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Genetics

Osmotic control of *kdp* operon expression in *Escherichia coli*

(potassium transport/*lac* fusion/turgor pressure)

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ABSTRACT **Turgor pressure, the difference in osmotic pressure across the inner membrane, has been found to regulate expression of the *kdp* operon in *Escherichia coli*. The *kdp* operon codes for a high-affinity repressible transport system for the uptake of potassium. We have studied the regulation of Kdp expression in a strain in which the gene for β-galactosidase, *lacZ*, was placed under control of the *kdp* promotor. Neither internal nor external K^+ concentrations directly controlled Kdp expression. Only when the external K^+ concentration was reduced to the point of limiting growth was the *kdp* operon expressed. An increase in external osmolarity at constant K^+ concentration, a procedure that reduces turgor pressure, caused expression of the *kdp* operon. As the magnitude of the osmotic shift was increased, corresponding to greater decreases in turgor pressure, the amount of Kdp expression also increased. The *kdp* operon thus appears to be controlled by changes in a physical force, the turgor pressure.**

Bacteria maintain an internal osmolarity significantly higher than that outside the cell. This positive difference between the internal and the external osmolarity, known as the turgor pressure, is required for growth and division. In *Escherichia coli*, the turgor pressure is maintained at approximately 3 atm (1 atm = 101 kPa) (1) by the accumulation of K^+ and a variety of metabolically produced anions (2). The primary role of K^+ in *E. coli* is the regulation of osmolarity (3), although it also serves to activate many cellular enzymes. The internal K^+ concentration is determined by the osmolarity of the medium and ranges from 0.1 M in very dilute media to 0.6 M in 1200 mosM media (3). Activation of cellular enzymes requires K^+ concentrations of less than 0.1 M (4), which is less than the lowest concentration ever found in *E. coli*.

To regulate internal osmolarity, *E. coli* accumulate K^+ by two distinct transport systems (5). Under most growth conditions, K^+ is taken up by the constitutive TrkA system, which has a high rate of transport and a low affinity for K^+ (5). At very low external K^+ concentrations or when the TrkA function has been impaired through mutation, K^+ is taken up by the high-affinity Kdp system, which has a K_m of 2 μM (5). This repressible system is ATP driven (6) and requires the expression of four closely linked genes (7, 8). Three of these genes, *kdpA*, *kdpB*, and *kdpC*, form an operon that codes for three inner membrane proteins (9). The fourth gene, *kdpD*, codes for a positive regulator and is located at the promoter-distal end of the *kdpABC* operon (8).

The *kdp* operon is repressed by growth in media of high K^+ concentration, and the K^+ concentration at which derepression occurs depends on the activity of the TrkA system. Growth in 5 mM K^+ medium represses the *kdp* operon in a wild-type strain but results in partial derepression in a strain that has an impaired TrkA function (5). These observations suggest that the ability to meet K^+ requirements can be detected and translated into a signal controlling Kdp expression. In this study, we examined the regulation of the *kdp* operon by introducing the *lacZ* gene into the *kdp* operon and using the synthesis of β-galactosidase as an indicator of Kdp expression. The findings suggest that turgor pressure regulates the expression of the *kdp* operon. Specifically, a decrease in turgor pressure is the signal to turn on expression. Our model implies a mechanism that couples mechanical forces to genetic regulation.

METHODS

Bacteria and Bacteriophages. The bacterial strains used are given in Table 1. Fusions of the *lacZ* gene into the *kdp* operon were made to provide an easy assay for Kdp expression. The specialized Mud(Ap,*lac*) transducing phage of Casadaban and Cohen (10) was used to fuse the *lacZ* gene into the *kdp* operon. Mud(Ap,*lac*) lysogens of strain TL1100 were obtained in liquid medium as described. To select for lysogens with insertions into the *kdp* operon, the cells were subjected to two rounds of penicillin selection in 0.1 mM K^+ medium as described (8). Strain TL1100 carries *trkA405* and *trkD1* mutations, which drastically reduce TrkA function. When *kdp* function is abolished by phage insertion, this strain becomes unable to grow at K^+ concentrations less than 10 mM (5). Such strains will therefore survive the penicillin selection in 0.1 mM K^+ medium and are candidates for fusions of *lacZ* to the *kdp* operon. A higher level of penicillin was used in these experiments (4000 units per/ml) to overcome the moderate resistance to penicillin coded by the Mud(Ap,*lac*) transducing phage. Kdp^- survivors were identified by the inability to grow on 5 mM K^+ medium and screened for β-galactosidase expression at low K^+ concentrations (20 mM), but not at high concentrations (115 mM), consistent with what is known about Kdp expression (5). Screening was done on minimal glucose plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside. The *kdp*::*lacZ* lysogens were further characterized as insertions into one of the three genes of the *kdp* operon by complementation with λpkdp-1 phages carrying mutations in one of the three genes (8). Chromosome mobilization studies (11) confirmed the location of the insertion, designated *kdpA5*::*lacZ*, used in this work.

Mud(Ap, *lac*) lysogens are temperature sensitive for growth because the prophage carries the c_{ts62} mutation, which makes the phage repressor thermolabile. Thus, derivatives of the Mud(Ap, *lac*) lysogens were constructed in which Mu was replaced by λp209, as described by Komeida and Ino (12). A *kdpA5*::*lacZ* temperature-resistant strain is designated by the suffix A—i.e., TL1105A. Derivatives of this strain with a wild-type TrkA system were obtained by introducing *trkA*$^+$ by transduction, and those with a wild-type Kdp system were obtained by introducing the *kdp*$^+$ F100 episome by conjugation.

Media. Minimal phosphate-buffered K115 and KO media and complex KML medium for genetic crosses have been de-

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Table 1. Bacterial strains

Strain*	Pertinent genotype†
TL1100	*kdp*$^+$ *trkA405*
TL1105 and TL1105A	*kdpA5*::*lacZ trkA405*
TL1106A	*kdpA5*::*lacZ trkA405* F100 *kdp*$^+$
TL1110 and TL1110A	*kdpA5*::*lacZ*
TL1111A	*kdpA5*::*lacZ* F100 *kdp*$^+$

* Strains designated with the suffix *A* are derivatives of the corresponding strain in which λp209 has replaced most Mu genes.
† All strains are Δ*lac thi trkD1 nagA rha*.

scribed (13). Medium osmolarities were maintained constant as the external K^+ was varied by the addition of compensating amounts of Na^+. Osmolarities were calculated by assuming an osmotic coefficient of 0.9 for all solutes. Intracellular K^+ concentrations for cells growing in these media were determined by interpolation of published data (3).

Assays. Assays for β-galactosidase activity were performed on toluene-treated cells as described (14). One unit of β-galactosidase liberates 1 nmol of *o*-nitrophenyl-β-D-galactoside per min at 37°C. Protein synthesis was measured by the rate of incorporation of radiolabeled isoleucine. For such measurements, cells were grown in minimal medium supplemented with isoleucine, leucine, threonine, and valine at 10 μg/ml. Before sampling, cells were resuspended in fresh medium and allowed to double. Simultaneous with the osmotic shift, L[4,5-^{3}H]isoleucine (1 μCi/ml; 1 Ci = 3.7×10^{10} becquerels; New England Nuclear) and isoleucine, leucine, threonine, and valine (each 2 μg/ml) were added. At intervals, samples were taken for determination of radioactivity incorporated into hot trichloroacetic acid-precipitable material. Growth rates were determined from turbidity measurements as described (7).

RESULTS

To simplify the study of the regulation of the *kdpABC* operon, we used the method of Casadaban (10) to fuse the *lacZ* gene to the operon and thus bring expression of β-galactosidase under *kdp* promotor control. This provides an easy enzymatic assay to monitor the expression of the *kdp* operon. Fusion of the *lacZ* gene to a *kdp* gene eliminates uptake of K^+ by the Kdp system and thus prevents it from altering regulation of its own expression. The *kdp*::*lacZ* fusions were obtained by use of the specialized transducing phage, Mud(Ap, *lac*). Integration of this phage in the proper orientation into an operon puts *lacZ* expression under control of the operon promotor. The strains used in this study (see Table 1) are derivatives of strain TL1105, which has the Mud(Ap, *lac*) phage integrated in the desired orientation in *kdpA*, the first gene of the *kdpABC* operon. Strain TL1105 also carries two *trk* mutations that limit uptake by the TrkA system to a low rate linearly proportional to the external K^+ concentration.

The relationship of Kdp expression to external K^+ concentration for several strains is shown in Fig. 1*A*. In strain TL1105, there was no expression at high external K^+ concentrations but, as this concentration decreased to less than 40 mM, expression of the *kdp* operon began. Expression reached a maximum rate at concentrations below 20 mM K^+. The growth rate of strain TL1105 began to decrease at K^+ concentrations less than 40 mM (Fig. 1*B*) due to its limited ability to take up K^+ (5). This correlation shows that expression of the *kdp* operon begins at approximately that K^+ concentration at which the growth rate becomes limited by the inability to accumulate K^+.

A different response to external K^+ concentration was seen in strain TL1110, which has a fully functional TrkA system in addition to the *kdpA5*::*lacZ* fusion. This strain accumulated K^+ much more effectively at intermediate concentrations. No limitation of growth occurred until the external K^+ concentration decreased to less than 10 mM, the same as the threshold concentration for the expression of the *kdp* operon. The expression of the *kdp* operon is thus not determined by the absolute concentration of K^+ in the medium. Rather, the operon is turned on whenever the uptake of K^+ becomes rate limiting for growth.

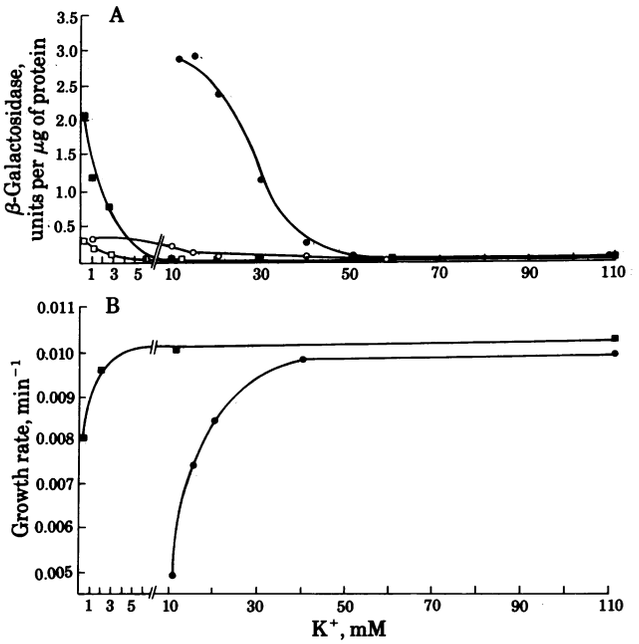

FIG. 1. Effect of external K^+ concentration on *kdp* operon expression and on growth rate of *kdp*::*lacZ* fusion strains in media of standard osmolarity. (*A*) Steady-state expression of β-galactosidase. (*B*) Growth rates in same experiments. Strains are as follows: ●, TL1105 (*trkA*$^-$); ■, TL1110 (*trkA*$^+$); ○, TL1106A (*trkA*$^-$ *F100*); □, TL1111A (*trkA*$^+$ *F100*). Experiments with TL1105 and TL1110 were at 28°C; those with TL1106A and TL1111A were at 37°C.

Strains TL1106A (*trkA*$^-$) and TL1111A (*trkA*$^+$) carry the wild-type *kdp* genes on an episome, in addition to the *kdpA5*::*lacZ* fusion. Expression of wild-type *kdp* genes in these strains contributes to net K^+ uptake at low external K^+ concentrations, so that the growth rate was not limited by K^+ concentrations above 0.1 mM. Although expression of the *kdp* operon was observed (Fig. 1*A*), it was at one-eighth the level of that in strains TL1105 and TL1110, which lack wild-type *kdp*

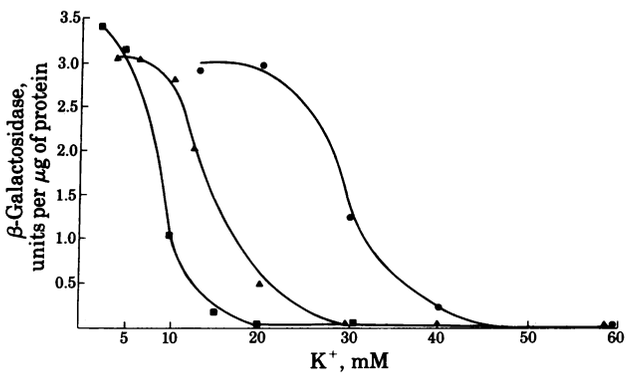

FIG. 2. Effect of internal K^+ concentration on Kdp expression. Steady-state expression of β-galactosidase as a function of external K^+ concentration for cells at three internal K^+ concentrations. Internal K^+ was controlled by varying external osmolarity. ●, 198 mosM, cell K^+ 225 mM; ▲, 99 mosM, cell K^+ 160 mM; ■, 45 mosM, cell K^+ 130 mM.

genes. Thus, low-level *kdp* expression can occur before an appreciable reduction in growth rate is seen. The reduced level of expression showed that function of the Kdp system alters its own expression. Higher levels of expression are seen only when the growth rate is K^+ limited. The external K^+ concentration at which expression begins corresponds to that found in the nonepisomal strains.

To study the role of internal K^+ in Kdp regulation, we used the fact that the internal K^+ concentration is determined by the external osmolarity (3). Cells of strain TL1105 growing in K115 medium or K115 medium diluted 2:1 or 4:1 with deionized water have internal K^+ concentrations of 225, 160, and 130 mM, respectively (3). The relationship between these internal K^+ concentrations and Kdp expression was examined as a function of external K^+ concentration (Fig. 2). In each medium, osmolarity was kept constant and ionic composition was varied by replacing K^+ with Na^+. If internal K^+, were the sole determinant of Kdp expression, one would expect expression only when the internal K^+ concentration fell below a critical value and to be independent of the external K^+ concentration. Such a response was not seen; instead, there was a more complex relationship involving the external K^+ concentration. As the external K^+ concentration decreased, a threshold value was reached, below which expression began. With further decreases, expression increased to a maximum rate. Similar responses were observed for all three internal concentrations, with variations only in the threshold concentration for Kdp expression. This variation reflects the decreased need for internal K^+ as the osmolarity decreases, which allows the impaired TrkA system to meet cellular K^+ needs. Internal K^+ is thus not the sole determinant of Kdp expression but rather appears to be a factor in a complex regulatory mechanism.

As the primary function of intracellular K^+ in bacteria is the maintenance of adequate turgor pressure, the role of turgor pressure in regulation of Kdp expression was examined. Cells of strain TL1105 growing in medium containing sufficient K^+ to completely repress Kdp expression were subjected to a transient increase in external osmolarity, corresponding to a decrease in turgor pressure, by the addition of 0.23 M glucose at constant external K^+ concentration (Fig. 3). After a lag of $\approx$10 min, Kdp expression began and proceeded rapidly. Later Kdp expression decreased and ultimately ceased as the cells adjusted to the new osmolarity by increasing the internal osmolarity through the accumulation of K^+. The differential rate of synthesis of kdp proteins increased rapidly following the osmotic pulse (see Fig. 3C). Thus, a decrease in turgor pressure at constant external K^+ concentration is sufficient to turn on expression of the *kdp* operon and appears to be the signal controlling expression. Ten other osmotically active sugars and hexitols (sucrose, maltose, sorbitol, mannitol, mannose, galactose, arabinose, trehalose, ribose, and xylose) and four salts (NaCl, Na_2SO_4, sodium 4-aminobutyrate, and sodium tris[hydroxymethyl] methyl glycine) gave comparable results when present at osmotically comparable concentrations (data not shown). Glycerol readily permeates the cell membrane (15) and therefore is not osmotically active. When 0.23 M glycerol was added to the medium, no stimulation of Kdp expression was seen, consistent with our model for regulation.

The magnitude of the osmotic shift determines the amount of Kdp expression. Kdp expression was examined as a function of time following the addition of glucose at constant K^+ concentration (Fig. 4). The greater the increase in osmolarity, the greater was the extent of Kdp expression. Increases in external osmolarity correspond to decreases in turgor pressure, so that the amount of Kdp expression is determined by the reduction in turgor pressure. As the magnitude of the osmotic shift increased, the time lag before commencement of Kdp expression also increased (see Fig. 4A). These lags can be attributed to variable periods of low protein synthesis (ref. 16, unpublished observations) that follow the shift; however, the times between the resumption of protein synthesis and the start of Kdp expression were comparable in all cases (see Fig. 4B). The longer lag times observed are thus not specific to expression of the *kdp* operon but are due to general effects on protein synthesis.

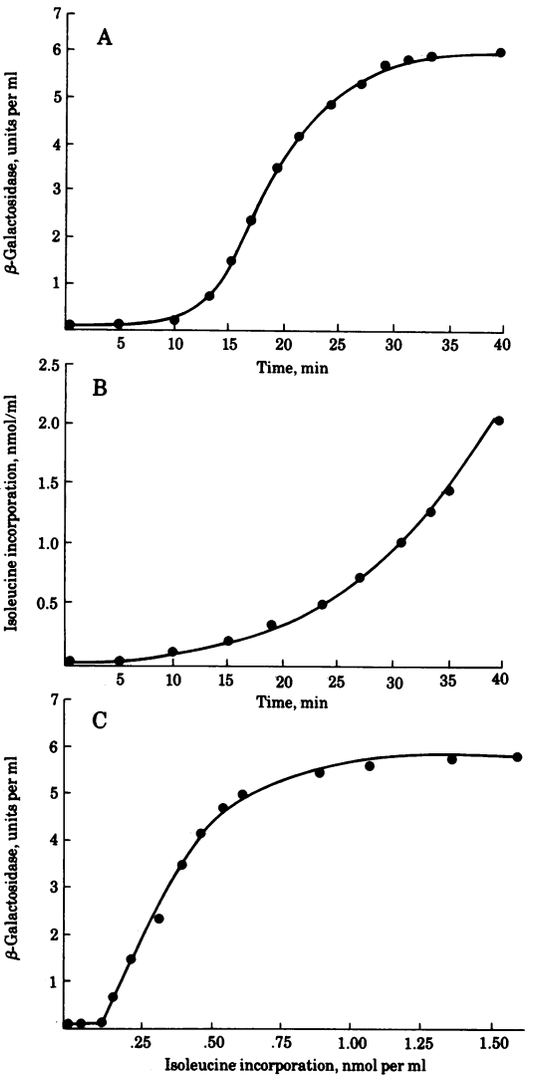

FIG. 3. Effect of shift in medium osmolarity on *kdp* operon expression and on protein synthesis in strain TL1105. At time zero, cells growing in 60 mM K^+ medium at 28°C received 0.11 vol 2.1 M glucose in 60 mM K^+ medium and radiolabeled isoleucine. (*A*) Expression of β-galactosidase as a function of time. (*B*) Incorporation of isoleucine as a function of time. (*C*) Differential plot of β-galactosidase vs. isoleucine incorporation.

DISCUSSION

We have fused the *lacZ* gene to the *kdp* operon of *E. coli* and shown that Kdp expression is determined by decreases in turgor pressure. Kdp expression depends on cellular need for K^+, and this cellular need is sensed through decreases in turgor pressure. This presents an example of an unusual effect—the control by a mechanical force, the differential osmotic pressure across the inner membrane, of the expression of a bacterial operon. The *kdp*::*lacZ* fusion creates a single mRNA in which synthesis is under control of the *kdp* promotor, but initiation of β-galactosidase translation remains under control of its own initiation

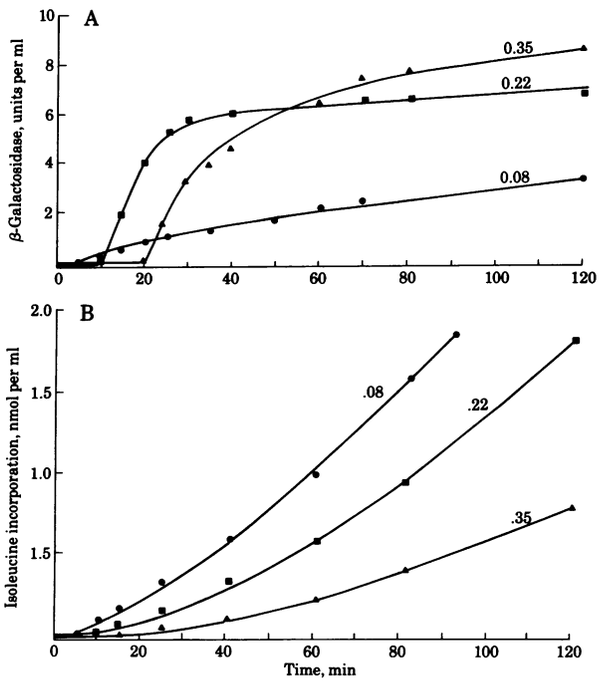

FIG. 4. Effect of magnitude of osmotic shift on *kdp* operon expression and on protein synthesis in strain TL1105. At time zero, cells growing in 60 mM K^+ medium at 28°C received radiolabeled isoleucine and 0.18, 0.11, or 0.04 vol of 2.1 M glucose in 60 mM K^+ medium to final glucose concentrations of 0.35 M (▲), 0.22 M (■), or 0.08 M (●). (*A*) Expression of β-galactosidase as a function of time. (*B*) Incorporation of isoleucine as a function of time.

region. Thus, our results show that the *kdp* operon is regulated at the transcriptional level, as is typical of most genes in *E. coli*. If there is translational control of the *kdp* operon in addition to transcriptional control, it would not be revealed by our studies.

Turgor pressure control of the *kdp* operon is most convincingly shown by experiments in which the turgor pressure is decreased by sudden increases in the osmolarity of the medium. As shown in Figs. 3 and 4, this shift in osmolarity is followed by expression of the *kdp* operon. The magnitude of Kdp expression is determined by the magnitude of the osmotic shift.

Control of expression by turgor pressure also explains the role of the external K^+ concentration in regulation of Kdp expression (see Figs. 1 and 2). For each strain examined, the K^+ concentration below which Kdp expression occurred correlates with the decrease in growth rate that results from inadequate uptake of K^+. This decrease in growth rate reflects cellular need for K^+ and is sensed by decreases in turgor pressure. When K^+ uptake is inadequate, the intracellular volume increases that accompany growth lead to a reduction in internal osmolarity. This reduction in internal osmolarity corresponds to a reduced turgor pressure and leads to Kdp expression. Low-level expression of the *kdp* operon occurs without significant decreases in growth rate in strains carrying a functional Kdp system. However, higher rates of Kdp expression in these strains are correlated with decreases in growth rate.

Control by the turgor pressure requires a transducer in the cell that senses turgor pressure and converts it into a genetic signal. Genetic evidence implicates the *kdpD* gene product in such control. The kdpD protein is considered to be a positive regulator of the *kdp* operon based on findings showing that (*i*) insertion mutations and most point mutations in *kdpD* prevent expression of the Kdp system and (*ii*) mutations to partial constitutive expression map in *kdpD*. Intracistronic complementation between some *kdpD* point mutations suggests that the functional product is oligomeric (8). By using the method of Ptashne (17) and a specialized transducing phage carrying *kdpD*, we have identified the product of this gene as a 90,000-dalton membrane-bound protein (unpublished observations).

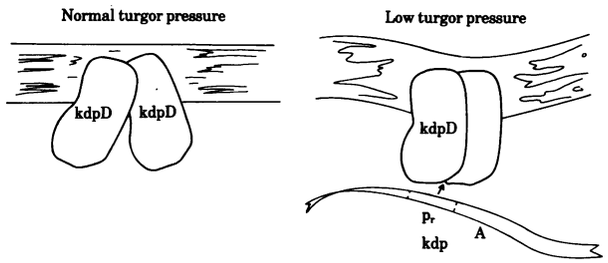

FIG. 5. Model for transduction of low turgor pressure signal to the *kdp* operon. The membrane-bound kdpD regulator undergoes a conformational change following membrane relaxation due to low turgor pressure. In this conformation, the kdpD protein interacts with the *kdp* promoter (p_r) to stimulate transcription.

We propose a model based on our information about the kdpD protein and about turgor pressure regulation of expression of the *kdp* operon. In this model, the kdpD protein is the turgor pressure sensor, which senses either the pressure across the membrane or the stretch in the plane of the membrane (Fig. 5). The conformational change produced by the mechanical forces accompanying a change in turgor pressure is communicated to a part of the protein that is free to interact directly or through an effector with the *kdp* promotor. In this model, the kdpD protein is a mechanical transducer that mediates *mechanogenetic* control.

In addition to controlling the expression of the *kdp* operon, turgor pressure plays an important role in regulating the function of the bacterial K^+ transport proteins themselves. When the turgor pressure is reduced by an increase in medium osmolarity, all K^+ transport systems of *E. coli* respond by increasing the net uptake of K^+ (3). This is presumably the primary event in osmoregulation of bacteria such as *E. coli*, in that the accumulated K^+ increases the internal osmolarity and restores the turgor pressure. The effect on transport proteins is immediate and does not require new protein synthesis. In the case of the Kdp system, one or more of the three *kdpABC* membrane proteins sense this mechanical change and adjust the uptake accordingly. Plant cells show similar stimulation of transport in response to reduced turgor pressure (18), suggesting the fundamental importance of this process. Turgor pressure thus regulates K^+ transport at two different levels; it controls both the expression of the *kdp* operon and the function of K^+ transport systems.

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