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Phages of *Staphylococcus aureus* and their impact on host evolution

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ABSTRACT

Most of the dissimilarity between *Staphylococcus aureus* strains is due to the presence of mobile genetic elements such as bacteriophages or pathogenicity islands. These elements provide the bacteria with additional genes that enable them to establish a new lifestyle that is often accompanied by a shift to increased pathogenicity or a jump to a new host. *S. aureus* phages may carry genes coding for diverse virulence factors such as Panton-Valentine leukocidin, staphylokinase, enterotoxins, chemotaxis-inhibitory proteins, or exfoliative toxins. Phages also mediate the transfer of pathogenicity islands in a highly coordinated manner and are the primary vehicle for the horizontal transfer of chromosomal and extra-chromosomal genes. Here, we summarise recent advances regarding phage classification, genome organisation and function of *S. aureus* phages with a particular emphasis on their role in the evolution of the bacterial host.

1. Introduction

The majority of both colonising and infectious *Staphylococcus aureus* isolates can be placed into a limited number of mostly pandemic clonal complexes (Lindsay, 2010; Lindsay and Holden, 2004; Melles et al., 2004). In general, *S. aureus* is regarded as a highly clonal species with a conserved core genome (Feil et al., 2003) that has evolved mainly through mutation, as illustrated by single-nucleotide polymorphisms detected by multilocus sequence typing (MLST) of selected housekeeping genes or through whole-genome sequencing. Thus, the diversity of the *S. aureus* species is mainly determined by the presence of mobile genetic elements, many of which are prophages or phage-related genomic islands. Both the horizontal transfer of most of the mobile elements and strain evolution are tightly linked to phages. First, phages can be mobilised and transferred to recipient strains. It is known that many accessory genes carried by phage genomes encode for staphylococcal virulence factors, which are important for the success of certain *S. aureus* strains. Second, phages support the induction, packaging and transfer of genomic islands. This interesting topic was recently reviewed (Christie and Dokland, 2012; Novick et al., 2010) and will not be the focus of the present review. Third, phage transduction is an efficient means to transfer not only extra-chromosomal mobile elements, such as plasmids, but also chromosomal markers (albeit with lower efficiency). In general, for *S. aureus,* it is believed that phages are the primary tool for diversification because the species is thought not to be naturally competent. However, this long-standing notion was recently challenged by the finding that at least under certain circumstances, an alternative sigma factor H can be expressed in subpopulations of bacteria in which a competence apparatus becomes activated to mediate the uptake of naked DNA (Morikawa et al., 2012). Interestingly, sigma factor H has also been shown to interact with the conserved promoter region of phage integrase (*int*) genes, which seems to result in the stabilisation of the lysogenic state (Tao et al., 2010). Thus, sigma factor H may serve as a regulatory tool involved in modulating horizontal evolution (at least under some thus-far-poorly-defined conditions), a topic that clearly needs further evaluation.

Here, we will first give a brief overview of previously used methods to classify *S. aureus* phages as well as basic insights into the genome structure of selected phages. Then, we will mainly focus on the impact of phages on the evolution of the bacterial host.

2. Serogroups and morphology of phages infecting *S. aureus*

All known *S. aureus* phages belong to the order Caudovirales (tailed phages), which are composed of an icosahedral capsid filled with double-stranded DNA and a thin filamentous tail. Based on the tail morphology, they can be further classified into three major families: Podoviridae, which have a very short tail; Siphoviridae, which have a long non-contractile tail; and Myoviridae, which have a long, contractile, double-sheathed tail, as shown in Fig. 1.

In early studies, *S. aureus* phages were compared, grouped and classified according to their reaction to polyclonal antiserum, which can neutralise phage infection. Based on the phage neutralisation tests, 39 phages were classified into six serogroups (Rountree, 1949). With more sera and more staphylococcal phage

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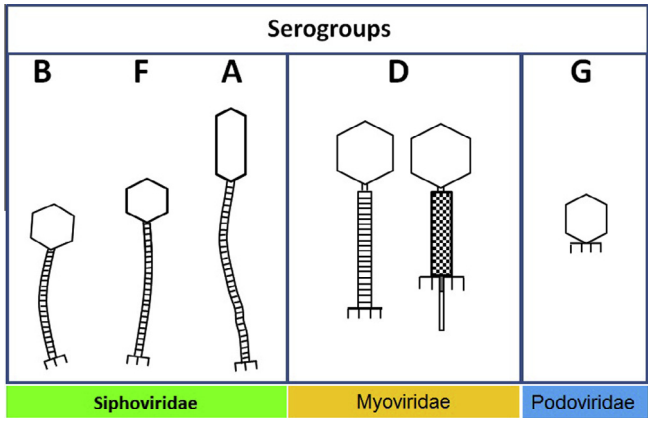

Fig. 1. Schematic representation of major groups of *S. aureus* phage. Modified according to (Brandis and Lenz, 1984).

isolates, a total of 11 serogroups (A–H and J–L) were defined (Rippon, 1952, 1956). Group E, J, and K phages were found to be specific for coagulase-negative staphylococci and avirulent to *S. aureus*. Most of the temperate phages infecting *S. aureus* could be assigned to serogroups A, B and F.

With the development of electron microscopy, morphology-based classification of *S. aureus* phages became feasible. Ackermann and Brandis et al. proposed a similar classification system including three major morphological groups, A–C or 1–3, which correspond to the phage families Myoviridae, Siphoviridae and Podoviridae, respectively (Ackermann, 1975; Brandis and Lenz, 1984). According to the proposal by Brandis and Lenz, all phages in morphological group 2 (Siphoviridae) have non-contractile tails. Based on a tail length longer or shorter than 200 nm and the shape of the head, staphylococcus phages in this group can be further assigned to subgroups 2.1, 2.2.1, and 2.2.2, which roughly correspond to serogroups B, A and F, respectively. While phages from serogroups B and F have isometric capsids, phages from serogroup A have distinct prolate heads (Fig. 1). Most serogroup B phages have tails shorter than 200 nm, while phages in serogroups A and F have tails longer than 200 nm. Phages in serogroups D and G seem unable to lysogenise host cells and can be assigned to Myoviridae or Podoviridae, respectively.

3. Genome organisation of *S. aureus* phages

The identification and differentiation of *S. aureus* phages has been continuously improved through the development of molecular biology techniques. Since the release of the full sequence of phi-PVL (Kaneko et al., 1998) there have been many genomes of staphylococcal phages and prophages fully sequenced. In a representative study (Kwan et al., 2005) based on the complete genomes of 27 phages, *S. aureus* phages are organised into three size classes: staphylococcal siphoviruses, with a genome size of 39–43 kb; podoviruses, with a smaller genome size of 16–18 KD; and myoviruses, with a genome size of 120–140 kb (for recent reviews, see (Deghorain and Van Melderen, 2012; Lobocka et al., 2012)).

The genome maps of four representative siphoviruses that belong to different serogroups (A, B and F) are shown in Fig. 2. The siphovirus genomes are usually organised into six functional modules: lysogeny, DNA replication, packaging, head, tail, and lysis. Nucleotide sequence analysis revealed that phage genomes of different serogroups share the most homology at the replication module. Two serogroup B phages, ϕ11 and ϕ80α, are among the best studied *S. aureus* phages – partially because both phages have very high transducing efficiency and were thus often used experimentally to transfer gene mutations between *S. aureus* strains. Although there are 50–70 ORFs encoded by each siphovirus genome, for the majority of the ORFs, we have only putative functional annotation. In the following part, only experimentally characterised staphylococcal phage genes will be reviewed. For an updated, expert annotated genome maps of representative phage gnomes please refer to Fig. 2.

3.1. Lysogeny module

As shown in Fig. 2, the integrase and regulator proteins, CI and Cro, respectively, are encoded in the lysogeny modules. The switch between lysogenic and lytic growth is most likely controlled by a molecular circuitry similar to that of the λ phage: The phage will remain in the lysogenic state if CI predominates but will be transformed into the lytic cycle if Cro predominates. In vitro analysis demonstrated that the ϕ11 CI-operator complex resembles those of lambdoid phages at the structural level. The mode of action of the ϕ11 CI, however, may be distinct from that of the repressor proteins of λ and related phages (Ganguly et al., 2009).

3.2. Module for DNA replication

Downstream of the lysogeny module, a dozen of the ORFs in the ϕ11 and ϕ80α genomes may be involved in the redirection of host DNA metabolism for phage DNA replication, as various DNA-binding motifs and putative nucleases could be identified in this region using *in silico* approaches. However, experimental proof is still lacking for most of the ORFs, with a few exceptions. In a recent study, three genes in this region were found to be involved in the mobilisation of *S. aureus* pathogenicity islands (SaPIs). SaPIs are a family of 14–27 kb genetic elements that usually stably reside in the *S. aureus* genome, similarly to prophages, and contain phage-like repressor, integrase and terminase genes but do not contain genes encoding for phage structural proteins. SaPIs also carry a variety of accessory genes including superantigen toxins, antibiotic resistance factors and other virulence factors. The excision and replication of SaPIs needs a helper phage. Both ϕ11 and ϕ80α are used as model helper phages to study SaPI mobilisation. Following the induction of a resident helper phage or superinfection by a helper phage, the SaPI genome is excised, replicated and packed into the structural proteins of the helper phage to form infectious SaPI particles whose capsid is usually 1/3 of the size of its helper phage (Christie and Dokland, 2012; Novick et al., 2010). The depression of different SaPIs requires different proteins from the helper phage. For example, SaPI1, SaPIbov1 and SaPIbov2 are depressed by Sri (ORF22), Dut (ORF32) and ORF15 of ϕ80, respectively (Tormo-Mas et al., 2010). Interestingly Sri was previously identified in ϕ77 as a DnaI-binding protein that inhibits host DNA replication (Liu et al., 2004), while *dut* codes for a dUTPase (Tormo-Mas et al., 2010).

Phage proteins that redirect bacterial metabolic pathways to the phage reproduction cycle have also been identified from the staphylococcal myovirus phages G1 and Twort. ORF67 from phage G1 interacts with the *S. aureus* RNA polymerase σ subunit and blocks cell growth by inhibiting transcription (Dehbi et al., 2009; Osmundson et al., 2012), whereas ORF240 from phage G1 binds tightly to the DNA sliding clamp and prevents both its loading onto DNA and its interaction with DNA polymerase C, leading to DNA replication arrest and cell death (Belley et al., 2006).

3.3. DNA Packaging and morphogenesis modules

In ϕ11 and ϕ80α, the packaging and head modules are localised between the DNA replication and tail module. Recently, the functions of several genes in this region were studied in detail. The RinA protein exerts a regulatory function – it binds to the operator situated upstream of the *terS* gene and activates the transcription of the late operon covering the morphogenesis and lysis modules (Ferrer et al., 2011). The transcription activator activity of both