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Tetracycline Resistance Element of pBR322 Mediates Potassium Transport

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The tetracycline resistance element of plasmid pBR322 partially complements the potassium transport defect of *Escherichia coli* K-12 mutants having markedly impaired K^+ transport. The plasmid increases K^+ transport. The Tn*10* element does not result in increased transport, demonstrating that the effect is not general for elements that increase resistance to tetracycline.

The ease of selection for antibiotic resistance elements has made such elements useful in the manipulation of genes. Antibiotic resistance is generally an additional, selectable trait, independent of and without effect on the traits and genes under study. We identified a situation in which a resistance element interferes with the analysis of chromosomal genes of *Escherichia coli*; the tetracycline element of pBR322 partially complements K^+ transport mutations.

Wild-type *E. coli* has two transport systems for the accumulation of K^+, the high-affinity, repressible Kdp system and the moderate-affinity, constitutive Trk system (3). In strain TK2205 (F^- *thi lacZ rha nagA kdpABC5 trkA405 trkD1*), Kdp function is abolished by a deletion and Trk transport is markedly impaired by two mutations. Potassium transport in this strain is so diminished that a K^+ concentration of ca. 25 mM is required to achieve a half-maximal growth rate in standard minimal media (Fig. 1; see reference 11). Wild-type strains grow well when no K^+ is added to minimal media (11). Strain TK2205 carrying pBR322 required 8 mM K^+ to achieve a half-maximal growth rate. Plasmids from which pBR322 is derived, pMB9 and pSC101, had a similar effect on growth, except that the effect of pSC101 was seen only in the presence of tetracycline (Fig. 1). Addition of tetracycline had no effect on the K^+ requirements of pBR322- or pMB9-bearing strains. Both pBR322 and pMB9 express tetracycline resistance constitutively, whereas resistance by pSC101 is inducible by the addition of the antibiotic (13). The correlation between the effect on K^+ requirements for growth and the regulation of tetracycline resistance of these plasmids suggests that the effect is caused by the tetracycline resistance elements of the plasmids. This conclusion was supported by the finding that derivatives of pBR322 having deletions of the tetracycline region from the *Eco*RI site to the *Sal*I site of the plasmid had no effect on K^+ requirement for growth, whereas plasmids having other defects (deletion of the beta-lactamase gene from the *Eco*RI to *Pst*I site or deletions of presumably silent regions from the *Ava*I to *Pvu*II site) produced an effect on growth identical to that of pBR322.

The growth effect shown in Fig. 1 for the pBR322-bearing strain was not exhibited by strains carrying Tn*10*, another tetracycline resistance element. Whether grown in the presence of tetracycline to induce resistance (6) or in the absence of the drug, the growth curves of strain TK2205 *srl*::Tn*10* were identical, within experimental error, to those of the strain without the insertion (Fig. 1).

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To examine the quantitative correlation between the extent of expression of tetracycline resistance and the effect on growth, the strength of the tetracycline promoter of pBR322 was altered. The promoter region was cut with the restriction endonuclease *Hin*dIII, and the staggered ends were blunted with the large (Klenow) fragment of *E. coli* DNA polymerase I and ligated. A contaminating nuclease produced aberrant plasmids containing small deletions in this region, as demonstrated by restriction analysis. Three such plasmids were no longer cut with *Hin*dIII, *Cla*I, or *Eco*RI, and the 206-base-pair *Hha*I fragment extending from residue 4260 to 103 (9, 12) was reduced in length by 30 to 120 base pairs. These plasmids were found to confer a moderately reduced resistance to tetracycline (Fig. 2), suggesting the expression of the tetracycline gene had been altered. Potassium requirement for growth (Fig. 2) was not significantly changed by the plasmid conferring a tetracycline resistance level of 25 μg/ml, a level of resistance much higher than that of plasmid-free strains. Thus, a high level of resistance was required for a noticeable effect on growth. The plasmids giving resistance to 50 μg and 75 μg of tetracycline per ml decreased the K^+ requirement in proportion to the drug resistance level. The correlation between the resistance level and the effect on growth supports the assertion that the tetracycline transport protein alters the K^+ requirement for growth.

The inability of strain TK2205 to grow at low K^+ concentrations has been inferred to be due to an inadequate rate of K^+ transport. The shift in growth curves on introduction of the pBR322 tetracycline resistance element implies an increase in the rate of K^+ uptake. Uptake in the strain without a plasmid showed the linear dependence on external K^+ concentration typical of this class of mutants (Fig. 3; see reference 11). In the presence of pBR322, transport was higher and no longer linearly dependent on K^+ concentration. The curve can be interpreted as the sum of the linear process seen in the absence of the plasmid and a saturable component with a K_m in the vicinity of 2 mM and a V_{max} of 1.2 μmol g^{-1} min^{-1}. The kinetic parameters were obtained by fitting a simple saturable function to the difference between the two curves of Fig. 3. The saturable component is presumably due to the tetracycline resistance element. The rate of K^+ transport associated with pBR322 is very low and is apparent only in a strain in which cellular K^+ transport ability is markedly reduced. In wild-type strains, K^+ uptake under similar conditions has a V_{max} of 550 μmol g^{-1} min^{-1} and a K_m of 1.5 mM (11).

The mechanism for tetracycline resistance is an active

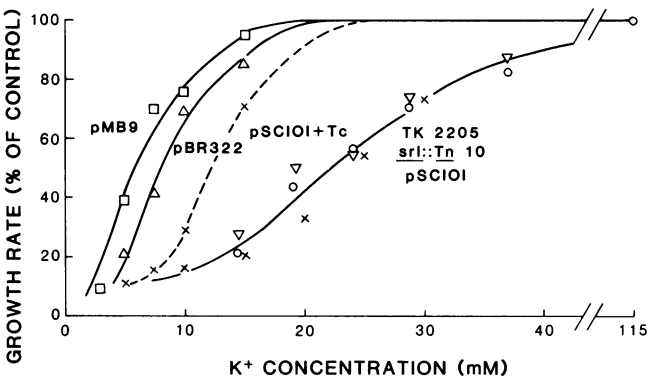

FIG. 1. Effect of tetracycline resistance elements on K^+ dependence of growth rate in TK2205. Cells were grown in glucose-minimal media (2) containing 115 mM K^+ and shifted to glucose-minimal media of the indicated K^+ concentration to a density of 10^8 cells per ml. Growth was monitored by periodic measurements of turbidity with a Bausch & Lomb Spectronic 20 colorimeter and is expressed as a percent of the growth rate at 115 mM K^+. The doubling time for all strains at 115 mM K^+ was 60 ± 3 min. The dashed curve indicates an experiment performed in the presence of 4 μg of tetracycline per ml. Resistance elements present: None (○); pMB9 (□); pBR322 (△), pSC101 (×), *srl*::Tn*10* (▽). The curve for the *srl*::Tn*10* strain in the presence of tetracycline was identical to that in the absence of the drug and is not indicated separately.

expulsion of the antibiotic (8). The evidence presented here suggests that the tetracycline resistance element of pBR322 mediates K^+ transport as well. We examined the level of tetracycline resistance as a function of K^+ concentration to determine whether there is any coupling between K^+ transport and tetracycline efflux mediated by the tetracycline element. In strain FRAG-1, a wild-type strain for K^+ transport, resistance varies marginally over the range 0.1 to 115 mM K^+. Because K^+ transport by the tetracycline element would be expected to be minimal at 0.1 mM K^+, the result implies that extrusion of tetracycline is not dependent on concomitant uptake of K^+ via the tetracycline resistance complex. Potassium has a paradoxical effect on tetracycline resistance in strain TK2205, with comparable levels of resistance at high and low K^+ concentrations and a two-fold increase in MIC at 10 mM K^+ at which growth rate is somewhat suboptimal (Fig. 1). A direct effect of K^+ on resistance is not totally excluded, but it is more probable that a variety of factors contribute to the complex effect of K^+ on resistance in strain TK2205.

Potassium transport is yet another transport phenomenon associated with the pBR322 tetracycline resistance element. Other examples were active extrusion of tetracycline (8), and increased sensitivity to, implying increased transport of, a variety of organic compounds (1, 7) and cadmium (4). We suggest that the transport system encoded by this tetracycline resistance element can mediate transport of these diverse substances as well as couple energy to the extrusion of tetracycline and the uptake of K^+.

Of the toxic organic substances to which tetracycline-resistant bacteria are selectively sensitive, the most effective are lipophilic acids (1). Such organic acids have been used to measure the membrane pH gradient in *E. coli*; the extent of acid accumulation increases progressively as the external pH falls below that in the cell (10). The organic acids which have an increased toxicity toward tetracycline-resistant bacteria are most effective when used at a low external pH. This argues that the mechanism of toxicity of these compounds may involve uptake. Chelation of membrane cations by these organic acids, as has been suggested (1), seems unlikely to be the reason for toxicity, since these acids would be less effective chelators at a lower pH.

As demonstrated in this report, neither K^+ nor tetracycline is necessary for the transmembrane movement of the other substrate, although some effect of K^+ on tetracycline resistance extrusion is possible. The transport kinetics are interpreted as the sum of two processes, facilitated diffusion by the residual K^+ transport system which is characterized by a linear dependence on external K^+ concentration (11) and a saturable component of transport due to an independent path for K^+ uptake provided by the tetracycline resistance transport protein. We think this is a simpler model than to suppose that the kinetics of Fig. 3 are due to an interaction of the tetracycline resistance protein of pBR322 with the residual K^+ transport system of strain TK2205, although the possibility of such an interaction cannot be eliminated from this data.

The fact that the Tn*10* element is not associated with increased K^+ transport is surprising. The MIC of strain TK2205 *srl*::Tn*10* to tetracycline is 300 μg/ml at 115 mM K^+ (data not shown) and is comparable to that of strain TK2205 carrying the pBR322 plasmid (Table 1). Tetracycline transport rates, shown to be proportional to resistance levels for these types of resistance elements in inverted vesicles (8), should be comparable in TK2205 carrying either the Tn*10* element or the pBR322 plasmid. This indicates that the absence of K^+ transport in the transposon-bearing strain is the result of differences between the transposon and the

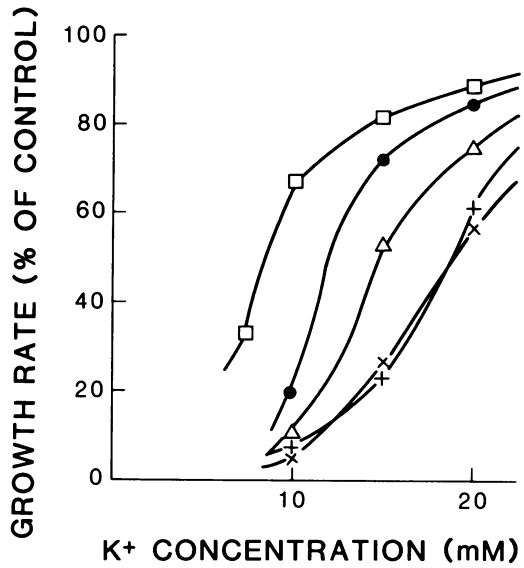

FIG. 2. Potassium dependence of growth of strain TK2205 carrying derivatives of pBR322 with altered tetracycline promoters. Plasmids were obtained as described in text. Growth data were obtained as described in the legend to Fig. 1. The MIC for tetracycline was used to indicate the degree of expression of tetracycline resistance. The MIC is the lowest concentration of antibiotic, increased by step-wise increments of 1.4-fold in concentration, which prevents visible growth after incubation for 35 doublings at 37°C in glucose-minimal media containing 115 mM K^+ inoculated with 10^4 cells per ml. Symbols (MICs): □, pBR322 (100 μg/ml); ●, pBR322Δ1 (75 μg/ml); △, pBR322Δ2 (50 μg/ml); +, pBR322Δ3 (25 μg/ml); ×, no plasmid (0.5 μg/ml).

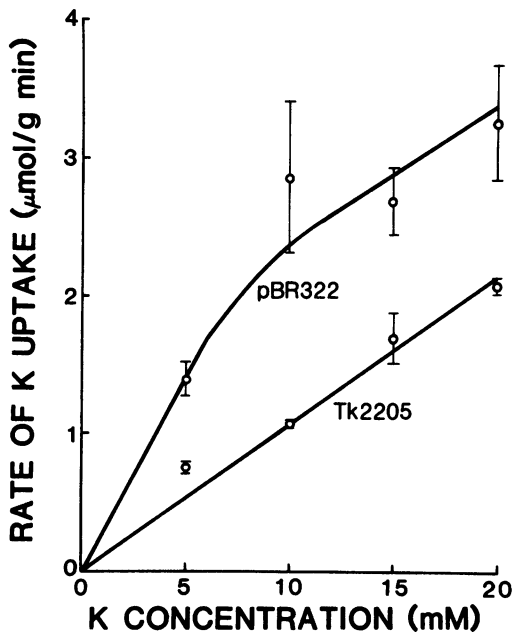

FIG. 3. Effect of pBR322 on kinetics of net K^+ uptake in strain TK2205. The initial rate of K^+ uptake was measured as previously described (11). Cells were depleted of K^+ by treatment with 2,4-dinitrophenol and allowed to equilibrate at 37°C, the temperature of subsequent uptake reactions, in K^+-free, NH_4^+-free buffer containing 0.2% glucose and 50 μg of chloramphenicol per ml for 30 min. Uptake reactions were initiated by the addition of K^+ to the appropriate concentration. At intervals, samples were collected on membrane filters and washed, and K^+ was determined by flame-emission photometry. Initial rates of uptake, expressed per gram of dry weight, were determined in three experiments; the data are means with standard deviations.

pBR322 elements and not due to an absence of transport systems. The homologous proteins from the pBR322 and the Tn*10* tetracycline resistance elements are ca. 45% identical at the amino acid sequence level (5). However, there are regions dispersed throughout the sequence in which nonconservative changes are evident, and two lengths of protein sequence, residues 170 to 220 and 20 residues at the carboxy terminus, are not homologous at all. The observations of this report that only the pBR322 tetracycline element confers K^+ transport capabilities suggests that these nonhomologous regions are important in determining the ability of the transport protein to affect increased K^+ transport.

TABLE 1. Effect of K^+ on MIC for tetracycline

K^+ (mM)	MIC (μg/ml)	
	TK2205(pBR322)	FRAG-1(pBR322)
0.1		200
5	200	
10	400	200
20	200	
40	200	
115	140	140

The ability of the tetracycline extrusion system associated with pBR322 to transport a variety of compounds with little or no similarity to tetracycline is a simple explanation for the diverse effects of this plasmid. The low substrate specificity predicts that other compounds may be able to use this pathway to enter bacteria.

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