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Moonlighting bacteriophage proteins derepress staphylococcal pathogenicity islands

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Abstract

Staphylococcal superantigen-carrying pathogenicity islands (SaPIs) are discrete, chromosomally integrated units of ~15 kilobases that are induced by helper phages to excise and replicate. SaPI DNA is then efficiently encapsidated in phage-like infectious particles, leading to extremely high frequencies of intra- as well as intergeneric transfer[1–3]. In the absence of helper phage lytic growth, the island is maintained in a quiescent prophage-like state by a global repressor, Stl, which controls expression of most of the SaPI genes[4]. Here we show that SaPI derepression is effected by a specific, non-essential phage protein that binds to Stl, disrupting the Stl–DNA complex and thereby initiating the excision-replication-packaging cycle of the island. Because SaPIs require phage proteins to be packaged[5,6], this strategy assures that SaPIs will be transferred once induced. Several different SaPIs are induced by helper phage 80α and, in each case, the SaPI commandeers a different non-essential phage protein for its derepression. The highly specific

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interactions between different SaPI repressors and helper-phage-encoded antirepressors represent a remarkable evolutionary adaptation involved in pathogenicity island mobilization.

Pathogenicity islands have a major role in spreading virulence genes among bacterial populations. A notable example are the phage-related pathogenicity islands of staphylococci, the SaPIs, which are responsible for the inter- as well as intrageneric spread of toxins—such as TSST-1 (toxic shock syndrome toxin) and other superantigens—through the exploitation of specific staphylococcal helper phages for high-frequency transfer within phage-encoded particles[7]. Stable maintenance of SaPIs in the absence of helper phage requires a SaPI-encoded repressor, Stl, which, like classic prophage repressors, binds to a region between two divergent promoters that initiate the major SaPI transcripts and thus inhibits expression of most of the SaPI genes[4]. Mutations inactivating *stl* cause SaPI excision and replication in the absence of a helper phage[4], suggesting that the primary regulatory function of the helper phage is to relieve Stl repression. To elucidate phage-mediated SaPI induction, we exploited the documented interference of SaPIs with the growth of their helper phages, which reduces phage burst size by 10–100 fold[1] and blocks plaque formation. We reasoned that phage mutants unable to relieve Stl repression would form plaques on a SaPI-containing strain because SaPI genes interfering with phage lytic growth would not be expressed. We used two different, well studied helper phages, 80α (GenBank accession NC_009526) and ϕ11 (NC_004615), and three different SaPIs—SaPI1, SaPIbov1 and SaPIbov2[7]. All SaPIs encode an Stl homologue, but these proteins are very poorly conserved (Supplementary Fig. 1). Whereas ϕ11 induces only SaPIbov1, 80α has been shown to induce at least five different SaPIs (SaPI1, SaPI2, SaPIbov1, SaPIbov2 and SaPIn1[7]) with widely divergent Stl proteins, raising the question of how the putative 80α derepressor protein could have such broad specificity.

Spontaneous 80α mutants able to form plaques on *Staphylococcus aureus* strain RN4220 containing SaPIbov1 were readily obtained. These mutant phages had lost the ability to mobilize the island, consistent with failure to relieve Stl-mediated repression. Eighteen independent SaPIbov1-resistant 80α mutants carried point mutations in open reading frame (ORF)32, a gene annotated as *dut* based on homology with dUTPases (Supplementary Fig. 2). Missense and nonsense mutations were obtained, suggesting that this gene was both necessary for SaPIbov1 induction and non-essential for the phage. This was confirmed by the introduction of an inframe deletion in 80α *dut*, which also eliminated SaPIbov1 mobilization but did not impair phage growth (Table 1). Similar results (Table 1 and Fig. 1a) were obtained by deleting the *dut* homologue of ϕ11, another phage that can mobilize SaPIbov1, confirming the role of *dut* in SaPIbov1 induction.

As mentioned earlier, 80α mobilizes a number of different SaPIs. Interestingly, the 80α SaPIbov-resistant *dut* mutants, which plate normally on a SaPIbov1-containing strain, were still unable to form plaques on strains containing either SaPI1 or SaPIbov2, and were undiminished in mobilization of either of these islands (Table 1). This observation raised a surprising possibility for helper-phage–SaPI specificity; namely, that phage 80α possesses further genes for derepressing these other SaPIs. Using the same selection strategy, 80α mutants resistant to SaPI1 and SaPIbov2 were isolated. SaPI1-resistant mutations were found in ORF22 (hereafter called *sri*), which encodes a DnaI binding protein that inhibits staphylococcal replication[8]. SaPIbov2-resistant mutations were found in ORF15, which encodes a small protein of unknown function. Construction of inframe deletions confirmed that these two extra phage genes were also non-essential and, once again, specific for inducing the SaPI on which they were selected (Table 1 and S.M.T., P. K. Damle, A.S. and G.E.C., unpublished data). Phage ϕ11, which cannot induce SaPI1 or SaPIbov2, lacks homologues of either of these non-essential 80α genes.

The cloned *dut* genes of 80α and ϕ11, as well as 80α ORF15, complemented the respective phage deletion mutants when expressed under inducing conditions from the Pcad promoter in expression vector pCN51[9] (Supplementary Tables 1 and 2). The cloned *dut* genes had no effect on SaPI induction by the phage mutants with defects in ORF15 or *sri* (data not shown). Similar complementation studies were not performed with 80α *sri* because of the toxicity of this gene to *S. aureus.* An alternative approach to studies of Sri activity will be reported elsewhere (M. Harwich and G.E.C., unpublished data).

Expression of the cloned genes in SaPI-containing strains demonstrated that *dut* and ORF15 were sufficient to induce their respective SaPIs. As shown in Fig. 1b (lanes 1 and 3), when overexpressed, the cloned ϕ11 and 80α *dut* genes induced SaPIbov1 excision and replication. Similarly, plasmid-encoded 80α ORF15 induced SaPIbov2 excision and replication (Supplementary Fig. 3). Finally, expression of 80α ORF15 allowed high-frequency SaPIbov2 transfer by ϕ11 (Supplementary Table 2), indicating that the absence of this gene in the ϕ11 genome is the cause of its inability to induce SaPIbov2.

We next investigated the relationship between dUTPase activity and SaPIbov1 induction. The aspartate at position 81 in ϕ11 Dut, predicted to be essential for activity[10], was replaced with alanine, and the lack of dUTPase activity was confirmed *in vitro* (Supplementary Table 3). This mutant protein (D81A) retained wild-type SaPIbov1 induction activity (Fig. 1b, c, lane 2), indicating that dUTPase activity, per se, is not responsible for SaPIbov1 induction. The protein encoded by the ϕ11 *dut* gene is required, however, as demonstrated by the lack of activity of a frameshift mutant (Fig. 1b, c, lane 5). Further evidence that the dUTPase and derepression activities are separate functions of the *dut* gene product was provided by one of the 80α *dut* mutants that had been selected for resistance to SaPIbov1 interference. This mutant, a D95E substitution, retained dUTPase activity (Supplementary Table 3) even though it was defective for SaPI derepression (Supplementary Table 1), confirming that the derepression and dUTPase activities are separate. Thus, Dut represents a true 'moonlighting' protein with two different and genetically distinct activities.

We expected Dut-mediated derepression to involve interference with *stl* expression or function rather than Stl cleavage, as Stl lacks the consensus cleavage motif common to phage repressors, and SaPI induction does not involve the SOS response[3] (see also Fig. 1c, lane 8). Using purified His-tagged proteins and a DNA probe consisting of the *stl-str* intergenic region, we showed first, by mobility shifts, that SaPIbov1 Stl binds to the site but ϕ11 Dut does not (Fig. 2a). This indicates that Dut does not act by competing with Stl for access to its regulatory binding site. Because this fragment includes the *stl* promoter, Dut also cannot act as a repressor of *stl* expression. Addition of Dut blocked the Stl-mediated gel shift in a dose-dependent manner (Fig. 2a, right), suggesting that derepression involves Dut binding to Stl. This predicted protein–protein interaction was confirmed by coexpression and affinity purification of His_6–$Stl_{SaPIbov1}$ and untagged Dut proteins. It was possible to co-purify a complex between His-$_6$–$Stl_{SaPIbov1}$ and $Dut_{\phi 11}$ (Fig. 2b, lane 1), whereas untagged $Dut_{\phi 11}$ alone did not bind to the resin (Fig. 2b, lane 2). $Dut_{\phi PH15}$, which does not derepress SaPIbov1 (Fig. 1b), did not co-purify with His_6–$Stl_{SaPIbov}$ (lane 3), confirming the specificity of the $Dut_{\phi 11}$–His_6–$Stl_{SaPIbov1}$ interaction. The identity of each of these bands was confirmed by amino acid sequencing and mass spectrometry. A similar interaction was observed with His-tagged 80α ORF15 and SaPIbov2 Stl (Fig. 2b, lane 4), as well as with 80α Sri and SaPI1 Stl (M. Harwich, A. Poliakov, J. Mobley and G.E.C., unpublished data), suggesting that the general mechanism of phage-induced SaPI derepression involves proteins that function as antirepressors, complexing with Stl to prevent it from binding to DNA.

If Dut acts by disrupting the binding of Stl to its target site, it should induce transcription of the Stl-repressed SaPI genes. This was confirmed using plasmid pJP674, which carries a β-lactamase reporter gene fused to xis, downstream of *str* and the Stl-repressed *str* promoter[4], and also encodes Stl (see Fig. 2c). Cloned *dut* genes were introduced on vector pCN51 and expression was tested in the presence or absence of an inducing concentration of $CdCl_2$. Induction of ϕ11 *dut*, but not PH15 *dut*, strongly increased β-lactamase expression from the *str* promoter (Fig. 2c). We conclude from these results that a SaPI-inducing Dut activates transcription by specifically disrupting the pre-formed Stl–DNA complex.

Insight into the possible domain involved in SaPIbov1 induction by Dut was provided by a comparison of predicted dUTPase sequences from various staphylococcal phages (Supplementary Fig. 2). This alignment showed high sequence similarity except for a central region of about 40 amino acids that was highly divergent among the *S. aureus* phage enzymes and was absent from the *S. epidermidis* phage PH15 dUTPase, which does not induce SaPIbov1 (Fig. 1b, c, lane 4 and Supplementary Table 1). Differential activity of the ϕ11 and 80α enzymes—which are fully conserved except for two residues in the amino-terminal region of the proteins and the divergent 40-aminoacid region, where they differ sharply (Fig. 3a)—suggested strongly that this region is involved in SaPIbov1 induction. The *dut* genes from these two phages had the same SaPIbov1 derepression activity when fully induced (Fig. 1b), but low constitutive expression of $dut_{80\alpha}$ in the absence of $CdCl_2$ failed to derepress SaPIbov1, although there was still full derepression by $dut_{\phi 11}$ under these conditions (Fig. 1c). Similar results in the absence of *dut* induction were seen in the complementation analysis reported in Supplementary Table 1. As the Dut protein levels produced from these constructs are comparable (Fig. 1c), the ϕ11 Dut is more effective than that of 80α in derepression of SaPIbov1. The difference in activity was mapped to the divergent region by exchanging the amino acids that differ between the ϕ11 and 80α Dut proteins and testing these derivatives for SaPIbov1 induction. Exchanging either of the two variable amino acids near the N terminus had no effect on derepression by either protein (Fig. 3b and Supplementary Table 1). However, when the divergent region was exchanged, the induction efficiency was transferred along with the exchanged amino acids (Fig. 3b and Supplementary Table 1). The possibility that differential expression of the two genes was responsible for the difference was ruled out by a western blot analysis (Fig. 3b), which confirmed that the two genes were expressed at the same levels.

The absence of this central divergent region from the PH15 enzyme suggested that it might be an independent domain involved in relief of SaPIbov1 repression. However, deletion of this entire region from $Dut_{\phi 11}$ inactivated both dUTPase activity and SaPI induction (not shown). Furthermore, insertion of the divergent region from $Dut_{\phi 11}$ (N91–I128) between residues I91 and V102 of Dut_{PH15} did not confer induction activity on the chimaeric protein and also eliminated its dUTPase activity, indicating that this region is involved in the overall structure of the protein. Further mutants and structural analysis will be required to elucidate fully the dual functions of these dUTPases and their interaction with SaPIbov1 Stl.

A similar difference was found for derepressors of SaPIbov2. Phage 85 does not induce SaPIbov2, but it does encode a homologue of 80α ORF15, designated ORF73 (Supplementary Fig. 4). Both 80α ORF15 and ϕ85 ORF73, when cloned in plasmid pCN51 and overexpressed, restored SaPIbov2 transfer in 80α ΔORF15 (Supplementary Table 2). However, with low basal expression, only 80α ORF15 fully complemented SaPIbov2 transfer, indicating that these phages, too, carry allelic variants of inducing genes with different affinity for the SaPI-encoded repressors. Analysis of these allelic variants (in progress) is likely to be informative of the induction mechanism.

The process by which related SaPIs have acquired the ability to exploit entirely unrelated phage proteins as antirepressors represents a remarkable evolutionary adaptation. A single phage protein may have been originally targeted; because SaPIs interfere with phage maturation, mutational modification of such a protein to escape from SaPI derepression could have a selective advantage for the phage. A second stage in SaPI evolution could have involved divergence of the SaPI repressor, enabling it to complex with a different phage protein. More extensive analysis of SaPI derepression and the role of phage genes may clarify this and other interesting issues that have been identified in this study.

METHODS

DNA methods

Probes for detection of phage and SaPI DNA in Southern blots were generated by PCR using primers SaPIbov1-112mE and SaPIbov1-113cB (SaPIbov1), Sip-16mB and Sip-10cE (SaPIbov2), and Orf-24-ϕ11-1mB and Orf-25-ϕ11-1c (ϕ11), which are listed in Supplementary Table 5. Labelling of the probes and DNA hybridization were performed according to the protocol supplied with the PCR-DIG DNA-labelling and chemiluminescent detection kit (Roche). Southern blots experiments were performed by standard procedures[11].

SaPI-resistant phage mutants were characterized by amplification of overlap-ping fragments by PCR and direct sequence analysis of gel-purified fragments. Sequencing was done by the Instituto de Biología Molecular y Celular de Plantas (IBMCP) and by Retrogen Inc.

Mobility shift assays

EMSAs were performed as described before[12] using purified His_6–Stl or/and His_6–dUTPase proteins and a DIG-labelled DNA fragment obtained by PCR using the oligonucleotides 19-20upbov2 and 19-20 dwbov (listed in Supplementary Table 5). In each case purified dUTPase, Stl or both were added to the DNA–protein-binding mixture.

Enzyme assays

β-Lactamase assays, using nitrocefin as substrate, were performed with cells in exponential growth phase as described[13], using a Thermomax (Molecular Devices) microtiter plate reader. β-Lactamase activities were recorded as initial slopes divided by cell density (maximum velocity (V_{max}))/OD_{650nm}.

dUTPase activity was assayed using His_6–dUTPase proteins purified after expression in E. coli. Enzyme assays were performed using the EnzCheck Pyrophosphate Assay Kit (Molecular Probes), as previously reported[14].

Plasmid construction

Plasmid constructs were prepared by cloning PCR products obtained with oligonucleotide primers as listed in Supplementary Table 5. All clones were sequenced by the Institute Core Sequencing Lab.

To introduce specific dut mutations into the phage, we used plasmid pMAD[15] for allelic exchange as previously described[3,4].

In the western blot assays, probing was carried out with anti-33Flag antibodies (Sigma), according to the protocol supplied by the manufacturer.

In-gel enzymatic digestion and mass fingerprinting

Protein bands of interest were analysed as previously described[6]. Mass spectroscopy of proteins was performed by the ProteoRed Institute, at the Centro de Investigación Príncipe Felipe.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Lindsay JA, Ruzin A, Ross HF, Kurepina N, Novick RP. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in Staphylococcus aureus. Mol. Microbiol. 1998; 29:527–543. [PubMed: 9720870]
2. Chen J, Novick RP. Phage-mediated intergeneric transfer of toxin genes. Science. 2009; 323:139–141. [PubMed: 19119236]
3. Úbeda C, et al. Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. Mol. Microbiol. 2005; 56:836–844. [PubMed: 15819636]
4. Úbeda C, et al. SaPI mutations affecting replication and transfer and enabling autonomous replication in the absence of helper phage. Mol. Microbiol. 2008; 67:493–503. [PubMed: 18086210]
5. Tallent SM, Langston TB, Moran RG, Christie GE. Transducing particles of Staphylococcus aureus pathogenicity island SaPI1 are comprised of helper phage-encoded proteins. J. Bacteriol. 2007; 189:7520–7524. [PubMed: 17693489]
6. Tormo MA, et al. Staphylococcus aureus pathogenicity island DNA is packaged in particles composed of phage proteins. J. Bacteriol. 2008; 190:2434–2440. [PubMed: 18223072]
7. Novick RP, Penades JR, Christie GE. The phage-related chromosomal islands of Gram-positive bacteria. Nature Rev. Microbiol. 2010 (in the press).
8. Liu J, et al. Antimicrobial drug discovery through bacteriophage genomics. Nature Biotechnol. 2004; 22:185–191. [PubMed: 14716317]
9. Charpentier E, et al. Novel cassette-based shuttle vector system for Grampositive bacteria. Appl. Environ. Microbiol. 2004; 70:6076–6085. [PubMed: 15466553]
10. Chan S, et al. Crystal structure of the Mycobacterium tuberculosis dUTPase: insights into the catalytic mechanism. J. Mol. Biol. 2004; 341:503–517. [PubMed: 15276840]
11. Ausubel, FM., et al. Current Protocols in Molecular Biology. John Wiley & Sons; 1990.
12. Úbeda C, et al. SaPI operon I is required for SaPI packaging and is controlled by LexA. Mol. Microbiol. 2007; 65:41–50. [PubMed: 17581119]
13. Ji G, Beavis R, Novick RP. Bacterial interference caused by autoinducing peptide variants. Science. 1997; 276:2027–2030. [PubMed: 9197262]
14. Varga B, et al. Active site of mycobacterial dUTPase: structural characteristics and a built-in sensor. Biochem. Biophys. Res. Commun. 2008; 373:8–13. [PubMed: 18519027]
15. Arnaud M, Chastanet A, Debarbouille M. New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, Gram-positive bacteria. Appl. Environ. Microbiol. 2004; 70:6887–6891. [PubMed: 15528558]

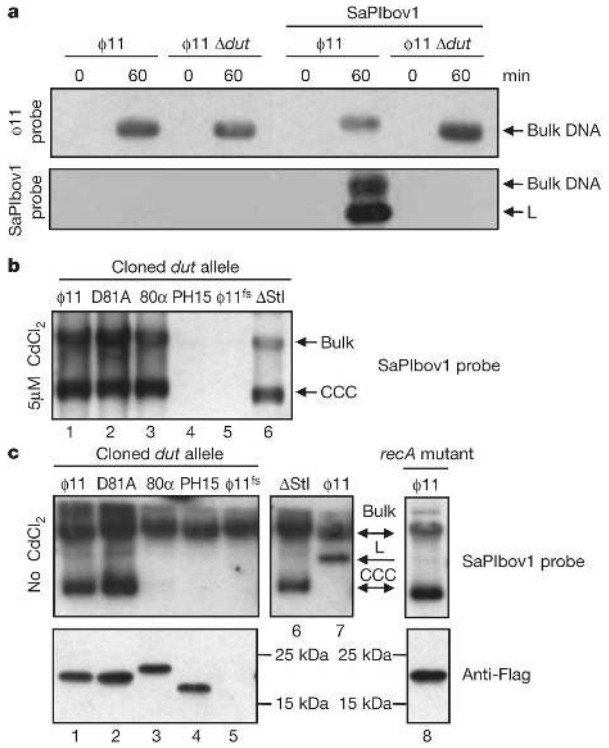

Figure 1. Induction of SaPIbov1 by different dut alleles

a, Southern blot of ϕ11 mutant lysates, from strains with (JP1794 and JP4125) or without (RN451 and JP4025) SaPIbov1 *tst::tetM*, as indicated. Samples were isolated 0 or 60 min after induction with mitomycin C, separated on agarose and blotted with a phage- or SaPIbov1-specific probe. Upper band is 'bulk' DNA, including chromosomal, phage and replicating SaPI; lower band is SaPI linear monomers (L) released from phage heads. **b**, SaPIbov1 excision and replication after induction of cloned *dut* genes from different staphylococcal phages. A non-lysogenic derivative of strain RN4220 carrying SaPIbov1 was complemented with plasmids expressing 3×Flag-tagged Dut proteins. One millilitre of each culture (optical density $(OD)_{540nm} = 0.3$) was collected 3 h after treatment with 5 μM $CdCl_2$ and used to prepare standard minilysates, which were resolved on a 0.7% agarose gel, Southern blotted and probed for SaPIbov1 DNA. Lane 1, JP6789; lane 2, JP6790; lane 3, JP6797; lane 4, JP6791; lane 5, JP6796; and lane 6, JP6772. In these experiments, because no helper phage is present, the excised SaPI DNA appears as covalently closed circular molecules (CCC) rather than the linear monomers that are seen following helper-phage-mediated induction and packaging (as in **c**, lane 7). **c**, SaPIbov1 excision and replication induced by constitutive expression of cloned *dut* genes. Lanes 1–6 are as in **b**, above. Lane 7, SaPIbov1 induction after mitomycin C treatment of a ϕ11 prophage (JP1794). Lane 8, induction by cloned ϕ11 Dut in a *recA* mutant (JP6773). The upper panel is a Southern blot probed for SaPIbov1 DNA; the lower panel is a western blot probed with antibody to the Flag tag carried by the proteins. kDa, kilodaltons.

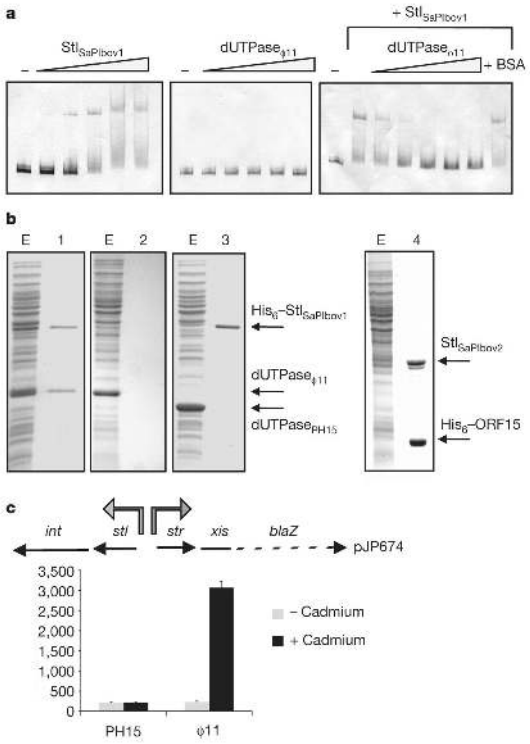

Figure 2. Phage-inducing proteins bind SaPI-encoded Stl proteins

a, dUTPase prevents $Stl_{SaPIbov1}$ from binding to the *stl–str* divergent region. Shown are electrophoretic mobility shift assays in which increasing concentrations of $Stl_{SaPIbov1}$ (0, 0.03, 0.06, 0.12, 0.24 and 0.48 μg; left), $dUTPase_{\phi 11}$ (0, 0.02, 0.04, 0.08, 0.16 and 0.2 μg; middle), or $Stl_{SaPIbov1}$ (0.12 μg) in the presence of $dUTPase_{\phi 11}$ (0, 0.02, 0.04, 0.08, 0.16 and 0.2 μg) or 10 μg BSA (right) were mixed with labelled DNA containing the SaPIbov1 divergent region. **b**, Affinity chromatography of dUTPase using His_6–$Stl_{SaPIbov1}$ (left), or affinity chromatography of $Stl_{SaPIbov2}$ using His_6–$ORF15_{\phi 80\alpha}$ (right). *E. coli* strains expressing the different pairs were isopropyl-β-D-thiogalactoside (IPTG)-induced and, after disruption of the cells, the expressed proteins were applied to a Ni^{21+} agarose column and eluted. The presence of the different proteins was monitored in the load (lanes E), flow-through, wash and elute fractions by Coomassie staining. Elution fractions (lanes 1, 2, 3 and 4) were concentrated 2.5-fold relative to the load. Lane 1, His_6–$Stl_{SaPIbov1}$ and $dUTPase_{\phi 11}$ (JP6760); lane 2, $dUTPase_{\phi 11}$ alone (JP6762); lane 3, His_6–$Stl_{SaPIbov1}$ and $dUTPase_{PH15}$ (JP6761); lane 4, His_6–$ORF15_{\phi 80\alpha}$ and $Stl_{SaPIbov2}$ (JP6763). **c**, Derepression of *str* transcription by dut expression. Top, schematic representation of the *blaZ* transcriptional fusion generated in plasmid pJP674. Bottom, strains containing pJP674- and pCN51-derivative plasmids expressing dut_{PH15} (JP5469) or $dut_{\phi 11}$ (JP5468) were assayed for β-lactamase activity in the absence of or 5 h after induction with 5 μM $CdCl_2$. Samples were normalized for total cell mass. Data are from an experiment in triplicate. Error bars represent s.d.

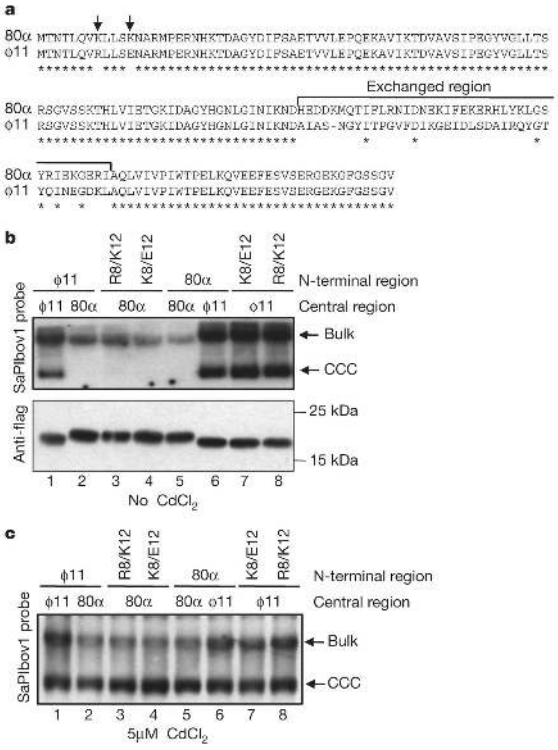

Figure 3. The level of SaPIbov1 inducing activity correlates with the central divergent region of Dut

a, Alignment of predicted staphylococcal phage dUTPase protein sequences from phages 80α and ϕ11. Black arrows indicate the two N-terminal variations between 80α and ϕ11. The bracket indicates the region that was exchanged between 80α and ϕ11 dUTPases. Asterisks indicate identical residues between phage 80α and ϕ11. **b**, **c**, SaPIbov induction and replication was measured in a non-lysogenic derivative of strain RN4220 carrying SaPIbov1 and plasmids expressing 3×flag-tagged Dut proteins containing substitutions of two amino acids in the N-terminal region or an exchange of the central region, as indicated. One millilitre of each culture (OD_{540nm} = 0.3) was collected in the absence of induction (**b**) or 3 h after treatment with 5 μM $CdCl_2$ (**c**) and used to prepare standard minilysates, which were resolved on a 0.7% agarose gel, blotted and probed for SaPIbov1 DNA or with antibody to the Flag tag. In both panels, lane 1, JP6789; lane 2, JP6794; lane 3, JP6793; lane 4, JP6800; lane 5, JP6797; lane 6, JP6795; lane 7, JP6798; lane 8, JP6799.

Table 1

Effects of phage mutations on SaPI replication and transfer

Phage	SaPIbov1			SaPIbov2			SaPI1		
	Rpl	SaPI titre*	ϕtitre†	Rpl	SaPI titre*	ϕtitre†	Rpl	SaPI titre*	ϕtitre†
80α	+	4.1×10^7	1.5×10^8	+	1.3×10^8	6.0×10^7	+	1.6×10^8	1.1×10^8
80α Δ*dut*	2	8.3×10^2‡	4.4×10^{10}	+	1.1×10^8	9.1×10^7	+	2.8×10^8	4.3×10^8
80α Δ*sri*	+	5.3×10^7	2.1×10^8	+	9.2×10^7	8.2×10^7	−	3.8×10^2‡	2.3×10^{10}
80α ΔORF15	+	3.9×10^7	1.8×10^8	−	5.4×10^3‡	3.0×10^9	+	4.1×10^8	4.1×10^8
ϕ11	+	1.8×10^7	6.3×10^6	−	ND	ND	−	ND	ND
ϕ11 Δ*dut*	−	9.2×10^2‡	9.4×10^8	−	ND	ND	−	ND	ND

The means of results from three independent experiments are presented. Variation was within ± 5% in all cases. Rpl, replication as determined by Southern blot. ND, not determined.

*Transductants ml^{-1} of lysate, using RN4220 as recipient.

†Plaques ml^{-1} of lysate, using RN4220 as indicator.

‡This frequency is typical of transfer by generalized transduction and is not SaPI-specific.