**zur**: a Zn\(^{2+}\)-responsive regulatory element of *Staphylococcus aureus*

Jodi A. Lindsay† and Simon J. Foster

A putative operon encoding a probable zinc-responsive regulatory element (*zur*) and components of an ABC-type transporter (*mreA mreB*) have been characterized in *Staphylococcus aureus*. The zur gene was inactivated but apparently this did not alter Zn\(^{2+}\) uptake. Expression of *mreAB zur* is at a low level under a range of ion conditions. To allow inducible expression of the operon, a construct was made placing it under the control of the IPTG-inducible P\(_{spac}\) promoter. Using this approach, it was shown that zur is able to repress expression of the entire operon in a Zn\(^{2+}\)-dependent manner, and that *mreA* and *mreB* are likely to be involved in high-affinity ion uptake. zur has no apparent role in pathogenicity in a lesion model of *S. aureus* infection.

**Keywords**: metals, transcription, zinc, fur, regulation

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**INTRODUCTION**

Metal-ion homeostasis is important for bacterial life. This is particularly so during pathogenicity, when iron availability is often limiting. The iron concentration within many bacteria is sensed by the Fur regulator, which controls the expression of many genes, including iron-uptake systems (Escolar et al., 1999). Iron transporters and Fur play an important role in virulence in a number of organisms (Litwin & Calderwood, 1993; Cornelissen et al., 1998; Janakiraman & Slauch, 2000; Bearden & Perry, 1999; Escolar et al., 1999). Like iron, free Zn\(^{2+}\) is found in low concentrations in vivo (10\(^{-10}\) M) (Magneson et al., 1987), and Zn\(^{2+}\) uptake is known to be necessary for *Haemophilus ducreyi* pathogenicity (Lewis et al., 1999).

Fur is found in virtually all pathogenic bacteria and also in many non-pathogens. More recently, it has been shown that some organisms contain multiple fur homologues (Bsat et al., 1998; Escolar et al., 1999). The Gram-positive soil microbe *Bacillus subtilis* is related to *Staphylococcus aureus*, and encodes three fur homologues (*fur, perR* and *zur*) and a homologue of the *Corynebacterium diphtheriae* iron-regulator *dtxR* (*mntR*). *B. subtilis* Fur binds Fe\(^{2+}\) and regulates the expression of iron-uptake genes (Bsat et al., 1998). PerR regulates the peroxide regulon and binds to Mn\(^{2+}\) or Fe\(^{2+}\) (Bsat et al., 1998), while Zur down-regulates two Zn\(^{2+}\)-uptake pathways in the presence of Zn\(^{2+}\) (Gaballa & Helmann, 1998). *mntR* binds Mn\(^{2+}\) and regulates Mn\(^{2+}\) transport (Que & Helmann, 2000).

*S. aureus* is an important bacterial pathogen causing significant morbidity and mortality, particularly in hospitals (where antibiotic-resistant strains are common). Under iron stress, *S. aureus* produces siderophores for the uptake of iron (Meiweis et al., 1990; Haag et al., 1994; Lindsay & Riley, 1994; Trivier et al., 1995; Courcol et al., 1997), unique cell-associated and exoproducts which may play a specific role in uptake (Domingue et al., 1989; Lindsay & Riley, 1994; Trivier et al., 1995; Courcol et al., 1997; Cockayne et al., 1998), and can access iron bound to transferrin (Modun et al., 1994, 1998; Lindsay et al., 1995; Modun & Williams, 1999). *S. aureus* has ORFs corresponding to *fur, perR, zur* and *dtxR* which are predicted to be involved in the regulation of these iron responses, and in the regulation of responses to other metal ions. It has been demonstrated that *fur* contributes to virulence and controls siderophore expression and the iron repression of several promoters (Horsburgh et al., 2001). *perR* is involved in oxidative stress and iron storage, and is also necessary for virulence (M. J. Horsburgh, M. O. Clements, H. M. Crossley, E. Ingham & S. J. Foster, unpublished). Previously, the *S. aureus* Fur protein had been overexpressed in *Escherichia coli*, and bound Fe and putative iron-responsive promoters (Xiong et al., 2000).
The characterization of the *S. aureus zur* locus is described here. The role and regulation of the genes *mreA*, *mreB* and *zur* have been investigated. Although we can demonstrate that the genes are involved in Zn$^{2+}$ regulation, their expression is poor and they are not responsible for all of the *S. aureus* responses to zinc.

**METHODS**

**Bacterial strains and growth conditions.** The bacterial strains used in this work are listed in Table 1. Transformations into strain RN4220 (Kreiswirth *et al*., 1983) were performed as described by Schenk & Laddaga (1992). The φ11 generalized transducing bacteriophage was used for transductions (Novick, 1991; Lindsay *et al*., 1998). *S. aureus* was grown on brain–heart infusion agar, using selection with tetracycline (Novick, 1991; Lindsay *et al*., 1998). Transducing bacteriophage was used for transductions (Novick, 1991; Lindsay *et al*., 1998). *S. aureus* were grown in LB, supplemented with ampicillin (50 μg ml$^{-1}$) where appropriate. Growth yields were compared using the two-sample independent-groups t-test (Dawson-Saunders & Trapp, 1990). DNA cloning was performed according to the method of Sambrook *et al*., 1989).

**Molecular methods.** Sequencing of the *mreAB zur* region was carried out using AmpliTaq DNA polymerase (Applied Biosystems) and was based on the dye terminator cycle sequencing method; the analysis was performed on an ABI DNA sequencer (Applied Biosystems).

**Construction of JL100 (zur::tc).** A PCR product spanning 1.46 kb of the *zur* gene and downstream regions was amplified using pSPW100 (Clements *et al*., 1999), as a template, and the primers 5′-CGTAGTGGGATCCGGTTGTAAGGGCGTTATCAG-3′ and 5′-CGTCACTGAAATGGCAATGTAGTCAG-3′ including the putative ribosome-binding site. The digested region was religated from pJL11 by restriction digestion with BglII and EcoRI and cloned into pUBS1 (Foster, 1991) to generate plasmid pJL10 (Table 1). Two divergent primers internal to the *zur* gene and 125 bp apart were designed: 5′-GGATCCCTCATCAGTTGTACCATGG-3′ and 5′-GGATCCGTTGTAAGGCGTTATC-3′. Using these primers, a 5 kb fragment of the *zur* gene, and downstream regions was amplified employing pJL10 as the template. This product was cleaved with BglII and MluI and ligated to the tc fragment. A 1.5 kb fragment spanning the tc (tetracycline resistance) cassette was purified from pDG1513 (Guerout-Fleury *et al*., 1995) after restriction digestion with BglII and MluI. Ligation of the tc cassette with the 4.5 kb pJL10-derived PCR product and transformation into E. coli DH5α resulted in pJL11 (Table 1); this contains a copy of the *zur* region with a 125 bp deletion in the *zur* gene which had been filled with a tc marker. The mutated *zur* region was religated from pJL11 by restriction digestion with BamHI and EcoRI and cloned into similarly digested pMUTIN4 (Vagner *et al*., 1998) to generate pJL12.

pJL12 was transformed into *S. aureus* RN4220, and a single crossover Campbell insertion of the plasmid into the chromosome was confirmed using Southern blotting. A phage lysate was generated from this strain and transduced into 8325-4. Clones were selected for tetracycline resistance and erythromycin sensitivity, indicating an outcross event. The correct construct containing a single copy of the region with a tc insertion in the *zur* gene was confirmed by Southern blotting (results not shown).

**Construction of JL110, JL111, JL120 and JL121.** JL110 (mreB::pMUTIN4) and JL120 (mreA::pMUTIN4) were constructed in a similar manner and are illustrated in Fig. 1. The primers 5′-TCGAGGAAACCTGTGATAGAGCCTTCAG-3′ and 5′-TCGAGGAAACCTGTTGATAGAGCCTTCAG-3′ were used to PCR-amplify a 502 bp fragment internal to *mreB*. The primers had restriction sites incorporated (underlined), allowing the product to be digested with HindIII and BamHI. Similarly, the primers 5′-TCGAGGATCCGTTGATAGAGCCTTCAG-3′ and 5′-TCGAGGAAACCTGTTGATAGAGCCTTCAG-3′ were used to amplify a 717 bp fragment of the 5′ end of the *mreA* gene, including the putative ribosome-binding site. The digested

**Table 1. Strains and plasmids used in this work**

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Description</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8325-4</td>
<td><em>S. aureus</em> wild-type strain</td>
<td>Novick (1967)</td>
</tr>
<tr>
<td>RN4220</td>
<td>Restriction-deficient</td>
<td>Kreiswirth <em>et al</em>., 1983</td>
</tr>
<tr>
<td>JL100</td>
<td>8325-4, zur::tc</td>
<td>This study</td>
</tr>
<tr>
<td>JL110</td>
<td>8325-4, mreB::pMUTIN4</td>
<td>This study</td>
</tr>
<tr>
<td>JL111</td>
<td>8325-4, mreB::pMUTIN4 zur::tc</td>
<td>This study</td>
</tr>
<tr>
<td>JL120</td>
<td>8325-4, mreA::pMUTIN4</td>
<td>This study</td>
</tr>
<tr>
<td>JL121</td>
<td>8325-4, mreA::pMUTIN4 zur::tc</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pUBS1</td>
<td>E. coli cloning vector</td>
<td>Foster (1991)</td>
</tr>
<tr>
<td>pDG1513</td>
<td>Contains tetracycline-resistance cassette (tc)</td>
<td>Guerout-Fleury <em>et al</em>., 1995</td>
</tr>
<tr>
<td>pMUTIN4</td>
<td>EryR, promoterless transcriptional lacZ vector with P&lt;sub&gt;spac&lt;/sub&gt; inducible promoter</td>
<td>Vagner <em>et al</em>., 1998</td>
</tr>
<tr>
<td>pJL10</td>
<td>pUBS1 with 1.5 kb fragment of zur and upstream regions</td>
<td>This study</td>
</tr>
<tr>
<td>pJL11</td>
<td>pJL10 with tc inserted into zur</td>
<td>This study</td>
</tr>
<tr>
<td>pJL12</td>
<td>pMUTIN4 with zur and tc regions from pJL11</td>
<td>This study</td>
</tr>
<tr>
<td>pJL110</td>
<td>pMUTIN4 with 502 bp mreB internal fragment</td>
<td>This study</td>
</tr>
<tr>
<td>pJL120</td>
<td>pMUTIN4 with 717 bp mreA, including 5′ end and putative RBS</td>
<td>This study</td>
</tr>
</tbody>
</table>
fragments were cloned into HindIII- and BamHI-cut pMUTIN4, generating plasmids pJL110 and pJL120, respectively (Table 1).

pJL110 and pJL120 were transformed into RN4220. As the plasmids could not replicate in S. aureus, a single crossover event resulted in stable insertions in the chromosome. Transduction was used to transfer the insertions into the 8325-4 background, with selection for erythromycin resistance (EryR), generating strains JL110 and JL120 (Fig. 1). All constructs were confirmed using both PCR and Southern blotting (results not shown).

The zur::tc mutation was transduced into JL110 and JL120, generating JL111 and JL121, respectively (Table 1).

Specialist media and growth conditions. CL medium was developed for this study. It is a completely defined medium based on CDM (Watson et al., 1998). The components of the media are (mg l−1): glucose (10000), Na2HPO4 (7000), KH2PO4 (300), adenine sulphate (20), guanidine.HCl (20), l-glutamic acid (2220), l-aspartic acid (2220), l-proline (2220), glycine (2220), l-threonine (2220), l-serine (2220), l-alanine (2220), l-lysine.HCl (560), l-isoleucine (560), l-leucine (560), l-histidine (440), l-valine (440), l-arginine (330), l-cystine (220), l-phenylalanine (190), l-tyrosine (170), l-methionine (170), l-tryptophan (60), pyridoxal (0.8), pyridoxamine.2HCl (0.8), d-pantothenic acid (0.4), riboflavin (0.4), nicotinic acid (0.4), thiamin.HCl (0.4) and biotin (0.02). Chelex 100 resin (10 g l−1; Sigma) was mixed for 6 h with the medium to remove ions, and the resin was then removed by filtration. S. aureus has an absolute requirement for magnesium, so CL medium was supplemented with 400 μM MgSO4. Staphylococcal siderophore detection medium (SSD) was prepared and used as previously described (Lindsay & Riley, 1994). When appropriate, SSD and CL media were supplemented with various ions, typically at the following concentrations: ZnCl2, 20 μM (or 0.02 or 0.2 μM); CaCl2, 400 μM; FeCl3, 20 μM; MnCl2, 20 μM; CoCl2, 200 μM; CuSO4, 200 μM; MbCl2, 200 μM; NiCl2, 200 μM.

Colonies from overnight cultures grown on CL agar plates (CL broth without Chelex treatment, supplemented with 400 μM MgSO4 and 1 %, w/v, agar) were inoculated into a CL preculture. Test cultures of 10 ml in 250 ml acid-washed glass flasks were inoculated with pre-culture to an OD600 of 0.002 and incubated at 250 r.p.m. and 37 °C. All experiments were repeated at least three times.

Assays. β-Galactosidase assays were performed as described by Chan & Foster (1998). One unit of β-galactosidase activity was defined as the amount of enzyme that catalysed the production of 1 pmol MU min−1 per OD600 unit. Siderophores were assayed by using the chrome azurol S liquid assay on supernatants from SSD cultures, as described by Lindsay & Riley (1994).

Pathogenicity study. A mouse- abscess model of infection was used, as described elsewhere (Chan et al., 1998). Briefly, bald mice were inoculated subcutaneously with 1 x 10⁸ bacteria, either 8325-4 (10 mice) or JL100 (10 mice). After 7 d, the mice were killed, their lesions were removed, and viable bacteria within the lesions were counted. Differences were compared using the Mann–Whitney U test.

Mass spectrometry. Cells (12 ml) from 24 h cultures were centrifuged, washed three times with deionized water, autoclaved and then dried. Cells were resuspended in 1 ml of M HCl, heated to 60 °C for 3 h, then left at room temperature overnight. Samples were spun in a microfuge for 10 min and supernatants were tested for ion concentrations by MS. The ions tested were Li, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Mo, Ba and Pb.

RESULTS

Sequencing and gene homology

Previous work in our laboratory had identified sodA of S. aureus (Clements et al., 1999). Further sequencing of the 3·8 kb region from pSPW100 revealed a putative operon containing three genes upstream of sodA. The
third gene showed greatest homology to Staphylococcus epidermidis ‘fur’ (87% at the amino acid level), although this gene was named before it became apparent that multiple fur homologues existed in some bacteria (Heidrich et al., 1996). The predicted protein contained all the conserved residues typical of a fur homologue (Achenbach & Yang, 1997). The S. aureus gene was 51% identical to both B. subtilis and Listeria monocytogenes zur, and so was tentatively labelled zur.

Upstream of zur are two genes predicted to encode the ATP-binding and membrane-bound components of an ABC transporter system, the strongest homology being to Zn\(^{2+}\) transporters and then to other metal-ion transporters (Fig. 1; Table 2). These have been designated mreA and mreb (membrane-metal-binding elements). The mreAB zur operon has a hairpin-loop structure downstream of zur (ΔG = −6.2 kJ mol\(^{-1}\)) and, suggesting that it is not transcriptionally linked to the downstream ORF, sodA, encoding a manganese-dependent superoxide dismutase (Clements et al., 1999). Upstream of mreA is the 3’ end of an ORF showing strong homology to the endonuclease IV family of Saccharomyces cerevisiae (40% amino acid homology over 105 aa) and Escherichia coli (38% identity over 101 aa). Endonuclease IV proteins are involved in DNA repair of free-radical damage, and contain Zn\(^{2+}\) and Mn\(^{2+}\) ions (Levin et al., 1991). The hairpin loop (ΔG = −5.4 kJ mol\(^{-1}\)) between the putative exonuclease IV gene and mreA suggests that they are in separate operons.

The organization of mreAB zur is identical to that seen in L. monocytogenes but different to that in B. subtilis (Table 2) and, surprisingly, in S. epidermidis (in which zur is monocistronic).

A consensus sequence upstream of mreA corresponding to the Fur box (Escolar et al., 1999) or to the putative B. subtilis Zur box (Gaballa & Helmann, 1998) was not identified.

**Characterization of the roles of zur and mreAB**

The zur gene was inactivated by partial deletion and insertion of a tetracycline-resistance cassette. First, the role of zur in Zn\(^{2+}\) susceptibility was tested. If the zur gene product bound Zn\(^{2+}\) and repressed Zn\(^{2+}\) uptake, zur mutant strains would be expected to be more susceptible to Zn\(^{2+}\) toxicity at high concentrations. However, there was no difference in the Zn\(^{2+}\) susceptibility of the two strains (data not shown). Secondly, the addition of 20 µM Zn\(^{2+}\) enhanced the growth of S. aureus 8325-4 in CL medium, suggesting that the growth of cells in unsupplemented media was limited by Zn\(^{2+}\)

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**Table 2. Amino acid homology of MreA and MreB to previously characterized ABC transporters of metal ions**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Locus*</th>
<th>ATP-binding protein (% identity to MreA)</th>
<th>Membrane-binding protein (% identity to MreB)</th>
<th>Ion transported</th>
<th>Regulated by (ion/regulator)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes</td>
<td>zurAM</td>
<td>47</td>
<td>51</td>
<td>?</td>
<td>Zn(^{2+})/†</td>
<td>Dalet et al. (1999)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ycd(H)</td>
<td>35</td>
<td>33</td>
<td>Zn(^{2+})</td>
<td>Zn(^{2+})/zur</td>
<td>Gaballa &amp; Helmann (1998)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>adcCB</td>
<td>34</td>
<td>30</td>
<td>Zn(^{2+})</td>
<td>?</td>
<td>Dintilhac et al. (1997)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ytg(A)</td>
<td>33</td>
<td>22/26</td>
<td>?</td>
<td></td>
<td>GenBank accession no. AF008220</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>mts(A)</td>
<td>36</td>
<td>26</td>
<td>Multiple cations including Zn(^{2+}), Fe(^{2+})</td>
<td>?</td>
<td>Janulczyk et al. (1999)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>psaBC</td>
<td>33</td>
<td>31</td>
<td>Mn(^{2+})</td>
<td>?</td>
<td>Novak et al. (1998)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>znu(A)</td>
<td>31</td>
<td>22</td>
<td>Zn(^{2+})</td>
<td>Zn(^{2+})/zur</td>
<td>Patzer &amp; Hantke (1998)</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>sitAB</td>
<td>33</td>
<td>26</td>
<td>?</td>
<td>Fe(^{2+})/</td>
<td>Cockayne et al. (1998)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>sir(A)</td>
<td>18</td>
<td>15</td>
<td>?</td>
<td>Fe(^{2+})/</td>
<td>Heinrichs et al. (1999)</td>
</tr>
</tbody>
</table>

* The ATP-binding proteins are always directly upstream of the corresponding hydrophobic membrane protein. Genes in parentheses represent the lipoprotein members of the ABC transporter operon (listed in order of appearance), and are not homologous to mreA or mreb.

† zurAMR of L. monocytogenes shows exactly the same gene organization as S. aureus mreAB zur.
strains were constructed (Lindsay & Riley, 1994). In no case could an altered phenotypic response between the wild-type and the zur::tc mutant strain or mreAB mutant strains be demonstrated.

*S. aureus* also possesses a Zn\(^{2+}\)-efflux mechanism (ZntRA) which is induced by very high concentrations of zinc (1·5 mM Zn\(^{2+}\); Xiong & Jayaswal, 1998). Mutants in zntA are unable to export toxic levels of Zn\(^{2+}\), and have an MIC of approximately 0·5 mM Zn\(^{2+}\); wild-type cells have an MIC of 5 mM. The mreAB zur operon did not appear to interfere with this process, as all strains had MICs in the region of 5 mM Zn\(^{2+}\). Therefore, zur, mreA and mreB genes do not appear to be involved in the export of Zn\(^{2+}\) out of cells.

The role of zur in pathogenesis was evaluated by comparing JL100(zur) with 8325-4 in a mouse skin-lesion model. No attenuation of virulence (compared to the wild-type strain) was seen (results not shown).

**Expression of mreA, mreB, zur**

Using Northern blotting, the zur transcript could not be detected. Consequently, it was also impossible to map the transcription start site. Strains with a lacZ reporter gene fused to either mreA or mreB were constructed (Fig. 3) and used to measure gene expression levels in growth media containing a range of metal-ion concentrations. Both constructs (JL110 and JL120) showed conducted, and siderophore production was measured (Lindsay & Riley, 1994). In no case could an altered phenotypic response between the wild-type and the zur::tc mutant strain or mreAB mutant strains be demonstrated.

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low-level (<50 MUG units) constitutive expression (results not shown). This suggested that the likely promoter controlling mreA and mreB upstream of mreA is weakly expressed in all of the growth conditions tested.

**Artificial expression of mreA, mreB and zur to reveal function**

To determine a potential function for the locus and to dissect the role of zur in regulation, the P_spac inducible promoter in strains JL110 and JL120 (Fig. 3) was used.

The P_spac promoter is repressed by the product of lacI encoded on the integrated plasmid. In the presence of IPTG, LacI inhibition of P_spac is relieved. Therefore, it was possible to induce expression of the operon by adding IPTG.

The entire mreAB zur operon (JL120) was expressed by using IPTG in low-ion conditions or in the presence of 20 μM Zn^{2+}, but had no effect on growth of the culture or on the cellular protein profile (results not shown). However, when lacZ activity was measured, the expression of mreA was strongly repressed by the presence of Zn^{2+}. JL120 grown in the presence of Zn^{2+} and IPTG produced significantly less β-galactosidase activity than the same strain grown without Zn^{2+} (P < 0.05; Fig. 3). This suggests that zur may repress transcription of the genes in a Zn^{2+}-dependent manner. No other metal ion was able to cause this repression of transcription.

To confirm that Zn^{2+}-dependent repression of mreA::lacZ is due to zur alone, two experiments were conducted. Using JL110 (mreB::lacZ; Fig. 1), in which mreB is inactivated, expression was still found to be repressed by Zn^{2+} when zur was induced by the presence of IPTG. Conversely, when mreA mreB zur expression was induced in strain JL121, in which zur has been inactivated, no Zn^{2+}-dependent repression was seen (P < 0.05; Fig. 3). Similar results were obtained using cultures of JL111. Therefore, the Zn^{2+}-dependent repression of mreAB zur is mediated by zur.

Addition of Zn^{2+} (20 μM) to CL results in an increased (almost double) growth yield of 8325-4 (Fig. 2). The potential role of mreA and mreB in this effect was analysed. All strains grew significantly better when supplemented with Zn^{2+} (P < 0.05); the exception to this was JL121 supplemented with IPTG, which showed enhanced growth without Zn^{2+} (asterisk in Fig. 2). In this case, mreA and mreB were induced in the absence of zur, and growth was significantly greater than that in the same experiment when zur was present (double asterisk in Fig. 2) (P < 0.05). JL110 and JL111 did not show enhanced growth in IPTG-induced cultures (Fig. 2), suggesting that both mreA and mreB are required. Therefore, mreAB may encode components of a ion-uptake pathway that only functions in the absence of zur. To determine if this enhanced growth was due to increased uptake of Zn^{2+} or another ion, MS on whole washed cells from cultures of JL121 with or without IPTG was carried out. However, there were no apparent differences in ion content in the two cell preparations.

**DISCUSSION**

Three genes predicted to be involved in Zn^{2+} regulation and Zn^{2+} uptake in S. aureus have been characterized. The zur gene product represses its own expression in the presence of excess Zn^{2+}. The MreA and MreB proteins are involved in enhanced growth in low-ion conditions.

The complex results regarding the ability of mreA and mreB to function in ion uptake can be explained as follows. Induction of mreAB (in the absence of zur) allows transport of either Zn^{2+} or another ion that compensates for Zn^{2+}. The fact that zur prevents this uptake could be due to Zur-mediated repression of an essential component of mreAB transport. Since most ABC-type transporters consist of three types of protein (Dintilhac et al., 1997; Gaballa & Helmann, 1998; Patzer & Hantke, 1998), it seems reasonable to predict that a third protein (a lipoprotein, equivalent to the Gram-negative substrate-binding protein) is encoded elsewhere on the S. aureus chromosome and that its expression is down-regulated by Zur. The fact that znuA encoding the lipoprotein component of an ABC transporter of Zn^{2+} in H. ducreyi is monocistronic (Lewis et al., 1999) supports this theory.

If mreA and mreB are components of a high-affinity Zn^{2+}-transport mechanism, the organization of this operon suggests that it forms a regulatory feedback loop. This would have the effect of maintaining expression of the operon at a constant level, with repression occurring in high-Zn^{2+} conditions when a high-affinity uptake mechanism would be unnecessary or even deleterious.

The role of zur in Zn^{2+} regulation appears to be markedly different in the Gram-positive bacteria studied so far. Whilst L. monocytogenes has an operon organization similar to that of S. aureus, it is expressed at a much higher level (Dalet et al., 1999), suggesting the putative transporter and Zur might play a greater role. Surprisingly, S. epidermidis zur is monocistronic (Heidrich et al., 1996), implying that the regulation of both itself and other Zn^{2+} transporters may be more flexible. The B. subtilis zur is also located on the chromosome separately from the two Zn^{2+}-transporter pathways that it regulates (Gaballa & Helmann, 1998). The implication is that zur is constitutively expressed in B. subtilis, thus allowing it to function solely according to the intracellular Zn^{2+} concentration. Therefore, these ion-regulatory pathways are more complex than predicted, as well as being species-specific.

S. aureus 8325-4 appears to respond to zinc by altering protein expression (in our assay conditions), although this activity is independent of mreAB zur. Similarly, under Zn-stressed conditions, such as in CL broth or on CL agar plates impregnated with a filter-paper disc containing EDTA, the mreAB locus in 8325-4 offered no
growth advantage. We conclude, therefore, that other Zn transporters and regulators function in our wild-type strain independently of mreAB zur.

This work emphasizes the care that must be taken in assigning roles for genes on the basis of sequence similarity or functional activity in related bacteria. If Zur had been overexpressed in E. coli, our results suggest that we would have detected Zn\(^{2+}\)-binding and promoter-binding activity, which might have led to the conclusion that Zur plays an important role in S. aureus Zn\(^{2+}\) homeostasis. It is therefore essential to use gene inactivation to determine the importance of such regulators unambiguously.

An understanding of the ability of S. aureus to respond to environmental ions might have implications for its ability to survive or induce infection. Whilst much has been written about the essential role of Fe\(^{3+}\) uptake and signalling in pathogenic bacteria (for a review, see Litwin & Calderwood, 1993), Zn\(^{2+}\) is also thought to be an essential nutrient for all cells, having important effects on the immune response and the activity of superantigens such as toxic shock syndrome toxin and enterotoxins produced by Streptococcus pneumoniae (PerR) repressors.

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