Genome hypermobility by lateral transduction

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Abstract

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-packaging pathway, but instead excise late in their lytic program. Here, DNA packaging initiates *in situ* from integrated prophages, and large metameric spans including several hundred kilobases of the *S. aureus* genome are packaged in phage heads at very high frequency. *In situ* replication prior to 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.

One Sentence Summary: Genetic and genomic analyses reveal the most powerful mode of bacteriophage-mediated gene transfer, lateral transduction.

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, where 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 (GT or ST, respectively)(*3-5*). GT is the process by which *pac*-type phages can package any bacterial DNA and transfer it to another bacterium, whereas ST is limited to the transfer of specific sets of genes. GT results from the recognition of *pac* site homologs (also called pseudo-*pac* sites)(*6*, *7*) in host chromosomal or plasmid DNA, by the *pac*-type headful mechanism (*2*). The *cos*-type phages typically are not involved in GT because the probability

of two *cos* site homologs being found in the host DNA at an optimal length apart is exceedingly rare (*2*).

The formation of ST particles is more complicated than that of the GT mechanism, and our current understanding is based on the classical λ phage model, where aberrant excision events join part of the prophage to bacterial genes adjacent to their attachment site (*attB*) in the excised DNA (*5*). Since ST is limited to restricted sets of genes, it is assumed that most phage-mediated HGT events are governed by the GT mechanism. However, aberrant prophage excision is not the only means of ST particle formation, and other mechanisms involving the initiation of DNA packaging from the un-excised viral genome (*in situ* packaging) have been proposed (*8*). Early studies showed that artificially generated mutants of *cos*- and *pac*-type phages (λ and P22, respectively) could package headfuls of viral DNA still connected to adjacent bacterial DNA. These particles were not mature and required *in vitro* nuclease treatment (in λ) and the attachment of purified phage tails to be fully infective (*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.

Prophages typically excise and circularize early after induction, and the ensuing replication forms head-to-tail concatemers that are packaged by the terminase machinery. The order of this sequence of events is important, because DNA packaging prior to 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*).

We have discovered that the resident prophages of *Staphylococcus aureus* have atypical lytic programs, because they do not excise until late in their life cycles. The potentially

detrimental effects of delayed excision are offset by *in situ* bidirectional replication, which creates multiple integrated genomes so that both *in situ* DNA packaging and phage maturation can proceed in parallel. As a result, staphylococcal phages naturally generate high titers of transducing particles in the process of wild type phage production.

Delayed prophage excision results in DNA packaging from integrated viral genomes.

In a previous study on phage transcriptional activators, by using tiling microarray analysis on a *S. aureus* strain lysogenic for phage 80α we showed that transcription of the excisionase gene (*xis*) was not activated until late (30-60 minutes) after induction (*16*). This result indicated that the 80α prophage may delay excision but is not defective for phage production. Therefore, we performed transcriptional profiling using 80α as a model and compared it with other staphylococcal phages, including ϕ11, ϕNM1, and ϕNM2. 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 minutes afterwards. Consistent with our previous findings, transcriptional activation of *xis* occurred in all the phages between 30 to 60 minutes after prophage induction (Fig. 1, Fig. S2). To correlate the onset of *xis* expression with prophage excision, the 80α and ϕ11 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 percent of integrated prophage. The percent of integrated 80α steadily declined after 30 minutes (Fig. S3), matching the timing of *xis* transcriptional activation and confirmed excision was delayed. Interestingly, after 60 minutes, the decrease in percent of 80α integration began to slow and that of ϕ11 began to increase (see below).

We reasoned that DNA packaging could initiate from the integrated genome because of the delay in prophage excision, and since 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 ϕ11. Phage *pac* sites are often embedded in structural *terS* genes and direct unidirectional packaging toward the 3' end of the gene (*1*); likewise for the staphylococcal phages (Fig. S4). Since the *ter*S genes are located near the center of the 80α and ϕ11 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^R) was inserted in the *S. aureus* chromosome 5 kb downstream of the ϕ11 *attB* site ($attB_{\phi 11}$), in a phage-free or ϕ11 lysogen, for both intact wild type (wt) and *terS* deleted mutants (Δ*terS*)(Fig. S5). A distance of 5 kb was chosen because it is well within a headful capacity for ϕ11 (~32 kb from the $terS_{\phi 11}$), while providing sufficient flanking DNA for homologous recombination in the non-lysogenic recipient host strain. Since the viral genome is extrachromosomal after infection, a non-lysogenic host was infected with ϕ11 to measure GT. To measure *in situ* packaging the ϕ11 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 ϕ11 infection of the non-lysogen transferred the Cd^R_1 marker at a frequency of 1E+2 to 1E+3 transductants ml^{-1} (Fig. 1B), consistent with GT frequencies of other chromosomal markers in *S. aureus* (*17*). In contrast, ϕ11 prophage induction transferred the Cd^R_1 at very high frequencies, three orders of magnitude greater than those observed for GT, in a $terS_{\phi 11}$-dependent manner (Fig. 1B). Since 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^R_1 marker. Other explanations include ST by an unusually efficient aberrant

excision mechanism, super-infection by phages released early in the lytic cycle, or as the transcriptomic analyses indicates, the prophage initiated packaging prior to excision.

For excision to be efficient, one possibility is that cryptic repeated sequences in both the ϕ11 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^{R}_{1} marker at 5kb distance downstream of the $attB_{80\alpha}$ in an 80α lysogen and tested for transfer of Cd resistance. Induction of the 80α lysogen resulted in high transfer frequencies like those from ϕ11 induction (Fig. 1C). Other phages including, ϕNM1 and ϕNM2, which use the same *attB* as ϕ11 and 80α, also showed high frequency transduction of the Cd^{R}_{1} markers 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* (N303D) mutant that is defective for recombination but not for LexA cleavage or the SOS response (*18*). Lysates from ϕ11 induction in a wt or *recA* (N303D) background transferred the Cd^{R}_{1} marker at comparable frequencies (Fig. S7), showing that recombination is not involved in Cd^{R}_{1} packaging. Moreover, polymerase chain reaction analysis of 100 Cd resistant colonies confirmed that the transductants of 80α prophage 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 the wt phage could initiate packaging from the un-excised genomes (*12*). Therefore, we induced ϕ11 in the presence of sodium citrate at 100 and 200 mM, which was sufficient to block all phage adsorption. This experiment showed

that the packaging and transfer frequencies of the Cd^R_1 marker was unaffected (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, we term lateral transduction.

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

The high frequency of Cd^R transfer by the lateral transfer mechanism indicated headful packaging occurs off the integrated prophage after induction of the lysogen. To test this possibility, we inserted two additional downstream markers (Cd^R_2 and Cd^R_3) within a headful capacity, two markers (Cd^R_4 and Cd^R_5) beyond a headful capacity, and one marker (Cd^R_6) upstream of the $attB_{\phi 11}$ in non-lysogenic strains or ϕ11 lysogens. Next, lysates resulting from ϕ11 infection of a non-lysogenic strain or from prophage induction were tested for marker transfer. As expected, in lysates from ϕ11 infection we observed low levels of transfer typical of GT (Fig. 2A). In contrast, lysates from ϕ11 induction resulted in high frequencies of transduction for all markers, located in the directionality of packaging i.e., Cd^R_{2-5} (Fig. 2A). Only minor reductions in the transfer frequencies of the Cd^R_4 and Cd^R_5 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 (Tet^R) cassettes upstream and downstream of the $attB_{\phi 11}$, and paired them with the previous Cd^R markers i.e., with Cd^R_6 or downstream Cd^R_{1-4}, respectively, in non-lysogenic strains or ϕ11 lysogens (Fig. 2B). Then we tested the lysates generated from ϕ11 infection or by prophage induction (GT or lateral transduction, respectively) for co-transduction of the two markers by selecting for Cd resistance and scoring for Tet^R. As expected for GT, we found

that for lysates generated by ϕ11 infection, all co-transduction frequencies were inversely proportional to their distance apart, where the shortest distances exhibited the highest percentage of co-transduction (Fig. 2B). However, when we tested lysates generated by ϕ11 induction, co-transduction 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 Cd^R markers downstream of the ϕ12 or DI *attB* sites in non-lysogenic or lysogenic strains. As expected, lysates of ϕ12 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 $terS_{\phi 11}$ gene and efficiently initiates the next headful.

Since the frequency of lateral transduction was so high from the first ϕ11 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 (Cd^R_2 and Cd^R_5) for the first two headfuls, and inserted five additional markers 10 kb into each successive headful i.e., seven in total, into non-lysogenic strains or ϕ11 lysogens. As an additional control to measure GT by prophage induction, a strain was generated in which the $attB_{\phi 11}$ was deleted so that ϕ11 could be lysogenized at a new $attB_{\phi 11}$ inserted at the SaPI 4 *attB*; note that in this strain, the ϕ11 prophage is not linked to the Cd markers. We found that for lysates generated by ϕ11 induction, lateral transduction transferred up to seven headfuls of markers at levels that were significantly higher than the frequencies observed for lysates generated from ϕ11 infection or induction from ϕ11 (S4) (Fig. 2C). Results obtained with phage 80α, showed that lateral transduction-mediated Cd^R marker transfer was much greater than GT for at least seven headfuls (Fig. 2D). In summary, these results show that lateral transduction can mediate high

frequency HGT of bacterial host DNA for several hundred kb 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 ϕ11 lysogenic derivatives and containing Cd^{R}_{2} with a streptomycin-resistant host recipient, and plated the mixture for cadmium resistance and streptomycin selection. As shown in Fig. S9, spontaneous induction of ϕ11 resulted in a steady increase in lateral transductants from 4, 8, and 24 hours (>4.0 E+03 transductants ml^{-1}). In contrast, spontaneous GT by the strain carrying the ϕ11 prophage integrated into the SaPI 4 *attB* (strain S4) was just slightly more than that of the ϕ11 (Δ*ter*S) 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 significant 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, first we 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 prior to prophage excision. We either infected non-lysogenic strains or mitomycin C-induced lysogenic derivatives of 80α (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 minutes, we quantified the reads corresponding to 80α and the host DNA adjacent to the $attB_{80\alpha}$ site and measured coverage relative to the average of the entire genome. Induction of the 80α lysogen showed strong amplification of 80α DNA (Fig. 3). Phage replication started before 60 minutes and was robust by 60-120 minutes post induction. Interestingly, host DNA flanking the 80α lysogen also showed significant amplification by 60-120 minutes, 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 ϕ11 began to increase 60 minutes after induction. Host DNA amplification was distinct from phage replication, as it decreased linearly away from the phage origin of replication, similarly to that observed for chromosomal replication (Fig. S10). In contrast, 80α infection of non-lysogenic strains showed strong amplification of phage DNA but not of host DNA. Similar results were observed for ϕ11, ϕ52A and several Newman phages (Fig. 3B-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 ϕ52a could be tightly regulated and inducible. To do this, we constructed a frame-shift mutant (ϕ52a-fs) of the ϕ52a *rep* gene (that controls bi-directional replication) so that the embedded origin of replication remained intact and the mutant could be complemented in trans with $rep_{\phi 52a}$ under the control of a tetracycline-inducible promoter. To test for lateral transduction, a Cd^R marker was inserted 5kb downstream of the $attB_{\phi 52a}$ site. Derivatives of these ϕ52a lysogenic strains were induced

with mitomycin C (t=0) and anhydrotetracycline was added at 0, 30, 60, 90, and 120 minutes for replicase expression. Because ϕ52a replicase mutants are unable to lyse open 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 $rep_{\phi 52a}$. Wild type and the ϕ52a-fs mutants were capable of high frequency lateral transduction of the Cd^R marker (Fig. 4), indicating that *in situ* replication is not required for lateral transduction. However, the ϕ52a-fs mutants were completely unable to produce plaque-forming units without complementation with $rep_{\phi 52a}$. More importantly, complementation of the ϕ52a-fs mutants only resulted in normal levels of phage production if $rep_{\phi 52a}$ was provided within 90 minutes of mitomycin C induction. This timing indicates that the ϕ52a-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

Based on 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 *S. aureus* pathogenicity islands (SaPIs) *attB* sites scattered throughout the *S. aureus* chromosome (*20*, *21*). Further analysis of the regions flanking the phage *attB* sites revealed that nearly all of the SaPIs and the three staphylococcal chromosomal islands (νSaα, νSaβ and νSaγ) were positioned such that they could be highly transferred by lateral transduction (Fig S13). Remarkably, the localisation of

the phage *att*B 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 νSaα, νSaβ, and νSaγ. Lateral transduction could provide a mechanism to mobilize these islands too. In fact we have already shown lateral transduction-mediated transfer of νSaγ by 80α in the Cd resistance transfer experiments, because the Cd^{R}_{2} marker in the second headful was inserted within this island (Fig. 2D). We have also directly tested for lateral transduction-mediated transfer of νSaα by prophage ϕ52a, and found that a Cd^{R} marker 83 kb from the Sa6 *attB* site 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 a hundred 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= 1.066-07, paired *Wilcoxon* non-parametric hypothesis test for comparison of matched samples and unpaired *t-test* p-value length of recombinant fragments = 0.0316) (Fig. S16). By contrast, the nearby regions of the Sa2 phage *attB* site 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*-value = 1.308e-08, unpaired Wilcoxon test for recombinant nucleotides $p\text{-value}_{Sa2}$ = 5.514e-07). This region can still be classed as a recombination hotspot and encompasses the gene encoding the surface giant protein Ebh, 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 genome-wide analysis of recombination in *S. aureus*, Everitt *et al.* found hot spots of recombination at insertion sites for mobile genetic elements (*25*). Moon *et al.* also observed phage-mediated transfer of virulence-associated genes located in the νSaβ island flanking the phage ϕSaBov 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. In this report, we have identified and characterized not just an additional mode of natural phage transduction, but potentially the most powerful. 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 λ phage, where the induction of *doc*L mutants, unable to excise, resulted in the production of non-infectious particles carrying bacterial DNA located to the right of the λ *cos* site (*8-10*). Although the model was similar to lateral transduction, the result was quite different, as *doc*L mutants were unable to produce viable phage and the transducing particles

required DNase treatment and the addition of purified tails to be infectious. In contrast, the staphylococcal phages naturally generate high titers of infectious transducing particles in the process of wild type phage production.

While late prophage excision is the first step in lateral transduction, the timing of *xis* expression has been only investigated in a few of phages. In most phage genomes the *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. Since both integrase and excionase are generally required for efficient excision, phages with 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. Since this mechanism is not exclusive of the *S. aureus* phages but widespread in nature, we anticipate lateral transduction will be a universal mechanism of gene transfer. This is currently under study.

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. Since 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, but which in return, provides selection to keep prophages intact and functional. We believe this mechanism sheds light on many genomic conundrums that have gone unexplained for decades in microbiology, such as the mosaicism of the packaging modules of phages, why phage-carried toxins and virulence factors are typically found on certain ends of phage genomes, how some chromosomal islands can be highly transferred, and the occurrence of

inexplicable hot spots 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.

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

Competing interests: Authors declare no competing interests.

List of Supplementary Materials:

Materials and Methods

Figures S1-S18

Tables S1-S4

Figure 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 strand. An 80α lysogen was treated with mitomycin C and samples were analyzed without induction (light blue) or at 30 (early genes, green) and 60 minutes (late genes, red) after induction. **(B and C)** Transfer of Cd resistance markers downstream of *attB* sites for **(B)** ϕ11 and a Cd^R_1 5 kilobase downstream of the $attB_{\phi 11}$ and **(C)** 80α and a Cd^R 4 kilobase downstream of the $attB_{80\alpha}$. Non-lysogenic strains (light blue) were infected (F) or the lysogenic wt (+, dark blue) and *terS* deletion (Δ, gray) strains were induced (D) with mitomycin C and the lysates tested for transduction into *S. aureus*. Transduction units (TrU) ml^{-1} were normalized by plaque forming units (PFU) ml^{-1} and represented as the TrU of an average phage titer (1E+9 PFU). TrU ml^{-1} for Δ*terS* were <10. Values are means ± SD (n = 3 independent samples).

Figure 2. Lateral transduction transfers large metameric spans of the bacterial chromosome at high frequencies by the headful packaging mechanism.

(A) ϕ11 tested for transfer of Cd^R markers upstream (Cd^R_6) of the $attB_{\phi 11}$, and downstream within ($Cd^R_{1,2,3}$) and beyond ($Cd^R_{4,5}$) a capsid headful capacity (HF). **(B)** Co-transduction frequencies for strains containing both a Cd^R and a Tet^R marker at varying distances apart. 100 Cd^R transductants from a ϕ11 infection (light blue) or lysogen induction (dark blue) were tested for Tet^R and the frequency represented as the (Tet^R/Cd^R) x 100%. The headful (HF) limit indicated does not account for marker expansion. **(C and D)** Transfer of Cd^R markers in seven successive headfuls for **(C)** ϕ11 and **(D)** 80α. **(A, C, and D)** Non-lysogenic strains

(light blue) were infected (F) or the lysogenic wt (+, dark blue), ϕ11 (S4, blue), and *terS* deletion (Δ, gray) strains were induced (D) with mitomycin C and the lysates tested for transduction into *S. aureus*. Strain ϕ11 (S4) has ϕ11 integrated at the SaPI 4 *attB* instead of the natural $attB_{\phi 11}$. Transduction units (TrU) ml^{-1} were normalized by plaque forming units (PFU) ml^{-1} and represented as the TrU of an average phage titer (1E+9 PFU). TrU ml^{-1} for Δ*terS* were <10. For all panels, values are means ± SD (n = 3 independent samples).

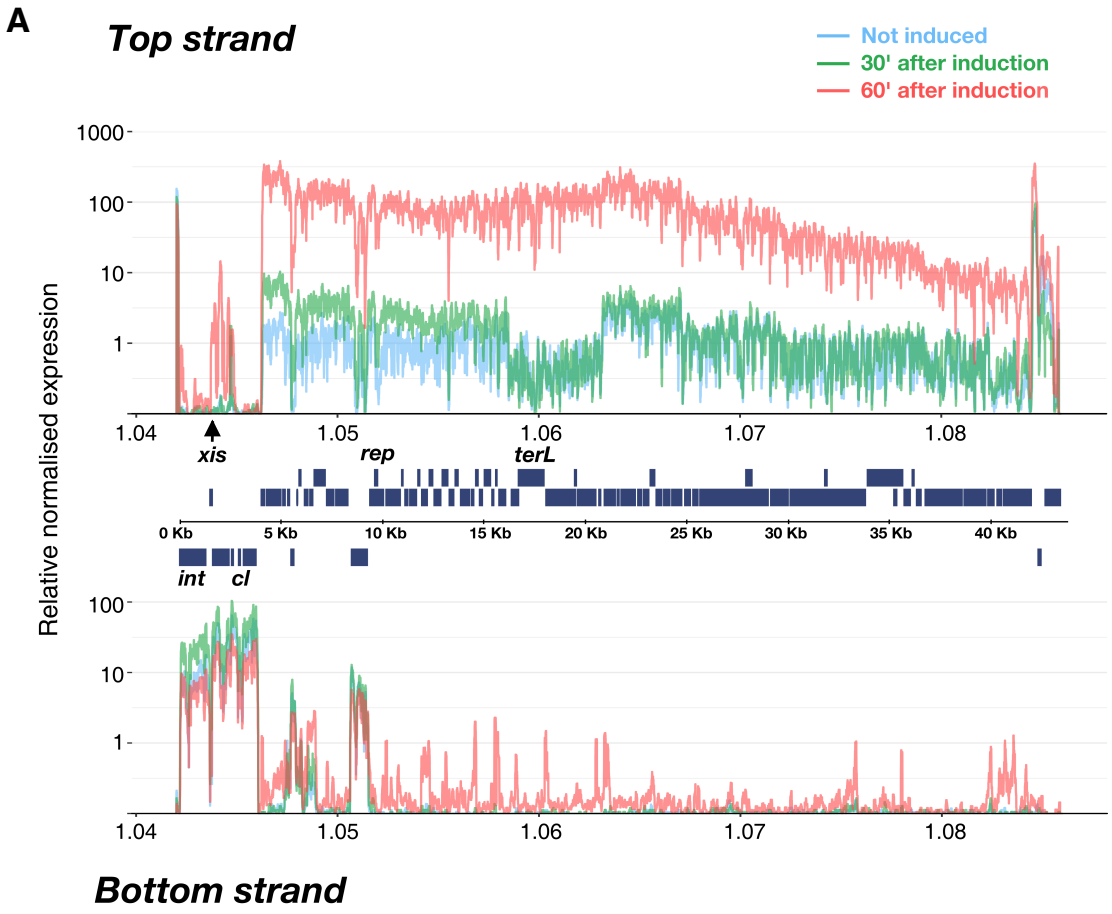
Figure 3. Staphylococcal phages replicate *in situ* before excision.

Relative abundance of phage genomic DNA and the chromosomal regions proximal to where they integrate for (A) 80α, (B) ϕ11, and (C) ϕ52a. Samples were analyzed at 0 (blue), 30 (light blue), 60 (orange) and 120 minutes (red) post-induction with mitomycin C. Shaded grey rectangles represent the location of the prophages in the *S. aureus* chromosome. The following strains were analyzed: 80α lysogen, ϕ11 lysogen or ϕ52A lysogen; derivatives of these strains carrying mutations in both the *ori* site and *rep* genes (80α *ori* deletion or ϕ11 *ori* deletion), non-lysogenic strain infected with 80α, and the non-lysogenic strain. For the 80α and ϕ52A lysogens, the t = 0 samples obscure the t = 30 samples.

Figure 4. *In situ* replication is required for phage reproduction but not for lateral transduction.

(A) Lysogens of ϕ52a and ϕ52a-fs ($rep_{\phi 52a}$ frame-shift) were induced with mitomycin C. Anhydrotetracycline was added at 0, 30, 60, 90, and 120 minutes for complementation by P_{tet}-$rep_{\phi 52a}$ or un-induced (U). The cells were mechanically lysed 2 hours after the addition of anhydrotetracycline and the lysates tested for plaque forming units (PFU) ml^{-1} on a *S. aureus* host that constitutively expresses $rep_{\phi 52a}$. The results are represented as the (PFU / PFU_{wt}) x

100%. **(B)** The same lysates in (A) were tested for lateral transduction of the Cd^R marker 5kb downstream of the $attB_{\phi 52a}$ site to *S. aureus.* The results are represented as transduction units (TrU) ml^{-1}. Values are means ± SD (n = 3 independent samples).


A
Top strand
Not induced
30' after induction
60' after induction
Relative normalised expression
1000
100
10
1
1.04
1.05
1.06
1.07
1.08
xis
rep
terL
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10 Kb
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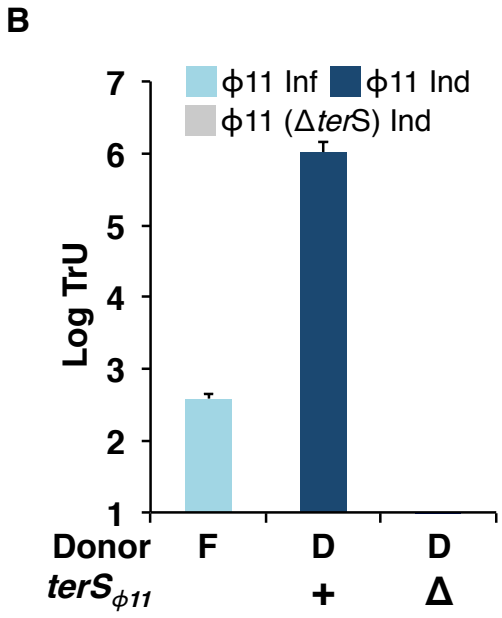
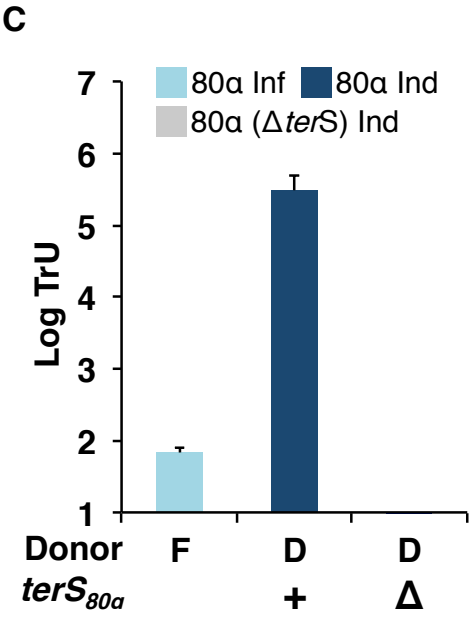
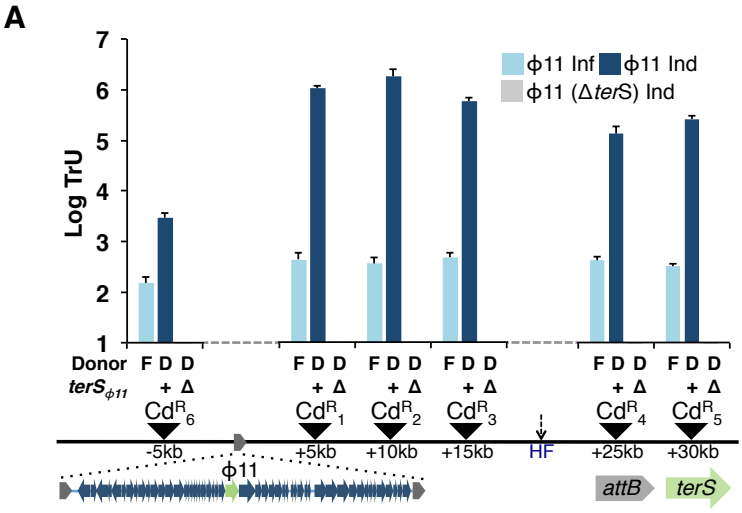
30 Kb
35 Kb
40 Kb
int
cI
Bottom strand


B
ϕ11 Inf
ϕ11 Ind
ϕ11 (ΔterS) Ind
Log TrU
7
6
5
4
3
2
1
Donor
terS ϕ11
F
D +
D Δ


C
80α Inf
80α Ind
80α (ΔterS) Ind
Log TrU
7
6
5
4
3
2
1
Donor
terS 80α
F
D +
D Δ


A
Log TrU
ϕ11 Inf
ϕ11 Ind
ϕ11 (ΔterS) Ind
Donor
terS ϕ11
F D D
+ Δ
CdR 6
CdR 1
CdR 2
CdR 3
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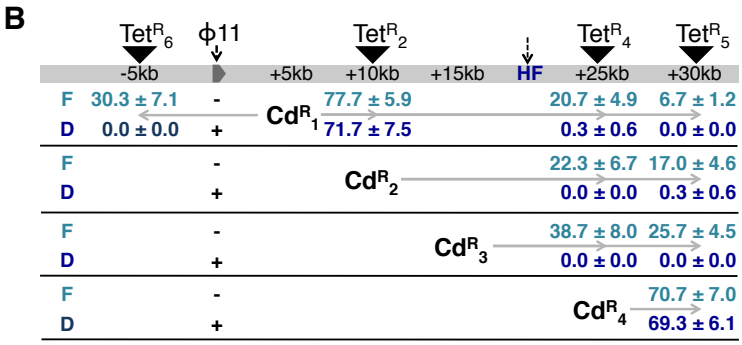
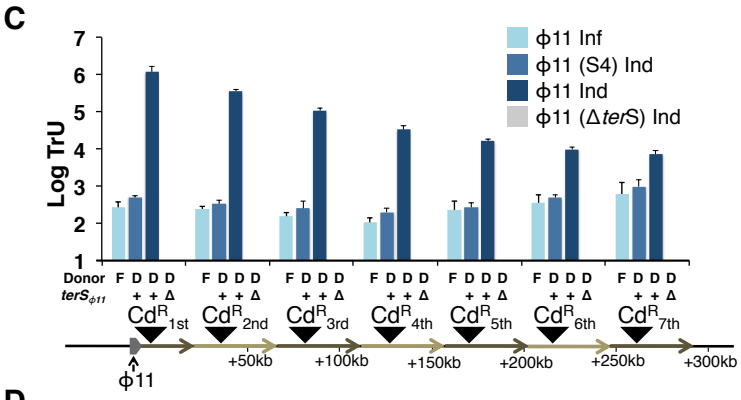
+15kb
HF
+25kb
+30kb
ϕ11
attB
terS


B
TetR 6
ϕ11
TetR 2
TetR 4
TetR 5
-5kb
+5kb
+10kb
+15kb
HF
+25kb
+30kb
F 30.3 ± 7.1 - CdR 1 77.7 ± 5.9 20.7 ± 4.9 6.7 ± 1.2
D 0.0 ± 0.0 + 71.7 ± 7.5 0.3 ± 0.6 0.0 ± 0.0
F - CdR 2 22.3 ± 6.7 17.0 ± 4.6
D + 0.0 ± 0.0 0.3 ± 0.6
F - CdR 3 38.7 ± 8.0 25.7 ± 4.5
D + 0.0 ± 0.0 0.0 ± 0.0
F - CdR 4 70.7 ± 7.0
D + 69.3 ± 6.1


C
Log TrU
ϕ11 Inf
ϕ11 (S4) Ind
ϕ11 Ind
ϕ11 (ΔterS) Ind
Donor
terS ϕ11
F D D D
+ + Δ
CdR 1st
CdR 2nd
CdR 3rd
CdR 4th
CdR 5th
CdR 6th
CdR 7th
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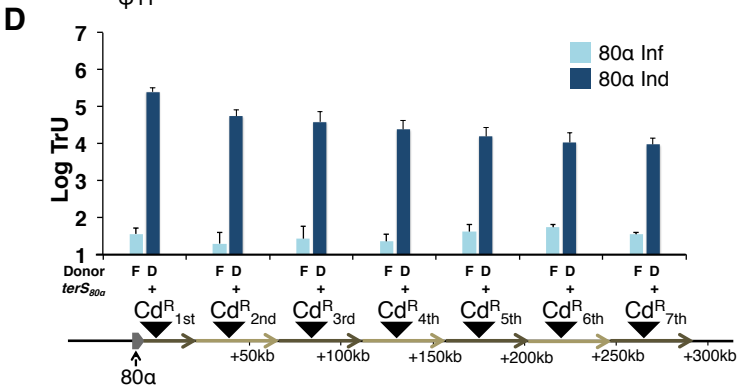
+50kb
+100kb
+150kb
+200kb
+250kb
+300kb


D
Log TrU
80α Inf
80α Ind
Donor
terS 80α
F D
+
CdR 1st
CdR 2nd
CdR 3rd
CdR 4th
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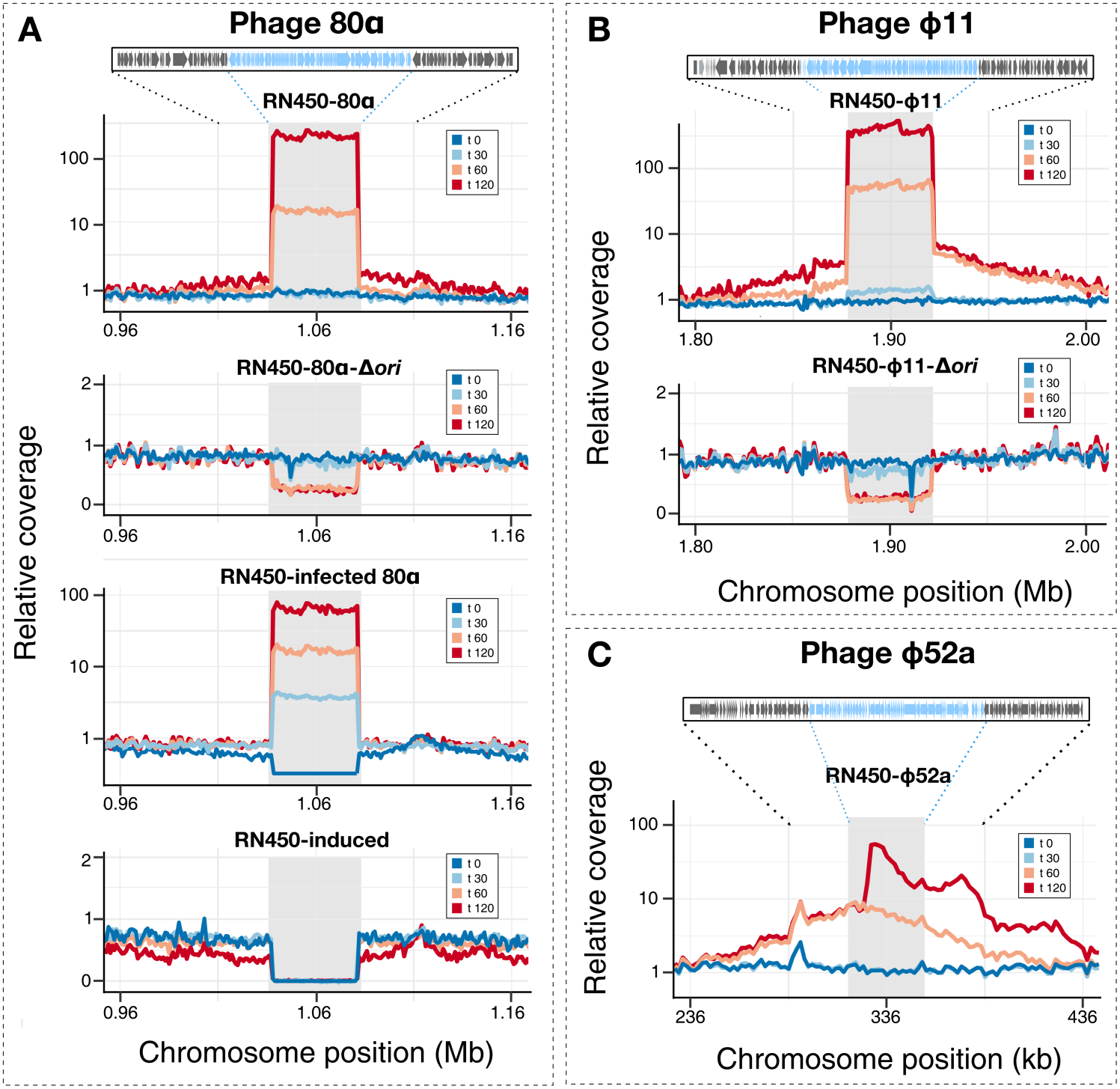
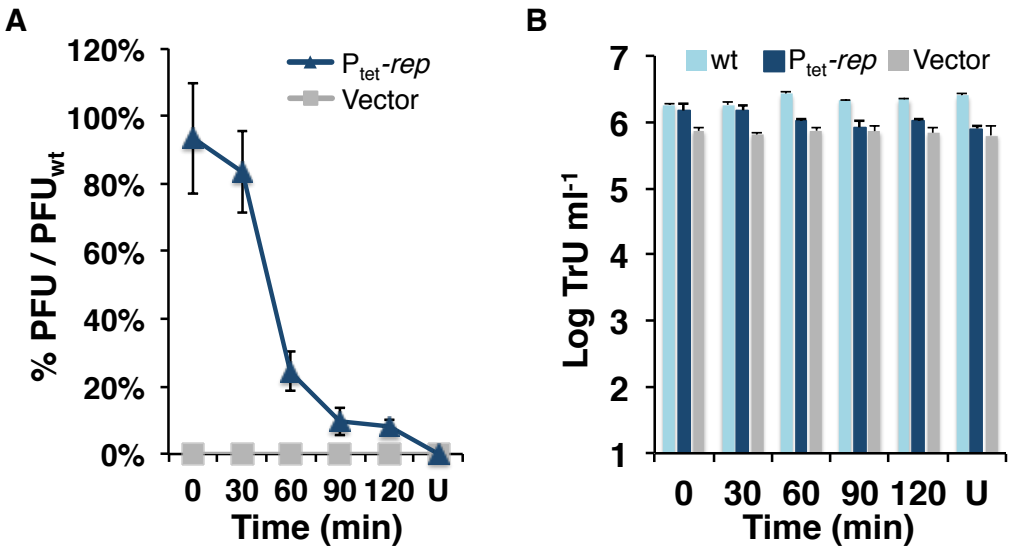
80α
+50kb
+100kb
+150kb
+200kb
+250kb
+300kb


A
Phage 80α
RN450-80α
RN450-80α-Δori
RN450-infected 80α
RN450-induced
t 0
t 30
t 60
t 120
Relative coverage
Chromosome position (Mb)
B
Phage ϕ11
RN450-ϕ11
RN450-ϕ11-Δori
Relative coverage
Chromosome position (Mb)
C
Phage ϕ52a
RN450-ϕ52a
Relative coverage
Chromosome position (kb)


A
120%
100%
80%
60%
40%
20%
0%
% PFU / PFU$_{wt}$
P$_{tet}$-*rep*
Vector
0 30 60 90 120 U
Time (min)
B
7
6
5
4
3
2
1
Log TrU ml^{-1}
wt
P$_{tet}$-*rep*
Vector
0 30 60 90 120 U
Time (min)