trans-Acting Mutations in Loci Other than *kdpDE* That Affect *kdp* Operon Regulation in *Escherichia coli*: Effects of Cytoplasmic Thiol Oxidation Status and Nucleoid Protein H-NS on *kdp* Expression

ABHIJIT A. SARDESAI[1] AND J. GOWRISHANKAR[1,2*]

Centre for Cellular and Molecular Biology, Hyderabad 500 007,[1] and Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500 076,[2] India

Transcription of the K^+ transport operon *kdp* in *Escherichia coli* is induced during K^+-limited growth by the action of a dual-component phosphorelay regulatory system comprised of a sensor kinase (integral membrane protein), KdpD, and a DNA-binding response regulator (cytoplasmic protein), KdpE. In this study, we screened for new *dke* (named *dke* for decreased *kdp* expression) mutations (in loci other than *kdpDE*) that led to substantially decreased *kdp* expression. One *dke* mutation was shown to be in *hns*, encoding the nucleoid protein H-NS. Another *dke* mutation was mapped to *trxB* (encoding thioredoxin reductase), and an equivalent reduction in *kdp* expression was demonstrated also for *trxA* mutants that are deficient in thioredoxin 1. Exogenously provided dithiothreitol rescued the *kdp* expression defect in *trxB* but not *trxA* mutants. Neither *trxB* nor *trxA* affected gene regulation mediated by another dual-component system tested, EnvZ-OmpR. Mutations in genes *dsbC* and *dsbD* did not affect *kdp* expression, suggesting that the *trx* effects on *kdp* are not mediated by alterations in protein disulfide bond status in the periplasm. Reduced *kdp* expression was observed even in a *trxB* strain that harbored a variant KdpD polypeptide bearing no Cys residues. A *trxB hns* double mutant was even more severely affected for *kdp* expression than either single mutant. The *dke* mutations themselves had no effect on strength of the signal controlling *kdp* expression, and constitutive mutations in *kdpDE* were epistatic to *hns* and *trxB*. These results indicate that perturbations in cytoplasmic thiol oxidation status and in levels of the H-NS protein exert additive effects, direct or indirect, at a step(s) upstream of KdpD in the signal transduction pathway, which significantly influence the magnitude of KdpD kinase activity obtained for a given strength of the inducing signal for *kdp* transcription.

Active uptake of K^+ in *Escherichia coli* and other enterobacteria is mediated by an inducible high-affinity transport system, Kdp, and at least three lower-affinity transport systems (TrkD [also called Kup], TrkG, and TrkH) that are constitutively expressed (reviewed in reference 46). The Kdp transporter is a P-type ATPase comprised of four polypeptides encoded by genes of the *kdpFABC* operon. It appears that the physiological role of Kdp is to permit growth of *E. coli* in medium containing a sufficiently low concentration of extracellular K^+ ($[K^+]_e$) that is not adequate for uptake through the constitutively expressed systems. The *kdp* operon is repressed under conditions of K^+-replete growth and the Kdp transporter activity is also inhibited under these conditions.

Transcriptional control of the *kdp* operon has mainly been studied in strains carrying *kdp-lac* operon fusions, and it is mediated by KdpD and KdpE (37, 52), a protein pair that is a member of the family of dual-component regulatory systems found in various prokaryotes (for a review, see reference 36). KdpD (the sensor kinase) is an integral protein of the inner membrane which, during K^+-limited growth, undergoes autophosphorylation on a cytoplasmic Asp residue; the phosphoryl group is then transferred to a His residue of the cytoplasmic

response regulator protein KdpE, and phospho-KdpE binds to an operator site immediately upstream of the *kdp* operon promoter to activate transcription of the operon (21, 33, 34, 50). KdpD and KdpE are the products of an independent *kdpDE* operon situated immediately downstream of *kdpFABC* (37).

Even though the components of the signal transduction pathway downstream of KdpD autophosphorylation have been well characterized, the exact nature of the signal involved in *kdp* regulation is not clear. Among the alternatives that have been proposed as the signals determining KdpD kinase activity are intracellular K^+ concentration ($[K^+]_i$), cell turgor, rate of transmembrane K^+ flux, or the combination of $[K^+]_e$ (or $[K^+]_i$) and osmotic strength of the medium (2, 12–14, 25, 27, 42, 49). Also not known is whether the signal acts directly on KdpD to modulate its kinase activity or indirectly via additional steps in the signal transduction pathway.

In this study, we employed approaches of insertional and localized mutagenesis to identify new loci that affect *kdp-lac* expression in *trans*. We found that mutations in *trxA* and *trxB*, encoding thioredoxin 1 and thioredoxin reductase, respectively, lead to a specific reduction in *kdp-lac* expression and that the reduction persists even in strains that express a cysteineless variant of the KdpD protein. We also found that a deficiency of nucleoid protein H-NS leads to down regulation of *kdp*. Data from epistasis experiments support the interpretation that the *trx* and *hns* mutations exert their effects on *kdp*

* Corresponding author. Mailing address: Centre for DNA Fingerprinting and Diagnostics, ECIL Road, Hyderabad 500 076, India. Phone: 91-40-7151344. Fax: 91-40-7155610. E-mail: shankar@www.cdfd.org.in.

TABLE 1. List of *E. coli* K-12 strains[a]

Strain	Genotype	Reference/source
MH225	Δ(*argF-lac*)U169 *rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25* Φ(*ompC-lacZ*) 10-25	15
TL1105A	*thi rha nagA* Δ*lac trkA405 trkD1 kdpA*::[Mu *lacZ*(λ)]	25
GJ1426	GJ1427 Δ*trxA*	This study
GJ1427	*thi rha nagA lacZ trkA405 kdp-200*::[λd*lac*(Ap)]	From GJ642 (reference 2)
GJ1428	GJ1427 *trxB30*::Tn*10*dTet	This study
GJ1429	GJ1427 Δ*trxA trxB30*::Tn*10*dTet	This study
GJ1430	GJ1427 *trxB*::kan	This study
GJ1431	GJ1427 *grxA*::kan *zbi*::Tn*10*	This study
GJ1438	MH225 *trxB30*::Tn*10*dTet	This study
GJ1439	MH225 *trxB*::kan	This study
GJ1441	MH225 Δ*trxA*	This study
GJ1442	*thi rha nagA lacZ trkA405 trkD1*Δ*kdpD kdp-204*::λp*lac*Mu55(Kan)	From TK2240 (reference 11), in two steps
GJ1442H	GJ1442 *hns-205*::Tn*10*	This study
GJ1442T	GJ1442 *trxB30*::Tn*10*dTet	This study
GJ1449	*thi rha nagA* Δ(*argF-lac*)U169 *trkA405 trkD1 kdp-200*::[λd*lac* (Ap)]	From TK2205 (W. Epstein), in several steps
GJ1449H	GJ1449 *hns-205*::Tn*10*	This study
GJ1449T	GJ1449 *trxB30*::Tn*10*dTet	This study
GJ1450	GJ1449 *kdp-205*	This study
GJ1450H	GJ1450 *hns-205*::Tn*10*	This study
GJ1450T	GJ1450 *trxB30*::Tn*10*dTet	This study
GJ1451	GJ1449 *kdp-207*	This study
GJ1451H	GJ1451 *hns-205*::Tn*10*	This study
GJ1451T	GJ1451 *trxB30*::Tn*10*dTet	This study
GJ1455	TL1105A *zci-3117*::Tn*10*Kan *hns-202*	This study
GJ1456	TL1105A *zci-3117*::Tn*10*Kan	This study
GJ1458	TL1105A *zci-506*::Tn*10 hns-202*	This study
GJ1459	TL1105A *zci-506*::Tn*10*	This study
GJ1461	TL1105A *hns-205*::Tn*10*	This study
GJ1469	GJ1427 *hns-205*::Tn*10*	This study
GJ1470	GJ1427 *hns-205*::Tn*10 trxB*::kan	This study
GJ1485	TL1105A *recB268*::Tn*10*	This study

[a] Genotype designations are as those in the work of Berlyn (4). All strains are F⁻. Allele numbers are given, where they are known. The *trxB30* and *hns-202* mutations are also referred to in the text as *dke-1* and *dke-2*, respectively. In the strains listed, the following mutations were transduced from strains previously described: *zci-3117*::Tn*10*Kan and *zci-506*::Tn*10* from CAG 18551 and CAG12169, respectively (47); *hns-205*::Tn*10* from PD145 (8); Δ*trxA* and *trxB*::kan from AD494 and WP570, respectively (7); *grxA*::kan and *zbi*::Tn*10* from A407 (44); Δ*kdpD* from TKV2208 (20); *recB268*::Tn*10* from JJC777 (5); and *kdp-200, kdp-205, kdp-207*, and *kdp-204*::λp*lac*Mu55(Kan) from GJ18, GJ618, GJ619, and GJ610, respectively (2).

regulation at a step(s) in the signal transduction pathway upstream of KdpD.

MATERIALS AND METHODS

Media and growth conditions. Unless otherwise specified, cultures for determinations of growth rates and β-galactosidase activities were grown at 30°C in phosphate-buffered media with reciprocally varying concentrations of Na⁺ and K⁺ that were prepared, as described previously (9), by mixing together 115 mM K⁺-phosphate medium with 115 mM Na⁺-phosphate medium in the appropriate proportion so as to achieve the desired [K⁺]ₑ. These media were supplemented with glucose and Casamino Acids (Difco) at 0.2 and 0.5%, respectively. Growth was monitored by measurement of absorbance at 600 nm. Medium KML (9) was used as the rich medium. Spectinomycin (Sp) was used at a final concentration of 50 μg/ml; other antibiotics and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were added at concentrations as specified previously (2).

Bacterial strains and plasmids. The *E. coli* K-12 strains employed in the study are listed in Table 1. The plasmids that were used included (i) pLG H-NS, a pSC101 replicon derivative which encodes Kanʳ and carries the cloned *hns*⁺ gene (54); (ii) pPV5-1 Cys⁺ and pPV5-1 Cys-less, which are both pMB9 (ColE1) replicon derivatives encoding Ampʳ and carrying variant versions of the *kdpD* gene under control of the *tac* promoter (the first has silent nucleotide substitutions that do not alter the amino acid sequence of the gene product and in the second, the codons for the six Cys residues in the native protein have all been altered to specify other amino acids [20]); and (iii) pBD-R511Q, which is also a pMB9 derivative encoding Ampʳ but which carries a *kdpD* variant (under control of a regulated *ara* promoter) with an altered codon 511 that specifies Gln instead of Arg (19). Additional plasmids pHYD704 and pHYD705 were constructed in this study from vector pCL1920 (pSC101 replicon, encoding Spʳ [26]) as described below; plasmid pHYD708 was constructed by the subcloning of a

*Hin*dIII-*Sac*I fragment carrying the *lacI*�q gene from pMJR1560 (48) into the corresponding sites of vector pCL1920.

Experimental techniques. The procedures for P1 transduction (13), generation of Tn*10*dTet transpositions employing phage λ 1323 (22), and in vitro DNA manipulations and transformation (45) were as described previously. The procedure for making a strain Δ*trxA* involved, first, the introduction of an *ilv*::Tn*10* or *ilv*::Tn*10*Kan marker, followed by a second P1 transduction to Ilv⁺ with a lysate prepared on an *ilv*⁺ Δ*trxA* strain; inheritance of Δ*trxA* was assessed by scoring for resistance to phage T7 (28). The method of Murgola and Yanofsky (32) was followed for localized mutagenesis of the 28-min chromosomal region, in which P1 phage propagated on the *zci-3117*::Tn*10*Kan strain GJ1456 was treated with hydroxylamine and then used to transduce TL1105A to Kanʳ. The specific activity of β-galactosidase in cultures grown to mid-log phase was measured by the method of Miller (30), and the values are reported in Miller units.

RESULTS

Isolation of *dke-1* and *dke-2* mutants. Strain TL1105A carries mutations in the *kdp*, *trkA*, and *trkD* genes (rendering it deficient in all the active transport systems for K⁺) and also a chromosomal *kdp-lac* fusion (25). Following whole-genome mutagenesis of a derivative of strain TL1105A with transposon Tn*10*dTet (22), we screened for clones that exhibited an altered *lac* expression phenotype on phosphate-buffered medium containing 20 mM [K⁺]ₑ and X-Gal. One mutant exhibiting reduced *lac* expression under these conditions was identified, and preliminary P1 transduction experiments (data not shown) permitted the conclusions that the *lac* expression
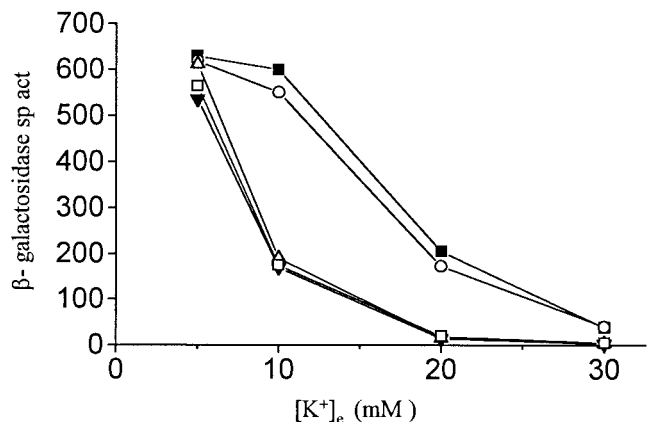
FIG. 1. β-Galactosidase specific activities (sp. act., expressed in Miller units [30]) in *kdp-lac* strains with *trx* or *grx* mutations, as a function of $[K^+]_e$ of the growth medium. ○, GJ1427 (parental); △, GJ1428 (*dke-1*; that is, *trxB30*::Tn*10*dTet); ▼, GJ1426 (Δ*trxA*); ■, GJ1431 (*grxA*); □, GJ1429 (Δ*trxA trxB30*::Tn*10*dTet).
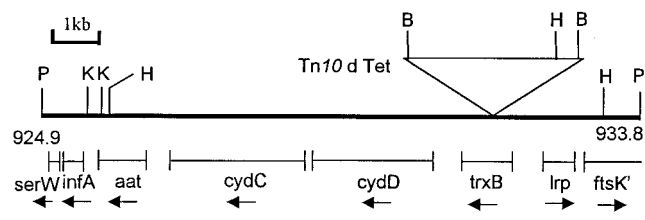

FIG. 2. Physical map of insert DNA in plasmids pHYD704 and pHYD705. Shown (with kilobase scale marked) is the restriction map of a *Pst*I (P) fragment, inserted in the two orientations in plasmids pHYD704 and pHYD705, respectively, for the enzymes *Bam*HI (B), *Hin*dIII (H), and *Kpn*I (K). The line in bold represents the alignment to the physical map of the *E. coli* chromosomal *Pst*I fragment that lies between kb coordinates 924.9 and 933.8 (43), and the inverted triangle represents the position of the *dke-1*::Tn*10*dTet insertion. The positions and transcriptional orientations of the different chromosomal genes that are carried on the insert are marked below the map.

phenotype was (i) 100% linked to Tet[r] and (ii) unlinked to the *kdpFABCDE* locus. The mutation was designated *dke-1* (named *dke* for decreased *kdp* expression). Comparison of the profiles of *kdp-lac* expression in an isogenic pair of strains, GJ1427 (*dke*[+]) and GJ1428 (*dke-1*::Tn*10*dTet), revealed that the reduction in *kdp-lac* expression in the latter was most pronounced (4- to 10-fold) at intermediate levels of $[K^+]_e$ (Fig. 1). The further characterization of *dke-1* is described below.

The *dke-2* mutant GJ1455 was also identified by screening on X-Gal-supplemented media derivatives of strain TL1105A, this time after localized mutagenesis of the 28-min region of the chromosome as described above. Our original rationale for undertaking this localized mutagenesis experiment was to examine whether missense mutations in *kch*, the gene encoding a putative K^{+} channel which maps to this chromosomal region (4, 43), could be identified that affect *kdp-lac* expression. However, the subsequent studies described below indicated that *dke-2* is not in *kch* but is an *hns* mutation.

Characterization of *dke-1* as a *trxB*::Tn*10*dTet insertion. A *Pst*I-digested chromosomal DNA library from a *dke-1*::Tn*10*dTet mutant derivative was established in the plasmid vector pCL1920. The Tn*10*dTet element is not digested with *Pst*I, and hence plasmid clones bearing the *dke-1*::Tn*10*dTet insertion (with flanking chromosomal DNA) were obtained following Tet[r] selection. Two plasmids, with identical 12-kb inserts (comprising 3 kb of Tn*10*dTet and 9 kb of chromosomal DNA) but in opposite orientations relative to the vector backbone, were identified and designated pHYD704 and pHYD705. When radiolabeled pHYD704 DNA was used to probe the ordered *E. coli* genome library in λ phage constructed by Kohara et al. (24), intense hybridization signals were obtained for phage clones 213 and 214 along with weaker signals for the flanking clones 212 and 215 (data not shown). These results indicated that the Tn*10*dTet insertion is situated in the 19.9- to 20.1-centisome region (43). Restriction mapping of the insert DNAs in plasmids pHYD704 and pHYD705 permitted the inference that the Tn*10*dTet insertion had occurred at kb-coordinate 930.8 of the *E. coli* physical map, that is, approximately at the junction of the proximal and middle thirds of the

trxB open reading frame, encoding thioredoxin reductase (Fig. 2). We were subsequently able to demonstrate that another well characterized *trxB*::kan insertion (7) is also associated with the phenotype of reduced *kdp* expression (see Fig. 3 and 6, curves for GJ1430). The new insertion mutation *dke-1* obtained in this study has been designated *trxB30*::Tn*10*dTet.

Effects of other perturbations in cellular thiol oxidation status on *kdp* expression. A characteristic feature of the *E. coli* cytoplasm is the absence of disulfide bonds in proteins. The reducing environment of the cytoplasm is maintained by the action of several reductant proteins, the three most effective of which are thioredoxin 1, thioredoxin 2, and glutaredoxin 1, which are encoded by *trxA*, *trxC*, and *grxA*, respectively (for a review, see reference 3). The first two proteins are substrates for thioredoxin reductase, while the last one derives its reducing potential from glutathione. Furthermore, of these three proteins, thioredoxin 1 and glutaredoxin 1 appear to be physiologically important during routine growth, whereas thioredoxin 2 is induced primarily under conditions of oxidative stress (41). Based on our identification of *trxB* as a *dke* locus, we tested the effects of other perturbations in cellular thiol oxidation status on *kdp* expression.

We found that a mutation in *trxA*, but not *grxA*, affected *kdp-lac* expression in a manner analogous to that described above for *trxB* (Fig. 1, curves for strains GJ1426 and GJ1431, respectively). A *trxB trxA* double mutant, GJ1429, showed a phenotype no more pronounced than either single mutant (Fig. 1). With increasing concentrations of dithiothreitol added to the culture medium, we noted a progressive restoration of *kdp-lac* expression in the *trxB trxA*[+] strains GJ1428 and GJ1430 but not in the *trxB*[+] *trxA* (GJ1426) or *trxB trxA* (GJ1429) derivatives (Fig. 3). The dithiothreitol supplementation experiment was done using concentrations of the reductant that were sublethal for the *trxA* and *trxB* strains (31).

We also examined whether the reported induction by oxidative stress of thioredoxin 2 (following the addition of H_2O_2 [41]) could rescue the *kdp* expression phenotype in a thioredoxin 1-deficient mutant, but the results were negative. The measured activities of β-galactosidase after growth in 20 mM $[K^+]_e$ medium, without and with H_2O_2 supplementation (added as a 5 mM pulse to cultures in early log phase followed by continued incubation for 90 to 180 min), for a pair of isogenic
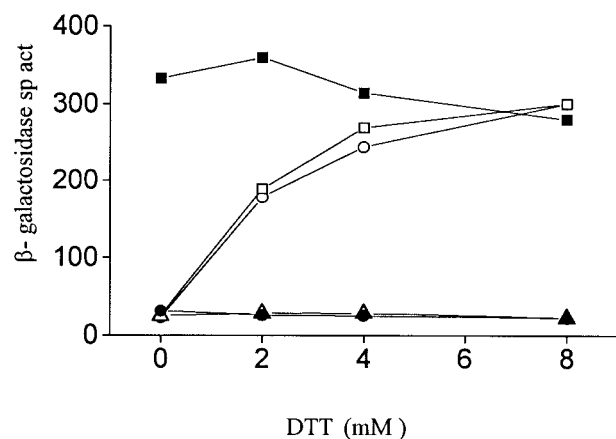
FIG. 3. β-Galactosidase specific activities (sp. act., expressed in Miller units [30]) in *kdp-lac* strains with *trx* mutations, as a function of dithiothreitol (DTT) supplementation of growth medium containing 15 mM $[K^+]_e$. ■, GJ1427 (parental); ○, GJ1428 (*trxB30*::Tn*10*dTet); □, GJ1430 (*trxB*::kan); △, GJ1426 (Δ*trxA*); ●, GJ1429 (Δ*trxA trxB30*::Tn*10*dTet).

FIG. 4. β-Galactosidase specific activities (sp. act., expressed in Miller units [30]) in *kdp-lac* Δ*kdpD* strain GJ1442 or its *trxB30*:: Tn*10*dTet derivative, GJ1442T, each carrying plasmids pPV5-1 Cys$^+$ or pPV5-1 Cys-less, encoding native KdpD or Cys-less KdpD, respectively, along with the *lacI*^{q}-bearing plasmid pHYD708. Cultures were grown in media with the indicated $[K^+]_e$. Histogram symbols: open, GJ1442/pPV5-1 Cys$^+$; striped, GJ1442/pPV5-1 Cys-less; solid, GJ1442T/pPV5-1 Cys$^+$; stippled, GJ1442T/pPV5-1 Cys-less.

kdp-lac strains were as follows: GJ1426 (Δ*trxA*), 6.8 and 7.6 units, respectively; and GJ1427 (*trxA*$^+$), 161 and 207 units, respectively.

Involvement of cytoplasmic, and not periplasmic, thiol oxidation status in *kdp* regulation. Taken together, the above data indicated that the reducing potential of thioredoxin 1, which is generated either by the action of endogenous thioredoxin reductase or following exogenous dithiothreitol supplementation, is necessary for optimal regulation of the *kdp* operon in *E. coli*. Thioredoxin reductase and reduced thioredoxin 1 are involved in thiol-disulfide isomerization reactions not only in the cytoplasm, where they act directly, but also in the periplasm where they act indirectly via another disulfide bond isomerase, DsbC (for reviews, see references 3 and 38). Some of the features identified for the perturbation in *kdp* regulation, notably, dithiothreitol rescue and absence of *grxA* effect, have been shown for phenotypes that are periplasmically determined (39, 40), and we therefore tested such a possibility further. We found, however, that *kdp-lac* expression was unaffected in *dsbC* or *dsbD* mutants (data not shown), which are otherwise known to be perturbed in periplasmic thiol-disulfide redox reactions (3, 38). Our results therefore suggest that it is the cytoplasmic thiol oxidation status dictated by thioredoxin reductase and reduced thioredoxin 1 which may be important in *kdp* regulation.

***trxB*-determined phenotype is also seen in a strain with Cys-less KdpD.** The response regulator KdpE is a small protein located in the cytoplasm with a lone Cys residue. On the other hand, the membrane-localized sensor kinase KdpD has six Cys residues, and we considered the possibility that inappropriate disulfide bond formation within or between the monomer subunits of KdpD in *trxB* and *trxA* mutants results in the abnormal signal transduction for *kdp* expression.

Jung et al. have created a gene encoding a variant KdpD protein with no Cys residues, which is nevertheless normal for *kdp* signal transduction in vivo (20). We constructed strain GJ1442 (and also its *trxB30* derivative, GJ1442T) that was
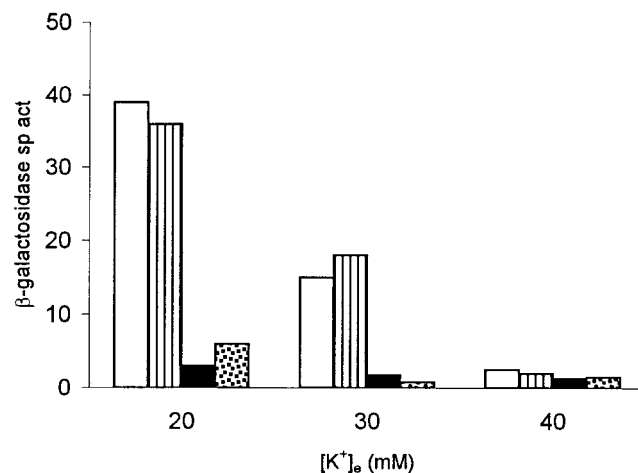
chromosomally Δ*kdpD kdpE*$^+$ and in which either the variant Cys-less KdpD protein or its normal counterpart could then be expressed (from the heterologous *tac* promoter) by introduction of the plasmids pPV5-1 Cys-less or pPV5-1 Cys$^+$, respectively. All derivatives also carried the *lacI*^{q} gene on plasmid pHYD708, in order to avoid the toxicity problems associated with otherwise massive overproduction of the KdpD proteins (reference 18 and data not shown).

The results presented in Fig. 4 indicate that the *trxB*:: Tn*10*dTet mutation was associated with a reduction in *kdp-lac* transcription both in the strain that was expressing native KdpD and in the strain expressing the Cys-less variant. This provided conclusive evidence that the *trxB* effect on *kdp* is not mediated through the Cys residues of KdpD.

It may be noted that in the experiment shown in Fig. 4, the induced level of *kdp-lac* expression in the *trxB*$^+$ control strains (with plasmid-borne *kdpD*) was itself lower than that normally obtained with haploid *kdpD*$^+$*E*$^+$ strains. Similar low values for *kdp* expression have been reported by Jung et al. (20), working with the same multicopy *kdpD* plasmids pPV5-1 Cys$^+$ and PV5-1 Cys-less, and Jung and Altendorf (18) have suggested that an optimal level of KdpD protein is a critical factor in signal transduction.

Absence of effect of *trxB* or *trxA* on another dual-component regulatory system. Osmolarity-dependent expression of the outer membrane protein gene *ompC* is under the control of a dual-component system consisting of the membrane-bound sensor kinase EnvZ and the cytoplasmic response regulator OmpR (reviewed in reference 36). In order to test whether the effects of perturbations in thiol-disulfide bond isomerization on *kdp* expression are specific to the particular dual-component regulatory system represented by KdpD and KdpE, we compared the levels of *ompC-lac* expression among *trxB*, *trxA*, and wild-type strains (Table 2). The basal level of *ompC* expression was unaffected in either of the mutant strains, and

TABLE 2. *ompC-lac* regulation in *trx* mutants[a]

Strain (*trx* genotype)	β-Galactosidase sp act at NaCl concn (M) of:		
	0	0.1	0.3
MH225 (*trx*⁺)	260	375	715
GJ1438 (*trxB30*::Tn*10*dTet)	281	398	648
GJ1439 (*trxB*::kan)	360	407	670
GJ1441 (Δ*trxA*)	399	533	709

[a] Specific activity (in Miller units [30]) of β-galactosidase was measured in cultures of the *ompC-lac* fusion strain MH225 and its *trx* mutant derivatives grown to mid-log phase in low-osmolarity K medium (13) supplemented with NaCl at the indicated concentrations.

there was not any significant alteration in the magnitude of transcriptional induction of the promoter at elevated osmolarity. These data also indicated that the observed decrease in β-galactosidase activity in the *kdp-lac* fusion strains with the *trxB* or *trxA* mutation is not the consequence of inappropriate disulfide bond formation in the reporter enzyme. We have also obtained evidence that expression of a *proU-lac* fusion or of the wild-type *lac* operon is not affected in the mutants (data not shown). We therefore conclude that there is indeed a specificity associated with the reduction of *kdp* expression in *trxB* and *trxA* mutants.

The *dke-2* mutation is an *hns* allele. As described above, the *dke-2* mutant was isolated following localized mutagenesis of the 28-min region of the chromosome. P1 transductional mapping experiments demonstrated that *dke-2* is 95% cotransducible with each of the inserts *zci-3117*::Tn*10*Kan and *zci-506*::Tn*10*, which constitute a cognate pair in the collection of Singer et al. (47); this pair has subsequently been mapped to lie in the *oppC* gene (35). The induced level of *kdp-lac* expression in the mutant was 10- to 15-fold lower than that in the *dke*⁺ control (Fig. 5). The *dke-2* mutation also conferred phenotypes of nonmotility as well as derepression of *proU-lac* expression (data not shown), which suggested (23, 54) that it is an allele of the *hns* gene, which maps close to *oppC* at 28 min and which
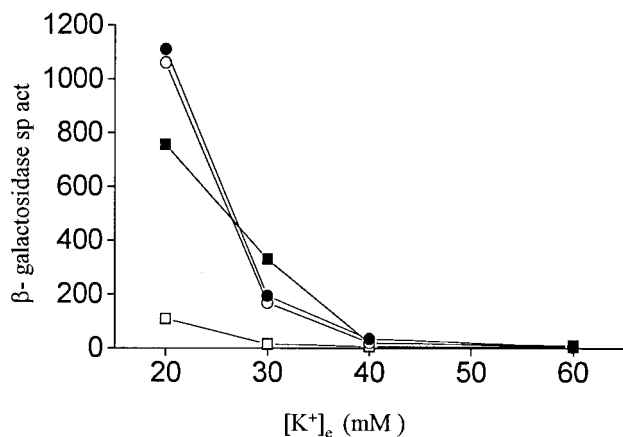

FIG. 5. β-Galactosidase specific activities (sp. act., expressed in Miller units [30]) in the isogenic *kdp-lac* strains GJ1459 (parental) and GJ1458 (*dke-2*; that is, *hns-202*) or their derivatives carrying the *hns*⁺-encoding plasmid pLG H-NS, as a function of [K⁺]ₑ of the growth medium. ■, GJ1459; □, GJ1458; ○, GJ1459/pLG H-NS; ●, GJ1458/pLG H-NS.
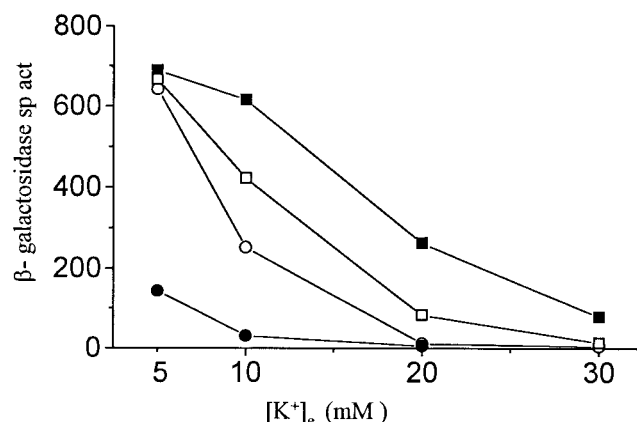

FIG. 6. β-Galactosidase specific activities (sp. act., expressed in Miller units [30]) in *kdp-lac* strains with *trxB* or *hns* mutations as a function of [K⁺]ₑ of the growth medium. ■, GJ1427 (parental); ○, GJ1430 (*trxB*::kan); □, GJ1469 (*hns-205*::Tn*10*); ●, GJ1470 (*trxB*::kan *hns-205*::Tn*10*).

encodes the nucleoid protein H-NS (4, 43). Introduction of the medium-copy-number plasmid pLG H-NS (carrying the *hns*⁺ gene) restored the *kdp-lac* expression profile in the mutant to that seen in the *dke*⁺ strain carrying the same plasmid (Fig. 5). Furthermore, a previously characterized *hns-205*::Tn*10* mutation (8) conferred a phenotype similar to *dke-2* on *kdp-lac* expression (Fig. 6; Table 3). These results support the conclusions that (i) *dke-2* is in *hns*, and (ii) null mutations in *hns* serve to reduce *kdp* transcription. The *dke-2* mutation has accordingly been designated *hns-202*.

Expression of *kdp-lac* in the *trxB*::kan *hns*::Tn*10* double mutant strain GJ1470 was reduced even more drastically than in either single mutant derivative (Fig. 6), suggesting that the two *dke* loci act additively in perturbing *kdp* regulation. A null mutation in *stpA*, the gene encoding the H-NS-like protein StpA that is believed to represent a molecular back-up of H-NS (55), by itself had no effect on *kdp* expression. An *hns stpA* double mutant was even more compromised for *kdp* regulation than was the *hns* single mutant (data not shown), but as explained below, interpretation of this finding is rendered difficult because of the poor growth rate observed with the double mutant strain (16, 55).

TABLE 3. Expression of *kdp-lac* in *trx kdpDE* double mutants[a]

dke mutation	β-Galactosidase sp act in presence of *kdpDE* mutation:			
	None	*kdp-205*	*kdp-207*	*kdpD*(R511Q)
None	250	713	890	423
trxB30::Tn*10*dTet	6	535	920	248
hns-205::Tn*10*	9	540	919	236

[a] The following sets of strains were used for assessment of *kdp-lac* expression (isogenic within each set and indicated, within parentheses, in the order of no *dke* mutation, *trxB* and *hns*): (i) no *kdpDE* mutation (GJ1449, GJ1449T, GJ1449H); (ii) with *kdp-205* (GJ1450, GJ1450T, GJ1450H); (iii) with *kdp-207* (GJ1451, GJ1451T, GJ1451H); and (iv) with *kdpD*-R511Q (derivatives of GJ1442, GJ1442T, and GJ1442H each transformed with plasmid pBD-R511Q). Cultures were grown to mid-log phase in medium containing 30 mM [K⁺]ₑ (supplemented with ampicillin in the case of derivatives carrying plasmid pBD-R511Q). β-Galactosidase specific activities are reported in Miller units (30).

***trxB* and *hns* effects on *kdp* are unrelated to alterations in growth rates.** It is known (2) that for a given $[K^+]_e$, *kdp* expression in a strain decreases with decreasing growth rates, ostensibly because lower rates of K^+ uptake suffice under these conditions (10). The following experiments demonstrated, however, that the *dke* nature of *trxB* may not be explained on this basis. Consistent with the findings of an earlier report (7), a *trxB* mutant GJ1428 grew just as well as its *trxB*⁺ parent GJ1427 (with doubling times of 40 min each) in medium with 20 mM $[K^+]_e$, that is, under the conditions where the mutation's effect on *kdp-lac* transcription is very pronounced (Fig. 1). Furthermore, supplementation of the cultures with 8 mM dithiothreitol led, as expected (31), to a reduction in growth rates of the two strains (with measured doubling times of 70 and 60 min, respectively), even as the level of *kdp* expression in the mutant was almost completely restored to that in the parent (data not shown; see also Fig. 3).

Mutations in *hns* are known to affect growth rate (55), and in order to examine whether the *hns* effect on *kdp* could be accounted for by such alterations we measured the doubling times and levels of *kdp-lac* expression in cultures of the parental strain TL1105A and of its derivatives carrying mutations in *hns* (GJ1461) or *recB* (GJ1485). The *recB* mutation was chosen as a control, as it effects a moderate growth rate reduction similar to that of the *hns* allele. The β-galactosidase activities (with culture doubling times in parentheses) for the parent, *hns*, and *recB* strains grown in 30 mM $[K^+]_e$ were 306 units (45 min), 24 units (55 min), and 150 units (55 min), respectively. These results indicate that the two mutations each had equivalent effects in reducing the growth rate of the parental strain, but the reduction in *kdp* expression was very much more pronounced in the *hns* mutant than it was in the *recB* derivative. Therefore, the *hns* effect on *kdp* transcription is not solely because of a concomitant decrease in the growth rate.

***kdpDE* constitutive mutations are epistatic to *trxB* and *hns*.** In order to establish epistasis relationships, we examined the effects of the *trxB*::Tn*10*dTet or *hns*::Tn*10* mutation on *kdp-lac* expression in strains carrying three different *trans*-acting mutations in the *kdpDE* locus. The latter included the *kdp-205* and *kdp-207* alleles described earlier (2), as well as a site-specific alteration in *kdpD* that results in an Arg⁵¹¹→Gln (R511Q) substitution in KdpD (19). Of these mutations, the *kdp-205* mutant exhibits a reduced sensitivity for repression of the *kdp* operon by $[K^+]_e$, while the other two are fully constitutive. We found that the elevated levels of *kdp-lac* expression conferred by the *kdpDE* mutations were largely unaffected by the *trxB* or *hns* mutations (Table 3). As further discussed below, these results suggest that *trxB* and *hns* exert their effects on *kdp* expression at a step(s) upstream of KdpD in the signal transduction pathway.

DISCUSSION

The mechanism of transcriptional activation of the *kdp* operon in *E. coli* by the protein pair comprised of the sensor kinase KdpD and response regulator KdpE is well established, although the nature of the signal during K^+-limited growth which leads to increased KdpD autophosphorylation is unclear. Different mutations could be expected to alter *kdp-lac* expression (for a given $[K^+]_e$) either by altering the strength of the environmental signal that is sensed by the cell in controlling *kdp* transcription (e.g., mutations in *trkA* or *trkD*) or by interfering with the signal transduction pathway (e.g., mutations in *kdpD* or *kdpE*). The hallmark of the former is that the change in *kdp-lac* expression in the mutant is inversely correlated with its growth ability in low-$[K^+]_e$ media. By this criterion, the mutations that have been identified in this study as reducing *kdp* expression (*trxB*, *trxA*, and *hns*) appear to do so by interfering with signal transduction rather than signal strength, because there is no concomitant increase of K^+-limited growth rates in the mutant cultures.

Cytoplasmic thiol oxidation status in *kdp* regulation. The observations made in this study, concerning the *trxB* and *trxA* mutants as well as the effects of exogenous dithiothreitol supplementation, support the proposal that reduced thioredoxin 1 is required for appropriate signal transduction in *kdp* regulation in vivo. This requirement apparently cannot be substituted by thioredoxin 2 or the glutaredoxins, nor does it involve the thiol-disulfide isomerase DsbC in the periplasmic compartment (whose functioning is dependent on availability of reduced thioredoxin 1). We therefore suggest that this requirement is cytoplasmic. To our knowledge, this is the first example of a thiol oxidation status-determined function in the cytoplasmic compartment that is absolutely dependent only on reduced thioredoxin 1 and also one that is affected to an equivalent extent by *trxB* and *trxA* mutations.

That the *trxB*- or *trxA*-mediated reduction in reporter enzyme activity in the *kdp-lac* fusion strains is not a consequence, for example, of inappropriate disulfide bond formation in the cytoplasmically localized β-galactosidase was established in control experiments involving *lacZ* expression from other promoters, including its native promoter. Also, regulation was not affected in another system (EnvZ-OmpR) involving similar phosphotransfer (as in *kdp*) between an autophosphorylated sensor kinase and a cytoplasmic activator protein, hence arguing for a specificity in the reduced thioredoxin 1 requirement for *kdp* regulation.

As mentioned above, the signal controlling *kdp* expression is not known, but several models suggest that this signal acts directly on membrane-bound KdpD to determine the latter's autophosphorylation activity (25, 27, 42, 49). Our data, on the other hand, from the experiments employing strains with the Cys-less KdpD variant protein as well as those testing epistasis with *kdpDE* mutations, suggest that the absence of reduced thioredoxin 1 interferes with a step in the *kdp* signal transduction pathway upstream of KdpD function. (Implied also in such an interpretation is the notion that cellular thiol status does not exert its effect on *kdp* regulation via KdpE [by formation or breakage, for example, of a disulfide bridge between two monomer subunits], because KdpE is downstream of KdpD in the signal transduction pathway.) To that extent, therefore, we believe that alternative models may need to be considered in which the effect of the signal (which is generated during K^+-limited growth) on KdpD activity is mediated or modulated by additional protein(s). Nevertheless, the exact mechanism by which reduced thioredoxin 1 participates in the signal transduction pathway remains to be determined.

Finally, it may be noted that the cytoplasmic thiol reductant glutathione has been shown in earlier studies both to accumulate during osmotic stress (when there is a concomitant cyto-

plasmic accumulation of K$^+$) (29) and to mediate gating of the K$^+$-efflux channels KefB and KefC (6). Glutathione-deficient strains, particularly those that are Kdp$^+$, exhibit abnormalities in the maintenance of [K$^+$]$_i$ (11). Cytoplasmic thioredoxin 1 has also been shown to leak out (through MscL channels) from cells subjected to an osmotic downshock (1). The relevance of any of these observations, however, to the findings described in this paper is unclear.

Nucleoid protein H-NS in *kdp* regulation. Mutants in *hns* are known to be pleiotropic (for a review, see reference 53); this study has identified an additional phenotype, that of a significant reduction in the induced levels of *kdp* transcription, for these mutants. H-NS is known more for its role as a global repressor protein (53), and there are only a limited number of identified promoters whose transcription is reduced in an *hns* null mutant (16, 17, 23). Interestingly, there exists a bent-DNA motif (which is also a high-affinity binding site for H-NS) that overlaps the binding site for phospho-KdpE immediately upstream of the *kdp* operon promoter (51), and one could therefore envisage a direct role for H-NS in providing an optimal chromatin configuration for transcription activation by phospho-KdpE. However, our results from the epistasis experiments with *kdpDE* argue (as for *trxB*) that the effect of *hns* on *kdp* is upstream of KdpD and is, therefore, almost certainly indirect. The precise mechanism of this indirect effect of H-NS remains to be elucidated.

In conclusion, we have shown in this study that in addition to the KdpD and KpdE regulator proteins, factors such as cytoplasmic thiol oxidation status and the nucleoid protein H-NS can significantly affect in vivo expression of the *kdp* operon. It may therefore be necessary to accommodate the roles of these factors as well in models that seek to explain the mechanism of signal transduction in *kdp* operon regulation.

REFERENCES

1. **Ajouz, B., C. Berrier, A. Garrigues, M. Besnard, and A. Ghazi.** 1998. Release of thioredoxin via the mechanosensitive channel MscL during osmotic downshock of *Escherichia coli* cells. J. Biol. Chem. **273:**26670–26674.
2. **Asha, H., and J. Gowrishankar.** 1993. Regulation of *kdp* operon expression in *Escherichia coli*: evidence against turgor as signal for transcriptional control. J. Bacteriol. **175:**4528–4537.
3. **Åslund, F., and J. Beckwith.** 1999. The thioredoxin superfamily: redundancy, specificity, and gray-area genomics. J. Bacteriol. **181:**1375–1379.
4. **Berlyn, M. K. B.** 1998. Linkage map of *Escherichia coli* K-12, edition 10: the traditional map. Microbiol. Mol. Biol. Rev. **62:**814–984.
5. **Bidnenko, V., M. Seigneur, M. Penel-Colin, M.-F. Bouton, S. D. Ehrlich, and B. Michel.** 1999. *sbcB sbcC* null mutations allow RecF-mediated repair of arrested replication forks in *rep recBC* mutants. Mol. Microbiol. **33:**846–857.
6. **Booth, I. R., M. A. Jones, D. McLaggan, Y. Nikolaev, L. S. Ness, C. M. Wood, S. Miller, S. Tötemeyer, and G. P. Ferguson.** 1996. Bacterial ion channels, p. 693–729. *In* W. N. Konings, H. R. Kaback, and J. S. Lolkema (ed.), Handbook of biological physics, vol. 2. Elsevier Science B.V., Amsterdam, The Netherlands.
7. **Derman, A. I., W. A. Prinz, D. Belin, and J. Beckwith.** 1993. Mutations that allow disulfide bond formation in the cytoplasm of *Escherichia coli*. Science **262:**1744–1747.
8. **Dersch, P., S. Kneip, and E. Bremer.** 1994. The nucleoid-associated DNA-binding protein H-NS is required for the efficient adaptation of *Escherichia coli* to a cold environment. Mol. Gen. Genet. **245:**255–259.
9. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108:**639–644.
10. **Epstein, W., and S. G. Schultz.** 1965. Cation transport in *Escherichia coli*. V. Regulation of cation content. J. Gen. Physiol. **49:**221–234.
11. **Ferguson, G. P., and I. R. Booth.** 1998. Importance of glutathione for growth and survival of *Escherichia coli* cells: detoxification of methylglyoxal and maintenance of intracellular K$^+$. J. Bacteriol. **180:**4314–4318.
12. **Frymier, J. S., T. D. Reed, S. A. Fletcher, and L. N. Csonka.** 1997. Characterization of transcriptional regulation of the *kdp* operon of *Salmonella typhimurium*. J. Bacteriol. **179:**3061–3063.
13. **Gowrishankar, J.** 1985. Identification of osmoresponsive genes in *Escherichia coli*: evidence for participation of potassium and proline transport systems in osmoregulation. J. Bacteriol. **164:**434–445.
14. **Gowrishankar, J.** 1987. A model for the regulation of expression of the potassium-transport operon, *kdp*, in *Escherichia coli*. J. Genet. **66:**87–92.
15. **Hall, M. N., and T. J. Silhavy.** 1981. The *ompB* locus and the regulation of the major outer membrane porin proteins of *Escherichia coli* K-12. J. Mol. Biol. **146:**22–43.
16. **Johansson, J., C. Balsalobre, S.-Y. Wang, J. Urbonaviciene, D. J. Jin, B. Sondén, and B. E. Uhlin.** 2000. Nucleoid proteins stimulate stringently controlled bacterial promoters: a link between the cAMP-CRP and the (p)ppGpp regulons in *Escherichia coli*. Cell **102:**475–485.
17. **Johansson, J., B. Dagberg, E. Richet, and B. E. Uhlin.** 1998. H-NS and StpA proteins stimulate expression of the maltose regulon in *Escherichia coli*. J. Bacteriol. **180:**6117–6125.
18. **Jung, K., and K. Altendorf.** 1998. Truncation of amino acids 12-128 causes deregulation of the phosphatase activity of the sensor kinase KdpD of *Escherichia coli*. J. Biol. Chem. **273:**17406–17410.
19. **Jung, K., and K. Altendorf.** 1998. Individual substitutions of clustered arginine residues of the sensor kinase KdpD of *Escherichia coli* modulate the ratio of kinase to phosphatase activity. J. Biol. Chem. **273:**26415–26420.
20. **Jung, K., R. Heermann, M. Meyer, and K. Altendorf.** 1998. Effect of cysteine replacements on the properties of the turgor sensor KdpD of *Escherichia coli*. Biochim. Biophys. Acta **1372:**311–322.
21. **Jung, K., B. Tjaden, and K. Altendorf.** 1997. Purification, reconstitution, and characterization of KdpD, the turgor sensor of *Escherichia coli*. J. Biol. Chem. **272:**10847–10852.
22. **Kleckner, N., J. Bender, and S. Gottesman.** 1991. Uses of transposons with emphasis on Tn*10*. Methods Enzymol. **204:**139–180.
23. **Ko, M., and C. Park.** 2000. H-NS-dependent regulation of flagellar synthesis is mediated by a LysR family protein. J. Bacteriol. **182:**4670–4672.
24. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
25. **Laimins, L. A., D. B. Rhoads, and W. Epstein.** 1981. Osmotic control of *kdp* operon expression in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78:**464–468.
26. **Lerner, C. G., and M. Inouye.** 1990. Low copy number plasmids for regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability. Nucleic Acids Res. **18:**4631.
27. **Malli, R., and W. Epstein.** 1998. Expression of the Kdp ATPase is consistent with regulation by turgor pressure. J. Bacteriol. **180:**5102–5108.
28. **Mark, D. F., and C. C. Richardson.** 1976. *Escherichia coli* thioredoxin: a subunit of bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA **73:**780–784.
29. **McLaggan, D., T. M. Logan, D. G. Lynn, and W. Epstein.** 1990. Involvement of γ-glutamyl peptides in osmoadaptation of *Escherichia coli*. J. Bacteriol. **172:**3631–3636.
30. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Missiakas, D., C. Georgopoulos, and S. Raina.** 1993. Identification and characterization of the *Escherichia coli* gene *dsbB*, whose product is involved in the formation of disulfide bonds in vivo. Proc. Natl. Acad. Sci. USA **90:**7084–7088.
32. **Murgola, E. J., and C. Yanofsky.** 1974. Structural interactions between amino acid residues at positions 22 and 211 in the tryptophan synthetase alpha chain of *Escherichia coli*. J. Bacteriol. **117:**444–448.
33. **Nakashima, K., A. Sugiura, K. Kanamaru, and T. Mizuno.** 1993. Signal transduction between two regulatory components involved in the regulation of the *kdpABC* operon in *Escherichia coli*: phosphorylation-dependent functioning of the positive regulator, KdpE. Mol. Microbiol. **7:**106–116.
34. **Nakashima, K., A. Sugiura, H. Momoi, and T. Mizuno.** 1992. Phosphotransfer signal transduction between two regulatory factors involved in the osmoregulated *kdp* operon in *Escherichia coli*. Mol. Microbiol. **6:**1777–1784.
35. **Nichols, B. P., O. Shafiq, and V. Meiners.** 1998. Sequence analysis of Tn*10* insertion sites in a collection of *Escherichia coli* strains used for genetic mapping and strain construction. J. Bacteriol. **180:**6408–6411.
36. **Ninfa, A. J.** 1996. Regulation of gene transcription by extracellular stimuli, p. 1246–1262. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin,

K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, D.C.

37. **Polarek, J. W., G. Williams, and W. Epstein.** 1992. The products of the *kdpDE* operon are required for expression of the Kdp ATPase of *Escherichia coli*. J. Bacteriol. **174:**2145–2151.

38. **Raina, S., and D. Missiakas.** 1997. Making and breaking disulfide bonds. Annu. Rev. Microbiol. **51:**179–202.

39. **Rietsch, A., D. Belin, N. Martin, and J. Beckwith.** 1996. An *in vivo* pathway for disulfide bond isomerization in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **93:**13048–13053.

40. **Rietsch, A., P. Bessette, G. Georgiou, and J. Beckwith.** 1997. Reduction of the periplasmic disulfide bond isomerase, DsbC, occurs by passage of electrons from cytoplasmic thioredoxin. J. Bacteriol. **179:**6602–6608.

41. **Ritz, D., H. Patel, B. Doan, M. Zheng, F. Åslund, G. Storz, and J. Beckwith.** 2000. Thioredoxin 2 is involved in the oxidative stress response in *Escherichia coli*. J. Biol. Chem. **276:**2505–2512.

42. **Roe, A. J., D. McLaggan, C. P. O'Byrne, and I. R. Booth.** 2000. Rapid inactivation of the *Escherichia coli* Kdp K$^+$ uptake system by high potassium concentrations. Mol. Microbiol. **35:**1235–1243.

43. **Rudd, K. E.** 1998. Linkage map of *Escherichia coli* K-12, edition 10: the physical map. Microbiol. Mol. Biol. Rev. **62:**985–1019.

44. **Russel, M., P. Model, and A. Holmgren.** 1990. Thioredoxin or glutaredoxin in *Escherichia coli* is essential for sulfate reduction but not for deoxyribonucleotide synthesis. J. Bacteriol. **172:**1923–1929.

45. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

46. **Silver, S.** 1996. Transport of inorganic cations, p. 1091–1102. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American

Society for Microbiology, Washington, D.C.

47. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.

48. **Stark, M. J. R.** 1987. Multicopy expression vectors carrying the *lac* repressor gene for regulated high-level expression of genes in *Escherichia coli*. Gene **51:**255–267.

49. **Sugiura, A., K. Hirokawa, K. Nakashima, and T. Mizuno.** 1994. Signal-sensing mechanisms of the putative osmosensor KdpD in *Escherichia coli*. Mol. Microbiol. **14:**929–938.

50. **Sugiura, A., K. Nakashima, K. Tanaka, and T. Mizuno.** 1992. Clarification of the structural and functional features of the osmoregulated *kdp* operon of *Escherichia coli*. Mol. Microbiol. **6:**1769–1776.

51. **Tanaka, K., S. Muramatsu, H. Yamada, and T. Mizuno.** 1991. Systematic characterization of curved DNA segments randomly cloned from *Escherichia coli* and their functional significance. Mol. Gen. Genet. **226:**367–376.

52. **Walderhaug, M. O., J. W. Polarek, P. Voelkner, J. M. Daniel, J. E. Hesse, K. Altendorf, and W. Epstein.** 1992. KdpD and KdpE, proteins that control expression of the *kdpABC* operon, are members of two-component sensor-effector class of regulators. J. Bacteriol. **174:**2152–2159.

53. **Williams, R. M., and S. Rimsky.** 1997. Molecular aspects of the *E. coli* nucleoid protein, H-NS: a central controller of gene regulatory networks. FEMS Microbiol. Lett. **156:**175–185.

54. **Williams, R. M., S. Rimsky, and H. Buc.** 1996. Probing the structure, function, and interactions of the *Escherichia coli* H-NS and StpA proteins by using dominant negative derivatives. J. Bacteriol. **178:**4335–4343.

55. **Zhang, A., S. Rimsky, M. E. Reaban, H. Buc, and M. Belfort.** 1996. *Escherichia coli* protein analogs StpA and H-NS: regulatory loops, similar and disparate effects on nucleic acid dynamics. EMBO J. **15:**1340–1349.