The Kdp-ATPase system and its regulation

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K^+, the dominant intracellular cation, is required for various physiological processes like turgor homeostasis, pH regulation etc. Bacterial cells have evolved many diverse K^+ transporters to maintain the desired concentration of internal K^+. In *E. coli*, the KdpATPase (comprising of the KdpFABC complex), encoded by the *kdpFABC* operon, is an inducible high-affinity K^+ transporter that is synthesised under conditions of severe K^+ limitation or osmotic upshift. The *E. coli kdp* expression is transcriptionally regulated by the KdpD and KdpE proteins, which together constitute a typical bacterial two-component signal transduction system. The Kdp system is widely dispersed among the different classes of bacteria including the cyanobacteria. The ordering of the *kdpA*, *kdpB* and *kdpC* is relatively fixed but the *kdpD/E* genes show different arrangements in distantly related bacteria. Our studies have shown that the cyanobacterium *Anabaena* sp. strain L-31 possesses two *kdp* operons, *kdp1* and *kdp2*, of which, the later is expressed under K^+ deficiency and desiccation. Among the regulatory genes, the *kdpD* ORF of *Anabaena* L-31 is truncated when compared to the *kdpD* of other bacteria, while a *kdpE*-like gene is absent. The extremely radio-resistant bacterium, *Deinococcus radiodurans* strain *R1*, also shows the presence of a naturally short *kdpD* ORF similar to *Anabaena* in its *kdp* operon. The review elaborates the expression of bacterial *kdp* operons in response to various environmental stress conditions, with special emphasis on *Anabaena*. The possible mechanism(s) of regulation of the unique *kdp* operons from *Anabaena* and *Deinococcus* are also discussed.

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1. Introduction

Potassium (K^+), the major intracellular cation, is essential for life in both prokaryotic and eukaryotic cells. The high intracellular concentration of K^+ in bacteria reflects an ancient strategy of living cells to accumulate K^+ and exclude Na^+ ion (Epstein 2003). K^+, being the most dominant intracellular cation, makes a major contribution to the turgor pressure of the cells. K^+ not only plays a vital role in bacterial osmotic adaptation (Epstein 1986) but is also important for pH regulation (Booth 1985), gene expression (Prince and Villarejo 1990) and activation of cellular enzymes (Suelter 1970). K^+, both as an extracellular signal and as an intracellular metabolic regulator, plays critical role in adaptation to stressful environments in bacteria (Csonka and Hanson 1991). The heterocystous, filamentous cyanobacterium *Anabaena* (used as a biofertilizer in rice paddy fields) shows a strong dependence on K^+ for normal growth and cellular functions like photosynthesis and nitrogen fixation (Alahari and Apte 1998). Therefore, study of K^+ metabolism is not only important to understand molecular basis of cyanobacterial stress tolerance, but is also crucial for development of novel cyanobacterial biofertilzers for use in adverse field conditions.

Despite the trace amounts of K^+ available in most environments (0.1–10 mM), bacteria maintain a high concentration of K^+ (0.1-0.6 M) within their cells (McLaggan *et al* 1994). Bacteria have evolved several K^+ uptake systems to maintain their internal K^+ levels. The activity of the K^+ transport systems is regulated in such a manner that there

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Abbreviations used: CTD, C-terminal domain; K^+, potassium; NTD, N-terminal domain; ORF, open reading frame; RR, response regulator; SK, sensor kinase.

is no futile cycling of K^+ across the membrane (Stumpe *et al* 1996). In *Escherichia coli*, the constitutively expressed K^+ transporters Trk and Kup (Epstein and Kim 1971) have low affinity for K^+ but are competent to maintain required levels of K^+ under normal physiological conditions. When *E. coli* cells are subjected to extreme K^+ limitation or osmotic upshock, the high affinity (K_m= 2 μM) Kdp-ATPase (Potassium dependent adenosine triphosphatase) is expressed (Laimins *et al* 1981), which can reduce K^+ concentration in the medium to as low as 50 nM (Stumpe *et al* 1996). Thus, the Kdp-ATPase is an efficient K^+ scavenging system that is expressed when other K^+ transporters cannot support cellular requirement for K^+.

E. coli Kdp-ATPase, the most widely characterized Kdp system, is made up of four protein subunits viz. KdpF, KdpA, KdpB and KdpC (Gassel *et al* 1999). The KdpF is important for stability, KdpA subunit binds and transports K^+, KdpB is similar to other P-type ATPases and contains the highly conserved phosphorylation site while the KdpC subunit plays a role in the assembly of the Kdp-ATPase complex. The KdpA, KdpB and KdpC subunits are absolutely essential for Kdp-ATPase function *in vivo* (Epstein 2003). In *E. coli*, the genes encoding the KdpFABC complex are organized in a single operon i.e. *kdpFABC* operon.

The regulatory proteins KdpD and KdpE, which belong to a family of sensor kinase and response regulator systems, control *kdpFABC* expression in *E. coli* (Polarek *et al* 1992). The *E. coli* KdpD (894 amino acids, 99 kDa) consists of a cytosolic N-terminal domain (NTD) and a cytosolic C-terminal domain (kinase domain) interconnected by the four transmembrane segments (Zimmann *et al* 1995). The transmembrane segments along with the C-terminal domain are hereafter referred to as CTD. The KdpE (225 amino acids, 25 kDa) is a cytosolic response regulator with DNA binding capability (Nakashima *et al* 1992).

2. Occurrence of Kdp system in bacteria

BLAST search with Kdp protein sequences shows that the Kdp-ATPase system is widely distributed among the Gram negative bacteria (e.g. *E. coli*, *Salmonella typhimurium* LT2, *Clostridium acetobutylicum*) the Gram positive bacteria (e.g. *Bacillus cereus* E33L, *Alicyclobacillus acidocaldarius*), cyanobacteria (e.g. *Synechocystis* PCC 6803, *Anabaena* sp. strain L-31) and the archea (e.g. *Halobacterium* sp. NRC-1, *Thermoplasma volcanium* GSS1). Walderhaug *et al* (1989) used *E. coli kdpABC* and the *kdpD* gene probes to screen chromosomal DNA (for homology with *E. coli kdp*) from different bacteria. The enterobacteria (*Shigella flexineri*, *Yersina pestis* etc.) showed good homology to *kdpABC* probes and the *kdpD* probe. Among the cyanobacteria, the unicellular cyanobacteria *Synechococcus* sp. strain 6301 and *Anacystis nidulans* showed homology to the *E. coli kdpABC* genes while the filamentous cyanobacteria (*Anabaena torulosa*, *Anabaena* sp. strain PCC7120 and *Anabaena* sp. strain L-31) did not show any homology to the *E. coli kdp* genes (Walderhaug *et al* 1989).

A Kdp-like system with high affinity for K^+ was identified from the bacterium *Alicyclobacillus acidocaldarius*. When allowed to grow in conditions of K^+ starvation, a 70 kDa protein showing cross-reactivity to the *E. coli* KdpB antiserum was induced in this bacterium (Bakker *et al* 1987). Subsequent studies have shown the high-affinity K^+-transporting ATPase from *A. acidocaldarius* to consist of 3 sub units KdpA, KdpB and KdpC (Hafer *et al* 1989). Phototrophic bacteria *Rhodobacter capsulatus* and *Rhodospirillum rubrum* produced a 70-75 kDa polypeptide that showed immunological cross-reactivity to *E. coli* KdpB antiserum when grown in conditions of limiting K^+ (Abee *et al* 1992a). Abee *et al* (1992b), have purified the Kdp-ATPase from *Rhodobacter sphaeroides* and shown that, like other Kdp-ATPases, it comprises of KdpA, KdpB and KdpC subunits. Recently, the KdpE homologue from *Listeria monocytogenes* has been shown to contribute to the growth of that bacterium during osmotic stress and low temperature (Bronstead *et al* 2003). The requirement of absence of K^+, for induction of *kdp* from 3 different strains of mycobacteria has also been demonstrated (Steyn *et al* 2003).

Earlier studies from our laboratory have shown nitrogen-fixing cyanobacteria, *Anabaena* spp. to possess a Kdp-like system. Under conditions of K^+ limitation, three strains of *Anabaena* (i.e. *Anabaena torulosa*, *Anabaena* sp. strain PCC 7120 and *Anabaena* sp. strain L-31) synthesized a 78 kDa protein that cross-reacted with the *E. coli* KdpB antiserum indicating the presence of Kdp homologs in *Anabaena* (Apte and Alahari 1994). This KdpB-like polypeptide was subsequently shown to be located in the membrane fraction of *Anabaena* (Alahari *et al* 2001). Recently, we have detected KdpB polypeptide from a unique, highly radio-resistant bacterium, *Deinococcus radiodurans*, employing the *E. coli* KdpB antiserum. The polypeptide was located in *D. radiodurans* membranes and was found only under conditions of K^+ limitation (Basu B, unpublished results).

3. Organization of *kdp* operons in bacteria

In several bacteria, genes encoding the structural subunits of the Kdp-ATPase (i.e. *kdpA*, *kdpB* and *kdpC*) are arranged in an operon while the genes encoding the regulatory KdpD/E proteins (i.e. *kdpDE*) constitute another operon (Walderhaug *et al* 1992; Treuner-Lange *et al* 1997). The ordering of the *kdpA*, *kdpB* and *kdpC* is relatively fixed but the *kdpD/E* genes show different arrangements in distantly related bacteria. The organization of *kdp* operons from different bacteria is shown in figure 1. The *E. coli kdp* operon consists of *kdpF*, *kdpA*, *kdpB*, and *kdpC*, arranged in that

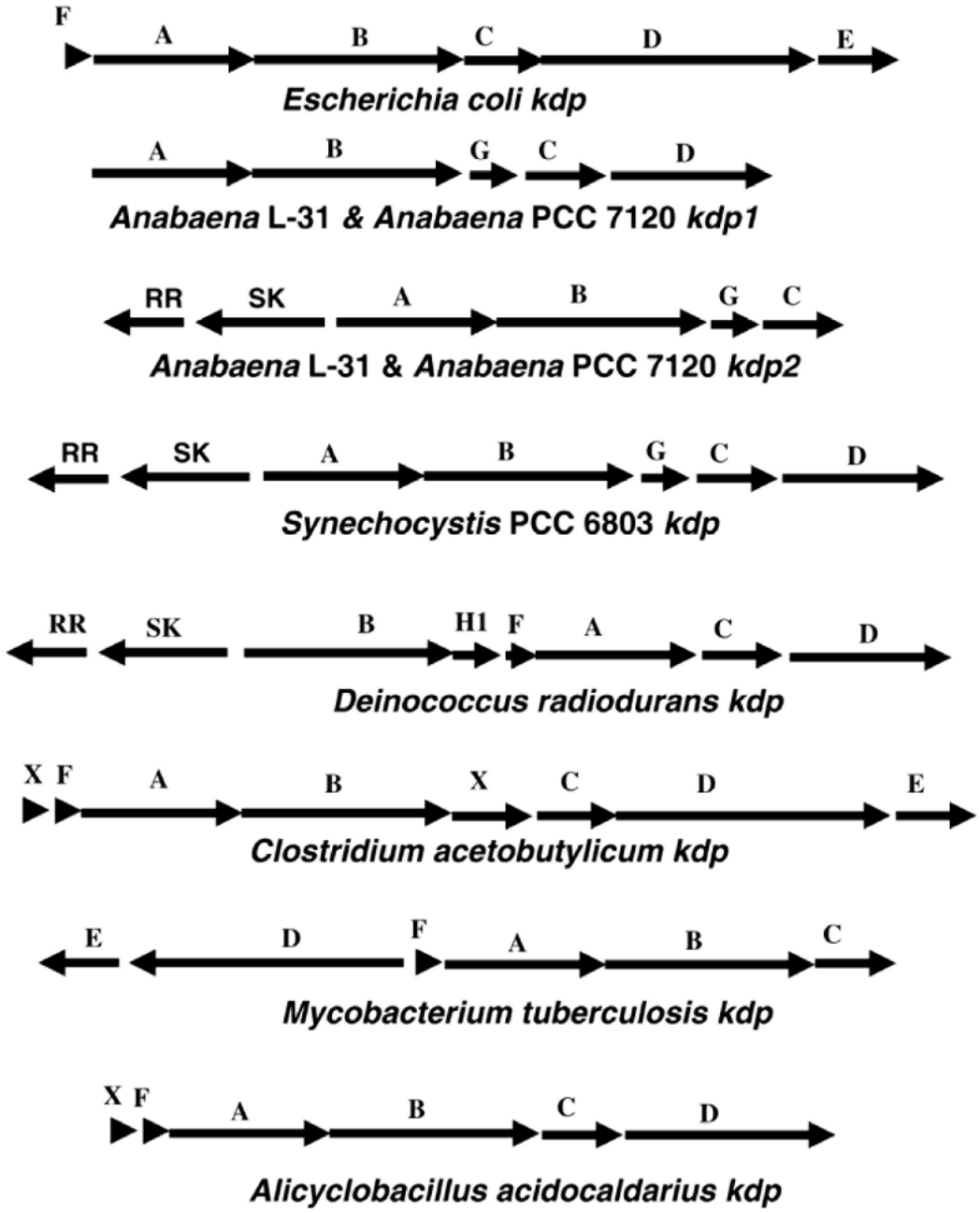

Figure 1. Organization of *kdp* operons in bacteria. The arrowheads indicate the direction of translation as determined from the nucleotide sequence. *E. coli* (Walderhaug *et al* 1992, Gassel *et al* 1999), *Anabaena* L-31 *kdp1*/*kdp2* (Ballal and Apte 2005), *Anabaena* 7120 *kdp1*/*kdp2* (Kaneko *et al* 2001), *Synechocystis* 6803 (Kaneko *et al* 1996), *Deinococcus radiodurans kdp* (White *et al* 1999), *Clostridium acetobutylicum kdp* (Treuner-Lange *et al* 1997), *Mycobacterium tuberculosis kdp* (Cole *et al* 1998), *Alicyclobacillus acidocaldarius kdp* (Schleussinger *et al* 2006).

order, while the *kdpDE* genes, which constitute a separate operon, are situated downstream of *kdp*C gene (Hesse *et al* 1984; Polarek *et al* 1992). In *Clostridium acetobutylicum*, the arrangement of *kdpABCDE* is similar to that of *E. coli* except that two additional ORFs, *kdpZ* and *kdpX* (both encoding hydrophobic peptides), are present before *kdpF* and *kdpD* respectively (Treuner-Lange *et al* 1997). The KdpZ, KdpY and KdpX are also shown to be a part of the functional Kdp complex from *C. acetobutylicum*. (Behrens *et al* 2001). In *Mycobacterium tuberculosis*, the arrangement

of the *kdpFABC* is similar to that of *E. coli* but the *kdpDE* genes are arranged in reverse orientation upstream of the *kdpA* gene (Cole *et al* 1998) (figure 1).

We employed a PCR based strategy to clone the *kdp* operon from *Anabaena* sp. strain L-31 (hereafter referred to as *Anabaena* L-31). The analysis of *kdp*-like PCR products showed the presence of not one but two distinct *kdp* operons in *Anabaena* L-31 (termed *kdp1* and *kdp2*) (Ballal and Apte 2005). All bacteria, wherein *kdp* has been identified, show the presence of single *kdp* operon. That *Anabaena* L-31 should possess more than one *kdp* operon is, therefore, interesting. Nucleotide sequence analysis of *kdp1* (GenBank accession No. AF213466) showed the presence of 5 open reading frames (ORFs) viz. *kdpA1*, *kdpB1*, *kdpG1*, *kdpC1* and *kdpD*. The *kdp2* operon (GenBank accession No. AY753299) showed the presence of 4 open reading frames ORFs i.e. *kdpA2*, *kdpB2*, *kdpG2* and *kdpC2*. While a *kdpF*-like gene was absent from the two *Anabaena* L-31 *kdp* operons, an additional ORF, *kdp*G, was found to be located at an unusual location between *kdpB* and *kdpC* (figure 1). The *Anabaena* L-31 *kdpG* appears to encode a hydrophobic protein with two transmembrane segments.

The *kdp* genes from *Anabaena* L-31 *kdp* operon showed only 55-70% nucleotide sequence identity to the corresponding genes from other *kdp* operon and indicated that the two *kdp* operons were not identical copies of each other. A naturally short *kdpD* ORF was observed downstream of *kdpC1*, while no such ORF was observed in the *kdp2* operon of *Anabaena* L-31 (figure 1). The *kdpD* ORF encoded a protein of only 365 amino acids corresponding to the KdpD-N terminal domain of *E. coli*; while the C-terminal histidine kinase domain that is critical for phosphotransfer reactions (Voelkner *et al* 1993) was missing from *Anabaena* L-31 KdpD. No *kdpE*-like gene was found in the vicinity of the two *kdp* operons.

Another filamentous, N_2-fixing cyanobacterium, *Anabaena* sp. PCC 7120 (Kaneko *et al* 2001) also possesses two *kdp* operons very similar to those found in *Anabaena* L-31. However, Kdp is not ubiquitous in cyanobacteria and some of them e.g. *Thermosynechococcus elongatus* BP-1 (*www.kazusa.or.jp/cyano/thermo*) do not contain *kdp* in their genome. Among other filamentous cyanobacteria whose genomes have been sequenced, *Nostoc punctiforme* (*http://genome.jgi-psf.org/draft_microbes/nospu/nospu*) and *Nodularia spumigena* CCY9414 (Accession No. AAVW00000000, unfinished genome sequence) both show presence of two *kdp* operons while *Anabaena variabilis* (*http://genome.jgi-psf.org/finished _microbes/anava/anava*) possess three *kdp* operons. Unicellular cyanobacteria like *Synechocystis* PCC6803 (*www.kazusa.or.jp/cyano/synechocystis*), *Synechococcus elongatus* (*http://genome.jgi-psf.org/finished_microbes/synel/syne*) and *Gloeobacter violaceous* (*www.kazusa. or.jp/cyano/gloeobacter*) possess only one *kdp* system. Thus, as per the information available today, unicellular cyanobacteria have single *kdp* system or none, whereas filamentous cyanobacteria have two or more.

The extremely radioresistant bacterium, *Deinococcus radiodurans*, shows a single *kdp* operon with a different gene arrangement i.e. *kdpB* is the first gene of the operon followed by a hypothetical ORF (*H1*), *kdpF*, *kdpA*, *kdpC* and finally by *kdpD* (in that order) (figure 1). Like cyanobacterial KdpD, *D. radiodurans kdpD* is also naturally truncated, encoding a protein similar to the *E. coli* KdpD-NTD while the *kdpE*-like gene is absent (White *et al* 1999). The *kdp* operon from the thermophilic bacterium, *Alicyclobacillus acidocaldarius*, displays a *kdpD* ORF that encodes a protein, resembling head to tail fusion of two KdpD-NTD equivalents, while the *kdpA*, *kdpB*, and *kdpC* are similar to those of other bacteria (GenBank Acc. No. AJ715821) (figure 1) Thus, a great variety in the number and the arrangement of genes exists among *kdp* operons of bacteria.

4. Expression of *kdp* operons in response to K^+ limitation

All the bacterial *kdp* operons investigated so far are repressed during growth in media of high external K^+ concentration ($[K^+]_e$), and there are no exceptions to this rule. Interestingly, of the two *kdp* operons present in *Anabaena* L-31, only *kdp2* (and not *kdp1*) expression could be detected as a 5.3 kb transcript on Northern blots (Ballal and Apte 2005). Most of the *kdp* expression related data, especially in *E. coli* and *S. typhimurium*, have come from the *kdp*-promoter-*lacZ* fusion experiments and very little information is available regarding the expression and size of the *kdp* transcripts (mRNA) *per se*. The observation that the *E. coli kdpF*, *kdpA*, *kdpB*, and *kdpC* genes do constitute an operon is based on the polar effects of λ integration into the *kdpA* gene (Rhoads *et al* 1978), but no full-length (*E. coli*) *kdpFABC* transcript has been experimentally demonstrated. The presence of a 5.3-kb signal clearly demonstrates that the *Anabaena* L-31 *kdpA2B2G2C2* does indeed constitute an operon, and that these genes are co-transcribed in response to K^+ limitation. Minor *kdpA1 or kdpC1* expression could be detected by the very sensitive RT-PCR technique. However, the amount of *kdpA1/C1* PCR products was several-fold lower than that obtained with *kdpA2* specific primers, again confirming that *kdp2* is the major *kdp* operon expressed under K^+ starvation in *Anabaena* L-31 (Ballal and Apte 2005).

In wild type *E. coli* and *C. acetobutylicum*, the *kdp* expression occurs when the external K^+ ($[K^+]_e$) falls below 2 mM and 4 mM respectively (Laimins *et al* 1981; Treuner-Lange *et al* 1997). In contrast, the *Anabaena* L-31 *kdp2* operon shows a much lower threshold of $[K^+]_e$ (50 μM), while no expression was observed above 0.1 mM $[K^+]_e$

(Ballal and Apte 2005). The *Synechocystis* PCC 6803 global gene expression studies have also shown the *kdp* operon to remain uninduced in BG-11 medium wherein the $[K^+]_e$ is 0.175 mM (Kanesaki *et al* 2002). The threshold of $[K^+]_e$ for *kdp* expression appears to be considerably low in cyanobacteria as compared to *E. coli* or *C. acetobutylicum.*

E. coli and *Salmonelella typhimurium* both have three major K^+ transporters viz. Kup, Trk and the Kdp (Stumpe *et al* 1996). The constitutively expressed Trk and Kup have a low affinity for K^+ (K_m=1.5 mM). Once the $[K^+]_e$ falls below 2mM, desired levels of K^+ cannot be maintained by the constitutive systems and the high affinity Kdp is induced. Apparently, K_m (for K^+) of low affinity transporters appears to determine the threshold of *kdp* induction in bacteria.

Cyanobacteria (e.g. *Synechocystis* PCC 6803 and *Anabaena* PCC 7120) and a few other bacteria possess another K^+ uptake system called Ktr (Berry *et al* 2003; Matsuda *et al* 2004). The Ktr, which is the major K^+ uptake system in *Synechocystis* PCC 6803 (Berry *et al* 2003), has a moderately high affinity (K_m=60 μM) for K^+ (Matsuda *et al* 2004). Therefore, it is possible that in these organisms, Kdp would be required at an external K^+ concentration where Ktr would be incapable of K^+ uptake (i.e. less than 60 μM external K^+). However, the effect of *ktr* deletion on the threshold $[K^+]_e$ required to induce *kdpABGC* expression in *Synechocystis* PCC 6803 (or *Anabaena*) remains to be determined.

The relative affinities of various bacterial K^+ transporters (for K^+) and the external K^+ that induces *kdp* expression seem to relate well to the ecophysiological K^+ concentration found in the habitats of different microbes. For e.g. in mammalian gut (the natural habitat of *E. coli* and other enteric bacteria) the K^+ concentration is always higher than 10 mM K^+, and *kdp* is only expressed as an emergency system when $[K^+]_e$ falls below 1 or 2 mM. Naturally occurring soil bacteria (e.g. *Anabaena*) normally encounter 0.1 to 5 mM $[K^+]_e$. The $[K^+]_e$ perceived to be as 'limiting K^+' is therefore expected to be several fold lower ($\simeq$ 50 μm). Evolution appears to have selected K^+ uptake systems (with appropriate K_m) and threshold of Kdp induction for different bacteria in a need based manner.

5. Effect of osmotic stress on *kdp* expression

Laimins *et al* (1981) showed that the $[K^+]_e$ that induced *kdp* expression during steady-state growth was dependent on the osmolarity of the medium. In *E. coli*, for a given $[K^+]_e$, higher *kdp* expression was correlated with high osmolarity. Addition of ionic or non-ionic impermeable solutes to *E. coli* cultures, grown at $[K^+]_e$ that repressed *kdp* expression, resulted in transient increase of *kdp* expression (Laimins *et al* 1981). Glycerol, which is freely permeable, did not cause such transient induction. As increase in osmolarity reduced turgor pressure, Laimins *et al* (1981) proposed that reduction in turgor pressure induced *kdp* expression.

The turgor model was challenged by several sets of data that were not consistent with it. Asha and Gowrishankar (1993) showed that only ionic solutes could induce steady state *kdp* expression in *E. coli* while non-ionic solutes failed to do so. A decrease in pH of the medium (at a given $[K^+]_e$) increased the magnitude of *kdp* expression (Asha and Gowrishankar 1993). It is known that uptake of other compatible solutes like L-proline and glycine-betaine increases in response to osmotic stress (Dunlap and Csonka 1985; Cairney *et al* 1985; May *et al* 1986). These compatible solutes are expected to restore cell turgor during growth in media of high osmolarity and consequently decrease *kdp* expression (Gowrishankar 1987). The accumulation of these osmolytes occurs during osmotic stress, but the level of *kdp* expression remains unaffected under these conditions (Asha and Gowrishankar 1993). This indicates that *kdp* expression does not depend on availability of other compatible solutes that are expected to restore turgor. (Gowrishankar 1987; Asha and Gowrishankar 1993). Malli and Epstein (1998) re-examined the effects of compatible solutes and of medium pH on expression of *kdp* in studies wherein growth rate was also measured. In all cases, there was a correlation between *kdp* expression and $[K^+]_e$ at which growth began to slow. Malli and Epstein (1998) have postulated that the decrease in growth rate because of K^+ limitation was due to reduction in turgor. Since addition of betaine did not increase turgor, *kdp* expression was not affected. On this basis, Malli and Epstein (1998) reasoned that the data on *kdp* expression were consistent with control by turgor pressure. To summarize, the role of turgor in *kdp* regulation still remains unequivocal.

Unlike *E. coli*, the *Anabaena* L-31 *kdp2* operon does not respond to increase in osmolarity imposed by PEG or sucrose, and exposure to NaCl in fact represses *kdp* expression (Ballal and Apte 2005). Another strain of *Anabaena*, *Anabaena torulosa*, too does not show *kdp* expression in response to osmotic stress mediated by NaCl or sucrose (Alahari *et al* 2001). Global-scale gene expression analysis of *Synechocystis* PCC 6803 has also shown that the *kdp* is not induced in response to osmotic upshock (Kanesaki *et al* 2002). Thus, cyanobacteria, in general do not induce *kdp* in response to osmotic stress.

In natural ecological niches, desiccation is one of the most common environmental stresses faced by cyanobacteria. Interestingly, *Anabaena* L-31 *kdp2* expression was observed in response to desiccation stress. Recently, *kdp2* operon from *Anabaena* PCC 7120 was also reported to be induced in response to desiccation stress (Katoh *et al* 2004). Desiccation results in the loss of water and causes dehydration of cells. The cells try to overcome loss of water by pursuing a set of responses that tries to restore acceptable level of water in cytoplasm (Bremer and Kramer 2000). These physiological

responses primarily include influx of K^+ into the cell followed by synthesis of compatible solutes like glycine betaine and trehalose (Csonka and Epstein 1996; Poolman and Glaasker 1998). The expression of KdpATPase, encoded by the *Anabaena* L-31 *kdp2* operon, may fulfill the increased requirement of K^+ that occurs during desiccation stress.

6. Mechanism of kdp induction

Transcriptional activation of the *kdp* operon is mediated by a membrane-bound sensor kinase KdpD, which upon perception of the appropriate stimulus undergoes autophosphorylation (Nakashima *et al* 1992) (figure 2). The phosphoryl group is subsequently transferred to the cytosolic response regulator KdpE (KdpE~P), thereby transforming it into a transcriptional activator. The KdpE~P binds to a specific DNA sequence upstream of the *kdpFABC* promoter and induces transcription of the *kdp* operon (Nakashima *et al* 1993).

The NTD, the transmembrane segments and a cluster of positively charged arginine residues in the C-terminal domain (CTD) form the input domain involved in stimulus perception by the KdpD (Heermann *et al* 2000). The NTD includes two motifs that are very similar to the classical ATP binding 'Walker A' and the 'Walker B' motif (Walker *et al* 1982) (figure 2). These sites have been proposed to play an important role in fine-tuning the *E. coli* KdpD activity. The KdpD-CTD, which shows a moderate sequence similarity to other sensor kinases (Polarek *et al* 1992), harbors a conserved histidine residue (His-673) where phosphorylation occurs (Voelkner *et al* 1993) (figure 2). The

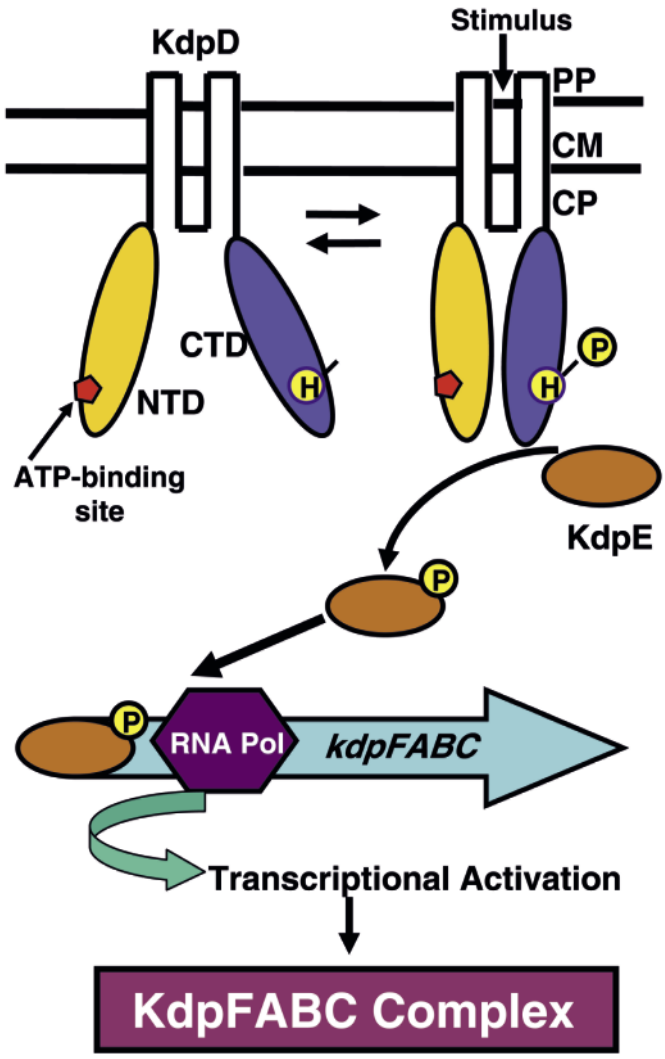

Figure 2. The KdpD-KdpE phosphorelay in *E. coli*. PP, periplasm; CM, cell membrane; CP, cytoplasm NTD, N′- terminal domain; CTD, C′- terminal domain; H, conserved histidine; P, phosphorylation.

phospho-KdpE phosphatase activity of the *E. coli* KdpD is significantly increased in presence of ATP (or its non-hydrolyzable analogs), whereas, other nucleotides (i.e. CTP, GTP etc.) have no effect (Jung and Altendorf 1998). The KdpD-NTD is known to modulate the *in vivo* and *in vitro* activity of the KdpD protein and is required for maximal *kdp* expression (Puppe *et al* 1996). In *E. coli*, the NTD deleted KdpD (KdpD/*Δ*NTD) responds poorly to osmotic stress or K^+ limitation *in vivo* and shows a deregulated phosphatase activity that is independent of the presence of ATP *in vitro* (Jung and Altendorf 1998).

7. The unique case of *Anabaena kdp* regulation

As mentioned earlier, the *Anabaena* L-31 *kdp1* operon and deinococcal *kdp* show the presence of a short version of *kdpD* (figure 1) resembling only the KdpD-NTD (inclusive of the Walker A and B sites). The *Anabaena* L-31 KdpD protein was shown to be constitutively expressed and located in the *Anabaena* membrane fraction. The *Anabaena* and deinococcal KdpD could bind to the ATP analog 8-azido-ATP indicating the presence of a functional ATP binding site (Ballal *et al* 2005; Basu *et al* unpublished results). These data suggest that these truncated KdpD proteins were likely to be functional proteins. As *Anabaena* L-31 is not amenable to genetic manipulation, the role of truncated KdpD was analyzed in *D. radiodurans*. Insertional mutagenesis of deinococcal *kdpD* had no effect on KdpB expression under K^+ deficiency (Basu *et al* unpublished results) suggesting that the *kdpD* gene may be inconsequential for *kdp* expression in that species.

The ability of *Anabaena* L-31 KdpD protein to interact with membrane anchored *E. coli* KdpD-CTD and to modulate its activity was tested. For this a chimeric "*anacolikdpD*" gene that expressed a protein carrying the first 365 amino acids of *Anabaena* L-31 fused to *E. coli* KdpD-CTD was constructed (Ballal *et al* 2002). *In vitro*, the Anacoli KdpD could get autophosphorylated and transfer the phosphoryl group to *E. coli* KdpE in a fashion very similar to the wild-type *E. coli* KdpD. Like the wild-type *E. coli* KdpD, the Anacoli KdpD showed a greater ability to dephosphorylate KdpE~P in presence of ATP- *γ*-S rather than in its absence (Ballal *et al* 2002). The Anacoli KdpD protein was also functional *in vivo* and could induce *kdp* expression in response to K^+ limitation and osmotic stress in *E. coli*.

The *E. coli* KdpD-NTD has been shown to complement a separately expressed and membrane anchored KdpD-CTD *in trans*, resulting in restoration of signal transduction *in vivo* (Hermann *et al* 2000). The ability of *Anabaena* KdpD to interact with *E. coli* KdpD-CTD was tested when the two proteins were independently expressed (i.e. in *trans*). The phosphatase activity of the *E. coli* KdpD-CTD increased in presence of separately expressed *Anabaena* KdpD and the *E. coli* KdpD could co-elute with *Anabaena* KdpD (Ballal *et al* 2005). These findings demonstrate the ability of the two proteins to interact in *trans*.

Recently, the KdpD-NTD (*per se*) of *E. coli* has been reported to support expression of *E. coli kdpFABC* operon, irrespective of K^+ concentration of the medium (Heermann *et al* 2003). This *kdp* expression requires the presence of KdpE, and the KdpD-NTD is thought to stabilize the binding of KdpE~P to its corresponding binding site in the *kdpFABC* promoter. It is suggested that low molecular weight phosphodonors like acetyl phosphate or carbamoyl phosphate phosphorylate KdpE *in vivo* in *E. coli* in absence of KdpD-CTD (Heermann *et al* 2003). However, this finding is contested by Rothenbucher *et al* (2006) who showed that a KdpD-NTD construct, which also contains the first two KdpD transmembrane segments, failed to induce *kdp* expression under extreme K^+ limitation in *E. coli.* Therefore, the role of *E. coli* KdpD-NTD *per se* or the short KdpD proteins from *Anabaena* and *Deinococcus* in *kdp* regulation remains uncertain.

The molecular mechanisms underlying the regulation of *kdp* operons in *Anabaena* L-31 or *Deinococcus radiodurans* remain enigmatic. It is likely that inability of *kdp* operon to respond to osmotic stress in organisms like *Anabaena*, may relate to the presence of naturally short KdpD protein. The transmembrane domains of the *E. coli* KdpD protein are implicated to play a role in sensing the transmembrane phenomena of turgor (Epstein 1992; Stumpe *et al* 1996). Experimental evidence also points to the fact that KdpD transmembrane domains are involved in signal perception (Sugiura *et al* 1994; Puppe *et al* 1996). As the cyanobacterial KdpD has no membrane-spanning segments, it seems unlikely to sense membrane stretch or turgor drop and therefore may not respond to osmotic stress.

It is equally possible that a sensor kinase/response regulator pair regulates the *kdp* expression in *Anabaena*, *Deinococcus radiodurans* and other bacteria that encode a naturally short KdpD (missing the histidine kinase domain). Some of these bacteria with a naturally short *kdpD* also show the presence of two ORFs encoding a histidine kinase and a response regulator immediately upstream of their *kdpABC* operon (figure 1). For example, in *Synechocystis* PCC 6803, ORF *sll1590* (also designated as *hik20*) coding for a sensor kinase (SK) and an ORF *sll1592* coding for a response regulator (RR), are located immediately upstream of the *kdpABGC* operon. The genomes of *Anabaena* PCC 7120 (Kaneko *et al* 2001) and *Anabaena* L-31 also show the presence of sensor kinase (ORF *alr3155*) /response regulator (ORF *alr3156*) genes, very similar to the *Synechocystis* PCC 6803 *sll1590* and *sll1592*, immediately upstream of the *kdp2* operon. It should be noted that in *Anabaena* L-31 and *Anabaena* PCC 7120, the short *kdpD* is located downstream

of *kdp1* operon while the sensor kinase/response regulator pair is located upstream of the *kdp2* operon (figure 1). A similar SK/RR pair is also encoded by two ORFs (DR0082 and DR0081 respectively) present upstream of the *Deinococcus radiodurans kdp* operon.

Even more striking is the fact that the SK protein resembles the *E. coli* KdpD-CTD i.e. it consists of N-terminal transmembrane segments connected to a cytosolic histidine kinase domain. It is very tempting to speculate that this sensor kinase/response regulator pair may be involved in the regulation of *kdp* expression. The short KdpD protein may interact with the SK and modulate its activity. The participation of the naturally short KdpD and the SK/RR regulatory pair in *kdp* expression is currently being explored in our laboratory.

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