Physical and Genetic Characterization of the Melibiose Operon and Identification of the Gene Products in *Escherichia coli*\

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We have identified hybrid plasmids carrying the melibiose operon of *Escherichia coli* in a colony bank of Clarke and Carbon (Tsuchiya, T., Ottina, K., Moriyama, Y., Newman, M., and Wilson, T. H. (1982) J. Biol. Chem. 257, 5125–5128). Using one of the plasmids as a starting material, the DNA fragments containing the melibiose operon were recombined in a vector pBR322. Restriction maps were prepared, and several DNA segments were subcloned into pBR322. Genetic complementation tests and recombination analyses using those plasmids and melA and melB mutants as well as biochemical analyses of mel mutants transformed with those plasmids enabled us to determine the physical location of promoter, melA, and melB on the DNA segment. The size of the melAB region was about 5,000 base pairs. Gene products were identified using maxicells harboring plasmids carrying the melibiose operon. The apparent molecular weight of the α-galactosidase (coded by melA) was about 50,000 and that of the melibiose carrier (coded by melB) was about 31,000, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The melibiose carrier was also identified as a 30,000-dalton protein in reconstituted proteoliposomes which possessed melibiose transport activity.

The melibiose operon of *Escherichia coli*, which is located at approximately 93 min on the revised genetic map, consists of at least two structural genes, melA and melB, which code for α-galactosidase and the melibiose transport carrier, respectively (1, 2). These two proteins are necessary for the metabolism of melibiose.

The genetic properties of the melibiose operon and biochemistry of the gene products are poorly understood. The gene organization of each gene is not known. It is known, however, that the melibiose operon is an inducible one (3). Catabolite repression on this gene expression and its release by cyclic AMP have been reported in *E. coli* (4) and in *Salmonella typhimurium* (5). Thus, the structure and the regulatory mechanism of this operon seem to be similar to those of the lactose operon which contains structural genes for β-galactosidase (lacZ) and the lactose transport carrier (lacY) (6). The lactose operon, however, contains one more structural gene for transacetylace (lacA). In addition, the structural gene for repressor (lacI) is present at the beginning of the lactose operon. At present, it is not clear whether or not the transacetylace gene is present in the melibiose operon. Also, it is not clear whether or not repressor for the melibiose operon is present. The gene products of the melibiose operon, α-galactosidase and the melibiose carrier, have not been identified, although α-galactosidase activity has been measured in whole cells and in cell extracts (8) and the melibiose carrier activity has also been measured in whole cells (3, 9), in membrane vesicles (9), and in reconstituted proteoliposomes (10). Our studies represent an attempt to obtain insight into the genetic and biochemical aspect of the melibiose system.

We describe here the construction of a series of recombinant plasmids carrying various portions of the melibiose operon. Restriction mapping, organization of genes, and identification of gene products of the melibiose operon of *E. coli* are also reported.

**EXPERIMENTAL PROCEDURES**

**Organism and Growth**—The *E. coli* strains utilized in these studies are listed in Table I. All strains are derivatives of K12 and most of them lack the lactose transport system. The α-galactosidase-negative strains and melibiose transport-negative strains were obtained from W3333-2 with nitrosoquamide mutagenesis (15) followed by penicillin treatment selection in the presence of melibiose. The recA derivative of each strain was obtained by mating with K16-99 (14). The identification of strains containing plasmids carrying the melibiose genes from the Clarke-Carbon collections (12) was described previously (10). Unless specified otherwise, cells were grown in L broth at 37 °C. For transport assays, cells were grown in minimal medium (17) supplemented with 1% Bacto-tryptone (Difco) and 10 mM melibiose when necessary and harvested at middle exponential phase of growth.

**Construction of Plasmids Carrying mel DNA**—The plasmid DNA of pLC25-33 was used as the starting material. Plasmid DNA was prepared by a published procedure (15). DNA fragments containing the mel genes were inserted into pBR322 plasmid DNA digested with identical restriction endonucleases. To determine restriction sites, these plasmids were subjected to single and multiple restriction enzyme digestion followed by agarose or acrylamide gel electrophoresis (19).

**Bacterial Transformation**—Competent cells were prepared from each strains and transformed according to the procedure of Mohlke and Doskocil (20).

**Genetic Complementation Assay**—Competent cells of mel mutants (and recA) were transformed with plasmids. The transformants were analyzed for acquisition of appropriate drug resistance concomitant with the ability to utilize melibiose.

**Recombination Assay**—Transformed cells were selected on L-agar

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1 An α-galactosidase specified by Raf-plasmid D1021 has been purified from *E. coli* (7). But properties of this enzyme are very much different from the α-galactosidase coded by genomic DNA.
containing either ampicillin or tetracycline in order to select for plasmid-carrying bacteria. Antibiotic-resistant colonies were then streaked on agar plates containing melibiose and checked whether or not melibiose recombinants appeared.

**Identification of Plasmid-coded Proteins in Maxicells**—Maxicells were prepared by the method of Sancar et al. (21) using transformed derivatives of N1790 (13). Cells, uninduced or induced with melibiose, were labeled for 60 min with ~[^35]S]methionine, washed, and subjected to SDS-polyacrylamide gel electrophoresis (22). The abbreviations used are: SDS, sodium dodecyl sulfate; TMG, methyl-0-D-thiogalactopyranoside.

**RESULTS**

**Cloning, Characterization, and Physical Mapping**—Previously, we had identified hybrid plasmids carrying *melAB* (JA200/pLC25-33, and JA200/pLC25-33) or *melA* (JA200/pLC16-37) (10) from Clarke-Carbon's colony bank (12) by genetic analysis. In those experiments, we assayed α-galactosidase activity and the transport activity of the melibiose carrier of these three cell lines and of the other three cell lines arbitrarily chosen from the Clarke-Carbon bank. Cells of JA200/pLC17-38, JA200/pLC25-33, and JA200/pLC16-37 showed several fold higher activity of α-galactosidase (data not shown). Strains JA200/pLC17-38 and JA200/pLC25-33 showed several fold higher activity of melibiose transport but JA200/pLC16-37 did not. These results are consistent with genetic results reported previously (10). Although pLC17-38 was shown to possess *melAB*, it has been reported that this plasmid might possess *recA* (12). The location of the *recA* gene on the linkage map is at approximately 58 min, which is very far from that of *melB* (1). We have not tested whether or not pLC17-38 carries the *recA* gene. Possible explanations for these unreasonable observations would be: 1) two different portions of chromosomal DNA were connected into one plasmid or 2) JA200/pLC17-38 we obtained was different from the original one. Plasmid pLC25-33 was used for subsequent experiments.

One of the striking properties of the melibiose carrier is that this carrier of wild type strain K12 is temperature-sensitive (3); the carrier is inactivated at 37 °C although it is stable at 30 °C. The chromosomal DNA segment of Clarke-Carbon plasmids was derived from *E. coli* CS520 (12). We have tested whether or not the melibiose carrier derived from this strain was temperature-sensitive. Cells of JA200/pLC25-33 was grown and induced with melibiose at various temperatures. Then, *Na*+-dependent TMG uptake, which is a characteristic of the melibiose system, was measured. Cells grown at 30 and 37 °C exhibited almost the same TMG transport activity (Fig. 1), but cells grown at 42 °C showed slightly lower activity (Fig. 1). Thus, the melibiose carrier derived from CS520 was not temperature-sensitive, being stable at 37 °C and fairly stable even at 42 °C. No *Na*+-dependent TMG transport was observed in JA200 (lacY*) when grown at 37 °C, indicating that the melibiose carrier of JA200 was temperature-sensitive.

Since pLC25-33 does not possess a drug resistance marker, it is not easy to test for the presence of this plasmid. Thus, we recloned the *melB* operon present in pLC25-33 to a cloning vector pBR322. DNA fragments digested with various restriction endonucleases were ligated to pBR322 DNA digested with identical enzymes, and new plasmids were obtained. Strains RA11r (*melA*, *recA*) and RE16r (*melB*, *recA*) were transformed with these plasmids and assayed for their ability to grow on melibiose (see below). Cleavage sites for various restriction enzymes were then determined with those plasmids (Fig. 2).

**Genetic Mapping of Promoter, melA, and melB**—Using *melA*, *recA* strain (RA11r) and *melB*, *recA* strain (RE16r), each new plasmid was subjected to a complementation test

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**TABLE I**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant properties</th>
<th>References</th>
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<tbody>
<tr>
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<td>9</td>
</tr>
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<td><em>melA</em>B*, lacZ-Y*</td>
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<td><em>melA</em>B*, recA*, lacZ-Y*</td>
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<td>RE16</td>
<td><em>melA</em>B*, lacZ-Y*</td>
<td>11</td>
</tr>
<tr>
<td>RE16r</td>
<td><em>melA</em>B*, recA*, lacZ-Y*</td>
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</tr>
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<tr>
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<td>K16-69</td>
<td>recA*</td>
<td>14</td>
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<tr>
<th>Strains</th>
<th>Relevant properties</th>
<th>References</th>
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<tr>
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</tr>
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<tr>
<td>N1780</td>
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<tr>
<td>K16-69</td>
<td>recA*</td>
<td>14</td>
</tr>
</tbody>
</table>

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The abbreviations used are: SDS, sodium dodecyl sulfate; TMG, methyl-0-D-thiogalactopyranoside.
enzymes were then determined with PstI, B, BamHI.

Plasmid pLC25-33 was cleaved with various restriction endonucleases, and the resulting DNA fragments were inserted into pBR322 DNA (pertinent restriction sites are shown on the right) digested with identical restriction enzymes. Cleavage sites for those restriction enzymes were then determined (S, SalI; E, EcoRI; H, HindIII; P, PstI; B, BamHI).

**Table II**

<table>
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<tr>
<th>Plasmids</th>
<th>RA11r (melA')</th>
<th>RE16r (melB')</th>
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<tr>
<td>pSTY3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pSTY21</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pSTY41</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pSTY81</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pSTY91</td>
<td>+</td>
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</tr>
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</table>

(Table II). Plasmid pSTY3, which carries the longest chromosomal DNA fragment among the newly constructed plasmids (Fig. 2), complemented both melA' and melB'. Plasmid pSTY21 complemented melA' but not melB'. Plasmids pSTY41, pSTY81, and pSTY91 showed the same complementation pattern with pSTY3. From these results, the following conclusions were obtained. 1) Genes melA and melB are located between the PstI site and the EcoRI site (Fig. 2) because this PstI-EcoRI fragment is common to pSTY41, pSTY81, and pSTY91. 2) Gene melA is located between the PstI site and the BamHI site (Fig. 2) because pSTY21 complements only melA'. Thus, at least part of the melB gene is located within the two BamHI sites. 3) The promoter of the melibiose operon is present in pSTY81 and pSTY41 since no functional promoter of pBR322 is available for inserted DNA when ligated to either the EcoRI site or the HindIII site of pBR322 (28). On the other hand, the promoter of the ampicillin gene is available when a DNA fragment is ligated to the PstI site, and the promoter of the tetracycline gene is available when ligated to the BamHI site or the SalI site. Thus, among the plasmids we obtained, the shortest plasmid carrying promoter, melA, and melB was pSTY91. This plasmid contained the EcoRI fragment of chromosomal DNA.

To assign the locations of promoter, melA, and melB more precisely, we have constructed several more plasmids from pSTY81 and pBR322. An EcoRI fragment of pSTY81, which contains promoter, melA, and melB, was digested with PstI or BamHI, and each fragment was isolated and then ligated to appropriate restriction sites of pBR322 (Fig. 3). Plasmids thus constructed were used to transform melA- (RA11, RA56), melB' (RE16, RE57), or melB" (W3133) strains. Using recA+ derivatives of these strains, complementation analysis was performed. Plasmid pSTY81-30 complemented melA- mutants (Table III), but pSTY81-20 and pSTY81-40 did not complement any strains tested. These results indicate that the EcoRI-BamHI fragment (Fig. 3) carries the promoter and the melA gene and that the melA gene is proximal to the promoter. Since pSTY81-20 did not complement melA' and melB', although melA and melB are located on the PstI-EcoRI fragment as mentioned above, it was concluded that the promoter of the melibiose operon was located between the EcoRI site and the PstI site of pSTY81-30. The recA+ cells of melA', melB', and melB" were transformed with pSTY81-20, pSTY81-30, or pSTY81-40 to confirm the location of melA and melB. The drug-resistant transformants were tested to see whether or not melibiose+ recombinants appeared at 37°C. From every strain (melA', melB', and melB"), melibiose+ recombinants appeared when transformed with pSTY81-20 (Table III). Melibiose+ recombinants appeared from melA- mutants and RE57 (melB') but not from RE16 (melB") and W3133 (melB") when transformed with pSTY81-30. Plasmid pSTY81-40 was capable of producing melibiose+ recombinants at 37°C when introduced into RE16 and W3133, but not others. In addition, melibiose+ recombinants were obtained when pSTY81-40 was introduced into several other melB mutants (data not shown). These results support the view that the melA is located between the PstI site and the BamHI site and that most portions of the melB gene are located between the BamHI site and the BamHI site (Fig. 3). Thus, promoter, melA, and melB were assigned on the restriction map, as shown in Fig. 3. The size of the PstI-BamHI fragment was about 1.7 kilobases and that of the BamHI-BamHI fragment was 1.1 kilobases. If the promoter exists at the left of the PstI site, melibiose transport activity in induced N1790/pSTY91 and N1790/pSTY81 showed similar high activities. Uninduced cells of N1790/pSTY91 and N1790/pSTY81 showed similar high activities. Uninduced cells of N1790/pSTY81 and N1790, however, showed very low activities. α-Galactosidase activity in induced N1790/pSTY91 and N1790/pSTY81 was several fold higher than that in induced N1790. Thus, cells bearing the plasmids showed a gene dosage effect. The melibiose carrier of N1790 seems to be temperature-sensitive, a characteristic of wild type K12, since melibiose transport activity in N1790 grown at 37°C was extremely low (Fig. 4).

**Identification of the Gene Products**—To identify the gene products of the melibiose operon, the maxicell method (21) was utilized. Polypeptides encoded by melAB genes were synthesized in vivo and were labeled with [35S]methionine. Maxicells harboring pSTY91, which carries melAB genes but...
Identification of melAB Gene Products in E. coli

For genetic complementation and recombination tests, competent cells of each mutant were transformed with each plasmid. recA- cells were used for complementation test, and recA+ cells were used for recombination test. The appropriate drug-resistant transformants were tested for their ability to grow on melibiose at 37 °C. + indicates that colonies appeared on an agar plate containing a minimal medium and 10 mM melibiose, and - indicates that no colony appeared.

### TABLE III

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>RA11 -recA</th>
<th>RA11 +recA</th>
<th>RA56 -recA</th>
<th>RA56 +recA</th>
<th>RE57 -recA</th>
<th>RE57 +recA</th>
<th>RE16 -recA</th>
<th>RE16 +recA</th>
<th>W3133 -recA</th>
<th>W3133 +recA</th>
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<tbody>
<tr>
<td>pSTY81-20</td>
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<td>-</td>
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### TABLE IV

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<tr>
<th>Cells</th>
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<th>Induced</th>
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<tr>
<td>α-Galactosidase</td>
<td>nmol/min·mg protein</td>
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</tr>
<tr>
<td>N1790</td>
<td>2.9</td>
<td>14.2</td>
</tr>
<tr>
<td>N1790/pSTY81</td>
<td>3.3</td>
<td>93.5</td>
</tr>
<tr>
<td>N1790/pSTY91</td>
<td>57.2</td>
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### FIG. 4

**Constitutive activity of the melibiose carrier in cells harboring pSTY91.** Cells were grown in the absence (open symbols) or in the presence (closed symbols) of melibiose in a minimal medium (17) supplemented with 1% Bacto-tryptone. Cells were washed and assayed for transport in the presence of 100 mM potassium phosphate, pH 7.0, 10 mM NaCl, and 0.1 mM ['H]melibiose. Circle, N1790; triangle, N1790/pSTY81; square, N1790/pSTY91.

not intact amp gene, produced a 50,000-dalton polypeptide and a 31,000-dalton polypeptide (Fig. 5A). Two major radioactive polypeptides of apparent Mr = 31,000 and 27,000 and a minor polypeptide of 50,000 daltons were detected on SDS-polyacrylamide gel when maxicells harboring pSTY81 were analyzed (Fig. 5A). Judging from radioactive polypeptide synthesized from pBR322, polypeptides with apparent Mr = 31,000 and 27,000 are β-lactamase (21). The larger one would be the precursor-possessing leader peptide. It was difficult to detect the 37,000-dalton polypeptide which was coded by the tet gene (21) under our experimental conditions. It seemed likely that the 31,000-dalton band produced from pSTY81 contained a gene product of the mel operon in addition to β-lactamase. In order to determine which polypeptide (50,000- or 31,000-dalton) was encoded by melA and which one by melB, we analyzed total cellular proteins of uninduced cells and of melibiose-induced cells, harboring pSTY81, on SDS-polyacrylamide gel and stained with Coomassie brilliant blue. A very dense band at 50,000 daltons was observed with induced cells but not with uninduced cells (Fig. 5B). This dense band disappeared when proteins in membrane vesicles were analyzed in a similar way (Fig. 5B). Namely, no difference in the 50,000-dalton band was detected between the two samples. Therefore, the 50,000-dalton protein is not a membrane protein. Since α-galactosidase is a soluble protein (8) and melibiose transport carrier is a membrane protein (9, 10),

![Fig. 4](http://example.com/f4.png)

**Fig. 4.** Constitutive activity of the melibiose carrier in cells harboring pSTY91. Cells were grown in the absence (open symbols) or in the presence (closed symbols) of melibiose in a minimal medium (17) supplemented with 1% Bacto-tryptone. Cells were washed and assayed for transport in the presence of 100 mM potassium phosphate, pH 7.0, 10 mM NaCl, and 0.1 mM ['H]melibiose. Circle, N1790; triangle, N1790/pSTY81; square, N1790/pSTY91.

![Fig. 5A](http://example.com/f5a.png)

**Fig. 5. Analysis of gene products by polyacrylamide gel electrophoresis.** A, maxicells were irradiated and labeled with L-[35S]methionine as described under "Experimental Procedures." A radioautogram of a polyacrylamide gel is shown. Lane 1, maxicells with no plasmid; lane 2, pBR322; lane 3, pSTY91; lane 4, pSTY81. B, total cellular proteins and membrane proteins of uninduced N1790/pSTY81 or melibiose-induced N1790/pSTY81 were analyzed on polyacrylamide gel and stained with Coomassie brilliant blue. Lane 1, cellular proteins of uninduced N1790/pSTY81; lane 2, cellular proteins of melibiose-induced N1790/pSTY81; lane 3, membrane proteins of uninduced N1790/pSTY81; lane 4, membrane proteins of melibiose-induced N1790/pSTY81. Molecular weight markers used were bovine serum albumin (66K, Mr = 66,000), ovalbumin (45K), DNase I (31K), and carbonic anhydrase (29K).
Identification of melAB Gene Products in E. coli

Fig. 6. Comparison of proteins of reconstituted proteoliposomes. Lane 1, membrane vesicles were prepared from melibiose-induced cells of RA11/pLC25-33. Membrane proteins were solubilized and then reconstituted into liposomes. Lane 2, membrane vesicles were prepared from uninduced cells of RA11/pLC25-33. Solubilization and reconstitution were performed. Proteins of both types of proteoliposomes were analyzed on 10% polyacrylamide gel. The molecular weight markers used were ovalbumin (45K, M, = 45,000), lactate dehydrogenase (35K), carbonic anhydrase (29K), and trypsin (23K). The arrow indicates the position of a band (about M, = 30,000) which is present in lane 1 but absent in lane 2.

It is reasonable to assume that the 50,000-dalton protein is α-galactosidase, the product of melA, and that the 31,000-dalton protein is the carrier, the product of melB. This conclusion was confirmed by the following experiment. The melibiose carrier was identified in reconstituted proteoliposomes. In these experiments, membrane proteins were solubilized with octyl glucoside from membrane vesicles prepared from uninduced cells and from induced cells and then reconstituted into liposomes, as reported previously (10). Melibiose transport activity was observed with reconstituted proteoliposomes derived from induced cells, but not with proteoliposomes derived from uninduced cells (data not shown). Proteins present in both types of proteoliposomes were then analyzed on SDS-polyacrylamide gel. About 30 major protein bands were detected. Among these, only one band was different in two types of proteoliposomes. A polypeptide with an apparent M, = 30,000 was present only in reconstituted proteoliposomes which were derived from induced cells (Fig. 6). This band was absent in proteoliposomes from uninduced cells. Thus, this band represents the melibiose carrier. This molecular weight is very close to the estimated value of the melibiose carrier described above.

DISCUSSION

We have constructed hybrid plasmids carrying the melibiose operon of E. coli using pBR322 as a vector. Several DNA segments of the melibiose operon were subcloned. By complementation analyses and recombination tests, we physically assigned promoter, melA, and melB on 4.6-kilobase DNA segment of the melibiose operon. Organization of the melibiose operon was very similar to that of the lactose operon. Namely, promoter, galactosidase gene, and carrier gene were present in this order in both operons. Because of the similarities in structure of melibiose and lactose and organization of the melibiose operon and the lactose operon, it is likely that these two operons were derived from a common evolutionary origin. To obtain insight into the homology of the two operons at a molecular level, we are trying to determine the nucleotide sequence of the melibiose operon. So far, we have determined the nucleotide sequence of several portions of the DNA segments. Just at the left of the PstI site (Fig. 3), we have found a promoter structure which is very similar to that of the lactose operon (29).

melA, the gene for α-galactosidase, is located just downstream of the promoter. The size of this gene seems to be much smaller than that of lacZ. The size of melAB is at most 3.0 kilobases and that of lacZ is also about 3.0 kilobases (30). The apparent molecular weight of α-galactosidase is 50,000, as mentioned above. On the other hand, the molecular weight of β-galactosidase has been reported to be about 116,000 (30, 31). If the molecular weight of α-galactosidase estimated from the mobility on SDS-polyacrylamide gel is close to the real value, the size of α-galactosidase is about a half of that of β-galactosidase.

The estimated size of the melB gene is at most 1.5 kilobases. Based on this size and on our observations that most of the melB mutants possessed defects in the BamHI-BamHI fragment and one melB mutant in a region upstream of the BamHI site (Table III),3 we predicted the region of melB as shown in Fig. 3. Now we are trying to determine the nucleotide sequence of melB, the gene for the melibiose carrier. Preliminary sequencing results revealed that the size of melB is about 1.4 kilobases.3 It should be pointed out that the molecular weight of a polypeptide coded by 1.4-kilobase DNA should be 51,000–52,000, and this value is quite different from an apparent molecular weight of the identified carrier shown in Figs. 5 and 6 (30,000–31,000). This discrepancy is not surprising, however, because a similar discrepancy has been reported with the lactose carrier. An apparent molecular weight of the lactose carrier estimated from analysis on SDS-polyacrylamide gel was about 30,000 (32) and that calculated from the nucleotide sequence was 46,500 (33). Since amino acid composition of the purified lactose carrier agreed very well with the composition predicted from the nucleotide sequence (34), it was believed that there was no processing of the polypeptide. Therefore, it is very likely that these two carrier molecules migrate very differently from most other proteins on SDS-polyacrylamide gel. It is thought that membrane proteins which are extremely hydrophobic bind large amounts of SDS and migrate faster than soluble proteins of a comparable molecular weight. Thus, we believe that the molecular weight of the melibiose carrier is about 51,000–52,000. This should be clarified after the completion of sequencing of melB.

It is not known whether or not a transacetylase gene which is present next to the carrier gene in the lactose operon is also present in the melibiose operon. We could not detect the third polypeptide in maxicell experiments (Fig. 5). This, however, does not necessarily mean the absence of the third structural gene.

It is also not known whether or not the gene for repressor

3 H. Yazyu, and T. Tsuchiya, unpublished results.
is present at the head of the promoter. Two lines of experiments are now under way to clarify this point. We are trying to isolate the melI mutant and trying to determine nucleotide sequence of this region.

Burstein and Kepes (8) have extracted α-galactosidase from a derivative of E. coli K12. The approximate molecular mass of the active form was about 200,000 daltons. We estimated the approximate molecular weight of the subunit of α-galactosidase as 50,000. Therefore, the active form of α-galactosidase would be tetramer. An α-galactosidase specified by the Raf-plasmid D1021 has been purified by Schmid and Schmitt (7). The reported molecular weight of the subunit is 82,000, and the active enzyme consists of four subunits (7). Furthermore, the active form of β-galactosidase of E. coli is tetramer (35). Thus, tetramer structure seems to be the common functional form for these galactosidases.

It should be noted that a constitutive promoter should be present next to the melibiose operon. A plasmid pSTY41 carries a chromosomal DNA fragment ligated to the HindIII site of pBR322 (Fig. 2). This insertion inactivates the promoter of the tet gene (28). For the expression of the tet gene, new promoter in inserted DNA segment is required. Although the promoter of the melibiose operon is present in this DNA segment, the expression of the operon is inducible. Since cells harboring pSTY41 was resistant to tetracycline even in the absence of melibiose, an inducer of the melibiose operon, the expression of the tet gene in pSTY41 should depend on a constitutive promoter which should be present between the EcoRI site and the HindIII site (Fig. 2, right). It is not known what operon is present in this region (1).

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