JOURNAL OF BACTERIOLOGY, Nov. 1971, p. 639-644
Vol. 108, No. 2

Printed in U.S.A.

Potassium Transport Loci in *Escherichia coli* K-12

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Received for publication 21 June 1971

Mutants of *Escherichia coli* K-12 requiring considerably elevated concentrations of potassium for growth are readily obtained as double mutants combining a *kdp* mutation with a mutation in one or more of five other loci. These loci are referred to as *trk*, for transport of K, because these mutations result in alterations in K transport. The *kdp* mutation is essential in the isolation and identification of this type of mutant; in a Kdp^+ strain, the presence of a *trk* mutation does not prevent growth of the strain in media containing very low concentrations of K. The *trk* loci are widely scattered over the *E. coli* chromosome; none of them is very near any other *trk* locus or near the *kdp* genes.

Cells of *Escherichia coli* K-12 accumulate potassium to achieve high cell concentrations of this ion. The accumulation of K has all of the properties expected of an active transport system: inhibition by metabolic poisons (14), saturation kinetics (5, 13), and the existence of K gradients much larger than can be explained as due to passive distribution in accordance with the membrane potential of the cells (15), or due to the property of the cell proteins to bind K somewhat preferentially to Na, the other monovalent cation present in high concentrations in the growth media (3). The cell K concentration of *E. coli* is dependent on the osmolality of medium, indicating that K plays a major role in the osmoregulation of these cells (5).

Continuing previous studies of this transport system, we isolated mutants requiring elevated concentrations of K for growth. Starting with a wild-type strain, only a single type of such mutant was obtained. This type of mutant was called Kdp, for K-dependent, and shows only a modest increase in K requirement (4). When a strain with a *kdp* mutation is used as parent, many more types of mutants are obtained. In this study we report on the isolation of such double mutants and on the mapping of the loci responsible for the different phenotypes. We refer to these loci as *trk* for transport of K, because all of them result in changes in K transport. A description of the transport properties of the mutants will be the subject of a separate report (W. Epstein and F. B. Waters, *manuscript in preparation*). A brief report of some of this work has been presented (W. Epstein, Biophys. Soc. Annu. Meet. Abstr., p. 225a, 1970).

MATERIALS AND METHODS

Media. The minimal media employed in this work are similar to those used in earlier studies (4) but include a wider range of K concentration and *p*H. The media are referred to by their K concentration; thus K115 medium contains 115 mM K, K10 contains 10 mM K, etc. K115 medium consists of: K_2HPO_4, 46 mM; KH_2PO_4, 23 mM; $(NH_4)_2SO_4$, 8 mM; $MgSO_4$, 0.4 mM; $FeSO_4$, 6 μM; sodium citrate, 1 mM; thiamine hydrochloride, 1 mg/liter; and glucose or another carbon source, 2 g/liter. K0 medium is similar, with equimolar amounts of the sodium salts replacing the potassium phosphates. K0 medium contains approximately 20 μM K due to contamination of the sodium salts. Media containing up to 10 mM K were prepared by adding 1 M KCl to K0; media with higher K concentrations were made by mixing suitable proportions of K0 and K115. These media have a *p*H of approximately 7.0 after autoclaving. The medium of *p*H 5.6 was made by altering the proportion of monobasic to dibasic phosphates in the medium while keeping the total osmolality constant. Amino acid-supplemented media contain 50 mg of each of the 18 natural L-amino acids per liter.

The elevated K requirements of the mutants necessitated some changes in other media and solutions. KML, a high-K version of ML medium (4), contains per liter: tryptone, 10 g; KCl, 10 g; and yeast extract, 5 g. Indicator media [MacConkey Agar Base and EMB Agar Base (Difco) containing 10 g of the desired sugar per liter] were modified by the addition of 50 mM KCl. The buffer used to suspend cells for bacteriophage P1kc absorption in cases where Trk was an unselected trait contained: KCL, 110 mM; $CaCl_2$, 5 mM; and $MgSO_4$, 1 mM.

Strains. The strains used in this work are listed in Table 1. Markers used in mapping are those from the strains listed in the table. The *ara* mutation used in mapping *trkC* was isolated after ultraviolet mutagenesis and plating on indicator medium.

TABLE 1. *Strains*

Strain	Mating type	Pertinent markers[a]	Origin
5333S	Hfr Hayes	*crp strA*^{r}	R. Perlman (10)
5336	Hfr Hayes	*cya*	R. Perlman (11)
AB1206	F-14		E. A. Adelberg (12)
AB2561	F$^-$	*ilv*	E. A. Adelberg
AT3201	F$^-$	*pdxA leu*	A. L. Taylor
CAE101	Hfr Hayes	*thr kdpABC5*	From XS-5 (4)
CAZ827	Hfr Hayes	*trp827*	D. Zipser
FRAG-5	F$^-$	*kdpABC5 lac gal rha thi*	From FRAG-1 (4)
JR100	F-41	*rec malA argG*/F-41 *argG*$^+$ *malA*$^+$	J. Davies
JR100C	F$^-$	*rec malA argG*	From JR100
V280	F$^-$	*aroE spc*^{r}	J. Davies
X135	F$^-$	*cysB pyrF*	E. Signer

[a] Only markers used in this study are listed. The abbreviations used are those of Taylor (16) except for: *cya*, locus affecting adenyl cyclase (18); and *crp*, locus for the adenosine 3',5'-monophosphate-binding protein (10).

Isolation of mutants. Mutagenesis with ultraviolet light or 2-aminopurine, and the subsequent penicillin selection and mutant identification, were performed just as in the isolation of the *kdp* mutants (4), except that media of different K concentrations were used. In some selections, the mutants were required to grow in K10, and penicillin selection was performed in K0.1 medium. In other selections, the cells were grown in K115 and treated with penicillin in K5 medium. In later work, a few selections were done that enrich for all of the different types of *trk* mutants: cells were grown in K115 medium and penicillin-treated in K0.1 medium.

Complementation. Complementation was examined in stable merodiploids utilizing the F-41 episome for the *trkA* and *trkB* loci. The procedure used was analogous to that described for the complementation studies of the *kdp* mutants (4). Mutant episomes, obtained after one cycle of penicillin in low-K medium in which the desired homozygous *trk*$^-$/*trk*$^-$ would not grow, were transferred to JR100C, a *rec* strain used for storage of the episomes and as donor in the matings with another *trk* mutant strain. The recipient *trk* strains were made *malA* by selecting spontaneous λ^r mutants that were Mal$^-$ and were complemented by the F-41 episome. The JR100C donor and the *trk malA* recipient were mated for approximately 60 min, and suitable dilutions were plated on K5 plates (for *trkA* mutations in a *trkD*$^-$ strain) or K0.1 plates (for *trkB* mutants) to test complementation, and on maltose K115 plates to obtain a measure of the frequency of episomal transfer.

Other methods. Crosses with Hfr × F$^-$ matings, or P1kc-mediated transduction were carried out as described (4), except for the changes in media necessitated by the higher K requirements of the Trk mutants. In general, all manipulations were carried out in high-K media (KML, or K115) to minimize any selective advantage the wild type might have.

RESULTS

Isolation of mutants. The rationale for the isolation of mutants was that, among mutants selected to require higher concentrations of K to achieve rapid growth rates, one should find some with defects in K transport. Initial attempts to obtain mutants with higher K requirements led to the isolation of only a single class of mutant, referred to as *kdp* for K-dependent. These mutants require approximately 10^{-4} M KCl to achieve half-maximal growth rates, whereas the growth rate of the wild type is constant down to K concentrations below 10^{-5} M. The Kdp$^-$ phenotype is due to a mutation in any one of four closely linked *kdp* genes located at 16 min on the map of *E. coli* (4). Attempts to obtain a mutant from wild-type parents that would require more K than do the *kdp* mutants were uniformly unsuccessful. However, when a *kdp* mutant strain was used as parent, mutants of the desired type were readily obtained. Thus it seems that more than one mutation is needed to obtain a wide variety of high-K-requiring mutants in *E. coli* K-12. This result is in accord with earlier experiments of Lubin, who found that mutants of *E. coli* K-12 requiring high concentrations of K could be obtained if selected in several steps of increasing K requirement (M. Lubin, *personal communication*).

All of the mutants described here were isolated by using FRAG-5 as the parental strain. FRAG-5 carries the *kdpABC5* mutation, a deletion affecting three of the four *kdp* cistrons (4). This deletion was chosen so that there would be no possibility of reversion at the *kdp* locus. Mutants were selected (see Materials and Methods) either for a modest increase in K requirement—no growth in K0.1, but growth in K10—or for a large increase in K requirement—no growth in K5 but growth in K115. Of the mutants obtained to date, we have identified five discreet classes and have mapped the mutations responsible for their defects. The genotypes and growth properties of these mutants are listed in Table 2.

TABLE 2. *Properties of the Trk mutants*

Class	No. studied	Genotype[a]	Growth on plates of indicated K conc[b]			
			0.1 mM	0.1 mM, pH 5.6	5 mM	115 mM
Trk$^+$			+	+	++	++
TrkA	2	*trkA*	0	+	+	++
TrkA/D	19	*trkA trkD*	0	0	0	++
TrkB	6	*trkB*	0	+	++	++
TrkC	3	*trkC*	0	0	++	++
TrkE	1	*trkE*	0	0	+	++

[a] All strains also carry the *kdpABC5* mutation.

[b] Growth was scored as follows: 0, no growth; +, slight to moderate growth; ++, good growth.

Role of the kdp mutation. In studying these mutants, we first investigated the role of the *kdp* mutation in the mutant phenotype. It was found that *kdp*$^+$ derivatives of all of the Trk mutants grew well on KO medium, indicating that, when a *trk* mutation is in a *kdp*$^+$ strain, the *trk* defect is not expressed in a way that can be detected by growth tests. This ability of *kdp*$^+$ to suppress the defects due to the *trk* mutations explains why it was not possible to obtain the desired type of mutants from wild-type parents. At least two mutations are necessary to obtain the desired mutants, and the mutagenic agents used in this work (ultraviolet irradiation and 2-aminopurine) do not produce large numbers of double mutations. We recently found that the *kdp* genes control the appearance of a transport system with very high affinity for K and that it is this transport system that allows cells of *E. coli* to grow rapidly in media containing low concentrations of K. We did not detect this system in earlier work (4), because the synthesis of this system is repressed when the cells are grown in media containing excess K (W. Epstein and F. B. Waters, *manuscript in preparation*).

Mapping the trk loci. Since the *kdp*$^+$ allele compensates for the K defect of the *trk* mutations, care had to be taken that *kdp*$^+$ recombinants were not obtained in mapping of the *trk* loci. This did not present an important problem in Hfr $\times$ F$^-$ crosses, because none of the *trk* loci is very near the *kdp* genes. However, in transductions in which *trk*$^+$ was the selected marker, the donor strain usually carried the same *kdpABC5* mutation that is in all of the *trk* mutants. In a few crosses in which a *kdp*$^+$ donor was used, the *trk*$^+$ *kdp*$^-$ recombinants were distinguished from the *trk*$^-$ *kdp*$^+$ class by the fact that the former do not grow on K0 plates, but the latter do. Only one or two mutations of each type were mapped. The others of that class were then shown to be identical either by complementation studies (for the *trkA* and *trkB* mutations) or by showing that other mutants of that class recombined only at very low frequency with the mutation that had been mapped, or showed similar linkage to nearby markers.

The map locations of the five *trk* loci are presented in Fig. 1, in which the linkage of *trkA* and *trkB* to nearby markers by transduction is also shown. For four of the mutant types (TrkA, TrkB, TrkC, and TrkE), a single mutation accounts for the difference between mutant and FRAG-5 parent, as indicated by the genotypes listed in Table 2. The *trkA* and *trkB* mutations are closely linked to *strA*. A series of three-factor crosses (Table 3) established the order: *spc-trkA-aroE* (cross A); *strA-spc-trkA* (cross B); and *crp-trkB-strA* (cross C). The recombinant classes are listed for these and the other three-point crosses presented in this study so that, given the order of markers drawn in the figures, the class of recombinant that requires four crossovers, and thus would be the least frequent, is shown in the last row of each cross. For

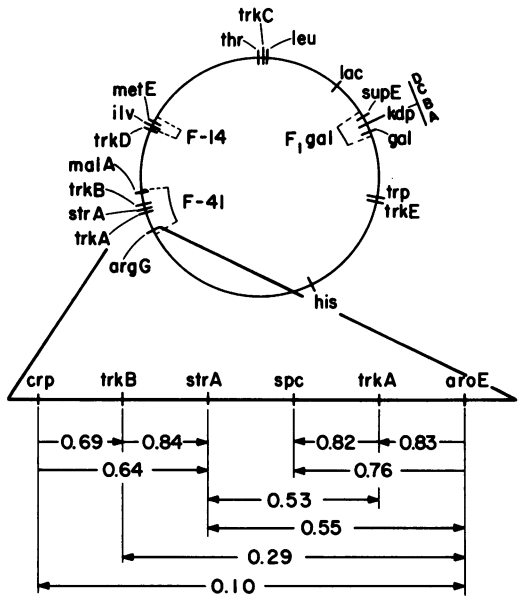

FIG. 1. *Location of the trk loci on the circular linkage map of E. coli. Reference loci are placed as on the map of Taylor (16). At the bottom, cotransduction frequencies between markers near trkA and trkB are shown. Arrows point from selected marker to scored unselected donor marker; double-headed arrows indicate the average of cotransduction frequencies measured in both directions. Distances indicated at bottom are arbitrary and not drawn to scale.*

TABLE 3. *Ordering of trkA and trkB with respect to adjacent loci by three-factor transductional crosses*

Cross A Donor: *spc*^{s} *trkA*$^-$ *aroE*$^+$ Selected marker: *aroE*$^+$	Recipient: *spc*^{r} *trkA*$^+$ *aroE*$^-$ No. scored: 309
Recombinant class	No.
spc^s *trkA*$^-$	227 (73%)
spc^r *trkA*$^+$	37 (12%)
spc^r *trkA*$^-$	40 (13%)
spc^s *trkA*$^+$	5 (2%)
Cross B Donor: *strA*^{s} *spc*^{r} *trkA*$^+$ Selected marker: *trkA*$^+$	Recipient: *strA*^{r} *spc*^{s} *trkA*$^-$ No. scored: 150
Recombinant class	No.
strA^s *spc*^{r}	81 (54%)
strA^r *spc*^{s}	21 (14%)
strA^r *spc*^{r}	44 (29%)
strA^s *spc*^{s}	4 (3%)
Cross C Donor: *crp*$^+$ *trkB*$^-$ *strA*^{s} Selected marker: *crp*$^+$	Recipient: *crp*$^-$ *trkB*$^+$ *strA*^{r} No. scored: 458
Recombinant class	No.
trkB$^-$ *strA*^{s}	293 (64%)
trkB$^+$ *strA*^{r}	140 (30%)
trkB$^-$ *strA*^{r}	22 (5%)
trkB$^+$ *strA*^{s}	3 (1%)

each of the crosses this is the least frequent type, confirming the order shown in the figure. This map order of the *trkA* and *trkB* markers is also the one most consistent with the cotransduction frequencies in this region and the mapping data of others (16).

The *trkC* locus is closely linked to the *pdxA* locus near 0.5 min on the map of *E. coli* (Fig. 2). Three-factor crosses (Table 4) showed the order: *thr-trkC-ara* (cross A); and *trkC-pdxA-ara* (cross B). The *pyrA* marker was not used in the mapping, but the closer linkage of *trkC* to *pdxA* (75%) than has been reported for the linkage of *pyrA* to *pdxA* (14%, reference 17) suggests that *trkC* is probably between *pyrA* and *pdxA*, as drawn in Fig. 2. In mapping the *trkC* locus, *trkC*$^+$ was not used as the selected marker because attempts to do so were frustrated by two problems. (i) All three of the TrkC mutant strains revert at a frequency of approximately 10^{-6}, and (ii) immediate plating of cells after absorption of transducing phages never resulted in an increase in the number of cells growing on K0.1 plates compared to the control. The failure to obtain transductants is probably due to the phenotypic dominance of *trkC*$^-$ over *trkC*$^+$, so that delayed plating is necessary to obtain *trkC*$^+$ transductants. It is not known whether the high reversion frequency of the TrkC mutant strains is due to reversion of the *trkC* mutation, or whether some type of suppressor mutations are responsible.

The TrkA/D mutants differ from the parental type in two mutations. These strains have a much higher K requirement than any of the other mutants (Table 2) and carry a *trkA* mutation which is in the same complementation group as the mutations in the TrkA mutants. The TrkA/D mutants carry an additional mutation at the *trkD* locus, resulting in loss of a function which partly compensates for the absence of the TrkA product. The *trkD* locus is closely linked to the *ilv* locus, with somewhat lesser linkage to *cya* and *metE*. The donor *trkD* marker was inherited as unselected marker in 82% of selected *ilv*$^+$ transductants, 61% of *cya*$^+$ transductants, and 34% of *metE*$^+$ transductants. Three-factor crosses established the order *trkD-ilv-cya* and *ilv-cya-metE* (Table 5). These results, placing *trkD* slightly counterclockwise from *ilv*, are supported by the fact that the *ilv*, *cya*, and *metE* loci are represented on the F-14 episome, whereas *trkD* is

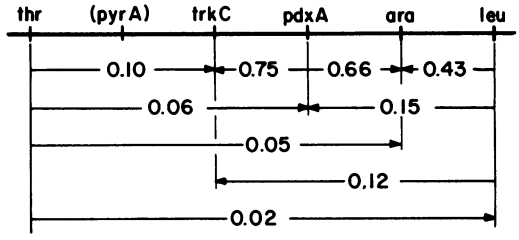

FIG. 2. *Cotransduction frequencies for markers in the vicinity of the trkC locus. Data are presented as in Fig. 1.*

TABLE 4. *Mapping of trkC by three-factor transductional crosses*

Cross A Donor: *trkC*$^+$ *ara*$^-$ *leu*$^+$ Selected marker: *leu*$^+$	Recipient: *trkC*$^-$ *ara*$^+$ *leu*$^-$ No. scored: 220
Recombinant class	No.
trkC$^-$ *ara*$^+$	121 (55%)
trkC$^-$ *ara*$^-$	72 (33%)
trkC$^+$ *ara*$^-$	23 (10%)
trkC$^+$ *ara*$^+$	4 (2%)
Cross B Donor: *trkC*$^-$ *pdxA*$^+$ *ara*$^+$ Selected marker: *ara*$^+$	Recipient: *trkC*$^+$ *pdxA*$^-$ *ara*$^-$ No. scored: 160
Recombinant class	No.
trkC$^+$ *pdxA*$^+$	10 (6%)
trkC$^+$ *pdxA*$^-$	129 (81%)
trkC$^-$ *pdxA*$^+$	19 (12%)
trkC$^-$ *pdxA*$^-$	1 (1%)

not. TrkD mutants are not complemented by F-14, and, when a P1kc lysate of strain AB1206 is used to transduce an *ilv*$^-$ recipient to *ilv*$^+$, all of the transductants retain the *trkD* marker of the recipient, regardless of whether the recipient is *trkD*$^+$ or *trkD*$^-$. This loss of linkage between *ilv* and *trkD* in AB1206 indicates that the chromosomal break which formed the F-14 episome occurred between *trkD* and *ilv* (12).

The *trkE* locus is near 25.5 min on the linkage map of *E. coli*. In transductional crosses in which *trkE* was an unselected donor marker, the donor *trkE* marker was found to be 10% linked with *trp*, 15% with *cysB*, and 61% with *pyrF*. A three-factor cross in which *cysB*$^+$ was the selected donor marker (Table 6) is most consistent with the order *cysB-pyrF-trkE*. From this cross it can be seen that *pyrF* is more closely linked to *cysB* than is *trkE*, indicating that *trkE* is not between *cysB* and *pyrF* and thus is almost certainly clockwise to *pyrF*. The presumed four-crossover class of recombinant (*pyrF*$^-$ *trkE*$^-$) is not as infrequent as is usually the case, perhaps owing to unusually high negative interference.

Complementation. Complementation at the *trkA* and *trkB* loci was studied in stable merodiploids as described above. For the *trkA* locus, a total of 19 mutations were tested. Episomes carrying six different *trkA* mutations were prepared and used to test complementation with each other and the other 13 mutations. The majority of the *trkA* mutations tested were originally isolated as mutants of the TrkA/D class. No complementation was observed between any of these mutations. Episomes carrying four of the six *trkB* mutations were prepared, and similarly no complementation was found for mutants at this locus. Complementation occurs between *trkA* and *trkB* mutations, as is expected for mutations in different genes.

TABLE 5. *Mapping of the trkD locus*

Cross A	
Donor: *trkD*$^-$ *ilv*$^+$ *cya*$^+$ Selected marker: *cya*$^+$	Recipient: *trkD*$^+$ *ilv*$^-$ *cya*$^-$ No. scored: 160
Recombinant class	**No.**
ilv$^+$ *trkD*$^+$	49 (31%)
ilv$^+$ *trkD*$^-$	98 (61%)
ilv$^-$ *trkD*$^+$	13 (8%)
ilv$^-$ *trkD*$^-$	0 (0%)
Cross B	
Donor: *ilv*$^+$ *cya*$^+$ *metE*$^-$ Selected marker: *ilv*$^+$	Recipient: *ilv*$^-$ *cya*$^-$ *metE*$^+$ No. scored: 159
Recombinant class	**No.**
cya$^+$ *metE*$^+$	68 (43%)
cya$^-$ *metE*$^+$	82 (51%)
cya$^+$ *metE*$^-$	9 (6%)
cya$^-$ *metE*$^-$	0 (0%)

TABLE 6. *Mapping of the trkE locus*

Donor: *cysB*$^+$ *pyrF*$^+$ *trkE*$^-$ Selected marker: *cysB*$^+$	Recipient: *cysB*$^-$ *pyrF*$^-$ *trkE*$^+$ No. scored: 160
Recombinant class	**No.**
pyrF$^+$ *trkE*$^+$	46 (29%)
pyrF$^+$ *trkE*$^-$	17 (11%)
pyrF$^-$ *trkE*$^+$	90 (56%)
pyrF$^-$ *trkE*$^-$	7 (4%)

DISCUSSION

In our search for K transport mutants, we have found that mutations in nine genes, clustered in six groups, result in an increased K requirement for growth. Since we have examined a rather small number of mutants, it is possible that additional loci affecting the K requirement will be found. Except for the four clustered *kdp* genes, each of the loci probably consists of a single cistron not very closely linked to other loci of this type. In view of the fact that no complementation occurred between the 19 *trkA* mutations, it is safe to conclude that this locus contains only a single cistron. A similar conclusion is likely for *trkB*, where no complementation occurred between the six mutation tests. Complementation studies of mutations at the other loci will require the isolation of sufficient numbers of each type of mutant to make such studies meaningful.

Transport studies now in progress indicate that all of the mutations which result in an increased K requirement for growth specifically alter K transport. The TrkB and TrkC mutants are primarily defective in the retention of K; the other mutants have reduced rates of K uptake as compared with wild-type cells (W. Epstein and F. B. Waters, *manuscript in preparation*).

We believe that our frequent isolation of the TrkA/D double mutants occurred through the spontaneous appearance of a *trkD*$^-$ mutation in one of the stocks of cells used in the isolation of mutants. The loss of the *trkD* function is virtually undetected in a *trkA*$^+$ strain, because the transport system associated with the *trkD* locus is a minor one, completely overshadowed by the major transport system associated with the *trkA* locus.

Two of the loci of mutations affecting K transport in *E. coli* are consistent with the mapping reported for mutations affecting K transport in *E. coli* B. A mutant of *E. coli* B isolated by Lubin has a defect in a location consistent with *trkC* (8) and is defective in the retention of K, just as are the TrkC mutants (7, 9). A mutant

isolated by Damadian which is defective in K uptake but which also loses K rather readily (2) has a defect mapping near the *gal* operon (1), a location consistent with that of the *kdp* genes.

ACKNOWLEDGMENTS

We thank J. Beckwith, J. Davies, B. Low, E. A. Adelberg, A. L. Taylor, R. Perlman, and E. Signer for providing strains, and Fred B. Waters for technical assistance.

This investigation was supported by grant GB 8470 from the National Science Foundation. W. Epstein is the recipient of a Public Health Service Career Development Award (GM 10725) from the National Institute of General Medical Sciences.

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