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The complete genomes of *Staphylococcus aureus* bacteriophages 80 and 80α—Implications for the specificity of SaPI mobilization

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ABSTRACT

Staphylococcus aureus pathogenicity islands (SaPIs) are mobile elements that are induced by a helper bacteriophage to excise and replicate and to be encapsidated in phage-like particles smaller than those of the helper, leading to high-frequency transfer. SaPI mobilization is helper phage specific; only certain SaPIs can be mobilized by a particular helper phage. Staphylococcal phage 80α can mobilize every SaPI tested thus far, including SaPI1, SaPI2 and SaPIbov1. Phage 80, on the other hand, cannot mobilize SaPI1, and ϕ11 mobilizes only SaPIbov1. In order to better understand the relationship between SaPIs and their helper phages, the genomes of phages 80 and 80α were sequenced, compared with other staphylococcal phage genomes, and analyzed for unique features that may be involved in SaPI mobilization.

Introduction

Remarkably, most or all of the toxinosis-causing bacterial toxins are encoded by mobile genetic elements, including prophages, plasmids, transposons, and pathogenicity islands (Novick, 2003), and horizontal gene transfer plays a major role in the dissemination of these virulence determinants. In the staphylococci, temperate phages carry a variety of known virulence factors (reviewed in Christie et al., 2010). In addition, certain staphylococcal phages have been implicated in the high frequency mobilization of a family of phage-related chromosomal islands (reviewed in Novick et al., 2010). The superantigen-encoding pathogenicity islands of *S. aureus* (SaPIs) are generally 15–18 kb in length and reside stably in their host chromosomes under the control of a master repressor (Ubeda et al., 2008). Following infection by particular helper bacteriophages, they are induced to excise and replicate autonomously, using a phage-like mode of replication (Ubeda et al., 2007a,b). They are then encapsidated in phage-like particles composed entirely of phage virion proteins (Tallent et al., 2007; Tormo et al., 2008) but with smaller capsids that accommodate the SaPI genome while excluding that of the phage (Ruzin et al., 2001). These SaPI-containing particles are capable of very high transfer frequencies not only among strains of *S. aureus* (Lindsay et al., 1998), but to other *Staphylococcus* sp. (Maiques et al., 2007) and also trans-generically to *Listeria monocytogenes* (Chen and Novick, 2009). One staphylococcal phage in particular, 80α, is capable of mobilizing a variety of SaPIs as well as enabling their transgeneric transfer. 80α was reportedly derived from one of the staphylococcal typing phages, 80, by selection for the ability to plate on strains of the NCTC 8325 lineage (R. Novick, 1967), and is in wide use as a generalized staphylococcal transducing phage. Phage 80, however, was originally reported to mobilize only one of the SaPIs thus far tested, SaPI2, which was not mobilized by 80α in initial studies (Lindsay et al., 1998).

We undertook the study of these two phages in an attempt to determine the genetic basis of their SaPI mobilization capacity, their SaPI mobilization specificities, and to clarify the origin of 80α. In this report, we present the sequences of the two phages, showing that 80α

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is very closely related to a different phage, staphylococcal typing phage 53, but is not closely related to 80. These results suggest that 80α is very unlikely to have originated as a mutant or restriction variant of 80, and is much more likely to represent a variant of 53, which was likely picked up as a contaminant in the initial study.

Studies with SaPIbov1 repressor mutants suggest that a primary determinant of specificity for SaPI mobilization is the ability to derepress the pathogenicity island (Ubeda et al., 2008), and recent work has identified different 80α proteins that interact specifically with the repressors of SaPIbov1, SaPI1 and SaPIbov2 (Tormo-Más et al., 2010; M.D. Harwich, SMT, A. Shrestha, KDL, P.K. Damle, A. Poliakov, J. A. Mobley and GEC, in prep). Consistent with this model, the comparison presented here of 80 and 80α indicates that there is no correlation between the morphogenetic functions provided by the helper phage and the specificity for SaPI mobilization. The ability of both 80 and 80α to mobilize SaPI2 and SaPIbov1, however, raises some interesting questions about SaPI packaging determinants.

Results

General features of the 80α and 80 genomes

The genome length of 80α is 43,864 bp, containing the information for approximately 73 ORFs of 50 or more codons (Supplementary Table 1), and is deposited in Genbank under accession number DQ517338. The genome length of 80 is 42,140 bp, with approximately 61 ORFs (Supplementary Table 2), and is deposited in Genbank under accession number DQ908929. The assembled sequences of both phages were topologically circular, consistent with the circular permutation expected from the proposed headful packaging of these phages. The genomes are displayed in Fig. 1, shown in the prophage orientation with the integrase gene at the left end. Both 80α and 80 belong to a class of related staphylococcal *Siphoviridae* that are highly mosaic but maintain a conserved organization of genes in functional modules (Kwan et al., 2005). While certain staphylococcal *Siphoviridae* encode virulence factors, such as staphylokinase, exfoliative toxin A, enterotoxin A, Panton-Valentine leukocidin, or the innate immune modulators SCIN and CHIPS (Winkler et al., 1965; Narita et al., 2001; Yamaguchi et al., 2000; Betley and Mekalanos, 1985; van Wamel et al., 2006), neither 80 nor 80α carries any known virulence factors.

80α is a variant of phage 53

Although 80α was isolated as a single plaque arising during an attempt to adapt phage 80 for growth on NCTC 8325 (R. P. Novick, 1963), it now appears that typing phage 53 was the most likely source of that plaque. Nearly 90% of the 80α genome is identical to the published sequence of 53, a phage that forms plaques on NCTC 8325 and may have been a contaminant of the 80 lysate used in that experiment (see Fig. S1). Most of the areas of divergence between 80α and 53 appear to be the result of recombination with ϕ11 and ϕ13, two of the three prophages in NCTC 8325 (Iandolo et al., 2002; R. Novick, 1967). The first 3000 bp of the 80α prophage would be exactly the same as the corresponding region of 53 except for a 1376 bp replacement that is identical to ϕ11 (Table 1). Two small blocks of sequence that are nearly identical (98%) to ϕ13 are found in the replication module. In the tail module, there is a 304 bp region that differs from the most recently published 53 sequence (Kwan et al., 2005; AY954952), yet its closest relative in Genbank is a different entry for 53 (Pantucek et al., 2004; AF513856). One of many plausible explanations for the differences between the two published 53 sequences is that recombination occurred between 53 and a prophage residing in the 53 propagating strain NCTC 8511. Two areas of divergence between 80α and 53 cannot be accounted for by simple recombination with known prophages in the propagating strain. A highly mosaic block from 3608 to 5271, affecting the immunity module, includes sequences unrelated to 53 or the NCTC 8325 prophages but highly conserved among other staphylococcal siphoviridae as well as a central region with similarity to both 53 and ϕ11, in which all but two nucleotides match one or the other genome and thus might have arisen by multiple recombination events between the two phages. The other divergent block, from 12121 to 12473, spans the C-terminus of ORF26 and the N-terminus of ORF27, two conserved genes of unknown function. The closest match in the database is ϕ11, but the sequence identity is only 91%.

Specificity of SaPI mobilization by helper phages

Because typing phage 53 is so closely related to 80α, we tested it for mobilization of SaPI1. We found that 53 could induce excision, replication, and transduction of SaPI1 *tst*::*tetM* at high frequency (Fig. 2 and Table 2). When we attempted to confirm that 53 would mobilize SaPI1 but not SaPI2, as had been reported for 80α, we

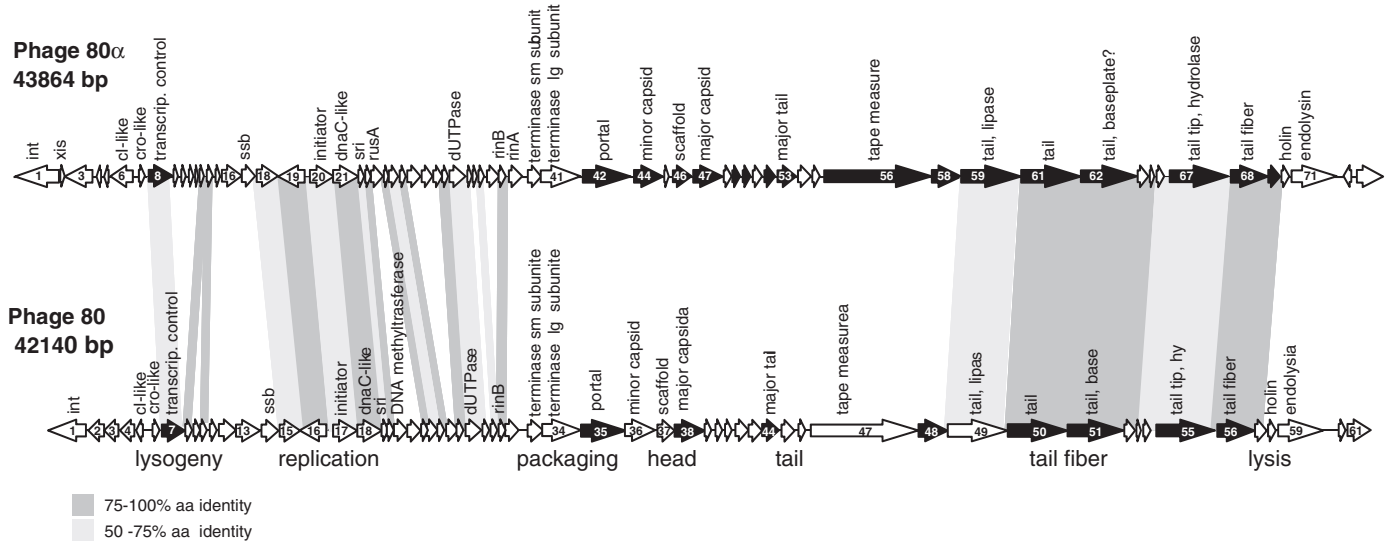

Fig. 1. Genomic maps of phages 80α and 80. Predicted open reading frames of at least 50 codons are indicated. Open reading frames shown in black are those for which gene products have been identified in phage virions by mass spectrometry. Genes encoding proteins with at least 50% amino acid identity are indicated by shaded regions between the two genomes; darker shading indicates amino acid identity of at least 75%.