Structural and functional characterisation of plant TIR domains and their role in plant innate immunity

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In plant innate immunity, the superfamily of nucleotide-binding leucine-rich repeat receptors (NLRs) are responsible for the recognition of pathogen effectors. A large family of NLRs contain TIR domains. Recent studies have shown that TIR domains of TIR-NLRs (TNLs) or TN/TIR-only proteins are able to cleave nicotinamide adenine dinucleotide (NAD^+) into a large variety of nucleotide containing products, collectively termed as immunomodulatory purine nucleotides (IPNs). These products were observed to be essential for the activation of downstream signalling eventually leading to localised cell death. However, the exact mechanism for the formation of each product and its associated substrates are still poorly understood. Additionally, TIR domains has also been found to form a filament along double stranded DNA and cleave DNA/RNA to produce 2′,3′-cAMP/cGMP. Given the multiple roles TIR domains play in plant innate immunity, my project aims to structurally and functionally characterise four different plant TIR domains (TX10, $TN11^{TIR}$, $RUN1^{TIR}$, $RPS4^{TIR}$). In support of the current hypothesis of TNLs roles in innate immunity, $RUN1^{TIR}$ and $RPS4^{TIR}$ has been found to produce the downstream signalling molecules using liquid chromatography-mass spectrometry (LC-MS). Additionally, while electrophoretic mobility shift assays (EMSA) on TIR domains demonstrate DNA binding activity, these TIR domains were unable to produce 2′,3′-cAMP/cGMP. Of the TIR domains investigated, only $RUN1^{TIR}$ has been found to produce filamentous structures upon incubation with DNA using negative staining electron microscopy. This suggest that 2′,3′-cAMP/cGMP may not be the main product of TIR-DNA/RNA binding, with its binding instead serving as scaffolds for as yet unknown mechanism. Future work will utilise different substrates and mutants in order to further current understanding of the conversion of NAD^+ to nucleotides as well as structural characterisation of the DNA-TIR filament complex.

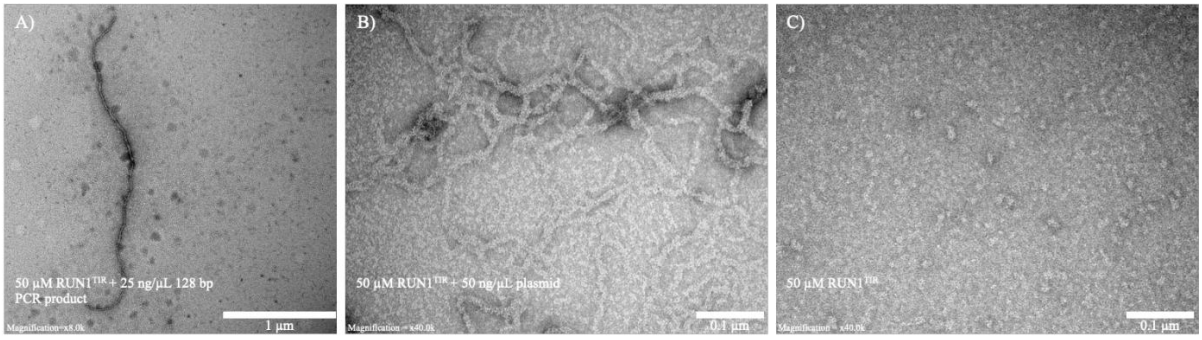

Figure 1. Negative stain electron microscopy images of $RUN1^{TIR}$ after 3 h incubation at room temperature. Formation of $RUN1^{TIR}$-DNA filament (~20 nm diameter) upon incubation with (A) 128 bp PCR product and (B) plasmid. (C) $RUN1^{TIR}$ monomers without DNA incubation.