Different Foci for the Regulation of the Activity of the KefB and KefC Glutathione-gated K\(^{+}\) Efflux Systems*  

(Received for publication, December 10, 1998, and in revised form, January 22, 1999)

Lorna S. Ness and Ian R. Booth‡

From the Department of Molecular and Cell Biology, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, United Kingdom

KefB and KefC are glutathione-gated K\(^{+}\) efflux systems in *Escherichia coli*, and the proteins exhibit strong similarity at the level of both primary sequence and domain organization. The proteins are maintained closed by glutathione and are activated by binding of adducts formed between glutathione and electrophiles. By construction of equivalent mutations in each protein, this study has analyzed the control over inactive state of the proteins. A UV-induced mutation in KefB, L75S, causes rapid spontaneous K\(^{+}\) efflux but has only a minor effect on K\(^{+}\) efflux via KefC. Similarly amino acid substitutions that cause increased spontaneous activity in KefC have only small effects in KefB. Exchange of an eight amino acid region from KefC (HALESDIE) with the equivalent sequence from KefB (HELETAID) has identified a role for a group of acidic residues in controlling KefC activity. The mutations HELETAID and L74S in KefC act synergistically, and the activity of the resultant protein resembles that of KefB. We conclude that, despite the high degree of sequence similarity, KefB and KefC exhibit different sensitivities to the same site-specific mutations.

The carboxyl-terminal domain contains a sequence highly similar to a Rossman fold (7, 8). A number of mutations that cause increased spontaneous activity in KefC have been characterized and fall in two regions: a region (the “HALESDEI” sequence) predicted to lie at the cytoplasmic face of the membrane domain and residues within, and adjacent to, the Rossman fold of the carboxyl-terminal domain (7–9). One mutation at the latter site alters the glutathione regulation of the KefC protein (9), but the specific mechanism of activation by the other lesions is not known.

The two *E. coli* glutathione-gated K\(^{+}\) efflux systems can be differentiated by their activation by MG (4). Methylglyoxal only weakly activates KefC, whereas KefB achieves almost maximum activity with this electrophile. In this study we sought to characterize the structural gene for KefB to determine the relatedness to KefC. The two proteins are similar at the sequence and organizational levels. However, the creation of equivalent mutations at a number of positions in KefB and KefC shows that the residues controlling the activation of the two systems are different.

**EXPERIMENTAL PROCEDURES**

*Reagents—*All chemical reagents were purchased from Sigma or BDH and were of analytical grade where possible. Chemicals used for preparation of complex growth medium were supplied by Oxoid. Restriction enzymes and *Taq* DNA polymerase were supplied by Boehringer. *Pfu* polymerase was obtained from Stratagene. The *Qiagen Plasmid Preparation Kits* were obtained from Qiagen. All primers used in this study were purchased from Genosys Biotechnologies Inc. The Wizard PCR Preps DNA Purification System was obtained from Promega. The PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit was obtained from Applied Biosystems Ltd.

*Bacterial Strains—*Bacterial strains used in this study are all derivatives of *E. coli* K-12 (Table I). Strains MJF270 and MJF276 were previously thought to carry an internal deletion in the *kefB* gene, as they were isolated as suppressors of the *kefB110* mutation. However, sequence analysis during the current work revealed that the strain carries two mutations, L75S and D157N, that together inactivate KefB.

**Growth and Cell Viability—**The growth medium used throughout was K\(_1\) where X is the concentration of K\(_2\) (10). Strains were grown overnight at 37 °C in K\(_{10}\) minimal medium supplemented with 0.04% (w/v) glucose and 1 μg/ml thiamine. Ampicillin (25 μg·ml\(^{-1}\)) was included if the strain carried a plasmid. Aliquots of 3 ml were washed in K\(_0\) buffer, suspended in 30 ml of K\(_0\) minimal medium containing 0.2% (w/v) glucose and 1 μg·ml\(^{-1}\) thiamine placed at 37 °C and the OD\(_{650}\) monitored over time. For analysis of cell viability the appropriate strains were grown as above and grown to early exponential phase (OD\(_{650}\) = 0.4) before diluting 10-fold into fresh prewarmed medium containing MG from a 540 mM stock solution. Cell viability was determined exactly as described previously (4).

**Potassium Efflux Experiments and Determination of Cytoplasmic pH—**Potassium efflux and cytoplasmic pH determinations were carried out as described previously (5, 6, 10) with cells grown at 37 °C in K\(_{10}\) minimal medium (10) supplemented with 0.2% (w/v) glucose and 1 μg·ml\(^{-1}\) thiamine. For the assay cells were washed and suspended in K\(_0\).

* This work was supported by The Wellcome Trust Grant 040174 and by Research Leave Fellowship 046289 (to I. R. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 44-1224-273152; Fax: 44-1224-273144; E-mail: gen11@abdn.ac.uk.

The abbreviations used are: NEM, N-ethylmaleimide; PCR, polymerase chain reaction; kb, kilobase(s).

*This paper is available on line at http://www.jbc.org*
buffer, which lacks ammonium sulfate and MgSO₄. To determine the intracellular K⁺ content of cells during growth, samples were centrifuged in KₐD₀ₐ₇ minimal medium to an OD₆₅₀ of 0.8–1.0 and 6–m were centrifuged through 200 following standard protocols (12).

Cloning of kefB—A 2.6-kb fragment encompassing the yheR and kefB genes was amplified by PCR from strain MJF277 using primers KeF3 and KeF4 (Table II), both of which had BamHI restriction sites incorporated at their 5' ends. The PCR products obtained were end-filled by treating with the Klenow enzyme, restricted with BamHI, ligated into similarly restricted plasmid pHG165 (11) to create plasmid pKefB, and transformed into strain JM109. Klenow treatment, restriction enzyme digestion, ligation, and transformation procedures were carried out following standard protocols (12).

DNA Sequencing—For DNA sequencing, the cloned yheR and kefB genes from plasmid pKefB and mutant plasmids were amplified in 450 ± 50 base pairs overlapping fragments, using primers designed specifically to complement the available yheR and kefB gene sequences from the E. coli genome project (13). The PCR products obtained were cleaned using Promega PCR DNA clean-up kit. A cycle sequencing reaction with each one of the primer pair used for amplification was performed using Applied Biosystems sequencing premix. The products were cleaned by ethanol precipitation and run on the Applied Biosystems 373A sequencer before being analyzed using the Applied Biosystems "Sequence Editor" program.

Site-directed Mutagenesis—To create plasmid pKefB-2, which carries an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed. Primer KefB8 (Table II) was designed such that it encompassed bases 774–792 of the kefB gene (amino acids 258–264 of the KefB protein) and contained one mismatch base T781A, which created an aspartate residue at position 262 of the KefB protein in place of the wild-type alanine residue (Table I), site-directed mutagenesis was performed.

All other mutant plasmids were obtained using the following method, which is based on a technique developed by Stratagene. Parental or wild-type plasmid DNA was purified from a strain that methylates its DNA (JM109 was used for this purpose), and this was used as template for 18 rounds of PCR using the appropriate mutagenic primers (Table II) and Pfu polymerase. Restriction with DpnI, an enzyme that restricts methylated DNA only, digests template DNA, while leaving amplified and, therefore, mutated DNA undigested. After transformation of the restricted PCR reactions into JM109, the majority of colonies obtained, therefore, should contain the desired mutant plasmid. Analysis of the putative mutants was by restriction enzyme digestion followed by DNA sequencing (see above).

RESULTS

Cloning of the kefB Gene from E. coli—The kefB locus at 75 min on the E. coli genetic map is required for K⁺ efflux elicited by...
by MG (4). Analysis of open reading frames in this region of the E. coli genetic map identified a sequence, ECOUW67_274 (P45522) with strong sequence similarity to KefC from E. coli and KefX from Hemophilus influenzae and Myxococcus xan-

thus. The predicted open reading frame is 601 amino acids (compared with 620 residues for KefC) shows 42% identity and 70% similarity at the amino acid sequence and exhibits similar domain organization to KefC. Another open reading frame ECOUW67_275 (P42621), yheR, overlapped kefB by a single base the 5' end. This gene arrangement is similar to that found for kefC, in which an upstream open reading frame, yabF, is required for the activity of the KefC protein (14). The putative yheR-kefB region was amplified and cloned into plasmid pHG165 to create pKefB (see “Experimental Procedures”) and transformed into strain MJF276 (KefB^-KefC^-). The cloned fragment was sequenced and confirmed to carry the same sequence as that deposited in the data base (13). The transformants were analyzed for electrophile-elicited K^+ efflux activity and for restoration of protection against MG.

Strain MJF276/pKefB rapidly lost 25% of the cell K^+ pool on suspension into K0 buffer (first time point 40 s after suspension in K0), and the pool declined to less than 50% of the control over a 25-min incubation (Fig. 1A). The K^+ pool of MJF276/pKefB was equal to, or greater than, that of MJF276 prior to suspen-
The KefB System of E. coli

sion in $K_0$ (705 ± 21 and 598 ± 51 μmol·g$^{-1}$ dry cell mass, respectively). Addition of MG caused more than 85% of the K$^+$ pool to be lost in the first 7 min of the incubation with the electrophile. The MG-elicited rate of efflux was considerably faster than that observed with strain MJF276 (KefB$^{+}$KefC$^{+}$) and MJF274 (KefB$^{-}$KefC$^{-}$), which carries a single chromosomal copy of the kefB gene (note that KefC makes little contribution to MG-elicited efflux) (Fig. 1B). It is notable that the initial rate of K$^+$ loss after addition of MG is slower than the maximum activity, which was achieved approximately 3–5 min after addition of the electrophile. Activation by NEM, which reacts spontaneously with glutathione to form the activator N-ethylsuccinimido-S-glutathione elicted high rates of K$^+$ efflux from MJF276/pKefB (Fig. 1C). The rate of K$^+$ efflux declined steadily as the K$^+$ pool declined. When compared with data for strains MJF274 (KefB$^{+}$KefC$^{+}$) and MJF277 (KefB$^{-}$KefC$^{-}$), which possess single copies of KefB, these data suggest a 10–12-fold increased expression of the KefB protein in MJF276/pKefB.

The cloned kefB gene provided full protection against MG. When incubated with 0.4 mM MG growth of E. coli cells was inhibited but strain MJF274 (KefB$^{+}$KefC$^{+}$) and MJF276/pKefB recovered and subsequent growth occurred at the same rate. Strain MJF276, which lacks functional KefB and KefC systems, also recovered but at a much slower rate (data not shown). When exposed to higher concentrations of MG cell systems, also recovered but at a much slower rate (data not shown).

**Fig. 3. Sequence alignment of the regions surrounding L75S (A), the HELETAID motif (B), and the Rossmann fold motif of KefB from known homologues (C).** KefB and KefC from E. coli (KefB E. coli and KefC E. coli), KefX from H. influenzae (KefX Haem), and KefX from M. xanthus (KefX Myx). Residues shaded black are conserved. Leu$^{75}$, Ala$^{262}$, and Val$^{428}$ in KefB are indicated (arrow).

When incubated with 0.4 mM MG growth of E. coli cells was inhibited but strain MJF274 (KefB$^{+}$KefC$^{+}$) and MJF276/pKefB recovered and subsequent growth occurred at the same rate. Strain MJF276, which lacks functional KefB and KefC systems, also recovered but at a much slower rate (data not shown). When exposed to higher concentrations of MG cell systems, also recovered but at a much slower rate (data not shown).

**Fig. 3. Sequence alignment of the regions surrounding L75S (A), the HELETAID motif (B), and the Rossmann fold motif of KefB from known homologues (C).** KefB and KefC from E. coli (KefB E. coli and KefC E. coli), KefX from H. influenzae (KefX Haem), and KefX from M. xanthus (KefX Myx). Residues shaded black are conserved. Leu$^{75}$, Ala$^{262}$, and Val$^{428}$ in KefB are indicated (arrow).
K0 buffer. stream regulatory regions. Time 0 indicates suspension of the cells in carry the yheR-kefB MJF111/pKefB; and MJF111/pKefB. Symbols: ● (A)
activity.

1 exhibits rapid K
E. coli family of proteins (Fig. 3) and the MJF111/pKefB-4 (V428A) (○), and pKefB-5 (V428A/HALESDIE) (△). All pKefB constructs carry the upstream transcription and translation sequences, the yheR gene, and the kefB gene.

and there was a slight reduction in NEM-elicted efflux (data not shown). Therefore, it is clear that this residue plays a less significant role than D264 in KefC.

V427A—Mutations in the Rossman fold of KefC (R416S and V427A) result in a similar phenotype to that seen with the L75S mutation in KefB (9). Val127 is conserved in the KefC family of proteins (Fig. 3) and the E. coli KefC mutant V427A exhibits rapid K+ efflux when present in single or low copy number (9). Strain MJF276/pKefB-111 (V427A), which is a monocopy plasmid based on pkC11 (Table I), failed to grow even in K120 medium suggesting that the K+ leak is too severe to allow growth. In contrast, only a small increase in spontaneous K+ leak was observed when the equivalent V428A mutation was introduced into KefB (Fig. 5; cf. pKefB and pKefB-4). Rates of MG-elicted efflux were rapid but showed no significant difference between pKefB and pKefB-4 (V428A) (data not shown).

The HALESDIE Sequence—Located between two highly conserved regions of KefC is a variable sequence HALESDIE that contains three acidic residues in all four known sequences (Fig. 3). Two UV-induced mutations in this region in E. coli KefC, D264A and E262K, enhance spontaneous K+ efflux (9). The KefB protein also has three acidic residues in the equivalent sequence (HELETAID), but also carries an alanine residue at position 262, echoing the D264A mutation in KefC. Therefore, we determined whether it was the presence of three acidic residues or their location at specific positions that controlled the activity of the KefC system. We exchanged the equivalent regions from KefB and KefC, namely the HELETAID and HALESDIE motifs, respectively, and measured the spontaneous and electrophile-induced rates of K+ efflux (Figs. 5 and 6, A and B). Replacement of the KefB HELETAID with KefC HALESDIE in plasmid pKefB-3 (Table I) had only a small effect on spontaneous efflux, enhancing the initial rate approximately 2-fold (Fig. 5). The mutation did not significantly affect the rate of electrophile-elicted efflux, which was faster than the spontaneous rate of K+ loss (data not shown). Combinations of the HALESDIE motif and V428A in KefB (pKefB-5) also led to higher spontaneous rates of K+ efflux, but the double change did not emulate the severity of the combination in KefC. Electrophile-elicted efflux was not significantly affected in the KefB mutant (data not shown). In contrast, in KefC, replacement of the HALESDIE sequence with HELETAID (plasmid pkC11-3) significantly enhanced the spontaneous K+ loss (Fig. 6A). This multiple change creates in KefC the D264A mutation but leaves three acidic amino acids in the motif. As a control, an equivalent plasmid pkC11-2 (KefC D264A) was created. Strain MJF276/pkC11-2 failed to grow in K120 medium, suggesting that the K+ leak overwhelms the uptake capacity of the strain. In contrast, MJF276/pkC11-3 (D264A), which has reduced expression of KefC due to a deletion 5′ to the structural gene, was able to grow normally in K120 medium (9). Strain MJF276/pkC11-3 (HELETAID), which recreates the D264A mutation but in a different context to pkC11-2 (KefC D264A) (Table I), grew normally in K120 medium and exhibited only a moderate K+ leak. These data suggest that the D264A mutation in plasmid pkC11-3 (HELETAID) is partially compensated by the presence of the three acidic residues in the motif.

![Fig. 4. L75S in KefB is important for channel regulation and activity. A, potassium efflux from the KefB leaky mutant, MJF111 (kefC::Tn10; kefB111; L75S). Symbols: ○, MJF111; ▲, MJF111 plus 3 mM MG; and □, MJF111 plus 0.5 mM NEM. The electrophile was added after 3 min (arrow). B, partial suppression of K+ efflux in strain MJF111/pKefB. Symbols: ●, spontaneous K+ efflux from Frag5 (KeF+ KefC-); ▲, Frag5/pKefB; ○, MJF111 (kefC::Tn10, kefB111; L75S); □, MJF111/pKefB; and △, MJF111/pkC11. Plasmids pKefB and pkC11 carry the yheR-kefB and yabF-kefC genes, respectively, and their upstream regulatory regions. Time 0 indicates suspension of the cells in K0 buffer.](image1)

![Fig. 5. The effect of HALESDIE on KefB regulation. Spontaneous K+ efflux was measured after suspension of the cells in K0 buffer. Symbols: MJF276 (KefB- kefC- ) transformed with: pKefB (wild-type KefB) (●), pKefB-3 (HALESDIE) (○), pKefB-4 (V428A) (○), and pKefB-5 (V428A/HALESDIE) (△). All pKefB constructs carry the upstream transcription and translation sequences, the yheR gene, and the kefB gene.](image2)
that at the first time point (approximately 40 s) the cells were completely depleted of K⁺ (Fig. 6A). These cells grew poorly and even in K120 medium achieved a rate that was only 78% of that of MJF276/pkC11 (μ = 0.6 h⁻¹ and 0.47 h⁻¹, for MJF276/pkC11 and MJF276/pkC11-5 (HELETAID + L74S), respectively. Thus, L74S acted synergistically with the HELETAID mutation. These data are consistent with the effect of the L75S mutation on KefB, which naturally possesses the HELETAID motif, and suggest that these two regions are critical to maintenance of the closed state of KefB.

**DISCUSSION**

These studies were undertaken to ascertain whether the amino acid residues critical to the regulation of two homologous K⁺ efflux systems were the same. KefB and KefC are 601 and 620 amino acid proteins, respectively, and are 42% identical and 70% similar in their sequences. The linker regions (amino acids 380–400 in KefB) are quite diverse and the major points of sequence deviation lie in the extreme carboxyl-terminal region. In view of their overall similarity, it was reasonable to expect that they might possess common regions responsible for the regulation of their activity. KefC is maintained in an inactive state even when present on a multi-copy plasmid, except in the presence of an activating electrophile (7). We have documented previously a number of KefC mutations that increase the spontaneous K⁺ efflux via this protein (9). The mutations substantially increased the rate of K⁺ loss from cells such that they could not grow in media low in K⁺ (K₀,2) (1, 9, 15). The mutations with the greatest effect on activity clustered to two sequences, the Rossman fold and HALESDIE, suggesting that these might be significant controlling regions in the protein. However, this study suggests that KefB and KefC have evolved different critical residues and that sequence conservation alone is not a guide to the identification of important sequences.

The HALESDIE region is different in KefB and KefC despite strong conservation in the flanking sequences (Fig. 3). Both proteins, and the KefX proteins of *H. influenzae* and *M. xanthus*, contain three acidic residues in this sequence, but it is noteworthy that their positions are not conserved. This study aimed to analyze the relative importance of position and sequence. Cells overexpressing the KefC D264A mutation in the HALESDIE context exhibit a much more profound growth defect in K120 medium than those where the mutation is surrounded by HELETAID, which retains the three acidic residues. The rate of spontaneous K⁺ loss in MJF276/pkC11-3 (KefC HELETAID) is similar to that observed previously in MJF276/pSM26 (9), which carries the kefC D264A mutation but expresses the KefC protein at an approximately 20-fold lower level. Consistent with this observation, MJF276/pkC11-2 (KefC D264A), which has high level expression of the KefC system, cannot grow in K120 medium. These data suggest that the context of the D264A mutation is a significant determinant of its impact on KefC activity and is consistent with the hypothesis that the number of acidic residues in the HALESDIE region of KefC is more important than their absolute position.

Five independent UV-induced mutations causing fast spontaneous K⁺ efflux in KefB were found to be L75S. The importance of Leu 75 is consistent with the observations on the L74S/HELETAID double mutant of KefC. Combination of L74S and HELETAID in KefC resulted in spontaneous efflux characteristics, resembling those of KefB (L75S). The KefB mutation is more severe than the change in KefC, since strain MJF276/pKefB-1 (L75S) could not grow in K120 medium, whereas MJF276/pkC11-5 (KefC L74S/HELETAID) grew, albeit with a reduced growth rate. The combination of the two mutations had a synergistic effect on spontaneous K⁺ loss via KefC (Fig. 6A). These data strongly suggest a possible interaction between
the region surrounding L75S and the HELETAID motif that leads to the maintenance of the protein in the closed state.

Acknowledgments—We are indebted to Wolf Epstein who initiated studies on KefB and isolated the original kefB mutants, to Dr. Guy Plunkett (Laboratory of Genetics, University of Wisconsin) who supplied sequence information for the kefB region prior to publication, and to Dr. Philip Carter (Department of Medical Microbiology) who supervised all the DNA sequencing.

REFERENCES

1. Booth, I. R., Epstein, W., Giffard, P. M., and Rowland, G. C. (1985) Biochimie (Paris) 67, 83–90
2. Meury, J., and Kepes, A. (1982) EMBO J. 1, 339–343
3. Elmore, M. J., Lamb, A. J., Ritchie, G. Y., Douglas, R. M., Munro, A., Gajewski, A., and Booth, I. R. (1990) Mol. Microbiol. 4, 405–412
4. Ferguson, G. P., Munro, A. W., Douglas, R. M., McLaggan, D., and Booth, I. R. (1993) Mol. Microbiol. 9, 1297–1303
5. Ferguson, G. P., McLaggan, D., and Booth, I. R. (1995) Mol. Microbiol. 17, 1025–1033
6. Ferguson, G. P., Nikolaev, Y., McLaggan, D., MacLean, M., and Booth, I. R. (1997) J. Bacteriol. 179, 1007–1012
7. Munro, A. W., Ritchie, G. Y., Lamb, A. J., Douglas, R. M., and Booth, I. R. (1991) Mol. Microbiol. 5, 607–616
8. Booth, I. R., Jones, M., McLaggan, D., Nikolaev, Y., Ness, L., Wood, C., Miller, S., Totemeier, S., and Ferguson, G. (1996) Handbook of Biological Physics (Könings, W. N., Kaback, H. R., and Lolkema, J. S., eds) Vol. 2, pp. 693–729, Elsevier Science Publishers B. V., Amsterdam
9. Miller, S., Douglas, R., Carter, P., and Booth, I. R. (1997) J. Biol. Chem. 272, 24942–24947
10. Epstein, W., and Kim, B. S. (1971) J. Bacteriol. 106, 639–644
11. Stewart, G. S. A. B., Lubinsky-Mink, S., Jackson, C. G., Cassel, A., and Kuhn, J. (1986) Plasmid 15, 172–181
12. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
13. Blattner, F. R., Plunkett, G., III, Mayhew, G. F., Perna, N. T., and Glassner, F. D. (1995) Escherichia coli Genome Entry; GenBank™ accession number U18997
14. Wood, C. M. (1996) A Molecular Analysis of the Potassium Eflux System KefC. Ph.D. thesis, University of Aberdeen, Aberdeen, UK
15. Douglas, R. M., Ritchie, G. Y., Munro, A. W., McLaggan, D., and Booth, I. R. (1994) Mol. Membr. Biol. 11, 55–61
16. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103–119