Activated bacterial TIR proteins: NADase activity and filamentous assemblies

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Toll/interleukin-1 receptor (TIR) domain-containing proteins have been found widely in animals, plants and bacteria, and functions relating to immune response by self-association and TIR-TIR interactions. Bacterial TIR proteins have been shown to have pathogenicity and anti-viral functions. During bacterial infection, some of these proteins act as virulence factors to inhibit immune responses by interfering with Toll-like receptor signalling. Additionally, some of these proteins possess NAD^+ nucleosidase activity, which is not only related to the virulence of pathogenic bacteria, but also plays an important role in bacterial anti-viral defence. Here, we report on our studies on two bacterial TIR domain-containing proteins: AbTir and PumA. Upon NAD^+ cleavage, *Acinetobacter baumannii* TIR domain-containing (AbTir) produces a signaling molecule that is a non-canonical variant of cyclic ADP ribose (v-cADPR). We characterized the products and revealing functions in antiviral defence in bacteria and suppression of plant immunity by bacterial effectors [1]. Furthermore, we found that addition of an inhibitor (3AD) of AbTir NAD^+ nucleosidase activity traps the protein in an enzyme-active state, which corresponds to filaments. Using cryo-EM, we determined the $AbTir^{TIR}$ filament structure, which is structurally analogous to filaments formed by mammalian Toll-like receptor adaptor proteins (Figure 1).

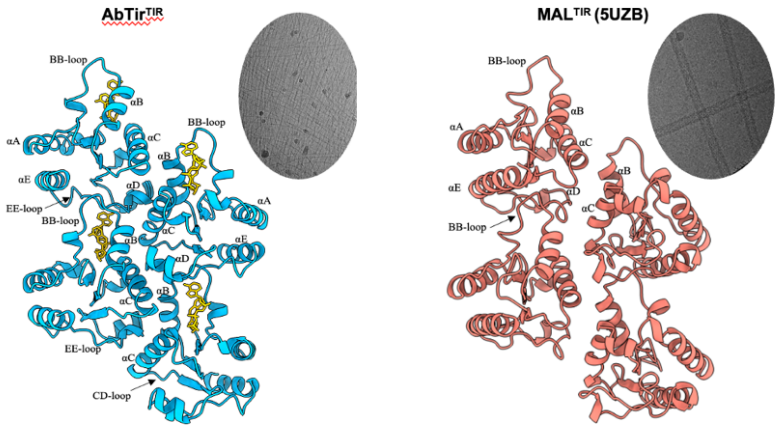

Figure 1. Similarity of filaments formed by the TIR domains from AbTir and the Toll-like receptor adaptor MAL.

Recent studies show that PumA, which is from the multi-drug resistant pathogen *Pseudomonas aeruginosa* PA7, is essential for the PA7 strain virulence [2]. We show that PumA also has NAD^+ nucleosidase activity. We also observed PumA TIR domain filaments upon incubation with 3AD. Further structural and functional studies will determine the structure of these filaments and investigate the role these assemblies play in bacterial virulence and anti-viral defence. Jointly, our studies provide the structural basis of the production of signaling molecules by TIR domains and form the foundation for the treatment bacterial infections.

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[2] Imbert P R C, Louche A, Luizet J B, et al. A Pseudomonas aeruginosa TIR effector mediates immune evasion by targeting UBAP 1 and TLR adaptors[J]. *The EMBO journal*, 2017, 36(13): 1869-1887.