−	+	+	−	−	−	+
OW15	A-15	++	++	++	−	−	−	++	+	−	−	−	±
OW16	A-16	++	++	++	−	−	−	+	+	−	−	−	+
OW17	A-17	++	++	++	−	−	−	+	+	−	−	−	±
OW18	A-18	++	++	++	−	−	−	++	++	−	−	−	+
OW19	A-19	++	++	++	−	−	−	+	++	−	−	−	+
OW20	A-20	++	++	++	−	−	−	+	++	−	−	−	+
OW21	A-21	++	++	++	−	−	−	++	++	−	−	−	+
OW22	A-22	++	++	++	−	−	−	++	++	−	−	−	±
OW23[b]	C-23	++	++	++	++	++	++	−	−	±	±	−	++
OW24	C-24	++	++	++	++	+	+	−	−	−	−	−	+
OW25	C-25	++	++	++	++	++	++	−	−	±	−	±	+
OW26[b]	C-26	++	++	++	++	++	++	−	−	±	±	±	++
OW27	C-27	++	+	++	+	+	++	−	−	−	−	−	⊖
OW28	C-28	++	+	+	+	±	+	−	−	−	−	−	++
OW29	C-29	++	++	++	++	+	+	−	−	±	−	−	+
OW30[b]	C-30	++	++	++	++	+	+	−	−	±	±	−	++
OW31	C-31	++	++	++	++	++	++	−	−	−	−	−	+
OW32	C-32	++	++	++	++	++	++	−	−	−	−	−	±
OW33	C-33	++	++	++	++	±	±	−	−	−	−	−	±
OW34	C-34	±	+	+	+	±	±	−	−	−	−	−	±
OW35	C-35	++	++	++	++	⊖	⊖	−	±	−	−	−	±
OW36[b]	C-36	++	++	++	+	+	++	−	−	−	−	−	+
OW37[b]	AC-37	++	+	++	−	−	−	±	−	−	−	−	+
OW38	AC-38	++	++	++	±	±	±	−	−	−	−	−	±
OW40[b]	AC-40	++	+	++	−	−	−	−	±	−	−	−	⊖
OW41	AC-41	++	++	++	−	−	−	−	−	−	−	−	++
OW43	AC-43	++	++	++	−	−	−	−	−	−	−	−	++
OW44	AC-44	++	++	++	−	−	−	−	−	−	−	−	⊖
OW39	(AC)-39	++	++	++	−	−	−	±	±	−	−	−	⊖
OW42	(AC)-42	++	+	+	−	−	−	±	±	−	−	−	±

[a] Complementation was carried out as described in the text. Symbols are defined as follows: ++, 75 to 100% of ring size when wild-type episome is in recipient; +, 50 to 75%; ±, 25 to 50 %; −, 0 to 25%.

[b] The haploid strain (not carrying any episome) makes a ring on galactose swarm plates enriched with the 11 amino acids (see media in Materials and Methods) although not on galactose swarm plates lacking this enrichment. However, this property does not seem to have affected the complementation. For purposes of deciding what gene a given mutation was in, ++ and + are considered a positive result, − a negative result, and ± an inconclusive result. Alleles 39 and 42 cannot be considered classified but are definitely not *mglB* mutants and so are denoted (AC). Examples of failure of complementation where a positive result was expected, denoted as partial dominance in the text, are circled. For this purpose, *mglABC-12* is considered to be a *mglB* mutation when on the episome since then it complemented some *mglA* and some *mglC* mutations. The fact that *mglABC-12* when on the episome complemented many *mglA*, *mglC*, and *mglAC* mutations but not when on the chromosome has not been explained. Possibly, the gene dosage of polypeptide from episome and chromosome are different.

were considered candidates for being galactose taxis mutants. Figure 1 shows a miniswarm plate having wild-type (with ring) and galactose taxis mutant (without ring) clones. Putative mutants were tested on galactose swarm plates and, as controls, on ribose, maltose, mannitol, and fructose swarm plates. Strains giving no ring on galactose swarm plates but showing wild-type rings on other sugar swarm plates were considered to be specifically defective in galactose ring formation.

Morphology of swarms. Strains mutant in galactose ring formation produced different morphologies of swarms on galactose swarm plates, although they never produced a wild-type ring. Figure 2 shows examples. Varieties of swarms included much spreading from the site of inoculation into a diffuse disk of bacteria (3 cases), limited spreading (4 cases), very modest growth at site of inoculation only (35 cases), and in one instance, formation of a slowly expanding faint thin ring.

Further tests of phenotype of these mutants, including capillary assays of chemotaxis and assays of transport of galactose, are presented in the accompanying investigation (14).

Complementation of mutants. To determine the number of genes involved specifically in galactose ring formation and to assign each mutation to a gene, complementation tests were performed. Episomes having mutations were introduced into recipients having the same or different mutations, as described in Materials and Methods. These F-ductants were tested on galactose swarm plates and the radius of any ring, if present, after about 24 h was measured. Examples are shown in Fig. 3.

On such a galactose swarm plate, mutant strains with the wild-type episome, or an episome having a mutation in a different gene from the mutation on the chromosome, should give rings, but strains having the same gene mutated on both episome and chromosome should not. In fact, strains in which complementation did not occur (no rings) usually showed spreading; this was partly due to growth on the 10 amino acid supplements since its replacement with the two required amino acids resulted in only little growth at the site of inoculation, but not spreading. The extra amino acids were included to make the test faster.

Besides mutants isolated in this study (Table 2), mutants previously identified as having defects in galactose taxis (AW520, AW526, AW543, AW550, and AW551) or galactose transport (W3092i 20SOK$^-$, and each of the above except AW551) were tested (Table 3).

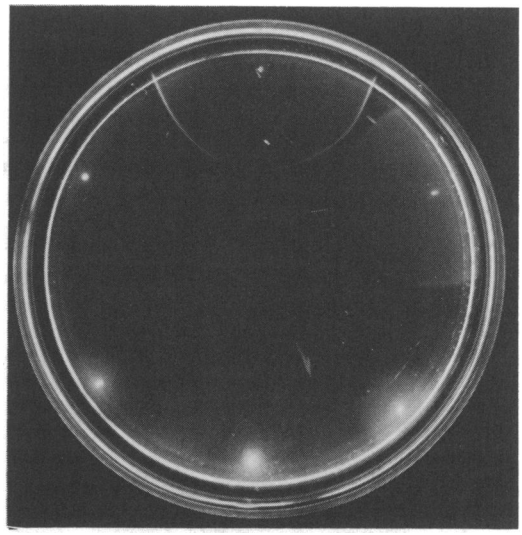

FIG. 2. *Galactose swarms of mgl$^-$ strains illustrating variety in swarm morphologies. The plate was prepared as described in Materials and Methods, with only required amino acids as supplements, and was inoculated with bacteria on toothpicks and incubated about 24 h at 35 C. Starting at the top and reading clockwise, the swarms are as follows: OW1 (mgl$^+$), OW7 (mglB$^-$), OW6 (mglB$^-$), OW28 (mglC$^-$), OW34 (mglC$^-$), and OW22 (mglA$^-$). OW7 shows much spreading from site of inoculation; OW6 and OW34 show limited spreading; OW28 later produced a faint, thin, ring but the photograph was taken too early to see it; OW22 showed growth only at the site of inoculation.*

The following observations may be made. (i) There are three genes for galactose ring formation, called *mglA*, *mglB*, and *mglC*. (ii) Of the newly isolated mutants, nine are in *mglA*, 10 are in *mglB*, 14 are in *mglC*, six affect *mglA* and *mglC*, two affect *mglA*, *mglB*, and *mglC*, and two are in *mglA* or affect *mglA* and *mglC*. (iii) Previously described mutants include mutants in *mglB* and in *mglA*, *mglC*. (iv) One previously described mutant, AW526, showed dominance when tested in the usual way in a *recA* background. (However, when tested in *rec*$^+$ background with two episomes having *mglA* defects, two having *mglB* defects, and two having *mglC* defects, it formed rings, probably from recombination, except when *mglB* mutant episomes were used, so that strain AW526 probably contains a *mglB* mutation.) (v) Finally, the mutation in strain OW12 gave a peculiar complementation pattern when on the episome. The data are presented in the right hand column of Table 2. This mutant episome complemented poorly with some chromosomal *mglA* and *mglC*

mutations but complemented with no *mglB* mutations. Hence, although the mutation is listed as a *mglA*, *mglB*, *mglC* mutation, it is really probably a *mglB* mutation, but shows partial dominance in many instances. Although it was not similarly tested, strain OW13 is assumed to be a *mglB* mutation as well since it has similar characteristics (Table 2) (see also Table 1 in accompanying investigation [14].

Map position of mutants. Kort, Reader, and Adler (in preparation) have mapped the *mgl* locus by interrupted mating experiments and by P1 transduction. It is located at approximately 41 min on the *E. coli* map (16). They used mutants with mutations in *mglB* in their study. Mutations in *mglA*, *mglB*, and *mglC* have roughly the same (about 33%) frequency of co-transduction with a mutation in fructose enzyme II of the phosphotransferase system. Therefore, it is likely that they are clustered and possibly constitute part or all of an operon.

Missense character of mutants. The mutants were all tested for being ambers by putting in F402 which has the *supD* suppressor, and F514 which has the *supU* suppressor. Their behavior on galactose swarm plates was assessed. None was found to be suppressible by these two suppressors, and hence all are presumed to be missense mutations. However, it is possible that some of the mutants might be ochre or "UGA" mutants or amber mutants not suppressible by *supD* or *supU*.

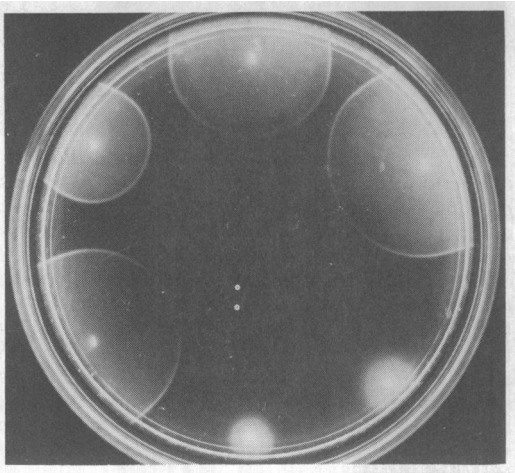

FIG. 3. *Complementation of mglA⁻ and mglB⁻ mutants. The galactose swarm plate contained the 10 amino acid supplements, lacking histidine, which was endogenously synthesized due to presence of the F131 episome. Starting at the top and reading clockwise, the mgl markers on episome and chromosome, respectively, were +/B⁻, +/A⁻, B⁻/B⁻, A⁻/A⁻, B⁻/A⁻, A⁻/B⁻. The last two swarms exemplify complementation, the first four being controls. The mglA⁻ marker was from strain OW22 and the mglB⁻ marker was from strain OW5.*

DISCUSSION

Complementation of a set of new mutants in galactose ring formation has shown that three genes are involved: *mglA*, *mglB*, and *mglC*. Because of the requirement of the galactose-binding protein for both galactose taxis (11) and transport (via the β-methylgalactoside permease) (2, 4) it is clear that galactose transport and taxis are closely connected and might share other components. Indeed, quantitative assays of chemotaxis and transport for galactose in the newly isolated mutants shows that *mglA*, *mglB*, and *mglC* all affect both galactose taxis and transport (14).

Identification of the mglB gene. Boos (5) has shown that EH3039 is a structural gene mutant in the galactose binding protein by virtue of its having an altered amino acid sequence and by virtue of the fact that further changes in sequence occurred upon reversion. Since the present study shows the defect in EH3039 to be in the *mglB* gene (Table 3), this

TABLE 3. *Complementation of previously described mutants*[a]

Strain	Gene	Mutation on episome										
		B 4	B 5	B 6	A 18	A 20	A 22	C 31	C 33	AC 41	AC 43	AC 44
AW520	B	−	−	−	+	+	+	+	±	+	+	+
AW543	B	−	−	−	++	+	++	+	+	+	++	++
AW550	B	−	−	−	++	++	+	+	+	++	++	++
AW551	B	−	−	−	++	++	+	++	+	++	++	++
EH3039 gal⁺	B	−	−	−	+	++	+	++	++	++	++	++
AW526	−	*	*	*	*	*	*	*	*	*	*	*
20SOK⁻ gal⁺	AC	+	+	++	−	−	−	−	−	−	−	−
W3092i gal⁺	AC	++	++	++	−	−	−	−	−	−	−	−

[a] Complementation was carried out as described in the text. Symbols are defined in the legend to Table 2. The asterisk means negative for wild-type episome and all mutant episomes tested (see text).

gene is the structural gene for the galactose-binding protein.

At least four other mutants previously studied for their defect in galactose taxis are now shown by this complementation study (Table 3) to be in *mglB*, the galactose-binding protein gene. These include strain AW551, which shows no galactose taxis but normal galactose transport; from its properties, Hazelbauer and Adler (11) considered it likely that AW551 was mutant in a gene whose product was involved in taxis but not in transport for galactose. However, Table 3 shows that the genetic defect in strain AW551 lies in *mglB*.

Nature of the mglA and mglC genes. Most of the newly isolated mutants had defects in only one gene, *mglA*, *mglB*, or *mglC*. However, six mutants were found that apparently had defects in both *mglA* and *mglC* and two were found that had defects in *mglA*, *mglB*, and *mglC*. It is not known whether these multiple mutations represent deletions, true multiple mutations, or point mutations showing partial dominance with all episomes tested. Evidence is presented, however, that at least one of the *mglA*, *mglB*, *mglC* mutations is actually a mutation in *mglB*. If so, this mutation shows partial dominance with all episomes having *mglA* or *mglC* mutations. Moreover, mutation *mglC 35* shows partial dominance over mutation *mglA 20* and *mglA 22*. Furthermore, the number of *mglA*, *mglC* mutants (6/43) seems too large to be accounted for by the existence of deletions or by two separate mutations, especially since no *mglA*, *mglB* or *mglB*, *mglC* mutants were found. Thus, many of the *mglA*, *mglC* mutations might similarly represent partial dominance mutations (i.e., each lies either in *mglA* or *mglC*) that show the effect with all *mglC* or *mglA* episomes, respectively, tested. Alternatively, if *mglA* and *mglC* are part of an operon, then the *mglAC* mutants might be nonsense mutants in one of these genes with polar effects on the other. The possibility remains, of course, that *mglA* and *mglC* are really just one gene in which there is extensive intragenic complementation. Until the corresponding polypeptides are isolated in vitro, it probably will not be possible to decide for certain whether there are one or two genes.

From studies of mutants in β-methylgalactoside permease, Boos (4) suggested the existence of components in addition to the galactose-binding protein, one being mutant in strain W3092i, whose galactose-binding protein is intact. The present study shows that W3092i is defective in *mglA*, *mglC* (Table 3), as is also the case for another β-methylgalactoside permease mutant previously studied, 20SOK$^-$.

The parental level of galactose-binding protein was found in the new *mglA*, *mglC*, and *mglA-mglC* mutants studied (OW19, OW22; OW23, OW27; OW37, OW39, OW42, respectively) when measured by the immunological assay of Boos (4) (M. Goy, unpublished data). This result indicates that *mglA* and *mglC* genes code for products other than the galactose binding protein.

The *mglA* and *mglC* genes, now newly recognized, make products that are involved in galactose ring formation, but these products now remain to be identified. Presumably, they interact in some way with the galactose-binding protein. In the accompanying investigation (14) we show that mutants in galactose ring formation can have defects in galactose taxis, transport, or both.

Finally, we wish to point out that since the use of the present method has allowed isolation of strains which have wild-type chemotactic responses but reduced transport (14), this is a new method for isolating transport mutants. It should be useful for obtaining transport mutants for any system in which chemotaxis is linked to a transport system.

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