Transcription of the phage-encoded Panton–Valentine leukocidin of \textit{Staphylococcus aureus} is dependent on the phage life-cycle and on the host background

Christiane Wirtz, Wolfgang Witte, Christiane Wolz and Christiane Goerke

\textsuperscript{1}Institut für Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Tübingen, Elfriede-Aulhorn-Str. 6, 72076 Tübingen, Germany
\textsuperscript{2}Robert-Koch-Institut Wernigerode, Burgstr. 37, 38855 Wernigerode, Germany

Panton-Valentine leukocidin (PVL) is a pore-forming, bi-component toxin secreted by \textit{Staphylococcus aureus} strains epidemiologically associated with diseases such as necrotizing pneumonia and skin and soft-tissue infections. Here we demonstrate that transcription of the phage-encoded PVL (encoded in the \textit{luk-PV} operon) is dependent on two major determinants: the phage life-cycle and the host chromosomal background. Mitomycin C induction of PVL-encoding prophages from different community-acquired MRSA strains led to an increase in the amount of \textit{luk-PV} mRNA as a result of read-through transcription from latent phage promoters and an increase in phage copy numbers. Failing prophage excision was reflected in a constant expression of \textit{luk-PV} as in the case of strain USA300, suggesting that \textit{\phi}Sa2USA300 is a replication-defective prophage. Additionally, we could show that \textit{luk-PV} transcription is influenced by the \textit{S. aureus} global virulence regulators \textit{agr} and \textit{sae}. We found a strong impact of the host background on prophage induction and replication when analysing PVL phages in different \textit{S. aureus} strains. For example phage \textit{\phi}Sa2mw was greatly induced by mitomycin C in its native host MW2 and in strain Newman but to a considerably lesser extent in strains 8325-4, RN6390 and ISP479c. This discrepancy was not linked to the SOS response of the bacteria since \textit{recA} transcription did not vary between the strains. These results suggest a fine tuning between certain phages and their host, with major impact on the expression of phage-encoded virulence genes.

INTRODUCTION

\textit{Staphylococcus aureus} causes a variety of local and systemic infections in humans and is one of the most important community-acquired and nosocomial pathogens. The versatility of this bacterium is due to its ability to produce a wide range of surface-exposed molecules which mediate interaction with the host cell, as well as several secreted virulence factors. \textit{S. aureus} necrotizing pneumonia and skin and soft-tissue infections, which can also affect young, immunocompetent persons, is described as a threat associated with community-acquired (ca)MRSA and MSSA strains bearing the Panton–Valentine leukocidin (PVL) genes (Diederen & Kluytmans, 2006; Gillet \textit{et al.}, 2002; Vandenesch \textit{et al.}, 2003). The possible contribution of PVL to the virulence of \textit{S. aureus} has been argued in studies using a variety of different animal models (Bubeck Wardenburg \textit{et al.}, 2008; Diep & Otto, 2008; Labandeira-Rey \textit{et al.}, 2007; Montgomery \textit{et al.}, 2008; Voyich \textit{et al.}, 2006; Wang \textit{et al.}, 2007). PVL is a bi-component, pore-forming cytotoxin that targets host defence cells such as human polymorphonuclear neutrophils, monocytes and macrophages (Genestier \textit{et al.}, 2005; Kaneko & Kamio, 2004; Prevost \textit{et al.}, 1995). The active form of PVL requires the assembly of two polypeptides, LukS-PV and LukF-PV, for which the corresponding genes (\textit{lukS-PV}, \textit{lukF-PV}) are carried by a prophage.

Although tightly linked to the phage genome and dependent on it for horizontal transfer, most of the phage-encoded virulence factors are integrated into the regulatory mechanism of the host. Examples of phage-related pathogenicity genes which are influenced by global \textit{S. aureus} virulence regulators are plasminogen activator staphylokinase (encoded by \textit{sak}), staphyloccocal complement inhibitor (SCIN), chemotaxis inhibitory protein (CHIPS) (Rooijakkers \textit{et al.}, 2006) and exfoliative

Abbreviations: ca, community-acquired; MRSA, meticillin-resistant \textit{Staphylococcus aureus}; MSSA, meticillin-sensitive \textit{Staphylococcus aureus}; PVL, Panton–Valentine leukocidin.
toxin A (ETA) (Sheehan et al., 1992). In a similar fashion, transcription of \textit{luk-PV} was shown to depend on the activity of the regulators \textit{agr}, \textit{sar} and \textit{sae} (Bronner et al., 2000; Voyich et al., 2009). Additionally, the composition of the growth medium (Bronner et al., 2000; Dumitrescu et al., 2007) and subinhibitory concentrations of different antibiotics exerted a strong effect on PVL expression (Dumitrescu et al., 2007; Stevens et al., 2007).

Many prophages are induced by environmental conditions that lead to DNA damage, including exposure to exogenous agents such as antibiotics (Wagner & Waldor, 2002). We could show that phages are mobilized during chronic lung infection of patients with cystic fibrosis, possibly due to the strong selective pressure exerted on the pathogen (Goerke et al., 2004, 2007). An enhanced expression of phage-encoded virulence genes after phage induction has been demonstrated for \textit{S. aureus} (Goerke et al., 2006a; Sumby & Waldor, 2003) and other bacteria (Wagner & Waldor, 2002). Here we show that transcription of \textit{luk-PV} is greatly enhanced upon induction of PVL-encoding phages. This was due to an increase in phage copy numbers and to the activation of latent phage promoters after mitomycin C treatment of lysogens. Additionally, the bacterial host exerted a strong effect on \textit{luk-PV} transcription and on prophage induction.

**METHODS**

**Bacterial strains and lysogens.** Bacterial strains used in this study are listed in Table 1. Phage lysates were obtained from the appropriate strains by adding 1 \(\mu\)g mitomycin C ml\(^{-1}\) to cells in the exponential growth phase (OD\(_{600}\) 0.8). After further incubation for 4 h, culture supernatants were sterilized using 0.45 \(\mu\)m pore diameter membrane filters (Millipore). In order to obtain lysogens we spotted phages on the target strains and selected colonies growing in the centre of a plaque. Single colonies were tested for phage integration by standard PCR using oligonucleotides specific for \textit{luk-PV} (\textit{luk-PV}: 5’-ACAGTGGTTTCAATCCTTCA-3’, \textit{luk-PV}-rev 5’-CTTTTGGATTTCGGCTTTGTA-3’) and by pulsed-field gel electrophoresis fingerprinting. All investigations in this study were performed with at least two independently obtained lysogens. MW2 lacking the \textit{hbl}-converting phage \textit{Sa3} was obtained by screening mitomycin-treated bacteria on sheep blood agar plates for \(\beta\)-haemolysin production. The respective colonies were picked and analysed by PFGE for restoration of the intact \textit{hbl} gene as described previously (Goerke et al., 2006a) and for retention of the PVL phage. MW2\textit{recA} was obtained by transduction using a \textit{fi1} lysate of strain ISP794\textit{recA}.

**Mitomycin treatment.** Strains were grown to the exponential growth phase (OD\(_{600}\) 0.8, 2 h) in CYPG medium (Casamino acids, 10 g l\(^{-1}\); yeast extract, 10 g l\(^{-1}\); NaCl, 5 g l\(^{-1}\); 0.5% glucose and 0.06 M phosphoglycerate) (Novick, 1991), at which point 300 ng mitomycin was added, followed by further incubation for 2 h.

**Quantitative real-time PCR for detection of extrachromosomal phage DNA.** For DNA isolation bacterial cells were disrupted with

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<tr>
<td><strong>Strain</strong></td>
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<tr>
<td>MW2 (USA400)</td>
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<tr>
<td>MW2-(\phi)Sa3mw</td>
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<tr>
<td>ISP794\textit{recA}</td>
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<tr>
<td>MW2\textit{recA}</td>
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<tr>
<td>USA300</td>
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<tr>
<td>02-02404</td>
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<tr>
<td>03-1816</td>
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<tr>
<td>8325-4</td>
</tr>
<tr>
<td>8325-4(\phi)Sa2mw</td>
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<tr>
<td>8325-4(\phi)Sa3mw</td>
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<tr>
<td>Newman</td>
</tr>
<tr>
<td>Newman(\phi)Sa2mw</td>
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<tr>
<td>s64c</td>
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<td>s64c(\phi)Sa2mw</td>
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<td>RN6390</td>
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<td>RN6390(\phi)Sa2mw</td>
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<td>ISP479c</td>
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<td>ISP479c(\phi)Sa2mw</td>
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<tr>
<td>ISP479c-\textit{sac}</td>
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<tr>
<td>ISP479c-\textit{sac}(\phi)Sa2mw</td>
</tr>
<tr>
<td>ISP479c-\textit{agr} (ALC14)</td>
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<td>ISP479c-\textit{agr}(\phi)Sa2mw</td>
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<td>N315</td>
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*Only phage-encoded virulence factors important for this study are listed in parentheses after the appropriate prophage.*
glass beads (Sigma-Aldrich) in a high-speed homogenizer (Savant Instrument) for 2 × 20 s at 6500 r.p.m. After heating for 2 min at 100 °C, 1:100 dilutions of the crude extracts were used. Quantification of the attP site of PVL-encoding phages and the S. aureus chromosomal gene gyr was performed by quantitative LightCycler PCR using the LightCycler SYBR Green kit (Roche Biochemicals). Copies of attP and gyr were determined using 10-fold serial dilutions of sequence-specific DNA standards as described previously (Goerke et al., 2006a). Briefly, DNA standards were generated by regular PCR and amplicons were quantified spectrophotometrically. Master mixes were prepared following the manufacturer’s instructions using oligonucleotides specific for attP (MW2circle2-for 5'-AAACAACCATGCGATA-3’ and MW2circle-rev 5'-ATTITGCCAAGGTGTTGTGC-3’) and gyr (Goerke et al., 2006a). At least two independent PCRs were performed for all samples. The specificity of the PCR was verified by ethidium bromide staining on 3% agarose gels and by sequencing of the amplicons (4base lab, Reutlingen, Germany).

**Northern analysis and quantitative RT-PCR.** For Northern analysis S. aureus strains grown overnight in CYPG were diluted to an initial OD_{600} of 0.05 in fresh medium and grown with shaking (222 r.p.m.) at 37 °C to the early exponential phase (T1, OD_{600} 0.8), late-exponential phase (T2, OD_{600} 1.9), and post-exponential phase (T3, OD_{600} 8). Bacteria were harvested by centrifugation and dissolved in 1 ml Trizol reagent (Invitrogen). RNA was isolated from the bacterial pellet and Northern blot analysis was done as described previously (Goerke et al., 2000). Specific primers for agr (RNAIII), sae, hla, recA, sak (Goerke et al., 2005, 2006a) and luk-PV (lukPV-for, lukPV-rev) were used to generate digoxigenin-labelled probes by PCR labelling (Roche Biochemicals). Quantification of luk-PV transcripts was performed by LightCycler RT-PCR as described previously (Goerke et al., 2005), employing primers lukPV-for and lukPV-rev.

**RESULTS**

**Transcription of luk-PV during growth**

To investigate transcription of the luk-PV operon during growth we carried out Northern blot analysis of RNA from four MRSA strains harbouring PVL-encoding phages. The strains selected represent typical cAMRSA clonal lineages of S. aureus: the prototypic cAMRSA MW2 and USA300 belong to MLST type ST1 and ST8, respectively. The two cAMRSA from the collection of the German reference centre for staphylococci at the RKI represent the European Clone ST80 (strain 02-02404) and the ST30 lineage (strain 03-1816). The lukS-PV and lukF-PV genes are transcribed as a single ~2.3 kb mRNA. In all four strains maximal expression was observed in the late-exponential growth phase and transcription dropped post-exponentially, with the exception of strain 03-1816, where mRNA levels remained equal (Fig. 1). The rapid decline in transcription in the stationary phase was unexpected for anagr-regulated gene (Bronner et al., 2000). Therefore, we also examined the expression of the hla gene, encoding the well-characterized agr-regulated toxin α-haemolysin; for this gene the expected continuous increase into the late exponential phase was observed (Fig. 1). Overall, strain MW2 produced considerably less hla than strains USA300, 02-02404 and 03-1816.

**Correlation between transcription of luk-PV and phage induction**

An increased transcription of phage-encoded staphylococcal enterotoxins and staphylokinase after induction of β-haemolysin-converting phages that integrate into the hlb gene had been detected previously (Goerke et al., 2006a; Sumby & Waldor, 2003). Activation of the SOS response by DNA-damaging agents led to the excision and replication of the phage, thereby increasing the amount of phage DNA template available for transcription. Similarly, expression of the phage-encoded luk-PV may also be linked to the phage life-cycle. Northern blot analysis revealed that mitomycin C treatment resulted in a marked increase in the amount of the ~2.3 kb luk-PV transcript in strains MW2 and 03-1816, but not in strain USA300 (Fig. 2a). Quantification of the luk-PV mRNA by quantitative RT-PCR led to the detection of 12 times more transcript in strain MW2 and 10 times more in strain 03-1816, whereas in USA300 the ratio remained equal. Mitomycin C treatment also resulted in the production of a higher-molecular-mass mRNA in both strains with enhanced luk-PV transcription. By Northern analysis employing probes hybridizing to different parts 5’ of luk-PV we could establish that the 4.9 kb transcript appearing in strain MW2 likely initiates from a region upstream of ORF MW1381 (data not shown). This is the putative holin gene of phage φSa2mw. Thus, the activation of latent phage promoters contributes to the increased expression of luk-PV.

RecA is the key enzyme of the bacterial SOS response. In all strains, a subinhibitory concentration of mitomycin led to increased recA expression (Fig. 2a). It should be noted that the recA transcription level did not differ between USA300 and the other strains and thus does not explain the differences in luk-PV transcription. In general, activated RecA leads to autolysis and resumption of the lytic cycle. To prove that PVL-prophage induction and subsequent increased luk-PV transcription is
The newly formed Phage induction was measured by a quantitative real-time PCR for the ratio of mitomycin-treated (300 ng ml$^{-1}$) to untreated cultures. Each analysis was performed in duplicate. The ethidium-bromide-stained gel is shown as a loading control. The numbers below the blots indicate the mean ratio ($\pm$ SEM) of luk-PV mRNA derived from mitomycin-positive culture to that derived from negative cultures as determined by quantitative RT-PCR. Values from two independent RNA isolations each were used to calculate the mean ratio.

In summary, in strains MW2, 03-1816 and USA300 phage induction and luk-PV expression are correlated: in the first two strains phage replication is linked to an enhanced luk-PV transcription from internal phage promoters as well; in the latter the failing phage excision is reflected in a constant low expression of luk-PV. However, Western blot analysis using specific antibodies against LukF-PV and LukS-PV revealed that elevated luk-PV transcription failed to result in increased amounts of protein.

**Phage induction is dependent on the bacterial host and is not influenced by co-infecting phages**

To analyse the influence of the host chromosomal background on luk-PV transcription, we lysogenized the phage-cured strain 8325-4 with $\phi$Sa$^{2}$MW and $\phi$03-1816. With real-time $attP$ PCR only a slight increase in phage particles could be detected for these lysogens after the addition of mitomycin (data not shown). Enhanced expression of recA was detected by Northern blot analysis (Fig. 3a), but only a weak increase of luk-PV transcription occurred in the 8325-4 background. Thus, although a clear SOS response could be elicited by the mitomycin treatment in this host, both phages seem to be only weakly inducible, which is reflected in a low increase of luk-PV transcripts.

One reason for the differences in luk-PV transcription and phage induction between the original and the 8325-4 lysogens could be the presence of co-infecting phages aiding PVL-phage excision. Strain MW2 also carries the hlb-converting phage $\phi$Sa3$^{3}$MW. Using a derivative of MW2 which was cured of $\phi$Sa3$^{3}$MW we detected the same enhanced luk-PV transcription (Fig. 3b) and phage excision (data not shown) after mitomycin treatment that was found in the original double lysogen. Thus, this PCR both the newly formed circular phage genomes after rejoining of $attP$ upon excision and all replicative forms of the phage can be detected. Quantification was performed with reference to the total amount of bacterial DNA represented by the chromosomal gyr (gyrase) gene. Phage excision and replication was clearly detectable in strains MW2 and 03-1816: mitomycin treatment resulted in a 690-fold and a 1080-fold increase, respectively, in newly formed $attP$ sites (Fig. 2b). In contrast, in strains USA300 and MW2recA no phage excision was observed. The negative result of the latter suggests a missing cleavage of the prophage repressor by activated RecA. To exclude a potential PCR failure in the non-reactive lysogen USA300 due to extensive sequence variations in the $attP$ sites of the prophages, we analysed the $attL/R$ sites of $\phi$SaUSA300. A perfect match of the $attP$ primers was found. Interestingly, replication of the hlb-converting phage of strain USA300, $\phi$Sa3USA300, was detectable by a real-time PCR specific for the $attP$ site of these phages (data not shown). Thus, the excision failure is a specific trait of $\phi$SaUSA300 and not due to a general dysfunctionality of the SOS response in strain USA300.

**reca-dependent we included a recA-defective derivative of strain MW2**. No increase in luk-PV transcription after mitomycin treatment could be observed in this mutant, indicating recA-dependent phage induction (Fig. 2a).

So far, the results indicate differences in phage induction and replication in the four lysogens MW2, USA300, 03-1816 and MW2recA. To test this, we performed quantitative real-time PCR using specific oligonucleotides which span the $attP$ sites of the excised PVL-encoding phages. In
co-infecting phages had no effect on PVL-phage induction and excision.

**Transcription of luk-PV and phage induction are dependent on the chromosomal background**

To further examine the impact of the bacterial background on PVL expression, the same phage, \( \phi \)Sa2mw, was used to lysogenize different hosts: Newman, N315, s64c and the 8325-4 derivative RN6390. First we analysed whether \( \phi \)Sa2mw is inducible in the different host backgrounds. In the original host MW2 and strain Newman the highest increase (482-fold and 473-fold, respectively) in newly formed \( \text{attP} \) sites of \( \phi \)Sa2mw was detected after mitomycin treatment (Fig. 4a). In N315 the increase was less pronounced (46-fold) and only a very slight increase was observed in strains s64c and RN6390 (2-fold and 7.5-fold, respectively).

In the next experiments we concentrated on strains MW2 and the \( \phi \)Sa2mw lysogens of N315 and RN6390 as those differing most in phage induction. We included a \( \phi \)Sa2mw lysogen of strain ISP479c, which like RN6390 is a derivative of strain 8325-4 (Adhikari et al., 2007). Mitomycin treatment resulted in an induction of the SOS system as demonstrated by the increased amount of \( \text{recA} \) transcript detected in Northern blot analysis (Fig. 4b). No differences in the level of \( \text{recA} \) expression could be detected between the strains. When \( \text{luk-PV} \) transcription was analysed, an increase in expression after mitomycin treatment was seen in strains MW2 and N315\( \phi \)Sa2mw (Fig. 4b). An activation of latent phage promoters was also seen here. No response to mitomycin could be detected in the \( \phi \)Sa2mw lysogens of strains RN6390 and ISP479c. Surprisingly, the latter lysogen, ISP479c\( \phi \)Sa2mw, showed a very high overall level of \( \text{luk-PV} \) expression independent of mitomycin induction. As a control the transcription of the staphylokinase was also determined in our strain collection since \( \text{sak} \) expression and phage replication are known to be linked (Goerke et al., 2006a; Sumby & Waldor, 2003). All...
of the strains analysed here harbour additional phages encoding this virulence factor (Table 1). Mitomycin treatment resulted in an increase in sak-specific mRNAs in all strains chiefly due to the activation of latent phage promoters, which can be deduced from the appearance of higher-molecular-mass bands in the Northern blot (Fig. 4b). Additionally, replication of the respective phages could be proven by real-time PCR (data not shown). Thus, the enhanced expression of both phage-encoded virulence factors, luk-PV and sak, after activation of the SOS response by mitomycin C is dependent on phage replication. However, whereas the sak-encoding, hlb-converting phages are induced in all strain backgrounds, the PVL-encoding phage φSa2mw is not.

**Influence of regulatory loci on luk-PV transcription**

Since the level of luk-PV transcription varied widely between strains, a strong influence of one or more host factors on PVL expression has to be assumed. Therefore, the activity of two global virulence regulators, sae and agr, was determined after mitomycin treatment. When RN6390 was compared with ISP479c the latter showed a higher sae transcription level, possibly explaining the discrepancies in luk-PV expression in these strains (Fig. 4b). It is consistent with this finding that the decrease in sae mRNA in RN6390 after mitomycin treatment was mirrored in a reduced luk-PV transcription. For further clarification we added a sae-replacement mutant of ISP479c to our investigation. The φSa2mw lysogen of ISP479c-sae displayed a dramatically diminished expression of luk-PV compared to the wild-type. Thus, the virulence regulator sae positively regulates luk-PV transcription. No differences in agr expression between the strains could be observed – with the exception of strain N315, which proved to be an agr non-producer (Somerville et al., 2003), explaining the very low level of luk-PV transcription. This was verified by analysis of an agr-replacement mutant of strain ISP479c, in which luk-PV mRNA was diminished compared to the agr-intact ISP479c lysogen, indicating that agr is a positive regulator of luk-PV, albeit to a lower extent than sae.

**DISCUSSION**

**Prophage induction leads to an enhanced expression of luk-PV**

*S. aureus* PVL is a pore-forming toxin secreted by strains epidemiologically associated with diseases such as necrotizing pneumonia and skin and soft-tissue infections (Gillet et al., 2002). PVL-encoding phages are found in virtually all MRSA strains that cause community-associated infections (Chambers, 2005; Vandenesch et al., 2003). Here we could show that transcription of PVL by *S. aureus* is dependent on two major determinants: the phage life-cycle and the host chromosomal background. Mitomycin C induction of PVL-encoding phages led to an enhanced transcription of the virulence factor. The increase in luk-PV mRNA was a result of read-through transcription from latent upstream phage promoters and an increase in phage copy numbers due to phage replication. The new luk-PV transcript appearing after the addition of mitomycin was probably initiated from a latent promoter upstream of the φSa2mw holin gene. This corresponds well with earlier results obtained with hlb-converting phages showing that an increase in sak transcription was due to the read-through from upstream phage promoters (Goerke et al., 2006a; Sumby & Waldor, 2003). In addition, phage replication was shown to play an essential role in the enhanced sak transcription (Sumby & Waldor, 2003). We could confirm the contribution of phage replication to luk-PV transcription by showing that the amount of luk-PV mRNA is directly linked to the quantity of extra-chromosomal phages: strongly replicating phages after mitomycin addition are strong PVL-producers (strain 03-1816); failing phage replication results in a constant luk-PV transcription (strain USA300). This link was emphasized when PVL-encoding phages were analysed in different host backgrounds. The weak induction of phages φSa2mw and φ03-1816 in strain 8325-4 was reflected in a small increase of luk-PV mRNA. For many bacterial species it has been shown that prophages are induced by environmental conditions and that induction is linked to enhanced production of phage-encoded virulence genes. In the case of *Escherichia coli* numerous epidemiological studies have detected an association between increased severity of infection and treatment with antibiotics (Wagner & Waldor, 2002). For *S. aureus* it could also be demonstrated that there is a link between induction of prophages and subsequent virulence modulation of the bacterium by antibiotics which are often used during the treatment of infections (Goerke et al., 2006a). A positive effect of β-lactam antibiotics on phage replication was recently described (Maiques et al., 2006). Subinhibitory concentrations of nafcillin (Stevens et al., 2007) and oxacillin (Dumitrescu et al., 2007) were found to enhance PVL expression, which is thought to contribute to worse outcomes of *S. aureus* infections after antibiotic therapy. The elevated PVL release observed in these studies might also be linked to an antibiotic-related induction of the prophage. However, in the current work the increase in luk-PV transcripts after mitomycin induction is not mirrored in a parallel increase in the PVL protein level. Sumby & Waldor (2003) were likewise unable to detect an increase in SEA production after mitomycin treatment, although φSa3ms prophage induction led to transcriptional upregulation of sea. A post-transcriptional regulation seems to influence toxin production.

**Prophage induction is dependent on RecA**

Induction of PVL phages was directly dependent on the initiation of the SOS system. The prophage repressor cl is generally inactivated by the key enzyme RecA, leading to
resumption of the lytic cycle. Lambda phage repressors are organized in two structural domains, the N-terminal domain responsible for contacts with the DNA and the C-terminal domain responsible for catalysing the autoproteolysis reaction. A linker region connects the two structural domains of the repressor. It contains a specific Ala (or Cys, Leu)-Gly cleavage site that is the target of C-terminal-domain-catalysed proteolysis (Koudelka et al., 2004; Little et al., 1980). Analysis of the cl-like repressor from φSa2mw revealed a structural organization dissimilar to that of the classical lambda repressors, making it difficult to envisage a RecA-dependent inactivation. However, we could show that in a recA-negative MW2 derivative PVL-phage induction is completely abolished, thus providing evidence of a sensitivity of the phage repressor to activated RecA. This suggests an alternative mode of cl inactivation in these PVL phages, for instance by a RecA-dependent activation of an anti-repressor as was described for a mobile element of Bacillus subtilis (Bose et al., 2008). Interestingly, other PVL-carrying phages (e.g. φSa2985PVL and φ108PVL) encode repressors which exhibit the typical lambda repressor structure (Ma et al., 2008).

**Influence of global virulence regulators on luk-PV transcription**

Besides the phage life-cycle, the in vitro growth conditions and the host chromosomal background exerted a strong effect on PVL production. Analysis of transcription during the growth of several caMRSA strains revealed a decline in the amount of luk-PV mRNA in the stationary phase, which is an uncommon expression pattern for a secreted, agr-regulated gene. Bronner et al. (2000) also observed a decline of expression post-exponentially when analysing S. aureus strain V8, whereas a strong transcription of luk-PV in the stationary phase up to 24 h was shown by another group (Stevens et al., 2007). One of our strains, 03-1816, also showed a prolonged expression of luk-PV into the post-exponential phase. Overall, the time-course and the level of luk-PV expression seem to vary considerably between different S. aureus strains, as was also shown by other groups (Hamilton et al., 2007; Said-Salim et al., 2005). Strains MW2 and USA300 in particular differed in the level of PVL expression. This was already observed by Montgomery et al. (2008), who showed that USA300 is more virulent than MW2 in a rat model of pneumonia. One reason for the observed strain-dependent PVL expression may be the activity of global virulence regulators. Several regulators like agr, sar and very recently sae have been identified as controlling luk-PV expression (Bronner et al., 2000; Voyich et al., 2009). Here we could confirm that the virulence regulator sae positively influences transcription: in the sae-knockout mutant ISP479c-sae, luk-PV-specific mRNA was no longer detectable. This was also reflected in the prominent expression of luk-PV in strain ISP479c compared to RN6390. As shown here these two strains as well as the caMRSA strains USA300 and MW2 (Geiger et al., 2008) vary clearly in sae expression, emphasizing the strong positive effect of sae on luk-PV transcription.

**Influence of the host background**

The host chromosomal background also had a strong impact on phage replication. Mitomycin C treatment resulted in a pronounced increase of φSa2mw excision and replication in the native MW2 background and in strain Newman. However, when we analysed φSa2mw induction in lysogens of strains 8325-4, RN6390 and ISP479c, we detected only a weak increase in phage copy numbers. This was not attributable to a failing initiation of the SOS system in these strains, since an obvious enhancement of recA transcription was observed after mitomycin addition. Another possible explanation for the discrepancies in prophage induction in different strain backgrounds may be the presence of additional phages in the host that complement vital phage functions. Helper phages were shown to be important for the mobilization of staphylococcal pathogenicity islands (Lindsay et al., 1998) and for the E. coli phage P4, which requires a P2 helper phage for its assembly, packaging and lysis of the host cell (Christie & Calendar, 1990). In our case strain MW2 also carries phage φSa3mw, whereas strain 8325-4φSa2mw is a single lysogen. However, curing the second prophage of strain MW2 had no impact on phage replication or luk-PV transcription. Additionally, strains RN6390 and ISP479c also harbour phage φ6390 (Goerke et al., 2006b), which was clearly able to excise and replicate, as shown by the increased number of sak transcripts after mitomycin treatment. Phage φSa2mw was not inducible in the same background. Differences in the relative inducibility of phages were also noticed for lambda phages of E. coli when spontaneous induction frequencies in double lysogens were compared (Livny & Friedman, 2004). In summary, there seems to be a strong link between the host background and the ability of a phage to resume the lytic cycle. How this interplay between a certain phage and its specific hosts functions remains to be elucidated.

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