
Haruki Tamura, Arisa Yamada, Hiroko Saito, Shigeo Murai, and Hirohisa Kato*

Department of Dental Pharmacology, School of Dentistry, Iwate Medical University, Morioka 020-8505, Japan

(Received 16 April 2004, accepted 29 June 2004)

The flanking region of the antigen I/II gene, \textit{paaA}, in \textit{Streptococcus cricetus} was examined using the gene-walking technique. In the region downstream of the \textit{paaA} gene, another antigen I/II gene designated as \textit{paaB} was found. The \textit{paaB} gene was disrupted at the alanine-rich region (A region) by a novel insertion sequence element, \textit{ISScr1}. \textit{ISScr1} is a member of the \textit{IS982} family and is composed of a 962-bp sequence and duplicated target DNA (the sequence 5’-TAGCTAAAT-3’) resulting from its insertion. To clarify the structural divergence of the two antigen I/II proteins (PAaA and PAaB), computational analysis of the \textit{paaB} gene was performed and the two structures were compared. The amino acid sequence homology indicated that PAaB resembled PAaA, but the middle region showed little similarity to that of PAaA. Phylogenetic analysis showed that PAaB was better classified in a major group with \textit{S. mutans} PAc and \textit{S. gordonii} SspA and SspB than with PAaA. The transcriptional expression of \textit{paaA} and \textit{paaB} was demonstrated by reverse transcription (RT)-PCR. In the region upstream of the \textit{paaA} gene, three genes homologous to the genes located in the region upstream of the \textit{S. sobrinus} antigen I/II gene (\textit{pag}) were found. Of the three genes, ORF3 showed homology to the \textit{par} gene encoding a transcriptional repressor for the \textit{pag} gene in \textit{S. sobrinus}. Therefore, ORF3 was designated the \textit{par} gene of \textit{S. cricetus}. Southern hybridization revealed that the \textit{par} gene of \textit{S. cricetus} was not found in other oral streptococci examined in this study.

Key words: antigen I/II, gene-walking, \textit{paaB}, \textit{par}, \textit{Streptococcus cricetus}

INTRODUCTION

Mutans streptococci have been implicated as causative organisms of dental caries in humans and experimental animals, and are divided genetically into seven groups, namely \textit{Streptococcus cricetus} (serotype a), \textit{S. rattus} (serotype b), \textit{S. mutans} (serotypes c/e/f), \textit{S. sobrinus} (serotypes d/g), \textit{S. downei} (serotype h), \textit{S. ferus} (serotype c), and \textit{S. macacae} (serotype c) (Hamada and Slade, 1980; Beighton et al., 1981). \textit{S. mutans} and \textit{S. sobrinus} have frequently been isolated from human dental plaques (Hamada and Slade, 1980), and \textit{S. cricetus} has been isolated from the oral cavities of hamsters, wild rats, and humans in North Africa (Loesche, 1986).

The cell surface components of mutans streptococci have been investigated as potential vaccine components against dental decay. Antigen I/II of \textit{S. mutans} is a high-molecular-weight protein antigen that is presumed to play a role in attachment of the streptococcal cells to acquired pellicles on tooth surfaces (Lee et al., 1989). Antigen I/II has been successfully used as a vaccine candidate for elimination of \textit{S. mutans} from the oral cavities of experimental animals and humans (Ma et al., 1987). This protein antigen of \textit{S. mutans} has been given various names, such as PAc (Okahashi et al., 1989), P1 (Crowley et al., 1993), and SR (Ogier et al., 1990). This cell surface protein contains two internal repeated amino acid sequences, the A region and the P region. The amino-terminal alanine-rich region, the A region, is involved in the interactions with salivary components (Lee et al., 1989; Crowley et al., 1993; Nakai et al., 1993). The activity of the adherence to saliva-coated hydroxyapatite resides in the central portion containing the proline-rich region, the P region (Crowley et al., 1993; Munro et al., 1993).

Antigen I/II homologs appear to be preserved in many oral streptococci (Ma et al., 1991) and the nucleotide
sequences of the antigen I/II homologs have been reported for *S. sobrinus* (LaPolla et al., 1991; Tokuda et al., 1991) and *S. cricetus* (Tamura et al., 2001). In addition, two copies of antigen I/II homologous genes (sspA and sspB) have been sequenced in *S. gordonii* (Demuth et al., 1996). *S. gordonii* is a member of the mitis group of streptococci and is the only oral *Streptococcus* species expressing two antigen I/II homologs so far reported.

Regulation of the expression of antigen I/II genes has been reported in *S. sobrinus* and *S. gordonii*. The par gene, located in the region upstream of the *S. sobrinus* antigen I/II gene (*pag*), has been identified as a negative transcriptional regulator of PAg (Takahashi et al., 1993). The expression of the sspA and sspB genes is modulated by environmental conditions (El-Sabaeny et al., 2000) and is up-regulated by saliva (Dû and Kolenbrander, 2000).

Information on various antigen I/II homologs from cariogenic bacteria may be useful for developing wide-range effective vaccines against dental caries. We identified an antigen I/II-homologous gene termed *paaA* in *S. cricetus* (Tamura et al., 2001). Little is known concerning the regulatory mechanism of the *paaA* gene expression in *S. cricetus*. In this study, we attempted to characterize the flanking regions of the *paaA* gene. Using the gene-walking technique, we identified another antigen I/II gene, *paaB*, which was interrupted by a novel insertion sequence element designated ISScr1. Furthermore, we identified the *S. sobrinus par* homolog in *S. cricetus*.

**MATERIALS AND METHODS**

**Bacterial strains and growth media.** The strains of the genus *Streptococcus* studied here are listed in Table 1. All streptococcal strains were grown anaerobically in brain heart infusion medium (Difco Laboratories, Detroit, USA) at 37°C. *Escherichia coli* JM 109 was grown in Luria-Bertani broth at 37°C.

**Gene-walking, DNA sequencing, and DNA analysis.** The region surrounding the *paaA* gene of *S. cricetus* E49 was sequenced using the gene-walking technique as described previously (Tamura et al., 2001). In brief, a Universal Genome Walker Kit (Clontech, Palo Alto, USA) was used to construct a series of adaptor-ligated genomic DNA fragments. The first internal primers were designed based on the nucleotide sequence of the *paaA* gene (Tamura et al., 2001): Primer U1, 5'-TGGCGTGTGCTGAATAGCGCATTGG-3'; Primer D1, 5'-GCTTCGACAGTGATTACGATCGACAGG-3'. To amplify the *paaA*-flanking region, polymerase chain reaction (PCR) amplification was performed using *Taq* DNA polymerase (Sawady, Tokyo, Japan) with gene-specific primers and the adaptor primer API provided in the kit. PCR products were gel-purified using a GeneClean II kit (Bio101, Vista, USA) and then directly sequenced using an automated DNA sequencer ABI 377XL (Applied Biosystems, Foster City, USA) and were cloned into pBluescript II SK+ (Stratagene, La Jolla, USA) with gene-specific primers and T-tailing as necessary for sequencing. The sequence data were assembled using Sequencher 3.1 software (Gene Codes Corp., Ann Arbor, USA). DNA sequences and deduced amino acid sequences were analyzed using the software MacVector 7.2 (Accelrys, Inc., San Diego, USA). The alignments of the amino acid sequences and phylogenetic analyses were performed with the Clustal X 1.81 program (Thompson et al., 1997). Protein similarity searching was performed using the algorithm of Altschul et al. (1997).

**Reverse transcription (RT)-PCR.** *S. cricetus* E49 cells in late-log phase were harvested and suspended in TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) supplemented with 8 mg lysozyme/ml and 100 U mutanolysin/ml and then incubated at 37°C for 1 h. Total RNA from *S. cricetus* lysates was isolated with an RNAeasy mini kit (Qiagen, Valencia, USA) and treated with RNase-free DNase I (Sigma, St. Louis, USA). The reverse transcription reaction was performed on 2 μg of total RNA with 200 U of *TrueScript II* reverse transcriptase (Sawady) and 0.5 μM random primer (9 mer). PCR amplification of cDNA was performed with SuperTaq DNA polymerase (Sawady) using primer pairs specific for *paaA* (5'-TAAGAGGCTTTGTTGTCGCC-3') and 5'-TTTACAGGCAAATTGTTGC-3') and for *paaB* (5'-

**Table 1. Strains of streptococci used in this study.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutans group</td>
<td></td>
</tr>
<tr>
<td><em>S. cricetus</em> E49</td>
<td>Serotype a</td>
</tr>
<tr>
<td><em>S. rattus BHT</em></td>
<td>Serotype b</td>
</tr>
<tr>
<td><em>S. mutans</em> MT8148</td>
<td>Serotype c</td>
</tr>
<tr>
<td><em>S. mutans</em> MT703R</td>
<td>Serotype e</td>
</tr>
<tr>
<td><em>S. mutans</em> MT557</td>
<td>Serotype f</td>
</tr>
<tr>
<td><em>S. sobrinus</em> SL1</td>
<td>Serotype d</td>
</tr>
<tr>
<td><em>S. sobrinus</em> OMZ65</td>
<td>Serotype g</td>
</tr>
<tr>
<td><em>S. dwonei</em> MFe28</td>
<td>Serotype h</td>
</tr>
<tr>
<td><em>S. ferus</em> 8S1</td>
<td>Serotype c</td>
</tr>
<tr>
<td>Mitis group</td>
<td></td>
</tr>
<tr>
<td><em>S. sanguinis</em> ATCC10556</td>
<td><em>S. sanguinis</em> type strain</td>
</tr>
<tr>
<td><em>S. oralis</em> ATCC10557</td>
<td></td>
</tr>
<tr>
<td><em>S. gordonii</em> ATCC10558</td>
<td><em>S. gordonii</em> type strain</td>
</tr>
<tr>
<td>Milleri group</td>
<td></td>
</tr>
<tr>
<td><em>S. anginosus</em> NCTC10713</td>
<td><em>S. anginosus</em> type strain</td>
</tr>
<tr>
<td><em>S. intermedius</em> GA1-1157</td>
<td></td>
</tr>
<tr>
<td><em>S. constellatus</em> ATCC27823</td>
<td><em>S. constellatus</em> type strain</td>
</tr>
</tbody>
</table>

**Information on various antigen I/II homologs from cariogenic bacteria may be useful for developing wide-range effective vaccines against dental caries.**
Identification of *S. cricetus paaB* and *par* genes

TGTTTGTAGAGGCTGCAC-3' and 5'-TCTGAGGTC-TGAACGGTCCC-3'). Settings were initially 94°C for 60 s and then 32 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 40 s. As a negative control, RNA without reverse transcriptase was used for RT-PCR. The PCR products were visualized by ethidium bromide staining in 3.0% agarose gels. The authenticity of products was confirmed by direct sequencing.

**Southern hybridization.** In the course of gene-walking, the *par* gene of *S. cricetus* was cloned into the EcoRV site of the pBluescript II SK (+) plasmid. The resultant clone, pBER5, was confirmed by sequencing both strands. Southern hybridization was performed using the DIG High Prime DNA Labeling and Detection Starter Kit I (Roche Diagnostics, Mannheim, Germany). Genomic DNA (5 µg) was digested with EcoRI restriction enzyme and separated on 0.7% agarose gels in 0.5x TBE buffer. DNA was transferred onto positively charged Hybond-N+ nylon membranes (Amersham Biosciences, Piscataway, USA) and U.V. cross-linked. The 0.5-kb *Stu*I fragment of pBER5 comprising the *par* gene was labeled with digoxigenin and used as the DNA probe. Hybridizations were performed at 42 and 32°C for 16 h, and then the membranes were separately washed according to the manufacturer's instructions (Roche Diagnostics). Hybridized fragments were visualized with a chromogenic alkaline phosphatase substrate NBT/BCIP after a 16-h incubation.

**RESULTS**

**Identification of the region adjacent to the paaA gene.** Using the gene-walking technique, we deter-
mined the nucleotide sequence of the region surrounding the paaA gene in *S. cricetus*. The region (18,442 bp) contained ten open reading frames (ORFs): three ORFs in the region upstream and six ORFs in the region downstream of the paaA gene (Fig. 1A). Nucleotide sequence analysis indicated that ORF7 (408 bp) and ORF8 (360 bp) overlapped by 35 bp. A potential stem-loop structure was identified near the termination codon of the paaA gene, as reported previously (Tamura et al., 2001). A BLAST search revealed that ORF1 (1,176 bp) and ORF2 (822 bp) exhibited significant homology with transaminase proteins (Sung et al., 1991) and proteins belonging to the glycosyltransferase family 8 (Campbell et al., 1997), respectively. ORF7 (408 bp) and ORF9 (429 bp) also showed homology to the MarR family of proteins identified as transcriptional regulators in a variety of important human pathogens (Sulavik et al., 1995). A homology search of the deduced amino acid sequences of the ten ORFs was performed with the data of the *S. mutans* genome project (Ajdić et al., 2002) and the results are summarized in Table 2. There was no gene homologous to *S. cricetus* ORF3 in *S. mutans*.

### Characteristics of ORF1, ORF3 (par), and ORF5.

A protein similarity search revealed that ORF1, ORF3 (552 bp), and ORF5 (573 bp) exhibited significant homology with three genes (*orf1*, *par*, and *orf3*) in the region upstream of the pag gene in *S. sobrinus* (Takahashi et al., 1993) and that ORF1 and ORF5 of *S. cricetus* showed homology to *orf1* (76.7% identical residues) and *orf3*

### Table 2. ORF proteins from *S. cricetus* and homologs in *S. mutans*.

<table>
<thead>
<tr>
<th>ORF (gene)</th>
<th>aa</th>
<th>Predicted function</th>
<th><em>S. mutans</em> homolog</th>
<th>Accession#</th>
<th>Identity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF1 (orf1)</td>
<td>391</td>
<td>transaminase</td>
<td>SMU.1312</td>
<td>AAN58989</td>
<td>25.6</td>
</tr>
<tr>
<td>ORF2 (orf2)</td>
<td>273</td>
<td>glycosyltransferase family 8</td>
<td>SMU.1039c</td>
<td>AAN58738</td>
<td>56.4</td>
</tr>
<tr>
<td>ORF3 (par)</td>
<td>183</td>
<td>transcriptional repressor of antigen I/II</td>
<td>-***</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ORF4 (paaA)</td>
<td>1653</td>
<td>cell surface protein antigen PAAa</td>
<td>SpaP (SMU.610)</td>
<td>AAN58348</td>
<td>54.7</td>
</tr>
<tr>
<td>ORF5 (orf5)</td>
<td>190</td>
<td>unknown</td>
<td>SMU.706c</td>
<td>AAN58437</td>
<td>40.8</td>
</tr>
<tr>
<td>ORF6** (paaB)</td>
<td>381</td>
<td>unknown</td>
<td>SpaP (SMU.610)</td>
<td>AAN58348</td>
<td>–</td>
</tr>
<tr>
<td>ORF7 (orf7)</td>
<td>135</td>
<td>transcriptional regulator</td>
<td>SMU.1969c</td>
<td>AAN59574</td>
<td>46.7</td>
</tr>
<tr>
<td>ORF8 (orf8)</td>
<td>119</td>
<td>unknown</td>
<td>SMU.1968c</td>
<td>AAN59573</td>
<td>66.7</td>
</tr>
<tr>
<td>ORF9 (orf9)</td>
<td>142</td>
<td>transcriptional regulator</td>
<td>SMU.1025</td>
<td>AAN58725</td>
<td>49.6</td>
</tr>
<tr>
<td>ORF10 (orf10)</td>
<td>127</td>
<td>unknown</td>
<td>SMU.1026</td>
<td>AAN58726</td>
<td>37.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percent identity of amino acid sequence to *S. mutans* homolog.

<sup>**</sup> ORF6 was disrupted by an insertion sequence ISScr1.

<sup>***</sup> –; None.

---

**A**

Pre A 199–223: `AHAAKSDYEAQLDQDLKAIQW`

A1 224–305: `THKNQYQAALAYCAKLERVKANGDKADAAYQNVKDKKAKNAEIAENAAAIKQRNADAKTYSAAVEQYADLIAAIQW`

A2 306–387: `ONKHNEAOYOAEKKGAYTELVARQVANKDAADAAYQNVKDKKAKNAEIAENAAAIKQRNADAKTYSAAVEQYADLIAAIQW`

Post A 388–403: `-----DLAEPYKKEYEEQ`

**B**

Pre P 765–771: `NPEPVPEPT`

P1 774–812: `KPEPKEYEVERKDEPAPEPVKFDPEPRTPTDPQPEPN`

P2 813–851: `KPEPKEYEVERKDEPAPEPVKFDPEPRTPTDPQPEPN`

P3 852–890: `KPEPKEYEVERKDEPAPEPVKFDPEPRTPTDPQPEPN`

Post P 891–999: `KPEPKEPNEP`

Post P2 900–920: `KPEPKEPNEPILKRFPGEPAVPVT`

Fig. 2. A. Sequence around the A region of the PaAB protein. The A region is composed of two 82-residue long repeats (A1, A2) and flanked by two abbreviated short repeats (Pre A, Post A). Each long repeat contains two inner short repeats and an abbreviated copy of this repeat, which are indicated by underlines and broken lines, respectively. The position of the integration of the ISScr1 element is indicated by a thick bar. B. Sequence around the P region. The P region is composed of three 39-residue long repeats (P1-3) and flanked by three short abbreviated repeats (Pre P, Post P1, and Post P2). Underlined sequences represent the inner short repeated sequences found in the long repeats. Shaded areas represent the frequently repeated amino acid sequence PTPP(T).
Identification of insertion sequence element ISScr1. DNA analysis revealed that ORF6 was disrupted by a new insertion sequence element designated ISScr1. In the ISScr1 sequence (962 bp in length), an ORF encoding 287 amino acids was found. The deduced amino acid sequence of the ORF showed homology to a putative transposase found in S. agalactiae ISS4d (88.5% amino acid identity) that belongs to the IS982 family (Spellerberg et al., 2000). ISScr1 carried imperfect inverted-repeat sequences (25 bp in length) at both ends and duplication of a 9-bp direct-repeat sequence (5'-TAGCTAAAT-3') generated on transposition (Fig. 1C).

Characteristics of ORF6 (paaB). To clarify the structural characteristics of ORF6 (paaB), the nucleotide sequence of the disrupted paaB gene was computationally reconstructed by deleting the nucleotide sequence of ISScr1. The nucleotide sequence of the hypothetical paaB gene consisted of 4,494 bp encoding a protein of 1,497 amino acid residues. The deduced amino acid sequence of PaaB included a signal sequence comprising 38 amino acid residues in the N-terminus and a cell-wall anchoring region comprising a cell-wall sorting signal (Schneewind et al., 1993) with the sequence LPQTG (residues 1,461-1,465) in the C-terminus. Two repetitive domains, the A region and the P region, were conserved in PaaB. In the A region, two tandem complete repeats (A1, A2) were found to be flanked by two incomplete repeats (Pre A, Post A) (Fig. 2A). Each 82-residue complete repeat consisted of two short repeated sequences and a partial short repeat, as in the case of the A region of PaaA (Tamura et al., 2001). In the P region, three complete 39-residue repeating units (P1-P3) were found adjacent to three incomplete repeats (Pre P, Post P1, and Post P2) (Fig. 2B). The complete repeating unit possessed two short decapetide repeats and the conserved sequence PTPP(T), like the P region of PaaA (Tamura et al., 2001).

Comparison of the deduced amino acid sequences of PaaB and PaaA. To examine the structural similarity between PaaB and PaaA, six portions of the molecule were compared. In contrast to PaaA, PaaB contained two complete repeat units in the A region and a longer middle domain (Fig. 3A). The P region and the C-terminal region showed high homology (78% and 68% identical residues, respectively). In contrast, the A region and the middle region exhibited low homology (31% and 29% identical residues, respectively).

Comparison of the deduced amino acid sequences of PaaB and antigen I/II members. The deduced amino
The acid sequence of PAaB was compared to those of antigen I/II proteins. Considerable sequence homology was found between PAaB and 
*S. mutans* PAc (64.3% identical residues) or *S. sobrinus* PAg (63.8% identical residues) or *S. gordonii* SspA (60.2% identical residues) or SspB (51.1% identical residues). Thus, PAaB showed close homology to PAc as well as PAg, and was more similar to SspA than SspB. As shown in Fig. 3B, a phylogenetic analysis of PAaB with the antigen I/II family of proteins showed that the antigen I/II proteins of *S. mutans* strains formed a specific subgroup and that the antigen I/II members of *S. sobrinus* strains made a single cluster. PAaB was positioned within a major cluster of antigen I/II proteins including both the *S. mutans* subgroup and the *S. gordonii* subgroup comprising SspA and SspB, whereas PAaA branched out of the major cluster including PAaB.

Comparison of the deduced amino acid sequences for PAaA and PAaB showed the least similarity (29% identical residues) in the middle domain (Fig. 3A). To examine whether this divergence was shared by antigen I/II proteins, amino acid sequence alignment of the middle regions of six members of antigen I/II proteins was performed (Fig. 4). The middle regions of antigen I/II proteins were divided into two groups: one group consisted of PAc, PAg, PAc, and PAaB; and the other of PAaA and SspB. Together, these findings suggest that the middle region of PAaB was derived from a discrete antigen I/II member, not from PAaA.

**RT-PCR analysis of the paaA and paaB genes.** To detect the transcripts of the paaA and paaB genes, RT-PCR was performed. As shown in Fig. 5, the PCR products generated with primers for the paaA (161 bp) and paaB (115 bp) genes were obtained only with reverse transcription. This finding indicates that the transcripts of both the paaA and paaB genes were expressed in *S. cricetus*.

**Distribution of sequences homologous to the ORF3 (par) gene.** To determine whether sequences homologous to the par gene of *S. cricetus* were present in other
streptococci, Southern hybridization was performed using a probe specific for the \textit{par} gene. Under high-stringency conditions, hybridization of the probe to 14-kb EcoRI fragments from \textit{S. cricetus} was detected (Fig. 6A, lane 1). On the other hand, no hybridization to EcoRI-digested genomic DNA fragments isolated from other streptococci occurred (Fig. 6A, lanes 2-15). Under low-stringency conditions, no hybridized fragments were detected in any species except for \textit{S. cricetus} (Fig. 6B).

**DISCUSSION**

**Computational analysis of PAaB.** Transcription of the \textit{paaA} and \textit{paaB} genes was detected by RT-PCR analysis (Fig. 5). Moreover, the expression of \textit{paaA} and \textit{paaB} was observed in the early-log phase (data not shown). These findings indicate that the \textit{paaB} gene was transcribed, as was the \textit{paaA} gene. Nevertheless, the product of the \textit{paaB} gene was a remnant polypeptide (381 residues) because of the insertion of \textit{IScr1}. The scarcity of remnant sequences in streptococcal species and \textit{S. cricetus} strains for antigen I/II genes or other genes has balked our attempts to determine the ancestral gene and evolution of antigen I/II genes. Therefore, we analyzed the \textit{paaB} gene computationally with the elimination of the \textit{ISScr1} element from the analysis. The putative PAaB consisted of a signal sequence region, two internal repeats, and a C-terminal cell wall anchoring region (Fig. 3A). Many antigen I/II proteins usually harbor three repeat units in each A and P region (Kelly et al., 1989; Nakai et al., 1993; Tamura et al., 2001). Hypothetically, the ancestral A region may have existed as many tandem short repeat units, and then tandem duplication occurred as the long repeat units that changed into the present stable long repeat units.

Phylogenetic analysis indicated that PAaB and PAA were positioned in distinct groups, whereas \textit{SspA} and \textit{SspB} of \textit{S. gordonii} formed a single cluster (Fig. 3B). In \textit{S. gordonii}, the divergence between \textit{SspA} and \textit{SspB} was observed in the middle region, whereas no deletion was found in the A region (Demuth et al., 1996). In \textit{S. cricetus}, the least homology was found in the A region and the middle region. Thus, it is suggested that the difference between PAaB and PAA of \textit{S. cricetus} resulted from the low homology in the A region and the middle region. Amino acid sequence alignment revealed that antigen I/II proteins were divided into two groups based on their middle regions (Fig. 4). Although the middle region corresponded to the variable region in \textit{S. mutans} (Kelly et al., 1989), the middle region of PAaB showed strong homology to that of PAc. The similarity between the central regions of SspA and PAc was demonstrated in a previous report (Demuth et al., 1996). Taken together, the findings suggest that members of each group may
share a particular portion of the ancestral genes.

**Comparison of the paaA flanking region of S. cricetus to the regions around the antigen I/II-homologous gene in streptococci.** The locus of the paaA gene in *S. cricetus* showed similarity with the region upstream of the pag gene in *S. sobrinus*. In contrast, the flanking region of the paaA gene differed from the corresponding region in *S. mutans*; nevertheless, all homologous genes except for par identified around the paaA gene in *S. cricetus* were preserved in *S. mutans* (Table 2). Ogier et al. (1991) reported the existence of a gene coding for a 40-kDa cell wall-associated protein in the region upstream of the sr gene in *S. mutans* OMZ175 (serotype f) and determined the distribution of this gene in *S. mutans* serotypes c, e, and f by DNA hybridization analysis. In accordance with that report, we found no gene encoding the 40-kDa cell wall protein in the flanking region of the paaA gene in *S. cricetus*. Thus, no significant sequence homology existed between the flanking region of the paaA gene of *S. cricetus* and that of the sr gene of *S. mutans*, as with the region upstream of the pag gene of *S. sobrinus* (Takahashi et al., 1993).

The nucleotide sequence analysis of the region upstream of the sspA gene in *S. gordonii* revealed that the gene positioned at 186 bases upstream of the sspA gene was homologous to the *E. coli* pgk gene encoding 3-phosphoglycerate kinase (El-Sabaeny et al., 2000). However, no gene homologous to the pgk gene was found in the sequenced region of *S. sobrinus* (Takahashi et al., 1993).

Putative regulator of gene expression of paaA and/or paaB in *S. cricetus*. The insertion sequence element is a transposable element and many insertion sequence elements promote the rearrangement of genomes by mechanisms including activation, movement, dissemination, and inactivation of genes (Mahillon and Chandler, 1998). Nucleotide sequence analysis revealed that a new insertion sequence, ISScr1, belonging to the IS982 family, existed in the paaB gene. It is suggested that ISScr1 may inactivate the paaB gene in *S. cricetus*. Interestingly, the *S. gordonii* strain M5 harbors ISSg1 in the intergenic region between sspA and sspB, whereas the DL1 strain does not (Demuth et al., 1997).

In *S. gordonii*, the expression of sspA and sspB is transcriptionally regulated by different promoters according to environmental conditions (El-Sabaeny et al., 2000). In addition, transcription of sspA and/or sspB is up-regulated in the presence of saliva (Dø and Kolbrander, 2000). In *S. cricetus*, the orf5 gene was positioned between the paaA and paaB genes. Therefore, it seems that the paaA and paaB genes are independently regulated by discrete promoters.

The deduced amino acid sequence of the ORF3 (par) gene showed homology to the *S. sobrinus* PAR (Fig. 1B). The par gene of *S. sobrinus* was characterized as a negative transcriptional regulator of PAg and is present in *S. sobrinus* serotypes d and g, but not in *S. mutans* serotypes e, f, and g (Takahashi et al., 1993). Southern hybridization with the probe for the *S. cricetus* par gene under low stringency conditions showed that no gene homologous to the *S. cricetus* par was observed in the streptococci used in this study (Fig. 6B). We surmised that the probe for *S. cricetus* par failed to detect it in *S. sobrinus* strains because of the low nucleotide sequence similarity (67.6%) of the *S. cricetus* par gene to the *S. sobrinus* par gene. The repression of the antigen I/II gene expression in cariogenic bacteria might be a strategy for inhibition of the attachment of the organisms to tooth surfaces, but further work will be required to substantiate this possibility.

This study was supported in part by a Grant-in-Aid to H. T. (No. 14771026) and a Grant-in-Aid for High Performance Biomedical Materials Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

**REFERENCES**


