ARTICLE

doi:10.1038/nature14667

Biogenesis and structure of a type VI secretion membrane core complex

Eric Durand[1,2,3,4,5]*, Van Son Nguyen[2,5]*, Abdelrahim Zoued[1]*, Laureen Logger[1], Gérard Péhau-Arnaudet[4], Marie-Stéphanie Aschtgen[1], Silvia Spinelli[2,5], Aline Desmyter[2,5], Benjamin Bardiaux[4,6], Annick Dujeancourt[3,4], Alain Roussel[2,5], Christian Cambillau[2,5], Eric Cascales[1] & Rémi Fronzes[3,4]

Bacteria share their ecological niches with other microbes. The bacterial type VI secretion system is one of the key players in microbial competition, as well as being an important virulence determinant during bacterial infections. It assembles a nano-crossbow-like structure in the cytoplasm of the attacker cell that propels an arrow made of a haemolysin co-regulated protein (Hcp) tube and a valine-glycine repeat protein G (VgrG) spike and punctures the prey's cell wall. The nano-crossbow is stably anchored to the cell envelope of the attacker by a membrane core complex. Here we show that this complex is assembled by the sequential addition of three type VI subunits (Tss)—TssJ, TssM and TssL—and present a structure of the fully assembled complex at 11.6 Å resolution, determined by negative-stain electron microscopy. With overall C_5 symmetry, this 1.7-megadalton complex comprises a large base in the cytoplasm. It extends in the periplasm via ten arches to form a double-ring structure containing the carboxy-terminal domain of TssM ($TssM_{ct}$) and TssJ that is anchored in the outer membrane. The crystal structure of the $TssM_{ct}$–TssJ complex coupled to whole-cell accessibility studies suggest that large conformational changes induce transient pore formation in the outer membrane, allowing passage of the attacking Hcp tube/VgrG spike.

In the environment, bacteria have evolved collaborative or aggressive mechanisms to communicate, exchange information and chemicals, or compete for space and resources[1–3]. One of the main weapons of bacterial conflicts is a multi-protein device called the type VI secretion system (T6SS) that is assembled in the attacker bacterium[4]. The T6SS is a versatile nanomachine that can deliver toxin proteins directly not only into prey prokaryotes but also into eukaryotic cells during bacterial infections[3,5–9]. Anti-host activities have been shown to inhibit phagocytosis and therefore to disable macrophages, while the anti-bacterial activities allow the bacterium to destroy competitors and to have a privileged access to the niche, to nutrients or to new DNA[3,9,10]. The T6SS is composed of 13 different proteins, encoded by genes that are usually clustered[11]. It assembles a tubular puncturing device that is evolutionarily, structurally and functionally similar to the tail of contractile bacteriophages. Its sheath is a tubular structure, hundreds of nanometres long, that extends in the cytoplasm and is built by the polymerization of TssBC building blocks[12–14]. It is assembled on an assembly platform, the baseplate[13,15–17], and maintained in an extended, metastable conformation[16–18]. The attacking arrow, wrapped by the sheath, comprises an inner tube that is built by stacked Hcp hexameric rings[19] and tipped by a puncturing spike composed of VgrG[20]. Upon contact with the prey, structural rearrangements of the sheath subunits induce its contraction and propulsion of the Hcp tube/VgrG spike towards the target cell, allowing toxin delivery[16,17,21]. The phage-like T6SS tail is anchored to the attacker cell membrane by a trans-envelope complex[22]. This membrane complex not only serves as a docking station but has been proposed as a channel for the passage of the inner tube after sheath contraction, thereby preventing membrane damage in the attacker[16,17]. The membrane core complex of the T6SS (that is, the minimal module required to function and conserved in all T6SS) is composed of the TssL and TssM inner-membrane proteins and the TssJ outer membrane lipoprotein[15–17,22–26]. These proteins are connected through a network of interactions between TssM and TssL, and TssM and TssJ[22,24,25]. Although the localization and topology of these subunits, their interactions and the crystal structures of the soluble domains of TssJ and TssL have been described[17,22–29], we still lack crucial information on the biogenesis and overall architecture of this complex and how it is used as a channel during T6SS action.

Localization, dynamics and biogenesis of the T6SS membrane core complex

We first sought to determine the assembly pathway of the entero-aggregative *Escherichia coli* (EAEC) T6SS membrane core complex. Strains producing fluorescently labelled T6SS membrane subunits were engineered. The sequence encoding the super-folder green fluorescent protein (sfGFP) was inserted upstream of the stop codon of the *tssJ* gene or downstream of the start codon of the *tssL* and *tssM* genes. In these constructs, the fusion proteins were produced from their native chromosomal loci. Hcp release and anti-bacterial assays demonstrated that the sfGFP–TssL and sfGFP–TssM fusion proteins were functional (Extended Data Fig. 1a). By contrast, strains producing TssJ–sfGFP or TssJ–mCherry had a non-functional T6SS (Extended Data Fig. 1b). Fluorescence microscopy analyses showed that sfGFP–TssL and sfGFP–TssM cluster at discrete positions at the cell periphery, in agreement with their membrane localization (Fig. 1a and Extended Data Fig. 1c). These foci are stable and static (Fig. 1a and Extended Data Fig. 1d). Statistical analyses further showed that one or two foci are observable in cells expressing the T6SS (Fig. 1b) and that these clusters are randomly distributed around the cell (Fig. 1c). Co-localization experiments with strains producing

[1]Laboratoire d'Ingénierie des Systèmes Macromoléculaires, Aix-Marseille Université - CNRS, UMR 7255, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France. [2]Architecture et Fonction des Macromolécules Biologiques, CNRS, UMR 7257, Campus de Luminy, Case 932, 13288 Marseille Cedex 09, France. [3]G5 Biologie structurale de la sécrétion bactérienne, Institut Pasteur, 25–28 rue du Docteur Roux, 75015 Paris, France. [4]UMR 3528, CNRS, Institut Pasteur, 25–28 rue du Docteur Roux, 75015 Paris, France. [5]AFMB, Aix-Marseille Université, IHU Méditerranée Infection, Campus de Luminy, Case 932, 13288 Marseille Cedex 09, France. [6]Unité de Bioinformatique Structurale, Institut Pasteur, 25–28 rue du Docteur Roux, 75015 Paris, France.
*These authors contributed equally to this work.