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Role of the *Streptococcus mutans gtf* Genes in Caries Induction in the Specific-Pathogen-Free Rat Model

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The role of each of the *Streptococcus mutans gtf* genes coding for glucan synthesis in cariogenesis was evaluated by using strain UA130 in the specific-pathogen-free (SPF) rat model system. Mutants defective in either or both of the genes required for insoluble glucan synthesis, the *gtfB* and *gtfC* genes, exhibited markedly reduced levels of smooth-surface carious lesions relative to that of the parental organism. Likewise, the mutant defective in the *gtfD* gene coding for the glucosyltransferase-S enzyme synthesizing water-soluble glucans also produced significantly fewer smooth-surface lesions than strain UA130. None of these mutations markedly altered the rate of sulcal caries induction relative to that of the parental organism. In addition, a mutant of strain UA130 defective in the *gtfA* gene was reexamined in the SPF rat model. In contrast to previous results from a gnotobiotic rat system, these mutants also induced significantly fewer smooth-surface carious lesions compared with that by strain UA130. These results suggest that all four genes are important for smooth-surface caries formation. Furthermore, these results are discussed relative to the differences in the diets utilized in the SPF and gnotobiotic rat model systems for assessing the virulence factors of *S. mutans*.

The ability of members of the mutans streptococci to induce dental caries is derived in part from their capacity to synthesize water-insoluble glucans (7). This cariogenic property is dependent upon the expression of extracellular glucosyltransferases (GTFs) by these organisms. Most strains of *Streptococcus mutans*, the principal etiological agent of human dental caries, harbor three distinct *gtf* genes expressing GTF activity (1, 5, 8, 9). Two of these genes, *gtfB* and *gtfC*, are tandemly arranged on the chromosomes of these organisms and express enzymes GTF-I and GTF-SI, respectively, synthesizing primarily water-insoluble glucans (1, 8). The third gene, *gtfD*, codes for the GTF-S enzyme synthesizing water-soluble glucans (9).

Despite the extensive literature describing the characterization of GTFs and their corresponding genes which has developed over the past two decades (17), the precise role of each of the GTFs in sucrose-dependent colonization of teeth has yet to be defined. Earlier experiments utilizing chemically induced mutants of mutans streptococci and rodent caries models suggested that insoluble glucan synthesis was important for smooth-surface caries formation (7). However, since the nature of these mutations was not defined, the interpretation of these results was somewhat equivocal. The construction of specifically defined mutants on the basis of the isolation of *S. mutans* genes allowed for more rigorous testing of the role of specific gene products in cariogenesis (14).

The results of in vitro assays have demonstrated that both the *gtfB* and *gtfC* gene products are required for sucrose-dependent colonization of hard surfaces by *S. mutans* GS5, while the *gtfD* gene is dispensable in this regard (1, 8, 9). Recently, experiments involving implantation of *S. mutans* strains specifically defective in insoluble glucan synthesis into gnotobiotic rats have demonstrated reduced smooth-surface caries induction (18). However, the reductions observed appeared to be much less than might be predicted on the basis of the results of in vitro experiments and earlier animal experiments employing chemically induced glucan synthesis-defective mutants of mutans streptococci (7). In addition, more recent results utilizing a naturally occurring strain of *S. mutans*, UA101, containing only a single *gtf* gene coding for a hybrid GTF synthesizing insoluble glucan suggested that the gnotobiotic rat model system utilizing diet 305 (UAB model), which contains 5% sucrose, may not be optimal for investigating the role of glucan synthesis in dental caries formation (28). Therefore, the present investigation was designed to utilize *S. mutans* mutants defective in each of the three *gtf* genes in a more glucan-dependent rat model system employing specific-pathogen-free (SPF) rats fed diet 2000, containing 56% sucrose (UR model). In addition, the role of the *gtfA* gene coding for sucrose phosphorylase activity (21) in caries induction was reexamined, since previous results (2) utilizing the UAB model indicated that *S. mutans* mutants defective in this gene produced wild-type levels of smooth-surface caries.

MATERIALS AND METHODS

Bacterial strains. *S. mutans* UA130 (2) and its *gtf* mutants (Table 1) were maintained and grown as previously described (1), while *Escherichia coli* strains were grown in the presence of the appropriate antibiotics in Luria-Bertani broth (1).

DNA manipulations. Chromosomal and DNA fragment isolation, restriction endonuclease cleavage, and DNA ligation were carried out as previously outlined (1). Insertional inactivation of the *S. mutans* UA130 *gtf* genes was carried out following transformation of strain UA130 with *S. mutans* GS5 *gtfB*, and *gtfD* DNA fragments containing intragenic erythromycin resistance (Em^r) and tetracycline resistance

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TABLE 1. Strains utilized in this study

Strain	Relevant property	Source
S. mutans		
UA130S	UA130 maintained in San Antonio, Tex.	P. Caufield[a]
UA130S(pTH10)	UA130S containing pTH10 (Em^r)	This study
UA130R	UA130 maintained in Rochester, N.Y.	P. Caufield[a]
GS5B	GS5 with inactive *gtfB* (Em^r)	1
GS5DD	GS5 with inactive *gtfD* (Tet^r)	9
UA130B	UA130S with inactive *gtfB* (Em^r)	This study
UA130C	UA130S with inactive *gtfC* (Em^r)	This study
UA130BC	UA130S with inactive *gtfB* and *gtfC* (Em^r)	This study
UA130DD	UA130S with inactive *gtfD* (Tet^r)	This study
UAB747	UA130 with inactive *gtfA* (Em^r)	2
UA130RR	UA130R isolated from SPF rats	This study
UA130SR	UA130(pTH10) isolated from SPF rats (Em^s)	This study
S. sobrinus 6715	Highly cariogenic in rats	4

[a] University of Alabama, Birmingham.

(Tet^r) genes (1, 9) (Fig. 1 and 2). Transformants generated following competence development (19) were identified on mitis salivarius agar plates containing either erythromycin (10 μg/ml) or tetracycline (4 μg/ml).

Gel electrophoresis. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (28). For detecting the GTF proteins, Western blotting with anti-GTF-I (15) or anti-GTF-S (provided by K. Fukushima, Nihon University, Matsudo, Japan) serum was carried out as recently described (28). DNA fragments were analyzed following electrophoresis on agarose gels (1).

Enzyme assays. GTF activities were determined with [glucose-U-^{14}C]sucrose (DuPont Co., Boston, Mass.) in the presence and absence of primer dextran T10 as previously described (13).

Virulence testing in the UR SPF rat caries model. SPF Sprague-Dawley rats (Kingston Facility, Charles River Breeding Laboratories) were utilized to test for caries induction as previously described (4). Animals (approximately 21 days old) were divided into groups of 12 animals each. All rats were fed diet 2000 containing 56% sucrose as well as drinking water containing 10% sucrose. After 5 weeks, the animals were killed by CO_2 asphyxiation. Verification of successful implantation of each strain was carried out by examining the GTF profiles of the recovered *S. mutans* strains following Western blotting (immunoblotting) with appropriate sera. Colonization as well as caries incidence was determined following analysis of variance as previously described (4).

RESULTS

Construction of mutants defective in glucan synthesis. Previous results from one of our laboratories (1, 8) have suggested that *S. mutans* GS5 mutants defective in the *gtfB* or *gtfC* genes do not colonize hard surfaces in vitro in the presence of sucrose. However, since strain GS5 has been demonstrated to exhibit low cariogenicity in rat model systems (6), it was necessary to construct *gtf* mutants in another cariogenic *S. mutans* strain, UA130 (2), for testing in vivo. By using insertionally inactivated copies of the GS5 *gtfB* and *gtfD* genes, it was convenient to construct UA130S mutants defective in each gene (Fig. 1 and 2). In addition, because of the high sequence homology of the *gtfB* and *gtfC* genes (25), it was also possible to isolate mutants defective in both genes as well as in each gene following homologous recombination (Fig. 1).

An examination of the GTF activities of the culture supernatant fluids as well as the cell-associated activities of the parental strain and the mutants verified the presence of the expected mutations (Table 2). For each strain, the majority of the GTF activity relative to the cell-associated activity was present in the culture fluids. The mutant defective in the *gtfB* gene, UA130B, synthesized wild-type levels of soluble glucan but exhibited only 69% of the wild-type capacity for insoluble glucan formation. Likewise, mutant UA130C with an insertionally inactivated *gtfC* gene also synthesized normal levels of soluble glucan and 84% of the parental levels of insoluble glucan. Therefore, as with strain GS5 (1), the *gtfB* product, GTF-I, appears to be more responsible for insoluble glucan synthesis than the *gtfC* gene product GTF-SI in strain UA130. Furthermore, the double mutant, UA130BC, was capable of normal synthesis of soluble glucan but insignificant production of insoluble glucan.

The mutant defective in primer-dependent soluble glucan synthesis following insertional inactivation of the *gtfD* gene, UA130DD, synthesized only very low levels of soluble glucan but near-normal levels of insoluble glucan in the presence of primer dextran T10 (Table 2). However, in the absence of primer the mutant produced approximately half of the level of insoluble glucan relative to that produced by the parental organism. This latter result suggested that some of the soluble glucan synthesized by the *gtfD* gene product, GTF-S, may be utilized for insoluble glucan synthesis as suggested by earlier investigations of mutans streptococci (11, 16).

The mutations were confirmed following Southern blot analysis of the chromosomal DNA of the mutants with the respective *gtf* gene probes (data not shown). Further confirmation of the nature of the mutations was obtained following Western blotting with anti-GTF-I and anti-GTF-S sera (Fig. 3). Because of the extensive amino acid homology between the GTF-I and GTF-SI enzymes (25), anti-GTF-I antibody detected two prominent bands in the culture fluids of UA130 (Fig. 3A, lane 2). However, the mutant defective in the *gtfB* gene (Fig. 3A, lane 3) exhibited only the lower-molecular-weight band, while the *gtfC* mutant (Fig. 3A, lane 1) showed a strong higher-molecular-weight band and a less intense

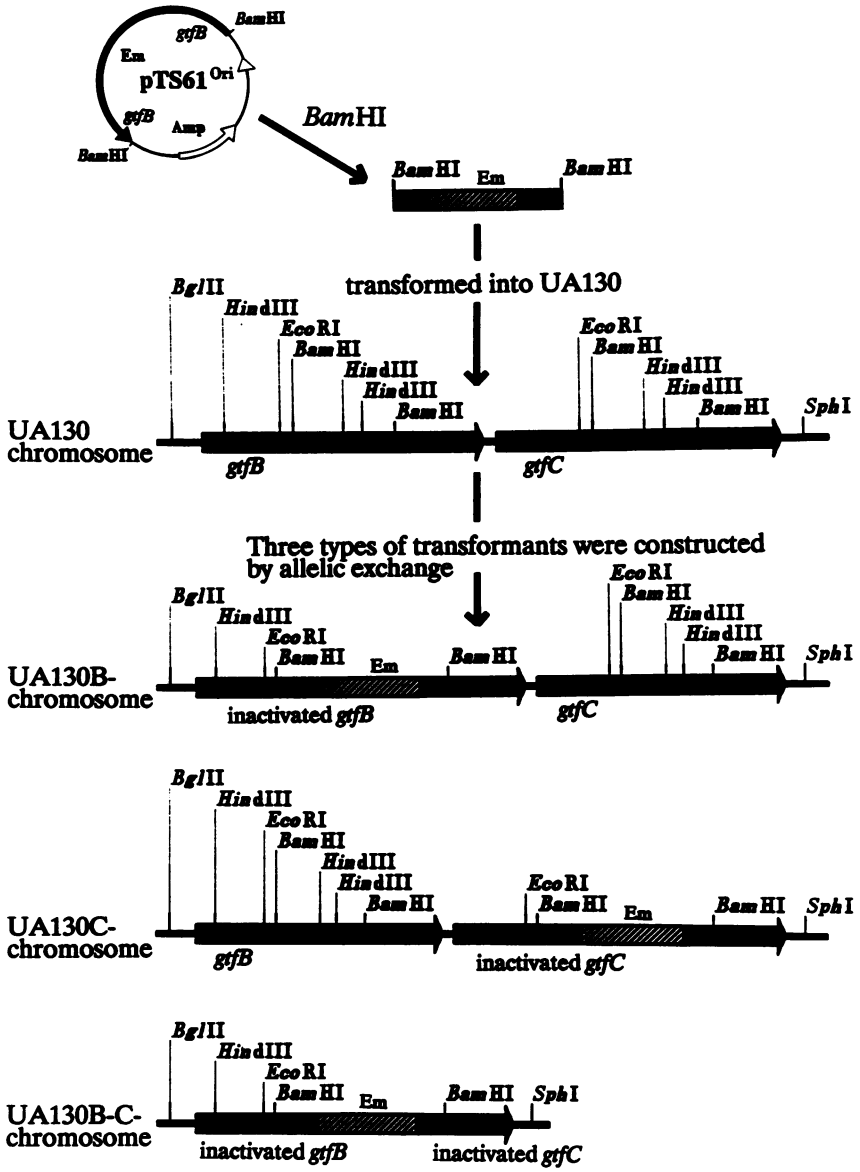

FIG. 1. Insertional inactivation of the *gtfB* and *gtfC* genes of strain UA130S. Plasmid pTS61 (1) containing an Em^r casette inserted into the *gtfB* gene from strain GS5 was utilized as the source of the fragment involved in allelic exchange.

low-molecular-weight band which would correspond to the truncated *gtfC* gene product.

Furthermore, neither of the major bands was detected in blots involving the culture supernatant fluids or cell-associated proteins from the mutant defective in both genes (Fig. 3B). These results were consistent with the predicted sizes of the two enzymes on the basis of nucleotide sequencing, which indicated that the GTF-I enzyme is slightly larger than the GTF-SI enzyme (25). Inactivation of the *gtfD* gene resulted in the disappearance of the positive band detected with anti-GTF-S serum (Fig. 3C). A weak lower-molecular-weight positive band was detected in culture fluids of the mutant which corresponds to the truncated GTF-S enzyme.

Caries induction by UA130 and its *gtf* mutants. Previous results utilizing *S. mutans* mutants defective in glucan synthesis have demonstrated a moderate reduction in smooth-surface caries in the UAB gnotobiotic rat model (18). In addition, since more recent results have suggested that this system may not be as sensitive for assessing the role of glucan synthesis in smooth-surface caries as the UR SPF model system (28), it was therefore essential to test each of the *gtf* mutants constructed in the present study in an animal caries system which was highly dependent upon glucan synthesis. Furthermore, since UA130 mutants defective in the *gtfA* gene coding for sucrose phosphorylase (21) exhibited wild-type levels of smooth-surface caries in the UAB model system (2), it was also of interest to reexamine this mutant in the UR model.

Implantation of parental strain UA130R into rats fed diet 2000 led to high rates of both sulcal and smooth-surface caries relative to those induced by cariogenic *Streptococcus sobrinus* 6715 (Table 3). However, parental strain UA130S harboring plasmid pTH10, a 5.0-kb *E. coli*-streptococcal shuttle plasmid containing the pC194 *ori* (10) expressing erythromycin resistance, exhibited high rates of sulcal caries but only moderate levels of smooth-surface caries. This

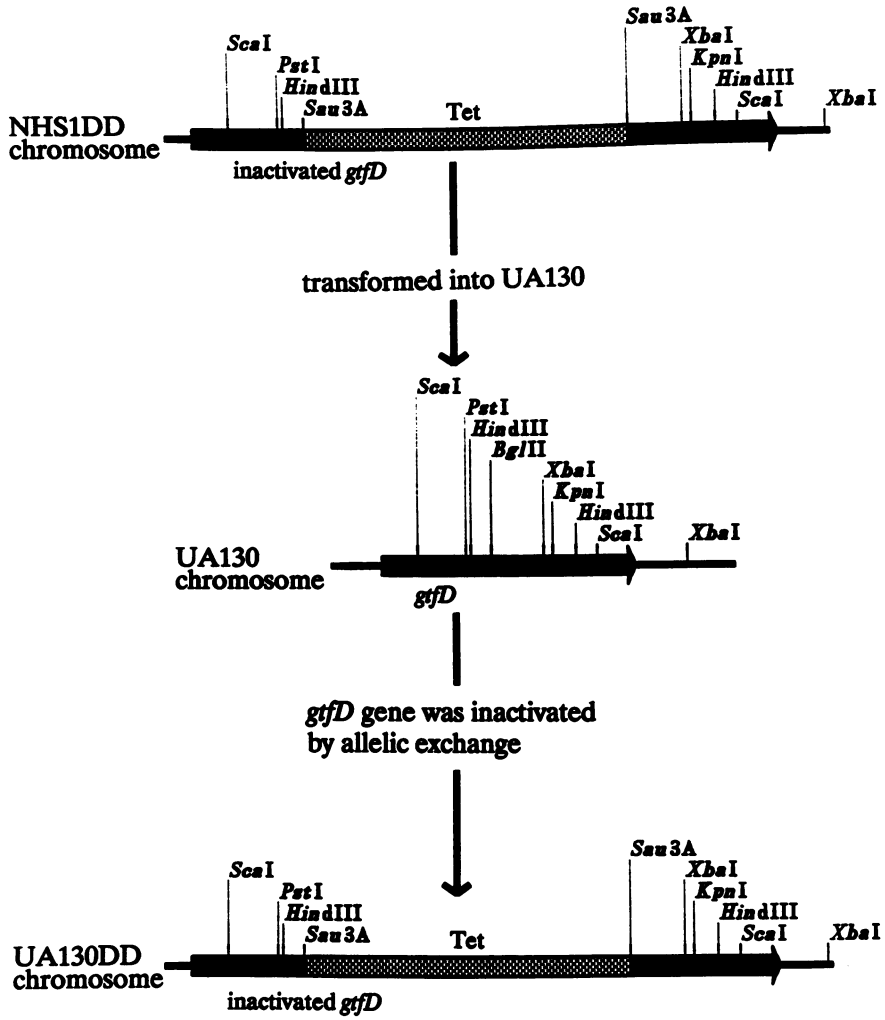

FIG. 2. Insertional inactivation of the *gtfD* gene of strain UA130S by allelic exchange. Chromosomal DNA from GS5DD was utilized to transform UA130S to Tet^r.

strain was utilized in order to obviate any potential effects of the expression of the Em^r gene on the results, since all of the mutants expressed this same gene. Examination of the UA130S(pTH10) colonies recovered following the termination of the experiment revealed that many of the isolates had lost the plasmid in the absence of antibiotic selection pressure. This observation suggested that the proper control for the *gtf* mutants in experiment 1 (Table 3) should be UA130R and not UA130S(pTH10).

Mutants defective in one (UA130B and UA130C) or both (UA130BC) of the *gtf* genes responsible for insoluble glucan synthesis exhibited marked reductions in smooth-surface caries relative to that from parental UA130 strains (Table 3, experiment 1). In addition, these mutants also displayed moderate reductions in sulcal caries. The reduction in the smooth-surface caries rates induced by the mutants were not reflected in the recovery of mutans streptococci from the teeth of the infected animals. Since specific sites on the teeth were not sampled for recovery, these numbers may not reflect the colonization of the *S. mutans* mutants on the smooth surfaces of teeth. In addition, the presence of insoluble glucan will influence the dispersion of plaque samples for quantitation of bacteria despite sonication of the samples.

UA130 mutant UAB747 (*gtfA*) also exhibited significantly fewer smooth-surface carious lesions than did the parental strain (Table 3, experiment 1). However, sulcal caries induction was not significantly affected by the mutation relative to that of UA130R. Since this same mutant induced wild-type levels of smooth-surface caries in the UAB model system (2), these results further indicate a difference in the utilization of the UAB and UR rat model systems for assessing the role of specific gene products in caries induction.

In order to verify that the lower rate of smooth-surface caries induction associated with strain UA130S(pTH10) relative to UA130R was not the result of a plasmid-independent defect in the former strain, both strains were reisolated from the animals utilized in the experiments whose results are shown in Table 3, experiment 1, and reinfected into SPF rats (Table 3, experiment 2). The former strain naturally cured of the plasmid during the course of the initial implantation was designated UA130SR and produced even higher rates of smooth-surface caries than did UA130RR, which is a reisolate of strain UA130R. These results indicated that the lower rate of smooth-surface caries observed in experiment 1 for UA130S(pTH10) (Table 3) was not the result of an inherent caries defect in the strain lacking the plasmid.

Previous results have also indicated that mutants of *S.*

TABLE 2. GTF activities of UA130 and its *gtf* mutants

Strain	Dextran T10	Synthetic activity (cpm/OD_{550})[a]			
		Insoluble glucan		Soluble glucan	
		Supernatant	Cell	Supernatant	Cell
UA130S	+	1,011	216	2,896	411
	−	1,182	207	432	182
UA130B	+	451	173	2,233	120
	−	698	180	414	61
UA130C	+	47	62	2,628	242
	−	995	25	722	125
UA130BC	+	44	42	2,222	0
	−	101	5	231	0
UA130S	+	710	162	1,805	4
	−	831	173	335	0
UA130DD	+	629	609	260	0
	−	425	110	147	50

[a] Each strain was grown to the mid-log phase in 5 ml of Todd-Hewitt broth in the presence (+) or absence (−) of dextran T10. After centrifugation, GTF activities in the cell and supernatant fractions were determined as described in the text. Each value was calculated relative to the culture optical density at 550 nm (OD_{550}).

mutans UA101 defective in the *gtfD* gene colonized hard surfaces in vitro as well as the parental organisms (8). However, the results of the SPF rat implantation experiments indicated that the UA130DD mutant produced significantly fewer smooth-surface lesions than the parental organisms (either UA130SR or UA130RR). Nevertheless, this mutant produced as many sulcal lesions as the parental strains.

DISCUSSION

The experiments described herein were primarily initiated in order to further define the role of each of the *S. mutans gtf* genes in cariogenesis. Since most strains of *S. mutans* contain three distinct *gtf* genes (5), insertional inactivation of each gene has allowed for the evaluation of the role of each gene product in sucrose-dependent colonization of hard surfaces as well as in cariogenesis. Mutants of *S. mutans* GS5 altered in either the *gtfB* or *gtfC* gene displayed reduced

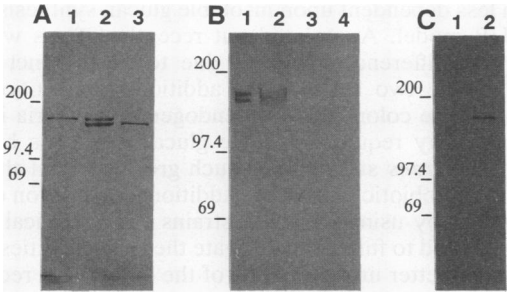

FIG. 3. Western blot analysis of the GTF enzymes from UA130S mutants constructed following allelic exchange. (A) Immunological detection of either *gtfB* or *gtfC* mutants. Lanes: 1, mixture of the cellular fraction (CF) and culture supernatant fluids (CS) of UA130C; 2, mixture of CF and CS of UA130S; 3, mixture of CF and CS of UA130B. (B) Immunological detection of double *gtfB gtfC* mutants. Lanes: 1, CF of UA130S; 2, CS of UA130S; 3, CF of UA130BC; 4, CS of UA130BC. (C) Immunological analysis of *gtfD* mutants. Lanes: 1, mixture of CF and CS of UA130DD; 2, mixture of CF and CS of UA130S.

ability to colonize hard surfaces in the presence of sucrose in vitro (18). In contrast, a mutant defective in the *gtfD* gene colonized such surfaces as well as the parental organism (9). These results were consistent with earlier in vitro and rodent model studies suggesting a crucial role for insoluble glucan synthesis in sucrose-dependent colonization of hard surfaces by mutans streptococci (7, 22).

Recent rat implantation studies utilizing mutated *S. mutans gtf* genes have also suggested an important role for the *gtfB* and *gtfC* genes in smooth-surface caries (18). These experiments utilizing strain V403 demonstrated that mutants defective in both the *gtfB* and *gtfC* genes as well as in all three *gtf* genes showed an approximately 20 to 30% reduction in smooth-surface caries relative to that induced by the parental strain when utilized in the UAB gnotobiotic model system (18). However, these mutations had a much less pronounced effect on sulcal caries. Mutants defective in only a single *gtf* gene were not examined in these experiments. The observed reductions in smooth-surface caries for the genetically engineered mutants were not as great as observed earlier with chemically induced mutants of *S. mutans* or *S. sobrinus* implanted into rats fed a diet containing higher concentrations of sucrose than was used in the UAB model system (25). More recently, the utilization of a naturally occurring isolate of *S. mutans*, strain UA101, has suggested that the choice of rat model systems can influence the outcome and interpretation of experiments designed to assess the role of a particular gene product in cariogenesis. Strain UA101, which is defective in insoluble glucan synthesis, produces as much smooth-surface caries as other cariogenic mutans streptococci in the UAB model (2) but significantly fewer smooth-surface lesions compared with those produced by some of these latter strains in the UR model system (28). Therefore, since these results have suggested that the UR model might be more appropriate for examining the role of the *gtf* genes in cariogenesis, the *gtf* mutants of strain UA130 constructed in the present study were examined in the latter animal model system.

The present results confirm and expand the observations from earlier in vitro studies (1, 8) suggesting that both the *gtfB* and *gtfC* genes are important for smooth-surface caries formation in the presence of the *gtfD* gene (Table 3, experiment 1). Inactivation of either gene reduced smooth-surface caries as much as the mutations affecting both genes. However, as observed in earlier studies (18, 25), these mutations produced less pronounced effects on the incidence of sulcal caries. The moderate levels of smooth-surface caries relative to UA130R observed with strain UA130S harboring plasmid pTH10 are compatible with a lower growth rate in the oral cavity for the plasmid-containing strains. The spontaneous loss of the plasmid suggested that growth of the plasmid-bearing strains might be inhibited in the absence of erythromycin. Such a reduction in the growth rate in the absence of antibiotic selective pressure prior to loss of the plasmid could be more critical for smooth-surface colonization than for sulcal colonization.

Both previous in vitro (9) and in vivo (28) results have suggested that the inactivation of the *S. mutans gtfD* gene coding for the primer-dependent GTF-S synthesizing water-soluble glucans would not significantly alter smooth-surface caries formation. However, mutant UA130DD induced significantly fewer smooth-surface lesions than did the parental organism (Table 3, experiment 2). Since the *gtfD* mutant utilized in the earlier UR rat model experiment was constructed in strain UA101, which induced only a very low rate of smooth-surface carious lesions in this system (28), the role

TABLE 3. Virulence of *gtf* mutants in SPF rats[a]

Group and mutant	Sulcal caries	Smooth-surface caries	CFU (10^7)	% Mutans streptococci
Expt 1				
1 (no infection)	24.3 (6.0)	1.5 (1.7)	1.5 (1.1)	0
2, 6715	38.2 (9.0)	31.3 (17.3)[b]	1.8 (1.9)	5.9 (5.5)
3, UA130S(pTH10)	38.3 (6.6)	14.4 (5.8)	5.4 (3.5)	49.6 (17.9)
4, UA130R	40.6 (7.8)	34.8 (18.2)[b]	6.0 (3.5)	33.5 (10.3)
5, UA130B	29.3 (6.5)[c]	6.3 (5.5)	3.6 (1.6)	47.8 (15.0)
6, UA130C	33.4 (6.9)	8.8 (5.7)	6.6 (4.8)	40.5 (16.2)
7, UA130BC	31.4 (5.5)[c]	7.4 (5.1)	4.1 (1.9)	41.6 (16.7)
8, UAB747	32.9 (5.4)[c]	19.0 (15.1)[d]	6.6 (2.7)	9.4 (8.3)
Expt 2				
9 (no infection)	27.5 (9.5)[e]	1.1 (1.9)	2.9 (1.0)	0
10, 6715	39.0 (6.4)	14.4 (14.1)	1.6 (1.7)	31.2 (7.5)
11, UA130RR	33.3 (8.5)	13.9 (11.0)	4.0 (1.7)	58.4 (11.1)
12, UA130SR	39.3 (15.3)	22.7 (19)	4.0 (3.7)	59.9 (28.4)
13, UA130DD	37.3 (8.2)	5.9 (5.9)[f]	5.6 (4.0)	52.6 (29.0)

[a] Standard deviations are given in parentheses.
[b] Significantly different from all other groups; $P \geq 0.05$.
[c] Significantly different from all groups except 6; $P \geq 0.05$.
[d] Significantly different from groups 5, 6, and 7; $P \geq 0.05$.
[e] Significantly different from all other groups.
[f] Significantly different from groups 1 and 4; $P > 0.0025$.

of this gene in smooth-surface colonization may have been obscured. Therefore, the GTF-S enzyme appears to be dispensable for in vitro colonization of hard surfaces but necessary for maximum development of smooth-surface carious lesions in the oral cavities of rats. This may reflect differences in the structure of the glucans required for colonization, plaque accumulation, or acidogenesis (27) in different environments. In addition, the present results suggest that extrapolation of in vitro results from sucrose-dependent colonization studies to cariogenesis in the oral cavities of animals should be made with some caution.

The role, if any, of the *gtfA* gene coding for sucrose phosphorylase activity (21) in the cariogenicity of *S. mutans* has not yet been documented. Mutants of strain UA130 defective in this gene produced as much sulcal and smooth-surface caries as the parental organism in the UAB gnotobiotic rat model (2). However, implantation of this same mutant, UAB747, in the UR rat model system resulted in significantly reduced smooth-surface caries formation relative to that by the parental strain (Table 3, experiment 1). Since the role of this enzyme in the caries process is unknown, it is not yet possible to explain these differences. However, in this regard, it is of interest that Pucci and Macrina (20) demonstrated that a *Streptococcus sanguis* strain transformed with the *S. mutans* LM7 *gtfA* gene apparently synthesized much more extracellular glucan than the nontransformed strain. Therefore, it is possible that this gene plays a previously unrecognized role in glucan synthesis by *S. mutans*. It is also worth noting that previous results with dextranase mutants of *S. mutans* yielded distinct results in the SPF and gnotobiotic rat model systems (24). These earlier results suggested that this enzyme is important for cariogenesis in the SPF system but not in gnotobiotic rats.

The reasons for the different results found in the two model systems quite likely reside in the composition of the diets used. Several investigators have shown that the level of smooth-surface caries induced in rats or hamsters (12) infected by mutans streptococci is related to the amounts of sucrose in the diet (22). Diet 2000 normally contains 56% sucrose; inclusion of smaller amounts results in reduced levels of caries (3). In contrast, diet 305, as used in the UAB model, contains only 5% sucrose; inclusion of 62% cornstarch results in a diet that is extremely adhesive when moist. Accumulation of the diet on the tooth surfaces may substitute for the synthesis of the glucan matrix of plaque and entrap organisms that might not otherwise adhere. Such entrapment would alleviate or greatly reduce the need for extracellular polysaccharides produced by mutans streptococci as virulence factors (23).

Despite the differences between the results from the utilization of genetically defined *S. mutans* mutants in the UAB and UR rat models described above, several common conclusions can be drawn from both systems. The earlier results with the UAB system (18) as well as the present results confirm the critical role of insoluble glucan synthesis in smooth-surface caries formation. In addition, sulcal caries induction by *S. mutans* does not appear to be as dependent upon such synthesis in both systems. However, it is now clear that smooth-surface caries induction in the UAB model is much less dependent upon insoluble glucan synthesis than in the UR model. As pointed out recently (28) as well as above, this difference could be due to the distinct diets utilized in the two systems. In addition, competition for smooth-surface colonization by endogenous bacteria in the UR model may require insoluble glucan synthesis by implanted *S. mutans* strains to a much greater extent than in the UAB gnotobiotic rat model. Additional evaluation of the two systems by using the same strains with identical diets will be required to further investigate these possibilities. It is likely that a better understanding of the differences recently uncovered in the two model systems will lead to a clearer definition of the virulence properties of the mutans streptococci and the relative role of diet in cariogenesis.

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REFERENCES

1. **Aoki, H., T. Shiroza, M. Hayakawa, S. Sato, and H. K. Kuramitsu.** 1986. Cloning of a *Streptococcus mutans* gene coding for insoluble glucan synthesis. Infect. Immun. **53:**587–594.
2. **Barletta, R. G., S. M. Michalek, and R. Curtiss III.** 1988. Analysis of the virulence of *Streptococcus mutans* serotype c *gtfA* mutants in the rat model system. Infect. Immun. **56:**322–330.
3. **Bowen, W. H.** Unpublished results.
4. **Bowen, W. H., K. M. Madison, and S. K. Pearson.** 1988. Influence of desalivation in rats on incidence of caries in intact cagemates. J. Dent. Res. **67:**1316–1318.
5. **Chia, J.-S., T.-Y. Hsu, L.-J. Teng, J.-Y. Chen, L.-J. Hahn, and C.-S. Yang.** 1991. Glucosyltransferase gene polymorphism among *Streptococcus mutans* strains. Infect. Immun. **59:**1656–1660.
6. **Fitzgerald, R. J., and H. K. Kuramitsu.** Unpublished results.
7. **Freedman, M. L., J. M. Tanzer, and A. L. Coykendall.** 1981. The use of genetic variants in the study of dental caries, p. 247–269. *In* J. M. Tanzer (ed.), Animal models in cariology. Proceedings of the Symposium and Workshop on Animal Models in Cariology. Information Retrieval Inc., Washington, D.C.
8. **Hanada, N., and H. K. Kuramitsu.** 1988. Isolation and characterization of the *Streptococcus mutans gtfC* gene, coding for synthesis of both soluble and insoluble glucan. Infect. Immun. **56:**1999–2005.
9. **Hanada, N., and H. K. Kuramitsu.** 1989. Isolation and characterization of the *Streptococcus mutans gtfD* gene, coding for primer-dependent soluble glucan synthesis. Infect. Immun. **57:**2079–2085.
10. **Kato, C., and H. K. Kuramitsu.** 1991. Molecular basis for the association of glucosyltransferases with the cell surface of oral streptococci. FEMS Microbiol. Lett. **79:**153–158.
11. **Koga, T., S. Sato, M. Inoue, K. Takeuchi, T. Furuta, and S. Hamada.** 1983. Role of primers in glucan synthesis by glucosyltransferases from *Streptococcus mutans* OMZ-176. J. Gen. Microbiol. **129:**751–754.
12. **Krasse, B.** 1965. The effect of caries-inducing streptococci in hamsters fed diets with sucrose or glucose. Arch. Oral Biol. **10:**223–230.
13. **Kuramitsu, H. K.** 1975. Characterization of extracellular glucosyltransferase activity of *Streptococcus mutans*. Infect. Immun. **12:**738–749.
14. **Kuramitsu, H. K.** 1993. Virulence factors of mutans streptococci: role of molecular genetics. Crit. Rev. Oral Biol. Med. **4:**159–176.
15. **Kuramitsu, H. K., and L. Ingersoll.** 1976. Immunological relationships between glucosyltransferases from *Streptococcus mutans* serotypes. Infect. Immun. **14:**636–644.
16. **Kuramitsu, H. K., and L. Wondrack.** 1983. Insoluble glucan synthesis by *Streptococcus mutans* serotype c strains. Infect. Immun. **42:**763–770.
17. **Loesche, W. J.** 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev. **50:**353–380.
18. **Munro, C., S. M. Michalek, and F. L. Macrina.** 1991. Cariogenicity of *Streptococcus mutans* V403 glucosyltransferase and fructosyltransferase mutants constructed by allelic exchange. Infect. Immun. **59:**2316–2323.
19. **Perry, D., and H. K. Kuramitsu.** 1981. Genetic transformation of *Streptococcus mutans*. Infect. Immun. **32:**1295–1297.
20. **Pucci, M. J., and F. L. Macrina.** 1985. Cloned *gtfA* gene of *Streptococcus mutans* LM7 alters glucan synthesis in *Streptococcus sanguis*. Infect. Immun. **48:**704–712.
21. **Russell, R. R. B., H. Mukasa, A. Shimamura, and J. J. Ferretti.** 1988. *Streptococcus mutans gtfA* gene specifies sucrose phosphorylase. Infect. Immun. **56:**2763–2765.
22. **Tanzer, J. M.** 1979. Essential dependence of smooth-surface caries on and augmentation of fissure caries by sucrose and *Streptococcus mutans* infection. Infect. Immun. **25:**526–531.
23. **Tanzer, J. M.** 1981. Discussion, p. 97–103. *In* J. M. Tanzer, ed. Animal models in cariology. Proceedings of the Symposium and Workshop on Animal Models in Cariology. Information Retrieval Inc., Washington, D.C.
24. **Tanzer, J. M., M. L. Freedman, and R. J. Fitzgerald.** 1985. Virulence of mutants defective in glucosyl transferase, dextran-mediated aggregation, and dextranase activity. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
25. **Tanzer, J. M., M. L. Freedman, R. J. Fitzgerald, and R. H. Larson.** 1974. Diminished virulence of glucan-synthesis defective mutants of *Streptococcus mutans* strains. Infect. Immun. **10:**197–203.
26. **Ueda, S., T. Shiroza, and H. K. Kuramitsu.** 1988. Sequence analysis of the *gtfC* gene from *Streptococcus mutans* GS-5. Gene **69:**101–109.
27. **van Houte, J., J. Russo, and K. S. Prostak.** 1989. Increased pH-lowering ability of *Streptococcus mutans* cell mass associated with extracellular glucan-rich material and the mechanism involved. J. Dent. Res. **68:**451–459.
28. **Yamashita, Y., W. H. Bowen, and H. K. Kuramitsu.** 1992. Molecular analysis of a *Streptococcus mutans* strain exhibiting polymorphism in the tandem *gtfB* and *gtfC* genes. Infect. Immun. **60:**1618–1624.