Genetic Analysis of Potassium Transport Loci in *Escherichia coli*: Evidence for Three Constitutive Systems Mediating Uptake of Potassium

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The analysis of mutants of *Escherichia coli* that require elevated concentrations of K⁺ for growth has revealed two new genes, *trkG*, near minute 30 within the cryptic *rac* prophage, and *trkH*, near minute 87, the products of which affect constitutive K⁺ transport. The analysis of these and other *trk* mutations suggests that high rates of transport, previously considered to represent the activity of a single system, named TrkA, appear to be the sum of two systems, here named TrkG and TrkH. Each of these two is absolutely dependent on the product of the *trkA* gene, a cytoplasmic protein associated with the inner membrane (D. Bossemeyer, A. Borchard, D. C. Dosch, G. C. Helmer, W. Epstein, I. R. Booth, and E. P. Bakker, *J. Biol. Chem.* 264:16403–16410, 1989). The TrkH system is also dependent on the products of the *trkH* and *trkE* genes, while the TrkG system is also dependent on the product of the *trkG* gene and partially dependent on the product of the *trkE* gene. It is suggested that the *trkH* and *trkG* products are membrane proteins that form the transmembrane path for the K⁺ movement of the respective systems. Two mutations altering the *trkA* product reduce the affinity for K⁺ of both TrkG and TrkH, indicating that changes in this peripheral protein can alter the conformation of the sites at which K⁺ is bound prior to transport. The TrkD system has a relatively modest rate of transport, is dependent solely on the product of the *trkD* gene, and is the sole saturable system for Cs⁺ uptake in this species (D. Bossemeyer, A. Schlösser, and E. P. Bakker, *J. Bacteriol.* 171:2219–2221, 1989).

The major cellular monovalent cation in bacteria, as in cells generally, is K⁺. This ion, accumulated by *Escherichia coli* to levels from about 100 mM to near 1 M (12, 27), serves to regulate cell osmolarity and to create an internal ionic environment favorable to cell enzymes (for a review, see reference 29). The amount of K⁺ accumulated is determined by osmotic needs. Cells growing in a medium of high osmolarity have high K⁺ pools, so that internal osmolarity rises with that of the medium. The size of the K⁺ pool appears to be controlled to maintain a relatively constant turgor pressure, the difference between internal and external osmotic pressures. When turgor is low, K⁺ uptake is stimulated, but when turgor is normal, K⁺ uptake is inhibited (12, 21, 26). Elevated levels of turgor stimulate K⁺ efflux (4, 22).

Cell K⁺ pools represent an equilibrium between the uptake and the efflux of this ion. Each of these processes appears to be mediated by multiple systems. Uptake, the more extensively studied process, is dependent on the activity of the repressible Kdp system and several constitutive Trk systems. Kdp is an ATP-driven system with a high affinity for K⁺ (29), and Kdp activity can compensate for deficits in Trk activity and thereby mask the effects of mutations on Trk. For studies of constitutive K⁺ transport, all strains carried deletions of parts of the Kdp structural genes to eliminate Kdp activity and the possibility of reversion to Kdp⁺ (10).

A genetic analysis of constitutive K⁺ uptake suggested the existence of two saturable systems, called TrkA and TrkD, and a system with a low rate of transport, called TrkF, with diffusion-limited kinetics (11, 26). This classification was based on the finding that *trkA* mutants lacked the major component of transport, a component with a $V_{\max}$ of 200 to 500 $\mu\text{mol g}^{-1} \text{min}^{-1}$ and a K_m for K⁺ of 1.5 mM. However, *trkA* mutants retained a minor component of transport which was abolished when a *trkD* mutation was present as well. Strains with mutations in both *trkA* and *trkD* retained only a low rate of uptake proportional to the external K⁺ concentration; the responsible system was called the TrkF system. No genes responsible for TrkF activity were identified. The *trkE* locus was represented by a single mutation that reduced uptake, but it was not clear whether it affected only TrkA or other systems as well.

The separate existence of TrkD was not strongly supported by the work mentioned above. It was therefore suggested that *trkD* may encode a component of the TrkA system (29). Recent work by Bossemeyer et al. (7) provided strong evidence that *trkD* encodes a separate transport system with a distinct substrate specificity. These authors showed that Cs⁺ uptake in *E. coli* was dependent on the *trkD* genotype of the strain. Regardless of the presence of mutations in other genes that affect K⁺ transport, all *trkD* mutants had a low rate of Cs⁺ uptake that was not saturable, while all *trkD*⁺ strains had a saturable rate of Cs⁺ uptake, with a K_m for Cs⁺ of about 5 mM. Further evidence indicating that *trkD* encodes a distinct transport system was the fact that a *trkA* mutant with a multicopy *trkD* plasmid had three- to fourfold-higher rates of Cs⁺ uptake (7) and of K⁺ uptake (6) than did a *trkA* mutant with only the single chromosomal copy of *trkD*.

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TABLE 1. Bacterial strains

Strain	Genotype ^a	Source or reference ^b
AB1157	<i>thr leuB proA hisG argE thi ara lacY galK xyl mtl rpsL tsx supE (rac)</i>	CGSC
AB1206	F14 <i>thi his proA lacY galK tfr rpsL</i>	CGSC
CAE153	Hfr Hayes <i>thi lacZ thr kdpABC5 trkD1 trkG82 zcj-230::Tn10</i>	Laboratory collection
CFE20	Hfr AB312 <i>thr leu nadA aroE trkD1 ilv::Tn10</i>	
DD1192	AB1157 <i>kdpABC5 trkD1 ilv::Tn10</i>	
FRAG5	<i>thi lacZ(Am) gal rha kdpABC5</i>	11
KL181	<i>pyrD his recA thi galK xyl mala rpsL</i>	CGSC
MR8301	Hfr KL14 <i>thi relA spoT polA zih-219::Tn10^c</i>	H. Wu
NK5151	<i>trpB83::Tn10</i>	CGSC
PLK1026	<i>trpR trpA his ilv pro arg thyA deoBC tsx zda-230::Tn10^c</i>	5
PLK1027	PLK1026 <i>zda-231::Tn10</i> instead of <i>zda-230::Tn10</i>	5
PLK1690	<i>ilv his zcj-2::Tn10^c</i>	5
TK1001	<i>lacZ gal lacZ kdpABC5 trkD1</i>	26
TK2076	<i>mal lacZ nadA trp pyrF kdpABC5 trkD1</i>	Laboratory collection
TK2088	TK2076 <i>trkE78 trkG88</i>	
TK2089	TK2076 <i>trkE79 trkG89</i>	
TK2090	TK2076 <i>trkE80 trkG90</i>	25
TK2091	TK2076 <i>trkG91 trkH1</i>	
TK2092	TK2076 <i>trkG92 trkH1</i>	
TK2205	<i>nadA lacZ kdpABC5 trkD1 trkA405</i>	25
TK2295	<i>nadA lacZ kdpABC5 trkA405</i>	
TK2654	<i>lacZ nadA trp kdpABC5 trkH1 trkD1</i>	
TK2685	TK2076 <i>trkG92</i>	Laboratory collection
TK2719	<i>lacZ nadA trp pyrF kdpABC5 trkD1</i>	
TK2721	TK2719 <i>pyrF⁺ trkE16</i>	
TK2729	TK2719 <i>pyrF⁺ trkE102</i>	Laboratory collection
X9136S	<i>thi rha gal lacZ aroE kdpABC5 trkD1 rpsL</i>	

^a All TK strains are also *thi rha*; all strains are F⁻, except as noted.

^b CGSC, *E. coli* Genetic Stock Center, Yale University; when no source is given, the strains were constructed or isolated for this work.

^c The letter designations of these insertions have been changed (allele numbers have been retained) to show the minute intervals in which they are located on the latest map of *E. coli* (3): *zci-2::Tn10* is *zci-2::Tn10*, *zci-230::Tn10* is *zda-230::Tn10*, and *zih-219::Tn10* is *zih-219::Tn10*.

Here we report the isolation and characterization of additional mutations that reduce Trk activity. These studies implicated a total of five genes that affect constitutive saturable K⁺ uptake in this species: *trkG* and *trkH*, characterized here, as well as *trkA*, *trkD*, and *trkE*, described earlier. Our results are most easily interpreted as supporting the existence of three different systems: the system capable of a low rate of transport, TrkD, dependent solely on the product of the *trkD* gene, and two systems capable of high rates of transport, TrkG and TrkH. The latter two were formerly lumped together as the single TrkA system. Both TrkG and TrkH are absolutely dependent on the product of the *trkA* gene but are distinguished by their dependence on other gene products and by minor kinetic differences.

MATERIALS AND METHODS

Bacterial strains and plasmids. Most of the strains of *E. coli* K-12 used in this work are listed in Table 1. As indicated by footnote *c* in Table 1, the nomenclature of the Tn10 insertions used has been changed to bring their letter descriptions in line with their positions on the latest map (3). Strain DD1192, a *kdpABC5 trkD1* derivative of *rac* mutant strain AB1157 (18), was constructed by introducing a *kdpA::lacZ* fusion from strain TL1138 by P1 transduction, with selection for Amp^r. Strain TL1138 is a 42°C survivor of *kdpA::Mu d (Ap lac)* fusion strain TL1102 (16) that retained the fusion and Amp^r marker of the parent. The *kdpA::lacZ* derivative was transduced to *kdp⁺ nadA* by transduction with a lysate of strain TK2247 (24; pertinent markers: *nagA nadA trkA405 trkD1*) and then to *nadA⁺ kdpABC5* with a lysate of strain FRAG-5 (11). The *trkD1* mutation was then

introduced by cotransduction with a Tn10 insertion in a selection for Tet^r with a P1 lysate of a *trkD1 ilv::Tn10* mutant strain.

Bacteria from which the *rac* prophage had been excised were selected with the use of λ rev as described by Binding et al. (5). The desired colonies were identified by the loss of a Tn10 insertion (*zda-230::Tn10*) in *rac* sequences (Fig. 1). Most of the strains in Tables 2 and 3 with additional combinations of mutations in different *trk* genes were constructed by transduction with the linked markers identified below or reported earlier (11). The *trk* genotype in most cases was determined by recovery of the allele in transductional crosses because the phenotype of the combinations was not known in advance; in many cases a given mutation had little or no apparent effect on the phenotype. The *trkA* genotype was changed or confirmed by cotransduction with *aroE*; the latter marker was introduced when needed by cotransduction with *rpsL* by use of a lysate of strain X9136S. In similar fashion, *trkE* mutations were introduced and the *trkE* genotype was confirmed by cotransduction with *pyrF*; the latter marker was introduced when needed in two steps, first by transduction to Tet^r (*trpB83::Tn10*) with a lysate of strain NK5151 and then by transduction to *trp⁺ pyrF* with a lysate of strain X135 (11). *trkG* mutations were introduced by cotransduction with the *zda230::Tn10* or *zda231::Tn10* insertion. The resulting genotype was confirmed by recovering the *trkG* allele by cotransduction with the insertion into a *trkD trkH* tester mutant strain. *trkH* mutations were introduced and tested by cotransduction with *metE*, the latter marker being introduced by cotransduction with the *zih219::Tn10* insertion (Fig. 2). *trkD* mutations were introduced and confirmed by cotransduction with the *ilv::Tn10*

FIG. 1. Map of the 28- to 31-min region of the *E. coli* chromosome, showing transductional linkage of *trkG* to nearby markers. The boxed area between minutes 30 and 31 represents *rac* sequences. Arrows point from selected to scored markers, with a minimum of 150 transductants scored in each cross. All frequencies for the Tn10 insertion mutations are for the presence of the insertion in the transductants scored.

insertion. Except as noted for a few *trkG* mutant strains, the strains used for growth and transport studies did not carry a Tn10 insertion.

The *trkA*-complementing plasmid pGH1 has been described previously (13). The *trkH*-complementing plasmid pJD306 (9a) was constructed by inserting into pSK⁺ (Stratagene) a 1.4-kb *EcoRI*-*HpaI* fragment derived from *trkH1*-complementing plasmid pWE211, a pBR322 derivative carrying a 4-kb *EcoRI* insert (6, 9b).

Media and bacterial growth. Complex KML medium and phosphate-buffered minimal media have been described previously (11). Minimal media are referred to by their K⁺ concentration (millimolar); thus, K5 medium contains 5 mM K⁺. Half ML medium contains (per liter) the following: tryptone (Difco), 5 g; yeast extract (Difco), 2.5 g; and NaCl, 5 g. Required amino acids at 25 mg/liter, vitamins at 1 mg/liter, Casamino Acids at 5 g/liter, and glucose or another carbon source at 2 g/liter in liquid media and 10 g/liter on agar media were added. Tetracycline hydrochloride at 10 mg/liter and sodium cefoxitin at 100 mg/liter were added as needed. Selection for the loss of tetracycline resistance was performed as described by Maloy and Nunn (20), with additional K⁺ added to the medium as dictated by the

requirements of the strain. Cells were grown with shaking at 37°C unless otherwise indicated.

Genetic methods. Generalized phage P1 transduction was performed as described previously (10). Conjugal crosses were performed with cells growing exponentially in KML medium at a density of 3×10^8 /ml. Equal numbers of recipient and donor cells were mixed, and the mating was allowed to continue at 37°C for various times without agitation. Matings were interrupted by vortexing the diluted mating mix, and suitable dilutions were plated on selective media. Some conjugal transfers of F plasmids were accomplished by spotting serial dilutions of the donor on a selective plate spread with the recipient.

Isolation of mutants. Five strains with mutations in the *trkE*, *trkG*, and *trkH* loci were isolated in strain TK2076 following UV irradiation with a General Electric germicidal lamp to a survival of 1 to 3%. The strains analyzed, TK2088 to TK2092 (Table 1), are *mal*⁺ transductants of the original mutants. Additional UV-induced *trkD* mutations were obtained in strain TK2295, *trkE* mutations were obtained in strain TK1001(pGH1), and additional *trkH* mutations were obtained in strain TK2685(pGH1). Recovery from mutagenesis and enrichment of mutants unable to grow at low K⁺ concentrations were done as in earlier work (11), except that cefoxitin rather than penicillin was used in the selection for *trkE* and *trkH* mutations because the parent was resistant to penicillin, owing to the presence of pGH1.

Complementation analysis of *trk* loci. Complementation of *trkE* and *trkG* mutations was examined in F123 merodiploids. The F plasmid was introduced into *nadA trp* strains carrying a *trkE* mutation (such as TK2721, constructed by cotransducing *trkE* with *pyrF*) or a strain carrying *trkH trkG* mutations (such as TK2092) (the *trkG89* and *trkG90* mutations were introduced into TK2654 by cotransduction with the *zda231::Tn10* insertion) in a mating with strain KL181 (F123), with selection for *trp*⁺ *his*⁺ exconjugants. These *trk/trk*⁺ diploids were subjected to one round of cefoxitin selection in K0.1 medium to enrich for *trk* homogenotes, and homogenotes were identified by their inability to grow in K0.1 medium and their ability to donate F123 to suitable recipients. The *trk* mutant plasmids were transferred to a *recA* mutant strain, either KL181 or TK2611 (24), and from the latter strain to other *trk* mutants by mating in KML medium. After the mating was done, the mixture was washed with K115 medium and about 10^3 cells were plated on K115 medium (selection for transfer of the plasmid *trp*⁺ marker) and on K0.1 medium (selection for *trk*⁺ as well as

TABLE 2. Role of *trkD* in the kinetics of net K⁺ uptake

Strain ^a	<i>trk</i> genotype	Transport kinetics	
		<i>K_m</i> (mM)	<i>V_{max}</i> (μmol g ⁻¹ min ⁻¹)
TK2734	A405	0.3 ^b	34 ^b
TK2205 ^c	A405 D1		
TK2687	E80 G90	0.4 ^b	39 ^b
TK2693 ^c	E80 G90 D1		
TK2689	G92 H1	0.4, 0.35 ^d	39, 37 ^d
TK2691 ^c	G92 H1 D1		
TK2697	A405 E80 H1 G92	0.25, 0.28 ^d	26, 30 ^d
TK2699 ^c	A405 E80 H1 G92 D1		

^a All strains have the *kdpABC5* deletion.

^b Data are from single measurements.

^c These strains have a slow rate of uptake that is linearly dependent on the external K⁺ concentration and is approximately 1 μmol g⁻¹ min⁻¹ at an external K⁺ concentration of 10 mM.

^d Data are from two separate measurements.

TABLE 3. Effect of other combinations of *trk* mutations and of two *trkA* alleles on growth and transport

Strain ^a	<i>trk</i> genotype	Growth K_m (mM)	Transport kinetics ^b	
			K_m (mM)	V_{max} ($\mu\text{mol g}^{-1} \text{min}^{-1}$)
FRAG5	Wild type	0.08	1–1.7 ^c	200–600 ^c
FRAG93	<i>D1 zda231::Tn10</i>	0.11	2.8, 4	380, 460
FRAG94	<i>D1 del(zda)</i> ^d		4	460
FRAG96	<i>D1 H1</i>	0.11	1, 1.1	370, 310
FRAG91	<i>D1 G92 zda231::Tn10</i>	0.11	2.6, 3	470, 490
FRAG92	<i>D1 G92 del(zda)</i>		3, 4	370, 455
FRAG97	<i>D1 E80</i>	2.4 ^e	1.3, 2	140, 120
FRAG98	<i>D1 E80 H1</i>	2.4	0.5, 0.8	170, 180
GH4002	<i>D1 A429</i>	8	8 $\pm$ 2	65 $\pm$ 8 ($n = 3$)
GH4027	<i>D1 A427</i>	1.5	7	57

^a Strains GH4002 and GH4027 were constructed from X9136S by transduction with P1 lysates of mutants carrying the respective *trkA* alleles. All other strains were constructed from FRAG5 by transduction as described in Materials and Methods.

^b Data are from single measurements, two measurements, or three measurements (values with standard deviations).

^c Kinetic data for the wild-type strain are ranges obtained in measurements over several years in two laboratories (6, 26).

^d We use the abbreviation *del(zda)* for the spontaneous mutation to the loss of tetracycline resistance selected for by resistance to fusaric acid (20) and isolated from the strain immediately above the strain carrying *del(zda)* in the table.

^e The growth rate of this strain in high- K^+ media is slightly reduced, as discussed in the text.

trp⁺). Complementation could be assumed to have occurred if equal numbers of colonies grew on both plates. Complementation analysis of additional *trkH* mutations was done as described below with pJD306, which carries *trkH*⁺.

The plaque-forming λ *rbs3* phage, which carries *trkD* and the genes of ribose catabolism on a 13.7-kb *EcoRI* insert, was used for complementation analysis of the *trkD* locus. Lopilato et al. (17) described the construction of this phage but did not name it. The plaque complementation assay used earlier in studies of *kdp* mutations (25) was used to identify phages carrying *trkD* mutations and to assay complementation. Strain TK2205 was infected with about 200 phages and plated on half ML medium. This strain grew very slowly on these plates to form a light lawn because this medium contained about 4 mM K^+ . Phage plaques were clear, except when the phage complemented the K^+ transport defect of the host, in which case there was dense growth, heavier than the uninfected lawn, at the center of each plaque. Ten independently isolated *trkD* mutants were lysogenized with λ *rbs3* by selection for colonies that grew on K1 medium. The prophage in these mutants was induced by brief exposure to UV light, and the resultant lysate was plated on the same *trkD* mutants with half ML medium. Phages that did

not complement were purified and plated on each of the other *trkD* mutants to determine whether any of the mutations complemented the others.

Other methods. The K^+ dependence of the growth rate and the initial rate of K^+ uptake in K^+ -depleted cells were determined as described earlier (26). Growth rates were measured at 37°C, while transport experiments were done at 30°C with cells grown at 30°C.

RESULTS

When a *kdp trkD* strain is mutagenized and mutants requiring higher K^+ concentrations for growth are selected, one obtains primarily strains with *trkA* mutations. In the course of screening several hundred UV-induced mutants of strain TK2076 that were unable to grow on K5 medium, we identified five that were not complemented by F141 (11) and therefore did not harbor *trkA* mutations. Strains TK2088 and TK2089 were independently isolated, while strains TK2090, TK2091, and TK2092 were from a single batch of mutants and could have been siblings. Our analysis showed that all carried at least one unique allele. All five were complemented to growth on K0.1 medium by F123, which carries

FIG. 2. Map of the 84.5- to 88.5-min region of the *E. coli* chromosome, showing transductional linkage of *trkH* to nearby markers. Data were obtained and are presented as for Fig. 1. Transduction frequencies in which the scored transductants had lost the *zih-219::Tn10* insertion are marked by an asterisk.

the 27.5- to 30.5-min segment of the *E. coli* chromosome (Lowplasmid). Complementation by the plasmid was consistent with mutations in *trkE* (11), but the inability of the new mutants to grow well on K5 medium distinguished them from the one *trkE* mutant identified earlier. Our analysis showed that all of the new mutants carried two mutations not found in parental strain TK2076 and that their phenotype was dependent on multiple *trk* mutations.

Identification of *trkG*. The locus responsible for the failure of mutants TK2091 and TK2092 to grow on K5 medium was not *trkE*, because we could not recover a *trkE* mutation in transductional crosses with the *trp* and *pyrF* loci, which were cotransduced with *trkE* at frequencies of 17 and 60%, respectively (11) (Fig. 1). Subsequent mapping work was done with mutant TK2092 (*trkG82*). Mating experiments suggested that *trkG* was near but clockwise from *pyrF*. A more precise location was implied by the analysis of pGH27, a plasmid obtained by shotgun cloning of *EcoRI* fragments of *E. coli* DNA into pBR322. This plasmid carries a 13.6-kb insert and complements strains TK2091 and TK2092 to growth on K0.1 (6, 14a). A comparison of the restriction map of this clone with that of the chromosome in the region of termination of DNA replication (8) showed that the cloned fragment was part of the *rac* sequences in this region.

The location of *trkG* in *rac* was confirmed by transductional linkage with three *Tn10* insertions in this region (Fig. 1). Two of these are in *rac*, while a third is adjacent to *rac*. Transductional linkage with the two insertions in *rac* is high, but transductional linkage with the insertion outside *rac* is low.

The identification of a locus in *rac* affecting K⁺ transport was puzzling, because this defective prophage is not present in some strains of *E. coli* K-12, such as AB1157 (18). The latter strain is therefore a natural *trkG* deletion mutant. We constructed DD1192, a derivative of AB1157 carrying the *kdpABC5* and *trkD1* mutations, expecting it to duplicate the phenotype of TK2092. However, DD1192 grew well on K0.1 medium. This result suggested the presence of at least one additional mutation in TK2092 affecting the K⁺ requirement of growth beyond those already identified. To confirm this idea, we introduced *trkG82* into strain TK2076 by cotransduction with the *zda-230::Tn10* and *zda-131::Tn10* insertions but obtained no (0 of 80 in each case) transductants unable to grow on K5 medium. As a control, we performed the same transductional cross with strain TK2654 (a *trkG*⁺ transductant of TK2092); over 50% of the transductants lost the ability to grow on K5 medium and thus reproduced the phenotype of TK2092. Subsequent analysis showed that these strains harbored a second mutation at a locus near minute 87 that is required for the phenotype of TK2092; we call this other locus *trkH*.

Mapping of *trkH*. In an interrupted mating between Hfr Hayes strain CAE153 and TK2092, recombinants able to grow on K1 medium were obtained quite late, after about 90 min, and were linked to *rha*. Sixteen of 19 *thr*⁺ *trkH*⁺ recombinants received the donor *rha*⁺ marker, while 19 of 21 *thr*⁺ *rha*⁺ recombinants were *trkH*⁺. A mating of Hfr strain CFE20 (transfers clockwise beginning at minute 68 [18]) with TK2092 indicated that *trkH* was probably between *ilv* and *rha*. Of 62 *thr*⁺ *leu*⁺ *ilv*::*Tn10* recombinants, 38 were *rha*⁺ and 48 were *trkH*⁺, both of the latter being donor markers. In transductional crosses, no linkage was observed with *ilv* (0 of 240), but there was 3% (11 of 315) cotransduction with *rha* and higher cotransductions with *metE* and with a *Tn10* insertion in this region (Fig. 2). This part of the chromosome is represented on the F14 plasmid and, as

predicted for recessive mutations, the wild-type plasmid complements strain TK2092 to growth on K1 medium.

Identification of *trkE trkG* double mutants. An analysis of strains TK2088, TK2089, and TK2090 showed that all carried *trkE* mutations, since a lesion leading to the inability to grow on K0.1 medium was 60% cotransducible with *pyrF*. However, these strains did not resemble the *trkE* mutant isolated earlier, because these strains required over 10 mM K⁺ to grow rapidly, while a *trkE trkD* mutant required only about 4 mM K⁺ (see below). The presence of a third *trk* mutation, in addition to *trkD* and *trkE*, in these strains was suggested by the finding that the introduction of other *trkE* mutations into strain TK2719 (a *trkE*⁺ *pyrF* derivative of TK2090) by cotransduction with *pyrF* resulted in strains, such as TK2090, unable to grow on K5 medium. A specific clue as to the other mutation was the observation that strain TK2090 was complemented to growth on K0.1 medium by plasmid pGH27 (6). Transduction of TK2090 with P1 lysates of *trkG*⁺ strains carrying either of the two *Tn10* insertions in *rac* (Fig. 1) yielded recombinants with the phenotype associated with *trkE* mutations in a *trkD* background: growth on K4 but not K0.1 medium. The ability of a known *trkG* mutation to reproduce the phenotype of TK2090 in a strain carrying a *trkE* mutation was demonstrated by the introduction of the *trkG82* mutation into strains TK2721 and TK2722 by cotransduction with the *zda-230::Tn10* insertion. Recombinants with a growth phenotype like that of strain TK2090 were recovered with a high frequency. All of the *trkE trkG trkD* mutant strains we examined grew slowly at 5 mM K⁺, something that neither *trkA trkD* nor *trkG trkH trkD* mutants did. Such growth suggested either that all *trkE* mutations are slightly leaky or that this combination of mutations does not completely abolish Trk activity.

Complementation studies of *trk* loci. To determine the number of genes at each locus, we isolated additional mutations at the *trkD*, *trkE*, and *trkH* loci as described in Materials and Methods. While *trkD* mutations are readily isolated in a *trkA* strain, *trkE* or *trkH* mutations are infrequently obtained when a *trkD* strain is used as a parent because over 90% of all mutations that abolish growth at 0.1 mM K⁺ are in *trkA*. To ensure that the selection did not enrich *trkA* mutants, we used strains carrying the multicopy *trkA* plasmid pGH1; strain TK1001(pGH1) was used to obtain *trkE* mutants, while strain TK2685(pGH1) was used to obtain *trkH* mutants.

All 10 *trkD* mutations were tested against each other with the plaque complementation assay and λ *rbs3* phage as described in Materials and Methods; no complementation between any of the mutations was observed. Four of the *trkE* mutations were placed on F123 and tested against each other and four other *trkE* mutations; no complementation was observed. Alleles 82, 89, and 90 at *trkG* were placed on F123 and tested against each other and the *trkG91* mutation; none of these diploids differed in phenotype from TK2092. Four other independent *trkH* mutations were complemented by pJD306, a plasmid with a 1.4-kb insert that complements the *trkH1* mutation. Therefore, each of these loci probably represents a single gene, although few mutations at *trkG* or *trkH* were tested, and the *trkH* mutations could represent more than one gene if each was relatively small. It has been shown that *trkA* represents only a single complementation group (11).

Growth and transport kinetics of *trk* mutants. To gain a clearer picture of the relationship of the *trk* genotype to transport, we characterized the transport of the strains listed in Tables 2 and 3. These include representatives of the new

mutants as well as strains with other combinations of *trk* mutations. In a few cases, we also report the growth K_m , the K^+ concentration required to achieve half the maximal growth rate in media containing high K^+ concentrations. Transport was measured as the initial rate of uptake in K^+ -depleted cells, a convenient assay in which the highest rates are achieved by all saturable systems studied. All of the strains that were able to grow at K^+ concentrations of 1 mM or lower had saturable uptake that fit simple saturable kinetics with linear double-reciprocal plots and so could be described by a K_m and a V_{max} . All of the strains with a growth K_m higher than 12 mM retained only the low rate of uptake that is linearly dependent on the external K^+ concentration, previously characterized in *trkA trkD* mutants, and referred to as the TrkF system (26). *trkE* mutants were unusual in that they fit neither of these categories and had unusual kinetics of uptake.

***trkD* encodes a separate K^+ uptake system.** A severely defective transport phenotype, as indicated by a requirement for more than 15 mM K^+ to attain half the maximal growth rate, could be achieved by at least three combinations of mutations (a *trkA* mutation, *trkE* and *trkG* mutations, or *trkG* and *trkH* mutations) provided that the strain was also *trkD* (Table 2). Transduction to *trkD*⁺ always resulted in a strain with a growth K_m of about 0.7 mM. This result led us to test whether the additional component of transport contributed by *trkD*⁺ was additive with other components. We made this comparison for the four *trkD*⁺ pairs of strains listed in Table 2. All four *trkD* derivatives were severely defective in transport, while the four *trkD*⁺ strains had a saturable component of uptake, with a K_m for K^+ in the range of 0.25 to 0.4 mM and a V_{max} ranging from about 25 to 40 $\mu\text{mol g}^{-1} \text{min}^{-1}$. There was unusually good agreement in the two strains when we did repeated assays on different days, but in other cases the variation in repeated experiments could be as high as 25%. Therefore, all of the data for the *trkD*⁺ strains can be considered identical within experimental error. Our results agree well with those of Bossemeyer et al. (6), who found a K_m for K^+ of 0.37 mM and a V_{max} of 27 $\mu\text{mol g}^{-1} \text{min}^{-1}$ in strain TK1110, identical in *trk* genotype to strain TK2734 in Table 2. We did not attempt to test for the additivity of the *trkD* component in strains in which activity in its absence was high, since it would have been difficult to quantitate a small increase due to the introduction of *trkD*⁺ over the high rate of transport in the *trkD* derivatives. Such a test might have been possible with *trkD*⁺ on a multicopy plasmid, in which case it would mediate uptake, with a K_m for K^+ of 0.1 mM and a V_{max} of 180 $\mu\text{mol g}^{-1} \text{min}^{-1}$, in strains otherwise defective in Trk activity (6).

A *trkD* mutation in a strain wild type for all other *trk* genes did have a discernible effect, as shown by a slightly higher growth K_m (Table 3). This result suggested that the TrkD system contributes in a modest way to increase uptake at low K^+ concentrations when the activity of the TrkA system is insufficient. Previous studies were unable to demonstrate an effect of TrkD on the kinetics of net K^+ uptake in a strain wild type for TrkA (26). As considered in Discussion, net K^+ uptake measurements are not necessarily indicative of the activity of K^+ uptake systems under physiological conditions of growth.

The additivity of the *trkD* component of transport supports the idea that this gene encodes a separate transport system that is independent of mutations at other *trk* loci. Therefore, the analysis described below of the effects of mutations at the other loci was performed with strains

carrying *trkD* mutations, so that the results would not be complicated by the activity of the TrkD system.

Effects of *trkG* and *trkH* mutations. During the isolation and mapping of mutations at the *trkG* and *trkH* loci, we were under the impression that the mutations alone (in the absence of the other mutation) had no effect on the K^+ requirement for growth and therefore presumably did not impair Trk activity. We examined this question in isogenic strains differing only at the locus under study. All of these strains grew with 0.1 mM K^+ , and all had high maximum rates of transport (Table 3), but there were some minor differences. The *trkH* mutant had a K_m for K^+ of 1 mM and a somewhat lower V_{max} , near 340 $\mu\text{mol g}^{-1} \text{min}^{-1}$, while isogenic strains that were either wild type for *trkH* and *trkG* or mutated only in *trkG* had an average K_m of 3.3 mM and an average V_{max} of 440 $\mu\text{mol g}^{-1} \text{min}^{-1}$. We thought that these results could be related to the presence of Tn10 in the latter group of strains, but the spontaneous deletion derivatives of these strains that had lost tetracycline resistance exhibited the same kinetics of transport (Table 3).

Unusual transport kinetics in *trkE* mutants. The kinetics of uptake in *trkE* mutants were abnormal, regardless of whether a *trkH* mutation was present or not. Uptake was linear with time for only a very short period, until cell K^+ rose to about 20% of the steady-state level (Fig. 3). After this short linear phase, uptake progressively slowed down and was still well below the steady-state level of the wild type 20 min after initiation. With other *trk* mutations, as illustrated by data for two other mutants shown in Fig. 3, uptake continued linearly with time until cell K^+ came close to reaching the steady-state level. The kinetics of the initial, linear phase of uptake (Table 3, strains FRAG97 and FRAG98) were only moderately reduced from those of wild-type strains. The long time required before cell K^+ reached normal levels probably accounted in large part for the fact that these mutants required a rather high concentration of K^+ , 2.4 mM, to attain half the maximal rate of growth despite only moderately impaired initial rates of uptake. It should be noted that the dependence of growth on the external K^+ of a *trkE* mutant had a much shallower slope than did that of any other mutant examined (26) (Fig. 4), indicating that even at rather high K^+ concentrations uptake was sufficiently impaired to remain rate limiting for growth. This conclusion is supported by the fact that cells of the *trkE* mutant growing in medium with 10 mM K^+ had a K^+ content of only $436 \pm 6 \mu\text{mol g}^{-1}$; during growth in medium with 115 mM K^+ , their K^+ content ($547 \pm 13 \mu\text{mol g}^{-1}$) was within the normal range for cells with a growth rate not limited by K^+ .

In a *trkE* mutant, a *trkH* mutation slightly enhanced transport, as can be seen by a comparison of strain FRAG98 with strain FRAG97 in Table 3. The V_{max} increased modestly, while the K_m was reduced. There also seemed to be a subtle effect on growth. While the rate of growth at intermediate K^+ concentrations, relative to that at 100 or 115 mM K^+ , was identical in *trkE* and *trkE trkH* mutants, the latter had a doubling time in high- K^+ media that was between 2 and 6 min shorter in four experiments in which the two strains were studied in parallel.

The various combinations of mutations examined in this work showed that the only combinations of *trk* mutations capable of abolishing saturable K^+ uptake in a *kdp trkD* background were those found in the five new mutants (strains TK2088 to TK2092), namely, *trkE* and *trkG* or *trkG* and *trkH*. In addition, mutations in *trkA* alone or in combi-

FIG. 3. Kinetics of net K^+ uptake at 30°C in *trkE* mutants as compared with other *trk* mutants and strains wild type for the TrkA system. Cells were depleted of K^+ by treatment with 2,4-dinitrophenol, and uptake was measured by adding glucose and K^+ as described previously (26). The K^+ concentrations used were 1 mM for strains FRAG91 and FRAG96 and 10 mM for strains FRAG97 and FRAG98. Note that the time scale is expanded for the first 4 min of each curve and that the onset of uptake for strains FRAG97 and FRAG98 has been displaced for clarity. Symbols: $\square$, FRAG96 (*trkH1*); $\circ$, FRAG91 (*trkG92*); $\circ$, FRAG97 (*trkE80*); $\triangle$, FRAG98 (*trkE80 trkH1*).

nation with any of the other mutations abolished saturable uptake.

Alleles with intermediate phenotypes. We have tacitly assumed that the mutations used in the above-described experiments were null alleles or close to null alleles because different mutations seemed to produce the same phenotype. The *trkA405* mutation appeared to be null under the conditions used here, since a *trkA* deletion had the same properties (12a). We did isolate two mutations in *trkA* that resulted in an intermediate phenotype. These two, *trkA427* and *trkA429*, resulted in indistinguishable kinetics of uptake (Table 3) but in different levels of dependence of the growth rate on the K^+ concentration (Fig. 4). The kinetic changes produced by these mutations, an increase in K_m combined with a decrease in V_{max} , suggested that they reduced affinity for K^+ . When each of these *trkA* alleles was tested in a *trkH* or a *trkG* background, the curves relating growth rate to K^+ concentration (data not shown) were the same as those for the *trkA* mutations alone (Fig. 4).

DISCUSSION

The five *trk* loci studied here appear to complete the genetic description of saturable and constitutive systems for K^+ uptake in *E. coli*. We were fortunate to have obtained double mutants, since only through these were we able to identify the *trkG* and *trkH* loci. Although UV light is considered a relatively mild mutagen, it frequently produces strains with multiple mutations, as once again demonstrated

here. Each of the five loci implicated in constitutive K^+ transport appears to represent only a single gene, although this idea has been demonstrated genetically only for *trkA* (11) and *trkE* and *trkD* loci (this study) and confirmed by a DNA sequence analysis of *trkA* (14). We suggest that these five genes together encode a total of three separable systems for the transport of K , systems that we propose to call TrkD, TrkG, and TrkH.

There is now good support for considering the TrkD system (also referred to as Kup [6]) as separate from the others and dependent on the product of only a single gene, *trkD*. We showed that transport due to *trkD*⁺ is additive, within experimental error, to residual transport in strains defective in other saturable uptake systems because of different combinations of *trk* mutations (Table 2). Bossemeyer and colleagues (6, 7) showed that only *trkD*⁺ strains had saturable uptake of Cs^+ and that the rate of uptake of both Cs^+ and K^+ increased when the gene dosage of *trkD* was increased. Not one of these criteria is proof of an independent system, but each one argues against a requirement for other gene products and hence against an interaction with another system. The unique cation substrate specificity of TrkD could represent a specificity-altering effect of a component of a multicomponent system. In the plasmid-encoded oxyanion export system studied by Hsu and Rosen, arsenite efflux requires only the ArsA and ArsB subunits, while arsenate efflux requires, in addition, the ArsC subunit (15). However, TrkD-mediated transport of K^+ (Table 2) or of

FIG. 4. Dependence of the growth rate on the K^+ concentration of the medium for strains defective in K^+ transport. Cells in the logarithmic phase of growth in medium containing 115 mM K^+ at 37°C were centrifuged, washed by centrifugation at room temperature, and transferred to medium with the indicated K^+ concentration at a density of about 10^8 cells per ml. The growth rate at 37°C was determined by periodic measurements of culture turbidity in a Spectronic 20 colorimeter (Bausch and Lomb). The growth rate of all strains at 105 mM K^+ was 58 ± 3 min. Symbols: Δ , TK1001 (*trkA*⁺); $\circ$, GH4027 (*trkA*427); $\blacktriangle$, GH4002 (*trkA*429); $\times$, FRAG97 (*trkE*80); $\square$, TK2205 (*trkA*405).

Cs^+ (7) was unaffected by mutations in any of the other genes studied, indicating that products of other known genes involved in K^+ transport are not required. The dependence of the rate of transport on the gene dosage of *trkD* argues against a requirement for other gene products but does not exclude other gene products if present in excess over the amount of the *trkD* gene product.

The results reported here have forced us to reexamine previous models and led us to propose the existence of two systems, each capable of high rates of transport. These two systems were previously grouped under the single name TrkA. The fact that mutations in *trkG* or *trkH* alone have only small, subtle effects on transport while mutations in *trkG* and *trkH* together abolish transport indicates that each is part of a separate system. We call these systems TrkG and TrkH, after the gene product uniquely required by each. The properties of these and other K^+ transport systems are summarized in Table 4.

The ancestral *E. coli* probably did not carry the *trkG* gene, since this gene is located within the sequence of *rac*, a cryptic lambdaoid prophage was not found in all K-12 strains (18). Analysis of a strain lacking *rac* would reveal only a single system with a high rate of uptake, TrkH, dependent on the *trkA*, *trkE*, and *trkH* genes. Mutations in *trkE* are not

quite equivalent to those in the other two genes, because all *trkE* alleles tested allowed slow growth at 5 mM K^+ . The rate of uptake required for such growth is estimated to be 0.3 to 0.5 $\mu\text{mol g}^{-1} \text{min}^{-1}$, too low to have been detected in our measurements. This trace uptake activity suggests either that all *trkE* mutations isolated are somewhat leaky or that there is a trace of activity in the absence of the *trkE* product. Both the *trkA* and the *trkH* products are absolutely essential for TrkH activity. Uptake mediated by the TrkH system differs little from uptake in a wild-type strain (Table 4): the affinity of TrkH for K^+ is slightly lower while the maximum rate of uptake is comparable whether measured in strains with single copies of each gene or in strains with the *trkH* gene on a multicopy plasmid.

We envision the *trkG* gene as an invader that conferred some advantage to the cell by providing a variant K^+ uptake system with a higher affinity for K^+ (Table 4). Since transport activity in a *trkG*⁺ strain is absolutely dependent on only one other gene, *trkA*, the minimal or "core" TrkG system is a complex of only the *trkG* and the *trkA* products. This core complex, when encoded chromosomally, is rather defective in transport (Table 3 and Fig. 3), but when *trkG* is on a multicopy plasmid, the kinetics of K^+ uptake are much closer to those of the wild type (6). The *trkA* product has

TABLE 4. Properties of saturable constitutive K^+ transport systems in *E. coli*

System	Composition ^a	Kinetics ^b		Unique property
		K_m (mM)	V_{max} ($\mu\text{mol g}^{-1} \text{min}^{-1}$)	
TrkA	A, E, G, and H	1–2	200–600	Composite activity of TrkG and TrkH systems, as in wild-type <i>E. coli</i> K-12
TrkD	D	0.33, 0.37	34, 27	Apparently consists of only a single protein subunit
TrkG (core)	A and G	1.5 ^c , 0.25	130 ^c , 205	Abnormal kinetics—slow to reach steady-state levels
TrkG (complete)	A, E, and G	1, 0.3	240, 230	
TrkH	A, E, and H	3, 2.2	450, 310	Trace activity in the absence of E

^a Products of the *trk* genes are referred to by the unique fourth letter of their genetic designation; i.e., A represents the *trkA* gene product, etc.

^b For TrkD, TrkG, and TrkH, the first value is the average based on the data in Table 3 and the second value is from Bossemeyer et al. (6).

^c These values are initial rates; they apply only to the first minute or so of uptake (Fig. 3).

been demonstrated to be a peripheral membrane protein (6), so that the *trkG* product must form the transmembrane subunit of the system. The DNA sequence of the *trkG* gene supports the inference that it is a membrane protein (3a, 9b). The complete or "holo" TrkG system has a third subunit, the *trkE* product, and with this composition it has a relatively high affinity for K⁺ and a maximum rate of uptake only slightly lower than that of TrkH (Table 4). Since the TrkH system and the complete TrkG system are very similar in function and each has three components, of which the *trkA* and *trkE* products are common, the *trkG* and *trkH* subunits can be considered to have analogous functions. Since we have already concluded that the *trkG* product forms a transmembrane protein, the *trkH* product should do the same. The *trkG* and *trkH* proteins could be homologous, although no homology of the genes at the DNA level was detected by Southern blotting under conditions of moderate stringency (9b).

The model of two systems normally containing *trkA* and *trkE* products as common subunits readily explains the pattern of membrane association of the *trkA* protein reported by Bossemeyer et al. (6). The *trkE* product and either the *trkG* product or the *trkH* product are the minimum requirements in combination with the *trkA* product for the formation of a "normal" system with a high rate of uptake, and these are the conditions required for efficient binding of the *trkA* product to membranes. In the absence of the *trkE* product, there was reduced binding, indicating that this subunit is not essential for binding. However, either the *trkG* product or the *trkH* product must be present; the presence of only the *trkE* product did not lead to membrane binding. This interpretation of the binding data is consistent with our suggestion that the *trkG* and *trkH* products are membrane proteins. The *trkE* protein could be a membrane protein that either forms a complex with the *trkG* or *trkH* protein to jointly form a binding site for the *trkA* protein or acts as an allosteric effector to allow the other subunit to bind the *trkA* protein with a high affinity. These functions do not require that the *trkE* protein be in the membrane; it could be a peripheral protein, as is the *trkA* protein. In the absence of the *trkE* protein, a binding site is present, but it has a relatively low affinity for the *trkA* protein.

The binding of the *trkA* product to other components of uptake systems can explain the finding that the *trkH* product reduced the rate of transport in a *trkE* mutant. We found that a *trkH* mutation increased the maximum rate of transport in a *trkE* mutant (Table 3 and Fig. 3); Bossemeyer et al. (6) observed reduced transport when extra copies of *trkH* were provided to a *trkE* mutant. This effect suggests that the *trkH* product is sequestering some rate-limiting component of the system. This component is most likely the *trkA* protein, since it is believed to be expressed at a rather low rate and can bind to membranes in the absence of the *trkE* protein (6). An increase in binding was not discernible from qualitative estimates of binding in a strain with a multicopy *trkH* plasmid, but if binding is weak it may be hard to measure.

The TrkG and TrkH systems appear to act independently, although when both are present, as in a wild-type strain, the total activity is less than the sum of each activity alone (Table 3). The lack of additivity does not appear to be due to limited amounts of the two common subunits, the products of the *trkA* and *trkE* genes, since strains diploid for each of these do not have an increased $V_{\max}$ for transport (14a, 26). More likely it is the export of protons and/or Na⁺ from the cell, needed to maintain charge balance during K⁺ uptake, that is limiting, since rates of K⁺ uptake are very high.

We chose to measure the kinetics of uptake in depleted cells because this method is both convenient and provides a measure of the maximum rate of transport. However, these conditions are not representative of those that apply during growth. Cells treated with 2,4-dinitrophenol have a low internal pH and a high Na⁺ content because the K⁺ lost is replaced mainly by Na⁺ and protons (26). During growth, cells take up K⁺ at a low rate, sufficient to maintain this ion at a constant concentration, into the cytoplasm, which has a very low Na⁺ content and a pH in the range of 7.6 to 7.9 (9, 23, 26, 28). Another difference is in turgor pressure, high during growth and presumably much reduced in depleted cells. Reduced turgor is well documented as a stimulus for K⁺ uptake (29), and changes in the internal pH and Na⁺ content may also be important factors in *E. coli*. In view of these differences in cell conditions, it should be no surprise that the kinetic data do not always correlate well with the K⁺ requirements for growth. In the presence of TrkG or TrkH or both, we never detected an effect of the TrkD system on the kinetics of K⁺ uptake into depleted cells (26), but the presence of TrkD was associated with a small but consistent reduction of the K⁺ requirements for growth (compare strains FRAG91, FRAG93, and FRAG94 with strain FRAG5 in Table 3). We presume that the TrkD system makes a relatively larger contribution to K⁺ uptake under conditions of growth than it does in depleted cells. There is also a poor correlation between the kinetic parameters as measured here and the transport requirements of growth. Rapidly growing cells have a K⁺ content of about 570 $\mu\text{mol g}^{-1}$ and a doubling time at 30°C of about 80 min. The rate of net K⁺ uptake required is about 5 $\mu\text{mol g}^{-1} \text{ min}^{-1}$, far below the $V_{\max}$ of any of the systems studied here, so that rapid growth ought to be achieved at K⁺ concentrations ranging from 2 to 20% of the K_m values shown in Table 3. However, the growth K_m is much higher than these values. The $V_{\max}$ under conditions of growth is probably much lower, and the K_m could also be rather different.

The unusual kinetics of uptake in the *trkE* mutant (Fig. 3) can be explained as a failure of the core TrkG system to respond appropriately to the needs of the cell. Initially, uptake is relatively fast, with a $V_{\max}$ about one-third that of the complete TrkG system, but this rate inflects rather abruptly when internal K⁺ reaches about one-fifth the level found in K⁺-replete wild-type cells (540 to 600 $\mu\text{mol g}^{-1} \text{ min}^{-1}$). Thereafter, uptake proceeds at a progressively slower rate. At K⁺ concentrations as high as 10 mM, well above the K_m for K⁺ of uptake during the initial, rapid phase (Table 3), internal K⁺ never achieves the levels found in wild-type cells but remains at about 80% those levels (Fig. 3 and Results), and we presume that this level does not allow normal rates of growth because turgor pressure remains low. One suggestion for the abnormal kinetics of the core TrkG system is that it requires both low turgor and a low internal pH for high activity and that the restoration of the internal pH occurs well before normal cell K⁺ pools are restored.

The two unusual *trkA* alleles studied here imply that this protein can alter the apparent affinity of TrkG and TrkH for K⁺. Since the K⁺-binding site is presumably on the externally facing surface of a membrane protein, the effect of mutations altering a cytoplasmic component indicates that the binding of the TrkA protein to the membrane proteins alters their conformation. There is an example of such an effect in the histidine transport system of *Salmonella typhimurium*. The *hisJ5625* mutation alters the periplasmic histidine-binding protein so that it still binds histidine but can no longer mediate transport. This defect can be suppressed by a

mutation in *hisP*, the gene for one of the three peptides of the membrane component of histidine transport (2). However, the *hisP* product is the ATP-binding peripheral membrane protein of the system, located at the inner face of the cytoplasmic membrane (1). Presumably, the altered *hisP* product can change the conformation of the membrane complex so that its periplasmic surface can interact productively with the *hisJ5625* mutant protein.

trkA427 and *trkA429* illustrate the discrepancy between the kinetics of uptake in K^+ -depleted cells and growth conditions. While the K^+ uptake kinetics are indistinguishable (Table 3), the K^+ requirements for growth, 1.5 and 8 mM, respectively, are quite different. The *trkA427* allele results in a biphasic growth curve (Fig. 4), suggesting that the V_{max} under growth conditions is relatively low and results in a plateau rate of growth over the range of about 5 to 20 mM K^+ . At high K^+ concentrations, the growth rate increases again over the range in which the TrkF system is effective (as illustrated in Fig. 4 by strain TK2205, which lacks all saturable systems). The TrkF system mediates uptake that appears to be linearly dependent on external K^+ and is unaffected by any *trk* mutations (26). As reported in Results, each *trkA* mutation seems to have the same effect when either the TrkG system or the TrkH system is present in the absence of the other, since the growth curves resulting from these mutations are indistinguishable from each other and from those of strains wild type for the *trkE*, *trkG*, and *trkH* genes (strains GH4002 and GH4027, respectively). This result suggests that the role of the *trkA* product in the TrkG system is very similar to its role in the TrkH system.

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