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A model for the regulation of expression of the potassium-transport operon, *kdp*, in *Escherichia coli*

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Abstract. The intracellular concentration of K^+ in *Escherichia coli* is known to be determined by osmolarity of the growth medium, and it is believed that the expression of the potassium-transport operon, *kdp*, is controlled by the turgor pressure differential between the cytoplasm and the extracellular environment. Several lines of evidence, however, argue against a *strict* turgor-regulation model for *kdp* expression. Instead, it is proposed here that *kdp* is controlled by *one fraction* of intracellular $[K^+]$, and that the size of this fraction is independent of the osmolarity of the culture medium.

Keywords. Potassium transport operon, *kdp; E. coli*; osmolarity; turgor-regulation model; intracellular potassium.

1. Introduction

Two independent uptake systems, Kdp and TrkA, are known to be involved in the active transport of K^+ in *Escherichia coli* (Rhoads *et al* 1976; Epstein and Laimins 1980). The Kdp transporter is a high-affinity, saturable system very specific for K^+; it is encoded by a set of genes organized in a single operon whose expression is K^+-repressible (Rhoads *et al* 1976, 1978; Epstein 1985). The TrkA system is more complex and less well-characterized; it is apparently constitutively expressed, and has a lower affinity but higher *Vmax* than Kdp for K^+ uptake (Epstein and Kim 1971; Rhoads *et al* 1976; Epstein and Laimins 1980). Except in conditions where the concentration of K^+ in the growth medium is $\lesssim$2mM when the *kdp* genes are induced, the major part of the intracellular K^+ requirement in *E. coli* is met by the TrkA transport system.

In addition to its requirement for the normal functioning of several enzymes, the ribosomal machinery etc., K^+ is also an important intracellular solute in osmoregulation, i.e., in the process of adaptation by cells to growth in high-osmolarity media. The steady-state intracellular concentration of K^+ increases more or less linearly with increasing osmolarity of the growth medium (Epstein and Schultz 1965; Meury *et al* 1985; Sutherland *et al* 1986), and it is believed that the reversal of plasmolysis and restoration of turgor consequent to K^+ accumulation is an essential component in the adaptation response (Epstein 1986; Sutherland *et al* 1986).

This paper is concerned with a review and interpretation of available evidence on the regulation of expression of the *kdp* operon (as studied in *kdp-lac* operon fusion strains), and of the role that this transport system plays during growth under conditions of K^+ limitation and of high osmolarity.

2. Possible models for *kdp* control

Three possible mechanisms by which the expression of the *kdp* operon is regulated – that is, three possible signals, to one or the other of which the operon is primarily responsive – have been so far considered They are briefly described and evaluated below.

2.1 *External* $[K^+]$

According to this model, as external $[K^+]$ is progressively reduced, a threshold is reached beyond which *kdp* expression begins and then increases progressively. Two observations, however, exclude this possibility: (a) The threshold at which induction occurs is not invariant and is, for example, shifted to the right in *trkA* mutants (Rhoads *et al* 1976; Laimins *et al* 1981; Gowrishankar 1985); and (b) for a given level of K^+ limitation, the magnitude of *kdp* gene expression at steady state is less in Kdp^+ than it is in Kdp^- strains (Laimins *et al* 1981). This suggests that Kdp functioning can serve to turn itself down, most probably in a feedback manner.

2.2 *Internal* $[K^+]$

The points (a) and (b) above can be explained by the assumption that it is the decrease in internal $[K^+]$ which serves as a determinant of *kdp* expression. With this assumption, however, it would be difficult to explain the induction of expression of *kdp-lac* during growth of cells in the presence of 0·3 M NaCl (Gowrishankar 1985; Sutherland *et al* 1986), conditions under which internal $[K^+]$ has in fact been shown to be markedly elevated even in Kdp^- strains (Sutherland *et al* 1986). Laimins *et al* (1981) have shown in an elegant series of experiments that increasing [NaCl] results in progressive shifting of the threshold of $[K^+]$-induction to the right, with respect to *kdp* expression.

2.3 *Turgor regulation*

In the model now current for explaining *kdp* regulation, it is suggested that *kdp* expression is responsive to cell turgor (i.e., the osmotic presure differential between the cytoplasm and the environment), which in turn can be independently and interactively affected by low external $[K^+]$ and by high external osmolarity (Epstein 1983, 1985, 1986; Laimins *et al* 1981). Such a model would account for all the observations above, as also for the finding of Laimins *et al* (1981) that *kdp* expression is transiently induced after the instantaneous addition of a variety of impermeable solutes to the growth medium, the magnitude of such induction being a function of the osmolar concentration to which the solute addition has been made.

Once again, however, certain expectations/predictions of a turgor-regulation model are not fulfilled in experiment:

(a) If turgor-regulation of *kdp* were important in homeostasis (i.e., for adaptation to osmotic stress), induction of *kdp* expression would be expected to be sustained (and not transient) during growth in the presence of water stress. The argument here is that under these conditions, intracellular potassium *content* increases at a

faster rate with increasing biomass in stressed than in non-stressed cells, and therefore requires continued functioning of the Kdp system. In other words, in all cases where a stress signal (in this instance, decrease in turgor) is involved in evoking a response (Kdp induction) that feeds back on the magnitude of the signal itself, in the new steady state (with increased intracellular $[K^+]$) neither can the signal become fully attenuated nor can the response return to pre-stress levels. It is known that intracellular water content/mg cell protein decreases during growth under water stress (Perroud and Le Rudulier 1985; Sutherland *et al* 1986), a finding which can be interpreted as support for the notion that turgor is not fully restored under these conditions (Strom *et al* 1986). The actual experimental observation with *kdp*, however, is that the induction of expression with osmolyte addition in $[K^+]$-rich media is only transient and is not a sustained response (Laimins *et al* 1981; Gowrishankar 1985; Sutherland *et al* 1986). In contrast, two other well-characterized systems that have also been postulated to be controlled by turgor pressure, *betA* (Landfald and Strom 1986; Styrvold *et al* 1986) and *proU* (Cairney *et al* 1985b; Dunlap and Csonka 1985; Gowrishankar 1985; Barron *et al* 1986), do indeed show steady-state induction upon growth in a variety of high-osmolarity media.

(b) If the current model is correct, the *kdp* system would be expected to be maximally sensitive to environmental changes that tend to perturb cell turgor under conditions wherein the latter is already being stressed by K^+-limitation. One would then expect that, under conditions of growth in moderate K^+-limitation such that the expression of *kdp* remains partially induced, the added imposition of osmotic stress (irrespective of the nature of impermeable osmolyte used) will serve to increase steady-state *kdp* expresion even further. Results with a large number of osmolytes tested (both non-ionic, or ionic such as choline chloride), however indicate that *kdp* expression is not further increased when any of them is present in a K^+-limiting growth medium (Gowrishankar 1985, and unpublished observations; Sutherland *et al* 1986). In the case of sucrose and of choline chloride, Sutherland *et al* (1986) have shown that the change in *kdp* expression with progressive K^+-limitation is *identical* in the presence and the absence of the added osmolyte. These observations throw serious doubt on a simple turgor-regulation hypothesis of *kdp* expression.

(c) It is known that transport systems for the uptake of other compatible solutes, L-proline and glycine betaine, are also induced and activated in response to osmotic stress (Csonka 1982; Dunlap and Csonka 1985; Cairney *et al* 1985a, b; Gowrishankar 1985, 1986; Gowrishankar *et al* 1986; Grothe *et al* 1986; May *et al* 1986). These compounds may, therefore, be expected to contribute to the restoration of turgor if (*and only if*) they are present in the growth medium and, consequently, affect the level of *kdp* expression in high-osmolarity media. Intracellular accumulation of these solutes in response to water stress has, in fact, been demonstrated (Measures 1975; Perroud and Le Rudulier 1985; Strom et al 1986; Sutherland et al 1986) with a concomitant reduction in the concentration of intracellular K^+ (Sutherland *et al* 1986); the level of *kdp* expression, however, was not affected under these conditions. Thus, in an experiment designed to measure the magnitude of *kdp* expression in high-osmolarity media with varying external $[K^+]$ in the presence or absence of added L-proline and glycine betaine, Sutherland *et al* (1986) have shown that *kdp* expression is related only to the concentration of

exogenous K^+, and is not dependent at all on the availability of other compatible solutes in the growth medium.

3. A new model

It would, therefore, seem that there is need for another model for *kdp* regulation. Any new model for this regulation must explain the known phenomenology of the 'steady-state' during growth under a variety of environmental conditions, for example observations such as the following: (i) *kdp* expression is induced by external K^+-limitation; (ii) *kdp* induction occurs at higher external $[K^+]$ for $TrkA^-$ than for $TrkA^+$ strains; (iii) increased external osmolarity is associated with a corresponding increase in intracellular $[K^+]$, irrespective of the nature of osmolyte used; and (iv) increased external osmolarity does not lead to the induction of *kdp* expression, the only exception being with the use of either NaCl or $(NH_4)_2SO_4$ as osmolyte in low-$[K^+]$ media (Gowrishankar 1985).

I should now like to propose the following as an explanation for the known phenomenology of *kdp* regulation. My model has three hypotheses:

(a) Under most conditions of osmotic stress, the intracellular accumulation of K^+ is driven not by Kdp but by another K^+-transport system. It is likely that the TrkA transporter fulfils the latter role, a possibility that is supported by earlier evidence that its activity is enhanced in response to elevated osmolarity (Meury *et al* 1985), and also by our observations that $TrkA^-$ Kdp^- cells are unable to grow in low-$[K^+]$ high-osmolar media (in respect of a variety of osmolytes used) whereas $TrkA^+$ Kdp^- cells are able to do so (Gowrishankar, unpublished).

(b) Intracellular K^+ exists in two interconnected 'compartments', one of which is osmotically active and increases with increasing external osmolarity, controlled by an osmosensor that is as yet not identified, whereas the other is not osmotically regulated. It is only the latter that serves as the primary signal in feedback regulation of expression of the *kdp* operon. This second 'compartment' may, for example, be that fraction of $[K^+]$ known to be associated with ribosomes and, perhaps, other macromolecules within the cell, whose concentration would be affected by changes in growth rate but not directly by osmolarity. Transient induction of *kdp* by osmolyte addition (Laimins *et al* 1981) may be explained as occurring indirectly, consequent to a shift of intracellular K^+ from the second into the first compartment above. A hypothesis for such compartmentation of K^+ into separate osmoregulatory and growth rate-regulated fractions in a related enterobacterium, *Aerobacter aerogenes*, was in fact earlier suggested by Tempest and Meers (1968).

(c) Sustained induction of *kdp* expression by the monovalent cations, Na^+ and NH_4^+ (Laimins *et al* 1981; Gowrishankar 1985; Sutherland *et al* 1986), when they are used as osmolytes at 0·3 or 0·4 M, is also indirect, and is consequent either to inhibition by them of the 'true' osmoregulatory K^+-uptake system, or to activation by them of a K^+-efflux route in the cell. The diversion of available intracellular K^+ into the 'osmotic' compartment under these conditions serves to deplete the concentration in the second compartment and so leads to sustained *kdp* induction.

4. Conclusions

In conclusion, the model proposed here is similar to the internal $[K^+]$-control model discussed in § 2·2 above, with a modification which assumes that the $[K^+]$ accumulated in response to osmotic stress is not seen by the *kdp* control mechanism. It is postulated that there are two events which are regulated by osmotic stress, namely the size of *one* of the two intracellular K^+ compartments and the activity of a *second* K^+-uptake system. It is likely that TrKa represents the latter transporter, but additional experiments are necessary to confirm this possibility.

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