JOURNAL OF BACTERIOLOGY, Feb. 1976, p. 467-474

Vol. 125, No. 2
Printed in U.S.A.

Mutant of *Escherichia coli* Defective in Response to Colicin K and in Active Transport

CHARLES A. PLATE

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received for publication 3 September 1975

A mutant of *Escherichia coli* has been isolated that grows poorly on succinate and exhibits a markedly reduced sensitivity to colicin K. This mutant is also deficient in the respiration-linked transport of proline and thiomethyl-β-D-galactoside but appears normal for the adenosine triphosphate-dependent transport of glutamine and arginine. A temperature-conditional revertant of the mutant grows on succinate and is sensitive to colicin K at 27 C, but fails to grow on succinate and is insensitive to colicin K at 42 C. Proline transport in the temperature-conditional revertant is reduced at 42 C when either glucose or succinate is used as energy source. Glutamine transport, on the other hand, is normal at 42 C with glucose as energy source, but is reduced with succinate, although not to the same extent as is proline transport. The lack of growth on succinate and the deficiencies in transport at 42 C are not due to a temperature-dependent lesion in either the electron transport chain or in Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase activity. Membrane vesicles prepared from the temperature-conditional revertant are impaired in proline transport at both 27 and 42 C. These findings suggest the existence in the cytoplasmic membrane of *E. coli* cells of a component, presumably protein, that is required for colicin K action and that functions in respiration-linked and, to a lesser degree, in adenosine triphosphate-dependent active transport systems. This protein may serve as the primary target of colicin K action.

The major physiological effects observed in cells of *Escherichia coli* treated with colicin K are inhibition of respiration-linked and adenosine triphosphate (ATP)-dependent active transport systems, the complete arrest of macromolecular syntheses, and reduction in the intracellular levels of ATP. Information on how these events are interrelated recently came from studies of the effects of colicin K on mutants of *E. coli* lacking Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase (ATPase) activity (24). ATPase-deficient mutants do not exhibit a decrease in ATP levels following colicin K treatment and, unlike normal *E. coli* cells, retain the ability to synthesize deoxyribonucleic acid, ribonucleic acid, and protein. The absence of ATPase activity, however, does not prevent killing by the colicin nor does it affect the inhibition of proline and glutamine transport, examples of respiration-linked and ATP-dependent transport systems, respectively (3, 4, 12). These findings taken collectively led to the hypothesis that the primary action of colicin K on the cytoplasmic membrane of *E. coli* cells is to de-energize a system that is essential to the functioning of both the respiration-linked and ATP-dependent active transport systems (24). It was further suggested that the ATPase mediates an abortive re-energization of this system, resulting in lowered ATP levels. The lowered ATP levels, in turn, would contribute in large measure to the arrest of macromolecular syntheses.

In an effort to verify and extend this hypothesis, I have sought mutants of *E. coli* that are altered in the system that putatively both serves as the primary target of colicin K action and is essential to the functioning of active transport systems. Such mutants might be expected to be altered both in their response to colicin K and in their active transport properties. One class of mutants possessing these characteristics is described in this communication.

MATERIALS AND METHODS

Bacteria and media. *E. coli* strain A279a (HfrH 3000 *thi*) was obtained from the S. E. Luria stock collection. Cultures were routinely grown in Ozeki medium base (20) supplemented with carbon source (0.4%) and thiamine (0.5 μg/ml). In some instances cultures were grown in LB broth (19) supplemented with glucose (0.4%). Solid Ozeki minimal and LB

media contained 1.5 and 1% agar, respectively.

Isolation of mutants. Nitrosoguanidine mutagenesis was carried out according to the procedure of Adelberg et al. (1). After mutagenesis, the cells were allowed to grow for approximately four generations in LB-glucose broth. Aliquots of this culture were then diluted into Ozeki medium base and spread on plates of Ozeki medium agar supplemented with glucose, thiamine, and neomycin sulfate (20 μg/ml) (8, 13). Colonies appearing on the neomycin plates were picked and scored for growth with glucose or succinate as sole carbon source and on LB-glucose plates containing $KClO_3$ (0.2%). Aerobic growth in the presence of $KClO_3$ has been reported to distinguish mutants of *E. coli* uncoupled for oxidative phosphorylation from electron transfer mutants (29). Isolates that grew on glucose and in the presence of $KClO_3$ aerobically, but did not grow on succinate, were tested for sensitivity to colicin K. Isolates showing resistance to colicin K were subsequently tested for sensitivity to colicins E1, E2, and E3.

Colicin sensitivity test. *E. coli* strains colicinogenic for colicins E1, E2, E3, or K were stabbed into LB-glucose plates and allowed to grow for 24 h at 37 C. The majority of the colicinogenic cells were removed with filter paper, and the remaining cells were killed by exposing the plates to chloroform vapors for 45 to 60 min. Aliquots of cultures to be tested for colicin sensitivity were inoculated into soft nutrient agar (14) supplemented with glucose (0.4%) and spotted over the colicinogenic stabs. After 18 h at 37 C, colicin sensitivity was indicated by the presence of a clear zone in the soft agar overlay.

Colicin K preparation. Purified colicin K was prepared as previously described (22).

Transport experiments with intact cells. Cultures were harvested by centrifugation, the cells were washed twice with Ozeki medium base, and the washed cells were resuspended at 5×10^8 cells/ml in Ozeki medium base containing the indicated energy source (0.4%) and chloramphenicol (100 μg/ml). Two methods for measuring transport have been used. (i) a 1.0-ml amount of cell suspension was transferred to a 25-ml flask and preincubated at the desired temperature for 3 to 10 min with shaking prior to addition of radioactive substrate. At intervals after addition of radioactive substrate, aliquots (0.2 ml) were filtered on nitrocellulose filters (pore size, 0.45 μm; Matheson-Higgins, Inc., Woburn, Mass.) and washed with 4 ml of Ozeki medium base at room temperature. After drying, the filters were placed in Omnifluor (New England Nuclear Corp.)-toluene, and ^{14}C radioactivity was counted in a liquid scintillation spectrometer at 83% efficiency. (ii) Aliquots (0.2 ml) of cell suspension were dispensed into tubes and preincubated at the desired temperature without shaking. At various times after addition of radioactive substrate, the incubation was terminated by rapid dilution and filtration. The washing and counting of the filters were carried out as described above.

Preparation of membrane vesicles and assay of transport activity. Membrane vesicles were prepared and assayed for transport activity as described by Kaback (11).

Oxygen uptake. Oxygen uptake was measured in a Warburg respirometer following standard techniques (30).

ATPase assay. Membrane vesicles were washed twice with 50 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.8) and were then resuspended in 2 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.1). ATPase activity was measured by the method of Kobayashi and Anraku (15).

Protein determinations. Protein was determined by the method of Lowry et al. (18), using bovine serum albumin as standard.

Chemicals. L-[U-^{14}C]proline, L-[U-^{14}C]glutamine, L-[U-^{14}C]arginine, and [1-^{14}C]thiomethyl-β-D-galactoside were obtained from New England Nuclear Corp. Uniformly ^{14}C-labeled α-methyl-D-glucoside was obtained from Calatomic. D-Lactate and 2-phosphoenolpyruvate were obtained from Calbiochem. ATP and neomycin sulfate were from Sigma Chemical Co. All other chemicals were reagent grade.

RESULTS

Properties of the colicin K-insensitive mutant B51. Selection for neomycin resistance has yielded various mutants of *E. coli* that are affected in different aspects of the energy-transducing system that couples respiration to ATP synthesis and to active transport (8, 13, 17, 27, 33). Since a primary consequence of colicin K action is the uncoupling of active transport systems from metabolic energy (6, 24), it seemed reasonable to search for colicin K target mutants among mutants of *E. coli* resistant to neomycin. A screening of neomycin-resistant mutants that grew on glucose, but not on succinate, for colicin K sensitivity yielded several that exhibited a decreased sensitivity to colicin K but that retained normal sensitivity to colicins E1, E2, and E3. These mutants did not appear to be affected in the receptor needed for colicin K adsorption since they retained sensitivity to the bacteriophage T6, a phage that shares a common receptor with colicin K. One of these colicin K-insensitive mutants, designated B51, and a temperature-conditional derivative of B51 have been studied in some detail.

The mutant B51 grew poorly on succinate plates (pinpoint colonies after 48 h at 37 C) and had a reduced growth rate with glucose at 37 C as compared with the parent strain A279a (1.9-h generation time for B51 versus 0.9-h generation time for A279a). In addition to showing a marked reduction in sensitivity to colicin K (Fig. 1), B51 cells also proved to be deficient in certain active transport systems. With glucose as energy source the accumulation of proline and thiomethyl-β,D-galactoside was markedly reduced in B51 cells as compared with cells of

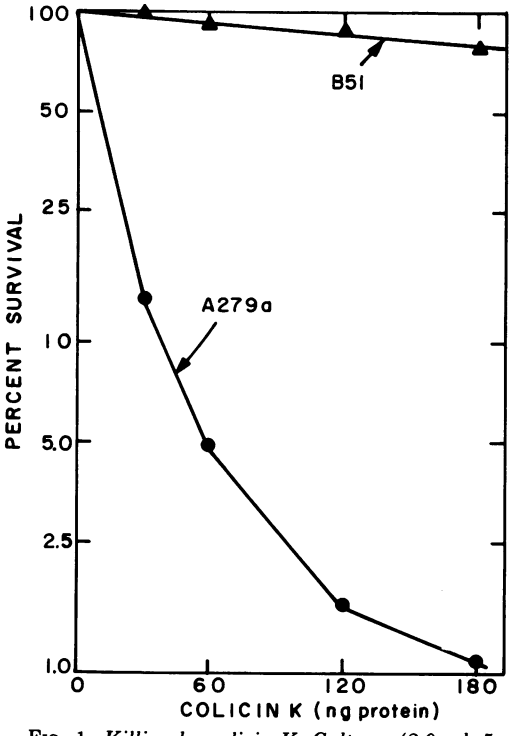

FIG. 1. *Killing by colicin K. Cultures (2.0 ml, 5 × 10^8 cells/ml) grown with glucose at 37 C were treated with the indicated amounts of colicin K for 15 min at 27 C. Viability assays were performed as previously described (23).*

the parent strain (Fig. 2A, B). Under similar conditions the ATP-dependent accumulation of glutamine or arginine appeared to be unaffected by the mutation in B51 cells (Fig. 2C, D).

To determine if the defect in transport, the decreased sensitivity to colicin K, and the poor growth on succinate were due to a single mutation in B51 cells, spontaneous revertants for normal growth on succinate were sought. One class of revertants was isolated from succinate plates at 37 C. In addition to having regained the ability to grow on succinate, these revertants exhibited a degree of colicin K sensitivity similar to that of the parent strain and had regained normal levels of proline transport.

A second type of spontaneous revertant, isolated from succinate plates at 30 C, exhibited a temperature-dependent phenotype. A representative revertant, designated B51-70, grew on either glucose or succinate at 30 C, but at 42 C it grew on glucose and only very poorly on succinate. B51-70 cells also manifested a temperature-conditional response to colicin K: an amount of colicin K that killed 84% of the cells in a B51-70 culture at 27 C gave little or no killing in a similar culture maintained and plated at 42 C. Finally, B51-70 cells exhibited a temperature-dependent defect in proline transport (see below).

The results obtained with these revertants of B51 support the conclusion that a mutation at a single site results in the inability of *E. coli* cells to utilize succinate normally as sole carbon source, markedly reduces their sensitivity to colicin K, and results in a deficiency in active transport.

Active transport in the temperature-dependent mutant B51-70. Different mechanisms of energy coupling have been shown to function in the active transport of proline and glutamine by *E. coli* cells. Proline transport is coupled to a form of energy that can be derived directly from electron transport and does not involve the formation of high-energy phosphate bonds (4, 12). Glutamine transport, on the other hand, requires ATP or some product derived from ATP (3, 4). The effect of the temperature-dependent lesion in B51-70 cells on these two amino acid transport systems is shown in Fig. 3 and 4.

With glucose as energy source, proline transport was only slightly reduced in B51-70 cells at 27 C (Fig. 3A), but was markedly reduced at 42 C as compared with parent cells (Fig. 3B). Similar results were obtained when succinate

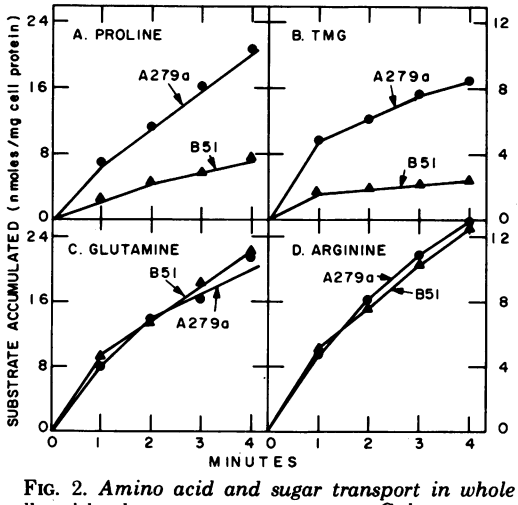

FIG. 2. *Amino acid and sugar transport in whole cells with glucose as energy source. Cultures were grown at 37 C and transport was measured at 27 C. Specific activity and the initial concentration of substrates used were as follows: (A) [^{14}C]proline, 10.6 μCi/μmol, 94 μM; (B) [^{14}C]thiomethyl-β-D-galactoside, 2.2 μCi/μmol, 100 μM; (C) [^{14}C]glutamine, 10.6 μCi/μmol, 94 μM; (D) [^{14}C]arginine, 10.6 μCi/μmol, 94 μM.*

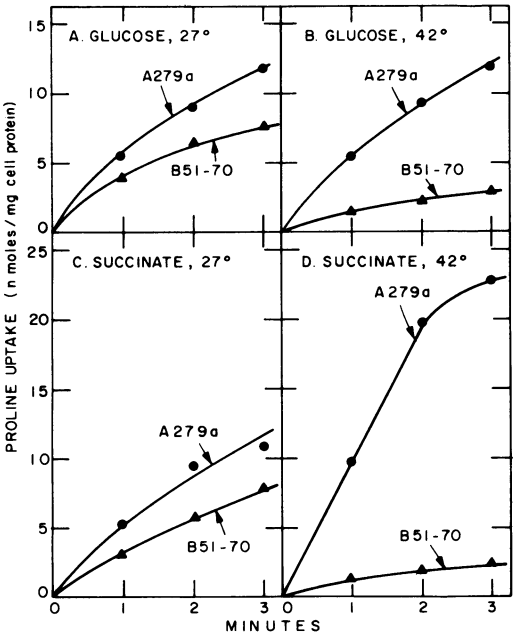

FIG. 3. *Proline transport with glucose and succinate as energy sources. Cells were grown at 27 C with either glucose or succinate as carbon source. The initial concentration of [^{14}C]proline used was 9.4 μM.*

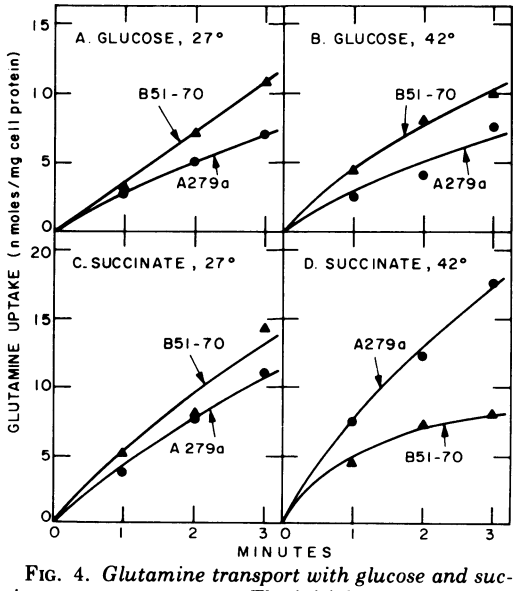

FIG. 4. *Glutamine transport with glucose and succinate as energy sources. The initial concentration of [^{14}C]glutamine was 9.4 μM.*

was the energy source (Fig. 3C, D). The inhibition of proline transport in B51-70 cells at high temperature was reversible; transferring the cells from 42 to 27 C in the absence of protein synthesis resulted in a complete restoration of proline transport activity within 10 min (data not presented).

In contrast to the findings with proline, glutamine was taken up by the mutant cells at a slightly enhanced rate as compared with the parent cells at both 27 and 42 C when glucose was the energy source (Fig. 4A, B). With succinate as energy source, the rate of glutamine uptake was normal in B51-70 cells at 27 C, but at 42 C it was approximately 50% lower than in the parent cells (Fig. 4C, D). That the reduced glutamine uptake at 42 C by succinate-grown B51-70 cells was not due to lowered intracellular ATP levels is shown by the data in Table 1. After a shift from 27 to 42 C, the ATP levels in B51-70 cells actually increased. The reason for this increase is not presently understood.

Thus the respiration-linked transport of proline appeared to be more sensitive to the temperature-dependent lesion in B51-70 cells than was the ATP-dependent transport of glutamine, although the latter transport system was also affected under certain conditions.

The temperature-dependent lesion in B51-70 cells also manifested itself by affecting proline retention at 42 C. B51-70 cells were allowed to accumulate radioactive proline to a steady-state level at 27 C and were then shifted to 42 C. The shift to the higher temperature resulted in a rapid efflux of proline from the B51-70 cells and the establishment of a new steady-state level considerably lower than that attained at 27 C (Fig. 5). This same temperature shift had little effect on the steady-state level of proline accumulation in the parent cells. These findings suggest that the temperature-dependent lesion in B51-70 cells affects the energy coupling of proline transport. It has been

TABLE 1. *Intracellular levels of ATP in succinate-grown A279a and B51-70 after a temperature shift from 27 to 42 C*

Time after temp shift[a] (min)	ATP (nmol/mg of cell protein)	
	A279a	B51-70
0	2.2	2.5
2	2.2	3.3
4	2.0	2.8
6	1.8	3.4
8	2.0	3.3
10	2.3	3.9
20	2.5	4.5

[a] Aliquots (0.5 ml) were removed from the cultures immediately before and at the times indicated after the cultures were shifted from 27 to 42 C. ATP was measured as previously described (24).

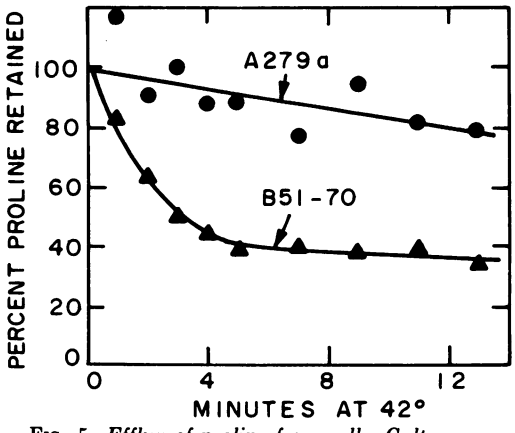

FIG. 5. *Efflux of proline from cells. Cultures were grown at 27 C with glucose as carbon source. Washed cells (2.0 ml) were pretreated with chloramphenicol (100 μg/ml) and were then incubated with glucose and [^{14}C]proline (9.4 μM) at 27 C for 15 min with shaking. A zero time sample (0.2 ml) was removed, and the cells were then transferred to a shaking water bath at 42 C. At the indicated times after the temperature shift, 0.2-ml aliquots were removed and filtered.*

shown that uncoupling of active transport systems by energy inhibitors results in a lowering of the steady-state K_m for exit and an increase in the rate of solute exit (16, 21, 32).

Respiration and ATPase activity in B51-70. The inability of B51-70 cells to grow at 42 C on a respiratory substrate such as succinate indicates the presence in these cells of a temperature-dependent lesion that affects either respiration or the coupling of respiration to energy metabolism. That respiration is functional in B51-70 cells at 42 C is shown by the data in Table 2. Oxygen consumption with glucose at 42 C was nearly the same for B51-70 cells as for cells of the parent strain A279a. With succinate, B51-70 cells utilize oxygen at a rate 60% of that of the parent cells. Thus the mutation in B51-70 cells does not appear to result in a temperature-sensitive component of the electron transport chain.

TABLE 2. *ATPase activity and oxygen consumption with strains A279a and B51-70*

Strain	O_2 consumption by intact cells at 42 C		ATPase activity in membranes at 42 C	
	Substrate	ng-atoms/mg of cell protein per min	Additions	μmol of Pi released/mg of membrane protein per 30 min
A279a	Glucose	231		0.61
	Succinate	272	Mg^{2+}	14.3
B51-70	Glucose	200		1.17
	Succinate	165	Mg^{2+}	20.6

Another possibility was that the lesion in B51-70 cells resided in the ATPase complex. Certain mutations affecting this enzyme system have pleiotropic effects on active transport in *E. coli* cells (2, 27-29, 31, 33; for contrasting results, see reference 25). Table 2 shows, however, that membranes prepared from parent and B51-70 cells contained comparable levels of ATPase activity at 42 C.

It appears, therefore, that the mutation in B51-70 cells must affect some as yet unspecified step that is essential to the coupling of electron transport to active transport and that is also necessary for the growth of *E. coli* cells on respiratory substrates.

Experiments with membrane vesicles. Membrane vesicles prepared from B51-70 cells grown on glucose at 27 C exhibited aberrant properties in proline transport when compared with vesicles prepared from the parent strain. Proline was accumulated to a lesser extent by the B51-70 vesicles at both 27 and 42 C, and increasing the temperature from 27 to 42 C did not stimulate the initial rate of uptake in the mutant vesicles as it did with the parent vesicles (Fig. 6A, B). Unlike the intact B51-70 cells, B51-70 membrane vesicles did not show a decrease in transport activity when the temperature was raised from 27 to 42 C. The low transport activity of the B51-70 vesicles was not due to decreased respiration of D-lactate: oxygen uptake with the parent vesicles at 42 C was 105 ng-atoms/min per mg of membrane protein, whereas with the mutant vesicles it was 203 ng-atoms/min per mg of membrane protein.

The membrane vesicles from B51-70 cells were also tested for the phosphoenolpyruvate-dependent uptake of α-methylglucoside, an uptake process that functions normally in intact cells of the mutant at both 27 and 42 C (data not presented). An increase in temperature from 27 to 42 C stimulated the rate of α-methylglucoside uptake in both the parent and mutant vesicles to nearly the same extent (Fig. 6C, D). The levels of α-methylglucoside accumulated by the mutant vesicles, however, were somewhat lower than those of the parent vesicles.

DISCUSSION

Results from studies with mutants of *E. coli* lacking energy-transducing ATPase activity suggested the hypothesis that the primary lesion resulting from colicin K action resides in a system that is essential to the functioning both of respiration-linked and ATP-dependent active

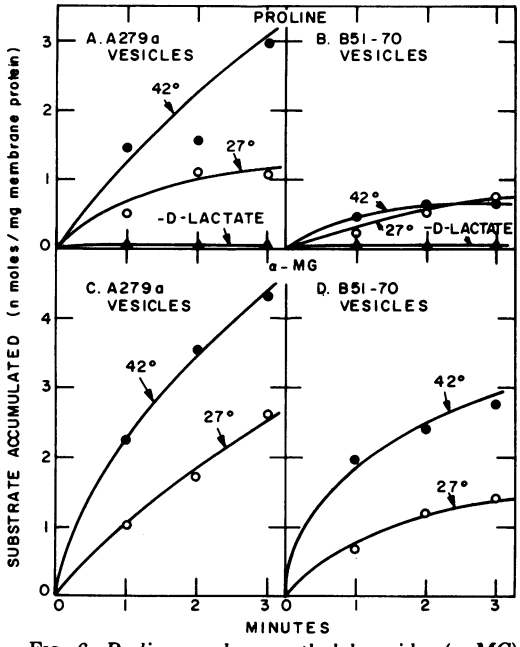

FIG. 6. *Proline and α-methylglucoside (α-MG) transport in membrane vesicles. For proline transport, 20 mM D-lactate was the energy source, and the initial concentration of [^{14}C]proline (100 μCi/μmol) was 10 μM. For α-MG uptake, 0.1 M 2-phosphoenolpyruvate was the energy source, and the initial concentration of [^{14}C]α-MG (50 μCi/μmol) was 33 μM.*

transport systems (24). Consistent with this hypothesis, the mutants B51 and B51-70 provide evidence for the existence within the cytoplasmic membrane of *E. coli* cells of a component, presumably protein, that functions in the energy coupling of respiration-linked active transport systems, may be required for ATP-dependent active transport systems, and is essential to the action of colicin K but not to that of colicins E1, E2, or E3. Conceivably, in normal *E. coli* cells this protein is rendered inactive by colicin K action and this inactivation results in an uncoupling of active transport systems. In the mutants B51 and B51-70 this protein may be modified in such a way that it is only partially functional in transport and does not respond to colicin K in a way that leads to the usual colicin K-triggered events.

Glutamine transport differs from proline transport in its requirement for ATP or some form of phosphate bond energy derived from ATP (3, 4). Yet colicin K inhibits glutamine transport with nearly the same kinetics as it inhibits proline transport, even under conditions where intracellular ATP levels are maintained (24). Furthermore, the establishment of strict anaerobic conditions with sodium dithionite markedly reduces both proline and glutamine transport, while reducing intracellular ATP levels by only 50% (10). These findings suggest that these operationally distinct active transport systems may have a common requirement essential for their optimal functioning. The degree to which they are dependent on this common element may be different, as indicated by the results of the present study. Whereas the effect of the mutation in B51 and B51-70 cells on colicin K sensitivity and the respiration-linked transport of proline is very pronounced, its effect on the ATP-dependent transport of glutamine is more subtle. Only when B51-70 cells are placed under conditions that severely restrict growth (i.e., transferring succinate-grown cells from 27 to 42 C) can the lesion be shown to have an inhibitory effect on glutamine uptake. It may prove significant in this regard that, in B51 and B51-70 cells, respiration-linked transport functions are not completely lost but rather are only reduced in activity. This raises the possibility that the lesion in the colicin K-insensitive mutants may be leaky and retains sufficient function for its role in glutamine transport (when glucose is present as energy source) but not for its role in proline transport. In these mutants, complete inactivation of the altered protein, such as may happen after colicin K action on normal cells, could have physiological consequences not evident in B51 and B51-70 cells.

The mutants reported in this study, while possessing an energy-uncoupled phenotype, have features that distinguish them from energy-uncoupled mutants of *E. coli* isolated in other laboratories. Two mutants of *E. coli* that lack Ca^{2+}, Mg^{2+}-activated ATPase activity, DL-54 (2, 29) and NR70 (26, 27), exhibit increased permeability to protons and deficiencies in active transport. Treating membrane vesicles prepared from these ATPase-deficient mutants with the ATPase inhibitor *N,N'*-dicyclohexyl carbodiimide results in the restoration of transport activity and also reduces proton permeability to the level characteristic of normal cells. The mutants reported in this communication differ from DL-54 and NR70 in at least three respects. First, the mutant B51-70 possesses normal levels of ATPase activity (Table 1). Secondly, treatment of B51-70 membrane vesicles with *N,N'*-dicyclohexyl carbodiimide under two sets of conditions (30 μM *N,N'*-dicyclohexyl carbodiimide, 10 min, 27 C and 70 μM *N,N'*-dicyclohexyl carbodiimide, 30 min, 27 C) did not result in the restoration of normal levels of proline transport (C. Plate, unpublished data). Thirdly, B51-70 cells do not appear to be

abnormally permeable to protons at either 27 or 42 C (M. Weiss and C. Plate, unpublished data). These findings suggest that the lesion in B51-70 cells does not lie within those portions of the ATPase complex that are required for hydrolytic activity or that govern proton permeability.

Failure to grow on succinate with retention of ATPase activity and of the ability to oxidize respiratory substrates such as succinate are properties that have been reported for several *E. coli* mutants, including the *uncB* mutant isolated by Butlin et al. (5), the *etc* mutant reported by Hong and Kaback (9), and the *ecf*^{ts} mutant recently reported by Lieberman and Hong (17). The properties of B51 and B51-70 are clearly different from those of an *uncB* mutant: the aerobic transport of proline is not reduced by the *uncB* mutation (7; C. Plate, unpublished data) and an *uncB* mutant is fully sensitive to killing by colicin K (C. Plate, unpublished data). It seems likely that the mutation in the colicin K-insensitive mutants is also distinct from the *ecf*^{ts} mutation reported by Lieberman and Hong (17). These investigators have stated that the *ecf*^{ts} mutation results in the inhibition of glutamine transport; this is not the case with the mutation in B51 and B51-70 cells, at least with glucose as energy source. In addition, membrane vesicles prepared from the *ecf*^{ts} mutant have been reported to exhibit normal transport properties at both the restrictive and permissive temperatures (17), whereas the vesicles prepared from B51-70 cells are abnormal for proline transport.

It is less clear that the colicin K-insensitive mutants reported in this study are distinct from the *etc* mutant described by Hong and Kaback (9). Clarification of this point should be possible when the mapping of the mutation in B51-70, currently in progress, is completed.

ACKNOWLEDGMENTS

I wish to thank S. E. Luria, J. L. Suit, and A. M. Jetten for helpful discussions throughout the course of this work. Technical assistance by Judy Fan is gratefully acknowledged.

This work was supported by grants to S. E. Luria from The National Science Foundation (GB 30575X) and the Public Health Service (AI 03038, National Insitiute of Allergy and Infectious Diseases).

ADDENDUM IN PROOF

J. Konisky (J. Bacteriol. **124**:1439–1446, 1975) has recently reported that a mutant of *E. coli* tolerant to colicins Ia and Ib and unable to grow on succinate also exhibits aberrant active transport properties.

LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. Altendorf, K., F. M. Harold, and R. D. Simoni. 1974. Impairment and restoration of the energized state in membrane vesicles of a mutant of *Escherichia coli* lacking adenosine triphosphatase. J. Biol. Chem. **249**:4587–4593.
3. Berger, E. A. 1973. Different mechanisms of energy coupling for the active transport of proline and glutamine in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:1514–1518.
4. Berger, E. A., and L. A. Heppel. 1974. Different mechanisms of energy coupling for the shock-sensitive and shock-resistant amino acid permeases of *Escherichia coli*. J. Biol. Chem. **249**:7747–7755.
5. Butlin, J. D., G. B. Cox, and F. Gibson. 1973. Oxidative phosphorylation in *Escherichia coli* K-12; the genetic and biochemical characterization of a strain carrying a mutation in the *unc B* gene. Biochim. Biophys. Acta **292**:366–375.
6. Fields, K. L., and S. E. Luria. 1969. Effects of colicins E1 and K on transport systems. J. Bacteriol. **97**:57–63.
7. Gibson, F., and G. B. Cox. 1973. The use of mutants of *Escherichia coli* K12 in studying electron transport and oxidative phosphorylation. Essays Biochem. **9**:1–29.
8. Gutnick, D. L., B. I. Kanner, and P. W. Postma. 1972. Oxidative phosphorylation in mutants of *Escherichia coli* defective in energy transduction. Biochim. Biophys. Acta **283**:217–222.
9. Hong, J.-S., and H. R. Kaback. 1972. Mutants of *Salmonella typhimurium* and *Escherichia coli* pleiotropically defective in active transport. Proc. Natl. Acad. Sci. U.S.A. **67**:3336–3340.
10. Jetten, A. M., and M. E. R. Jetten. 1975. Energy requirement for the initiation of colicin action in *Escherichia coli*. Biochim. Biophys. Acta **387**:12–22.
11. Kaback, H. R. 1971. Bacterial membranes, p. 99–120. *In* W. B. Jacoby (ed.), Methods in enzymology, vol. 22. Academic Press Inc., New York.
12. Kaback, H. R. 1974. Transport studies in bacterial membrane vesicles. Science **186**:882–892.
13. Kanner, B. I., and D. L. Gutnick. 1972. Use of neomycin in the isolation of mutants blocked in energy conservation in *Escherichia coli*. J. Bacteriol. **111**:287–289.
14. Kennedy, C. K. 1971. Induction of colicin production by high temperature or inhibition of protein synthesis. J. Bacteriol. **108**:10–19.
15. Kobayashi, H., and Y. Anraku. 1972. Membrane-bound adenosine triphosphatase of *Escherichia coli*. I. Partial purification and properties. J. Biochem. (Tokyo) **71**:387–399.
16. Koch, A. L. 1964. The role of permease in transport. Biochim. Biophys. Acta **79**:177–200.
17. Lieberman, M. A., and J.-S. Hong. 1974. A mutant of *Escherichia coli* defective in the coupling of metabolic energy to active transport. Proc. Natl. Acad. Sci. U.S.A. **71**:4395–4399.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. Luria, S. E., J. N. Adams, and R. C. Ting. 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348–390.
20. Nagel de Zwaig, R., and S. E. Luria. 1967. Genetics and physiology of colicin-tolerant mutants of *Escherichia coli*. J. Bacteriol. **94**:1112–1123.
21. Osborn, M. J., W. L. McLellan, Jr., and B. L. Horecker. 1961. Galactose transport in *Escherichia coli*. III. The effect of 2,4-dinitrophenol on entry and accumulation. J. Biol. Chem. **236**:2585–2589.
22. Plate, C. A. 1973. Effects of temperature and of fatty acid

substitutions on colicin K action. Antimicrob. Agents Chemother. **4**:16-24.
23. Plate, C. A., and S. E. Luria. 1972. Stages in colicin K action, as revealed by the action of trypsin. Proc. Natl. Acad. Sci. U.S.A. **69**:2030-2034.
24. Plate, C. A., J. L. Suit, A. M. Jetten, and S. E. Luria. 1974. Effects of colicin K on a mutant of *Escherichia coli* deficient in Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase. J. Biol. Chem. **249**:6138-6143.
25. Prezioso, G., J.-S. Hong, G. K. Kerwar, and H. R. Kaback. 1973. Mechanisms of active transport in isolated bacterial membrane vesicles. XII. Active transport by a mutant of *Escherichia coli* uncoupled for oxidative phosphorylation. Arch. Biochem. Biophys. **154**:575-582.
26. Rosen, B. P. 1973. β-Galactoside transport and proton movements in an adenosine triphosphatase-deficient mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. **53**:1289-1296.
27. Rosen, B. P. 1973. Restoration of active transport in an Mg^{2+}-adenosine triphosphatase-deficient mutant of *Escherichia coli*. J. Bacteriol. **116**:1124-1129.
28. Rosen, B. P., and L. W. Adler. 1975. The maintenance of the energized membrane state and its relation to active transport in *Escherichia coli*. Biochem. Biophys. Acta **387**:23-36.
29. Simoni, R. D., and M. K. Shallenberger. 1972. Coupling of energy to active transport of amino acids in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **69**:2663-2667.
30. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1959. Manometric techniques. Burgess Publishing Co., Minneapolis.
31. Van Thienan, G., and P. W. Postma. 1973. Coupling between energy conservation and active transport of serine in *Escherichia coli*. Biochim. Biophys. Acta **323**:429-440.
32. Winkler, H. H., and T. H. Wilson. 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biol. Chem. **241**:2200-2211.
33. Yamamoto, T. H., M. Mevel-Ninio, and R. C. Valentine. 1973. Essential role of membrane ATPase or coupling factor for anaerobic growth and anaerobic active transport in *Escherichia coli*. Biochim. Biophys. Acta **314**:267-275.