Genome hypermobility by lateral transduction

John Chen1*, Nuria Quiles-Puchalt2*, Yin Ning Chiang3*, Rodrigo Bacigalupe3, Alfred Fillol-Salom2, Melissa Su Juan Chee1, J. Ross Fitzgerald3, José R. Penadés2,4,5†

Genetic transduction is a major evolutionary force that underlies bacterial adaptation. Here we report that the temperate bacteriophages of Staphylococcus aureus engage in a distinct form of transduction we term lateral transduction. Staphylococcal prophages do not follow the previously described excision-replication-packing pathway but instead excise late in their lytic program. Here, DNA packaging initiates in situ from integrated prophage genomes, and large metamic spans including several hundred kilobases of the S. aureus genome are packaged in phage heads at very high frequency. In situ replication before DNA packaging creates multiple prophage genomes so that lateral-transducing particles form during normal phage maturation, transforming parts of the S. aureus chromosome into hypermobile regions of gene transfer.

Bacteriophages are the most abundant gene-transfer particles, and phage transduction is generally regarded as the most important mechanism of horizontal gene transfer (HGT) between bacterial cells. HGT is of considerable importance in medicine because it is the major route by which bacteria acquire virulence factors and antibiotic resistance.

Prophages are phage genomes that are integrated into bacterial chromosomes and replicate passively along with the host genome. Mature phages are produced in the lytic cycle during host cell infection or lysogenic induction, when rapid viral DNA replication and capsid assembly lead to formation of infectious particles that are released after cell lysis (fig. S1). Transducing particles are also produced during the phage lytic cycle, when bacterial DNA can also become packaged into newly formed procapsids. The acquisition of host DNA by transducing particles depends on the packaging mechanism. Most packaging begins with the cleavage of concatemeric DNA, generated by rolling-circle replication of the phage genome. A phage-specific packaging site (pac or cos) is recognized by the phage small terminase (TerS), which forms hetero-oligomers with the phage large terminase (TerL) to process DNA into procapsids (1). To complete DNA packaging, pac-type terminases make a non-specific sequence cut when capsid “headful” capacity (i.e., slightly longer than a genome unit length) has been reached. In an alternative mechanism, cos-type terminases require a second cos site for terminal cleavage and thus package precise genome monomers (2).

Phage-mediated HGT is known to occur by either generalized or specialized transduction...

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**Fig. 1. DNA packaging initiates from the pac site within integrated prophage genomes.** (A) Transcriptomic analysis of the early and late genes from phage 80α for the positive (top) and the negative (bottom) DNA strands. An 80α lysogen was treated with mitomycin C, and samples were analyzed without induction (light blue) or at 30 min (early genes, green) and 60 min (late genes, red) after induction. Expression was normalized by the number of aligned reads. (B and C) Transfer of Cβ markers downstream of attB sites for (B) φ11 and a Cβ 5 kb downstream of the attBφ11 and (C) 80α and a Cβ 4 kb downstream of the attB80α. Nonlysogenic strains (light blue) were infected (Inf or F) and the lysogenic WT (+, dark blue) and terS deletion (Δ, gray) strains were induced (Ind or D) with mitomycin C, and the lysates were tested for transduction into S. aureus. Transduction units (TrU) per milliliter were normalized by plaque forming units (PFU) per milliliter and represented as the log TrU of an average phage titer (1 × 109 PFU). TrU per milliliter amounts for ΔterS were <10. Values are means (n = 3 independent samples). Error bars indicate standard deviation.
The formation of ST particles is more complicated than that of the GT mechanism, and our current understanding is based on the classical phage model, in which aberrant excision events join part of the prophage to bacterial chromosome at high frequencies by the headful packaging mechanism. GT results from the recognition of viral genome (in situ packaging) have been proposed (6). Early studies showed that artificially generated mutants of cos- and pac-site phages (λ and P22, respectively) could package headfuls of viral DNA still connected to adjacent bacterial DNA. These particles were not mature and of viral genome. The order of this sequence showed that transcription of the excisionase gene (xis) was not activated until late (30 to 60 min) after induction (8–10). Other studies focusing on headful packaging showed that completed ST particles could be formed in vivo from “locked-in” prophage hybrids and mutants that were unable to excise; however, these experimental systems also did not produce viable phage (11–13). Therefore, most integrated phages are probably capable of ST, but the role of in situ packaging, if any, remains uncertain for normal phage production. Propagation typically excise and circularize early after induction, and the ensuing replication forms head-to-tail concatamers that are packaged by the terminase machinery. The order of this sequence is important because DNA packaging before excision and replication would split the viral genome and render the phage nonviable. Accordingly, most phages, including the λ and P22 phages, follow this temporal program (14, 15).

Delayed prophage excision results in DNA packaging from integrated viral genomes

In a previous study on phage transcriptional activators, using tiling microarray analysis on a S. aureus strain lysogenic for phage 80a, we showed that transcription of the excisionase gene (xis) was not activated until late (30 to 60 min) after induction (16). This result indicated that the 80a prophage may delay excision but is not defective for phage production. Therefore, (+) lysogenic; —, nonlysogenic strain. (C and D) Transfer of Cdr markers in seven successive HFs for (C) φ11 and (D) 80a. For (A), (C), and (D), nonlysogenic strains (light blue) were infected (Inf or F) and the lysogenic WT (dark blue) strains were infected (Ind or D) with mitomycin C, and the lysates were tested for transduction into S. aureus. Strain φ11 (S4) integrated at the SaPI4 attB instead of the natural attB11, TrU per milliliter was normalized by PFU per milliliter and represented as the log TrU of an average phage titer (1 x 10^3 PFU). TrU per milliliter amounts for ΔterS were <10. Error bars indicate standard deviation. For all panels, values are means (n = 3 independent samples).
Consistent with our previous findings, transcriptional activation of the 80α phage and host-DNA packaging, a cadmium-resistant cassette (Cd\(^5\)) was inserted in the S. aureus chromosome 5 kb downstream of the \(\phi 11\) attB site (attB\(_{511}\), in a phage-free or \(\phi 11\) lysogen, for both intact wild-type (WT) and terS deleted mutants (ΔterS) (fig. S5). A distance of 5 kb was chosen because it is well within a headful capacity for \(\phi 11\) (~32 kb from the terS\(_{511}\)) and also provides sufficient flanking DNA for homologous recombination in the nonlysogenic recipient host strain. Because the viral genome is extrachromosomal after infection, a nonlysogenic host was infected with \(\phi 11\) to measure GT. To measure in situ packaging, the \(\phi 11\) lysogenic derivatives were induced with mitomycin C, and the resulting lysates were tested as donors of cadmium resistance to S. aureus.

The lysates resulting from \(\phi 11\) infection of the nonlysogen transferred a Cd\(^5\) marker (Cd\(^5\)) at a frequency of 100 to 1000 transductants ml\(^{-1}\) (Fig. 1B), consistent with GT frequencies of other chromosomal markers in S. aureus (17). By contrast, \(\phi 11\) prophage induction transferred the Cd\(^5\) at very high frequencies, three orders of magnitude greater than those observed for GT, in a terS\(_{511}\)-dependent manner (Fig. 1B). Because expected low levels of GT were observed, it was unlikely that an exceptionally strong pseudo-pac site was directing high-frequency transfer of the Cd\(^5\) marker. Other explanations include ST by an unusually efficient aberrant excision mechanism, superinfection by phages released early in the lytic cycle, or, as the transcriptomic analyses indicates, the prophage initiated packaging before excision.

For excision to be efficient, one possibility is that cryptic repeated sequences in both the \(\phi 11\) genome and in the adjacent bacterial chromosome result in excision that generates ST particles. Changing the phage and/or the attB site should abolish this activity. First, we inserted a Cd\(^5\) marker 5 kb downstream of the attB\(_{511}\) in an 80α lysogen and tested for transfer of cadmium resistance. Induction of the 80α lysogen resulted in high transfer frequencies like those from \(\phi 11\) induction (Fig. 1C). Other phages—including \(\phi\)NMI and \(\phi\)NM2, which use the same attB as \(\phi 11\) and 80α—also showed high-frequency transfer of the Cd\(^5\) marker after prophage induction (fig. S6). Changing the phage and attB location did not abolish excision, and so it seemed unlikely that an aberrant excision mechanism was responsible for the high-frequency transfer.

To rule out recombination, we engineered a recA A37→Asp mutant (recA\((N303D)\)) that is defective for recombination but not for LexA cleavage or the SOS response (18). Lysates from \(\phi 11\) induction in a WT or recA\((N303D)\) background transferred the Cd\(^5\), marker at comparable frequencies (fig. S7), showing that recombination is not involved in Cd\(^5\) packaging. Moreover, polymerase chain reaction analysis of 100 cadmium-resistant colonies confirmed that the transductants of 80α

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Fig. 3. Staphylococcal phages replicate in situ before excision. (A to C) Relative abundance of phage genomic DNA and the chromosomal regions proximal to where they integrate for (A) 80α, (B) \(\phi 11\) and (C) \(\phi 52a\). Samples were analyzed at 0 (blue), 30 (light blue), 60 (orange), and 120 min (red) after induction with mitomycin C. Relative coverage is the DNA relative to the average bacterial genomic coverage (excluding phages). Shaded gray rectangles represent the location of the prophages in the S. aureus chromosome. The following strains were analyzed: 80α lysogen (RN450-80α), \(\phi 11\) lysogen (RN450-\(\phi 11\)), and \(\phi 52a\) lysogen (RN450-\(\phi 52a\)); derivatives of these strains carrying mutations in both the ori site and rep genes [80α ori deletion (RN450-80α-\(\Delta\)ori) or \(\phi 11\) ori deletion (RN450-\(\phi 11\)-\(\Delta\)ori)]; a nonlysogenic strain infected with 80α (RN450-infected 80α); and the nonlysogenic strain (RN450-induced). For the 80α and \(\phi 52a\) lysogens, the t = 0 min samples obscure the t = 30 min samples.

We performed transcriptional profiling using 80α as a model and compared it with other staphylococcal phages, including \(\phi 11\), \(\phi\)NMI, and \(\phi\)NM2. Lysogenic derivatives were treated with mitomycin C to elicit the SOS response, which activates the resident prophages, and total RNA for RNA-seq analysis was isolated before prophage induction and at 30 and 60 min afterward. Consistent with our previous findings, transcriptional activation of \(xis\) occurred in all the phages between 30 and 60 min after prophage induction (Fig. 1 and fig. S2). To correlate the onset of \(xis\) expression with prophage excision, the 80α and \(\phi 11\) lysogens were induced under the same conditions, but instead of RNA, we isolated total chromosomal DNA for whole-genome sequencing. At each time point, we identified the sequencing reads corresponding to empty attB sites (which gave a measure for excised prophage) and the reads covering attL sites (i.e., the left end of the integrated prophage) and represented the results as the percentage of integrated prophage. The percentage of integrated 80α steadily declined after 30 min (fig. S3), matching the timing of \(xis\) transcriptional activation and confirming that excision was delayed. Interestingly, after 60 min, the decrease in percentage of 80α integration began to slow and that of \(\phi 11\) began to increase (see below).

We reasoned that DNA packaging could initiate from the integrated genome because of the delay in prophage excision and because expression of the \(xis\) gene overlaps with the phage DNA-packaging module (late operon) (Fig. 1 and fig. S2). To test this, we first identified the pac sites of packaging initiation for 80α and \(\phi 11\). Phage pac sites are often embedded in structural terS genes and direct unidirectional packaging toward the 3’ end of the gene (1), and likewise for the staphylococcal phages (fig. S4). Because the terS genes are located near the center of the 80α and \(\phi 11\) prophage genomes, unidirectional packaging initiated in situ can only reach headful capacity (~105% of a phage genome or ~46 kb) by including the adjacent host DNA (fig. S5). Thus, to test for in situ prophage and host-DNA packaging, a cadmium-resistance cassette (Cd\(^5\)) was inserted in the S. aureus chromosome 5 kb downstream of the \(\phi 11\) attB site (attB\(_{511}\), in a phage-free or \(\phi 11\) lysogen, for both intact wild-type (WT) and terS deleted mutants (ΔterS) (fig. S5). A distance of 5 kb was chosen because it is well within a headful capacity for \(\phi 11\) (~32 kb from the terS\(_{511}\)) and also provides sufficient flanking DNA for homologous recombination in the nonlysogenic recipient host strain. Because the viral genome is extrachromosomal after infection, a nonlysogenic host was infected with \(\phi 11\) to measure GT. To measure in situ packaging, the \(\phi 11\) lysogenic derivatives were induced with mitomycin C, and the resulting lysates were tested as donors of cadmium resistance to S. aureus.

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phage induction did not have integrated defective prophages linked to the transferred marker. Together, these results show that aberrant excision is unlikely to be involved.

Next, we determined if phages released early after SOS induction could superinfect the remaining cells to initiate packaging from the resident prophage genome. A previous study showed that Salmonella typhimurium carrying deleted P22 prophages that lost lysogenic immunity could be superinfected and that the WT phage could initiate packaging from the unexcised genomes ([27]). Therefore, we induced λ11 in the presence of sodium citrate at 100 and 200 mM, which was sufficient to block all phage adsorption (Fig. S8). These results rule out a mechanism by which early released phages superinfect the remaining cells for in situ packaging. Hence, neither GT nor ST are involved, and we propose here the existence of a distinct mechanism of natural transduction that we term lateral transduction.

**Lateral transduction of large spans of the bacterial genome by the headful packaging mechanism**

The high frequency of Cd8 transfer by the lateral transfer mechanism indicated that headful packaging occurs off the integrated prophage after induction of the lysogen. To test this possibility, we inserted two additional downstream markers (Cd8p1 and Cd8p2) within a headful capacity, two markers (Cd8p3 and Cd8p4) beyond a headful capacity, and one marker (Cd8p5) upstream of the attB11 in nonlysogenic strains or λ11 lysogens. Next, lysates resulting from λ11 infection of a nonlysogenic strain or from prophage induction were tested for marker transfer. As expected, in lysates from λ11 infection, we observed low levels of transfer typical of GT (Fig. 2A). By contrast, lysates from λ11 induction resulted in high frequencies of transduction for all markers, located in the directionality of packaging, that is, Cd8p1 to Cd8p5 (Fig. 2A). Only minor reductions in the transfer frequencies of the Cd8p3 and Cd8p4 markers were observed, indicating either that packaging did not occur by the classical headful mechanism or that initiation on the next successive headful was highly efficient.

To test whether packaging occurred by the headful mechanism, we inserted tetracycline-resistance (Tet8) cassettes upstream and downstream of the attB11 and paired them with the previous Cd8 markers—that is, with Cd8p1 or downstream Cd8p3 to Cd8p4, respectively—in nonlysogenic strains or λ11 lysogens (Fig. 2B). Then we tested the lysates generated by λ11 induction or by prophage induction (GT or lateral transduction, respectively) for cotransduction of the two markers by selecting for cadmium resistance and scoring for Tet8. As expected for GT, we found that for lysates generated by λ11 infection, all cotransduction frequencies were inversely proportional to their distance apart, and the shortest distances exhibited the highest percentage of cotransduction (Fig. 2B). However, when we tested lysates generated by λ11 induction, cotransduction was observed for markers within a headful, but even very close markers were completely unlinked when they were separated by the predicted headful limit (Fig. 2B). Next, we determined if cos phages could also mediate lateral transduction by testing for the transfer of Cd8 markers downstream of the λ12 or phage DI attB sites in nonlysogenic or lysogenic strains. As expected, lysates of λ12 or DI produced by infection or lysogenic induction did not transfer cadmium resistance, showing that cos phages do not mediate lateral transduction. These results confirm our model that headful packaging initiates from the terS11 gene and efficiently initiates the next headful.

Because the frequency of lateral transfer was so high from the first λ11 headful and because packaging initiated so efficiently for the second headful (Fig. 2A), we reasoned that the packaging machinery could continue for many headfuls before diminishing into the low levels of GT. To test this, we used previous markers (Cd8p5 and Cd8p6) for the first two headfuls and inserted five additional markers 10 kb into each successive headful—that is, seven in total—into nonlysogenic strains or λ11 lysogens. As an additional control to measure GT by prophage induction, a strain was generated in which the attB11 was deleted so that λ11 could be lysogenized at a new attB11 inserted at the S. aureus pathogenicity island 4 (SaPI 4) attB (strain S4); in this strain, the λ11 prophage is not linked to the Cd8 markers. We found that for lysates generated by λ11 induction, lateral transduction transferred up to seven headfuls of markers at levels that were substantially higher than the frequencies observed for lysates generated from λ11 infection or from λ11 strain (strain S4) (Fig. 2C). Results obtained with phage 80u showed that lateral transduction-mediated Cd8 marker transfer was much greater than GT-mediated transfer for at least seven headfuls (Fig. 2D). These results show that lateral transduction can mediate high-frequency HGT of bacterial host DNA for several hundred kilobases before the frequencies diminish and smooth out into the basal levels of GT.

To simulate a more natural test for HGT, we assayed for lateral transduction resulting from spontaneous lysogenic induction. To test this, we mixed intact cells carrying λ11 lysogenic derivatives and containing Cd8p5 with a streptomycin-resistant host recipient and plated the mixture for cadmium resistance and streptomycin selection. As shown in fig. S9, spontaneous induction of λ11 resulted in a steady increase in lateral transductants from 4, 8, and 24 hours (>4000 transductants ml−1). By contrast, spontaneous GT by the strain carrying the λ11 prophage integrated into the SaPI 4 attB (strain S4) was just slightly more than that of the λ11 (ΔterS) negative control and of spontaneous streptomycin resistance of the donor strain. These results show that lateral transduction is a powerful mode of HGT that promotes considerable levels of genetic exchange, even in natural conditions of rare spontaneous lysogenic induction.

**In situ bidirectional replication enables phage maturation**

Phage production requires early genome excision, which is at odds with the in situ lateral transduction-packaging mechanism. In situ replication, understood as the ability some prophages have to initiate replication before excision, could create sufficient genomic redundancy to enable both lateral transduction and phage maturation to proceed in parallel. In support of this hypothesis, λ and P22 mutants defective for excision have been observed to replicate in situ ([10], [19]). Moreover, transcriptional analysis of the staphylococcal phages shows that the genes required for phage replication are expressed early, before xis transcription (Fig. 1 and fig. S2).

To test for in situ replication, we first determined whether the staphylococcal prophages exhibit escape replication—a phenomenon whereby the bacterial genome adjacent to occupied...
attB sites is amplified, owing to the initiation of bidirectional (theta) replication before prophage excision. We infected nonlysogenic strains or mitomycin C-induced lysogenic derivatives of 80a (carrying the WT or the rep-ori mutant prophage and thus incapable of replicating) in S. aureus and collected the total chromosomal DNA for whole-genome sequencing. At 0, 30, 60, and 120 min, we quantified the reads corresponding to 80a and the host DNA adjacent to the attB site and measured coverage relative to the average of the entire genome. Induction of the 80a lysogen showed strong amplification of 80a DNA (Fig. 3). Phage replication started before 60 min and was robust by 60 to 120 min after induction. Interestingly, host DNA flanking the 80a lysogen also showed considerable amplification by 60 to 120 min, confirming that the phage was still integrated and that escape replication had indeed amplified these regions. This phenomenon also explains the earlier observation (fig. S3) that the percentage of integrated 52a began to increase 60 min after induction. Host DNA amplification was distinct from phage replication, because it decreased linearly away from the phage origin of replication, similarly to that observed for chromosomal replication (fig. S10). By contrast, 80a infection of nonlysogenic strains showed strong amplification of phage DNA but not of host DNA. Similar results were observed for 52a, 52a, and several Newman phages (Fig. 3, B and C, and fig. S11). Hence, many staphylococcal prophages can initiate replication in situ.

To determine the role of in situ replication in lateral transduction and phage production, we designed a system in which the replication of 52a could be tightly regulated and inducible. To do this, we constructed a frameshift mutant (52a-fs) of the 52a rep gene (that controls bidirectional replication) so that the embedded origin of replication remained intact and the mutant could be complemented in trans with rep52a under the control of a tetracycline-inducible promoter. To test for lateral transduction, a Cd marker was inserted 5 kb downstream of the attB site. Derivatives of these 52a lysogenic strains were induced with mitomycin C (time (t) = 0 min), and anhydrotetracycline was added at 0, 30, 60, 90, and 120 min for replication expression. Because 52a replicase mutants are unable to lyse their host cells, owing to the lack of viral DNA replication, we used mechanical disruption to release all intracellular particles 2 hours after the addition of inducer. To assay for lateral transduction, the resulting phage lysates were tested as donors of cadmium resistance to S. aureus. For phage production, the lysates were tested for plaque formation on a recipient S. aureus that constitutively expressed rep52a. The WT and 52a-fs mutants were capable of high-frequency lateral transduction of the Cd marker (Fig. 4), indicating that in situ replication is not required for lateral transduction. However, the 52a-fs mutants were completely unable to produce plaque-forming units without complementation with rep52a. More importantly, complementation of the 52a-fs mutants only resulted in normal levels of phage production if rep52a was provided within 90 min of mitomycin C induction. This timing indicates that the 52a-fs mutants were incapable of being complemented at later time points, presumably because in situ packaging had compromised the integrity of the genomes. These results are consistent with the model (fig. S12) that early in situ replication provides genomic redundancy so that both in situ DNA packaging and excision (followed by phage maturation) can proceed in parallel.

Lateral transduction drives genome organization and evolution

On the basis of the high frequencies of lateral transduction, we speculated that the regions adjacent to phage attB sites in the direction of packaging could serve as platforms for high-frequency mobility for any DNA element. There are 10 phage and 5 SaPI attB sites scattered throughout the S. aureus chromosome. The further analysis of the regions flanking the phage attB sites revealed that nearly all of the SaPIs and the three staphylococcal chromosomal islands (vSa, vSa, and vSey) were positioned such that they could be highly transferred by lateral transduction (fig. S13). Notably, the localization of the phage attB sites and the directionality of the phage packaging suggest that most of the bacterial chromosome could also be mobilized by lateral transduction (fig. S13).

Although the SaPIs are well-characterized, highly mobile parasites of helper phages (22), specific mechanisms of transfer have not been identified for most of the chromosomal islands of all bacterial species, including vSa, vSa, and vSey. Lateral transduction could provide a mechanism to mobilize these islands, too. Indeed, we have already shown lateral transduction-mediated transfer of vSey by 80a in the cadmium-resistance transfer experiments, because the Cd marker in the second headful was inserted within this island (Fig. 2D). We have also directly tested for lateral transduction-mediated transfer of vSa by prophage 52a and found that a Cd marker 83 kb from the 52a attB site (SaS) was transferred at frequencies three orders of magnitude greater than those observed for GT (fig. S14). These results indicate that lateral transduction is a general mechanism for the high-frequency transfer of mobile genetic elements and pathogenicity islands in S. aureus.

To investigate the impact of lateral transduction on genome structure, gene content, and genetic variability, we compared 140-kb regions upstream and downstream of the Sa6 phage attB site from 100 complete S. aureus genomes. We found that the upstream regions were more highly conserved than the downstream regions, in terms of both gene synteny and gene similarity (fig. S15). This was primarily due to the presence of other phages and SaPIs downstream of the attB site. In addition, the number of predicted recombinant fragments in the conserved genes downstream of the attB site was significantly higher compared with those upstream (\( P = 1.066 \times 10^{-7} \) for paired Wilcoxon nonparametric hypothesis test for comparison of matched samples, and \( P = 0.0316 \) for unpaired t test for length of recombinant fragments) (fig. S16). By contrast, the nearby regions of the 52a attB site (Sa2) showed little gene variability and retained high levels of conservation, both upstream and downstream (fig. S17). However, in these regions, recombination is significantly much greater distal to one headful downstream of the attB site (fig. S18; paired Wilcoxon test \( P = 1.308 \times 10^{-7} \), unpaired Wilcoxon test for recombinant nucleotides \( P = 5.514 \times 10^{-7} \)). This region can still be classified as a recombination hotspot and encompasses the gene encoding the surface giant protein Ebh, which is involved in adhesion, bacterial envelope stability, and pathogenesis of staphylococcal infections (23, 24). Variants of Ebh are associated with increased sensitivity to certain antibiotics and reduced virulence (24). In a genome-wide analysis of recombination in S. aureus, Everett et al. found hotspots of recombination at insertion sites for mobile genetic elements (25). Moon et al. also observed phagemediated transfer of virulence-associated genes located in the vSa island flanking the phage vSaBov insertion site, without elucidating the mechanism (26). We found that HGT induced by lateral transduction has a major measurable impact on S. aureus genome structure and evolution: First, by promoting gene mobilization, lateral transduction leads to gain and loss of new functions, and second, it provides source material on which homologous recombination can act to generate genetic variability.

Discussion

Of the three modes of bacterial gene transfer (i.e., transformation, conjugation, and transduction), phage transduction is often regarded as the primary driving force of microbial evolution. Here we have identified and characterized not just an additional mode of natural phage transduction but potentially the most powerful one. In our model (fig. S12), bidirectional replication creates multiple integrated prophages so that lateral transduction and normal phage maturation can proceed in parallel. Interestingly, a similar model was proposed long ago for \( \lambda \) phage, where the induction of docL mutants, unable to excise, resulted in the production of noninfectious particles carrying bacterial DNA located to the right of the \( \lambda \) cos site (8–10). Although the model was similar to lateral transduction, the result was quite different, because docL mutants were unable to produce viable phage and the transducing particles required deoxyribonuclease treatment and the addition of purified tails to be infectious. By contrast, the staphylococcal phages naturally generate high titers of infectious transducing particles in the process of wild-type phage production.

Although late prophage excision is the first step in lateral transduction, the timing of \( xis \) expression has only been investigated in a few phages. In most phage genomes, the integrase...
(int) and xis genes are located in tandem and transcribed together, but for most staphylococcal phages, these genes are opposed, so that they are transcribed from different promoters. Because both integrase and excisionase are generally required for efficient excision, phages that have opposed int and xis genes or that differentially regulate these genes could be candidates for lateral transduction.

We have demonstrated here that the headful mechanism is essential for lateral transduction. Because this mechanism is not exclusive of the S. aureus phages but is widespread in nature, we anticipate lateral transduction will be a universal mechanism of gene transfer. This is currently being studied.

Because lateral transduction can promote the efficient transfer of several hundred kilobases, these spans essentially become large platforms of high-frequency gene transfer for any DNA element located within their boundaries. Considering that bacterial chromosomes often contain multiple prophages, this mode of transduction can transmit a large portion of the bacterial genome at exceptionally high frequencies in a single lytic event. Thus, lateral transduction creates high-volume channels of genetic exchange among hosts, which, in return, provides selection to keep prophages intact and functional. We believe this mechanism sheds light on many unexplained hotspots for recombination in bacterial genomes, just to name a few. Thus, our results indicate that phage-mediated lateral transduction is an extremely powerful force driving both bacterial and phage evolution.

REFERENCES AND NOTES


ACKNOWLEDGMENTS

Funding: This work was supported in part by the Singapore Ministry of Education (T1-201550-13), National Medical Research Council (BNIG15may013), and National University of Singapore (start-up funds) to J.C.; by a project grant (BB/I013873/1) and institute strategic grant funding (ISG: BB/E020021/3) from the Biotechnology and Biological Sciences Research Council (UK) to J.R.F.; by grant MR/M003876/1 from the Medical Research Council (UK), BB/S002873/1 from the Biotechnology and Biological Sciences Research Council (BBSRC, UK), and ERC-ADG-2014 Proposal no. 670932 Dut-signal (from EU) to J.R.P.; and by Wellcome Trust 201531/Z/16/Z to J.R.F. and J.R.P.

Author contributions: J.C. and J.R.P. conceived the study. J.C., N.Q.-P., Y.N.C., M.S.J.C., and A.F.-S. conducted the experiments. J.C. and J.R.P. wrote the manuscript. Competing interests: The authors declare no competing interests.

Data and materials availability: All data and code to understand and assess the conclusions of this research are available in the main text, supplementary materials, and the European Bioinformatics Institute repository via EBI accession number PRJEB27527.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/362/6411/207/suppl/DC1

Materials and Methods

Fig. S1 to S4

References (27–49)

15 March 2018; accepted 14 August 2018

10.1126/science.aat5867
Pathologizing *Staphylococcus*, fast

Bacteriophages are the main vehicle for gene swapping in bacteria, notoriously of pathogenicity islands and antibiotic resistance genes. Chen *et al.* noticed that the *Staphylococcus aureus* prophages do not excise from their host's genome until very late in their life cycles (see the Perspective by Davidson). Thus, the phage DNA is amplified while embedded in the bacterial chromosome. The resulting concatamers are processively packed into virus capsules while still integrated in the host chromosome. Each virion is only set loose when the capsule has reached physical capacity—a process called “headful” packaging. In situ amplification maximizes viral replication, and the headful mechanism means adjacent bacterial-host DNA also gets grabbed to fill the capsule. This process ensures that host genes are transmitted along with the phage.

*Science*, this issue p. 207; see also p. 152