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## Dispatch

### Microbiology: And *Amoebophilus* Invented the Machine Gun!

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**Bacterial contractile injection systems are fascinating particles that use a spring-like mechanism to inject an effector-loaded needle into target cells. A recent study shows that the intracellular bacterium *Amoebophilus asiaticus* uses arrays of contractile structures to escape from the amoeba phagosome.**

To compete effectively for access to nutrients, bacteria have developed an arsenal of weapons that target other microbial cells. These include: contact-dependent growth inhibition (CDI) systems, which are deployed at the cell surface and act as grenades that are pinned out and thrown inside the target bacterial competitor [1]; bacteriocins, which are released into the extracellular milieu and act as mines [2]; and Type VI secretion systems (T6SS), which resemble spear guns that use a spring-like mechanism to inject effectors into the target [3]. The T6SS is part of a larger family of so called contractile injection systems (CIS) that include prototypical bacteriophages such as T4 and Mu [4], and extracellular CIS such as R-pyocins [5], anti-feeding prophages [6], and *Photorhabdus* virulence cassettes [7] (Figure 1). CIS can also form arrays such as metamorphosis-associated contractile assemblies [8] (Figure 1). These structures comprise a common tail core: a needle composed of an internal tube made of stacks of hexameric rings tipped by a spike, and wrapped by a contractile sheath that assembles on a multiprotein complex called baseplate (Figure 2). CIS inject effectors into target cells and contribute to bacterial competition, pathogenesis, establishment of symbiosis, or modification of the host.

In a recent study published in *Science* [9], Martin Pilhofer's and Matthias Horn's teams report the identification and exquisite description of a new contractile structure in the obligate intracellular amoeba symbiont *Amoebophilus asiaticus*. The gene cluster encoding this structure was previously identified on the *A. asiaticus* genome but its role and its architecture

were not defined [10]. Here, the authors first used electron cryotomography to visualize this contractile injection system. However, imaging cytoplasmic structures of intracellular bacteria by electron cryotomography is a challenge as the quality of the tomographs rely on the sample thickness. The authors successfully used a focused ion beam to mill the surface and obtain thinner samples [11]. In addition to providing access to intracellular bacteria, surface milling bypasses the requirement to genetically induce cell-size reduction by deletion of genes that control mid-cell positioning of the divisome [12], and therefore might be of specific interest for strains that are not genetically tractable. Using this technical *tour de force*, this study revealed that these contractile structures form barrel-like arrays. With the exception of the previously described metamorphosis-associated contractile assemblies, this organization is unique for CIS. How do these structures associate with each other to form these highly organized arrays? In the metamorphosis-associated contractile assemblies, tails are connected by a net, however such fibers have not been observed in *A. asiaticus*. Rather, the organization of the hexagonal arrays seems to be controlled by baseplate–baseplate interactions. One question that arises when examining this barrel-like architecture is: why is it important to form arrays? In T6SS and R-pyocins, a few effectors are translocated at each firing event, and hence one may hypothesize that arrays will help to efficiently deliver tens or hundreds of effectors without the need to re-assemble a new CIS. However, this hypothesis suggests that all *A. asiaticus* tail sheaths of a barrel will contract simultaneously. This is clearly not the case, as the published tomographs show that arrays comprise both extended and contracted tails [9], demonstrating that there is no coordination of the contraction events. In addition, the absence of a ClpV-like AAA<sup>+</sup> ATPase encoded within the *A. asiaticus* CIS gene cluster suggests that these structures are not recycled once contracted. However, it remains possible that a housekeeping AAA<sup>+</sup> ATPase might be hijacked to disassemble and recycle sheath subunits. Interestingly, CISs share a common core (a common ancestor?) but have evolved in different flavours, by the recruitment of new functional modules (Figure 2). In addition to the common core, extracellular CISs such as R-pyocins and contractile bacteriophages, comprise fibres to bind and recognize host cell receptors; bacteriophages possess a capsid head to pack the genetic material as well as capsid/tail connectors. By contrast, T6SS have captured a trans-envelope membrane complex, and have adapted one of the baseplate subunits to specifically recognize the membrane complex and thus to properly orient the CIS toward the cell exterior [13,14]. In T6SS, the membrane complex serves as a docking station for the CIS and as a channel for the passage of the needle and hence prevents self-injury during sheath contraction [13]. CISs are highly modular structures, and are in constant evolution by the

addition of new subunits or domains. For example, some T6SS have integrated a peptidoglycan-binding motif to stably anchor the membrane complex [15], whereas others hijack peptidoglycan-modifying enzymes to locally degrade the cell wall for proper insertion of the membrane complex [16,17]. Similar to the prototypical T6SS, *A. asiaticus* CIS arrays are anchored to the membrane and oriented toward the exterior, via interactions between the baseplate and a membrane complex or a membrane anchor [9]. However, no gene encoding a putative membrane complex nor a membrane protein is present in the gene cluster. Understanding how the CIS arrays are connected to the membrane, and the origins of this putative membrane anchor are exciting questions yet to be answered

Phylogenetic analyses have shown that the *A. asiaticus* CIS arrays are more closely related to the metamorphosis-associated contractile assemblies and anti-feeding prophages than the T6SS [9]. Indeed, a number of subunits such as tape measure and tail terminator proteins are found in extracellular CISs but absent in T6SS. However, the absence of release of the *A. asiaticus* CIS in the milieu and the presence of a membrane anchor are specific to T6SS. Hence, the *A. asiaticus* is likely to represent a missing link between extracellular CIS and T6SS.

The next challenge will be to define the role of the *Amoebophilus* CIS. Based on the correlation between expression levels, infection stages and amoeba infection rates, as well as on the presence of CIS arrays at the sites of contact with the phagosomal membrane, Böck *et al.* have proposed that the *A. asiaticus* CIS are necessary for the early stages of amoeba infection, and more specifically in phagosome escape [9]. This observation raises several questions about the mechanism of action of the *A. asiaticus* CIS. How does *A. asiaticus* sense the phagosome membrane? What determines the site of array assembly? And what is the signal that triggers firing? From a functional perspective, there are also questions related to the functional role(s) of this unique structure: What are the effectors? How do they impact the phagosome and how are they loaded?

What we also learn from this study is that there are many more contractile injection machines to discover, each with their own specificity. Interestingly, genes encoding tail components could be found in many genomes but the structure and function of these putative CISs remain unknown [18]. As exemplified by this work and by the recent discovery of new cellular assemblies [19], electron cryotomography is a powerful technique to identify novel structures and to gain information on their architecture [20], but classical genetic and biochemical

approaches are then required to define their functions.

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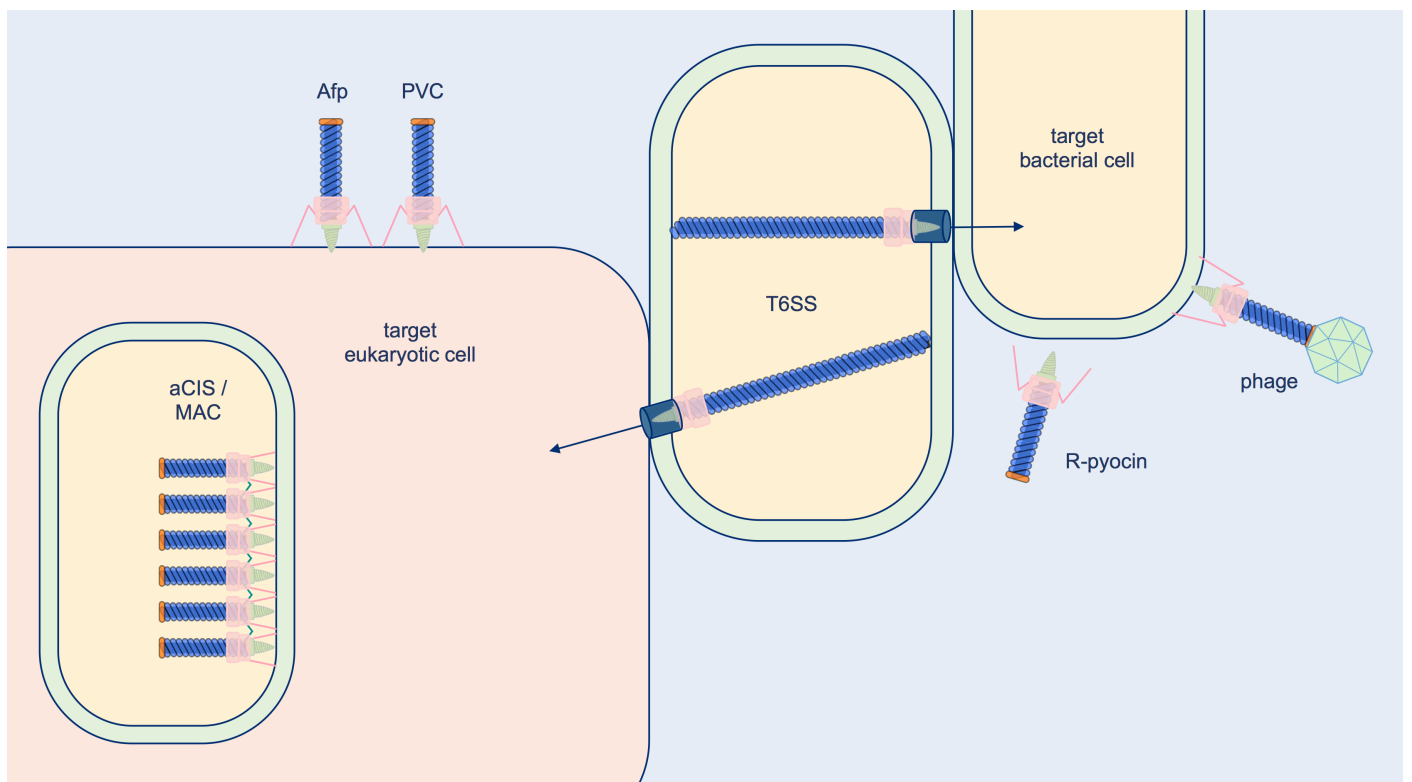
## Legend to Figures

**Figure 1. Schematic representation of known contractile injection systems.** The different CIS (Type VI secretion system (T6SS), phage, R-pyocin, anti-feeding prophage (Afp), *Photorhabdus* virulence cassette (PVC), metamorphosis-associated contractile assemblies (MAC) and *Amoebophilus asiaticus* CIS (aCIS) are represented, as well as their mode of action and their target cells.

**Figure 2. Commonalities and diversity in contractile injection systems.** The CIS common core (kit) is represented, as well as functional modules required for the assembly of specific structures.

## In Brief

**Contractile injection systems are fascinating particles that use a spring-like mechanism to inject an effector-loaded needle into target cells. A recent study shows that the intracellular bacterium *Amoebophilus asiaticus* uses arrays of contractile structures to escape from the amoeba phagosome.**



# Order your CIS toolkit now !

## the CIS kit



spike



baseplate



tube



sheath

to build a T6SS, order CIS kit + 1 and 4.

to build a phage, order CIS kit + 1, 3 and 5.

to build a pyocin, order CIS kit + 2 and 5.

to build an Afp, order CIS kit + 2 and 5.

to build a MAC, order several CIS kits + 2, 5 and 6.

## Accessories

(to be ordered separately)



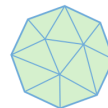
membrane complex  
(to stay at home)  
delivered with TssK adaptor.

1



fibers (to stably interact  
with your host)  
pack of 6


2



capsid  
(to store your DNA)

3



AAA+ ATPase   
(to recycle the sheath)  
pack of 6

4



ruler (to control  
your tail length)  
available in various dimensions

5



net (to combine  
your CISs)

6