

Presentation Abstract

Abstract Title: Mapping the Packaging Cleavage Determinants in the Phage-dependent *Staphylococcus aureus* Pathogenicity Island SaPI1

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Abstract: Members of the SaPI family of *Staphylococcus aureus* pathogenicity islands are highly mobile, superantigen-encoding genetic elements that depend upon specific helper bacteriophages for their horizontal transfer. Following helper phage infection, these elements excise, replicate, and package their genomes in virions provided by the helper phage. One of the novel features of this process is the use of a SaPI-encoded small subunit of terminase, which replaces the phage small terminase subunit and redirects terminase packaging specificity to SaPI DNA. The SaPI genome, like that of the helper phage, is packaged from concatemers by a headful packaging mechanism, resulting in encapsidated DNA molecules that exhibit terminal redundancy and partial circular permutation. In this study we have localized the site of initial cleavage in SaPI1, a prototype member of this family, and have defined a small region of the SaPI1 genome sufficient for specific packaging. The cleavage site was initially localized to an intergenic region upstream of SaPI1 operon 1 by identification of submolar fragments in restricted SaPI1 virion DNA. Ligation of linkers to the ends of SaPI virion DNA, followed by amplification and sequencing of the linker/SaPI1 junction, further defined the sites of initial cleavage. A fragment encompassing this region was cloned into a plasmid vector co-expressing SaPI1 small terminase and shown to confer high frequency plasmid transduction by helper phage 80 α . The critical determinants for SaPI1-specific packaging were further defined by deletion analysis of the cloned fragment. These results demonstrate that the sequence required for SaPI1-specific packaging maps to a small region upstream of the promoter for SaPI1 operon 1. This is strikingly different from the *pac* site used by the helper phage terminase, which, like other phage *pac* sites, maps to within the

small terminase gene itself.

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