Regulation of kdp Operon Expression in Escherichia coli: Evidence against Turgor as Signal for Transcriptional Control

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Kdp, an inducible high-affinity K⁺ transporter in Escherichia coli, is encoded by genes of the kdpABC operon, and its expression is regulated by the products of kdpD and kdpE. Loss of cell turgor has been proposed to be the signal which induces kdp expression (L. A. Laimins, D. B. Rhoads, and W. Epstein, Proc. Natl. Acad. Sci. USA 78:464–468, 1981). We reexamined kdp expression during steady-state growth under a variety of conditions and were able to confirm earlier observations which had indicated that it is primarily affected by the concentration of K⁺ in the medium and by mutations in genes encoding various K⁺ transporters in E. coli. Changes in pH of the culture also altered kdp expression. In all of these cases, an increase in [K⁺] of the medium repressed the operon. Several ionic solutes induced steady-state kdp expression (but to differing extents), whereas nonionic solutes had no effect, indicating that kdp expression is not determined by osmolarity of the growth medium. kdp expression during steady-state growth was shown also to be unaffected by the accumulation of other intracellular compatible solutes such as trehalose or glycine betaine, which would be expected to restore cell turgor during growth in high-osmolarity media. Two mutants that are defective in perception of the signal regulating kdp were isolated, and the mutation in each of them was mapped to the kdpDE regulatory locus. Analysis of kdp expression in one of these mutants provided additional evidence against the turgor regulation model. On the basis of these data, we discuss alternative candidates that might serve as the signal for control of kdp operon transcription.

Potassium is the major cation present within all cells, including bacteria such as Escherichia coli. The intracellular K⁺ concentration ([K⁺]ₐ) in E. coli under normal growth conditions is around 150 mM, and this level is believed to be required for the optimal activity of many cellular enzymes, including those engaged in protein synthesis, for the regulation of intracellular pH, for DNA-protein interactions, and for controlled dissipation of the proton motive force (4, 12). In addition, [K⁺]ₐ has been shown by several workers to increase progressively with increase in osmoticity of the growth medium and is therefore thought to be an important determinant of cell turgor (6, 7, 12, 15, 44).

To cope with environments that vary widely in their concentrations of extracellular K⁺ ([K⁺]ₑ), E. coli uses many transport systems with differing affinities for K⁺. The best characterized among them is Kdp, an inducible high-affinity transporter encoded by genes of the kdpABC operon (13, 38). The expression of kdp is induced by low [K⁺]ₑ and is repressed by excess [K⁺]ₑ. kdp expression is subjected to positive regulation by the products of two genes, kdpD and kdpE, which are organized as an operon immediately downstream of, and transcribed in the same direction as, the kdpABC structural genes (35, 38). Analysis of the nucleotide sequences of kdpD and kdpE had suggested that they constitute the sensor and response elements, respectively, of a two-component control system (47), similar to that described for other operons in E. coli (41). More recently, biochemical evidence supporting the view that KdpD is a sensory protein kinase exhibiting autophosphorylation and KdpE phosphotransfer characteristics and that KdpE binds to DNA immediately upstream of the promoter for the kdpABC structural genes, in a region which is necessary in cis for normal regulation of the operon, has also been obtained (30, 31, 43). The deduced amino acid sequence of KdpD also suggests that it is a transmembrane protein, and cell fractionation studies indicate that it is resident in the cytoplasmic membrane (31, 47).

Under conditions in which [K⁺]ₑ is not limiting and the Kdp transporter genes are repressed, E. coli uses three major constitutively expressed, low-affinity K⁺ uptake systems: TrkG, TrkH (both together formerly called TrkA, which is the designation used in this report), and TrkD (5, 11, 14, 39). Mutations in trkD abolish the function of the TrkD porter, whereas mutations in trkA render defective the function of TrkA (that is, both TrkG and TrkH). In mutants defective in one or more Trk systems, kdp is expressed at intermediate [K⁺]ₑ, as well (17, 23, 39). The transport activity of each of the K⁺ transporters, Kdp, TrkD, and TrkA, has been shown to be increased by an increase in osmoticity of the assay medium (10, 27, 37).

The environmental factors governing regulation of the kdp operon were first studied by Laimins et al. (23), using kdp-lac operon fusion strains. Their investigations revealed that during steady-state growth, kdp induction for each strain occurs at that [K⁺]ₑ at which the growth rate decreases because of K⁺ limitation. They also showed that the [K⁺]ₑ at which kdp was induced during steady-state growth was dependent on osmolarity of the medium; kdp-lac expression occurred at a higher [K⁺]ₑ in cultures grown at high osmolarity than in those grown at low osmolarity. In another experiment, they showed that upon addition of ionic or nonionic impermeable solutes to cultures grown at a [K⁺]ₑ sufficient to repress kdp, an instantaneous transient increase in transcription of kdp occurred. Glycerol, which is freely permeable across the cell membrane, did not cause this transient induction. From these results, Laimins et al. (23) proposed that turgor pressure is the signal for regulation of kdp expression and that growth in K⁺-limiting conditions imposes a turgor stress on the cells. That kdp transcription

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increases transiently upon instantaneous loss of impermeable solutes has also been confirmed recently by Sugiyama et al. (43).

Other work, reported both from our laboratory (17) and by Sutherland et al. (44), showed that whereas ionic solutes were able to induce steady-state kdp-lac expression in a range of [K+]o tested, nonionic solutes failed to do so. This result appeared to be inconsistent with the turgor regulation model of kdp expression (8, 9, 18), and we therefore decided to reexamine in detail the mechanism of kdp regulation. Toward this end, we have used kdp-lac operon fusion strains to analyze kdp expression under a variety of conditions and in different genetic backgrounds. The results of this study provide evidence against the hypothesis that turgor is the signal controlling kdp transcription.

MATERIALS AND METHODS

Chemicals and media. All antibiotics, medium constituents, and chemicals were obtained from commercial sources and were at least of reagent grade. Unless otherwise specified, the nutrient medium used was LB (29), and the defined growth medium was minimal A (29) with glucose as the C source. The nutrient medium was used for growth of all kdp-lac strains was 30°C. Any sugar as a C source was used at a final concentration of 10 mM. Stock solutions of sugars used as osmolytes were filter sterilized before use. Three other categories of media were used in this study.

(i) K medium (17) contained the following per liter: KH₂PO₄, 1 mM; (NH₄)₂SO₄, 1.5 mM; MgCl₂, 0.08 mM; FeSO₄, 0.5 mg; Casamino Acids (Difco), 5 g; and thiamine, 2 mg (pH adjusted to 7.0 with free Tris base). The contribution of Casamino Acids to [K+]o was about 350 μM. In Na-substituted K medium, NaH₂PO₄ (1 mM) was substituted for KH₂PO₄. Lactose-K medium was similar to K medium but had lactose instead of Casamino Acids as the C source.

(ii) Medium MM (6, 7) contained morpholinopropanesulfonic acid (MOPS), 40 mM; tricine, 4 mM; NH₄Cl, 9.52 mM; KH₂PO₄, 1.32 mM; MgCl₂, 0.523 mM; Na₂SO₄, 0.276 mM; FeSO₄, 0.1 mM; and trace micronutrients (pH adjusted to 7.0 with NaOH). Glucose was used as the C source.

(iii) Phosphate-buffered media with reciprocally varying concentrations of Na⁺ and K⁺ were prepared as described earlier by Epstein’s laboratory (14) by mixing together suitable proportions of 115 mM K⁺-phosphate medium (K115Na0) and 115 mM Na⁺-phosphate medium (K0Na115), each containing, as appropriate, (K or Na₂) HPO₄, 46 mM; (K or Na)H₂PO₄, 23 mM; (NH₄)₂SO₄, 8 mM; MgSO₄, 0.4 mM; FeSO₄, 6 μM; sodium citrate, 1 mM; thiamine hydrochloride, 1 mg/liter; and glucose or other carbon source. According to the nomenclature used in this report, K0Na55 medium, for example, refers to a mixture of the two media in a 60:40 ratio.

Antibiotics were used at the following final concentrations (micrograms per milliliter): ampicillin, 50; chloramphenicol, 50; kanamycin, 15 in low-osmolarity medium and 50 in other media; and tetracycline, 15 and 5 in rich and minimal media, respectively. The indicator dye 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) was used in plates at a final concentration of 25 μg/ml.

Bacterial strains, phages, and plasmids. All bacterial strains were derivatives of E. coli K-12 and are listed in Table 1. Phase P1kc was from our laboratory collection. Phages λMu507 (26), λplacMu55 (26), and Λ1105 (48) have been described earlier. Λ1105 is a vector for achieving high-frequency transposition of an otherwise transposition-defective Tn10-derived insertion element encoding Kanr (48), which is referred to below as Tn10Kan.

Plasmid pJG1, encoding Tetr Ampr, is a PBR322 derivative with a 6.3-kb EcoRI-HindIII fragment bearing the trkD gene as well as the rbsA gene (5) and was obtained from J. Beckwith. Plasmid pHYD94 (19), encoding Cm⁺, was used in some conjugation experiments to provide a convenient counterselectable marker in the recipient strain. ApλlacMu55(Kan) mutagenesis. One new lac operon fusion in each of the two operons, kdp and proU, was obtained during the course of this work as follows. The recA lacZ strain GJ602 was cotransformed with the lac fusion phage λplacMu55 and the helper phage λP5M07, and clones with random transposition of and lysogenization by the former phage were selected as Kan' colonies as described by May et al. (26).

From among 2,000 Kan' colonies screened, one strain, GJ602.1, was identified as a kdp-lac fusion derivative on the basis of (i) inducibility of lac expression in the strain by NaCl, (ii) its repressibility by K+, and (iii) its map position (data not shown). The mutation in this strain was designated kdp-204::λplacMu55(Kan).

Another strain, GJ602.2, was identified in the same experiment as a proU::lac derivative, because (i) it displayed an osmoreponsive Lac⁺ phenotype identical to that of strains with other proU::lac alleles characterized earlier (17), and (ii) the lac fusion concerned was shown in P1 transduction experiments to map to the proU locus at 58 min (data not shown). This allele was designated proU610::λplacMu55(Kan).

The kdp-204::lac and proU610::lac mutations were subsequently transferred from GJ602.1 and GJ602.2, respectively, into other strain backgrounds by P1kc transduction, using Kan' as a marker for selection.

Transpositions of Tn10Kan. Random transpositions of Tn10Kan into the chromosome were generated following infection of strains with Λ1105 as described previously (48).

Isolation of mutants altered in kdp regulation. A strain with the kdp-205 mutation and another with the kdp-207 mutation were both isolated as explained below in a single experiment whose original aim had been to obtain transposon-insertion-generated mutants altered in the expression of kdp-lac. Random insertions of Tn10Kan were generated in GJ18 by infection with Λ1105, and a double selection was imposed on the population of infected cells by plating for colonies on kanamycin-supplemented lactose-K minimal medium. (GJ18 has sufficiently low β-galactosidase activity in K medium that it is unable to grow on lactose as the sole C source under these conditions [17]). Further studies with two of the mutants obtained by this procedure showed that in either instance, the mutation conferring increased lac expression was not linked to the Kan' phenotype but was instead closely linked in P1kc transduction to the kdp-lac(Ap) allele of GJ18 itself. The mutations were designated kdp-205 and kdp-207; for further characterization, isogenic derivatives of MC4100 carrying each of these two alleles along with kdp-lac(Ap) were constructed by P1kc transduction (with selection for Amp') and were named GJ618 and GJ619, respectively.

Transduction and conjugation. The methods for P1kc transduction and conjugation were as described earlier (17, 29).

β-Galactosidase assays. The specific activity of β-galactosidase in cultures was measured, after treatment with chloroform and sodium dodecyl sulfate, by the method of Miller
TABLE 1. Strains of E. coli K-12

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype*</th>
<th>Derivation or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC4100</td>
<td>Δ(argF-lac)U169 rpsL150 relA1 araD139 fliB5301-deoC1 ptsF25</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>TL1105A</td>
<td>lac thi nagA rha trkA405 trkD1 kdpA5-lac</td>
<td>23</td>
</tr>
<tr>
<td>RJ70</td>
<td>MC4100 galpF::Tnl0</td>
<td>45</td>
</tr>
<tr>
<td>UE60</td>
<td>MC4100 otsA::Tnl0</td>
<td>3</td>
</tr>
<tr>
<td>GJ18</td>
<td>MC4100 kdp-200::Xadac(Ap)</td>
<td>17</td>
</tr>
<tr>
<td>GJ602</td>
<td>lac+ lacZ ΔM15 rspsL150 relA1 araD139 fliB5301-deoC1 ptsF25 recA rrl::Tnl0</td>
<td>From MC4100 by Plk transduction in two steps</td>
</tr>
</tbody>
</table>

* Genotype designations are those described by Bachmann (1). Allele numbers have been given where they are known. With the exception of GJ636, all strains listed are F−. The allele kdp-200::Xadac(Ap) represents a lac fusion into the kdpABC operon and was derived as a stabilized version of a MudI(lac Ap) insertion in kdp (17). The trkD204 mutation has also been referred to as sta-1 in the text.

(29), and the values are expressed in the units defined by Miller.

RESULTS

Effects of [K+]s and pH on steady-state kdp expression. The expression of kdp during steady-state growth was measured as a function of [K+]s in two kdp-lac operon fusion strains, GJ610 (trkA4 trkD2) and TL1105A (trkA trkD). Consistent with earlier reports (17, 23, 44), kdp-lac expression in the former increased with progressive K+ limitation, and such induction was further enhanced in the trkA trkD mutant, TL1105A (Fig. 1).

We examined the effects of addition of various solutes on steady-state kdp-lac expression in cells growing in K medium. In the course of experiments that made use of fermentable sugars as solutes, we found that pH itself was a factor affecting kdp expression. For example, at [K+]s < 10 mM, kdp expression in TL1105A was induced approximately 20-fold in the presence even of 0.2 M glycerol (a permeable solute) compared with that in its absence (data not shown). The pHs of the culture media at the time of assay were 7.0 and 7.7, respectively, for cultures growing exponentially in the presence and absence of glycerol. kdp induction by glycerol did not occur in the galF::Tnl0 strain GJ647, which cannot utilize glycerol (45) and in which the pH change associated with growth was not different between the glycerol-supplemented and -unsupplemented cultures (data not shown). Induction of the medium, in the presence of the medium, was also obtained in TL1105A when we used glucose, mannitol, or autoclaved sucrose as a solute added to the growth medium; some of the sucrose was presumably hydrolyzed to glucose and fructose during the autoclaving process (15).

These findings led us to systematically examine the effect of pH on kdp induction at a variety of [K+]s and in strains

FIG. 1. β-Galactosidase specific activities in kdp-lac strains as a function of pH and of solutes added to the growth medium. GJ610 (trkA4 trkD2; closed symbols) and TL1105A (trkA trkD; open symbols) were grown to mid-log phase in media containing defined concentrations of KCl added to Na-substituted K medium and K medium, respectively, and that were unsupplemented (●) or supplemented with 0.4 M sucrose (□) or 0.25 M NaCl (△).
was also studied. In the growth media of varying [K\(^+\)]\(_e\), in the presence and absence of isoosmotic concentrations of sucrose (0.4 M) or NaCl (0.25 M). NaCl supplementation was associated with a marked induction of kdp expression during steady-state growth, whereas sucrose did not have any significant effect (Fig. 1), confirming earlier observations made by one of us (17) and by Sutherland et al. (44).

We also tested whether the addition of different ionic solutes induced steady-state kdp-lac expression in G1610. Many positively charged ionic species (added to a final concentration of 0.2 M in the K medium used for growth) induced kdp during steady-state growth but to different degrees; compared with the basal value of 20 U in K medium, the specific activity of β-galactosidase was highest with Na\(^+\) or NH\(_4\)\(^+\) supplementation (around 315 U), followed by Tris, arginine (at pH 7), and Mg\(^+\), which gave, respectively, 242, 200, and 110 U. In all of these instances, induction did not occur in media with high [K\(^+\)]\(_e\). Lysine (at pH 7; 26 U) did not induce kdp expression in K medium, whereas Rb\(^+\) (4 U) and, as expected, K\(^+\) itself (3 U) repressed it. The different extents to which the osmolar concentrations of ionic and nonionic solutes induced kdp expression during steady-state growth suggest that their effects on kdp induction may not depend on the osmotic properties of the solutes. The repressing effect of Rb\(^+\) is also consistent with its reported ability to substitute for K\(^+\), both for growth (24) and as a substrate for the various K\(^+\) transporters in E. coli (5).

To test the effect of nonionic solutes on instantaneous induction of kdp, sucrose or D-arabinose (each dissolved in K60Na55 medium) was added, to a final concentration of 0.25 M, to a culture of TL1105A grown to log-phase in K60Na55 medium. The specific activity of β-galactosidase remained constant at 8 U for 10 min following solute addition, rose to a maximum of 35 U at 20 min, and gradually declined thereafter. Thus, instantaneous induction of kdp upon addition of nonionic solutes was confirmed, but the magnitude was less than that reported by Laimins et al. (23).

**An osmosensitive mutation does not affect steady-state kdp-lac expression.** Intracellular accumulation of solutes other than K\(^+\) salts contributes to the restoration of turgor in cells growing in high-osmolar conditions. In E. coli, increased synthesis of trehalose occurs in response to growth at elevated osmolality. The products encoded by the otsA and otsB genes are required for the osmotically regulated synthesis of trehalose, and mutations in either of the genes lead to an osmosensitive phenotype (3, 16, 42). We reasoned that if the hypothesis (12, 23) of inverse correlation between [K\(^+\)]\(_e\) in the growth medium and turgor stress sensed by the cells was correct, an ots mutant may be expected to suffer turgor stress (and therefore to express kdp-lac) at a higher [K\(^+\)]\(_e\) than would the corresponding ots\(^+\) strain. We examined kdp-lac expression in the isogenic strains TL1105A (ots\(^+\)) and G1649 (ots\(^+\)::Tn10) after growing them in K medium supplemented with 0.4 M sucrose and with varying [K\(^+\)]\(_e\). The range of [K\(^+\)]\(_e\) chosen for study corresponded to the heel of the induction curve for TL1105A shown in Fig. 1, because it is in this range that expression of kdp-lac in the strain is expected to be most sensitive to perturbations in signal strength. The introduction of the otsA mutation did not have any significant effect on kdp-lac expression over this range of [K\(^+\)]\(_e\) (Fig. 3A).

**Effect of glycine betaine on kdp-lac expression.** E. coli cells

![Graph showing the dependence of kdp-lac expression on pH of the growth medium.](image-url)
have two porters, ProU and ProP, for the active transport and intracellular accumulation of glycine betaine during growth in media of elevated osmolarity (8); each of these is believed to independently contribute to the alleviation of turgor stress under these conditions if, and only if, glycine betaine is provided in the growth medium. We exploited this fact to devise a test of the kdp turgor regulation model: the accumulation of glycine betaine in cells growing with 0.4 M sucrose over a range of [K+]e should serve to partially relieve turgor stress and consequently reduce kdp expression. We found, however, that the kdp-lac induction profile of TL1105A growing in K medium supplemented with 0.4 M sucrose and a range of [K+]e (as had been used above with the otsA derivative) was not significantly affected by the addition of 1 mM glycine betaine to the growth medium (Fig. 3A). The results of a control experiment demonstrated that the addition of 0.4 M sucrose induced proU-lac expression in the kdp trkA trkD strain GJ644 to 90% of normal (at the lowest [K+]e used as the data point in Fig. 3A); the addition of 1 mM glycine betaine also had the expected feedback-repressive effect (8) on such induction (data not shown). These data ruled out the possibility (44) that proU expression itself could be significantly impaired at these low values of [K+]e in TL1105A.

**Growth rate effect on kdp expression.** One criticism of the experiments described above that looked at the effects of otsA or of glycine betaine on kdp expression is that they were done in K medium, which contains 0.5% Casamino Acids. It is possible that the presence in Casamino Acids of other osmoprotective compounds like L-proline (7, 8, 17) might have masked the true effects of trehalose and glycine betaine. We therefore repeated both sets of experiments by using defined glucose-MBM supplemented with 0.4 M sucrose as the basal growth medium. We observed once again that at no value of [K+]e, did either the otsA mutation or glycine betaine have the predicted effects of, respectively, increasing or decreasing kdp-lac expression (Fig. 3B). In fact, a consistent two- to fourfold alteration of expression in the opposite direction was observed in both instances (Fig. 3B). An essentially similar pattern of results (but with all curves shifted to the right) was also obtained when 0.25 M NaCl was substituted for 0.4 M sucrose in the experiments described above (Fig. 3C).

We considered the possibility that the observed reverse effect (from that expected) of otsA and of glycine betaine on kdp-lac expression in MBM-growth media was secondary to differences in growth rates of the different cultures, for the following reasons. First, it is known that the intracellular accumulation of glycine betaine or of trehalose is associated with an increase in growth rate in high-osmolarity media (7, 8, 16, 19, 34, 42). Second, Epstein and Schulz (15) had earlier shown that a direct relationship between [K+]e and the richness of the C source used for growth possibly exists in E. coli. Finally, in cultures grown at different dilution rates in a chemostat, Tempest and Meers (46) also observed a correlation between growth rate and intracellular K+ content per gram (dry weight) of cells.

We, therefore, examined whether kdp expression might also be influenced by changes in culture growth rate at constant [K+]e, pH, and osmolarity. TL1105A was grown to log-phase in K30Na85 medium supplemented with each of the following C sources (arranged in decreasing order of the corresponding growth rates): glucose plus Casamino Acids, glucose, maltose, and glycrol. The specific activities of β-galactosidase in these cultures were, respectively, 500, 250, 30, and 5 U, providing support for the notion that steady-state kdp expression is enhanced at elevated growth rates and that this is a sufficient explanation for the data depicted in Fig. 3B and C. We emphasize that the effect described above is obtained when growth rate is altered by means other than K+ limitation; alterations in growth rate obtained by varying the [K+]e have exactly the opposite kind of effect on kdp-lac expression (23).

**Glycine betaine accumulation abolishes correlation between K+ limited growth and kdp induction.** In cultures grown in low-osmolarity medium, Laimins et al. (23) have shown that there is a close correlation between the [K+]e, at which growth rate is decreased and that at which steady-state kdp-lac induction occurs. In our experiments with glycine betaine addition to cultures grown at high osmolarity with varying [K+]e, we observed that this correlation breaks down (Fig. 4). Thus, the growth rates of TL1105A grown in
kdp-lac strain; and kdp-lac(Ap) fusion

As discussed similar expression enzyme assay As described shown results that MC4100 to prepared on different concentrations of added KCl. Each was then subcultured 1:100 in the same medium, and optical density was monitored as a function of time in a Klett-type colorimeter. The symbols △, ○, and □ correspond respectively to added K⁺ concentrations of 25, 30, and 40 mM in panel A and 35, 45, and 55 mM in panel B. Aliquots for enzyme assay were removed at approximately 5 h, and the measured specific activity (units) of β-galactosidase for each culture is marked.

Sucrose-supplemented media at three values of [K⁺]ₑ were similar (Fig. 4), although the magnitude of kdp-lac expression was quite different among the three cultures. Similar results were obtained in NaCl-supplemented media (Fig. 4), as have also been reported earlier by Sutherland et al. (44). As discussed below, these data also support the conclusion that kdp transcription is not controlled by turgor.

Isolation and characterization of kdp-lac regulatory mutants. As described above, GJ618 and GJ619 were obtained as kdp-lac(Ap) strains carrying, respectively, the additional kdp-205 and kdp-207 mutations which conferred increased lac expression after growth in K medium. When P1kc lysates prepared on GJ618 and GJ619 were used to transduce MC4100 to Amp', more than 90% of the transductants (out of approximately 500 scored in each cross) exhibited a Lac phenotype similar to that of the respective mutants, whereas the remainder had the phenotype of GJ18, the wild-type kdp-lac strain; the mutations in both GJ618 and GJ619 were thus shown to be closely linked to, yet separable from, the kdp-lac(Ap) fusion itself. Transduction experiments with strains carrying Tn10 insertions on either side of the kdp locus (from the collection of Singer et al. [40]) indicated that both kdp-205 and kdp-207 were situated to the counterclockwise (that is, promoter-distal) end of the kdpABC genes (data not shown), to the region where the trans-acting regulatory genes of the operon, kdpD and kdpE, have earlier been mapped (35, 38).

The map positions of the kdp-205 and kdp-207 mutations were each further confirmed as follows. The F-prime plasmid F(Ts)lac114 was used to mobilize the chromosome of a kdp-lac(Ap) strain, GJ636, into the respective kdp-205 and kdp-207 mutant strains GJ650 and GJ651. It is known that chromosome transfer would be initiated within the region of homology shared between the lac region of the F-prime plasmid and that at the kdp locus (at 16 min) and that markers from the promoter-distal end (that is, counterclockwise) of kdp-lac would be transferred as early markers in conjugation (20). Recombinants were selected for a proximally located Tn10 insertion allele at 11.5 min on the donor chromosome, and Cm' (confirmed by plasmid pHYD94 in the recipient) was used for contraselection. Approximately 60% of Tef' exconjugants in both crosses (out of 400 scored on K medium plates supplemented with X-Gal) had also recombined at the kdp-205 and kdp-207 loci, respectively; none of 70 Tef' exconjugants tested in either cross had recombined for a Tn10Kan allele located distally, at 31 min on the donor linkage map. These results clearly indicated that the kdp-205 and kdp-207 alleles were transferred as proximal markers in the conjugation experiments and thus established their map position to the kdpDE region.

The profile of steady-state kdp-lac expression in GJ618 as a function of varying [K⁺]ₑ in the growth medium is depicted in Fig. 5. A comparison of the results for GJ618 with those for the wild-type kdp-lac strain GJ18 indicates that the patterns of K⁺ repression of kdp expression are similar, although the overall curve for GJ618 is shifted far to the right. Furthermore, the addition of NaCl to the culture medium or introduction of the trkA mutation into the strain evoked similar patterns of change in kdp-lac expression in

FIG. 4. Growth rates of TL1105A in glucose betaine-supplemented media of varying [K⁺]ₑ. Cultures of TL1105A were grown overnight in glucose-MBM medium supplemented with 1 mM glucose betaine, either 0.4 M sucrose (A) or 0.25 M NaCl (B), and different concentrations of added KCl. Each was then subcultured 1:100 in the same medium, and optical density was monitored as a function of time in a Klett-type colorimeter. The symbols △, ○, and □ correspond respectively to added K⁺ concentrations of 25, 30, and 40 mM in panel A and 35, 45, and 55 mM in panel B. Aliquots for enzyme assay were removed at approximately 5 h, and the measured specific activity (units) of β-galactosidase for each culture is marked.

FIG. 5. Effect of kdp-205 on kdp-lac expression. β-Galactosidase specific activities in cultures of GJ618 (kdp-205; ○) and GJ18 (kdpD⁺ E⁺; ●) are plotted as a function of [KCl] added to Na-substituted K medium. Also shown are curves corresponding to cultures of GJ652 (kdp-205 trkA) grown in the same medium (△) and of GJ618 grown in medium supplemented with 0.25 M NaCl (□).
GJ618 and in the wild-type kdp-lac strain (Fig. 5). The addition of glycine betaine to a high-osmolar medium also did not alter kdp-lac expression in GJ618 (data not shown).

The kdp-lac induction profile of GJ618 is apparently similar to that of a trkA trkD mutant such as TL1105A. However, unlike the situation in the latter strain, in which enhanced expression of kdp-lac (compared with that in the trkA+ derivative) at any particular [K+]o was associated with a concomitant reduction in growth rate (23), in GJ618 was not accompanied by a decrease in growth rate. The growth rates of GJ618 and of GJ18 at any of various [K+]o were identical to one another (data not shown). The significance of these results with respect to the turgor regulation model for kdp is discussed below.

In contrast to that in GJ618, the expression of lac in GJ619 was constitutive (around 800 U) under all conditions and at all values of [K+]o tested (data not shown). It may be noted that the maximal level of kdp expression observed in both GJ618 and GJ619 was approximately twofold higher than that in GJ18.

Isolation and characterization of a trkD gain-of-function mutant. Strains doubly mutant in kdp and trkA are extremely crippled for K⁺ uptake and consequently grow slowly on most media. In the course of routine maintenance of a kdp lac strain (GJ617), a mutant, GJ616, was identified in which a presumed compensatory genetic alteration had resulted in the following changes in phenotype. (i) β-Galactosidase activity after growth in K medium was reduced to 6 U in GJ616, from 187 U in GJ617; consequently, GJ616 colonies were white on X-Gal-supplemented K medium, whereas those of GJ617 were blue on these plates. (ii) The presence of the trkA and kdp mutations in GJ617 renders it sensitive to growth at 42°C in K medium supplemented with 0.4 M NaCl (17), whereas GJ616 was able to grow under these conditions. The mutation in GJ616 conferring the new phenotype was not linked to either kdp or trkA (data not shown) and was tentatively designated sta-1 (suppressor of trkA).

To map the sta-1 locus, random transpositions of Tn10 were generated in MC4100, and a P1 lysate prepared on a population of these cells was used in transduction into GJ616, with selection for Kanr on X-Gal-supplemented medium. Approximately 1% of the Kanr transductant clones were blue on these plates, and upon further characterization, one of these latter clones was shown to have a Tn10 insertion which was 70% cotransducible with sta-. Standard procedures (40) were then used to map sta-1 to 84 min, 90% cotransducible with ilv in the gene order bgl-sta-1-rbs-ilv (data not shown).

The trkD locus is also located in the region between bgl and rbs and encodes one of the constitutive K⁺ uptake systems in E. coli. We used an indirect test of marker rescue (25) to examine whether sta-1 was allelic to trkD. Plasmid pJG1, carrying the cloned trkD gene and the adjacent rbsA gene (5), was introduced by transformation into the sta-1 mutant GJ632 by selection for Tet'. A P1kc lysate prepared on GJ632 (pJG1) was then used to transduce GJ642 (kdp-lac trkA sta-) to Tet'. The majority of transductants had acquired the original pJG1 plasmid and were dark blue on X-Gal-supplemented K medium indicator plates; however, approximately 1% of the colonies were pale blue on these plates, a phenotype characteristic of the sta-1 mutant. Plasmid preparations from representative colonies of the two classes of transductants were used in subsequent transformation into GJ642 to demonstrate that the difference in lac expression in the latter class was indeed the result of an alteration in the plasmid molecules carried in them. The simplest explanation for these results would be to assume that sta-1 is a dominant allele of trkD and that the pale blue colonies represent derivatives in which the mutation has been transferred by homologous recombination onto plasmid pJG1 (25). The sta-1 mutation has accordingly been redesignated trkD204.

The availability of the zie-901::TnlO insertion near the trkD204 locus and of zhe-904::TnlO near trkA enabled construction of a panel of isogenic kdp-lac strains that were trkA+ trkD+, trkA trkD204, trkA+ trkD204, and trkA trkD+. Measurement of β-galactosidase activity as a function of [K+]o in each of these strains (Fig. 6) indicated that the trkD204 mutation shifts the K⁺ repression curve leftward in both trkA+ and trkA backgrounds; that is, even though it was originally obtained in a trkA strain, its effect on kdp-lac occurs independently of the presence of the trkA mutation.

DISCUSSION

Mutations affecting kdp expression. Different mutations could affect kdp-lac expression either (i) by altering the strength of the environmental signal that is sensed by the cell in controlling kdp transcription, in which case the signal-transduction pathway, mediated by the products of kdpDE is itself normal; or (ii) by interfering with the latter pathway. The data from this study and from earlier studies (17, 23) indicate that mutations in the K⁺ transporter genes kdpABC, trkA, and trkD (including the gain-of-function trkD204 allele) alter kdp-lac expression by the first mechanism. The hallmark of all these mutants is that there continues to be a close correlation between the value of [K+]o at which growth limitation begins and that at which kdp-lac induction is observed (23). Various environmental factors that affect kdp expression, including pH, growth temperature (17), and the presence of other cationic solutes such as Na⁺ or NH₄⁺ in the culture medium, also do so by altering the strength of the signal. In all of these cases, kdp induction correlates with a decrease in growth rate, and both effects are reversible with K⁺ supplementation.

On the other hand, mutants GJ618 and GJ619 appear to be
defective in the signal transduction pathway of \textit{kdp} regulation. The mutation in either strain maps in \textit{kdpDE}, and there is no correlation between the value of [K\textsuperscript{+}]\textsubscript{i} that becomes growth limiting and that at which sustained \textit{kdp-lac} expression occurs. Of the two mutants, GJ619 remains fully constitutive at all values of [K\textsuperscript{+}]\textsubscript{i}, tested and appears to be the first trans-acting \textit{kdp} regulatory mutant of its kind that has been reported and characterized. In contrast, the \textit{kdp-205} mutation in GJ618 confers a partially constitutive phenotype; a far weaker signal is sufficient to induce \textit{kdp} expression in this mutant than in the isogenic \textit{kdpD\textsuperscript{+}E\textsuperscript{+}} strain.

An alternative explanation for the phenotype observed in GJ618 would be that the mutant regulatory protein (KdpD) requires a particular minimum [K\textsuperscript{+}]\textsubscript{i} to adopt a wild-type conformation and that this requirement has no relationship with the inducing signal per se. However, such a possibility is excluded by our observation that the curve describing \textit{kdp-lac} expression as a function of [K\textsuperscript{+}]\textsubscript{i} in the \textit{kdp-205} strain is shifted to the right by addition of Na\textsuperscript{+} to the growth medium or by introduction of the \textit{trkA} mutation. Thus, the [K\textsuperscript{+}]\textsubscript{i} at which repression is established in GJ618 is not invariant but can be perturbed to much the same extent as in the \textit{kdpD\textsuperscript{+}E\textsuperscript{+}} parent by factors which are known to influence signal strength in the latter.

**Is turgor the signal for \textit{kdp} regulation?** Several results obtained by us and by workers elsewhere are discordant with the proposal (23) that \textit{kdp} expression is turgor regulated, and these are listed below. Some of the arguments presented here have also been discussed earlier by one of us (18).

(i) The expression of \textit{kdp-lac} during steady-state growth was markedly induced by the presence of ionic solutes in the growth medium but was unaffected by nonionic solutes. The different ionic solutes tested also showed a gradation in the extent to which they induced \textit{kdp} expression. If loss of cell turgor were the inducing signal, equimolar concentrations of various impermeable solutes would be expected to induce \textit{kdp} expression to similar extents.

In this context, one line of evidence reported by Laimins et al. (45) is that the type of turgor regulation of \textit{kdp} was that steady-state expression of \textit{kdp-lac} for a given low [K\textsuperscript{+}]\textsubscript{i}, was reduced when medium osmolarity was decreased; in their experiment, however, osmolarity was lowered by progressive dilution of the predominantly Na\textsuperscript{+}-containing growth medium, and we believe now that their results can be explained on the basis of Na\textsuperscript{+}-specific effects on \textit{kdp} expression, described above and earlier (17, 44).

(ii) Accumulation of glycine betaine via ProU and ProP did not reduce \textit{kdp} expression at any value of [K\textsuperscript{+}]\textsubscript{i}. Glycine betaine is expected to contribute to restoration of cell turgor during adaptation of cells to high-osmolar conditions, and its instantaneous accumulation is known, for example, to be able to trigger K\textsuperscript{+} loss from K\textsuperscript{+}-replete cells through turgor-activated efflux pathways (2, 28).

(iii) Furthermore, our results indicate that although \textit{kdp-lac} is induced with decreasing [K\textsuperscript{+}]\textsubscript{i}, during growth in glycine betaine-supplemented high-osmolarity media, the growth rates of the different cultures under these conditions were similar to each other (Fig. 4). The latter observation lends support to the notion that various properties of the intracellular steady-state (including ionic strength, concentrations of compatible solutes, and turgor) could not have been different between the various cultures.

(iv) The absence of trehalose accumulation at high osmolality in an \textit{ots} mutant also did not increase \textit{kdp-lac} expression compared with that in an isogenic \textit{ots\textsuperscript{+}} strain. Since trehalose is another compatible solute whose intracellular accumulation is expected to contribute to restoration of turgor (42), one would have expected to observe such an increase in case of a turgor-regulated gene.

(v) As argued above, the strength of the environmental signal regulating \textit{kdp-lac} in the partially constitutive \textit{kdp-205} mutant GJ618, for any particular combination of growth conditions, is the same as that for the isogenic \textit{kdpD\textsuperscript{+}E\textsuperscript{+}} strain GJ18; the mutant is altered only in perception of the signal. The results with GJ618 (Fig. 5) indicate that the strength of the transcription-activating signal in a strain with defective Kdp and functional TrkA and TrkD porters does not remain constant for [K\textsuperscript{+}]\textsubscript{i} > 2 mM (as may have been inferred from the GJ18 results) but indeed progressively decreases with increase in [K\textsuperscript{+}]\textsubscript{i} even in the range between 10 and 30 mM. It is unlikely that there would be a concomitant increase in cell turgor during steady-state growth over this range of [K\textsuperscript{+}]\textsubscript{i}.

(vi) The finding that steady-state \textit{kdp} expression at sufficiently high [K\textsuperscript{+}]\textsubscript{i} is not affected by the osmolarity of the medium had earlier been taken to imply that cell turgor is completely restored in cultures adapted to growth at elevated osmolarity (12, 23, 44). It has not been possible to perform direct measurements of turgor pressure in \textit{E. coli}, but two lines of circumstantial evidence suggest that contrary to the assumption above, cell turgor during steady-state growth under K\textsuperscript{+}-replete conditions is indeed lower at elevated osmolarity than at low osmolarity (whereas \textit{kdp} expression itself remains unchanged). First, in those bacteria in which turgor pressure has been directly measured from the value of external hydrostatic pressure required to collapse intracytoplasmic gas vesicles, an inverse correlation between cell turgor during steady-state growth and osmolarity of the medium has been demonstrated (22, 36). Second, several workers have observed that the steady-state values for intracellular and cytoplasmic water volumes of \textit{E. coli} (expressed per gram [dry weight]) decrease with increasing medium osmolarity (6, 7, 10, 15, 34, 42, 44); this has been interpreted as evidence for a progressive decrease in cell turgor under these conditions, with a consequent reduction in stretch of the elastic cell envelope (9, 21, 42).

On the basis of the arguments above, we conclude that the signal involved in regulation of \textit{kdp} expression is not cell turgor. One observation that then remains unexplained is induction of \textit{kdp-lac} in response to a sudden osmotic up-shock. We suggest that this occurs by a mechanism unrelated to the true physiological signal, reflecting perhaps a transient perturbation of KdpD activity consequent to plasmolysis.

**Consideration of, and constraints on, alternative models.** The facts that the strength of the environmental signal controlling \textit{kdp} expression is influenced primarily by [K\textsuperscript{+}]\textsubscript{i} and that it is perturbed by mutations in each of three different K\textsuperscript{+} transporter loci (\textit{kdpABC}, \textit{trkA}, and \textit{trkD}) suggest that the signal must be related to [K\textsuperscript{+}]\textsubscript{i} or to the process of transmembrane K\textsuperscript{+} transport. Three alternative possibilities may be considered, but each has its own limitations in providing a complete explanation.

The first is that [K\textsuperscript{+}]\textsubscript{i} itself serves as the signal. The argument against this has been that steady-state [K\textsuperscript{+}]\textsubscript{i} can be altered by changes in osmolarity of the growth medium (6, 7, 15, 44), under conditions in which \textit{kdp-lac} expression remains constant. In its defense, however, several other reports suggest that [K\textsuperscript{+}]\textsubscript{i} increases only transiently when cultures are subjected to osmotic up-shock and that it subsequently returns to a value close to that obtaining at low
osmolarity (10, 32, 33); furthermore, the growth rate of *E. coli* in high-osmolarity media has been shown not to be affected either by K⁺ limitation (32) or under conditions in which K⁺ is unable to accumulate intracellularly (33).

The second possibility is that intracellular K⁺ is compartmentalized (18, 46) and that [K⁺] of one such compartment is the signal for kdp regulation. Recent reports by Wiggins (49) on the inhomogeneity of intracellular water and by Cayley et al. (6) postulating the polyelectrolyte model for distribution of cytoplasmic K⁺ indicate the possible existence of distinct K⁺ compartments within the cell, but the molecular details of such a model remain undefined.

The third candidate for the signal merits consideration because of the results that we have obtained with the regulatory mutant GJ618. The data from this strain impose a severe constraint on any model for kdp regulation, because they support the following conclusion: the strength of the signal which activates kdp expression (even in a kdpD'E' strain) progressively decreases over a range of increasing [K⁺], across which homeostatically controlled parameters, such as turgor, [K⁺], or [K⁺] in any subcellular compartment, are expected to remain constant (12). Our observation that kdp expression in a wild-type strain grown in glycine betaine-supplemented media is altered at varying [K⁺], despite a constant growth rate is also formally analogous to the behavior of GJ618 and once again argues against a steady-state determinant serving as the signal for kdp regulation.

It is possible, therefore, that the signal is related to the specific rate of transmembrane K⁺ influx (expressed per unit [dry weight] of cells), such that the operon is activated whenever the influx rate is decreased. Because the influx rate is expected to increase monotonically with increasing [K⁺], with K⁺ and turgor homeostasis under these conditions being achieved by concomitant increases in influx rates (2, 28), such a model would provide an explanation for the observations with both GJ618 and glycine betaine-supplemented media. The effects of solutes such as Na⁺ or NH₄⁺ and of pH (that is, H⁺) on kdp induction may also be explained on the assumption that each of these cationic species decreases the rate of K⁺ influx at a given [K⁺]. However, some mechanism must exist by which the influx rates through each of the Kdp, TrkA, and TrkD transporters are integrated to provide a single measure of signal strength, and this remains as a major untested assumption of the model.

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