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The *Staphylococcus aureus* Pathogenicity Island 1 Protein gp6 Functions as an Internal Scaffold during Capsid Size Determination

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Staphylococcus aureus pathogenicity island 1 (SaPI1) is a mobile genetic element that carries genes for several superantigen toxins. SaPI1 is normally stably integrated into the host genome but can become mobilized by "helper" bacteriophage 80α, leading to the packaging of SaPI1 genomes into phage-like transducing particles that are composed of structural proteins supplied by the helper phage but having smaller capsids. We show that the SaPI1-encoded protein gp6 is necessary for efficient formation of small capsids. The NMR structure of gp6 reveals a dimeric protein with a helix–loop–helix motif similar to that of bacteriophage scaffolding proteins. The gp6 dimer matches internal densities that bridge capsid subunits in cryo-electron microscopy reconstructions of SaPI1 procapsids, suggesting that gp6 acts as an internal scaffolding protein in capsid size determination.
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Introduction

Mobile genetic elements, such as bacteriophages, plasmids and pathogenicity islands, are commonly involved in horizontal gene transfer and genome evolution in bacteria.[1,2] *Staphylococcus aureus* is normally a harmless inhabitant of human skin and mucosal epithelia but is also associated with serious systemic and local infections.[3] A number of virulence determinants and antibiotic resistance genes have been linked to mobile genetic elements traded within the staphylococcal population,[4] such as the staphylococcal cassette chromosome *mec* (SCC*mec*) elements that carry genes for antibiotic resistance.[5]

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Abbreviations used: SaPI, *Staphylococcus aureus* pathogenicity island; dsDNA, double-stranded DNA; cryo-EM, cryo-electron microscopy; MS, mass spectrometry; HSQC, heteronuclear single quantum coherence; HMQC, heteronuclear multiple quantum coherence; 2D, two-dimensional; 3D, three-dimensional; 4D, four-dimensional; NOESY, nuclear Overhauser enhancement spectroscopy; NOE, nuclear Overhauser enhancement; PDB, Protein Data Bank.

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Bacteriophage ϕETA2 carries the gene for exfoliative toxin A,[6] and bacteriophage ϕSa3USA carries genes (*lukGH*) for the leukocidin that aids in the selective lysis of neutrophils.[4,7]

S. aureus pathogenicity islands (SaPIs) are a family of 14- to 27-kb mobile genetic elements that harbor genes for superantigen toxins, antibiotic resistance genes and other virulence factors involved in host invasion.[8,9] Acquisition of up to three SaPIs in combination with other mobile genetic elements has led to the proliferation of aggressive bacterial strains that are resistant to multiple antibiotics.[10–13] Certain SaPIs have also been shown to pass intergenerically between *S. aureus* and the food pathogen *Listeria monocytogenes*.[14] A detailed description of the mechanisms by which such elements are mobilized is therefore critically important to understand the spread and establishment of pathogenicity in *S. aureus*.

SaPI mobilization is intimately associated with specific bacteriophages (helper phages).[9,15] Though normally stably integrated, SaPIs are induced when a helper phage enters the lytic cycle, leading to the production of phage-like transducing particles that carry the SaPI genome but are composed of structural proteins supplied by the helper phage.[16–18] One of the more well characterized SaPIs, SaPI1, has a 15.2-kb genome encoding 21 open reading frames, including genes for the toxic shock syndrome toxin and enterotoxins K and Q.[9,19] SaPI1 can be mobilized by helper phage 80α, a temperate, tailed double-stranded DNA (dsDNA) bacteriophage in the *Siphoviridae* family (order *Caudovirales*).[20] 80α can act as the helper phage for several additional SaPIs, including SaPI2, SaPIbov1 and SaPIbov2.[9] Bacteriophage 80α capsids have $T=7$ icosahedral symmetry and are made of 415 copies of an HK97-like capsid protein, the product of gene *47* (gp47).[21] Like other dsDNA phages, the 80α capsid is assembled as an empty precursor called the procapsid. Procapsid assembly requires a scaffolding protein (gp46) that acts as a chaperone and forms an internal core in the procapsids.[16,21] DNA is packaged into the procapsids by a headful mechanism through the portal vertex, leading to scaffolding removal and capsid expansion.[21] The DNA packaging is carried out by the terminase complex, which consists of oligomers of the small (TerS) and large (TerL) terminase subunits (gp40 and gp41, respectively).

Specificity between 80α and SaPI1 is manifested at multiple levels. During mobilization, the 80α protein Sri (gp22) acts as an antirepressor for the SaPI1 repressor Stl (SaPI1 gp22).[22,23] Derepression is followed by excision and replication, which are independent of 80α gene products,[24] but SaPI1 DNA packaging requires a SaPI1-encoded TerS protein (gp3).[25] Strikingly, SaPI1 capsids are smaller (46 nm) than the regular 80α capsids (63 nm), reflecting a corresponding difference in genome size.[16,21,26] In the absence of SaPI1 TerS, only partial 80α genomes are packaged.[25] This process of capsid size determination resembles that of the *Escherichia coli* helper/satellite phage system P2/P4, wherein the external scaffolding protein Sid encoded by the P4 satellite redirects the assembly of the P2 helper phage capsid protein gpN from a $T=7$ pathway to one resulting in a smaller $T=4$ capsid.[27–29] By analogy, it is assumed that SaPI1 encodes one or more factors that shunt the 80α structural proteins to an alternate assembly pathway.

We previously showed that SaPI1 procapsids contained the SaPI1-encoded protein gp6 (8.3 kDa) in addition to a full complement of 80α-encoded structural proteins.[16] gp6 was present in an estimated 48 copies per SaPI1 procapsid. Another SaPI1-encoded protein, gp7 (22.8 kDa), was present as a minor component.[16] The genes corresponding to the equivalent two proteins were implicated in size determination in SaPIbov1.[24] In SaPI1, these two proteins are thus likely candidates for the presumed capsid size determination factors. Both gp6 and gp7 are adjacent and co-conserved among many members of the SaPI family (Fig. 1).[9] Here, we show that the SaPI1 protein gp6 plays a critical role in the efficient formation of small isometric capsids from 80α-encoded structural proteins. The NMR structure of gp6 shows a dimer reminiscent of bacteriophage scaffolding proteins.[30,31] These dimers match internal densities in the cryo-electron microscopy (cryo-EM) reconstruction of SaPI1 procapsids, suggesting that gp6 acts as an internal scaffolding protein during capsid size determination.

Results

SaPI1 gp6 is required for the efficient formation of small capsids

We previously identified gp6 as one of the prime candidates for a size determination factor for SaPI1.[16] Its 10 C-terminal residues are homologous to those of the 80α scaffolding protein gp46 (Fig. 1). To investigate the possible role of gp6 in scaffolding and capsid size determination, we deleted *orf6* from the SaPI1-harboring 80α lysogen RN10628, generating the strain ST100 (Table S1). When this strain was induced with mitomycin C, it produced procapsids and virions, similar to a normal wild-type induction, that were separated on CsCl gradients. The protein composition of these particles was normal, and although the virions contained neither gp6 nor gp7, as expected for mature transducing particles,[17] gp7 was found associated with the procapsid fraction by mass spectrometry (MS) analysis.

Cryo-EM of the SaPI1 Δ*orf6* virion fraction revealed a mixture of empty and full particles in various sizes and shapes (Fig. 2a). The isometric shells clustered in two size classes centered on

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