Aust. J. Biol. Sci., 1974, **27**, 331–40

Characterization and Intragenic Position of Mutations in the Gene for Galactoside Permease of *Escherichia coli*

J. Langridge

Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, A.C.T. 2601; and Department of Genetics, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra City, A.C T. 2601.

Abstract

To test certain models for the structure of the gene for galactoside permease and its product, appropriate mutants of *E. coli* were isolated after mutagenic treatment, partially characterized and mapped. The 69 mutants found to be in the permease gene were mapped by crosses with episomal deletions which divided the gene into 13 regions.

Both facilitated diffusion, measured by the uptake of *o*-nitrophenyl β-galactoside, and active transport, measured by the uptake of radioactive methyl β-thiogalactoside, were examined in the mutants. The results gave no evidence for separate cistrons coding for each activity. This conclusion was supported by the lack of demonstrable complementation between all pairs of point mutations tested.

Previous experiments had suggested that the galactoside permease protein may have two substrate-binding sites, but the failure of attempts to select corresponding mutants does not support the suggestion.

The data indicate that the gene for galactoside permease consists of only one cistron coding for a single protein molecule responsible for facilitated diffusion and that active transport additionally requires the products of other genes outside the lactose operon.

Introduction

The transport of β-galactosides in *Escherichia coli* is at least partly under the control of the *Y* gene of the lactose operon. Evidence suggests that the galactoside permease can carry out both facilitated diffusion, where certain galactosides are transported independently of metabolic energy (Koch 1964), and active transport, which requires a continual supply of metabolic energy (Kepes 1957). Facilitated diffusion, as measured by the entry of *o*-nitrophenyl β-galactoside, appears to be a function of a particular membrane component, the M protein (Carter *et al.* 1968). This protein, which has been isolated and partially purified, has been shown to be a product of the *Y* gene (Fox *et al.* 1967). However, it is uncertain if the specification of the M protein is the sole function of this gene. A mutant isolated by Wilson *et al.* (1970) had unimpaired transport of *o*-nitrophenyl β-galactoside, although it was incapable of active transport. The responsible mutation was found to be located in the *Y* gene (Wilson *et al.* 1972), which suggests that this gene has transport functions in addition to the specification of the M protein.

There are therefore at least two possible structures for the *Y* gene; either it is a single cistron coding only for the M protein or it consists of two cistrons, one concerned with the M protein and the other with another necessary component of the active transport system. A mutational study of *Y* gene structure in relation to the

functioning of the galactoside permease has therefore been made in an attempt to discriminate between the two models.

Materials and Methods

All mutations of the galactoside permease (*Y*) gene were induced in *E. coli* K-12 Hfr Hayes, strain 3000. The mutants were of several origins. The majority (42 mutants), numbered from 3020 to 3664, were isolated on lactose–triphenyltetrazolium plates from bacteria treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as described previously (Langridge 1968). A second experiment yielded 14 additional mutants numbered from 43 to 456. Six mutants (26, 34, 42, 168, 170, 230) were isolated on melibiose–triphenyltetrazolium plates at 42°C after treatment with *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine. A further seven mutants (P, V, X, N, R, P678, W1-4) were from the collection of Drs J. Monod and F. Jacob of the Institut Pasteur, Paris.

Table 1. Partial or complete deletions of the gene *Y* for galactoside permease

Deletion	Genotype[A]	Extent of deletion	Reference or origin
W3133	F⁻ *thi str*	Deletion of *lac-Y*	Cook and Lederberg (1962)
RV/FMS37	F⁻ Δ*lac thi str*/F′ MS37	Partial deletion of *lac-Z* and *lac-Y*	Malamy (1966)
MS1019	F⁻ *thi thr leu arg str*	Internal deletion of *lac-Y*	Malamy (1966)
1	F⁻ *thi thr leu arg str*	Internal deletion of *lac-Y*	Institut Pasteur
X65	F⁻ *thi thr arg leu str*	Internal deletion of *lac-Y*	Institut Pasteur
RV	F⁻ *thi str*	Deletion of *lac* operon	Institut Pasteur
G9	F⁻ *thi pro-C str*	Internal deletion of *lac-Y*	This paper
G11	F⁻ *thi pro-C str*	Internal deletion of *lac-Y*	This paper
X_2/FX_2	F⁻ Δ*lac thi thr leu arg his str*/F′ Δ*lac*	Partial deletion of *lac-Z*; complete deletion of *lac-Y* and probably *lac-A*	Institut Pasteur

[A] Gene symbols according to Taylor and Trotter (1972).

The origins of the permease deletions used in mapping are given in Table 1. For purposes of mapping all the F⁻ deletions were transferred to episomes and maintained as homogenotes. The deletion homogenotes tend to lose their episomes and must be reisolated from single colonies by test-crossing each time they are used. Crosses were made by the superimposition of drops of mutant and deletion bacteria on agar plates containing mineral salts, thiamin and lactose. The mutations are difficult to map because many mutants grow too well on lactose for recombinants to be easily visible. The best procedure was to grow the mutants to be crossed in mineral medium containing glucose to give maximum catabolite repression and to plate the crosses on a reduced lactose concentration (0·1 mg/ml).

For the study of complementation, homogenotes of two mutations, located towards the ends of *Y*, were constructed. The mutations were first transferred to a female background by crossing to an F⁻ stock containing a wild-type lactose operon and a mutation in the closely linked *pro-C* gene. Recombinants that were proline-positive and lactose-negative were selected and tested for the presence of the introduced mutation by backcrosses to the appropriate parent. Heterogenotes containing the mutation in the chromosome and a wild-type episome were selected on lactose–tetrazolium plates following sexduction. The mutation homogenotes were then obtained by streaking the heterogenotes on lactose–tetrazolium plates, selecting red segregants and testing them by further crosses. Complementation tests were made by crossing the homogenotes to selected F⁻ permease mutants and plating for heterogenotes on eosin–methylene blue plates. The plates contained, in addition to yeast extract and peptone, eosin Y (0·4 g/l), methylene blue (0·065 g/l) and lactose (10 g/l). If complementation occurs the colonies of heterogenotes develop, after 18 h incubation at 37°C, a diffuse red colour that is darker than that of the parents. Colonies were identified as containing heterogenotes by the appearance of dark-red recombinant sectors after about 48 h incubation.

Facilitated diffusion of galactosides by galactoside permease mutants was measured from the rate of hydrolysis of *o*-nitrophenyl β-galactoside in intact cells. The galactoside permease is half-saturated by a concentration of 10^{-3}M *o*-nitrophenyl β-galactoside (Kepes and Cohen 1962), whereas about 3×10^{-4}M is required to half-saturate the β-galactosidase (EC 3.2.1.23). The hydrolysis rate of *o*-nitrophenyl β-galactoside is therefore limited by the rate of uptake of the substrate. Cells were grown for 18 h in medium composed of yeast extract and peptone containing isopropyl β-thiogalactoside (10^{-3}M) as inducer. They were then reinoculated into fresh medium with inducer and grown for 4 h to an absorbance at 420 nm of about 0·5. Galactoside permease activity was assayed at 28°C on samples of 2·5 ml. Cells treated with galactosyl thiogalactoside (10^{-3}M), which inactivates the permease by strong competitive inhibition, provided a control to allow for passive diffusion of substrate and leakage of β-galactosidase. The details of estimation followed the method of Crandall and Koch (1971). Assays were made in duplicate and the mean values were expressed as a percentage of wild-type uptake.

The capacity of the permease mutants for active transport was measured from their ability to concentrate radioactive methyl β-thiogalactoside (Crandall and Koch 1971). Cultures for assay were first grown overnight at 37°C in mineral medium containing glycerol (0·2%) and isopropyl β-thiogalactoside (10^{-3}M). They were then diluted 20-fold with fresh medium, also containing isopropyl β-thiogalactoside, and grown to a density of about 5×10^8 cells/ml. The cells were centrifuged at 4°C, resuspended in fresh mineral–glycerol medium and kept on ice. A sample of 1·2 ml of bacterial suspension was added to 100 µl of solution containing ^{14}C-labelled methyl β-thiogalactoside (2·5 µCi/ml) together with unlabelled methyl β-thiogalactoside (3·5 mg/ml) and incubated for 10 min at 37°C. The cells of 1-ml aliquots of the reaction mixture were collected on membrane filters and the radioactivity of the dried filters counted in a liquid scintillation counter. A control for unspecific absorption was provided by mutant W3133, which has a deletion of most of the *Y* gene. Levels of galactoside permease, which are the means of duplicate determinations, are expressed as percentages of the wild-type activity.

Results

Isolation and Characterization of Mutants

Most of the permease mutants were obtained by selection of red colonies on plates containing yeast extract–peptone medium with triphenyltetrazolium chloride and either lactose or melibiose (an α-galactoside). On this medium mutant colonies unable to ferment the sugar appear red because of the reduction of triphenyltetrazolium to a red insoluble formazan. Selected bacteria were tested for growth on glucose, lactose and melibiose and assayed for β-galactosidase activity. Mutants of the galactoside permease gene are expected to grow on glucose but not on melibiose at 42°C or on lactose at any temperature. In addition to transporting melibiose by the galactoside permease of the lactose system, strain K-12 of *E. coli* has a specific permease for the uptake of melibiose, but this is not synthesized at 42°C. Growth on melibiose at 42°C is therefore negligible if the galactoside permease of the lactose operon is inactive.

In the larger experiment 473 red mutants were isolated. Of these, 171 failed to grow on glucose and therefore contained mutations in essential functions outside the lactose operon. A further 156 mutants, although red, grew on lactose and could not be mapped; they were either leaky mutants of the lactose operon or had been mutated in other ways to allow formazan formation. Other mutants were in the β-galactosidase gene (59), were mucoid mutants (11), or affected permease function but failed to map in the *Y* gene (14). All of these mutants were discarded, leaving 62 mutants in which only the galactoside permease gene was affected. Only 42 of these mutants could be mapped because some (10) had too high a rate of reversion, some (9) grew too well on lactose to be mapped, and a further mutant became mucoid. With additional isolations 69 independent mutations of the *Y* gene were accumulated and mapped.

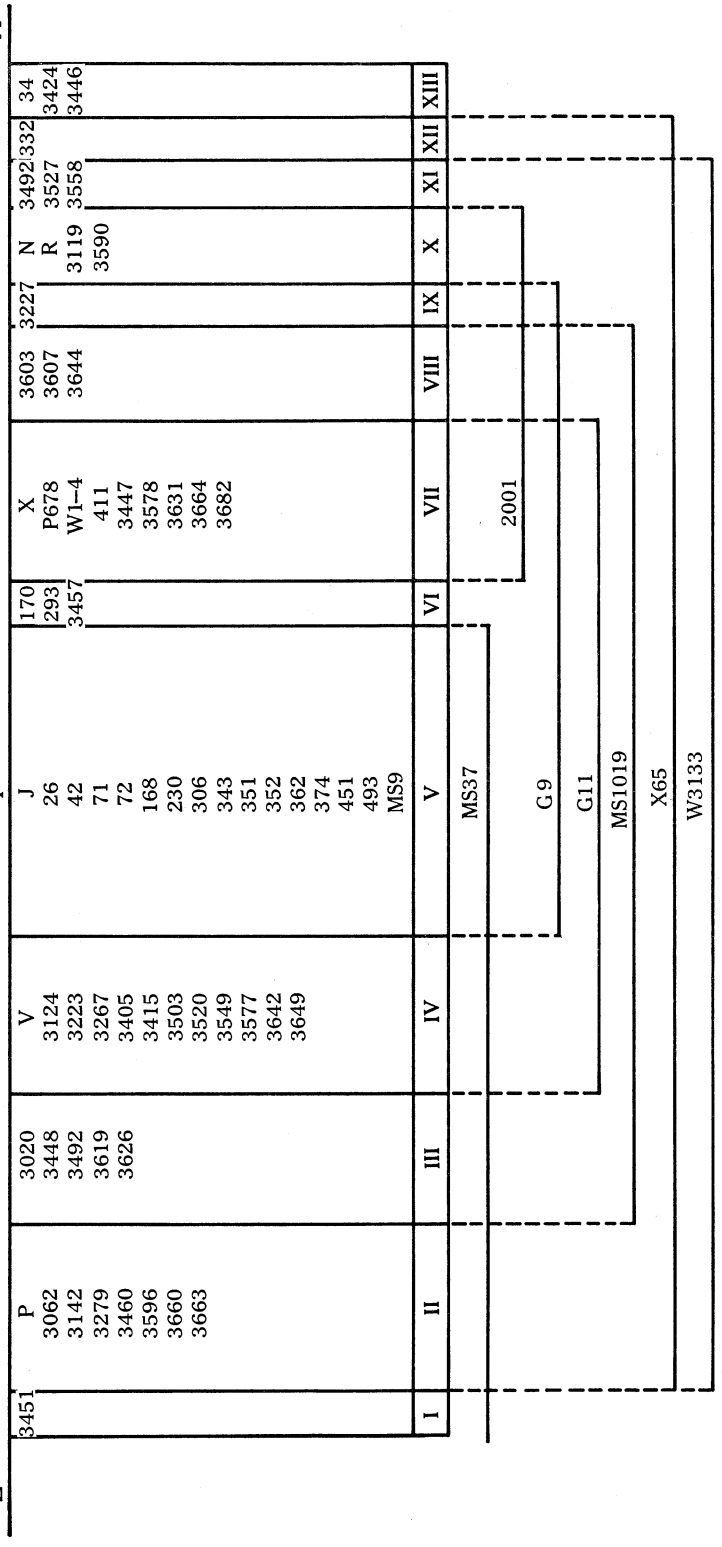

Fig. 1. Map of the gene for galactoside permease (*Y*) flanked proximally by the gene for β-galactosidase (*Z*) and distally by the gene for galactoside acetyltransferase (*A*). The roman numerals identify 13 regions of the gene defined by the deletions drawn and labelled in the lower part of the figure. The size of each region roughly corresponds to the number of mutations it contains. The mutations located within each deletion region are shown as numbers or letters.

It was found previously (Langridge and Campbell 1969) that with mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine of a lactose-constitutive strain, only about 2% of lactose-negative mutants carried mutations in the gene for galactoside permease. As will be shown later, many permease mutants grow reasonably well on lactose if an inducer of the lactose operon is supplied. Most permease-negative mutants seem unable to grow on lactose because they cannot induce the formation of β-galactosidase. Supporting this conclusion is the finding that, from the inducible strain used in the present experiments, about 50% of lactose-negative mutants had impaired galactoside permease. Forty-eight suspected permease-negative mutants previously isolated from a constitutive strain were tested, but all except 10 grew too well on lactose for mapping to be possible. Crossing tests showed that only 3 of the 10 mutations were located in the *Y* gene and, as these failed to recombine with point mutations, they evidently were large deletions of *Y*.

Attempts were made to isolate permease mutants in a constitutive strain by selecting for resistance to *p*-nitrophenyl β-thiogalactoside (1 mM). This substance enters the cell by the galactoside permease, inhibits the induction of the galactose pathway enzymes and kills the cells by an unknown mechanism. Forty-three resistant mutants were recovered but all proved to be large deletions removing most of the *Y* gene. Two of the deletions (G9 and G11) were used in the mapping of point mutations.

Intragenic Mapping of Permease Mutations

Mutants that fail to grow on both lactose at 37°C and melibiose at 42°C may have polar mutations occurring in the *Z* gene that prevent translation of the permease part of the messenger RNA, or mutations in the *Y* gene, or mutations in genes elsewhere that are involved in permease formation or activity. The polar *Z* mutations were previously eliminated by assays for β-galactosidase.

Initial tests to decide if the remaining mutations belonged to the *Y* gene were made by crosses with X_2/FX_2, which deletes about two-thirds of the *Z* gene, all of the *Y* gene and probably all of the *A* gene (for galactoside acetyltransferase, EC 2.3.1.18), and with W3133/FW3133, which removes most of the *Y* gene without affecting *Z* and *A*. Mutations that failed to recombine with both deletions or that recombined only with W3133/FW3133 were considered to be in *Y*, while the 14 that recombined with X_2/FX_2 were obviously in other genes.

The *Y* gene mutations were mapped by systematic crossing with all available episomal deletions and also used to identify further deletions and to define the length of the deletions. These crosses eventually resulted in a consistent pattern for the positions of deletions and point mutations as drawn in Fig. 1. The deletions divide the *Y* gene into 13 regions, indicated by roman numerals, each of which has been given a size roughly corresponding to the number of mutations in that region.

Suppression of Permease Mutations

Since it is necessary to assay for the two types of transport activity in the mutants to decide if the *Y* gene codes for more than one function, any chain-terminating mutants must be identified. If more than one function exists, a polar chain-terminating mutation in the proximal cistron could abolish all functions although located in the sequence corresponding to only one of them.

Suppressibility of all mutations was tested for by crossing the mutants to F^- stocks containing amber suppressors (*sup D* and *sup E*), ochre suppressors (*sup B* and

sup C) and an opal suppressor (of strain CAJ64). By the criterion of mutant growth on lactose following crosses to the suppressors, only 10 of 63 mutants tested were chain-terminating. Six mutants were UAG (amber), one was UAA (ochre) and three were UGA (opal). This low frequency of chain-terminating mutations (16%) is in marked contrast to that previously found for the β-galactosidase gene (93%) (Langridge and Campbell 1969). There are two possible causes of the difference. The proportion of chain-terminating mutations may be low because most amino acid replacements in the permease inactivate its function. Alternatively, the function of the permease protein may not tolerate the amino acids inserted by the suppressors and the crossing tests therefore fail to detect certain chain-terminating mutations. The latter possibility was examined by selecting on lactose plates for revertants of each permease mutation and testing for the presence of suppressors in the revertants by infection with chain-terminating mutants of bacteriophage T4. The bacteriophage mutants carried the three types of chain-terminating mutation in the lysozyme gene. If the reversion of a particular permease mutation has occurred by a further mutation to give a suppressor gene, the revertants will support the growth of an appropriate bacteriophage mutant and bacterial cell lysis will result. The original permease mutation must therefore have been a chain-terminating one, although not responding to the amino acids put in by the suppressors of the bacterial stocks. Two revertants of each mutant were tested for the presence of suppressors.

These tests established that a further four mutants were amber, two were ochre and three were opal, which raises the frequency of chain-terminating mutations to 30%. This relatively low frequency of chain-terminating mutations, together with the fact that about half of these mutations did not respond to the test suppressors, indicate that the permease activity is particularly sensitive to amino acid replacement, whether by mutation or by suppression.

Tests for Two Functions of the Y *Gene*

The discovery of a mutant by Wilson *et al.* (1970) that accumulates *o*-nitrophenyl β-galactoside but not lactose suggests that, in addition to controlling facilitated diffusion, the *Y* gene has a second function concerned with active transport which is the one inactivated by the mutation. In such a case, two groups of mutants are expected. Members of the first group could carry out facilitated diffusion because they contain the M protein, but would be lactose-negative because they are unable to couple energy to the uptake system. They are therefore expected to be able to catalyse the entry of *o*-nitrophenyl β-galactoside but not take up methyl β-thiogalactoside, which requires metabolic energy. Members of the second group should lack the M protein and thus be unable to transport both *o*-nitrophenyl β-galactoside and methyl β-thiogalactoside.

This expectation was tested by assaying the permease mutants for both facilitated diffusion, by measuring *o*-nitrophenyl β-galactoside uptake, and active transport, by measuring the uptake of radioactive methyl β-thiogalactoside. All of the mutants mapped in Fig. 1 were assayed for both functions with the exception of nine mutants that were lost. The results are shown in Table 2.

They do not support the proposal that facilitated diffusion and active transport are functions that can be separated by mutation. Allowing for the chain-terminating mutants, which may have a polar effect, there is no region containing mutants able to transport *o*-nitrophenyl β-galactoside but not methyl β-thiogalactoside. Only one

mutant (number 71) was of the type described by Wilson *et al.* (1970) and it was located in about the middle of the gene.

Table 2. List of mutants arranged in order of map position and showing extent of facilitated diffusion and active transport

Facilitated diffusion was measured as uptake of *o*-nitrophenyl *β*-galactoside (ONPG) and active transport as uptake of methyl *β*-thiogalactoside (TMG). Ability of mutants to be induced is indicated by their growth on lactose in the presence of isopropyl *β*-thiogalactoside (IPTG). Growth scores: ++ good growth with both levels of IPTG; + good growth with 10^{-2}M, poor with 10^{-4}M IPTG; ± fair growth with 10^{-2}M, none with 10^{-4}M IPTG; − no growth with either level of IPTG. Wild type = strain 3000

Map region	Mutant	ONPG uptake (% of wild type)	TMG uptake (% of wild type)	Growth on lactose + IPTG	Map region	Mutant	ONPG uptake (% of wild type)	TMG uptake (% of wild type)	Growth on lactose + IPTG
I	3451 (UAG)	26·6	6·1	−	V	352	28·2	3·6	−
II	P	9·3	3·5	++		362	0	1·8	+
	3062	7·6	1·3	±		374	4·9	0	++
	3142 (UAG)	21·5	0·2	−		451	6·4	6·3	−
	3279 (UAG)	4·4	4·4	−		493	0	0·2	−
	3460	0	0	−	VI	170	0	4·6	+
	3596	9·6	0	−		293	0	0	++
	3660	5·7	0	−		3457	4·7	3·3	−
III	3448	6·0	2·5	±	VII	X	12·5	0	+
	3492	5·8	3·0	+		P678	2·1	2·7	−
	3619	9·7	27·8	±		W1-4	4·9	7·9	−
	3626	0	18·6	−		411	0	5·0	++
IV	V	0	3·3	−		3447	0	0	−
	3223 (UGA)	0	8·7	−		3578	0	5·7	−
	3267	0	0	−		3631	8·5	10·5	++
	3405	5·6	2·6	+		3664	2·0	0	−
	3415	11·1	2·2	+	VIII	3603	0	1·3	−
	3503	0	1·5	−		3607	16·0	8·8	±
	3520	13·5	3·6	++		3644	0·9	17·2	+
	3577	22·1	0	++	X	N	5·6	0	−
	3642	1·5	3·7	−		R	17·0	0	−
V	26	3·8	0	−		3119	0	0	±
	42	6·8	2·1	+		3590	0	0	+
	71	142·0	11·3	+		3527	5·8	2·2	−
	72	2·0	7·3	±	XI	3558 (UGA)	16·3	3·3	−
	168	0	14·3	+	XII	332 (UAA)	0	0	−
	230	10·2	3·6	−	XIII	34 (UAG)	8·8	4·6	−
	306 (UAG)	11·8	8·8	+		3424 (UAA)	39·0	6·3	−
	343	0	0·9	−		3446	5·4	3·8	−
	351	0	24·9	−					

If the *Y* gene were composed of more than one cistron, a heterogenote of mutations in different cistrons is expected to show complementation. To test this possibility, two mutations N and P were made into homogenotes as described in the Methods. Since mutation P makes the normal amount of galactoside acetyltransferase and N makes 90% of the normal level, both mutations are probably missense as required for complementation testers. The episomes containing these mutations were transferred by sexduction to a range of F^- mutations located in different positions in *Y*, and examined for complementation. None of the heterogenotes gave any clear evidence of complementation, supporting the previous interpretation of the *Y* gene as a single cistron.

It has been mentioned by Kennedy (1970) that an inducible mutant capable of facilitated diffusion but not active transport is defective in the induction of the enzymes

of the lactose operon. To test if such mutations occur at specific positions in the *Y* gene, the permease mutants were examined for their ability to grow on plates containing mineral salts and lactose in the presence of the inducer, isopropyl β-thiogalactoside at two levels (10^{-2} and 10^{-4}M). The results are shown in Table 2.

Although none of the mutants will grow on lactose alone, the presence of the inducer reveals two classes of mutant. About half of the mutants grow reasonably well, but the other half fails to grow on lactose even when induced. However, there is no correlation of growth with map position or with capacity for facilitated diffusion or active transport.

Tests for Two Substrate-binding Sites in Galactoside Permease

When bacterial cells are treated with sulphydryl-reacting substances like *o*-chloromercuribenzoate (Kepes 1960) or *N*-ethylmaleimide (Fox and Kennedy 1965), the transport activity of the galactoside permease is markedly inhibited. Inactivation by *N*-ethylmaleimide can, however, be prevented by preincubation of the cells with certain substrates of the galactoside permease. Good protection is provided by melibiose and galactosyl thiogalactoside, but lactose and *o*-nitrophenyl β-galactoside afford little or no protection (Kennedy 1970). Since both lactose and melibiose are actively transported into the bacterial cell via the galactoside permease in quantities sufficient for growth, the specificity of protection against alkylation suggests that the permease protein or proteins have two distinct binding sites. One site may contain a reactive sulphydryl group and bind melibiose amongst other substrates, while the other site may lack a sulphydryl group and bind lactose and certain other galactosides.

This model predicts a class of mutants that transports lactose but not melibiose and another class in which melibiose but not lactose is taken up. If both types of mutant could be obtained, the position of the two presumed binding sites could then be determined by intragenic mapping of the corresponding mutations. Attempts were made to test the model by the isolation of the two predicted classes of mutant.

In the first experiment, devised to obtain mutants that would take up lactose but not melibiose, 545 mutants were isolated at 42°C from plates containing melibiose and triphenyltetrazolium chloride. An incubation temperature of 42°C was used because at this temperature the α-galactoside permease of *E. coli* K-12 is not synthesized and melibiose enters the cell only by the permease system of the lactose operon. Of these mutants, 243 grew on melibiose and were discarded, leaving 302 mutants that failed to grow on melibiose at 42°C. On testing the melibiose-negative mutants for growth on lactose, 122 grew well at 37°C and were further examined for α-galactosidase (EC 3.2.1.22) activity. Thirty-two of these mutants possessed the α-galactosidase and 102 mutants lacked this enzyme, thus explaining their growth on lactose but not melibiose at 42°C. The 32 mutants that were melibiose-negative, lactose-positive and that possessed α-galactosidase were crossed with the episomal deletions X_2/FX_2 and W3133/FW3133 to test if the mutations were located in *Y*. These crosses showed that all but two of the mutants carried mutations outside the *Y* gene and were therefore not of the required type. Their nature is not known. The remaining two mutants, 42 and 293, mapped in the middle of the *Y* gene in regions V and VI respectively (Fig. 1). Their phenotypes do not, however, fit the expectation for mutations inactivating the melibiose binding site. The mutants grow on lactose and glucose, but not on melibiose, galactose or glycerol and the lactose-positive character appears to be adversely sensitive to high temperature (42°C).

The reverse experiment, the isolation of mutants that grow on melibiose but not on lactose, was also unsuccessful. Following mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, 524 mutants were isolated on lactose–tetrazolium plates at 37°C. Of these mutants, 167 were lactose-negative and 32 of these were melibiose-positive at 42°C. Further testing gave only four mutants that were lactose-negative, melibiose-positive and contained β-galactosidase. Crosses both with deletions and point mutations of the *Y* gene showed that these mutants lacked all trace of the gene. They probably were able to grow on melibiose at 42°C because of a second mutation to give a temperature-stable α-galactoside permease.

Discussion

The presence of facilitated diffusion but not active transport in the mutant described by Wilson *et al.* (1970) suggested that the *Y* gene may be composed of two cistrons. No evidence has been found in the present study, either from intragenic mapping or from complementation tests, to support this suggestion, although an examination of induction ability shows that at least two functional classes of mutant occur. It is possible that different types of mutants would be obtained by selection for lack of growth on low concentrations of lactose. This was attempted, but the mutants were found to be similar to those obtained by selection on normal levels of lactose.

Similarly, previous studies of substrate protection of the permease protein against alkylation could be interpreted as indicating two substrate-binding sites in the uptake system. Although attempts to obtain the postulated mutants were unsuccessful, this does not prove that there are not separate binding sites for lactose and melibiose. However, over 1000 mutants were examined in the experiments, so it seems likely that the substrate protection patterns described by Kennedy (1970) have some other explanation.

All the data obtained from the genetic and biochemical experiments are consistent with a model of the *Y* gene as a single cistron coding for the M protein of Carter *et al.* (1968). This protein can, by itself, carry out facilitated diffusion, but for active transport it must interact with the products of other genes not in the lactose operon. The numerous mutations affecting galactoside uptake that were found not to be in the *Y* gene may be of the latter type.

A variant of this conclusion concerns the possibility that the colinearity found between the map position of mutations and specific subfunctions in β-galactosidase (Langridge 1968) does not hold for such proteins as galactoside permease. While all the information for the conformation and function of β-galactosidase is probably contained in the *Z* gene, the permease protein, which is embedded in a membrane, may partly depend on information in other genes that make the membrane subunits in contact with the permease. There does not appear to be any way at present by which this possibility could be tested.

A marked difference between the mutations of the galactoside permease gene and the β-galactosidase gene (Langridge and Campbell 1969) is the relative rarity of chain-terminating mutations in the former. This has been commented on elsewhere (Langridge 1974). In conjunction with other evidence it was interpreted as indicating that mutations leading to amino acid substitutions are much more frequently expressed in genes specifying metabolic enzymes than in genes like *Y* whose products form part of a structural complex.

Acknowledgments

I am grateful to Ms M. Caldersmith and Ms M. Moens for competent technical assistance. I thank Dr M. Malamy of Tufts University for many of the permease deletions and Dr B. Bridges, University of Sussex, Dr P. Bergquist, University of Auckland, and Dr G. Streisinger, University of Oregon, for supplying bacteriophage strains.

References

Carter, J. R., Fox, C. F., and Kennedy, E. P. (1968). Interaction of sugars with the membrane protein components of the lactose transport system of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **60**, 725–32.

Cook, A., and Lederberg, J. (1962). Recombination studies of lactose non-fermenting mutants of *Escherichia coli* K-12. *Genetics* **47**, 1335–53.

Crandall, M., and Koch, A. L. (1971). Temperature-sensitive mutants of *Escherichia coli* affecting β-galactoside transport. *J. Bacteriol.* **105**, 609–19.

Fox, C. F., and Kennedy, E. P. (1965). Specific labelling and partial purification of the M protein, a component of the β-galactoside transport system of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **54**, 891–9.

Fox, C. F., Carter, J. R., and Kennedy, E. P. (1967). Genetic control of the membrane protein component of the lactose transport system of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **57**, 698–705.

Kennedy, E. P. (1970). The lactose permease system of *Escherichia coli*. In 'The Lactose Operon'. (Eds J. R. Beckwith and D. Zipser.) pp. 49–92. (Cold Spring Harbor: New York.)

Kepes, A. (1957). Metabolismé oxidatif lié au fonctionnement de la galactoside-perméase d'*Escherichia coli*. *C. R. Seances Soc. Biol. Fil.* **244**, 1550–3.

Kepes, A. (1960). Études cinétiques sur la galactoside-perméase d'*Escherichia coli*. *Biochim. Biophys. Acta* **40**, 70–84.

Kepes, A., and Cohen, G. N. (1962). Permeation. In 'The Bacteria'. (Eds I. C. Gunsalus and R. Y. Stanier.) Vol. 4, pp. 179–221. (Academic Press: New York.)

Koch, A. L. (1964). The role of permease in transport. *Biochim. Biophys. Acta* **79**, 177–200.

Langridge, J. (1968). Genetic evidence for the disposition of the substrate binding site of β-galactosidase. *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1260–7.

Langridge, J. (1974). Mutation spectra and the neutrality of mutations. *Aust. J. Biol. Sci.* **27**, 309–19.

Langridge, J., and Campbell, J. H. (1969). Classification and intragenic position of mutations in the β-galactosidase gene of *Escherichia coli*. *Mol. Gen. Genet.* **103**, 339–47.

Malamy, M. H. (1966). Frameshift mutations in the lactose operon of *E. coli*. *Cold Spring Harbor Symp. Quant. Biol.* **31**, 189–201.

Taylor, A. L., and Trotter, C. D. (1972). Linkage map of *Escherichia coli* strain K-12. *Bacteriol. Rev.* **36**, 504–24.

Wilson, T. H., Kusch, M., and Kashket, E. R. (1970). A mutant in *Escherichia coli* energy-uncoupled for lactose transport: a defect in the lactose operon. *Biochem. Biophys. Res. Commun.* **40**, 1409–14.

Wilson, T. H., Kashket, E. R., and Kusch, M. (1972). Energy coupling to lactose transport in *Escherichia coli*. In 'The Molecular Basis of Biological Transport'. (Eds J. F. Woessner and F. Huijing.) pp. 219–47. (Academic Press: New York.)

Manuscript received 29 November 1973