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scbA from *Streptococcus crista* CC5A: an Atypical Member of the *lraI* Gene Family

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A new member of the *lraI* family of putative adhesin genes was cloned, from *Streptococcus crista* CC5A, and sequenced. The gene, *scbA*, appears to be part of an ABC transport operon and encodes a putative peptide of 34.7 kDa. The protein contains a signal sequence with residues 17 to 21 (L-A-A-C-S) matching the consensus sequence for the prolipoprotein cleavage site of signal peptidase II. ScbA is 57 to 93% identical, at the amino acid level, with the five previous sequenced members of the LraI family. Surprisingly, ScbA does not exhibit adhesion properties characteristic of the other LraI proteins. Strain CC5A bound poorly to saliva-coated hydroxyapatite and did not coaggregate with *Actinomyces naeslundii* PK606. An *scbA* insertion-duplication mutation that abolished expression of ScbA was created. There was no difference in fibrin binding between this mutant and wild-type CC5A. Since it is possible that ScbA could play a role in corncob formation between *S. crista* and *Fusobacterium nucleatum*, this property was examined. The mutant strain retained the ability to form corncobs. On the basis of the lack of adhesin properties it appears that ScbA is an atypical member of the LraI family.

Streptococcus crista and *Fusobacterium nucleatum* form distinct bicellular communities in mature human dental plaque. These communities have a morphological appearance similar to that of an ear of corn and thus have been called corncobs. Electron micrographs clearly show that the attachment between these bacteria occurs via unique tufts of fimbriae on the lateral surface of *S. crista* (17). Our efforts have focused on understanding the molecular basis of the attachment of the streptococci to the fusobacteria. Attachment could be mediated directly through structural components of the fimbriae or by an adhesin associated with the tips of the fimbriae. Recently, we found that *S. crista* is naturally transformable and have applied this property to the genetic analysis of the adhesins that mediate corncob formation (5). The ability to transform *S. crista* CC5A has also allowed us to investigate to what extent previously described oral streptococcal adhesins may be involved in corncob formation.

Andersen and coworkers (1) described experiments in which an oligomer probe, specific for a well-studied class of streptococcal adhesin genes, hybridized to genomic DNA from *S. crista* CC5A. This group of highly conserved cell surface receptors has been named the LraI (lipoprotein receptor antigen I) protein family (14). To date, the nucleotide sequences of five genes from this group (*ssaB* from *Streptococcus sanguis* 12 [11], *fimA* from *Streptococcus parasanguis* FW213 [7], *scaA* from *Streptococcus gordonii* PK488 [16], *psaA* from *Streptococcus pneumoniae* R36A [22], and *efaA* from *Enterococcus faecalis* EBH1 [18]) have been obtained from gram-positive species and compared. Three distinct functions for this group of proteins have been described: (i) the SsaB and FimA polypeptides are involved in the adherence of *S. sanguis* and *S. parasanguis*, respectively, to salivary pellicle components (14), (ii) antibodies directed against ScaA block coaggregation between *S. gordonii* PK488 and *Actinomyces naeslundii* PK606 (15), and (iii) genetic studies have indicated that FimA is necessary for the binding of *S. parasanguis* to fibrin monolayers (2). The functions of the other putative adhesin proteins, PsaA and EfaA, have not been determined.

In spite of the fact that all the members of the LraI protein family appear to be lipoproteins, some evidence is available to suggest that these proteins are secreted and can attach to fimbrial structures on the streptococcal cell surface (8, 19). Additional clues concerning the function of this family of proteins comes from their genetic organization on the chromosome. It has been reported that *scaA* and *fimA* are located in ABC transport operons (8, 16), where the gene products are thought to function as extracellular solute binding components.

A homolog (ScbA) of the LraI protein was identified in *S. crista*. We hypothesize that because of its composition it could have the potential to function as an adhesin in corncob formation. However, our experiments indicate that ScbA does not play a major role in corncob formation or in other binding activities previously associated with the oral streptococci.

MATERIALS AND METHODS

Strains, plasmids, and culturing conditions. *S. crista* CC5A and *F. nucleatum* ATCC 10953 have been described previously (17). The streptococci were grown in brain heart infusion (BHI) or Todd-Hewitt broth (Difco Laboratories, St. Louis, Mo.) at 37°C for 18 h. *F. nucleatum* was grown anaerobically in BHI broth supplemented with 0.2% yeast extract and 0.05% L-cysteine. *A. naeslundii* PK606 was grown in BHI broth at 37°C for 18 h. *Escherichia coli* JM109 [*endA recA1 gyrA96 thi hsdR17*(r_K^- m_K^+) *relA1 supE44* Δ(*lac-proAB*) F′ *traD36 proAB lacI*^{q}ZΔM15] and *E. coli* LE392 [F^- *hsdR514* (r_K^- m_K^-) *supE44 supF58 lacY1* or Δ(*lacIZY*)*6 galK2 galT22 metB1 trpR55* λ^-] were grown in L broth. The sources of the bacterial strains and plasmids used in this study are listed in Table 1, and the construction of the plasmids is described in the Results section.

Transformation and selection of mutants. *S. crista* was made competent as described previously (5). A description of the method for achieving maximum competence by using the sanguis group of streptococci will be reported separately. However, in brief 1 μg of pSCBA003 DNA was added to 330 μl of a strain CC5A cell culture that had reached an A_{660} of 0.09. This absorbance value was reached approximately 5 h after inoculation. The cells were incubated for 1 h at 37°C to allow expression of kanamycin resistance and plated on BHI plates containing 200 μg of kanamycin per ml. The MIC of kanamycin for wild-type CC5A was previously determined to be 32 μg/ml.

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source (reference)
Strains		
S. crista CC5A	Wild-type strain used in corncob studies	Laboratory collection (17)
S. sanguis 34	Positive control strain for coaggregation with actinomyces	Laboratory collection (4, 15)
A. naeslundii PK606	Coaggregation partner for testing one function of ScbA	Paul Kolenbrander (4)
F. nucleatum ATCC 10953	Corncob partner for testing one function of ScbA	Laboratory collection (17)
S. crista CC5A-r1	Insertion-duplication mutant of strain CC5A; Kan^r Spc^r	This study
S. crista CC5A-r2	Insertion-duplication mutant of strain CC5A; Kan^r Spc^r	This study
E. coli JM109	Host strain for plasmids EMBL3-scbA, pSCBA001, pSCBA002, and pSCBA003	Promega
E. coli LE297	Host strain for the λEMBL3::CC5A DNA library	Promega
Plasmids		
EMBL3-scbA	λ lysogen containing an 11-kb fragment (*scbA* locus) from strain CC5A	This study
pDL278ΔP	Derivative of the *E. coli*-streptococcus shuttle vector, pDL278, in which the gram-positive origin of replication is deleted	This study
pSCBA001	7-kb *Pst*I-*Sal*I fragment (*scbA* locus) from EMBL3-scbA cloned in pCR-ScriptSK+	This study
pSCBA002	1.8-kb *Hinc*II fragment (*scbA* locus) from pSCBA001 subcloned in pDL278ΔP	This study
pSCBA003	Insertion of an ΩKm-2 element in the *Stu*I site of pSCBA002	This study

Recombinant DNA techniques. Isolation of *S. crista* DNA has been described previously (5). Restriction endonuclease digestions were carried out according to the manufacturer's instructions. Plasmid DNA isolation, ligations, and transformation of *E. coli* were performed as described elsewhere (21).

Southern blotting. The 30-base oligonucleotide, scbA1709 (5′-TACATCTGG GAAATCAACACCGAAGAAGAA-3′), was labeled at the 5′ end with [γ-^{32}P] dATP (>5,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Hybridizations were carried out at 50°C for 17 h according to the method of Church and Gilbert (3). The hybridization buffer consisted of 1% bovine serum albumin, 1 mM EDTA, and 0.5 M Na_2HPO_4 (pH 7.2). The blot was incubated in 2× SSC (1× SSC is 150 mM NaCl plus 15 mM $Na_3C_6H_5O_7 \cdot 2H_2O$) at 50°C for 5 min, and this was followed by a 30-min incubation in 2× SSC containing 1% sodium dodecyl sulfate (SDS) at 50°C. Blots were air dried and exposed to DuPont NEF-493 Reflection autoradiography film at −70°C.

All other probes were labeled with [α-^{32}P]dATP (6,000 Ci/mmol; Amersham Corp.) by using a random primer labeling kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Southern blots were prehybridized for 2 h at 42°C and then hybridized overnight, at the same temperature, in 50% formamide. Usually 2×10^6 cpm of probe DNA (specific activity, 2×10^7 cpm/μg of DNA) was used. Following hybridization, the blots were washed once in 2× SSC containing 0.1% SDS for 15 min at room temperature and this was followed by a second wash in 0.1× SSC containing 0.1% SDS for 1 h at 42°C. Autoradiography was done as described above.

PCR. Oligomers for PCR priming and sequencing were made at the Nucleic Acid Facility, University of Pennsylvania. The *scbA* gene was amplified with primers scbA582 (5′-GGAAGCTTTTGATATGTGGATTAGTATTGG-3′) and scbA2124 (5′-GTCTCCATTCATGTTTGATAGC-3′) and CC5A genomic DNA by using the Gene-Amp kit (Perkin-Elmer, Norwalk, Conn.). The buffer included with the kit was used without any modifications. The genomic DNA was melted at 94°C for 1 min, primers were annealed at 42°C for 1 min, and extension was at 72°C for 2.5 min. This was repeated for 25 cycles, after which a 2.5-min 72°C soak was performed. The products were extracted with chloroform before further manipulations. The primers scbA1487 (5′-GGAAGCTTGGTATCATCTA TGCGCAAAATA-3′) and scbA1946 (5′-GGAAGCTTGATTGTATTTCATC ATGCTGTAG-3′) were used to amplify a portion of the *scbA* gene.

Sequencing. PCR products were ligated into pGEM-T (Promega, Madison, Wis.), and doubled-stranded plasmid DNA template was sequenced with the Sequenase version 2.0 kit (United States Biochemical, Cleveland, Ohio). The plasmid pSCBA001, containing the wild-type *scbA* gene, was used as a template in automated cycle sequencing. The primers scbA582, scbA1073 (5′-CTGCTTT TGCTGGCTTTTGTCGGC-3′), scbA1409 (5′-GTAAATCACGTCCACACC-3′), scbA1487, scbA1674 (5′-TTGAAGCAACCTTCGCTG-3′), scbA1709, scbA1946, and scbA2124 were used in the sequencing reactions. Automated cycle sequencing reactions were conducted by the Genetics Core Facility at the University of Pennsylvania by using an Applied Biosystems, Inc. 373A sequencer with the Stretch upgrade.

λEMBL3 library of strain CC5A. Strain CC5A genomic DNA (80 μg) was digested with *Sau*3AI (1 U) for 20 min. The digest was phenol extracted, resuspended in 250 μl of 10 mM Tris-HCl–1 mM EDTA (pH 8.0) (TE), heated at 70°C for 10 min, cooled to room temperature, and loaded onto a 10 to 40% sucrose gradient. The gradient was centrifuged at 26,000 rpm in a Beckman SW28.1 polyallomer tube for 23 h at 20°C. Thirty-two 500-μl fractions were collected and analyzed on a 0.7% agarose gel, and one fraction containing fragments in the 15- to 20-kb size range was ethanol precipitated. An aliquot of the DNA was ligated to dephosphorylated λEMBL3 arms digested with *Bam*HI, and the sample was packaged (Packagene System; Promega). The CC5A library had a titer of 6×10^5 PFU/ml.

Western blotting (immunoblotting). Bacteria were grown overnight in 10 ml of BHI broth, washed twice in 10 mM Tris-HCl (pH 8.0), and suspended in 0.5 ml of lysis buffer (2% SDS, 8 mM Tris-HCl [pH 6.8], 10% glycerol). The samples

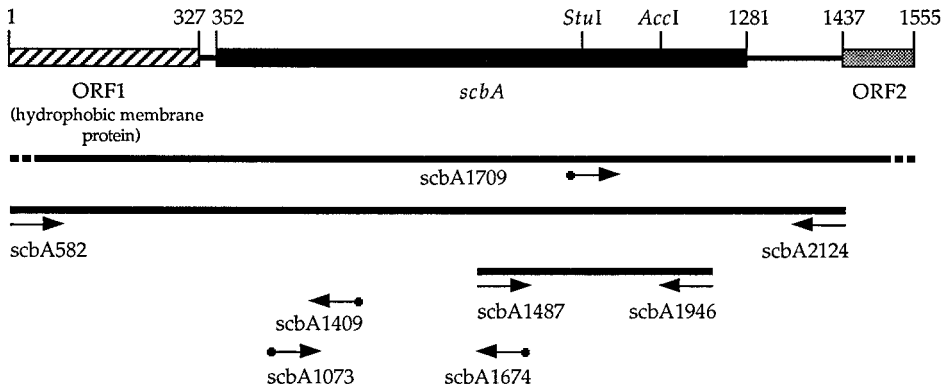

FIG. 1. Physical map of the *scbA* locus. Two partial ORFs (ORF1 and ORF2) flank the *scbA* gene. The primers used for PCR and DNA sequencing are depicted. The regions detected by hybridization using scbA1709 or amplified by PCR are designated by the solid bars. The arrows show the direction of the primers. The hybridization-positive fragment identified with scbA1709 extended beyond the limits of the *scbA* locus shown in the figure. This is shown by the dashed lines. All of the primers shown were used in sequencing reactions.

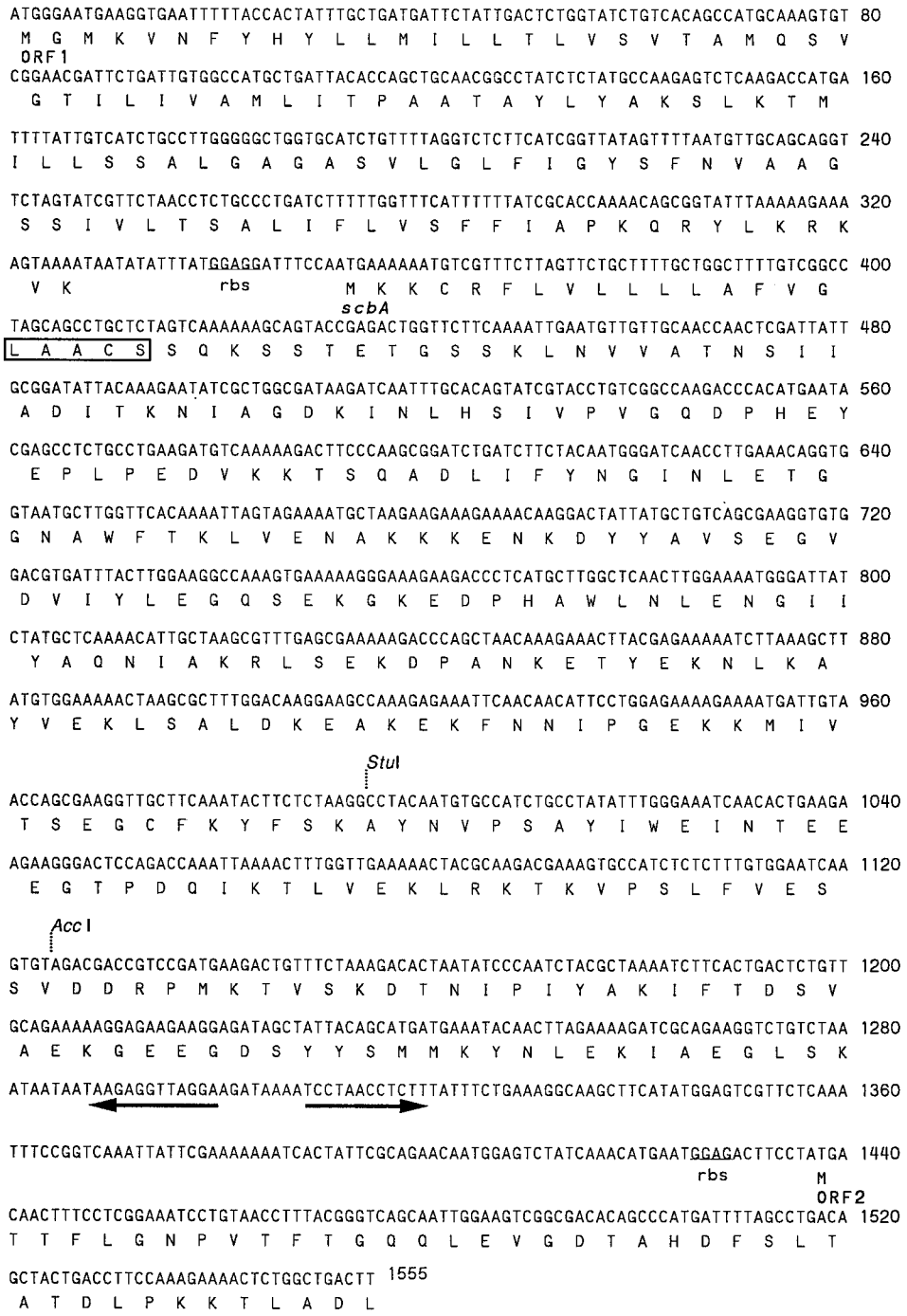

FIG. 2. Complete nucleotide sequence of the *scbA* gene from *S. crista* CC5A. A box surrounds the putative lipoprotein signal peptidase II cleavage site. The consensus sequence is L-X-A-C-X (23). Putative ribosomal binding sites (rbs) are underlined. A potential rho-independent terminator sequence for the *scbA* gene is marked by arrows.

were boiled for 20 min, and 40 μl was applied to a 17.5% acrylamide gel. Following electrophoresis, the gel was stained with Coomassie brilliant blue. Samples were run on a duplicate gel, and a Western blot was prepared as described previously (24). The blot was treated with anti-SsaB rabbit immunoglobulin G (kindly provided by Barry McBride, University of British Columbia [11]) at a 1:500 dilution. Bound antibody was detected with peroxidase-conjugated anti-rabbit immunoglobulin G (Boehringer Mannheim Biochemicals) used at a 1:1,000 dilution.

Binding assays. The corncob assay was performed as described previously (17). The coaggregation of *S. crista* and *A. naeslundii* was performed as described by Cisar et al. (4). The binding of wild-type *S. crista* CC5A and ScbA-defective mutants to saliva-coated hydroxyapatite was performed according to a previously described method (6).

Fibrin binding was performed, with several modifications, as described by Burnette-Curley et al. (2). Fibrin layers were made in polystyrene petri dishes (50 by 9 mm) by adding 0.125 ml of human thrombin (25 U/ml; Sigma Chemical Co., St. Louis, Mo.) to 0.5 ml of human fibrinogen (1 mg/ml; Sigma). The fibrin layer was allowed to set for 2 h at 37°C and was then washed with phosphate-buffered saline (PBS). *S. crista* CC5A-r1 and CC5A-r2 were grown overnight and diluted 1:10 in fresh BHI containing 100 μg of kanamycin per ml. These cells were then grown anaerobically with 5% CO_2 until the optical density of the cultures at 660 nm reached 0.600. The cells were washed twice with PBS and suspended at a

Percent Similarity

1	2	3	4	5	6	7	8		
	92.9	87.5	78.6	78.8	56.5	13.1	32.4	1	ScbA
		89.1	78.0	77.8	54.5	14.7	32.8	2	ScaA
			78.6	79.1	56.5	16.7	32.8	3	SsaB
				91.6	57.8	16.2	31.4	4	FimA
					58.8	17.0	31.1	5	PsaA
						15.3	34.1	6	EfaA
							16.0	7	FimA HI0119
								8	FimA HI0362

FIG. 3. Similarity matrix comparing members of the LraI protein family. The nucleotide sequences, from which the amino acid sequences were deduced, were obtained from GenBank under the accession numbers M37189 (*fimA*), M63481 (*ssaB*), L11577 (*scaA*), and L19055 (*psaA*) and from the Institute for Genomic Research (Gaithersburg, Md.) under the accession numbers HI0119 and HI0362 (*fimA*). The deduced EfaA amino acid sequence was obtained from Lowe et al. (18). A pairwise matrix was obtained by using the MegAlign program from the LaserGene molecular biology package (DNAStar, Inc., Madison, Wis.).

concentration of 10^8 cells per ml. The cells (1 ml) were added to the fibrin layers and incubated for 30 min at 37°C without agitation. Nonadherent cells were removed, and the fibrin layers were washed four times with PBS. The fibrin layers were dissolved with 0.5 ml of a 2.5% solution of trypsin, and aliquots were spread on BHI agar plates. Colonies were counted, and the percent adherence was calculated as the number of CFU divided by 10^8 and multiplied by 100. Four independent experiments were performed, and each strain was run in triplicate. The data were analyzed by Student's *t* test.

Nucleotide sequence accession number. The *scbA* sequence has been deposited in GenBank under the accession number U46542.

RESULTS

Cloning of *scbA*. A 30-base oligomer probe (scbA 1709) (Fig. 1), obtained from the sequence of the *ssaB* gene, hybridized to a single 4.1-kb *Pst*I fragment from strain CC5A chromosomal DNA. This confirmed the observation of Andersen et al. (1) that an *ssaB* gene fragment hybridized to *S. crista* DNA. PCR primers scbA582 and scbA2124 were made to a consensus sequence from the 5′ and 3′ ends of the *scaA* (16) and *ssaB* (11) genes and were used to amplify approximately 1.5 kb of strain CC5A genomic DNA. The PCR product was ligated to the pGEM-T vector, and a partial sequence was obtained to confirm the cloning of the *ssaB* gene homolog (*scbA*). The

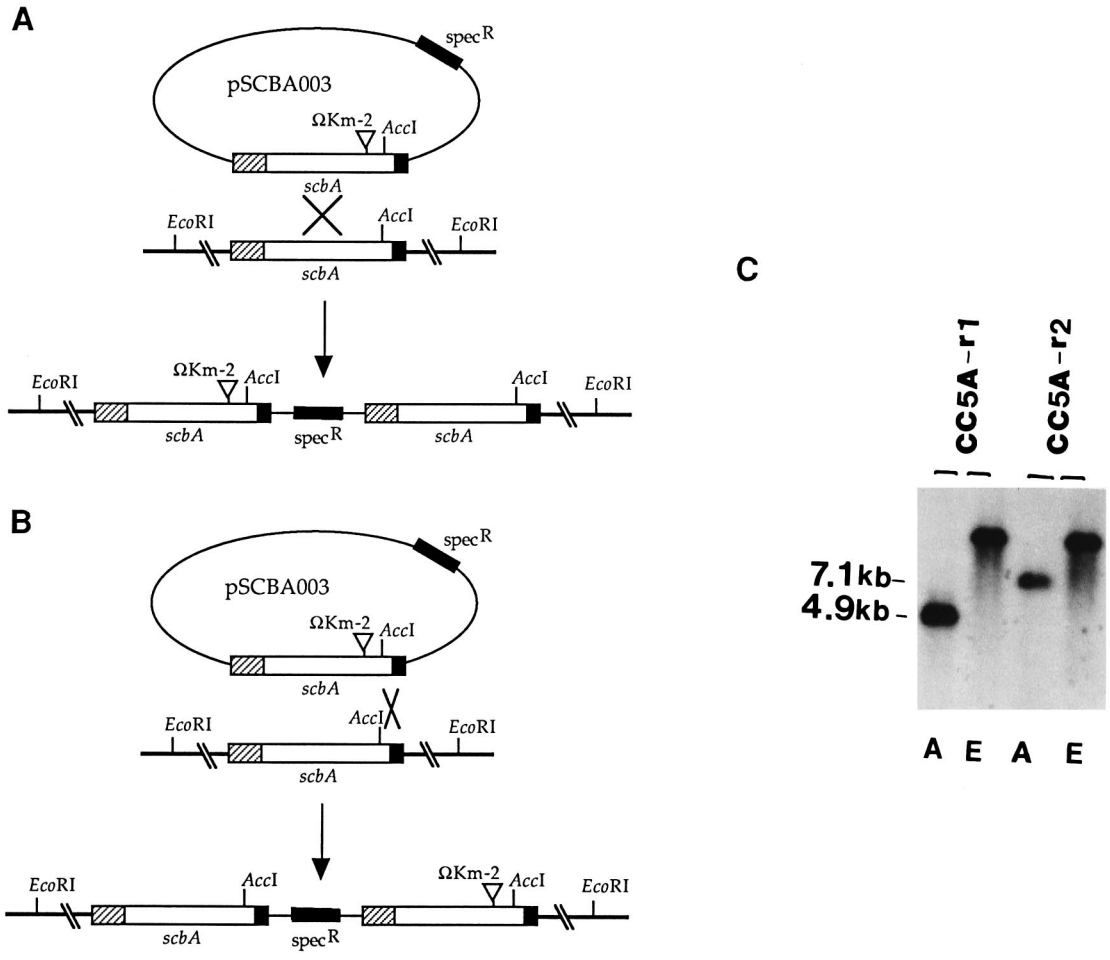

FIG. 4. Schematic representation of the insertion-duplication mutations obtained with pSCBA003. Two possible outcomes of a single-crossover event are depicted as configurations A (A) and B (B). The *scbA* gene is designated by the open box. The striped and solid boxes represent strain CC5A DNA that resides immediately upstream and downstream, respectively, of the *scbA* gene. × indicates the location of the crossover. The locations of the ΩKm-2 insertion element are shown by the inverted triangles. (C) The Southern blot confirming the configurations is shown. Insertion mutants CC5A-r1 and CC5A-r2 represent configurations A and B, respectively. Chromosomal DNA from each of these mutants was digested with either *Acc*I (lanes A) or *Eco*RI (lanes E). The blot was probed with pDL278ΔP.

FIG. 5. Western blot analysis of the expression of ScbA in the insertion-duplication mutants. Whole-cell lysates of strain CC5A and mutants CC5A-r1 and CC5A-r2 were run on the blot. The blot was probed with monospecific anti-SsaB serum at a 1:500 dilution and peroxidase-conjugated mouse anti-rabbit immunoglobulin G at a 1:1,000 dilution.

PCR primers scbA1487 and scbA1946 and DNA from *S. crista* CC5A were used to make a hybridization probe. The PCR product represented a sequence internal to the *scbA* gene.

A λEMBL3::CC5A library was constructed and screened with the hybridization probe to obtain the wild-type *scbA* gene. The wild-type gene was isolated to avoid sequencing errors that can arise because of the use of a PCR product as the DNA template. Three hybridization-positive plaques were identified from among 2,000 plaques. One of these (EMBL3-scbA) was chosen for further analysis and found to contain an insert of approximately 11.0 kb. A 7.0-kb DNA fragment that hybridized to the internal *scbA* PCR probe was cloned into pCR-ScriptKS+ to produce pSCBA001. This plasmid was then used as a template to obtain 1,555 bp of DNA sequence from both strands of the *scbA* gene region.

Sequence analysis of *scbA*. The 1,555-bp DNA fragment contained the entire *scbA* gene as well as two partial open reading frames (ORFs). These partial ORFs flanked the *scbA* gene. The *scbA* gene begins at nucleotide 352 and ends at nucleotide 1281 (Fig. 2). A potential ribosome binding site was found immediately upstream of the start codon at nucleotides 340 to 344. A potential stem-loop structure, followed by three adenines, indicative of a rho-independent terminator, was located immediately downstream from *scbA*.

The deduced amino acid sequence of the ScbA protein contains 310 residues having a combined molecular mass of 34,728 Da. The protein contains a 20-amino-acid hydrophobic signal sequence with residues 17 to 21 (L-A-A-C-S) matching the consensus sequence for the prolipoprotein cleavage site of signal peptidase II (23). The remainder of the protein is relatively hydrophilic.

Compared with the five members of the LraI protein family, from gram-positive bacteria, ScbA has an amino acid identity ranging from 56.5%, with EfaA from *E. faecalis*, to 92.9%, with ScaA from *S. gordonii* (Fig. 3). The two LraI protein homologs (HI0119 and HI0362) from *Haemophilus influenzae* (9) have 13.1 and 32.4% identity, respectively, with the ScbA protein.

The partial ORF upstream of *scbA* codes for a conceptual peptide of 109 residues that matches 100% with the hydrophobic membrane protein reported for *S. gordonii* PK488 (16). The 40-residue partial ORF downstream of *scbA* codes for a conceptual peptide that has 92% amino acid identity with ORF4 from the *scaA* locus of *S. gordonii* PK488 (16). The genetic organization of the sequenced *scbA* region also resembles that described for the *ssaB* locus in *S. sanguis* (11) and the *fimA* locus in *S. parasanguis* (7).

Construction and expression of *scbA* mutants. A 1.8-kb *Hin*cII fragment that contains the entire *scbA* gene from pSCBA001 was subcloned into pDL278ΔP to form pSCBA002. The pDL278ΔP vector carries a spectinomycin resistance marker and is unable to replicate in gram-positive host strains. An ΩKm-2 element (20) that codes for kanamycin resistance (*aphA-3*) and contains transcriptional and translational stops in all three reading frames was inserted into a unique *Stu*I site that is present at position 643 in the *scbA* sequence (Fig. 2). This insertion disrupted the ScbA protein at amino acid residue 215, which is located near the beginning of the B2 region defined by Jenkinson (14). The resulting plasmid, pSCBA003, was used to transform wild-type CC5A. Transformants were selected on kanamycin and were subsequently screened for resistance to spectinomycin. Of 146 kanamycin-resistant transformants, 137 were also spectinomycin resistant, indicating that the plasmid was integrated as a result of an insertion-duplication event. The remainder of the spectinomycin-sensitive transformants were assumed to arise from a double-crossover event that would eliminate the vector sequence and yield allelic replacements. However, Southern blots showed that the pDL278ΔP vector was still present in the spectinomycin-sensitive transformants. No allelic replacement mutants were recovered in three independent transformation experiments. All transformants resulted from single-crossover insertion-duplication mutations that could have either of two possible configurations (Fig. 4). One configuration, configuration A, results from recombination upstream of the ΩKm-2 cassette (Fig. 4A), and the other configuration, configuration B, results from recombination downstream of the ΩKm-2 cassette (Fig. 4B). The configurations were distinguishable on Southern blots when genomic DNA from the transformants was digested with *Acc*I and hybridized with pDL278ΔP. Configurations A and B resulted in single hybridizing bands of 4.9 and 7.1 kb, respectively (Fig. 4C, lanes A). The difference in size between the two hybridizing bands is due to the presence of the 2.2-kb ΩKm-2 element in the configuration B fragment. *Eco*RI-digested genomic DNA for each recombinant strain was included on the Southern blot. There are no *Eco*RI sites within the 1.8-kb *Hin*cII insert fragment; therefore, a single hybridization-positive fragment of equal size was expected for each recombinant. This is shown in Fig. 4C (lanes E).

A representative of each class of transformant (CC5A-r1 or CC5A-r2) was grown, and whole-cell extracts were prepared for Western blot analysis. Monospecific anti-SsaB immunoglobulin G was used to detect the ScbA protein in the transformants (Fig. 5). Wild-type strain CC5A (Fig. 5, lane CC5A) and a configuration B transformant (Fig. 5, lane CC5A-r2) each expressed a 35-kDa protein. This same protein was not expressed in configuration A transformants (Fig. 5, lane CC5A-r1).

Binding studies. To test whether the expression of the *scbA* gene contributed to the potential binding activities of *S. crista*, the wild-type strain and the transformants CC5A-r1 and CC5A-r2 were examined in several adherence assays. The transformants CC5A-r1 and CC5A-r2 failed to show any reduction in corncob formation with *F. nucleatum* relative to that of wild-type CC5A. In contrast to *S. sanguis* 34, wild-type strain CC5A did not coaggregate with *A. naeslundii* PK606. Furthermore, binding isotherms revealed that *S. crista* CC5A

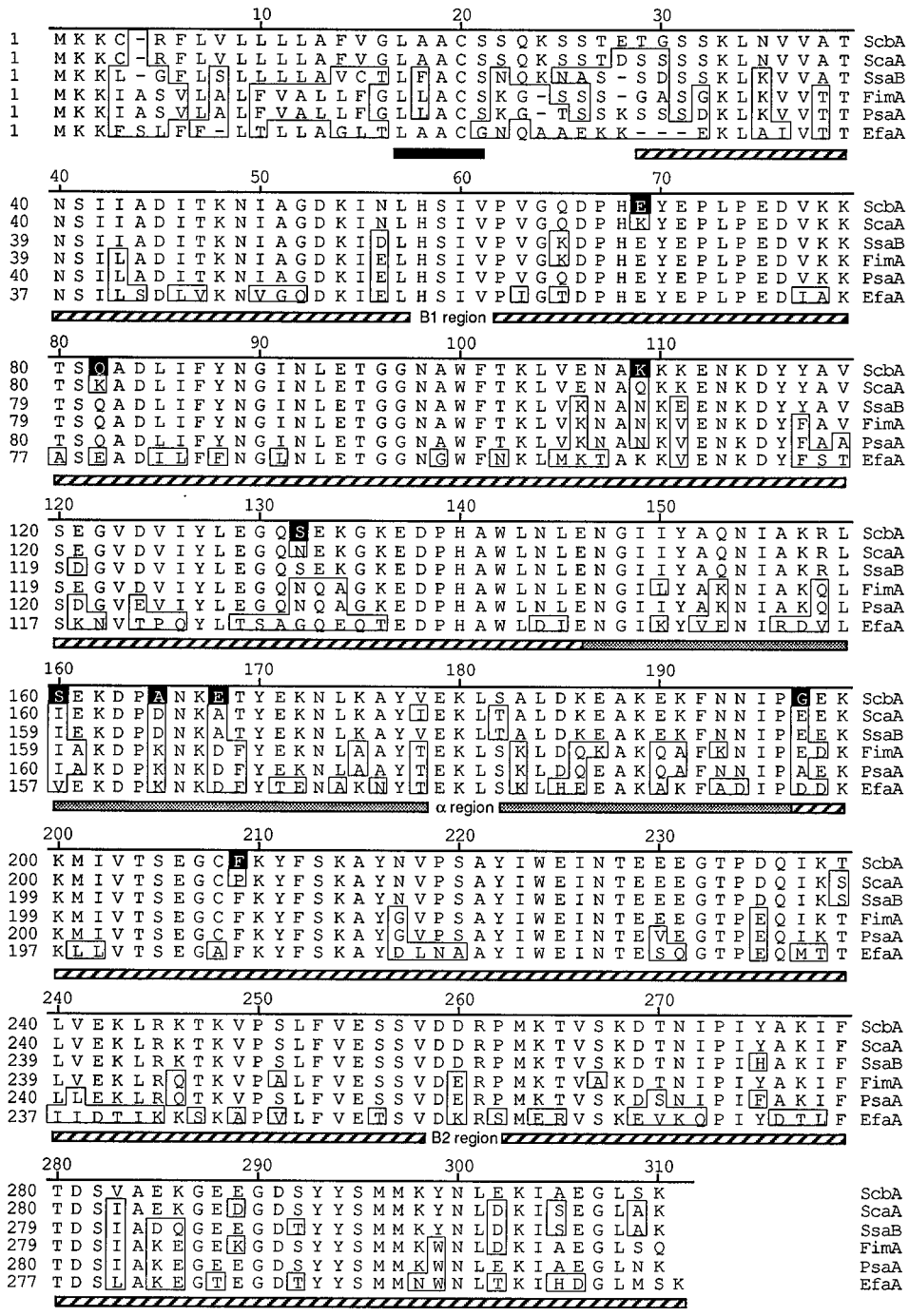

FIG. 6. Deduced amino acid sequence alignment of six members of the LraI protein family. Residues that differ from those in the ScbA sequence are boxed. Residues shown in the filled boxes are functionally nonconserved substitutions between the ScbA and ScaA sequences. The solid bar marks the prolipoprotein cleavage recognition site for signal peptidase II. The striped and hatched bars designate the theoretical β-sheet and α-helical regions of the proteins, respectively (14). The sequences were aligned by using the MegAlign program from the LaserGene molecular biology package. The deduced amino acid sequences were obtained as described in the legend to Fig. 3.

appears to have a low affinity to saliva-coated hydroxyapatite compared with its binding to uncoated hydroxyapatite (data not shown). To test the role of ScbA in adherence to fibrin, the transformants CC5A-r1 or CC5A-r2 were added to fibrin layers. The percent adherence of CC5A-r1 (nonexpressing transformant) was 8.5% ± 6.2%, and that of CC5A-r2 (expressing transformant) was 6.3% ± 3.5% (values are means ± standard errors of the means). This difference was not statistically significant.

DISCUSSION

A new member of the *lraI* adhesin gene family, *scbA* from *S. crista* CC5A, was cloned and sequenced. A comparison of the deduced amino acid sequence of ScbA with those of the other members of the family reinforces previous observations that there is a high degree of conservation among these proteins in gram-positive bacteria. To date only two genes in gram-negative bacteria appear to be related to the *lraI* family. Both of

these genes, designated *fimA*, were found during a search of the complete nucleotide sequence of the *H. influenzae* Rd genome (9). The deduced amino acid sequences from the genes having the identification numbers HI0119 and HI0362 are 13 and 32% identical, respectively, to *scbA*. Since this relationship is based on sequence comparisons we do not know if the *H. influenzae* gene products exhibit binding properties similar to those attributed to the gram-positive LraI proteins.

The *S. crista* polypeptide is most similar to ScaA from *S. gordonii* PK488. These two species are closely related biochemically and genetically, as judged by nucleic acid hybridization studies among the oralis group of streptococci (12). ScbA and ScaA are 93% identical at the amino acid level and have identical leader peptide sequences. This domain is divergent among other LraI members. The relatedness between these species is emphasized when sequences adjacent to the putative adhesin genes of the respective loci are compared. The partial ORFs identified immediately upstream and downstream of the *scbA* gene potentially encode polypeptides that are nearly identical with their counterparts in *S. gordonii* PK488. This operon in *S. gordonii* is analogous to the protein-dependent transport systems found in a wide range of bacterial species (13). ScbA appears to represent the binding protein that is typical of these transport systems. The binding protein is usually modified with lipid and associated with the outer region of the cytoplasmic membrane in gram-positive cells (23).

Three functions have been experimentally assigned to members of the LraI family and are consistent with the exposure of these proteins on the cell surface (8, 15). FimA and SsaB have been reported to be responsible for adhesion to the salivary glycoprotein pellicle. This is a reasonable function for these proteins since *S. sanguis* and *S. gordonii* are known to be early colonizers of the tooth surface during plaque initiation. Interestingly, *S. crista* strains appear to be the exception since they have not been found to be prevalent among the early colonizers. This observation is reinforced by binding studies that indicate that strain CC5A has a low affinity for saliva-coated hydroxyapatite.

A second property, uniquely ascribed to ScaA and of special interest in our studies, is that this protein appears to mediate coaggregation between *S. gordonii* and *A. naeslundii* PK606. We reasoned that ScbA might also promote the coaggregation of *S. crista* and *A. naeslundii* and, in addition, contribute to corncob formation with *F. nucleatum*. However, when CC5A and *A. naeslundii* PK606 were examined in a test tube coaggregation assay, no aggregation was observed. Furthermore, a mutation that knocked out the expression of *scbA* had no effect on corncob formation with *F. nucleatum*. More recently, the FimA protein has been associated with the ability of *S. parasanguis* FW213 to bind to fibrin films on the basis of the observation that FimA-defective mutants demonstrate a statistically significant reduction in binding (2). We found that loss of expression of *scbA* caused no reduction in binding of the bacteria to fibrin.

It appears that *S. crista* CC5A expresses a member of the LraI protein family but lacks the three major reported functions associated with these proteins. This is in spite of the fact that the protein homolog is 93% identical with ScaA. It is possible that one or several of the 20 amino acid differences between ScbA and ScaA may be critical for coaggregation with actinomyces. However, 11 of the 20 amino acid differences between ScbA and ScaA are functionally conserved substitutions while 3 of the remaining 9 amino acid differences, at positions 69, 82, and 209, predominate in the other LraI protein sequences (Fig. 6). It is interesting that four of the nonconserved substitutions, at positions 160, 165, 168, and 197, reside in the proposed solute binding site (α region [14]) and are generally unique to the ScbA protein. These differences could account for the apparent lack of binding functions of ScbA.

We have used insertion-duplication mutagenesis to test our initial hypothesis that the ScbA protein participates in corncob formation between *S. crista* CC5A and *F. nucleatum*. Concurrently, this mutagenesis approach has allowed us to test the role of the gene product in binding to fibrin. Mutations in the *scbA* gene were not required to confirm other possible adhesin properties since the wild-type strain CC5A demonstrates a low affinity for the salivary pellicle and coaggregates poorly with actinomyces. However, these experiments have established that this member of the LraI protein family is not an important streptococcal adhesin. The fact that *S. crista* can be readily manipulated genetically allows us to explore the molecular basis for the apparent functional differences between the ScbA protein and the other members of the LraI protein family.

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