

SHORT COMMUNICATION

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

John Chen¹, Nuria Carpena^{2,3}, Nuria Quiles-Puchalt^{3,4}, Geeta Ram¹, Richard P Novick¹ and José R Penadés³

¹Skirball Institute Program in Molecular Pathogenesis and Departments of Microbiology and Medicine, New York University Medical Center, New York, NY, USA; ²Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia, Spain; ³Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK and ⁴Universidad Cardenal Herrera CEU, Moncada, Valencia, Spain

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.

The ISME Journal (2015) 9, 1260–1263; doi:10.1038/ismej.2014.187; published online 14 October 2014

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

Correspondence: J Chen or RP Novick, Skirball Institute Program in Molecular Pathogenesis and Departments of Microbiology and Medicine, New York University Medical Center, New York, NY 10016, USA or JR Penadés, Institute of Infection, Immunity and Inflammation, University of Glasgow, 120 University Place, Sir Graeme Davies Building, Glasgow G12 8TA, UK.
E-mail: john.chen@med.nyu.edu or Richard.Novick@med.nyu.edu or JoseR.Penades@glasgow.ac.uk

Received 15 May 2014; revised 22 July 2014; accepted 4 August 2014; published online 14 October 2014

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, ϕ 12 and ϕ SLT, 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 $1 \mu\text{g ml}^{-1}$ DNase I and RNase A, filter sterilized ($0.2 \mu\text{m}$ 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. xylosum*, *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 ϕ 12 phage mutant in the small terminase (*terS*) gene (strain JP11012), using plasmid pJP1511 (Supplementary Table 2). The TerS protein is essential for ϕ 12 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 ϕ 12 and ϕ SLT to parasitize and form plaques on *S. xylosum*, *S. epidermidis* and *L. monocytogenes* strains. As shown in Supplementary Figure 2, phages ϕ 12 and ϕ SLT 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,

Table 1 Intra- and intergeneric SaPIbov5 transfer^a

Donor strain			
Phage	SaPI	Recipient strain	SaPI titre ^b
ϕ 12	SaPIbov5	<i>S. aureus</i> JP4226	8.3×10^4
		<i>S. epidermidis</i> JP829	2.4×10^4
		<i>S. epidermidis</i> JP830	4.7×10^4
		<i>L. monocytogenes</i> SK1351	6.6×10^3
		<i>L. monocytogenes</i> EGDe	2.1×10^4
		<i>S. xylosum</i> C2a	7.1×10^4
ϕ 12	SaPIbov5 Δ cos	<i>S. aureus</i> JP4226	<10
		<i>S. epidermidis</i> JP829	<10
		<i>S. epidermidis</i> JP830	<10
		<i>L. monocytogenes</i> SK1351	<10
		<i>L. monocytogenes</i> EGDe	<10
		<i>S. xylosum</i> C2a	<10
ϕ 12 Δ terS	SaPIbov5	<i>S. aureus</i> JP4226	<10
		<i>S. epidermidis</i> JP829	<10
		<i>S. epidermidis</i> JP830	<10
		<i>L. monocytogenes</i> SK1351	<10
		<i>L. monocytogenes</i> EGDe	<10
		<i>S. xylosum</i> C2a	<10
ϕ SLT	SaPIbov5	<i>S. aureus</i> JP4226	4.1×10^3
		<i>S. epidermidis</i> JP829	1.1×10^3
		<i>S. epidermidis</i> JP830	2.1×10^3
		<i>L. monocytogenes</i> SK1351	3.6×10^2
		<i>L. monocytogenes</i> EGDe	3.1×10^3
		<i>S. xylosum</i> C2a	4.0×10^3
ϕ SLT	SaPIbov5 Δ cos	<i>S. aureus</i> JP4226	<10
		<i>S. epidermidis</i> JP829	<10
		<i>S. epidermidis</i> JP830	<10
		<i>L. monocytogenes</i> SK1351	<10
		<i>L. monocytogenes</i> EGDe	<10
		<i>S. xylosum</i> C2a	<10

Abbreviation: SaPI, *Staphylococcus aureus* pathogenicity island.

^aThe means of results from three independent experiments are shown. Variation was within $\pm 5\%$ in all cases.

^bNo. of transductants per ml induced culture.

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.

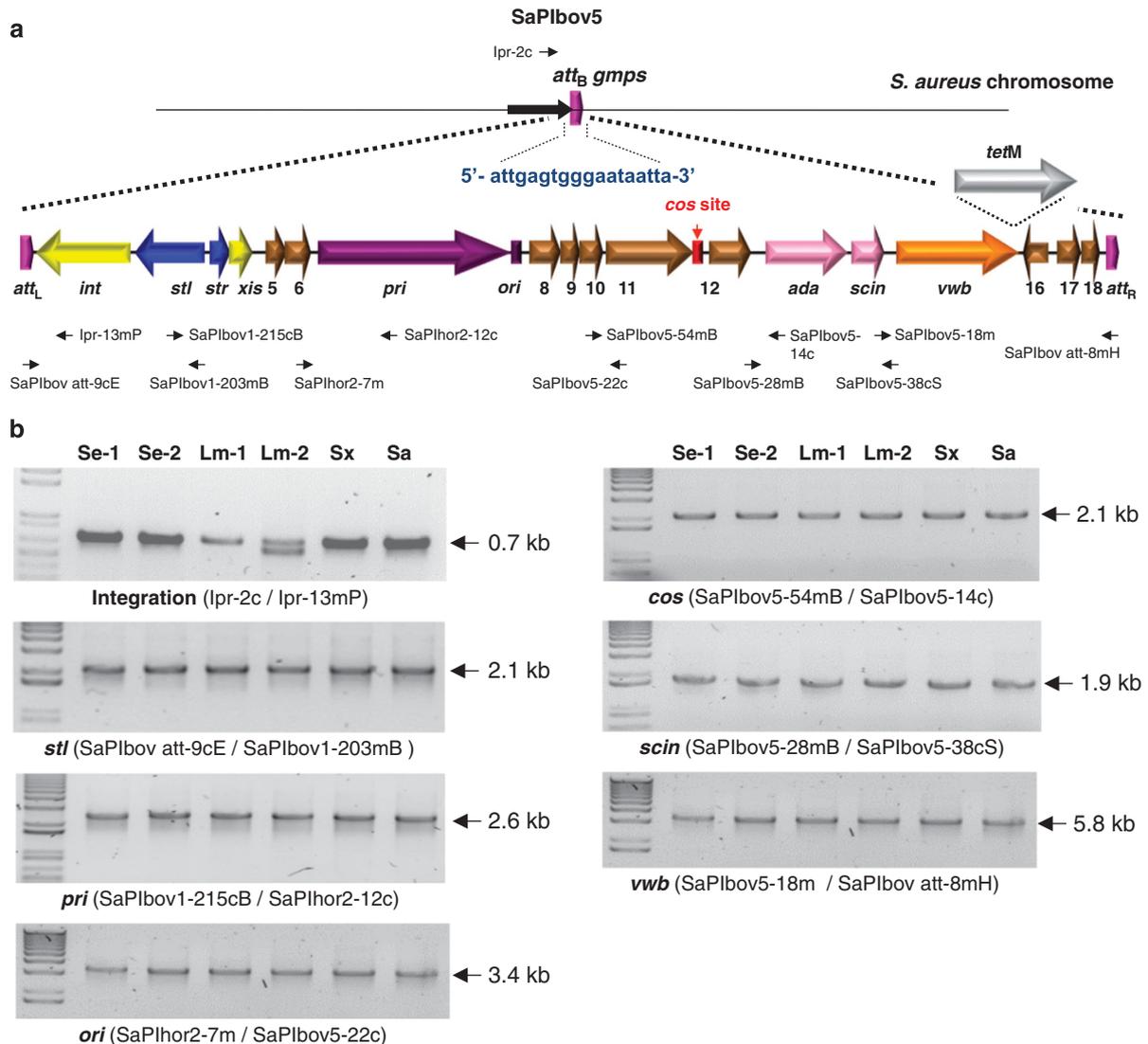


Figure 1 (a) Map of SaPIbov5. Arrows represent the localization and orientation of ORFs greater than 50 amino acids in length. Rectangles represent the position of the *ori* (in purple) or *cos* (in red) sites. Positions of different primers described in the text are shown. (b) Amplimers generated for 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. xylosum* 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 *attC* 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.

Acknowledgements

We thank R Calendar for gifts of strains and phages. This work was supported by grants Consolider-Ingenio CSD2009-00006, BIO2011-30503-C02-01 and Eranet-pathogenomics PIM2010EPA-00606 to JRP, from the

Ministerio de Ciencia e Innovación (MICINN, Spain), and grant R01AI022159 to RPN and JRP, from the National Institutes of Health.

References

- Chen J, Novick RP. (2009). Phage-mediated intergeneric transfer of toxin genes. *Science* **323**: 139–141.
- Maiques E, Ubeda C, Tormo MA, Ferrer MD, Lasa I, Novick RP *et al.* (2007). Role of staphylococcal phage and SaPI integrase in intra- and interspecies SaPI transfer. *J Bacteriol* **189**: 5608–5616.
- Novick RP, Christie GE, Penadés JR. (2010). The phage-related chromosomal islands of Gram-positive bacteria. *Nat Rev Microbiol* **8**: 541–551.
- Quiles-Puchalt N, Carpena N, Alonso JC, Novick RP, Marina A, Penadés JR. (2014). Staphylococcal pathogenicity island DNA packaging system involving *cos*-site packaging and phage-encoded HNH endonucleases. *Proc Natl Acad Sci USA* **111**: 6016–6021.
- Rao VB, Feiss M. (2008). The bacteriophage DNA packaging motor. *Annu Rev Genet* **42**: 647–681.
- Ruzin A, Lindsay J, Novick RP. (2001). Molecular genetics of SaPI1—a mobile pathogenicity island in *Staphylococcus aureus*. *Mol Microbiol* **41**: 365–377.
- Tormo-Más MÁ, Donderis J, García-Caballer M, Alt A, Mir-Sanchis I, Marina A *et al.* (2013). Phage dUTPases control transfer of virulence genes by a proto-oncogenic G protein-like mechanism. *Mol Cell* **49**: 947–958.
- Tormo-Más MÁ, Mir I, Shrestha A, Tallent SM, Campoy S, Lasa I *et al.* (2010). Moonlighting bacteriophage proteins derepress staphylococcal pathogenicity islands. *Nature* **465**: 779–782.
- Ubeda C, Maiques E, Barry P, Matthews A, Tormo MA, Lasa I *et al.* (2008). SaPI mutations affecting replication and transfer and enabling autonomous replication in the absence of helper phage. *Mol Microbiol* **67**: 493–503.
- Ubeda C, Maiques E, Tormo MA, Campoy S, Lasa I, Barbé J *et al.* (2007). SaPI operon I is required for SaPI packaging and is controlled by LexA. *Mol Microbiol* **65**: 41–50.
- Uchiyama J, Takemura-Uchiyama I, Sakaguchi Y, Gamoh K, Kato S-I, Daibata M *et al.* (2014). Intragenus generalized transduction in *Staphylococcus* spp. by a novel giant phage. *ISME J* **8**: 1949–1952.
- Viana D, Blanco J, Tormo-Más MÁ, Selva L, Guinane CM, Baselga R *et al.* (2010). Adaptation of *Staphylococcus aureus* to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein. *Mol Microbiol* **77**: 1583–1594.
- Winstel V, Liang C, Sanchez-Carballo P, Steglich M, Munar M, Bröker BM *et al.* (2013). Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens. *Nat Commun* **4**: 2345.

Supplementary Information accompanies this paper on The ISME Journal website (<http://www.nature.com/ismej>)