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The Adhesion-Associated *sca* Operon in *Streptococcus gordonii* Encodes an Inducible High-Affinity ABC Transporter for Mn^{2+} Uptake

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ScaA lipoprotein in *Streptococcus gordonii* is a member of the LraI family of homologous polypeptides found among streptococci, pneumococci, and enterococci. It is the product of the third gene within the *scaCBA* operon encoding the components of an ATP-binding cassette (ABC) transporter system. Inactivation of *scaC* (ATP-binding protein) or *scaA* (substrate-binding protein) genes resulted in both impaired growth of cells and >70% inhibition of $^{54}Mn^{2+}$ uptake in media containing <0.5 μM Mn^{2+}. In wild-type and *scaC* mutant cells, production of ScaA was induced at low concentrations of extracellular Mn^{2+} (<0.5 μM) and by the addition of ≥20 μM Zn^{2+}. Sca permease-mediated uptake of $^{54}Mn^{2+}$ was inhibited by Zn^{2+} but not by Ca^{2+}, Mg^{2+}, Fe^{2+}, or Cu^{2+}. Reduced uptake of $^{54}Mn^{2+}$ by *sca* mutants and by wild-type cells in the presence of Zn^{2+} was abrogated by the uncoupler carbonylcyanide *m*-chlorophenylhydrazone, suggesting that Mn^{2+} uptake under these conditions was proton motive force dependent. The frequency of DNA-mediated transformation was reduced >20-fold in *sca* mutants. The addition of 0.1 mM Mn^{2+} to the transformation medium restored only partly the transformability of mutant cells, implying an alternate role for Sca proteins in the transformation process. Cells of *sca* mutants were unaffected in other binding properties tested and were unaffected in sensitivity to oxidants. The results show that Sca permease is a high-affinity mechanism for the acquisition of Mn^{2+} and is essential for growth of streptococci under Mn^{2+}-limiting conditions.

Streptococci, pneumococci, and enterococci are human commensals colonizing a variety of human epithelial cell surfaces and have the ability to cause both superficial and life-threatening diseases. The colonization and virulence determinants of these organisms are under close scrutiny for the development of novel vaccines and inhibitors. The adhesion of streptococcal bacteria to host epithelial cells, connective tissue matrix and serum proteins, salivary components, and other bacterial cells in the environment is mediated for the most part by cell surface proteins (26, 38). Many of these proteins are anchored to the cell wall via a specialized C-terminal amino acid sequence (33). In addition, gram-positive bacteria express surface proteins that are lipid modified at the N terminus and that are associated with the outer face of the cytoplasmic membrane (36). The best characterized of these proteins in streptococci are the binding-protein components of ATP-binding cassette (ABC)-type membrane transport systems involved in the uptake of peptides (1), oligopeptides (25), and multiple sugars (32).

Several laboratories independently characterized similar surface proteins produced by different streptococcal species and implicated in bacterial cell adhesion to salivary glycoproteins (17, 31), *Actinomyces naeslundii* (2), and fibrin (7). Members of this protein family, designated LraI (24), have now been identified in six species of streptococci (6, 9, 37) and *Enterococcus faecalis* (28). In *Streptococcus gordonii*, which colonizes tooth and mucosal surfaces, the LraI protein is a prominent surface antigen (2) and is designated ScaA (approximate molecular mass, 35 kDa). In *Streptococcus parasanguis*, protein homolog FimA is a candidate vaccinogen against endocarditis (37), while in *Streptococcus pneumoniae* (pneumococcus) the PsaA protein is a virulence determinant (6). In each instance the LraI polypeptide is encoded by the third gene of a tricistronic operon encoding the components of an ABC transporter.

The *sca* operon in *S. gordonii* PK488 and DL1 comprises three genes that are transcribed from a promoter upstream of *scaC* (3). The *scaC* gene encodes an ATP-binding protein (251 amino acid [aa] residues), *scaB* encodes a transmembrane component (278 aa residues) which presumably dimerizes (22), and *scaA* encodes a lipoprotein (310 aa residues) (27). Downstream of *scaA* is an open reading frame (*ORF4*) encoding a protein of 163 aa residues with an amino acid sequence that is 52% identical overall to that of periplasmic thiol peroxidase of *Escherichia coli* (8), which scavenges superoxide and peroxide ions. There is evidence from transcript analysis of a homologous locus in *S. parasanguis* that *ORF4* (designated *ORF3* in *S. parasanguis*) is transcribed both from its own promoter and by readthrough of *fimA* (*scaA*) (16). Upstream of *scaC* and divergently transcribed from an overlapping promoter is a gene (*ORF6*) encoding a Zn^{2+}-dependent endopeptidase (Fig. 1).

Recently, in *S. pneumoniae* another ABC transporter operon (*adc*) (12) with sequence similarity to *psa* was discovered. Evidence indicates that the Adc permease is a high-affinity transporter for Zn^{2+} and is necessary for DNA-mediated transformation of pneumococcus (11). Furthermore, it was shown that

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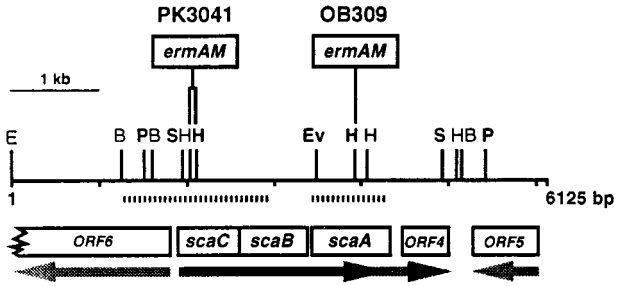

FIG. 1. Structure of the *sca* locus in *S. gordonii* PK488 and delineation of the coding sequences and directions of transcription (arrows). Sites of insertion of *ermAM* to generate *scaC* (PK3041) and *scaA* (OB309) mutants of *S. gordonii* DL1 are indicated. Segments amplified by PCR for subsequent insertional inactivation are shown as striped bars. Base pair numbers are in accordance with sequence L11577 deposited in GenBank. Abbreviations of restriction enzymes: B, *Bgl*II; E, *Eco*RI; Ev, *Eco*RV; H, *Hin*dIII; P, *Pst*I; S, *Sal*I. Sites confirmed to be present in both PK488 and DL1 are shown in boldface type.

expression of the *psa* operon was associated with a Mn^{2+} requirement (11). We have characterized further the function of the *sca* operon in *S. gordonii* and now provide evidence that this operon encodes the components of a high-affinity Mn^{2+} transporter that is inhibited by Zn^{2+}. This is the first report of a Mn^{2+} permease in oral streptococci that appears to be necessary both for growth of cells in environments with low concentrations of Mn^{2+} and for DNA-mediated transformation. Putative Mn^{2+}-binding lipoprotein ScaA, which is inducible under Mn^{2+}-limiting conditions, would thus provide a vital mechanism for the acquisition of Mn^{2+} by streptococci for growth and survival in the human host.

MATERIALS AND METHODS

Bacteria and growth media. *S. gordonii* DL1 (Challis) and PK488 are wild-type strains (2, 25). Strain OB309 (*scaA1*::*ermAM*) was an isogenic derivative of DL1 and is similar to strain OB470 (*scaA2*::*tet*) (23) but contains an *ermAM* cassette (25) replacing the *tet* cassette inserted into *scaA*. Strain PK3041 was an isogenic derivative of DL1 which contained an *ermAM* insertion in *scaC*. This strain was generated by cloning a 1,659-bp PCR amplimer obtained from genomic PK488 DNA with primers corresponding to regions between base pairs 1335 and 1362 and between base pairs 2969 and 2994 within the *sca* locus (GenBank accession no., L11577), replacing an internal 134-bp *Hin*dIII-*Hin*dIII fragment with *ermAM* (Fig. 1), and transforming the resistance determinant into *S. gordonii* DL1 by double crossover of the erythromycin resistance determinant into the coding region of the chromosomal gene (10, 23, 25). Candidate transformants for chromosomal gene conversion in the *sca* locus were confirmed by Southern blot analysis using *scaA* or *scaC* and *ermAM* as probes (25).

Bacterial cultures were grown without shaking at 37°C in brain heart infusion-yeast extract (BHY) medium, tryptone-yeast extract (TY)-glucose medium, or defined medium containing amino acids, vitamins, and glucose (25) with adjustments to concentrations of Mn^{2+} and Zn^{2+}, etc., as described below. Growth was assessed by measuring the optical density at 600 nm and by determining the numbers of CFU on BHY agar.

Protein extraction, antisera, and immunoblotting. Proteins were extracted from bacterial cells following treatment with mutanolysin (10) and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Antiserum was raised in rabbits to recombinant ScaA protein purified from inclusion bodies obtained from *E. coli* BL21 expressing the *scaA* gene cloned in pBluescript KS(+). Western blots of proteins were probed with antibodies to ScaA or HppA (25) at a dilution of 1:500 and were developed with peroxidase-conjugated secondary antibodies (25).

$^{54}Mn^{2+}$ uptake assay. Mid-exponential-phase cells in TY-glucose medium were harvested by centrifugation and resuspended in fresh TY-glucose medium at a density of 2×10^9 cells/ml. Assays were initiated by the addition of 0.5 μCi (75 pmol) of $^{54}Mn^{2+}$/ml and nonradioactive $MnCl_2$ where appropriate. To test the effects of $ZnSO_4$, other cations, or carbonylcyanide *m*-chlorophenylhydrazone (CCCP) on $^{54}Mn^{2+}$ uptake, cells were preincubated for 5 min with these compounds before the addition of the label. To test 2-deoxyglucose (dGlc) inhibition of $^{54}Mn^{2+}$ uptake, bacterial cells were resuspended in TY-dGlc medium and preincubated at 37°C for 10 min. Assays were performed at 37°C, and samples were removed at intervals into ice-cold 0.1 M LiCl. Cells were collected by vacuum filtration (25), and radioactivity was determined by scintillation counting at the ^{32}P window setting (34). As noted for *E. coli* (34), significant and reproducible uptake of $^{54}Mn^{2+}$ by streptococcal cells was achieved by utilizing buffered growth medium (TY-glucose).

Transformation. Competent cells were prepared in TY-glucose medium containing 5% (vol/vol) heat-inactivated fetal bovine serum and transformed to streptomycin resistance (St^r) with DNA isolated from a streptomycin-resistant derivative of *S. gordonii* DL1 as described previously (25). Competence-stimulating peptide (CSP) pherotype 1 (21) was synthesized and kindly supplied by L. S. Håvarstein (Agricultural University of Norway, Ås).

RESULTS

***S. gordonii* exhibits a *sca* operon-dependent growth requirement for Mn^{2+}.** Sequence analysis, restriction mapping, and blot hybridization all suggest that the genetic structures of the *sca* loci are similar in *S. gordonii* PK488 and DL1. Mutants of *S. gordonii* DL1 with the *scaA* or *scaC* genes inactivated were generated by insertion of the *ermAM* cassette into the respective coding sequences (Fig. 1). Production of the ScaA protein was abolished in the strain OB309 *scaA* mutant as determined by immunoblotting and by whole-cell enzyme-linked immunosorbent assay with ScaA-specific antibodies (23) (data not shown). In contrast, production of the ScaA protein by PK3041 (*scaC*::*ermAM*) cells was apparently unaffected (Fig. 2), suggesting that *ermAM* did not carry a strong transcriptional terminator, thus permitting readthrough of *scaBA*. This is consistent with the previously observed nonpolar effects of *ermAM* insertion into operons (1).

Both *scaA* and *scaC* mutants showed an increased lag phase and reduced growth rates in BHY and TY-glucose media. Growth yields of the strains in these media and in defined medium containing 0.1 μM Mn^{2+} were approximately 50% of that of the wild type (Fig. 3). Maximal growth of *sca* mutants in these media was restored by the addition of >1 μM Mn^{2+} (Fig. 3). Changing the concentrations of other ions such as Fe^{3+}, Ca^{2+}, and Mg^{2+} in the defined medium did not reveal any further growth differences between the wild type and mutants. The addition of ≥40 μM Zn^{2+} led to 40% growth inhibition of wild-type cells and to 80% inhibition of growth of *sca* mutants in defined medium containing 0.1 μM Mn^{2+} (Fig. 3). These results suggested that *scaC* and *scaA* genes were necessary for the activity of a high-affinity Mn^{2+} uptake system that was inhibitable by Zn^{2+}. The addition of Zn^{2+} to Mn^{2+}-replete

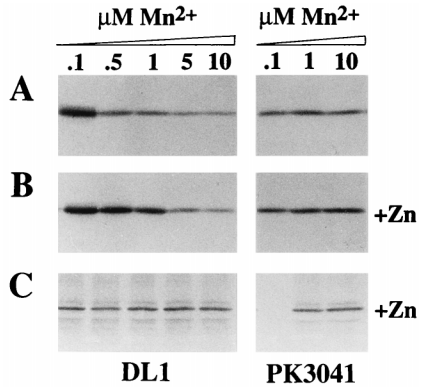

FIG. 2. Immunoblot detection of ScaA expression in wild-type (DL1) or *scaC* mutant (PK3041) cells in defined media containing Mn^{2+} (0.1 to 10 μM) in the absence (A) or presence (B) of 20 μM Zn^{2+}. Cultures were grown for 12 h at 37°C and the cells were harvested. Surface proteins were extracted, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and blotted onto nitrocellulose. Panels A and B show reactions with ScaA antibodies, and as a control Panel C shows a reaction with antibodies to HppA oligopeptide-binding lipoprotein from *S. gordonii* (25). Protein loadings were equalized (50 μg per lane) except for PK3041 cells grown in 0.1 μM Mn^{2+} with Zn^{2+}, where <10 μg of protein was loaded because of the 80%-reduced growth yield of cells (see text and Fig. 3).

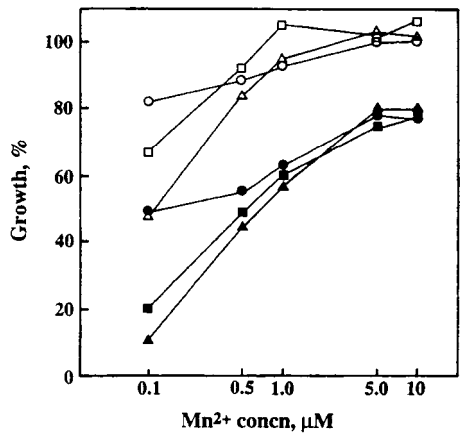

FIG. 3. Growth yields of wild-type and *sca* mutant strains after 16 h of incubation at 37°C in defined media containing different concentrations of Mn^{2+} (0.1 to 10 μM) in the absence (open symbols) or presence (filled symbols) of 40 μM Zn^{2+} (as $ZnSO_4 \cdot 7H_2O$). Data are expressed as percentages of the growth yield of wild-type cells in 10 μM Mn^{2+} without added Zn^{2+}, where 100% was equivalent to an optical density at 600 nm of 2.69 (5.4×10^9 cells/ml). Symbols: circles, DL1 (wild type); triangles, OB309 (*scaA1*::*ermAM*); squares, PK3041 (*scaC*::*ermAM*).

medium was less inhibitory, with growth yields of all strains of about 80% of the wild type in the absence of Zn^{2+} (Fig. 3). Therefore *S. gordonii* contains, in addition to a Sca-dependent Mn^{2+} uptake system, one or more lower-affinity uptake systems that allow growth of cells under Mn^{2+}-replete conditions.

ScaA is induced by growth-limiting Mn^{2+} concentrations. Production of ScaA protein by wild-type (DL1) cells was induced at an [Mn^{2+}] of ≤0.5 μM (Fig. 2A). Production of ScaA was enhanced further in the presence of 20 μM Zn^{2+} (Fig. 2B). Levels of HppA oligopeptide-binding lipoprotein did not change under these different growth conditions (Fig. 2C). Production of ScaA protein by PK3041 cells was enhanced at all Mn^{2+} concentrations, although production at 0.1 μM Mn^{2+} was less than in the wild type (Fig. 2A). At the lowest [Mn^{2+}] tested (0.1 μM) the growth of a PK3041 culture in 20 μM Zn^{2+} was reduced by 80% (Fig. 3), only small amounts of total cell protein were obtained for analysis, and HppA was not detectable on immunoblots (Fig. 2C). Under these conditions the ScaA protein was highly expressed (Fig. 2B). These data show that ScaA production in wild-type cells is under Mn^{2+} control and, furthermore, that ScaA production may be influenced by a Zn^{2+}-responsive regulator.

***S. gordonii* expresses at least two uptake systems for Mn^{2+}.** Uptake of $^{54}Mn^{2+}$ by wild-type cells was dependent upon the extracellular [Mn^{2+}] and was saturable as expected for active transport (Fig. 4A). The apparent K_m for uptake was estimated to be 0.1 to 0.3 μM. Mutants OB309 and PK3041 both showed about 70%-reduced uptake of $^{54}Mn^{2+}$ in 0.5 μM Mn^{2+} (Fig. 4B). Inhibition by Zn^{2+} of $^{54}Mn^{2+}$ uptake by wild-type cells was dose dependent, and at 80 μM Zn^{2+}, the uptake of $^{54}Mn^{2+}$ was equivalent to that of the *sca* mutants (Fig. 4B). Divalent cations Mg^{2+}, Ca^{2+}, Hg^{2+}, Cu^{2+}, and Fe^{2+} had no effect on $^{54}Mn^{2+}$ uptake, while there was a slight enhancement with 50 μM Fe^{3+} (data not shown).

In the presence of dGlc, $^{54}Mn^{2+}$ uptake by wild-type cells was reduced by 50%, which is consistent with a direct ATP requirement for transport (Fig. 4C). The addition of CCCP, which causes a collapse of the proton gradient, did not inhibit uptake (Fig. 4C). However, $^{54}Mn^{2+}$ uptake in the presence of 40 μM Zn^{2+} was abrogated by CCCP, suggesting that a major component of the lower-affinity $^{54}Mn^{2+}$ uptake system was proton motive force dependent. In accordance with these interpretations, the uptake of $^{54}Mn^{2+}$ in 0.5 μM Mn^{2+} by *scaA* and *scaC* mutants was slightly inhibited by Zn^{2+}, was reduced by 50% in the presence of dGlc, and was abolished by the addition of CCCP (Fig. 4D).

Transformation in *S. gordonii* is Sca dependent. Wild-type cells of *S. gordonii* at the early exponential phase of growth exhibited approximately 0.7% transformability (Fig. 5). Cells of OB309 and PK3041 grew more slowly in transformation medium (which was low in Mn^{2+}) and their transformabilities were reduced by >20-fold (Fig. 5). The addition of CSP to *sca* mutants growing in non-Mn^{2+}-supplemented medium did not affect their growth rates but increased their transformabilities (Fig. 5), with *scaA* mutants being more responsive than *scaC* mutants (Fig. 5), suggesting that *sca* mutations affect CSP production. Growth rates of *sca* mutants were restored to the wild-type rate and their transformabilities were improved by the addition of 10 μM Mn^{2+} to the transformation medium. However, transformation frequencies of *sca* mutants were only 10 to 20% of wild-type levels even at 100 μM Mn^{2+} (Fig. 5). This indicates that Sca permease is also required for another

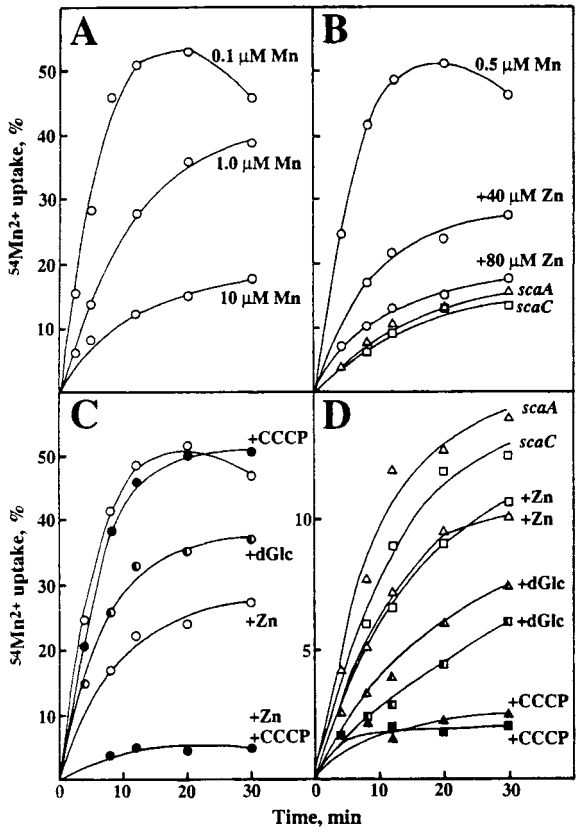

FIG. 4. $^{54}Mn^{2+}$ uptake by wild-type or *sca* mutant cells. (A) Uptake by wild-type cells (open circles) in the presence of nonradioactive Mn^{2+} (as $MnCl_2 \cdot 4H_2O$); (B) uptake in 0.5 μM Mn^{2+} by wild-type cells (open circles) and *scaA* (open triangles) and *scaC* (open squares) mutant cells and the effect of Zn^{2+} on uptake by wild-type cells; (C) uptake in the presence of 0.5 μM Mn^{2+} by wild-type cells (control; open circles) or in the presence of 50 mM dGlc and the effect of the addition of 100 μM CCCP (solid circles) in the absence or presence of 40 μM Zn^{2+}; (D) effect of Zn^{2+}, dGlc, or CCCP on uptake in 0.5 μM Mn^{2+} by *scaA* (triangles) and *scaC* (squares) mutants. Values are plotted as percentages of initial $^{54}Mn^{2+}$ uptake, where 100% uptake was equivalent to 3.8 pmol of $Mn^{2+}/10^8$ cells.

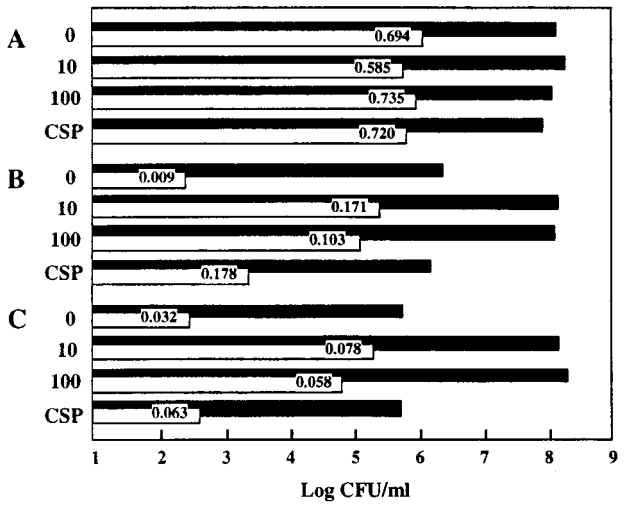

FIG. 5. Growth and transformability to St^r of a wild-type strain (A) and *scaA* (B) and *scaC* (C) mutant strains in transformation medium (<0.1 μM Mn^{2+}) without (0) or with Mn^{2+} (10 or 100 μM) or with CSP (400 ng/ml added 20 min prior to the addition of 50 ng of St^r-transforming DNA/ml). Early-exponential-phase cells were incubated with DNA for 30 min, 10 μg of DNAse I/ml was then added, and the cultures were incubated for a further 90 min before the numbers of total CFU (filled bars) and St^r CFU (open bars) were determined by viable-cell agar plate count. Values are percentages of St^r transformants.

(undefined) stage in the transformation process that is not related directly to Sca-mediated uptake of Mn^{2+}.

Phenotypic effects of *sca* mutations. Despite the evidence for ScaA and homologous proteins in other streptococci being involved in bacterial adhesion (24), OB309 and PK3041 mutant cells were not affected in their abilities to adhere to *A. naeslundii* cells or to an experimental salivary glycoprotein pellicle (data not shown). An important Mn^{2+}-requiring enzyme in streptococci is superoxide dismutase, which is essential for aerobic growth (29) and for the removal of inhibitory superoxide ions. Accordingly, growth rates of a wild-type strain and *sca* mutants in medium containing 0.1 μM Mn^{2+} were measured under anaerobiosis, with aeration, or in the presence of 5 mM paraquat, a superoxide ion generator. The anaerobic culture doubling time of the wild-type strain (50 min) was increased to 120 min under aeration and to 80 min by the addition of paraquat. Neither aeration nor the addition of paraquat had any significantly greater inhibitory effects on the growth rates of the *sca* mutants (data not shown), suggesting that these cells were no more sensitive to oxidants than wild-type cells.

DISCUSSION

There is currently a resurgence of interest in studying the requirement of prokaryotic and eukaryotic cells for trace metals as important cofactors in many cellular processes. For example, Zn^{2+} is estimated to be required for the activities of more than 300 proteins, but only recently has the first bacterial ABC transporter for Zn^{2+} been described (11). An ABC transporter for Ni^{2+} in *E. coli* was reported and was shown to be necessary for nickel-containing hydrogenase activity (30). The requirement of Fe^{2+} and Fe^{3+} for growth of bacterial pathogens is well documented, and a variety of iron-capturing and iron uptake systems that are essential for the growth of bacteria in the iron-depleted host environment have been characterized (14). Group A streptococci exhibit a growth requirement for iron (15). The addition of Mn^{2+} to the growth medium was required for optimal growth of *Streptococcus cricetus* (13) and *S. pneumoniae* (11); Mn^{2+} was shown to be essential for the glucan-associated adhesion of some mutans group streptococcal species (5) and for pneumococcal transformation (11). We now provide evidence for a high-affinity Mn^{2+} transport system in *S. gordonii* that is necessary for the growth of cells in low-Mn^{2+}-concentration environments and for DNA-mediated transformation. The genes encoding the Mn^{2+} transporter system are found in *S. gordonii*, *S. parasanguis*, and *S. pneumoniae*. Furthermore, since sequences highly similar to that of the *scaA* gene are found in *S. sanguis*, *Streptococcus crista*, and *E. faecalis*, it seems likely that the transporter functions in all these organisms.

In *S. gordonii*, production of the ScaA protein is induced by Mn^{2+} depletion and by the addition of Zn^{2+}. The uptake data suggest that Zn^{2+} competes with Mn^{2+} for uptake and therefore would act to reduce the effective intracellular concentration of Mn^{2+}. Growth of *sca* mutants in 0.1 μM Mn^{2+} was still possible but was not possible in the presence of Zn^{2+}. This indicates the activity of a second lower-affinity transporter that also is Zn^{2+} sensitive. This might be equivalent to the Adc Zn^{2+} transporter recently characterized for *S. pneumoniae*, which was proposed to also be able to transport Mn^{2+} (11). Furthermore, the uptake inhibition data suggest that there is a third Mn^{2+} transporter, essentially proton motive force dependent, that mediates the uptake of and satisfies the growth requirement for Mn^{2+} under Mn^{2+}-replete conditions and that is not significantly inhibited by Zn^{2+}. The notion of a combination of specific metal ion transporters working to cover all eventualities fits well with recent data on Mn^{2+} transport in other systems. For example, uptake of Mn^{2+} by the cyanobacterium *Synechocystis* occurs via a high-affinity ABC transporter, which has sequence similarity to Sca, and also through a lower-affinity system (4). Similarly, *Saccharomyces cerevisiae* contains at least two Mn^{2+} transport systems with differing affinities for Mn^{2+} (35).

The expression of ScaA is up-regulated in a low concentration of Mn^{2+}, and therefore it is likely that transcription of the *sca* operon is under the control of a Mn^{2+}-responsive regulator. Transcript analyses of the *sca* operon (3) and of the analogous operon in *S. parasanguis* (16) have shown a single polycistronic mRNA, consistent with operon structure. In addition, evidence was obtained for transcriptional readthrough of downstream *ORF3* (16) (*ORF4* in Fig. 1), the product of which shows sequence homology to a thiol peroxidase. It is possible then that under Mn^{2+}-limiting conditions, up-regulation of *sca* transcription may result not only in increased ScaA production and Sca transporter activity but also in increased production of thiol peroxidase. Since superoxide dismutase activity in streptococci is dependent upon Mn^{2+} (20, 29), increased production of thiol peroxidase under Mn^{2+}-limiting conditions might provide streptococcal cells with additional protection against oxidant stress. This could account for *sca* mutants being no more sensitive to oxidants than the wild type. It is also possible that the *ORF4* promoter may be under direct Mn^{2+}-responsive regulation.

The fact that *S. gordonii* cells exhibit a growth requirement for Mn^{2+} could present a problem for the growth of the organism within the human host, where most available Mn^{2+} is complexed with albumin and transferrin (19). We suggest that the Sca transporter operates in vivo as an Mn^{2+}-scavenging system. While ScaA is a lipoprotein and is presumably tethered to the cytoplasmic membrane, it has been shown that streptococcal lipoproteins may not remain held simply within the immediate confines of the membrane permease. Lipoproteins have been detected in extracellular culture fluid (36), and in

particular there is evidence that FimA may be found distal to the membrane and associated with cell surface fimbriae (16). We suggest that the LraI proteins, by binding Mn^{2+} or Mn^{2+} complexes, may provide a means of concentrating environmentally depleted Mn^{2+} within the bacterial community.

Since substrates generally show a fast association with binding proteins and since there is slow dissociation of substrate, it is possible that Mn^{2+} may be "shuttled" in a community or may be brought membrane proximal to the permease. Furthermore, since Mn^{2+} is not held tightly by albumin (19) it may be sequestered directly by ScaA from albumin or shuttled first through proteins and glycoproteins on the surfaces of other bacterial cells or other host proteins. If the binding protein can bind Mn^{2+} that is complexed with albumin or other proteins, then this process may be revealed to have an adhesive function, which may account for the consistent evidence that the ScaA-like (or LraI family) polypeptides are adhesins (24). Since streptococci bind many serum proteins (26) and produce a number of proteases, it may be that Mn^{2+} is also released from protein-bound complexes as a result of bacterial proteolytic activity. Although it remains speculative as to how LraI polypeptides function in vivo it would appear that Sca and related transporters in streptococci are essential for growth of the bacteria in low-Mn^{2+}-concentration environments. This could account for the reduced ability of *S. parasanguis fim* mutants to cause endocarditis (7) and for the virulence defects of pneumococcal *psa* mutants (6).

Growth of *psaA* mutants of *S. pneumoniae* is stimulated by Mn^{2+} in media containing 0.3 μM $MnSO_4$ (11). However, in contrast to the inhibition of growth by Zn^{2+} observed with *S. gordonii*, an excess (33-fold) of Zn^{2+} had no inhibitory effect on the growth of *psaA* mutants in media containing 1.2 μM Mn^{2+}. While both *S. gordonii* and *S. pneumoniae* appear to exhibit Mn-dependent DNA-mediated transformation, only pneumococcal transformability could be totally restored by the addition of 3 μM Mn^{2+}. These points underpin the possibility that among streptococci common systems may play alternate roles in bacterial cell physiology and that this may be especially true for the ABC-type transporters, the activities of which may impinge on multiple cell functions.

Within a 5.2-kb putative transport-related operon (*tro*) of *Treponema pallidum* subsp. *pallidum*, the first gene, *troA*, encodes a ScaA homolog (18). Four of the six potential gene products of the operon are homologous to proteins found in ABC systems associated with metal ion transport. Of interest is a fifth putative protein, TroR, which has a sequence similar to those of gram-positive iron-activated repressor proteins (DesR, DtxR, IdeR, and SirR). These results provide further evidence that the ScaA-like (LraI) proteins are part of a large family of substrate-binding proteins (cluster 9) (12) serving transporters associated with metal ion uptake and with Mn^{2+} (or Fe^{2+})-regulated gene expression in bacteria. This emphasizes the significance of our findings that ScaA was induced under Mn^{2+}-depleted conditions and indicates a direct link between the transport of Mn^{2+}, environmental sensing of trace ions, and gene regulation in streptococci.

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