Journal of Industrial Microbiology (1995) 15, 186–192

Genetic analysis of adherence by oral streptococci

HF Jenkinson

Department of Oral Biology and Oral Pathology, University of Otago, PO Box 647, Dunedin, New Zealand

Streptococci are one of the most successful bacterial colonizers of the human body and are major components of oral biofilms. The bacterial cells express multiple cell-surface adhesins that are responsible for the ability of streptococci to adhere to a wide range of substrates which include salivary and serous proteins, epithelial cells and other bacterial cells. Analysis of adherence-defective mutants has indicated the importance of high molecular mass wall-associated polypeptides and of enzymes catalyzing extracellular glucan polysaccharide synthesis to the adherence and accumulation of oral streptococci. The analysis of isogenic mutants of streptococci, generated through insertional inactivation (or allelic exchange), has confirmed the essential roles of specific surface polypeptides both to adhesive processes and to correct assembly of the cell wall layers.

Keywords: oral streptococci; adherence; *Streptococcus* adherence; genetics of adherence

There are more than thirty species of *Streptococcus* associated with infections of humans and animals. Broadly speaking, the genus consists of the pyogenic (β-haemolytic) streptococci, the oral streptococci, and several other more-or-less related species. The human oral streptococci comprise the 'mutans group' organisms (*S. mutans*, *S. sobrinus*, *S. rattus*, *S. cricetus*), the 'oralis group' organisms (*S. oralis*, *S. sanguis*, *S. gordonii*, *S. mitis*, *S. anginosus*, *S. constellatus*, *S. intermedius*, *S. parasanguis*, *S. crista*) and the 'salivarius group' bacteria (*S. salivarius*, *S. vestibularis*, *S. thermophilus*). The mutans group streptococci are found almost exclusively in dental plaque on oral hard surfaces (eg tooth enamel) while the oralis group bacteria, as well as being present in dental plaque [14], are found in the nasopharynx, throat, alimentary tract and urogenital tract where they can cause a variety of infections. The salivarius group of streptococci are found associated with the oral mucosa, on the dorsum of the tongue, and in saliva. The preferences of the individual species for colonizing different sites is owing to, at least in part, the abilities of the cells to adhere preferentially at those sites [52]. Adhesins (proteins) on the bacterial cell surface mediate the adherent interactions with host tissues and other microorganisms and serve as colonization factors. The ability to cause disease is attributed to the production of so-called virulence factors. These include extracellular products such as haemolysins, erythrogenic toxins, hyaluronidase, and streptokinase which cause damage to host tissues. The process of cell adherence, though, could be considered a prerequisite for streptococcal colonization. Because adherence is a primary event in initiation of infections, there is considerable interest in defining the molecular mechanisms involved in order to devise anti-colonization strategies.

Site-specific colonization by streptococci results from interplay of host receptor and bacterial adhesin expression. While the possession of a particular adhesin may allow a bacterium to attach to a surface expressing the appropriate receptor, adherence of the bacterium may be determined ultimately by the availability of receptors. In the oral cavity, receptor availability is dependent on numerous factors. These include the types of carbohydrate ligands present on glycoproteins and glycolipids (which are host-specific), salivary flow rate and composition, and the numbers and types of other microorganisms present. All these factors influence the adherence of the streptococci. Eventually a more complete understanding of the colonization processes will lead to new ways to disrupt or prevent formation of oral biofilms.

This article will consider how genetics specifically has helped in understanding more about some of the adherence processes that are critical to colonization of the human body by streptococci. However, to place an accurate perspective on the evolution of the subject of streptococcal adherence, it is necessary to summarize what is known about adherence mechanisms in general, and to stress that many of the adhesin-receptor interactions have been well-characterized without the aid of genetic techniques.

Streptococcal cell adherence

Much of our current knowledge about how streptococci adhere comes from *in vitro* binding assays of bacterial cells to various substrata. Studies of oral streptococci have concentrated on, predictably perhaps, the abilities of these organisms to adhere to saliva-pellicle coated surfaces such as hydroxylapatite. Members of the oralis and mutans groups of streptococci vary widely in their affinities of binding to saliva-coated surfaces. For strains of *S. gordonii*, *S. oralis* and *S. sanguis*, adherence involves both 'specific' (lectin-like) interactions of bacterial adhesins with salivary components, and 'non-specific' binding through physiochemical forces [3] of which hydrophobicity is thought of as being highly significant [9]. Mutans group streptococci adhere generally less well to salivary glycoprotein pellicles than do oralis group streptococci [46]. Instead, adherence of *S. mutans* and *S. sobrinus* is largely dependent upon the synthesis of, and binding to, water-insoluble glucan polysaccharides [18]. With the development of more sophisti-

Correspondence: Dr Howard F Jenkinson, Department of Oral Biology and Oral Pathology, University of Otago, PO Box 647, Dunedin, New Zealand
Received 21 November 1994; accepted 14 February 1995

cated protein separation techniques came the identification of specific components within human salivary secretions that are bound by bacteria. Oral streptococcal cells were shown to bind to glycosylated proline-rich proteins, mucins and α-amylase on nitrocellulose blots of salivary proteins separated by gel electrophoresis [43] and to purified acidic proline-rich proteins adsorbed to hydroxylapatite [17]. These and other experiments have identified several major salivary components that act as pellicle receptors for the oral streptococci (Figure 1).

Whole-cell binding assays have demonstrated further that streptococci bind many of the components of human serum and that some species adhere with high affinity to extracellular matrix components, particularly fibronectin (Figure 1). Such binding reactions are thought to be significant for establishing more deep-seated infections caused by pyogenic streptococci, and in streptococcal endocarditis. The streptococci also have an extraordinary capacity to bind to eukaryotic cells and to other prokaryotic cells. Most epithelial cell types carry receptors for streptococci and, as already mentioned, receptor structure and level of expression determines streptococcal cell tropism for host tissue sites. Highly significant for the development of oral biofilms is the ability of many species, especially within the oralis group, to bind with high affinity to other oral microbial cells. This phenomenon of intermicrobial adhesion, originally termed coaggregation, is believed to be responsible to a greater or lesser extent for the temporal accretion of microorganisms in the formation of dental plaque [34]. The streptococci are amongst the earliest colonizers of salivary pellicle and their ability to support adherence of many other oral organisms (Figure 1) suggests they act as a primary focus of plaque development [34]. It must be evident from the aforementioned therefore that the *Streptococcus* could be considered a microbial hub in the wheel of adherent interactions between host cells, tissue components, secretions and microbial cells (Figure 1).

Despite the wealth of information now available on the receptors for streptococcal adhesion, there has been slow progress identifying and characterizing the bacterial adhesins responsible for binding of cells to these receptors. There are probably two main reasons for this. First, the structure of the streptococcal cell wall layers cannot be easily dissected because they are complex in molecular composition and the adhesins are difficult to remove intact. Second, genetic analysis of function involving gene cloning, transfer and mutagenesis in streptococci is only just coming into its prime. Adhesins that have been successfully identified are typically either proteins with high affinity for a specific substrate (eg fibronectin), or proteins that make up a major component of the cell wall. Purified proteins from the cell walls of streptococci that act as adhesins include immunoglobulin-binding proteins [6] and fibronectin-binding protein [49] of *S. pyogenes*, salivary agglutinin-binding antigen I/II proteins in oral streptococci [25] and the glucan-binding proteins of mutans group streptococci [2,35]. Information on the *in vitro* binding properties of these various polypeptides has been crucial to general appreciation of the roles that cell-surface associated proteins play in adhesion. However, *in vitro* demonstration of binding activity of an isolated protein does not necessarily imply that the activity is significant in cell adherence. Genetic analysis provides one means of extending information on the roles of specific proteins in adhesion. If the gene encoding a putative adhesin is inactivated, and if this results in deficient adherence function, then evidence is strong that the gene product is involved in adherence.

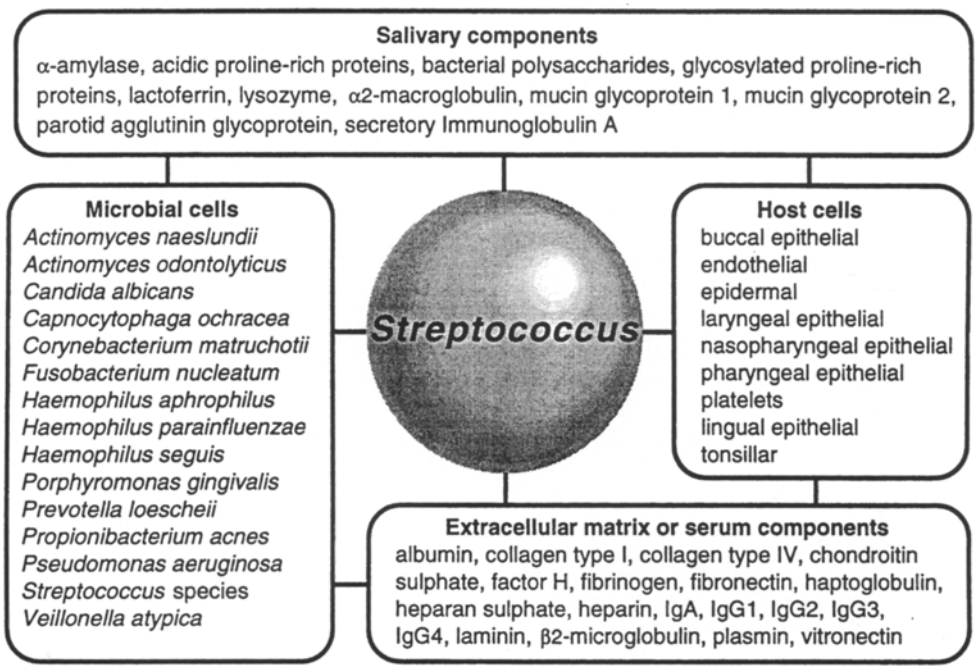

Figure 1 Summary of the adherent interactions displayed by streptococci with host and bacterial components. Not all species of streptococci exhibit all the binding reactions indicated. The diagram was constructed from data within several key references [6,23,34,35,46] and from other work alluded to in the text

Analysis of spontaneously- or chemically-derived mutants

The isolation and phenotypic characterization of adherence-defective mutants of oral streptococci has given significant insights into adherence mechanisms. The adherence properties of a spontaneously-isolated mutant strain of *S. sanguis* strain 12 [41] provided support for previous evidence that adherence of *S. sanguis* to pellicle involved positive cooperativity between binding sites [45]. The mutant strain was proposed to lack an adhesin that recognized a neuraminidase-sensitive pellicle receptor [41] but to express normally another adhesin for a separate pellicle receptor.

The isolation of adherence-defective mutants of oralis group streptococci lacking cell surface fibrils [16] or fimbriae [15] indicated that these structures might be involved in adherence of cells both to salivary molecules and to other bacteria [20]. Various chemically-induced mutants of *S. salivarius* altered in surface fibril morphology were unable to bind to buccal epithelial cells or coaggregate with *Veillonella* [54]. These observations led to the demonstration that *S. salivarius* HB cells normally expressed three structural classes of cell-surface fibril, two of which carried separate adhesive functions [54,55]. There is, however, no convincing demonstration that in other species of oral streptococci, adhesins are carried on surface structures [20]. What has become evident though from biochemical analyses of adherence-defective mutants is that production of high molecular mass cell wall proteins is associated with cell-surface hydrophobicity [40] and adherence of bacteria to salivary pellicle [10].

Studies of mutant strains of *S. mutans* and *S. sobrinus* that were altered in extracellular polysaccharide production were paramount in establishing that glucan production by the mutans group streptococci was essential for adherence of these bacteria to smooth surfaces and to cause dental caries [18]. A class of adherence-defective mutants of *S. sobrinus* were dextranase-negative [44]. The complexities of extracellular glucan metabolism in mutans group streptococci can now be better accounted for a decade later as a result of gene cloning and directed mutagenesis. This subject has been reviewed recently [35].

Lastly, spontaneous mutants of streptococci defective in coaggregation with oral *Actinomyces* species have allowed both an analysis of the mechanisms and of the molecules involved in coaggregation. The ability of coaggregation-defective mutants of *S. oralis* H1 to bind to some *A. naeslundii* strains but not to others established that at least two types of surface components were involved in coaggregation [29]. The isolation of a coaggregation-defective mutant of *S. gordonii* PK488 allowed identification of a protein denoted ScaA [32] present in coaggregating strains but not in mutants [31].

Streptococcal molecular genetics

The presentation of nearly 200 papers at the 4th International Conference on Streptococcal Genetics held in Santa Fe, NM, USA, in May 1994 exemplified the upsurge of research into the genetics of streptococci. The basis of genetic analysis requires that DNA can be introduced into an organism. This is achieved with ease for strains of *S. pneumoniae, S. gordonii* and *S. mutans* which are naturally-competent for DNA uptake and genetic transformation. Only recently, though, has the technique of electroporation [51] permitted genetic analysis of otherwise non-transformable streptococci [13]. The ability to electrically induce DNA uptake into virtually any *Streptococcus* opens up the study of the genetics of adherence of these bacteria. The initial aims of these studies will be to mutagenize the genes encoding adhesins.

Transposon-mediated insertional mutagenesis is not a technique that has been used popularly for the study of streptococcal adherence. This is perhaps surprising given the broad host range of the gram-positive conjugative transposons [7] and the development of new vectors which allow isolation of chromosomal sequences flanking transposon insertion [4]. However it is not certain that transposon mutagenesis really offers any advantages over other methods of non-directed mutagenesis to identify adhesins. A major stumbling block is in selection of the appropriate mutants for study. It is eminently clear that adherence of streptococci to many substrates involves multiple adhesin-receptor interactions [23]. Therefore, if adherence-defective mutants are selected these will most likely contain mutations that have pleiotropic effects—multiple phenotypic differences. These various problems in analysis are to some extent overcome by cloning and mutagenesis of genes encoding putative adhesins. This is a most direct, and as it turns out, highly informative, way of establishing both the identities and functions of adhesins.

Isogenic adhesion-defective mutants of streptococci

Data from whole-cell adherence assays, ligand-binding assays of purified cell-surface proteins, and biochemical analyses of adherence-defective mutants, all have provided candidate proteins for adhesins in streptococci. Many genes encoding cell-surface proteins have now been cloned, sequenced and the activities of their products analyzed. It is not pertinent to list all these here, but to consider those genes encoding surface polypeptides that have been mutated in order to determine the roles of their products in adherence. The results of these experiments have not only demonstrated adhesin function but have been more illuminating in showing that structurally closely-related proteins produced by different species of streptococci may have different binding activities.

The first published cloning and insertional inactivation of an oral streptococcal adhesin gene was that involving the SpaP surface antigen of *S. mutans* [37]. Inactivation of the *spaP* gene with a tetracycline-resistance determinant (*tetM*) resulted in mutant cells that were deficient in binding to salivary agglutinin glycoprotein, a previously-identified receptor in parotid saliva for *S. mutans* [12]. Similar experiments with another serotype *c* strain of *S. mutans* in which the PAc protein (96% identical amino acid sequence to SpaP [36]) gene was inactivated by insertion of *ermAM* (an erythromycin-resistance determinant) indicated that PAc$^-$ mutants were less hydrophobic than the wild-type strain and displayed much-reduced binding to experimental salivary pellicle [28]. These data demonstrated the importance of SpaP and PAc (both members of the antigen I/II family of polypeptides produced by many species of oral streptococci [25,36]) to the adherence of *S. mutans* cells.

The purified antigen I/II polypeptide denoted Ssp5 in *S. gordonii* M5 also binds salivary agglutinin glycoprotein, apparently through recognition of sialic acid residues [8]. When the gene encoding surface protein SspA in *S. gordonii* DL1 (corresponding to the Ssp5 protein in strain M5) was insertionally inactivated, mutants were defective in binding agglutinin [27], confirming the *in vitro* binding data. Unlike PAc$^-$ mutants of *S. mutans*, SspA$^-$ mutants of *S. gordonii* were not altered in hydrophobicity or in binding to salivary pellicle. Furthermore, SspA$^-$ mutants were instead deficient in coaggregation with *Actinomyces naeslundii* [27]. These gene inactivation experiments have revealed that binding of *S. gordonii* to pellicle *in vitro* is independent of SspA expression and therefore that antigen I/II polypeptides possibly have different functions in different streptococci.

Studies in which gene cloning and inactivation have been utilized to determine adhesin function are summarized in Table 1. Most significantly, experiments involving insertional inactivation were party to resolving some, but by no means all, of the questions as to the role of cell-surface M proteins in adherence of *S. pyogenes*. M proteins are multifunctional molecules that bind human serum proteins [48]. Inactivation of the structural gene for M protein (*emm 6.1*) in *S. pyogenes* generated M$^-$ mutants that were unaltered in their binding to fibronectin, and unaffected in binding to buccal or tonsillar epithelial cells [5]. However the same M$^-$ mutants were subsequently found to be deficient in adherence to cultured human laryngeal cells [53]. These conflicting results may be explained by suggesting that the human cell types carried different receptors. Alternatively, differences in culturing conditions of the bacteria in the two studies may have altered the relative M protein-requirements for adherence. Protein F on the surface of *S. pyogenes* is an adhesin that binds fibronectin [49]. When a mutation in the *prtF* gene, encoding protein F, was introduced into the *S. pyogenes* chromosome the mutant strain lost ability to adhere to fibronectin and to respiratory epithelial cells [21]. This demonstrates unequivocally that protein F is a major fibronectin-binding adhesin in *S. pyogenes*.

In the mutans group streptococci, cloning and inactivation of the *gtf* genes encoding glucosyltransferases has confirmed the essential role of these glucan-synthesizing enzymes in bacterial adherence and accumulation [35]. Inactivation of the *gtfB* and *gtfC* genes in *S. mutans* abolished the ability of cells to adhere to smooth surfaces *in vitro* [42] (Table 1). On the other hand, inactivation of the *ftf* gene encoding fructosyltransferase [50] or of the *gtfD* gene [19,42] had no effect on adherence.

In the examples cited, mutations have been targeted to genes which encode products that have been previously assigned *in vitro* binding or enzymatic functions. Gene disruption has also allowed identification and characterization of hitherto unknown adhesins. In *S. gordonii*, experiments involving insertional inactivation revealed that a high molecular mass cell-surface polypeptide (approximately 260 kDa) denoted CshA was necessary for cell-surface hydrophobicity and for coaggregation of streptococci with *A. naeslundii* [38]. Further genetic analyses indicated that a second polypeptide (CshB, approximately 245 kDa) highly similar to CshA was also produced [39]. Loss of either of

Table 1 Phenotypic effects of some gene inactivations in streptococci

Streptococcus strain	Inactivated gene(s)	Mature protein product(s) and molecular mass[a]	Cell adherence phenotype	Reference
S. pyogenes JRS4	*prtF*	Surface protein 67 kDa	Deficient binding to fibronectin and to hamster respiratory epithelial cells	[21]
S. pyogenes JRS4	*emm6.1*	Fibrillar M6 surface protein 49 kDa	No effect on adherence to human buccal or tonsillar epithelial cells	[5]
S. pyogenes JRS4	*emm6.1*	Fibrillar M6 surface protein 49 kDa	Deficient adherence to cultured human laryngeal epithelial cells	[53]
S. mutans NG5	*spaP*	Wall-associated protein 166 kDa	Reduced binding to parotid salivary agglutinin glycoprotein	[37]
S. mutans MT8148	*PAc*	Wall-associated protein 163 kDa	Reduced binding to experimental salivary pellicle	[28]
S. mutans GS-5	*wapA*	Wall-associated protein 45 kDa	Reduced sucrose-dependent adherence to smooth surfaces	[47]
S. mutans LT11	*AgA (wapA)*	Wall-associated protein 45 kDa	No effect on adherence to experimental salivary pellicle	[22]
S. mutans LT11	*AgB*	Wall-associated protein *c* 185 kDa	Reduced adherence to experimental salivary pellicle, increased adherence to glucan pellicle	[22]
S. mutans V403	*gtfB, gtfC*	Glucosyltransferases 162, 149 kDa	Abolished sucrose-dependent adherence to smooth surfaces	[42]
S. mutans V403	*ftf*	Fructosyltransferase 84 kDa	No effect on adhesion	[50]
S. gordonii DL1	*sarA*	Peptide binding protein *c* 76 kDa	Deficient binding to serum proteins and coaggregation with *A. naeslundii*	[26]
S. gordonii DL1	*sspA*	Cell-surface protein *c* 200 kDa	Deficient adherence to salivary agglutinin and to *A. naeslundii*	[27]
S. gordonii DL1	*cshA*	Wall-associated protein 259 kDa	Deficient binding to *A. naeslundii*	[38]
S. gordonii DL1	*cshB*	Cell-surface protein *c* 245 kDa	Reduced binding to *A. naeslundii*	[39]

[a]Calculated from inferred amino acid sequence or estimated from SDS-polyacrylamide gel electrophoresis (indicated by *c*)

these polypeptides from the cell surface through gene inactivation affected coaggregation of streptococcal cells with *A. naeslundii* but not their adherence to experimental salivary pellicle [39].

Pleiotropic effects of gene disruption

Evidence that a polypeptide is an adhesin includes the following: 1) antibodies to the polypeptide block adhesion of cells; 2) adhesive strains express the polypeptide on their cell surface; 3) the purified polypeptide blocks cell adhesion; 4) inactivation of the gene encoding the polypeptide results in adherence deficiency. In practice frequently one or more of these effects cannot be demonstrated and, especially with single gene inactivations, interpretations of adhesin function are not always clear. For example a nil effect on phenotype following inactivation of a gene encoding a putative adhesin is possible. This could arise because loss of the product is genetically or metabolically compensated for by the cells. Such effects are encountered with gene knockouts in eukaryotic systems [11]. Alternatively, pleiotropic effects may arise when the inactivated gene concerned normally encodes a polypeptide that is either a crucial component in the assembly of the cell wall layers, or is participant in a genetic regulatory system.

Two examples of pleiotropic effects following gene inactivations in streptococci are as follows. The first concerns the inactivation of the *sarA* gene in *S. gordonii* which encodes a protein SarA [24] similar to the oligopeptide-binding protein AmiA in *S. pneumoniae* [1]. Knockout of the *sarA* gene resulted in loss of SarA polypeptide from the cell surface, alteration in amount and exposure of several other cell wall proteins, and deficiencies in cell binding to salivary and serum components and to *A. naeslundii* [26]. These effects are not yet fully explained but one possibility is that *sarA* is part of a regulatory network of genes involved in determining cell-surface properties. The second example of pleiotropic effects following gene inactivation comes from work with the genes encoding antigens A and B of *S. mutans*. Inactivation of the gene encoding antigen B (an antigen I/II polypeptide similar to SpaP and PAc) caused a defect in adherence of *S. mutans* to salivary pellicle [22] as expected from previous studies (Table 1) as well as a reduction in amount of antigen A. Inactivation of the gene (*wapA*) encoding antigen A had no effect on adherence but did affect the ability of cells to be aggregated by dextrans [22, 47]. Interestingly, loss of antigen A (and to a lesser extent antigen B) from the cell surface resulted in release of lipoteichoic acid from the cells [22]. A loss of polysaccharide from the cell surface of *S. gordonii* was apparent also following disruption of the *cshA* gene encoding a coaggregation adhesin [38]. Thus certain wall-associated polypeptides are necessary for assembly of other proteins into wall layers and for retention of cell-surface polysaccharides.

Disruption of genes encoding cell-surface polypeptides may therefore have a variety of effects on the structure of the cell wall layers. These possibilities are considered in Figure 2. Loss of a 'scaffolding' protein might cause release of cell surface polysaccharides which are known to act as receptors in some interbacterial coaggregations [33] and which may assist in adherence of streptococci to epithelial cells [23]. Another possibility is that the loss of a polypeptide following gene disruption leads to unmasking of an underlying or secondary adhesin (Figure 2). This kind of effect has been suggested to account for the unexpected coaggregation properties of mutants of *S. oralis* [30]. Clearly, before assigning adherence function to a protein on the basis of gene disruption experiments, careful consideration of possible pleiotropic effects must be taken into account.

Compensatory effects resulting from gene disruption

An aforementioned possibility is that inactivation of the gene encoding a putative adhesin has a benign effect. One obvious explanation for this is that the protein does not have an adherence function. However, other possible explanations are that the loss of the protein might lead to up-regulation of compensatory pathways, or that the protein is one of two or more proteins with duplicated functions. Recently, evidence has been obtained that compensation effects may occur as a result of inactivation of genes encoding surface proteins in streptococci. CshA and CshB polypeptides are coaggregation adhesins and are expression products of separate genetic loci in *S. gordonii*. The CshA polypeptide is held at the cell surface via a specialized amino acid sequence at the carboxyl-terminal end of the polypeptide [38]. CshA and CshB are normally present on the cell surface in approximately similar amounts. When expression of *cshA* was disrupted by insertion of an antibiotic resistance marker at the carboxyl-terminal coding end of the gene, a truncated form of CshA missing the cell-wall anchor sequence was synthesized by the cells. Accordingly CshA polypeptide was not able to be held at the cell surface and was lost into the culture medium [38]. These cells were defective in coaggregation with *A. naeslundii* although they carried seemingly normal amounts of CshB on their surface [39]. However, when *cshA* was inactivated by insertion of an antibiotic resistance marker into the amino-terminal coding region of the gene, CshA polypeptide was no longer produced in any detectable form. These cells were still coaggregation-deficient but expressed vastly increased amounts of CshB polypeptide on their surfaces [39]. Although not yet formally proved, it seems likely that in the absence of CshA polypeptide, up-regulation of *cshB* expression occurred. Clearly though, increased amounts of CshB polypeptide did not wholly compensate for the coaggregation deficiency caused by loss of CshA.

Hidden complexities

It is now firmly established that streptococcal cells possess multiple adhesins and that these contribute to their success in colonizing animal hosts. Biochemical and immunological experiments have revealed that these adhesins are diverse in structure and binding specificity. Gene cloning and inactivation experiments have shown that streptococcal adhesins can have more than one adhesive function and that adhesin genes can be duplicated. Gene inactivation studies have revealed hidden complexities. These include the possible networking of adhesin gene expression (exemplified by preliminary studies of *cshA* and *cshB* gene expression in *S.*

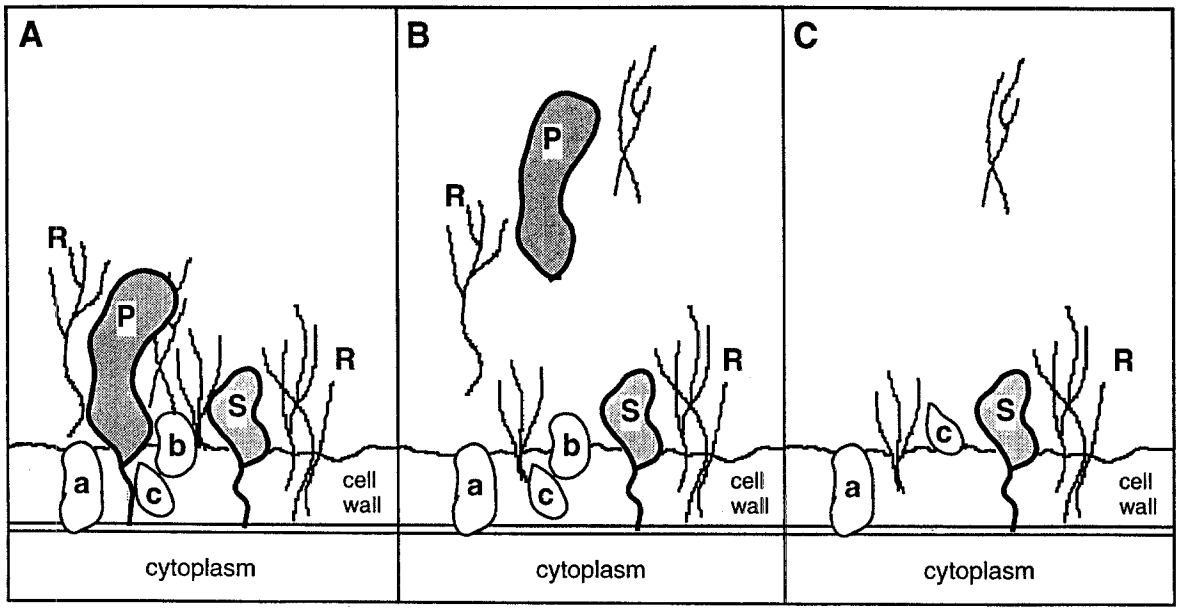

Figure 2 Cross-sectional diagram of the oral streptococcal cell surface layers showing possible structural and functional effects of disrupting expression of the gene encoding a cell-wall polypeptide. In (A), a cell-wall-anchored polypeptide adhesin (denoted P) acts also as a scaffold for presentation of cell-surface polysaccharides (denoted R). A secondary adhesin (S) is masked by cell-surface components. Polypeptides denoted (a), (b) and (c) are accessory proteins associated perhaps with secretion and/or function of (P). In (B), loss of (P) from the cell surface because of mutation in the protein anchoring region (see text) results in release of polysaccharides (R), exposure of adhesin (S) and modification of cell adherence properties. Accessory polypeptides remain ligated within the structure of the cell wall layers. In (C), synthesis of polypeptide adhesin (P) is abolished. Polysaccharides are released and (S) is unmasked as in (B), but in the absence of (P) the accessory proteins are released or degraded resulting in further modifications of cell-surface structure. Evidence for these effects occurring is described in the text

gordonii) and the dependence of cell wall assembly on the presence of certain structural polypeptides.

In *Escherichia coli* and some other *Enterobacteriacae*, adhesins are assembled into complexes on the cell surface and may be associated with fimbrial or non-fimbrial structures. The components of these adhesin complexes are encoded by gene clusters [46]. In the oral streptococci it is not apparent yet whether or not the adhesins are assembled into complexes, however the evidence so far, albeit limited, suggests that the genes encoding the cell-surface adhesins and structural proteins are not clustered. Nonetheless, the tightly-regulated expression of these genes and the proper assembly of their products into the cell wall layers is crucial to the presentation of adhesins necessary for colonization. It is thus proving an immense challenge to define the mechanisms of streptococcal adherence at the molecular level.

Acknowledgements

I thank colleagues in the laboratory especially R McNab for helpful discussions. Research of the author is supported by the Wellcome Trust, UK, the Health Research Council of New Zealand and the New Zealand Dental Research Foundation Trust Board.

References

1 Alloing G, P de Philip and J-P Claverys. 1994. Three highly homologous membrane-bound lipoproteins participate in oligopeptide transport by the Ami system of the gram-positive *Streptococcus pneumoniae*. J Mol Biol 241: 44–58.
2 Banas JA, RRB Russell and JJ Ferretti. 1990. Sequence analysis of the gene for the glucan-binding protein of *Streptococcus mutans* Ingbritt. Infect Immun 58: 667–673.
3 Busscher HJ, MM Cowan and HC van der Mei. 1992. On the relative importance of specific and non-specific approaches to oral microbial adhesion. FEMS Microbiol Rev 88: 199–210.
4 Camilli A, DA Portnoy and P Youngman. 1990. Insertional mutagenesis of *Listeria monocytogenes* with a novel Tn*917* derivative that allows direct cloning of DNA flanking transposon insertions. J Bacteriol 172: 3738–3744.
5 Caparon MG, DS Stephens, A Olsén and JR Scott. 1991. Role of M protein in adherence of group A streptococci. Infect Immun 59: 1811–1817.
6 Cleary P and D Retnoningrum. 1994. Group A streptococcal immunoglobulin-binding proteins: adhesins, molecular mimicry or sensory proteins? Trends Microbiol 2: 131–136.
7 Clewell DB and SE Flannagan. 1993. The conjugative transposons of gram-positive bacteria. In: Bacterial Conjugation (Clewell DB, ed), pp 369–393, Plenum Press, New York.
8 Demuth DR, EE Golub and D Malamud. 1990. Streptococcal-host interactions. Structural and functional analysis of a *Streptococcus sanguis* receptor for a human salivary glycoprotein. J Biol Chem 265: 7120–7126.
9 Doyle RJ, M Rosenberg and D Drake. 1990. Hydrophobicity of oral bacteria. In: Microbial Cell Surface Hydrophobicity (Doyle RJ and M Rosenberg, eds), pp 387–411, American Society for Microbiology, Washington, DC.
10 Elder BL and P Fives-Taylor. 1986. Characterization of monoclonal antibodies specific for adhesion: isolation of an adhesin of *Streptococcus sanguis* FW213. Infect Immun 54: 421–427.
11 Erickson HP. 1993. Gene knockouts of c-*src*, transforming growth factor β1, and tenascin suggests superfluous, nonfunctional expression of proteins. J Cell Biol 120: 1079–1081.
12 Erickson T and J Rundegren. 1983. Characterization of a salivary agglutinin reacting with a serotype *c* strain of *Streptococcus mutans*. Eur J Biochem 133: 255–261.
13 Fenno JC, A Shaikh and P Fives-Taylor. 1993. Characterization of allelic replacement in *Streptococcus parasanguis*: transformation and homologous recombination in a 'nontransformable' streptococcus. Gene 130: 81–90.

14 Frandsen EVG, V Pedrazzoli and M Kilian. 1991. Ecology of viridans streptococci in the oral cavity and pharynx. Oral Microbiol Immunol 6: 129–133.
15 Fives-Taylor PM and DW Thompson. 1985. Surface properties of *Streptococcus sanguis* FW213 mutants nonadherent to saliva-coated hydroxyapatite. Infect Immun 47: 752–759.
16 Gibbons RJ, I Etherden and Z Skobe. 1983. Association of fimbriae with the hydrophobicity of *Streptococcus sanguis* FC-1 and adherence to salivary pellicles. Infect Immun 41: 414–417.
17 Gibbons RJ, DI Hay and DH Schlesinger. 1991. Delineation of a segment of adsorbed salivary acidic proline-rich proteins which promotes adhesion of *Streptococcus gordonii* to apatitic surfaces. Infect Immun 59: 2948–2954.
18 Hamada S and H Slade. 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev 44: 331–384.
19 Hanada N and HK Kuramitsu. 1989. Isolation and characterization of the *Streptococcus mutans gtfD* gene, coding for primer-dependent soluble glucan synthesis. Infect Immun 57: 2079–2085.
20 Handley PS. 1990. Structure, composition and functions of surface structures on oral bacteria. Biofouling 2: 239–264.
21 Hanski E and M Caparon. 1992. Protein F, a fibronectin-binding protein, is an adhesin of the group A streptococcus *Streptococcus pyogenes*. Proc Natl Acad Sci USA 89: 6172–6176.
22 Harrington DJ and RRB Russell. 1993. Multiple changes in cell wall antigens of isogenic mutants of *Streptococcus mutans*. J Bacteriol 175: 5925–5933.
23 Hasty DL, I Ofek, HS Courtney and RJ Doyle. 1992. Multiple adhesins of streptococci. Infect Immun 60: 2147–2152.
24 Jenkinson HF. 1992. Adherence, coaggregation, and hydrophobicity of *Streptococcus gordonii* associated with expression of cell surface lipoproteins. Infect Immun 60: 1225–1228.
25 Jenkinson HF. 1994. Cell surface protein receptors in oral streptococci. FEMS Microbiol Lett 121: 133–140.
26 Jenkinson HF and RA Easingwood. 1990. Insertional inactivation of the gene encoding a 76-kilodalton cell surface polypeptide in *Streptococcus gordonii* Challis has a pleiotropic effect on cell surface composition and properties. Infect Immun 58: 3689–3697.
27 Jenkinson HF, SD Terry, R McNab and GW Tannock. 1993. Inactivation of the gene encoding surface protein SspA in *Streptococcus gordonii* DL1 affects cell interactions with human salivary agglutinin and oral actinomyces. Infect Immun 61: 3199–3208.
28 Koga T, N Okahashi, I Takahashi, T Kanamoto, H Asakawa and M Iwaki. 1990. Surface hydrophobicity, adherence, and aggregation of cell surface protein antigen mutants of *Streptococcus mutans* serotype *c*. Infect Immun 58: 289–296.
29 Kolenbrander PE. 1982. Isolation and characterization of coaggregation-defective mutants of *Actinomyces viscosus, Actinomyces naeslundii*, and *Streptococcus sanguis*. Infect Immun 37: 1200–1208.
30 Kolenbrander PE and RN Andersen. 1985. Use of coaggregation-defective mutants to study the relationship of cell-to-cell interactions and oral microbial ecology. In: Molecular Basis of Oral Microbial Adhesion (Mergenhagen SE and B Rosan, eds), pp 164–171, American Society for Microbiology, Washington, DC.
31 Kolenbrander PE and RN Andersen. 1990. Characterization of *Streptococcus gordonii (S. sanguis)* PK488 adhesin-mediated coaggregation with *Actinomyces naeslundii* PK606. Infect Immun 58: 3064–3072.
32 Kolenbrander PE, RN Andersen and N Ganeshkumar. 1994. Nucleotide sequence of the *Streptococcus gordonii* PK488 coaggregation adhesin gene, *scaA*, and ATP-binding cassette. Infect Immun 62: 4469–4480.
33 Kolenbrander PE, N Ganeshkumar, FJ Cassels and CV Hughes. 1993. Coaggregation: specific adherence among human oral plaque bacteria. FASEB J 7: 406–413.
34 Kolenbrander PE and J London. 1993. Adhere today, here tomorrow: oral bacterial adherence. J Bacteriol 175: 3247–3252.
35 Kuramitsu HK. 1993. Virulence factors of mutans streptococci: role of molecular genetics. Crit Rev Oral Biol Med 4: 159–176.
36 LaPolla RJ, JA Haron, CG Kelly, WR Taylor, C Bohart, M Hendricks, J Pyati, RT Graff, JK-C Ma and T Lehner. 1991. Sequence and structural analysis of surface protein antigen I/II (SpaA) of *Streptococcus sobrinus*. Infect Immun 59: 2677–2685.
37 Lee SF, A Progulske-Fox, GW Erdos, DA Piacentini, GY Ayakawa, PJ Crowley and AS Bleiweis. 1989. Construction and characterization of isogenic mutants of *Streptococcus mutans* deficient in major surface protein antigen P1 (I/II). Infect Immun 57: 3306–3313.
38 McNab R and HF Jenkinson. 1992. Gene disruption identifies a 290 kDa cell-surface polypeptide conferring hydrophobicity and coaggregation properties in *Streptococcus gordonii*. Mol Microbiol 6: 2939–2949.
39 McNab R, HF Jenkinson, DM Loach and GW Tannock. 1994. Cell-surface-associated polypeptides CshA and CshB of high molecular mass are colonization determinants in the oral bacterium *Streptococcus gordonii*. Mol Microbiol 14: 743–754.
40 Morris JE, N Ganeshkumar and BC McBride. 1985. Cell surface components of *Streptococcus sanguis*: relationship to aggregation, adherence, and hydrophobicity. J Bacteriol 164: 255–262.
41 Morris JE and BC McBride. 1984. Adherence of *Streptococcus sanguis* to saliva-coated hydroxyapatite: evidence for two binding sites. Infect Immun 43: 656–663.
42 Munro C, SM Michalek and FL Macrina. 1991. Cariogenicity of *Streptococcus mutans* V403 glucosyltransferase and frutosyltransferase mutants constructed by allelic exchange. Infect Immun 59: 2316–2323.
43 Murray PA, A Prakobphol, T Lee, CI Hoover and SJ Fisher. 1992. Adherence of oral streptococci to salivary glycoproteins. Infect Immun 60: 31–38.
44 Murchison H, S Larrimore and R Curtiss III. 1981. Isolation and characterization of *Streptococcus mutans* mutants defective in adherence and aggregation. Infect Immun 34: 1044–1055.
45 Nesbitt WE, RJ Doyle, KG Taylor, RH Staat and RR Arnold. 1982. Positive cooperativity in the binding of *Streptococcus sanguis* to hydroxylapatite. Infect Immun 35: 157–165.
46 Ofek I and RJ Doyle. 1994. Bacterial Adhesion to Cells and Tissues. Chapman & Hall, New York.
47 Qian H and ML Dao. 1993. Inactivation of the *Streptococcus mutans* wall-associated protein A gene (*wapA*) results in a decrease in sucrose-dependent adherence and aggregation. Infect Immun 61: 5021–5028.
48 Retnoningrum DS and PP Cleary. 1994. M12 protein from *Streptococcus pyogenes* is a receptor for immunoglobulin G3 and human albumin. Infect Immun 62: 2387–2394.
49 Sela S, A Aviv, A Tovi, I Burnstein, MG Caparon and E Hanski. 1993. Protein F: an adhesin of *Streptococcus pyogenes* binds fibronectin via two distinct domains. Mol Microbiol 10: 1049–1055.
50 Schroeder VA, SM Michalek and FL Macrina. 1989. Biochemical characterization and evaluation of virulence of a fructosyltransferase-deficient mutant of *Streptococcus mutans* V403. Infect Immun 57: 3560–3569.
51 Trevors JT, BM Chassy, WJ Dower and HP Blaschek. 1992. Electrotransformation of bacteria by plasmid DNA. In: Guide to Electroporation and Electrofusion (Chang DC, BM Chassy, JA Saunders and AE Sowers, eds), pp 265–290, Academic Press, London.
52 van Houte J. 1982. Colonization mechanisms involved in the development of the oral flora. In: Host-Parasite interactions in Periodontal Diseases (Genco RJ and SE Mergenhagen, eds), pp 86–97, American Society for Microbiology, Washington, DC.
53 Wang J-R and MW Stinson. 1994. M protein mediates streptococcal adhesion to HEp-2 cells. Infect Immun 62: 442–448.
54 Weerkamp AH, HC van der Mei and RSB Liem. 1986. Structural properties of fibrillar proteins isolated from the cell surface and cytoplasm of *Streptococcus salivarius* (K^+) cells and nonadhesive mutants. J Bacteriol 165: 756–752.
55 Weerkamp AH, PS Handley, A Baars and JW Slot. 1986. Negative staining and immunoelectron microscopy of adhesion-deficient mutants of *Streptococcus salivarius* reveal that the adhesive protein antigens are separate classes of cell surface fibril. J Bacteriol 165: 746–755.