Chapter 5. Model for control of \textit{kdp} transcription

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As described in the previous chapters, many of the results obtained in this study provide evidence against the turgor-model for \(kd\)p regulation. If not turgor, what is the signal which regulates \(kd\)p expression? In this chapter, alternative possibilities for the signal and the constraints placed on such possibilities are discussed. A model invoking \(K^+\)-flux rate as the signal controlling \(kd\)p is proposed.

5.1. CONSTRAINTS ON ALTERNATIVE MODELS

The fact that the strength of the environmental signal controlling \(kd\)p expression is influenced primarily by \([K^+]_e\), and that it is perturbed by mutations in the genes encoding each of three different \(K^+\)-transporters Kdp, TrkA, and TrkD would suggest that the signal must be related to \([K^+]_i\), or to the process of transmembrane \(K^+\)-transport. Three alternative possibilities may be considered, but each has its own limitations in providing a complete explanation.

The first possibility is that \([K^+]_i\) itself serves as the signal. The argument against this is the finding by various research groups that steady-state \([K^+]_i\) increases with increasing osmolarity of the growth medium, and that this increase is modulated also by the ability of these cells to accumulate other compatible solutes such as glycine betaine, L-proline or trehalose (24, 25, 48, 142). On the other hand, the results obtained in this study clearly indicate that \(kd\)p expression is not appropriately altered under such conditions (i.e., steady-state \(kd\)p expression is not affected by osmotic strength of the medium nor by the accumulation of other compatible solutes).

Nevertheless, there is some evidence in support of \([K^+]_i\) itself being the signal. In direct contradiction to the results of work cited above, several other reports suggest that \([K^+]_i\) increases only transiently when cultures are subjected to osmotic upshock, and that it subsequently returns to a value close to that obtaining at low osmolarity (39, 108, 109). In accord with this conclusion, Ohwada and Sagisaka (108) have reported that \(E.coli\) cultures grown under conditions of \(K^+\)-excess and of \(K^+\)-limitation, when subjected to osmotic
upshock, differ only in the duration of lag-phase before resumption of growth, and that the subsequent growth rates in the two cultures are identical; similarly, Ohyama et al (109) have shown that *E. coli* can adapt to osmotic upshock even in the presence of a protonophore-uncoupler (under which conditions there is no increase in \([K^+]_i\)), and once again it is only the lag-phase which is prolonged by such treatment. If these views were correct, \([K^+]_i\) itself remains as a plausible candidate for the signal controlling *kdp* expression. Moreover, the growth rate of *E. coli* in high-osmolarity media has been shown not to be affected either by \(K^+\)-limitation (108) or under conditions where \(K^+\) is unable to accumulate intracellularly (109).

The second possibility for the signal regulating *kdp* expression merits consideration in case it is established that steady-state \([K^+]_i\) does indeed increase with increasing osmolarity. In that event, how does one reconcile the notion that *kdp* regulation is related to \([K^+]_i\) but not to medium osmolarity during steady-state growth? It was to account for this apparent paradox that Gowrishankar had postulated that intracellular \(K^+\) might be compartmentalized (57); interestingly, Tempest and Meers (144) had made the same suggestion, for somewhat similar reasons, twenty-five years ago. In neither case, however, was the nature of such intracellular compartment(s) clearly specified. Two recent reports, by Wiggins (153) and Cayley et al. (25) respectively, indicate the possible existence of distinct \(K^+\) compartments within the cell.

Wiggins (153) has postulated that intracellular water is not homogeneous but exhibits a continuum of densities, with the most dense (least ordered) layer adjacent to the surfaces of polyanions (nucleic acids, proteins and acidic phospholipids in the membrane) and the least dense (most ordered) some distance away; furthermore, cations such as \(K^+\) exhibit differential partitioning behaviour in water regions of differing densities. In a more explicit formulation, Cayley et al. (25) have proposed the polyelectrolyte model for distribution of \(K^+\) in the *E. coli* cytoplasm, according to which \([K^+]_i\) may be considered as the sum of two components: one, a solution of \(K^+\)/polyanions (approximately 50% dissociated), whose
concentration is independent of osmolarity; and the other, an almost completely ionized solution of potassium glutamate, whose concentration (i) increases with increasing osmolarity and (ii) is reduced when glycine betaine or L-proline are permitted to accumulate in the cytoplasm of high-osmolarity-grown cells. Cayley et al. (25) further state that the concentration of K⁺ bound to polyanions (referred to herein as [K⁺]ₜ and representing the non-dissociated fraction of the first component above) would remain constant at all osmolarities of the growth medium. It is conceivable then, that [K]ₜ (or a component thereof) is the signal regulating kdp expression as also the activities of all K⁺-transporters so far considered to be turgor-regulated. However, the molecular details of such a model invoking compartmentalisation of [K⁺]ₜ remain undefined.

Notwithstanding the discussion above, two findings from the present study provide major arguments against the notion that [K⁺]ₜ or [K⁺]ᵢ is the signal for kdp regulation, i.e., against both possibilities 1 and 2. The first of these relates to the behavior of the 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⁺]ₑ across which homeostatically controlled parameters, such as turgor, [K⁺]ᵢ, or [K⁺]ₜ, in any intracellular sub-compartment, are expected to remain constant (44, 49). The observation that kdp expression in glycine betaine-supplemented media is altered at varying [K⁺]ₑ despite a constant growth rate (another finding from the present study) is 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 therefore suggested that a third novel possibility be considered in which K⁺-flux rate serves as the signal regulating kdp expression; a model incorporating this suggestion is further described below.
5.2. HYPOTHESIS FOR THE kdp REGULATORY SIGNAL

According to this third possibility, the kdp regulatory signal is related to the specific rate of transmembrane K\(^+\)-influx, (expressed per unit dry weight of cells), such that the kdpABC operon is activated whenever the flux rate is decreased. Because the influx rate is expected to increase monotonically with increasing $[K^+]_e$ (with K\(^+\)- and turgor-homeostasis under these conditions being achieved by concomitant increases in efflux rates, as further discussed below), such a model would provide an explanation for observations with GJ618 and glycine betaine-supplementation. The effects of solutes such as Na\(^+\) or NH\(_4^+\), 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 $[K^+]_e$. This hypothesis will be consistent with the fact that the KdpD sensor protein is resident in the cytoplasmic membrane (106, 149), and may also explain the observation that the kinetics of KdpD autophosphorylation in vitro are in part determined by the presence or absence of KCl in the assay medium (106).

This model would require that some mechanism exists by which the influx rates through each of the Kdp, TrkA and TrkD transporters are integrated to provide a single measure of signal strength. How could such integration be achieved? It is suggested that the various K\(^+\)-transporters in E. coli all share a common subunit protein, the gene for which has not so far been identified because null mutations in it would be lethal. This shared subunit is proposed to interact with the KdpD sensor protein in transmitting the flux rate signal from each of the transporters in order to activate or repress kdp operon transcription.

For potassium ion homeostasis at high $[K^+]_e$, this model would invoke the functioning of distinct turgor- or $[K^+]_b$-activated K\(^+\)-efflux system(s), ion transport through which is not part of the kdp regulatory signal. Besides the KefB and KefC efflux systems that have been genetically characterised (8, 46) in E. coli, there is believed to be a third one which is turgor-activated (8) and perhaps a fourth which mediates efflux in response to
alkalinization of the cytoplasm (8). The characteristics of the turgor-activated efflux system which have so far been determined are compatible with the requirements of this model.

5.2.1. Can the shared subunit represent a K⁺ channel?

In earlier papers (122, 123), Epstein's group had suggested that K⁺-uptake in *E.coli* might occur through a single type of K⁺-channel, and that each of the genetically defined uptake systems (Kdp, TrkA and TrkD) might represent interactions of particular sets of gene products with this channel in order to confer characteristic properties of kinetics and energetics on the transport process; the transport activity (TrkF) that remains in the triple-mutant strain would then represent the basal activity of this channel. Subsequently, these workers (40, 49) and others (131) appear to have moved away from this notion to one where each transport system represents a self-contained transporter, but reconstitution studies have so far not been reported to support the latter claim.

The idea of a common K⁺-channel is attractive because the signal for *kdp* transcription could then be related to the rate of K⁺ movement through this channel. The integration of the flux rates for the three uptake systems could be achieved by the association of this channel with each of them. Kdp, TrkA and TrkD would be distinct energy-generating or energy-coupling assemblies which confer specific energetic and kinetic properties to the uptake process via the channel. The rate of such ion-flux would depend on (i) whether the Kdp, TrkA and TrkD transport systems are functional or defective, and (ii) the [K⁺]ₑ, even when the latter is in the high range. It is also assumed that this rate is affected by environmental factors (such as pH, temperature and concentration of other cationic solutes), that are known to affect strength of the regulatory signal in a [K⁺]ₑ-dependent manner, but that it is independent of osmolarity of the medium during steady-state growth. The induction of *kdp* following osmotic upshock might reflect a direct and transient activation of membrane-associated KdpD consequent on plasmolysis, whereas regulation by growth rate may be explained on the assumption that the latter influences the density of K⁺-channels in the membrane.
The residual transport activity, designated TrkF, in triple-mutant strains such as TL1105A could represent passive uptake of K\(^+\) through the channel, driven by the membrane potential. This would explain the increased [K\(^+\)]\(_\text{e}\)-requirement for growth of these strains at low pH, when the membrane is relatively depolarized (16). All the available data on TrkF-mediated transport (44, 97, 122, 123) are compatible with this notion.

According to the channel model, one would expect that KefB- or KefC-mediated efflux also occurs through the common K\(^+\)-channel, and that the signal for regulation of \textit{kdp} expression is related not to unidirectional influx rate but to the net influx rate of K\(^+\). The data on the \textit{kefB} and \textit{kefC} mutants above are not so easy to interpret, however, because null mutants in these genes have no overt growth phenotype whatsoever (not even the expected sparing of [K\(^+\)]\(_\text{e}\)-requirement for growth of \textit{kdp} mutants, [8]), and hence the true physiological functions of KefB and KefC are unknown (43). One major argument against a common channel subserving K\(^+\) transport is the observation that TrkD transports Cs\(^+\); channels, at least in eukaryotic cells, are known to be size-selective in terms of the ions they transport.

### 5.2.2. Distinction between channels and transporters—present dogma

Classically transporters and channels are believed to be distinct entities and some of the characteristics used to distinguish between them are discussed below. (a) Transporters are generally considered to be enzyme-like, interacting stoichiometrically with their substrate and undergoing defined conformational changes during each transport cycle. Transporters allow translocation of substrate by alternating access to binding sites first to one side of the membrane and then to the other. In contrast, channels are more akin to holes which, when functional, are simultaneously open at both sides of the membrane. They allow non-stoichiometric passage to molecules with appropriate characteristics. (b) The turnover number of transporters (i.e., the number of molecules of substrate that can be transported per second) is necessarily restricted by enzyme-like conformational changes. On the other hand, channels are ultimately diffusion limited, and have very high turnover numbers. (c) Channels
do not use energy for transport and simply facilitate equilibration of substrate in response to concentration and electrochemical gradients. Transporters, however, can utilize energy to concentrate substrate against a gradient. (d) Channels are usually gated (for example by a ligand or by voltage) alternating between open and closed states whereas transporters are not gated. (e) Channels, in contrast to transporters, are extremely selective with respect to the substrate they transport (64).

5.2.3. Can channels exist in the *E. coli* cytoplasmic membrane?

Two important implications of the model discussed above are that K+ channels exist in the *E. coli* cytoplasmic membrane and that the K+ transporters in *E. coli* should have both transporter properties as well as channel properties. How likely are these possibilities? The first of these is discussed here while the second possibility is discussed in the following section.

As mentioned above, a crucial property used to distinguish between channels and transporters is turnover number. The turnover number for channels is usually very high, in the range of $10^4$-$10^8$ substrate molecules transported per second per protein molecule. On the other hand, transporters usually have, on an average, turnover numbers of up to $10^3$ per second (3). Maloney (87) has postulated that given the facts that channels pass ions rapidly and that bacteria are extremely small-sized (compared to eukaryotes), the existence of bacterial channels, if at all they do occur, would place them at risk of uncoupling i.e., a collapse of the proton gradient. He has argued that because of this reason, channels in bacteria are restricted to the outer membrane, in contrast to the situation in eukaryotes where channels often reside in the plasma membrane. Notwithstanding Maloney's argument, there have been several recent reports on the presence of ion channels in the cytoplasmic membranes of bacteria. Delcour et al (38) have described the reconstitution of a voltage-sensitive, cation-selective ion channel of *E. coli* into liposomes. The same group of workers have also described studies using patch-clamp techniques on mechanosensitive channels of giant *E. coli* spheroplasts (90). Furthermore, using electrophysiological techniques on
isolated inner membrane preparations, Simon and Blobel (134) have reported the identification of K+ channels in *E. coli*, but these have not been further defined or characterized. These reports therefore provide evidence for the presence of ion channels in the cytoplasmic membranes of bacteria, although the properties of prokaryotic channels may differ from those of eukaryotes.

5.2.4. Can transport systems display both transporter and channel properties?

In a recent thought-provoking review, Ames and Lecar (3) have discussed the analogy between channels and transporters. They contend that the distinction between channels and transporters is somewhat blurred as both systems have essential features in common. They also argue that channels with turnover numbers lower than $10^4$ might exist and may not be detectable with the present technology of single channel measurement. Recent studies on eukaryotic transport systems have revealed the existence of functionally distinct transporter and channel activities residing in the same protein. Examples of such systems come from two classes of proteins: the ABC (ATP-binding cassette) transporters and the P-type ATPases. Transport function in each of the ABC transporters is coupled to ATP hydrolysis. The P-glycoprotein, a human multidrug resistant ABC transporter is known to transport drugs using energy from ATP hydrolysis. It has also recently been found to have a functionally distinct volume-regulated Cl⁻ channel activity (54, 146). Although ATP hydrolysis is not required for channel activation, ATP binding appears to be obligatory for the purpose (54). A second example is the CFTR protein (alterations in which lead to the inherited disorder cystic fibrosis), which belongs to the family of ABC transporters by virtue of its homology with members of this class, and yet has been shown to be a cAMP-regulated Cl⁻ channel (5). Surprisingly, unlike any other channel, CFTR reportedly needs ATP hydrolysis for its channel function (6). An associated transporter function has also been postulated (64), but is yet to be identified for this protein.
As is the case for ABC transporters, transport catalysed by the P-type ATPases is also coupled to ATP hydrolysis. This class of proteins (which includes Kdp transporter as well, see Chapter 1) derives its name from the fact that a phosphorylated protein intermediate is formed during the transport process (whereas there is no evidence for such a mechanism operating in ABC transporters). Among the P-type ATPases, the mammalian Na\(^+\) K\(^+\)-ATPase which transports sodium and potassium ions has been recently shown also to have certain properties characteristic of channels (52). The ATPase1 gene from *Plasmodium falciparum* codes for the longest reported cation ATPase and is another example of a protein postulated to perhaps have both transporter and channel function. The amino acid sequence of the conserved cytoplasmic domains of the latter protein shares homology on the one hand with members of the P-type ATPase family and, on the other, with hydrophobic segments of eukaryotic K\(^+\)-channel proteins (77). These two examples provide the first link between the cation ATPases and the superfamilies of ion channels. It has been suggested that P-type cation ATPases arose as an "evolutionary mosaic" when a primitive ATP hydrolysing domain combined with different types of ion channels (77). Another example of a carrier with dual functions is A23187, which is a calcium ionophore. Recently, Balasubramanian et al. (9), have shown that this ionophore can form channels with properties similar to gramicidin when incorporated in lipid bilayers.

Ames and Lecar (3) have argued that an incipient channel would be an ancestral characteristic of all membrane transport entities; such an ancestral system may evolve as a full-fledged channel or as a transporter or may exhibit dual properties.

5.3. SUMMARY OF NEW MODEL FOR CONTROL OF *kdp* TRANSCRIPTION

In the new model for *kdp* expression, the regulatory signal is related to specific rate of transmembrane K\(^+\)-influx, such that the *kdp* operon is activated whenever the flux rate is decreased. It is suggested that the various K\(^+\)-transporters in *E. coli* all share a common subunit protein. This shared subunit, perhaps a channel, is proposed to interact with the KdpD sensor protein in integrating and transmitting the flux rate signal from each of the
transporters in order to activate or repress kdp operon transcription. The genetic predictions of this model would be that (a) null mutations in the gene encoding the putative shared subunit protein would be lethal, (b) these mutations can be identified as conditional lethal (temperature-sensitive) mutations, and that such strains would exhibit kdp-lac derepression upon shift to the restrictive temperature even in high-[K+]e media.