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Structure of the capsid size-determining scaffold of "satellite" bacteriophage P4

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Satellite phage P4 is an 11.6 kb replicon that exists as a plasmid or stably integrated into the genome of its host, *Escherichia coli*, and parasitizes helper phages like P2 (33.6 kb, family *Myoviridae*) to achieve its own transduction. P4 is derepressed in the presence of P2 undergoing lytic replication (or derepresses the P2 prophage to trigger lytic replication), is excised from the host genome if necessary, and replicates in the host cytoplasm alongside P2. Normally, the $T = 7$ isometric icosahedral procapsids of P2 are assembled from the P2 major capsid protein, gpN, an internal scaffolding protein, gpO, and the portal protein, gpQ. However, in the presence of P4, the P4-encoded size determination protein (Sid) forms an external dodecahedral scaffolding cage that redirects capsid assembly, resulting in smaller $T = 4$ P4 procapsids of approximately 1/3 the volume of the normal P2 procapsids. The reduction in volume prevents the relatively large genome of P2 from being packaged into the smaller procapsids, making them suitable only for packaging of P4. After assembly, the Sid protein dissociates from the capsid, the procapsids expand and mature, the P4 genome is packaged, and the capsids are decorated with the P4-encoded polarity suppression protein (Psu). The hijacking of phage-like particles with altered capsid size has been termed molecular piracy and has also been observed in the genetically unrelated *Staphylococcus aureus* pathogenicity islands (SaPIs), which achieve capsid size redirection by distinct mechanisms [1-2]. SaPIs are a subset of the phage-inducible chromosomal islands (PICIs), which have been discovered in diverse Gram-positive and Gram-negative bacterial hosts [3].

Previous studies identified size responsiveness (*sir*) mutations clustered in a region of gpN denoted the *sir* loop that allow P2 to escape size redirection. N mutation sensitive (*nms*), or "super-Sid", mutations localized near the C-terminus of Sid act as second-site suppressors of the *sir* mutations and restore size redirection [1,4]. Atomic models of gpN built into a cryo-electron microscopy (cryo-EM) single particle reconstruction of the P4 procapsid later revealed that the *sir* loop of gpN interacts with Sid, suggesting that the *sir* and *nms* mutations disrupt and restore, respectively, Sid binding to gpN in the procapsid [4]. However, the structure of Sid could not be determined, leaving the details of these interactions—and its mechanism of capsid size redirection—unclear. In honor of Dr. Michael Rossman and his research on the unusual phage ϕX174, which assembles its procapsid with an external scaffolding protein, we present here our structure of the Sid external scaffolding protein and its mechanism of capsid size redirection.

We produced P4 procapsids *in vitro* from co-expressed gpN, protease-deficient mutant gpO, and Sid. Single particle reconstruction with icosahedral symmetry averaging of images of this sample reached near-atomic resolution, but the Sid density was attenuated and uninterpretable. We hypothesized that dissociation of Sid from the procapsid could occur partially, resulting in procapsids with incomplete dodecahedral scaffolding cages, so we employed icosahedral symmetry expansion followed by masked 3D classification to colocalize all possible copies of Sid bound to the procapsid in the dataset and sort out any instances where Sid was missing. This allowed a reconstruction to near-atomic resolution without symmetry averaging that included continuous density for the entire Sid protein [5].

Sid is almost entirely α-helical and forms knotted homodimers that bind via their C-terminal helices (α6) to the *sir* loops of two gpN subunits of each hexamer in the P4 procapsid. As expected, the *sir* mutations occur within the interface between the *sir* loop and Sid α6, disrupting Sid binding. However, none of the *nms* mutations occur within the interface, indicating that they do not directly counteract the *sir* mutations [5]. Instead, the two *nms* mutations shown to be most effective in restoring capsid size redirection add positively charged sidechains in Sid α6 near a highly negatively charged section of the *sir* loop containing no known *sir* mutations [5,6]. Thus, these *nms* mutations likely restore capsid size redirection by establishing new

electrostatic interactions that circumvent typical gpN-Sid binding [5]. This also explains the prior observation that the *nms* mutations lack allele specificity in suppressing *sir* mutations [6].

Each Sid monomer includes an elongated coiled-coil stem domain formed by helices α1 and α2 [5]. The only known structure resembling Sid is that of P4 Psu, which has an identical fold despite low sequence homology [5,7]. Sid differs from Psu in the length of its stem domain, which is extended by approximately 20 residues in both α1 and α2. This extension allows the stem domain tips from Sid homodimers bound to adjacent gpN hexamers to come into contact, forming trimeric interfaces stabilized by electrostatic interactions. By fitting density for the Sid dimer bound to a gpN hexamer from the P4 procapsid to a previous reconstruction of the P2 procapsid, we simulated the hypothetical organization of the Sid scaffold onto the larger $T = 7$ procapsid lattice. This demonstrated that the trimeric interaction of Sid stem domain tips is prohibited in the larger procapsid and suggested a model for the function of Sid. Sid homodimers capture gpN hexamers early in assembly, while their organization is still to be determined, and link the hexamers through the interaction of the Sid stem domain tips. The resulting configuration of gpN hexamers cannot be extended to form $T = 7$ procapsids, redirecting assembly towards the $T = 4$ organization of the smaller P4 procapsids instead [5]. This mechanism of capsid size redirection is unrelated to that employed by SaPIs, indicating that diverse molecular pirates have convergently evolved to achieve a common strategy through different means [1,8].

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