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SHORT COMMUNICATION

Intra- and inter-generic transfer of pathogenicity island-encoded virulence genes by *cos* phages

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Bacteriophage-mediated horizontal gene transfer is one of the primary driving forces of bacterial evolution. The *pac*-type phages are generally thought to facilitate most of the phage-mediated gene transfer between closely related bacteria, including that of mobile genetic elements-encoded virulence genes. In this study, we report that staphylococcal *cos*-type phages transferred the *Staphylococcus aureus* pathogenicity island SaPIbov5 to non-*aureus* staphylococcal species and also to different genera. Our results describe the first intra- and intergeneric transfer of a pathogenicity island by a *cos* phage, and highlight a gene transfer mechanism that may have important implications for pathogen evolution.
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Classically, transducing phages use the *pac* site-headful system for DNA packaging. Packaging is initiated on concatemeric post-replicative DNA by terminase cleavage at the sequence-specific *pac* site, a genome slightly longer than unit length is packaged, and packaging is completed by non-sequence-specific cleavage (reviewed in Rao and Feiss, 2008). Generalized transduction results from the initiation of packaging at *pac* site homologs in host chromosomal or plasmid DNA, and typically represents ~1% of the total number of phage particles. In the alternative *cos* site mechanism packaging is also initiated on concatemeric post-replicative DNA by terminase cleavage at a sequence-specific (*cos*) site. Here, however, packaging is completed by terminase cleavage at the next *cos* site, generating a precise monomer with the cohesive termini used for subsequent circularization (Rao and Feiss, 2008). Although *cos* site homologs may exist in host DNA, it is exceedingly rare that two such sites would be appropriately spaced. Consequently, *cos* phages, of which lambda is the prototype, do not engage in generalized transduction. For this reason, *cos*-site phages have been preferred for possible phage therapy, since they would not introduce adventitious host DNA into target organisms.

The *Staphylococcus aureus* pathogenicity islands (SaPIs) are the best-characterized members of the phage-inducible chromosomal island family of mobile genetic elements (MGEs; Novick *et al.*, 2010). SaPIs are ~15 kb mobile elements that encode virulence factors and are parasitic on specific temperate (helper) phages. Helper phage proteins are required to lift their repression (Tormo-Más *et al.*, 2010, 2013), thereby initiating their excision, circularization and replication. Phage-induced lysis releases vast numbers of infectious SaPI particles, resulting in high frequencies of transfer. Most SaPI helper phages identified to date are *pac* phages, and many well-studied SaPIs are packaged by the headful mechanism (Ruzin *et al.*, 2001; Ubeda *et al.*, 2007). Recently, we have reported that some SaPIs, of which the prototype is SaPIbov5 (Viana *et al.*, 2010), carry phage *cos* sequences in their genomes, and can be efficiently packaged and transferred by *cos* phages to *S. aureus* strains at high frequencies (Quiles-Puchalt *et al.*, 2014). Here we show that this transfer extends to non-*aureus* staphylococci and to *Listeria monocytogenes*.

Since the *pac* phages transfer SaPIs to non-*aureus* staphylococci and to the Gram-positive pathogen

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Listeria monocytogenes (Maiques *et al.*, 2007; Chen and Novick, 2009), we reasoned that *cos* phages might also be capable of intra- and intergeneric transfer. We tested this with SaPIbov5, into which we had previously inserted a tetracycline resistance (*tetM*) marker to enable selection, and with lysogens of two helper *cos* phages, ϕ12 and ϕSLT, carrying SaPIbov5 (strains JP11010 and JP11194, respectively; Supplementary Table 1). The prophages in these strains were induced with mitomycin C, and the resulting lysates were adjusted to 1 μg ml^{-1} DNase I and RNase A, filter sterilized (0.2 μm pore), and tested for SaPI transfer with tetracycline selection, as previously described (Ubeda *et al.*, 2008). To test for *trans*-specific or *trans*-generic transduction, coagulase-negative staphylococci species and *L. monocytogenes* strains were used as recipients for SaPIbov5 transfer, respectively, as previously described (Maiques *et al.*, 2007; Chen and Novick, 2009). As shown in Table 1, SaPIbov5 was transferred to *S. xylosus, S. epidermidis* and *L. monocytogenes* strains at frequencies only slightly lower than to *S. aureus*. PCR analysis demonstrated that the complete island was transferred to the recipient strains and integrated at the cognate att_B site in the host chromosome (Figure 1 and Supplementary Table 2). In contrast, deletion of the SaPIbov5 *cos* site (strains JP11229 and JP11230) did not affect SaPI replication (Supplementary Figure 1), but completely eliminated SaPIbov5 transfer (Table 1). To rule out the possibility that other mechanisms of gene transfer were involved in this process, we generated a ϕ12 phage mutant in the small terminase (*ter*S) gene (strain JP11012), using plasmid pJP1511 (Supplementary Table 2). The TerS protein is essential for ϕ12 and SaPIbov5 DNA packaging, but not for phage-mediated lysis (Quiles-Puchalt *et al.*, 2014). As expected, this mutation abolished SaPIbov5 transfer (Table 1). Taken together, these results show that intra- and intergeneric transfer of the island was *cos* phage mediated. Furthermore, SaPI proteins, such as integrase (Int) and potentially toxins, can be expressed and functional in non-*aureus* strains.

Because plaque formation is commonly used to determine phage host range, we next determined the ability of phages ϕ12 and ϕSLT to parasitize and form plaques on *S. xylosus, S. epidermidis* and *L. monocytogenes* strains. As shown in Supplementary Figure 2, phages ϕ12 and ϕSLT can parasitize and form plaques on their normal *S. aureus* hosts, but are completely unable to lyse the non-*aureus* strains. Therefore, as previously observed with *pac* phages (Chen and Novick, 2009), these results indicate that the overall host range of a *cos* phage may also be much wider if it includes infection without plaque formation.

Previous studies have demonstrated *pac* phage-mediated transfer of MGEs between *S. aureus* and other bacterial species (Maiques *et al.*, 2007; Chen and Novick, 2009; Uchiyama *et al.*, 2014); however, no previous studies have described the natural intra- or intergeneric transfer of pathogenicity islands by *cos* phages. As bacterial pathogens become increasingly antibiotic resistant, lytic and poorly transducing phages, such as *cos* phages, have been proposed for phage therapy, on the grounds that they would not introduce adventitious host DNA into target organisms and that the phages are so restricted in host range that the resulting progeny are harmless and will not result in dysbiosis of human bacterial flora. Because plaque formation was once thought to determine the host range of a phage, the evolutionary impact of phages on bacterial strains they can transduce, but are unable to parasitize, has remained an unrecognized aspect of phage biology and pathogen evolution. Our results add to the recently recognized concept of 'silent transfer' of pathogenicity factors carried by MGEs (Maiques *et al.*, 2007; Chen and Novick, 2009) by phages that cannot grow on the target organism. They extend this capability to *cos* phages, which have hitherto been unrecognized as mediators of natural genetic transfer.

Table 1 Intra- and intergeneric SaPIbov5 transfer[a]

Donor strain			
Phage	*SaPI*	*Recipient strain*	*SaPI titre*[b]
ϕ12	SaPIbov5	*S. aureus* JP4226	8.3×10^4
		S. epidermidis JP829	2.4×10^4
		S. epidermidis JP830	4.7×10^4
		L. monocytogenes SK1351	6.6×10^3
		L. monocytogenes EGDe	2.1×10^4
		S. xylosus C2a	7.1×10^4
ϕ12	SaPIbov5 Δ*cos*	*S. aureus* JP4226	<10
		S. epidermidis JP829	<10
		S. epidermidis JP830	<10
		L. monocytogenes SK1351	<10
		L. monocytogenes EGDe	<10
		S. xylosus C2a	<10
ϕ12 Δ*ter*S	SaPIbov5	*S. aureus* JP4226	<10
		S. epidermidis JP829	<10
		S. epidermidis JP830	<10
		L. monocytogenes SK1351	<10
		L. monocytogenes EGDe	<10
		S. xylosus C2a	<10
ϕSLT	SaPIbov5	*S. aureus* JP4226	4.1×10^3
		S. epidermidis JP829	1.1×10^3
		S. epidermidis JP830	2.1×10^3
		L. monocytogenes SK1351	3.6×10^2
		L. monocytogenes EGDe	3.1×10^3
		S. xylosus C2a	4.0×10^3
ϕSLT	SaPIbov5 Δ*cos*	*S. aureus* JP4226	<10
		S. epidermidis JP829	<10
		S. epidermidis JP830	<10
		L. monocytogenes SK1351	<10
		L. monocytogenes EGDe	<10
		S. xylosus C2a	<10

Abbreviation: SAPI, *Staphylococcus aureus* pathogenicity island.
[a]The means of results from three independent experiments are shown. Variation was within ±5% in all cases.
[b]No. of transductants per ml induced culture.

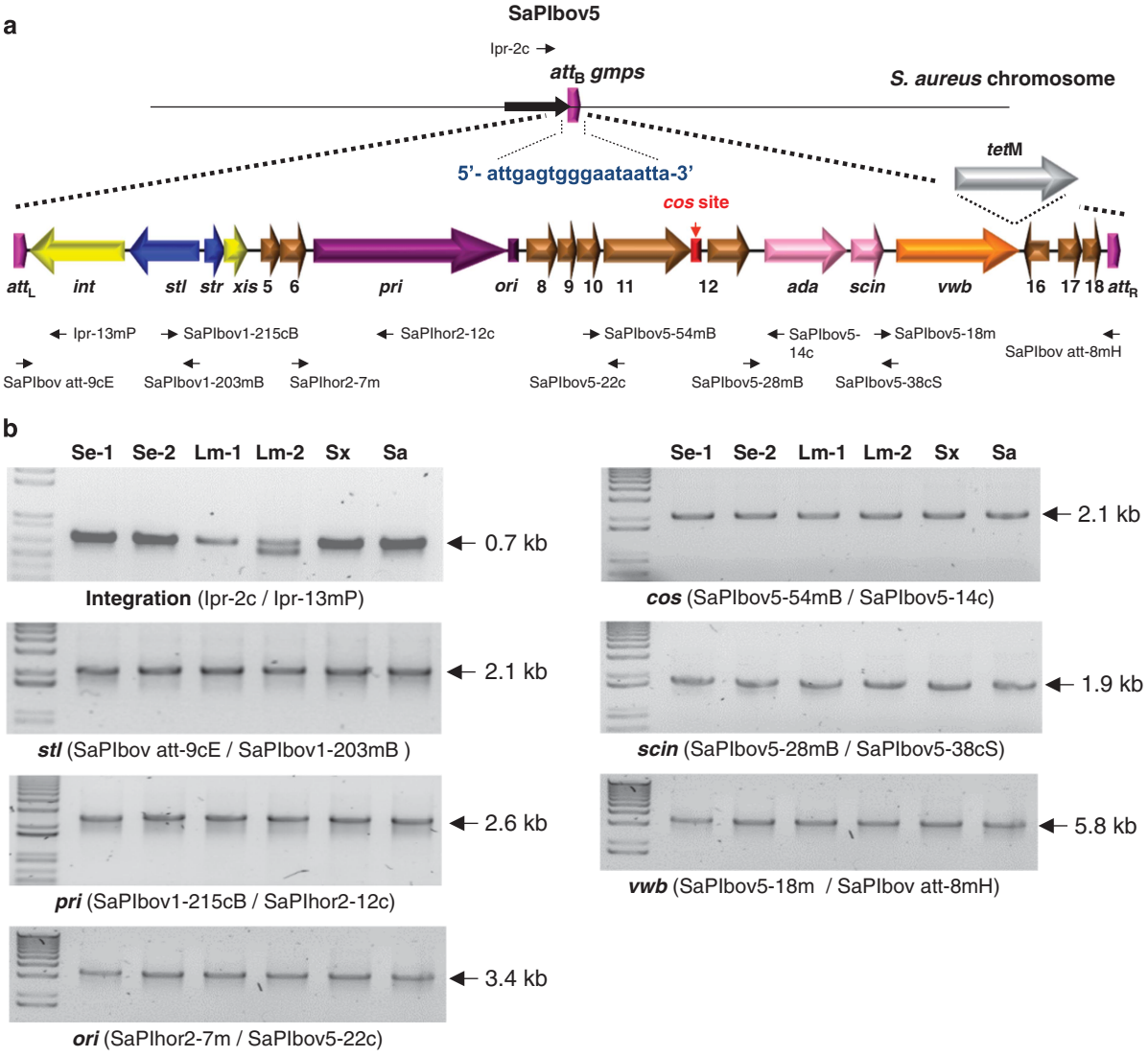

Figure 1 (**a**) Map of SaPIbov5. Arrows represent the localization and orientation of ORFs greater than 50 amino acids in length. Rectangles represent the position of the *ori* (in purple) or *cos* (in red) sites. Positions of different primers described in the text are shown. (**b**) Amplimers generated for detection of SaPIbov5 in the different recipient strains. Supplementary Table 2 lists the sequence of the different primers used. The element was detected in *S. epidermidis* JP829 (Se-1), *S. epidermidis* JP830 (Se-2), *L. monocytogenes* SK1351 (Lm-1), *L. monocytogenes* EGDe (Lm-2), *S. xylosus* C2a (Sx) and *S. aureus* JP4226 (Sa).

The potential for gene transfer of MGEs by this mechanism is limited by the ability of *cos* phages to adsorb and inject DNA into recipient strains, and also by the presence of suitable attachment sites in recipient genomes. However, since different bacterial genera express wall teichoic acid with similar structures, which can act as bacteriophage receptors governing the routes of horizontal gene transfer between major bacterial pathogens, horizontal gene transfer even across long phylogenetic distances is possible (Winstel *et al.*, 2013). In addition, our previous results also demonstrated that the SaPI integrases have much lower sequence specificity than other typical integrases, and SaPIs readily integrate into alternative sites in the absence of the cognate att_C site, such that any bacterium that can adsorb SaPI helper phage is a potential recipient (Chen and Novick, 2009). Thus, we anticipate that *cos* phages can have an important role in spreading MGEs carrying virulence and resistance genes. We also predict that *cos* sites will be found on many other MGEs, enabling *cos* phage-mediated transfer of any such element that can generate post-replicative concatemeric DNA.

Conflict of Interest

The authors declare no conflict of interest.

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