Abstract

Toll-like receptors (TLRs) detect pathogens and endogenous danger, initiating immune responses that lead to the production of pro-inflammatory cytokines. Recruitment of signalling adaptors such as MyD88, MAL, TRIF and TRAM to the TLR requires Toll/interleukin-1 receptor (TIR)-domain interactions. Except for TLR3, all TLRs utilize the MyD88-dependent pathway, and TLR3 and endosomal TLR4 use the TRIF-dependent pathway. However, knowledge of how receptors recruit downstream adaptor proteins and the structural basis of these interactions remains limited, only BB-loop has been identified to play a key role in the interface. Here we show that TRAM TIR domains spontaneously and irreversibly form filaments in vitro. The TRAM TIR domain also forms co-filaments with TLR4 TIR domains. A 5.5 Å-resolution cryo-EM structure shows a stable TRAM filament involving two parallel strands of TIR-domain subunits in a BB-loop-mediated head-to-tail arrangement. The two-stranded head-to-tail arrangement of TRAM TIR filaments is similar to that observed for TIR domain assemblies of MAL and MyD88, which have previously been shown to signal through a mechanism involving cooperative assembly formation. A BB-loop mutant that prevents filament formation validates the key role of the BB loop in filament formation. Using this mutant, we determined the crystal structure of TRAM TIR to 3 Å-resolution. The BB-loop of the crystal structure adopts a different conformation to that observed in the filament structure and may represent a monomeric non-signalling state. Cell-based signalling assays are used to validate TRAM TIR assembly interfaces. This information is crucial for a mechanistic understanding of TLR signalling, the development of therapeutic strategies, and understanding of the molecular basis of the consequences of the human disease of adaptor polymorphic variants.