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# Genetic Diversity in Temperate Bacteriophages of *Streptococcus pyogenes*: Identification of a Second Attachment Site for Phages Carrying the Erythrogenic Toxin A Gene

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**Bacteriophage T12, the prototypic bacteriophage of *Streptococcus pyogenes* carrying the erythrogenic toxin A gene (*speA*), integrates into the bacterial chromosome at a gene for a serine tRNA (W. M. McShan, Y.-F. Tang, and J. J. Ferretti, Mol. Microbiol. 23:719–728, 1997). This phage is a member of a group of related temperate phages, and we show here that not all *speA*-carrying phages in this group use the same attachment site for integration into the bacterial chromosome. Additionally, other phages in the group use the same serine tRNA gene attachment site as phage T12 and yet do not carry *speA*. The evidence suggests that recombination between phage genomes has been an important means of generating diversity and disseminating virulence-associated genes like *speA*.**

One of the most intriguing characteristics of infections caused by group A streptococci (GAS; *Streptococcus pyogenes*) is the periodic shift in severity and predominant clinical syndromes (12, 13). The genetic basis for these phenotypic shifts in GAS natural populations has been investigated in several recent studies, and the results have shown that considerable allelic variation exists at the DNA level in both bacterial and phage-associated genes as well as in linkage between specific genes and clinical syndromes (2, 6, 9, 11, 16), more than could be reasonably expected to result from accumulated random mutations between genetically isolated individuals. Two of the three principal means that bacteria use to transfer DNA are transformation and conjugation, neither of which has been shown to occur naturally in the GAS. However, the third vehicle for genetic exchange, transduction, is of particular importance because of the high rate of carriage of temperate bacteriophages in *S. pyogenes* (8).

Recombination between phage genomes is a well-documented means of generating diversity and expanding the host range of a phage to new strains of a bacterial species; additionally, the presence of cryptic phages or complete phages of a different immunity group resident in the host genome can provide additional reservoirs of genes (4). Two principle determinants control phage host range: a surface receptor for phage adherence on the targeted bacterium and the presence of a specific DNA sequence in the bacterial chromosome that is recognized by the phage integrase at the bacterial attachment site. Although its surface receptor is still unknown, bacteriophage T12, which carries the structural gene for erythrogenic toxin A (*speA*, also known as “streptococcal pyrogenic exotoxin A”), is known to integrate by site-specific recombination into a gene for a serine tRNA (*attB*), completing the downstream half of the gene by a 96-base duplication in the phage genome (10). Previous work in our laboratory has demonstrated the existence of at least three *speA*-containing phages with distinct genomes (17), T12 being prototypic (18). We here demonstrate that not all *speA*-carrying GAS phages use the same attachment site and that recombination of functional modules between genomes has most likely led to the diversification of this family of phages.

Yu and Ferretti (17), in surveying 300 GAS strains for the presence of *speA* and a second phage T12-specific sequence by DNA hybridization, found that 24% of the strains that hybridized to the phage T12-specific probe were negative for *speA*. As an extension of our recent studies into the integration of phage T12 (10), we reexamined a number of those strains as well as phage ϕ49, a *speA*-bearing phage with a larger genome (40 kb, compared to 36 kb for phage T12) and a physical map distinct from that of phage T12, to determine what association might exist between the use of the specific bacterial attachment site for phage T12 (the serine tRNA gene) and *speA* carriage. Integration of phage T12 into the *S. pyogenes* genome is easily detected by the change in hybridization pattern at the bacterial attachment site (*attB*) (Fig. 1A). With uninfected bacteria, one band, on which is located the native serine tRNA gene, is observed (Fig. 1A, lane 2), but integration of phage T12 into *attB* results in the appearance of two new bands, resulting from the insertion of the 36-kb phage genome (lane 3). The results revealed that not all *speA*-carrying bacteriophages share *attB* with T12: while ϕ49 is *speA* positive (Fig. 1C, lane 4), it does not integrate into the T12 *attB* site (Fig. 1A, lane 4). However, use of the T12 *attB* site does not invariably correlate with carriage of *speA*. For example, ϕ436 recombines with the bacterial chromosome at the serine tRNA gene (Fig. 1A, lane 5) and has the same integrase gene as phage T12 (lane 5) but does not contain *speA* (lane 5). The deduced phenotypes of the three phages are summarized in Table 1. We previously demonstrated that phage 270, a *speA*-bearing phage that shares the T12 integrase gene and attachment site (10), has a smaller

TABLE 1. Phenotypes of bacteriophages T12, ϕ49, and ϕ436

| Bacteriophage | Presence of *speA* | Integration site | Integrase |
|---|---|---|---|
| T12 | + | Serine tRNA | $Int_{T12}$ |
| ϕ49 | + | Unknown | $Int_{\phi 49}$[a] |
| ϕ436 | − | Serine tRNA | $Int_{T12}$ |

[a] Uncharacterized.

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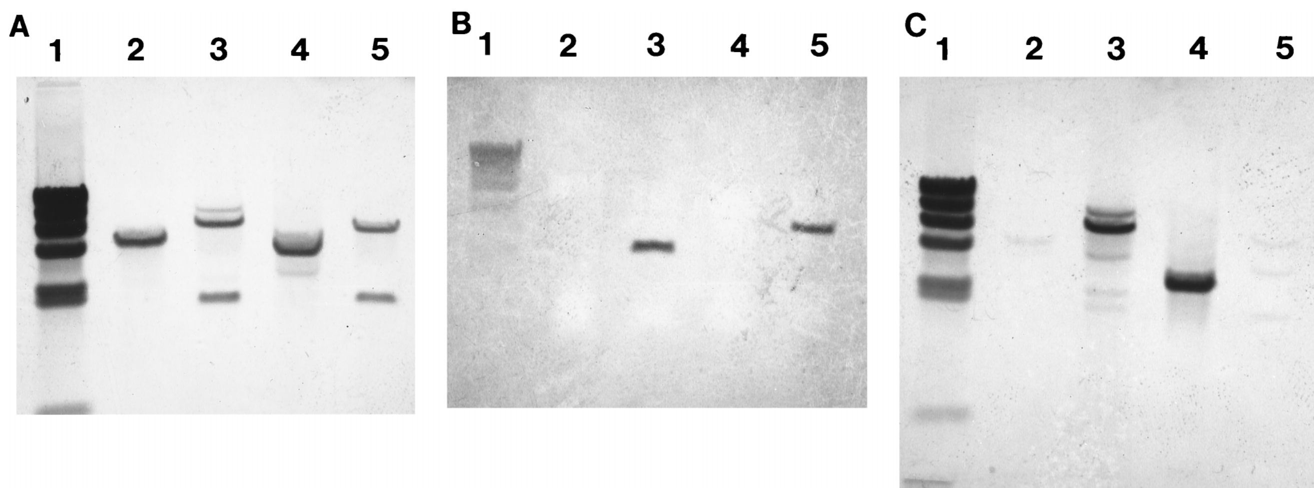


FIG. 1. Identification of a second *att* site for *speA*-carrying GAS bacteriophages. *Hin*dIII-digested chromosomal DNA from T25$_3$c (negative for T12 and *speA*), either uninfected (lanes 2) or infected with temperate phage T12 (*speA* positive), ϕ49 (*speA* positive), or ϕ436 (*speA* negative) (lanes 3 to 5, respectively), was separated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized with probes for the T12 attachment site (*attB*) (A), the T12 integrase gene (*int*) (B), and the erythrogenic toxin A gene (*speA*) (C). *Hin*dIII-digested lambda DNA was used as the molecular weight marker (lanes 1). Probes were labelled with digoxigenin by PCR amplification of cloned DNA fragments of those genes with Boehringer Mannheim Biochemicals Genius reagents, and hybridization was detected according to the manufacturer's instructions. The sequences of these genes have been previously described (10, 15).

genome (32 kb) and a different physical map (17). These results support Botstein's modular theory of phage genomes, where modules (i.e., genes and regulatory elements) are recombined between phage genomes to generate functional and host range diversity (3).

Understanding genetic variation in temperate GAS bacteriophages can provide clues to the distribution and prevalence of phage genes, especially the virulence-associated toxins. The data presented here are consistent with the genomic diversity observed in the well-characterized lambdoid phages (reviewed by Casjens et al. [5]), in which genomes of contemporary GAS phages have resulted from recombination of functional modules (e.g., integrative functions or tail genes). Significantly, intergenic regions which flank the functional genes are often the most highly conserved, providing regions for homologous recombination that result in the reassortment of functional units (3). In Fig. 2, possible sites of recombination between phage T12 and ϕ49 and ϕ436 in such intergenic regions are shown, accounting for the observed gene distributions and reflecting a diversity similar to that observed in lambdoid phages.

Selection for successful recombinant phages probably occurs both at the level of the phage and at the level of the host.

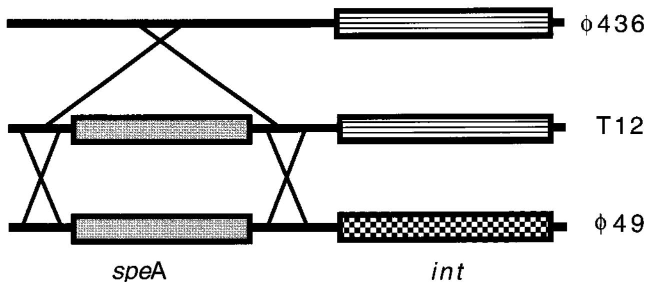


FIG. 2. Comparison of the genomes of GAS bacteriophages T12, ϕ49, and ϕ436 from the region upstream of *speA* through *int*. The gene arrangements shown are the order found in the circular, unintegrated phage genomes, with possible regions of recombination indicated.

Successful combinations of phage genes will produce viruses that may infect the predominant GAS strains and thus maintain their presence in the current bacterial population. Additionally, those phages that carry the alleles of virulence-associated genes that are most advantageous to the streptococcus will also tend to increase in frequency, as the host bacterium is better able to establish and maintain a human infection. Kehoe et al. (9) suggested that the prevalence of the *speA1* allele may indicate that this allele is evolutionarily older than *speA2* and *speA3*; however, such a view does not take into account the typical degree to which recombination has reassorted phage genes to generate novel genomes (1, 5, 7, 14). We believe that the most accurate interpretation of the prevalence of given GAS phage alleles is that natural selection has favored those variants in the populations studied. Probably the prevailing environmental factors that influence the interaction of the human host with the streptococcus combined with the interactions of bacterium with the phage dictate the dominant constellation of genes and/or alleles.

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