The product of the *ybdE* gene of the *Escherichia coli* chromosome is involved in detoxification of silver ions

Sylvia Franke, Gregor Grass and Dietrich H. Nies

**INTRODUCTION**

Silver (Ag⁺) combines a high toxicity with relatively frequent occurrence in natural ecosystems and a long tradition of medical use (Nies, 1999). The toxic effects of silver on bacteria have been investigated for more than 60 years and many silver-resistant bacteria have been isolated (Yudkins, 1937; Nies, 1999). However, the molecular background of silver resistance has only recently become clear. In Gram-positive bacteria, Ag⁺ resistance may be the result of efflux catalysed by a P-type ATPase which is also responsible for the export of Cu⁺ (Solioz & Odermatt, 1995). In Gram-negative bacteria, a plasmid-encoded silver resistance determinant mediates efflux of Ag⁺ by the RND-driven SilCBA transport system (Gupta et al., 1998, 1999), and physiological evidence for a chromosomally encoded silver efflux system in *Escherichia coli* has also been reported (Li et al., 1997).

Previously known CBA transport systems for heavy metal cations include the Czc, Cnr and Ncc systems, with Czc being best characterized. Czc provides resistance to Co²⁺, Zn²⁺ and Cd²⁺ in the Gram-negative bacterium *Ralstonia metallidurans* CH34 (previously *Alcaligenes eutrophus*; Brim et al., 1999; Mergeay, 2000; J. Goris, P. De Vos, D. Janssens, M. Mergeay & P. Vandamme, unpublished). The CzcCBA efflux pump (Nies et al., 1989; Rensing et al., 1997) is composed of three subunits. CzcA transports the cations across the cytoplasmic membrane. The protein has been purified (Goldberg et al., 1999) and shown to be an inner-membrane proton–cation antiporter. CzcA belongs to the RND protein superfamily (TC 2.A.6.1.1; Saier, 2000) of proton-driven sym- and antiporters (Tseng et al., 1999). CzcB, a membrane fusion protein, and CzcC, an outer-membrane-associated protein, may transport the cations across the periplasmic space and the outer membrane to the outside (Rensing et al., 1997).

The total *E. coli* genome contains seven genes encoding RND proteins. We have demonstrated that one of them, *ybdE* (gb|AE000162.1|), is involved in chromosomal silver resistance. The YbdE protein is similar to CzcA from *R. metallidurans* CH34 (Nies et al., 1989) and SilA from *Salmonella typhimurium* (Gupta et al., 1999). Like the czcA or silA genes, *ybdE* is preceded by a gene encoding a membrane fusion protein, *ylcD*, and a gene encoding a putative outer-membrane-associated protein, *ylcB*. Between *ylcB* and *ylcD* is located the small ORF *ylcC*, which is homologous to a small ORF in the *sil* operon at the same respective location. Finally, the genes for a two-component regulatory system are located adjacent to the putative *ylcBCD–ybdE* operon.

**Transcription of the ybcZ–ylcA ylcBCD–ybdE region of the *Escherichia coli* K38 chromosome was analysed by Northern RNA–DNA hybridization, RT-PCR and primer extension. Transcription of a dicistronic ybcZ–ylcA mRNA and a tricistronic ylcBCD–ybdE mRNA was induced by silver and was initiated from the sigma-70 promoters ylcAp and ylcBp. Expression of β-galactosidase activity from a φ(ylcBp–lacZ) operon fusion was also induced by Ag⁺ and Cu²⁺, but not by Zn²⁺. In-frame deletion of *ybdE* from the chromosome yielded a silver-sensitive *E. coli* mutant strain which did not differ in its copper resistance from its wild-type strain. On the other hand, deletion of the copA gene for the copper-exporting P-type ATPase CopA resulted in copper sensitivity, but not in silver sensitivity. A Δ*ybdE ΔcopA* double mutant strain behaved towards copper as the ΔcopA strain and towards silver as the Δ*ybdE* strain. Thus, in *E. coli*, the YlcBCD–YbdE system may be involved in silver- but not in copper resistance, and CopA may be involved in copper- but not in silver resistance.**

**Keywords:** heavy metal resistance, cation efflux, silver resistance, RND family
The ybcZ gene encodes the putative sensor and ylcA the predicted response regulator (Blattner et al., 1997). This paper describes the initial characterization of this bacterial silver resistance system.

**METHODS**

**Bacterial strains and growth conditions.** Tris-buffered mineral salts medium (Mergeay et al., 1985) containing 2 g glucose or sodium gluconate l\(^{-1}\) and 1 g yeast extract l\(^{-1}\) (TGY medium) was used to cultivate *E. coli* wild-type strain K38 (Tabor & Richardson, 1985), its derivatives EC756 ∆ybdE, EC774 ∆copA, EC773 AybdE ∆copA, and the Lac\(^{-}\) strain JM83 (Vieira & Messing, 1982). Luria broth (LB; Sambrook et al., 1989) was used as complex medium. When resistance to silver was being tested, NaCl was omitted from the LB medium and the cultures were incubated in the dark. Stock solutions of AgNO\(_3\), CdCl\(_2\), ZnCl\(_2\), CoCl\(_2\), NiCl\(_2\) and CuCl\(_2\) were 1 M in water and were sterilized by filtration. The stock solution of CuCl\(_2\) was in HCl (370 g l\(^{-1}\)). Solid Tris-buffered media contained 20 g agar l\(^{-1}\). β-Galactosidase activity was determined in permeabilized cells as published previously (Nies et al., 1992) with 1 U defined as the activity forming 1 nmol o-nitrophenol min\(^{-1}\) at 30 °C (Parde et al., 1959; Ullmann, 1984).

**Genetic techniques.** Standard molecular genetic techniques were used (Nies et al., 1987; Sambrook et al., 1989). Total RNA of *E. coli* was isolated as published by Oelmüller et al. (1990), van der Lelie et al. (1997) and Große et al. (1999). Amount and quality of total RNA was determined spectrophotometrically at 260 and 280 nm.

**Strain constructions.** To prevent any polar effect mediated by deletion of ybdE, the gene was exchanged against a small ORF encoding a polypeptide of 20 amino acids. The first 10 amino acids encoded by this small ORF were identical with the 10 amino-terminal amino acids of YbdE, and the last 8 amino acids were identical with the last, carboxy-terminal amino acids of that protein. Positions 11 and 12 were Glu and Phe, encoded by the hexanucleotide recognition sequence GAATTC of the restriction endonuclease *EcoRI*. Thus, the 500 bp upstream of ybdE were amplified by PCR (Table 1, primers 1 and 2), and this fragment ended with the 30 bp coding sequence for the first 10 amino acids of the respective gene, followed by an *EcoRI* hexanucleotide. Secondly, the

**Table 1. Oligonucleotide primers used**

<table>
<thead>
<tr>
<th>No.</th>
<th>Use</th>
<th>DNA sequence, 5' → 3' direction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ybdE, deletion, 5' part; ylcD–ybdE, RT-PCR</td>
<td>AAAGGATCCGGAGGCCGCTAAAACCGGGC</td>
</tr>
<tr>
<td>2</td>
<td>ybdE, deletion, 5' part; ylcD–ybdE, RT-reaction</td>
<td>AAAGAATTCCACCGAGCGACAATAACCAT</td>
</tr>
<tr>
<td>3</td>
<td>ybdE, deletion, 3' part; ybdE–pheP, RT-PCR</td>
<td>AAAGAATTCTCGCACCAGACATCGGGTACGG</td>
</tr>
<tr>
<td>4</td>
<td>ybdE, deletion, 3' part; ybdE–pheP, RT-reaction</td>
<td>AAAGGATCCGCAAAGTGGGCAATGAACCG</td>
</tr>
<tr>
<td>5</td>
<td>copA, deletion, 5' part</td>
<td>AAA GGATCC CGG CGT TAA GTG AGT GAA ATT GG</td>
</tr>
<tr>
<td>6</td>
<td>copA, deletion, 3' part</td>
<td>AAA GAATTC GCA GTG ACC GCA GGA CAG G</td>
</tr>
<tr>
<td>7</td>
<td>copA, deletion, 3' part</td>
<td>AAA GAATTC GTG AGT AAC GCC AAC CCG TTG</td>
</tr>
<tr>
<td>8</td>
<td>copA, deletion, 3' part</td>
<td>AAA GAATCC AAA ACA TCG CTT CAT CTC GTA GGC</td>
</tr>
<tr>
<td>9</td>
<td>ylcA, primer extension; ylcAB, RT-PCR</td>
<td>GGTTAAGCTGAGCCGGCGTGTGTC</td>
</tr>
<tr>
<td>10</td>
<td>ylcB, primer extension; ylcAB, RT-reaction</td>
<td>CCGTCTGAGGCTGACTGAG</td>
</tr>
<tr>
<td>11</td>
<td>ybcZ, RT-reaction</td>
<td>GCGTGGAGGGCGATGCAAAGAAGGC</td>
</tr>
<tr>
<td>12</td>
<td>ybcZ, RT-PCR</td>
<td>GTGTTTACGCCGCAGTTCAAGAA</td>
</tr>
<tr>
<td>13</td>
<td>ybcZ–ylcA, RT-reaction</td>
<td>GCTTGGCGAGGTTCGAGGTTG</td>
</tr>
<tr>
<td>14</td>
<td>ybcZ–ylcA, RT-PCR</td>
<td>TCTGCCTGACGCCGCAAATGTCAC</td>
</tr>
<tr>
<td>15</td>
<td>ylcBCD, RT-reaction</td>
<td>GCCGCAGAAATAATACCCGCC</td>
</tr>
<tr>
<td>16</td>
<td>ylcBCD, RT-PCR</td>
<td>CTGATGGCCGAGCGCCTTCTTTA</td>
</tr>
<tr>
<td>17</td>
<td>ybdE, RT-reaction</td>
<td>TCCGCTTCGCCGTGTTAGTTC</td>
</tr>
<tr>
<td>18</td>
<td>ybdE, RT-PCR</td>
<td>GCGTTTTCACAGTTTGGCGC</td>
</tr>
<tr>
<td>19</td>
<td>Cloning of ylcBp</td>
<td>ATAGGATACCTAAATTCTGTTAGTTATGCCG</td>
</tr>
<tr>
<td>20</td>
<td>Cloning of ylcBp</td>
<td>CAGGGATCCATTTCCTCCGCGATTTGCCC</td>
</tr>
</tbody>
</table>

* Restriction endonuclease sites are underlined.

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500 bp downstream of ybdE were amplified by PCR (Table 1, primers 3 and 4), and this fragment started with the EcoRI recognition sequence and the last 24 bp of the respective gene. Both fragments were fused by EcoRI restriction and ligation, cloned, verified by DNA sequencing, and finally cloned into pKO3 (Link et al., 1997). The resulting plasmid was used for mutation in E. coli K38 as described by Link et al., (1997), giving strain EC756 ΔybdE. The mutant genotype was verified by PCR and Southern DNA–DNA hybridization. The AcopA mutation was generated in exactly the same fashion in both strains (primers 5–8), the K38 wild-type giving EC774 ΔcopA ΔybdE strain EC773 ΔybdE ΔcopA.

For the complementation assay, the ylcBp–ylcBCD–ybdE region was amplified by PCR from genomic DNA of E. coli wild-type strain K38 using primers 4 and 20 (Table 1). The resulting DNA was cloned into vector plasmid pGEM-T-easy (Promega), giving plasmid pECD590.

For construction of the reporter gene fusion, the ylcBp promoter was amplified by PCR (primers 19 and 20, Table 1) from chromosomal DNA of E. coli strain K38 and cloned upstream of a promoterless lacZ gene (N. Peitzsch & D. H. Nies, unpublished) in plasmid pKO3 (Link et al., 1997).

Northern DNA–RNA hybridization. Northern (RNA) blot analysis was performed as published by Große et al., (1999) by fractionation of RNA samples on agarose/formaldehyde gel (15 g agarose l−1), followed by transfer to a positively charged Qibnrame nylon filter (Qiagen) using a pressure blot (Posiblot; Stratagene). For quantitative analysis, the same amount of total RNA (40 µg) was loaded into each well of the same gel, and this was verified by ethidium bromide staining after electrophoresis. After prehybridization for 3 h at 55 °C in hybridization buffer (Engel-Blum et al., 1993), the filters were hybridized for at least 14 h at 55 °C in the same buffer. The filters were probed with PCR fragments representing parts of ylcZ–ylcA (positions 1339–1813, Fig. 1) and ybdE (positions 5738–6339). The DNA fragments were labelled with digoxigenin by random priming using the DIG-DNA labelling kit (Roche). After several washing steps, the filters were developed with the DIG-luminescent detection kit (Roche).

Reverse transcription. Cells of E. coli K38 cultivated in Tris/glucose/yeast extract medium were incubated for 10 min with 15 µM Ag⁺ or without silver. Total RNA was isolated from these cells and digested with 1 U RNase-free DNase I (Roche) (µg RNA)−1 for 2 h at 37 °C (Große et al., 1999). Twenty picomoles of the 3’ antisense primer (Table 1) was mixed with 2 µg (RT-PCR) or 10 µg (primer extension) of total RNA in 12 µl water, heated for 10 min at 70 °C and quickly cooled on ice. Buffer [4 µl; 250 mM Tris/HCl (pH 8.3), 375 mM KCl, 15 mM MgCl₂], 2 µl 100 mM DTT and 1 µl containing 10 mM of each of the four dNTPs were added. After incubation at 50 °C for 2 min, 200 U Superscript II reverse transcriptase (Gibco BRL) was added, and incubation was continued for 60 min at 50 °C. The reaction was stopped by heating at 80 °C for 5 min.

Primer extension experiments. Primer extension analysis was performed by a modification of a standard protocol (Sambrook et al., 1989; Große et al., 1999) using fluorescein-labelled oligonucleotides and an automated ALF DNA Sequencer (Pharmacia), as described previously. The fluorescein-labelled 3’ antisense primers (Table 1, primers 9 and 10) were complementary to the corresponding gene regions. The cDNA (obtained by reverse transcription as described above) was vacuum-dried and suspended in 4 µl H₂O and 4 µl ALF stop solution (Pharmacia). Following heat denaturation, the sample was loaded onto a 7% polyacryl-
amide sequencing gel. In parallel, a sequencing reaction was performed with the same fluorescein-labelled primer and a DNA fragment containing the respective gene region. The transcription start site was determined by comparison of the retention time of the primer extension reaction with that of the sequencing reaction.

**RT-PCR experiments.** Reverse transcription was carried out with 3' antisense primers (see Fig. 1 for positions, and Table 1 for sequences). PCR amplification was performed with 0-1 vol. of the reverse transcription reaction in a mixture containing 50 mM Tris/HCl (pH 9.0), 20 mM ammonium sulphate, 20 pmol 5' sense primer (see Fig. 1 for positions, and Table 1 for sequences), 0-1 mM of each dNTP, 1.5 mM MgCl₂ and 1 U Taq polymerase (Roche) in a final volume of 50 μl. After a ‘hot start’ (2 min, 96 °C), the amplification profile used was: denaturation at 96 °C for 30 s, annealing at 50–58 °C for 20 s, extension at 72 °C for 30 s, final extension at 72 °C for 2 min; 30 cycles were performed. The amplification was performed with a mineral oil overlay in a Trio-Thermoblock (Biometra). Negative controls were: no templates, DNA and RNA without silver (lanes 2 and 4) or for 10 min in the presence of 15 μM Ag⁺ or without silver (Fig. 2). In both cases, smears may indicate unstable mRNAs. The ybdE-specific smear had an upper

![Fig. 2. Northern DNA–RNA hybridization experiments. Total RNA was isolated from cells of E. coli K38 cultivated in TGY medium without silver (lanes 2 and 4) or for 10 min in the presence of 15 μM Ag⁺ (lanes 1 and 3). After electrophoresis on a denaturing gel, the RNA was blotted and hybridized with an ybdE-specific DNA probe (PCR fragment extending from base pair 5738 to 6339, lanes 1 and 2) or an ybdE–ybcA-specific DNA probe (PCR fragment extending from base pair 1339 to 1813, lanes 3 and 4). The arrows on the right indicate the sizes of markers in nucleotides. The position of the 16S and the 23S rRNAs are shown by the arrowheads on the left. Each experiment was done at least twice with similar results.](image)

![Fig. 4. Induction of the ylcBp promoters. The predicted ylcBp promoter was cloned into the low-copy vector pKO3 (Link et al., 1997) upstream of a promoterless lacZ gene. E. coli cells of the Lac strain IMB3 (Vieira & Messing, 1982) carrying these plasmids were cultivated in TGY medium without silver (○, both panels), in the presence of 5 (□) or 10 (■) μM AgNO₃ (a), or in the presence of 5 (△) or 10 (▲) μM CuCl₂ or 5 (▲) or 10 (△) μM ZnCl₂ (b; note the different scales on the y axis compared to a). Each experiment was done at least twice with similar results.](image)
boundary at a size below 6900 nt, which was close to the size of the predicted transcript of an ylcBCD–ybdE operon (6250 nt, Fig. 1). An upper boundary was not visible for the ybcZ–ylcA transcript (Fig. 2). Densitometric analysis revealed for the ylcBCD–ybdE mRNA a value of 33 arbitrary units after induction with silver, and ≤7·4 in the non-induced control (Table 2). The induction quotient was ≥4·5. The values for ybcZ–ylcA were 28 arbitrary units (induced) and 1·9 (uninduced, quotient 14). Thus, both mRNAs were highly unstable and both were induced by 15 µM Ag⁺.

Using RT-PCR, the presence of continuous transcripts in the ybdE region was analysed (Fig. 1b). A negative RT-PCR result (Fig. 1b, lane 3) indicated no transcript shared between ylcA and ylbC, which are transcribed in different directions from a common promoter region (Fig. 1). RT-PCR products gave evidence for transcripts spanning the ybcZ–ylcA (Fig. 1b, lanes 1 and 2) as well as the ylcBCD–ybdE region (Fig. 1b, lanes 4–7). The latter transcript may even include the ybcZ–ylcA and of a ylcBCD–ybdE at least tetracistronic mRNA in cells incubated with silver.

The promoters

Using primer extension experiments, the start sites upstream of ylcB and ylcA were determined (Fig. 3). Transcription of both genes started 10–20 bp upstream of the predicted RBS. The −10 regions of both promoters, TAAAGT (ylcBp) and TAGAAT (ylcAp), were similar to the consensus motifs of sigma-70 promoters (TATAAT; Rosenberg & Court, 1979). Conservation of the −35 site with respect to the sigma-70 consensus (Rosenberg & Court, 1979) was weaker for ylcBp (CGGAAA) than for ylcAp (TTGCCA). Thus, in terms of conservation of the sigma-70 consensus motif, ylcAp should be a stronger promoter than ylcBp. Around position −47 of ylcBp, the inverted repeat 2283-GCCAAAATGACAAATTTCATCTTTTCTG was identified, which might be an upstream activating sequence used by YlcA. This sequence has similarity to inverted repeats upstream of promoters pcoAp, pcoEp, silCp, silEp, copAp and copHp, genes involved in copper and silver resistance (Nies & Brown, 1998; Gupta et al., 1999).

Table 2. Induction of a Φ(ylcBp–lacZ) operon fusion by copper and silver

<table>
<thead>
<tr>
<th>Promoter*</th>
<th>Inducer</th>
<th>Maximum increase (−fold uninduced control)†</th>
<th>Maximum level (−fold uninduced control)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>ylcBp</td>
<td>AgNO₃ (5 µM)</td>
<td>5·04</td>
<td>3·69</td>
</tr>
<tr>
<td>ylcBp</td>
<td>AgNO₃ (10 µM)</td>
<td>3·75</td>
<td>2·54</td>
</tr>
<tr>
<td>ylcBp</td>
<td>CuCl₂ (5 µM)</td>
<td>18·7</td>
<td>14·2</td>
</tr>
<tr>
<td>ylcBp</td>
<td>CuCl₂ (10 µM)</td>
<td>20·0</td>
<td>20·5</td>
</tr>
<tr>
<td>ylcBp</td>
<td>CuCl₂ (100 µM)</td>
<td>33·6</td>
<td>27·7</td>
</tr>
<tr>
<td>ylcBp</td>
<td>ZnCl₂ (5 µM)</td>
<td>0·82</td>
<td>0·85</td>
</tr>
<tr>
<td>ylcBp</td>
<td>ZnCl₂ (10 µM)</td>
<td>1·21</td>
<td>0·97</td>
</tr>
<tr>
<td>None</td>
<td>–</td>
<td>0·65</td>
<td>0·93</td>
</tr>
<tr>
<td>None</td>
<td>AgNO₃ (5 µM)</td>
<td>0·45</td>
<td>0·83</td>
</tr>
<tr>
<td>None</td>
<td>CuCl₂ (100 µM)</td>
<td>0·14</td>
<td>0·91</td>
</tr>
</tbody>
</table>

*None, promoterless lacZ genes.
† Increase in β-galactosidase activity in U h⁻¹ (mg dry wt)⁻¹ (Fig. 4) divided by the respective value [ylcBp promoter, no inducer (◇) in both parts of Fig. 4] obtained for the uninduced control of the operon fusion Φ(ylcBp–lacZ).
‡ The highest β-galactosidase activity reached after induction divided by the uninduced control value [24·2 U (mg dry wt)⁻¹].

The ylcBp promoter is regulated by heavy metal cations

When the ylcBp promoter was cloned upstream of a promoterless lacZ gene, reporter gene expression of the Φ(ylcBp–lacZ) fusion could be induced by 5 µM Ag⁺ (Fig. 4a). The maximum increase in velocity was fivefold the increase in the uninduced control (Table 2) and reached a fourfold higher maximum level compared to the uninduced control. Induction by 10 µM Ag⁺ was somewhat weaker (Fig. 4a), probably due to the toxicity of the metal at this concentration.

Surprisingly, induction of ylcBp by 100 µM Cu²⁺ was very strong (Table 2). At similar concentrations, induction by copper was about fourfold stronger than induction by silver (Table 2). Zn²⁺, on the other hand, had no effect on this promoter (Fig. 4b). There was also no increase in β-galactosidase activity when the ylcBp promoter was omitted from the construct (Table 2).

The ybdE gene is involved in resistance to silver

In the chromosome of E. coli strain K38, the ybdE gene was deleted in-frame giving E. coli strain EC756. In a filter-disk inhibition zone assay, strain EC756 was more sensitive to Ag⁺, Co²⁺, Cu⁺ and Cu²⁺ than wild-type
strain K38 (data not shown); however, the differences were minimal. The strains did not differ in their sensitivity to other metals or organic substances (ethidium bromide, tetracycline, SDS; data not shown).

On solid LB (Sambrook et al., 1989) agar plates without NaCl, wild-type strain K38 grew in the presence of 25 μM Ag⁺, but EC756 did not (data not shown). There was no difference in resistance to Co²⁺, Cu²⁺ or Cu⁺ on solid media (data not shown). In liquid culture, strain EC756 ΔybdE was more sensitive to Ag⁺ compared to its wild-type E. coli strain K38 (Fig. 5a). The deletion could be complemented in trans by the complete ylcBp–ylcBCD–ybdE gene region (Fig. 5b).

Since the ylcBCD–ybdE operon was induced by copper, EC756 and wild-type K38 were also compared in liquid LB medium in the presence of various amount of Cu²⁺; however, there was no difference between the strains (Fig. 5c). The gene for the Cu⁺-exporting P-type ATPase CopA (Rensing et al., 2000) was also deleted from wild-type K38 (giving strain EC774 ΔcopA) and EC756 ΔybdE (giving the double mutant strain EC773 ΔybdE ΔcopA). In the presence of silver, the growth of EC774 ΔcopA was comparable to wild-type K38 (Fig. 5a), but EC773 ΔybdE ΔcopA was more sensitive to Cu²⁺. The double mutant strain EC773 was as silver-sensitive as strain EC756 ΔybdE and as copper-sensitive as EC774 ΔcopA (Fig. 5).

**DISCUSSION**

The ylcBCD–ybdE operon provides only a low level of silver resistance to E. coli and it is therefore an open question whether Ag⁺ is the sole or main substrate of the YlcB–YbdE efflux system. Since copper was a strong inducer of this determinant, Cu⁺ rather than Ag⁺ could have been this main substrate. However, growth of the ΔybdE deletion strain and its wild-type did not differ in the presence of various concentrations of copper.

The CopA P-type copper ATPase was shown to detoxify Cu⁺ in E. coli (Rensing et al., 2000) and increased expression of CopA may therefore compensate for the effect of the missing ybdE gene on copper resistance. Thus, ΔcopA deletions were introduced into wild-type K38 and the ΔybdE deletion strain. The resistance phenotypes displayed by the resulting set of single and double mutant strains in comparison with the wild-type (Fig. 5) ruled out any significant involvement of YbdE in copper detoxification and of CopA in silver detoxification, although both determinants were gratuitously induced by the other monovalent heavy metal cation (Rensing et al., 2000). Secondly, the Cu²⁺ cations in the growth medium were indeed reduced to Cu⁺ in the cytoplasm as expected (Nies, 1999) or no effect of the ΔcopA deletion on Cu²⁺ resistance would have been observed.

To study transcriptional regulation of ybdE, the only currently known substrate, Ag⁺, was used as the inducer. Both operons, ybcZ–ylcA and ylcBCD–ybdE, are transcribed in different directions from an overlapping promoter/operator region. A possible binding site for YlcA was identified, at position −47 with respect to the ylcBp promoter and −42 with respect to ylcAp. With the membrane-bound histidine kinase sensor YbcZ, these components are sufficient to explain the observed activation of ylcBCD–ybdE transcription by Ag⁺ and Cu⁺ cations.
Since this publication was submitted, another group has characterized the ylc gene region under a different aspect (Munson et al., 2000). The data provided by these authors show that the YbcZ–YlcA two-component system regulates not only the ylcBp promoter, but also the promoter pcoEp of a plasmid-bound copper resistance determinant. However, the main copper resistance gene, pcoA, is only partially regulated by YbcZ–YlcA. The transcriptional start site of the ylcB gene (Munson et al., 2000) differs just by 1 base from the start site given here.

The authors speculate (Munson et al., 2000) that the genes of the ylc region may be involved in copper resistance in E. coli, but they provide no data for this assumption. In contrast, the data shown in Fig. 5 clearly indicates that the ybdE gene is not involved in copper resistance. If this gene mediates heavy metal resistance at all, it should be silver resistance. The renaming of the ylc genes as cus is therefore highly premature; if these genes are renamed, a name such as ‘agr’ for silver resistance (agrSR agrCFBA) would be more appropriate.

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