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Structure and Function of the *Staphylococcus aureus* Bacteriophage 80α Baseplate

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Staphylococcus aureus is an opportunistic human pathogen [1], and a public health threat due to the increased incidence of antibiotic resistance. Transduction by bacteriophages is the main mechanism of transfer of genes encoding virulence factors and antibiotic resistance in *S. aureus* [2]. Specific "helper" phages are also capable of high frequency mobilization of *S. aureus* pathogenicity islands (SaPIs), disseminating the virulence factors the SaPIs carry [3]. Phage 80α is a model of *S. aureus*-infecting siphoviruses and is capable of both generalized transduction and specialized transduction of SaPIs [4]. The phage baseplate is used for recognition and adhesion to target cells; however, very little is known about these structures in staphylococcal phages.

80α tails were produced from a mutant phage with a deletion of residues 2-13 of the scaffolding protein (Fig. 1a). Cryo-electron microscopy was carried out using an FEI Titan Krios electron microscope with a DE-20 Direct Detection Device at the Biological Science Imaging Resource at Florida State University. Three-dimensional reconstruction was done using EMAN and EMAN2 [5] with six-fold (c6) symmetry applied. The resolution of the final model (Fig. 1c) was 8.8 Å according to the 0.143 FSC criterion, limited by the small number of particle images used (7000).

The 80α baseplate is encoded by at least six genes (Fig. 1b). Some of these can be identified by comparison with other phages [6]. HHpred matched 80α gp58 to the crystal structure of *Bacillus subtilis* phage SPP1 distal tail protein (Dit) [7], which best fit into the upper core of the baseplate reconstruction (Fig. 1d). gp59 is thought to correspond to the tail spike protein (Tal) of *Lactococcus lactis* phage p2 [8], but could not be reliably fitted to the baseplate reconstruction. 80α gp61 is nearly identical to *S. aureus* phage ϕ11 receptor binding protein (RBP) gp45, and its crystal structure[9] fits into six distinctive peripheral features of the baseplate. 80α gp62 (central tail fiber; FibC) matches the N-terminal domain of SPP1 baseplate upper protein (BppU) [10], followed by a coiled-coil region that could be fitted into the only extended fibrous features of the baseplate reconstruction, and a C-terminal region that also matched the ϕ11 RBP. 80α gp67 matches several hydrolytic enzymes and may constitute part of the unassigned baseplate core. 80α gp68 includes the same N-terminal region as FibC, followed by a collagen-like triple helix. This protein most likely forms additional outer tail fibers (FibO), but could not be distinguished in the baseplate reconstruction.

ϕ11 adsorption to *S. aureus* requires either α- or β-GlcNAc residues attached to wall teichoic acids, suggesting these are receptors for ϕ11 and presumably for 80α [11]. As expected, an 80α Δ*orf61* strain lacking RBP was not viable. A Δ*orf62* strain lacking FibC was also not viable, suggesting that host recognition and adsorption involves more than RBP alone. Both strains make aberrant baseplates that appear to lack both FibC and RBP. FibO may play a role in host recognition, as well, but an 80α Δ*orf68* mutant lacking FibO was viable and formed an essentially normal baseplate. The presence of both RBP and two types of tail fibers distinguishes the phage 80α baseplate from baseplates of other better described Gram-positive phages. A better structural and functional description of the 80α baseplate and

its proteins will be important to understand the role of phages in defining bacterial virulence and opens for the possibility to engineer phages for therapeutic purposes.

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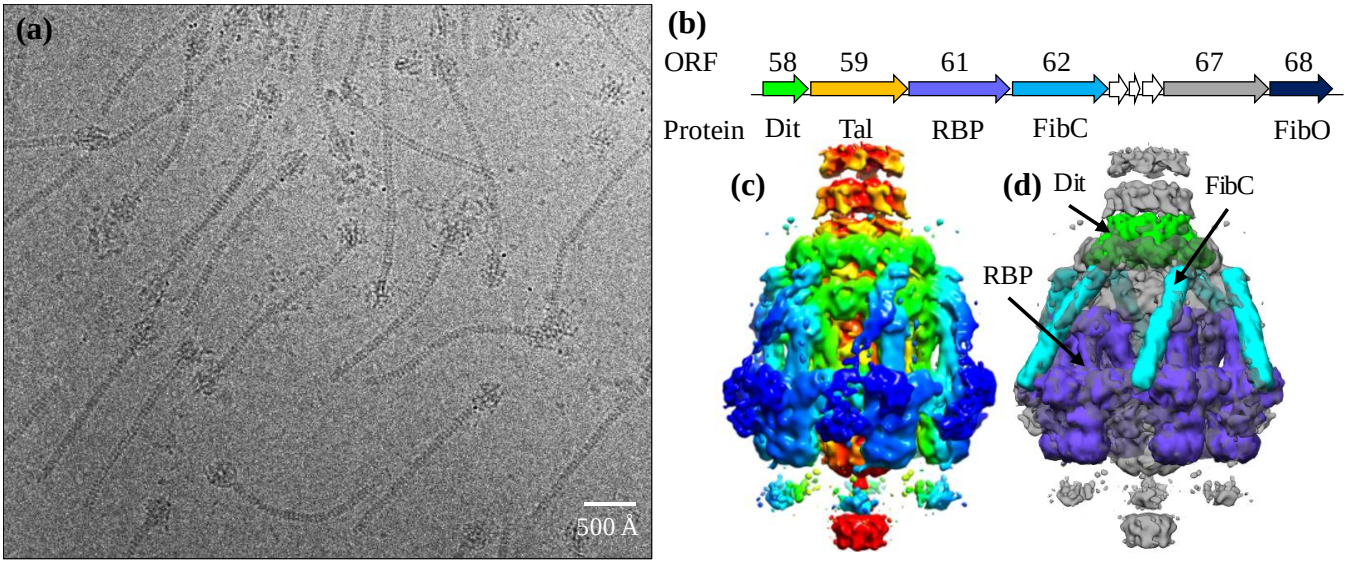

Figure 1. 80α baseplate genes and cryo-EM reconstruction with expected protein locations. **(a)** Typical cryo-EM micrograph of 80α with Δ2-13 scaffolding protein **(b)** 80α genes encoding distal tail protein (Dit), tail-associated lysin (Tal), receptor binding protein (RBP), central fiber (FibC), and outer fiber (FibO). White arrows correspond to possibly unexpressed open reading frames (ORFs). The gray arrow denotes unknown localization. **(c)** 8.8Å model colored radially in Chimera[12] for clarity. The top is the direction of the tail. **(d)** PDB models for Dit (2X8K), RBP (5EFV), and coiled-coil region of FibC (4LIN) best fit the reconstruction with correlations 0.82, 0.84, and 0.78, respectively.

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