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A MUTANT OF *ESCHERICHIA COLI* K_{12} ENERGY-UNCOUPLED FOR LACTOSE TRANSPORT

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SUMMARY

A mutant (X71-54), isolated from X71 ($i^-z^+y^+a^-$), was found to be defective in the accumulation of thiogalactosides but showed no loss of membrane carrier activity. An increased rate of exit from the mutant accounted for its inability to accumulate normally. This exit pathway was confined to lactose analogs as normal accumulation was observed for D-fucose, α-methyl glucoside, L-leucine, L-lysine and α-aminoisobutyric acid.

The cotransduction of the lactose operon and the energy uncoupled property into a lactose-deleted strain indicates that the defect is in or near the lactose operon. The probability that the defect resides in the y gene was strengthened by the observation that the transport activity of the mutant was abnormally sensitive to sulfhydryl-group reagents such as *N*-ethylmaleimide and *p*-chloromercuribenzoate.

These data are consistent with the hypothesis that energy coupling reduces the exit rate of galactosides from the cell by reducing the affinity of the carrier for the substrate at the inner border of the plasma membrane, having no effect on entry.

INTRODUCTION

The mechanism by which metabolic energy is coupled to active transport of lactose across the bacterial cell membrane remains poorly understood. Experiments utilizing metabolic inhibitors suggested that energy coupling prevented exit of the substrate from the cell, having no effect on the entry rate. Such conclusions came from data obtained on both the lactose[1–5] and galactose[1] transport systems. Since these views have recently been questioned by a number of workers[6–8], a re-evaluation of the hypothesis appeared appropriate.

Isolation of a mutant which was functionally energy-uncoupled from lactose transport would provide a system to test the several hypotheses. Two such mutants have now been obtained. The first mutant (ML 308-22)[9] was isolated from ML 308

Abbreviations: TMG, thiomethyl-β-D-galactopyranoside; IPTG, thioisopropyl-β-D-galactopyranoside; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; TDG, D-galactopyranosyl-β-D-thiogalactopyranoside; XG, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; Sm, streptomycin; Nal, nalidixic acid; y^{UN}, the y gene which is presumed to be defective in the energy uncoupled mutant X71-54; K_t, concentration of substrate which gives one-half the maximum transport rate (analogous to K_m of enzyme kinetics).

with the use of a procedure of MÜLLERHILL *et al.*[10] which selects for cells unable to accumulate β-galactosides. This mutant possessed a severe defect in its ability to accumulate four different thiogalactosides without a reduction in membrane carrier activity (assayed by three independent methods). The mutant possessed an abnormally rapid efflux of β-galactosides while it showed no diminution of influx, consistent with the hypothesis that energy coupling reduces the rate of exit by reducing the affinity of the carrier for substrate on the inner surface of the membrane. Since no genetic studies were possible with the mutant similar mutants were sought in the K_{12} strain.

Utilizing the same selection procedure as that described above an energy-uncoupled mutant (X71-54) was isolated from a K_{12} strain (X71). This communication describes the properties of this mutant. A preliminary report of this work has appeared elsewhere[11].

MATERIALS AND METHODS

Chemicals

Thio [^{14}C]methyl-β-D-galactopyranoside, [1-^{14}C]acetyl-CoA, L-[^{14}C]leucine, L-[^{14}C]lysine and α-[1-^{14}C]aminoisobutyric acid were obtained from New England Nuclear Corp. α-Methyl-D-[^{14}C]glucoside and D-[6-^{3}H]fucose were purchased from Calbiochem. [^{3}H]Thiodigalactoside was kindly provided by Dr. Eugene P. Kennedy. Non radioactive thiomethyl-β-D-galactopyranoside (TMG), thioisopropyl-β-D-galactopyranoside (IPTG), *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and *p*-chloromercuribenzoate were obtained from Calbiochem Corp. D-Galactopyranosyl-β-D-thiogalactopyranoside (TDG) was purchased from Mann. *p*-Chloromercuribenzoate sulfonic acid was obtained from Sigma. Thio-*p*-nitrophenyl-β-galactoside and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) were obtained from Cyclo Chemical Co.

Bacteria

The parental strain X71 ($i^-z^+y^+a^-$, $proC^-$, try^-, B_1^-, F^-, Sm^R) was derived[12] from X5072, a transacetylase positive strain (from Dr. Jon Beckwith). X71-316 ($i^-z^+y^-a^-$) was isolated from X71 by the thio-*p*-nitrophenylgalactoside procedure of MÜLLERHILL *et al.*[10] followed by selection of lactose-negative (white) clones on MacConkey plates. RV/F'MS367 (Δlac Sm^S/F'$i^+z^-y^-a^-$) and RV (Δlac, Nal^R, F^-) were kindly provided by Dr. Michael Malamy.

Growth of cells

Cells were grown to exponential phase in minimal Medium 63 (ref. 13) supplemented with NaCl (0.29 %) *plus* casein hydrolysate (tryptone, Difco) at a concentration of 1 % *plus* supplement (L-tryptophan (10 μg/ml), L-proline (100 μg/ml) and thiamine (0.5 μg/ml)). Growth was monitored by the increase in absorbance of the culture in a Klett–Summerson colorimeter with a No. 42 filter. Cells were harvested by centrifugation at 4° for 10 min at 12000 × g, and washed with cold minimal Medium 63. Cells were resuspended in Medium 63 (pH 5.8).

Assays

Active transport of TMG was measured[4] by exposing washed cells to [^{14}C]TMG for various periods of time at 25° followed by separation of the cells from the medium

on a Millipore filter (0.65 μm pore size) which was subsequently placed in liquid-scintillation vials and dissolved in Bray's solution for counting. Intracellular concentrations of TMG were calculated assuming that 1 ml of a cell suspension with an absorbance of 100 Klett units (No. 42 filter) contained 0.6 μl of cell water. There were 2.7 μl of cell water per mg of dry wt. (ref. 4). A suspension of cells which reads 100 Klett units contains $6 \cdot 10^8$ cells/ml.

ONPG entry into cells was measured by exposing a cell suspension (usually 5 ml of a final absorbance of approx. 20 Klett units/ml) to different concentrations of ONPG at 37°. From the 5-ml mixture 2 ml were removed at 30 sec and placed into 4 ml of ice-cold (0.6 M) Na_2CO_3. After 10 min and 30 sec (or other desired times) another 2-ml sample was withdrawn and placed in carbonate. After centrifugation the color was determined with a Klett with a No. 42 filter. To determine the rate of entry *via* pathways other than the lactose transport system control experiments were carried out with either TDG (10 mM) or formaldehyde (10 mM)[2]. The TDG-sensitive (or formaldehyde-sensitive) entry rate was taken as a measure of the lactose carrier.

Thiogalactoside transacetylase was assayed by the method of FOX AND KENNEDY[14]. Heat-treated cells (70° for 5 min) were incubated in the presence of IPTG and [^{14}C]acetyl-CoA. [^{14}C]Acetyl-IPTG was measured after separation from the coenzyme A on a Dowex-1 (formate) column.

Isolation of mutant

Non-mutagenized cells of X71 were grown overnight in Medium 63 containing 0.2 % glucose. Cells (at a final concn. of $1 \cdot 10^6$ cells/ml) were incubated in Medium 63 containing the following: 3 mM thio-*p*-nitrophenyl-β-galactoside, 0.5 mM thioisopropyl-β-galactoside, 0.4 % glycerol and supplement (10 μg/ml L-tryptophan, 100 μg/ml L-proline, and 0.5 μg/ml thiamine). No visible growth occurred after 24 h but at 48 h good growth was observed. Cells were spread on MacConkey lactose indicator plates and pink clones were picked (parent was red under these conditions). Each clone was spotted on an agar plate containing amino acids, triphenyltetrazolium chloride and [^{14}C]TMG[12]. Sterile filter paper was pressed on the surface of the agar, removing a portion of each clone onto the paper. After drying, the paper was exposed to X-ray film for several days. Parental cells appeared black on the developed film while transport negative cells appeared as a very faint grey spot. The mutant X71-54 appeared distinctly less dark than the parent on the X-ray film but darker than the transport-negative control. Cells from the original TMG-plates were then assayed for TMG accumulation and ONPG entry rate.

Transfer of the lactose operon of mutant X71-54 (and parent) to a lactose deleted strain by F-duction

RV/F'MS367 (Δlac, Sm^S/F'$i^+z^-y^-a^-$) was mated with X71-54 ($i^-z^+y^{UN}a^-$, Sm^R,F^-). The i^-/F'i^+ diploid (54A) was selected as a light blue clone on XG plates containing streptomycin. When 54A had grown in rich medium and been replated on XG plates, it gave rise to recombinants which were dark blue (i^-). Several of these i^- clones were purified and found to possess only one copy of the *z* gene. High-frequency mating to a lactose-deleted strain RV (Nal^R) indicated that the F-factor contained the lactose operon. The lactose F-ductants were identified as red clones on

MacConkey plates containing nalidixic acid. In each cross about half of the clones were lactose positive. Several independent F-ductions were carried out with both mutant (X71-54) and parent (X71). The properties of the F-ductants are shown in Table V.

RESULTS

Defect in accumulation

When a suspension of the mutant cells (X71-54) was exposed to 0.15 mM thiomethylgalactoside at pH 5.8 the cells accumulated only about one fifth as much sugar as the parental cell (X71). A very similar defect was noted with thiodigalactoside as substrate (Fig. 1). This defect in ability to accumulate was less severe at pH 7 and disappeared entirely at pH 8.2 (Fig. 2). The effect of pH on the accumulation process of mutant and parent was very similar to that previously found for another energy-uncoupled mutant (ML 308-22). In this latter mutant TMG accumulation was less than 10 % of the parental strain when tested at pH 5.8 (unpublished results).

The effect of incubation temperature on TMG accumulation was tested. The

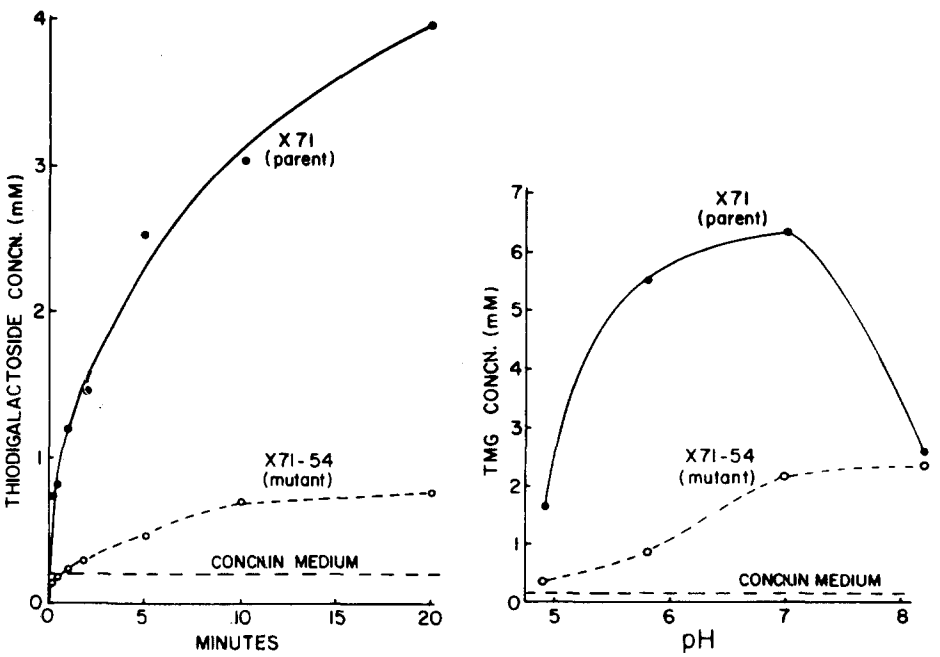

Fig. 1. Accumulation of thiodigalactoside by X71 and X71-54. Cells in logarithmic phase were centrifuged and washed once with 0.1 M phosphate buffer (pH 5.8) containing 1 mM mercaptoethanol. Cells (A = 190) were incubated at 25° in 0.1 M phosphate buffer containing 0.2 mM (0.1 μC/ml) [^{3}H]thiodigalactoside *plus* 1 mM mercaptoethanol. Samples (0.5 ml) were withdrawn, cells filtered, washed and counted.

Fig. 2. Effect of pH on steady-state accumulation of TMG by X71 and X71-54. Cells in logarithmic phase were centrifuged and washed once. Cells (A = 150) were incubated in 0.1 M phosphate buffer of the indicated pH in the presence of [^{14}C]TMG (0.14 mM; 0.1 μC/ml). Samples (0.5 ml) were taken at various time intervals, the cells filtered, washed and counted. The 10-min value was used since the steady state had been reached.

maximum steady-state level of TMG was observed at 25° for both parent and mutant (a phenomenon known from the work of KEPES[16]). The accumulation defect by the mutant, however, was evident at all temperatures. An experiment at 4° is shown in Fig. 3.

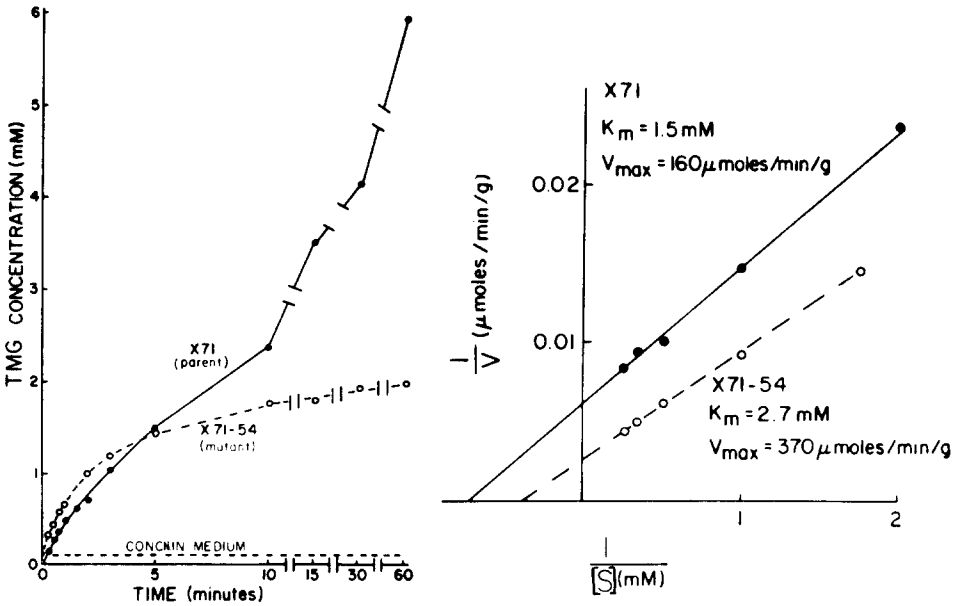

Fig. 3. TMG uptake by X71 and X71-54 at 4°. Cells in logarithmic phase were centrifuged and washed with Medium 63 (pH 5.8). Cells (A = 150) were incubated in 6.5 ml of Medium 63 (pH 5.8) containing [^{14}C]TMG (0.1 mM; 0.05 μC/ml) and 0.5 mM mercaptoethanol at 4°. Samples (0.5 ml) were withdrawn, cells filtered, washed and counted.

Fig. 4. Effect of concentration on ONPG entry into X71 and X71-54. Cells in logarithmic phase were centrifuged and washed with 0.1 M phosphate buffer (pH 5.8). Cells (A = 21) were incubated at 37° in 0.1 M phosphate buffer (pH 5.8) containing ONPG at different concentrations. Samples were removed at 30 sec and again at 10 min and 30 sec placed in 0.6 M Na_2CO_3 centrifuged and yellow color determined. At each concentration of ONPG a parallel experiment was carried out in the presence of 10 mM formaldehyde (to estimate entry *via* pathways other than the lactose-transport system). The formaldehyde-sensitive entry was used for the calculations given in the figure.

The possibility that the α-galactoside transport system ("TMG II" transport system of PRESTIDGE AND PARDEE[17]) was involved in the defect was investigated. Mutant cells grown at 42°, a temperature which inactivates the α-galactoside transport system, showed the same defect in accumulation observed when cells were grown at 37°. Furthermore, a y$^-$ derivative of X71 (X71-316) grown either at 37 or 42° was unable to accumulate TMG, confirming the absence of the α-galactoside transport system under these conditions.

Presence of membrane carriers

Several assays for membrane carriers were carried out. The first of these was the rate of entry of the chromogenic galactoside ONPG. This sugar enters the cell *via* the β-galactoside transport system and is then split by β-galactosidase to yield

galactose and *o*-nitrophenol which is yellow in color. Since membrane transport is the rate limiting step in the *in vivo* hydrolysis of ONPG, this method has been extensively used as an assay for carriers. Fig. 4 shows the results of a representative experiment. The means for the K_t values in five experiments were 1.4 mM for X71 and 2.5 mM for X71-54; mean values of v_{max} in the same experiments were 176 μmoles/min per g for X71 and 319 μmoles/min per g for X71-54. It should be noted that the ONPG entry measured in these experiments was *via* the lactose transport system as it was blocked by the specific inhibitor thiodigalactoside (TDG). The small TDG-resistant ONPG entry was subtracted from the total rate. In some experiments inhibition of transport was carried out with formaldehyde (rather than TDG) after the suggestion of KOCH[2].

A second assay for membrane carriers was with TMG as substrate. The initial rate of entry of TMG into the mutant was greater than the parent at 4° (Fig. 3). This low temperature was chosen in order to slow the rate of entry sufficiently to obtain reliable initial rates of entry. In experiments carried out at 25° (such as that in Fig. 1) the earliest point taken (15 sec) does not represent an initial rate of entry. The experiment in Fig. 3 shows both the rapid initial rate of entry in the mutant and the defect in steady-state accumulation during later time periods.

Another assay with TMG as substrate was that of counterflow[18,19]. This assay involved monitoring uptake of [^{14}C]TMG into washed cells preloaded with non-radioactive TMG and completely energy-uncoupled with NaN3. Under these conditions exit of entering radioactive sugar is blocked temporarily by the high intracellular

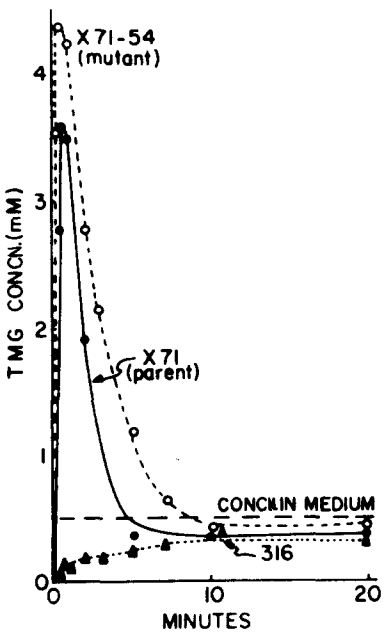

Fig. 5. TMG counterflow by X71, X71-54 and X71-316. Cells in logarithmic phase were centrifuged and washed with Medium 63 (pH 5.8). Cells (A = 200) were incubated in 5 ml of Medium 63 (pH 5.8) containing 30 mM NaN_3, 20 mM TMG and 1 mM mercaptoethanol for 45 min at 25°. Cells were centrifuged, supernatant carefully poured off and the pellet resuspended in 5 ml of Medium 63 (pH 5.8) containing [^{14}C]TMG (0.5 mM; 0.2 μC/ml) and 30 mM NaN_3. Samples (0.5 ml) were taken at the times indicated, the cells filtered, washed and counted.

concentration of non-radioactive molecules. In such an experiment the initial rate of entry is directly proportional to the number of membrane carriers. The peak of the counterflow curve is displaced to the right if the number of carriers is reduced. In the experiment of Fig. 5 the initial rate of entry into the mutant cell was slightly greater than that in the parent and the time to the peak was similar for mutant and parent. The significance of the apparent delay in the return of the mutant counterflow curve is not clear. Whether this is due to experimental variation or to factors not yet understood remains for further study. This semiquantitative assay indicated that there was increased (or normal) carrier activity in the mutant cell.

TABLE I

INHIBITION OF ONPG ENTRY BY VARIOUS INHIBITORS

Inhibitors were preincubated 6 min with cells at 37° prior to addition of ONPG (1 mM).

Inhibitor	*Concn. (mM)*	*Inhibition (%)*	
		X71	*X71-54*
N-Ethylmaleimide	0.1	67 (4)	92 (2)
	0.05	50 (4)	77 (3)
p-Chloromercuribenzoate	0.005	62 (3)	97 (3)
	0.001	13 (4)	96 (4)
Iodoacetamide	2	51 (3)	80 (3)
	1	35 (3)	67 (3)
p-Chloromercuribenzoate sulfonic acid	0.02	97 (3)	98 (3)
	0.002	4 (3)	53 (3)
Formaldehyde	10	94 (4)	92 (4)

Inhibition by sulfhydryl-group binding reagents

The effect of a variety of inhibitors was tested. Table I shows that four different sulfhydryl-binding reagents showed a consistently greater inhibition of ONPG entry into the mutant compared with the parent. For example, 0.001 mM *p*-chloromercuribenzoate inhibited transport into the mutant (X71-54) 96 % while inhibiting that in the parent (X71) by only 13 %.

Summary of genotype

The enzymes β-galactosidase and transacetylase were assayed to determine possible alterations of components of the lactose operon other than that of transport. A comparison was made between the mutant (X71-54) and the parent (X71) as well as its parent (X5072). Table II shows that the levels of these two enzymes were similar in the uncoupled mutant and its parent (X71).

Note should be made of the fact that the previously reported high β-galactosidase activity associated with the uncoupled transport property in ML 308-22 could not be reproduced in a recent reinvestigation of this question. The mutant (ML 308-22) possessed exactly the same enzyme activity as its parent (ML 308). Thus the energy-uncoupled property in both the ML and K_{12} mutants is not associated with abnormalities in β-galactosidase or transacetylase.

TABLE II

LACTOSE OPERON OF THE MUTANT AND PARENT

X5072 is the parent of X71 which differs only in the loss of transacetylase. All three cells are constitutive for the lactose operon.

	X5072 (%)	*X71* (%)	*X71-54* (%)
β-Galactosidase	100	100	108
TMG accumulation	100	100	20
ONPG entry rate (v_{max})	100	100	180
Transacetylase	100	5	5

TABLE III

EXIT RATE OF TMG FROM X71 AND X71-54

Cells were preloaded with TMG to the same intracellular level by exposing X71 to 0.16 mM [^{14}C]TMG and X71-54 to 2.2 mM [^{14}C]TMG. Under these conditions the intracellular concentration of TMG was approx. 10 mM in both cells. Loaded cells were centrifuged, supernatant poured off and tube wiped dry. Pellet was resuspended with 100 ml Medium 63 containing 10 mM cold TMG at 4°. Ten ml samples were removed, the cells filtered and counted. Intracellular concentrations were calculated and data expressed as % loss of radioactivity in 45 sec. Values are the mean of 4 experiments.

	Exit of TMG (% loss of intracellular TMG in 45 sec)
X71	9
X71-54	38

Increased exit rate

The mutant possessed a distinctly faster exit rate compared with the parent. Table III shows that the mutant lost 38 % of its TMG under conditions where only 9 % was lost from the parent. Exit was measured in the presence of non-radioactive TMG in the external medium to block the recapture by the membrane carrier of radioactive molecules from the periplasmic space[4,20]. Preliminary experiments indicate that exchange diffusion in mutant 54 is not abnormally rapid and thus does not account for the rapid exit under these conditions.

The question arises as to whether the increased exit pathway was a non-specific leak or whether it was mediated by the lactose transport system. If a non-specific leak were present then a defect should be noted for the accumulation of all small molecules. A careful study was made of the transport of D-fucose, α-methyl-glucoside, L-leucine, L-lysine and α-aminoisobutyric acid by parental and mutant cells. No defect in accumulation could be detected in the mutant.

Growth properties

Mutant cells gave rise to clones slightly smaller than those of the parental type on agar plates with 0.2 % lactose. When cells were grown on MacConkey 0.2 % lactose plates the parental clones were slightly redder than those of the mutant. A

TABLE IV

GROWTH OF CELLS IN LACTOSE, GLYCEROL AND AMINO ACIDS

The growth medium was pH 5.8.

	Doubling time (min)			
	Lactose		*Amino acids* (0.2%)	*Glycerol* (0.4%)
	25 mM	*0.25 mM*		
X71	60	60	42	120
X71-54	60	150	42	120
X71-316	No growth		—	120

TABLE V

TRANSFER OF "UNCOUPLED" PROPERTY WITH THE LACTOSE OPERON TO LACTOSE-DELETED STRAIN BY MEANS OF F-DUCTION

The TMG concentration represents that attained at the steady state (10 min) in Medium 63 (pH 5.8) at 25°. Entry rate of 1 mM ONPG was measured at 37° and is expressed in terms of cell wet wt.

Organism	*F' donor*	*Recipient*	*TMG uptake (mM)*	*ONPG entry (μmoles/min per g)*
RV/F'X71 (II)	X71Δlac/F'X71	RVΔlac	8.9	69
RV/F'X71 (III)	X71Δlac/F'X71	RVΔlac	8.1	72
RV/F'X71 (VI)	X71Δlac/F'X71	RVΔlac	7.7	79
RV/F'X71 (XIII)	X71Δlac/F'X71	RVΔlac	7.2	76
RV/F'54 (V)	54Δlac/F'54	RVΔlac	1.3	73
RV/F'54 (VI)	54Δlac/F'54	RVΔlac	1.3	71
RV/F'54 (VII)	54Δlac/F'54	RVΔlac	1.3	72
RV/F'54 (XI)	54Δlac/F'54	RVΔlac	1.2	75
X71*	—	—	4.8	76
X71-54*	—	—	1.1	98

* Mean values of 7 determinations.

striking observation was the failure of mutant clones to ferment melibiose at 37°, a sugar utilized by the parent X71. This was the subject of another communication[21].

When cells were grown in minimal medium with lactose as the sole source of energy and carbon the mutant was found to grow normally in 25 mM lactose but showed slow growth at 0.25 mM substrate (Table IV). Normal growth was observed in amino acids and in 0.4 % glycerol.

F-duction experiments

The lactose operon of the mutant cell was transferred to a lactose-deleted strain by means of F-duction (see MATERIALS AND METHODS). In control experiments a similar transfer was performed with the parental cells. In all cases tested the uncoupled property was associated with the lactose operon (Table V).

DISCUSSION

Since the discovery of active transport of lactose by *Escherichia coli*[22] several speculations have been made concerning the role of energy coupling. Two basic hypotheses have been suggested: the first states that energy is required for entry of substrate[6,7,16,23] while the second proposes that energy coupling is required to prevent exit, having no effect on the entry step[1–4].

In 1957 it was clearly stated by COHEN AND MONOD[24] that the uncouplers azide and 2,4-dinitrophenol had little effect on entry of galactosides (especially ONPG) while completely blocking accumulation of thiogalactosides. These observations were confirmed in several laboratories[1,2,4]. This evidence suggested that energy coupling affected exit and not entry. This view was strengthened by the finding[4] that azide dramatically increased the affinity of the carrier for TMG during exit thus increasing the rate of loss of the sugar from the cell to the external medium. It was therefore proposed[3,4] that at the outer surface of the cell the membrane carrier (product of the y gene) bound the substrate with a high affinity, the carrier-substrate complex migrated to the inner surface of the cell where an energy coupling mechanism reduced the affinity of carrier for substrate, releasing it into the cell. The empty carrier migrated to the outer surface spontaneously regaining its high affinity once again.

Since these conclusions have been recently questioned[6–8] it was important to seek independent confirmation of the hypothesis without the use of metabolic inhibitors. The isolation and study of energy uncoupled mutants provide fresh evidence which relates to this question. The two mutants, one from the ML strain[9] and the other from a K_{12} strain, show remarkably similar properties. They demonstrate a severe defect in accumulation of thiogalactosides but possess somewhat greater membrane carrier activity than their respective parental cells. That the defect resides in abnormally rapid exit is provided by direct experimental evidence. It was demonstrated that this rapid exit pathway was not a non-specific leak (nor the α-galactoside transport system) but specific for substrates of the lactose transport system.

Three lines of evidence, none of which alone is conclusive, indicate that the defect resides in the membrane transport protein. The genetic evidence consists of the co-transduction of the lactose operon and the uncoupled property into lactose-deleted recipients by either the bacteriophage P_1 (ref. 11) or the F-factor (Table V). As the strain (X71-54) is transacetylase-negative this portion of the operon is eliminated as a possible source of the energy coupling property. A second piece of evidence implicating the transport protein in the defect is the marked increased sensitivity of the mutant transport system to agents which bind to sulfhydryl groups (Table I). The third piece of evidence is the possibility that the marked increase in the mutant's v_{max} of entry is due to a structural modification of the transport protein. The alternative possibility that increased numbers of membrane-transport proteins are present in the plasma membrane is not an attractive hypothesis since there is no evidence for an increased production of β-galactosidase. The possibility that normally only 50% of the transport protein molecules are incorporated into the membrane and that the mutant allows complete incorporation of such molecules cannot be ruled out. These three pieces of evidence taken together suggest that the defect in the mutant is in the y gene which codes for an abnormal transport protein.

Energy coupling affecting entry

There is no question that the activity of the lactose-transport system can be modified under a variety of circumstances. The mechanism best understood at the present is the oxidation of the essential sulfhydryl groups of the transport system. KENNEDY[25] has discussed this process and indicated that not only protection but reversal of the oxidation can be brought about by the addition of sulfhydryl-containing compounds such as mercaptoethanol.

KOCH[7] has discovered that glucose-grown cells depleted of substrate and required to transport α-methylglucoside will lose lactose transport acitivity. Most of the original activity may be regained by addition of glucose. This loss of activity is apparently not due to oxidation of sulfhydryl groups and deserves further study. These two types of reversible inactivations of the carrier protein are undoubtedly important but are in no way incompatible with the views expressed in this paper concerning energy coupling.

Physiclogical function of active transport

The manner in which energy coupling of lactose transport could provide a selective advantage for *E. coli* has never been clearly stated. If the coupling has no effect on entry how can energy coupling provide a useful mechanism for the cell to survive in competition with its neighbors?

The study of these two mutants has provided important new information on this question. The energy uncoupled mutants do, in fact, grow perfectly normally on high lactose concentrations which probably lead to intracellular concentrations above the K_m of β-galactosidase. When the external concentration is reduced, however, to 0.25 mM the mutant X71-54 grows more slowly and the ML 308-22 will not grow (while the parent grows well). Thus, when external lactose concentrations fall well below the K_m of the β-galactosidase, energy coupling (and presumably accumulation of lactose) is necessary for growth. Expenditure of metabolic energy is not thermodynamically necessary when external lactose concentrations are very high. A fascinating unresolved question is whether cells growing on a high concentration of lactose can uncouple energy supplies from transport, conserving metabolic energy while permitting the energy stored in the concentration gradient to drive sugar into the cell (*via* the membrane carriers).

The second selective advantage of active transport of lactose is the possibility that the process of induction is facilitated by the capacity of the cell to accumulate substrate. Data have been presented showing that the energy-uncoupled X71-54 grows very poorly on melibiose at 37° because the cell cannot efficiently induce the α-galactosidase[21]. In the absence of the α-galactoside transport system at 37° melibiose enters *via* the lactose system. The energy-uncoupled lactose transport system presumably cannot accumulate melibiose to sufficiently high levels to permit induction of the α-galactosidase. Preliminary data suggest the possibility that energy coupling is important for the induction of the lactose operon by lactose.

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