The *Xanthomonas campestris* type III effector XopJ proteolytically degrades proteasome subunit RPT6 to prevent salicylic acid-mediated signalling

Abstract

XopJ is a type III effector protein from *Xanthomonas campestris* pv. *vesicatoria* that interferes with plant immune responses. Previous work reported that XopJ targets the proteasomal subunit RPT6 *in planta* to suppress proteasome activity. Mutation of the catalytic triad as well as of the N-terminal myristoylation motif in XopJ abolished its proteasome-inhibiting ability, suggesting that enzymatic activity and membrane anchoring are required for proteasome inhibition. XopJ-mediated manipulation of the host proteasome interferes with salicylic acid (SA)-dependent defense response to attenuate onset of necrosis. However, it was not clear how XopJ acts mechanistically to inhibit the proteasome and SA-signaling. Co-expression and BiFC studies reveal that only membrane-localized XopJ is able to recruit RPT6 to punctuate structures at the plant plasma membrane being reminiscent of lipid rafts. Further analysis of the RPT6-XopJ complex shows that once localized to the membrane, RPT6 is degraded by XopJ *in planta*, dependent on its myristolyation motif and catalytic activity. In vitro activity measurement using a generic substrate demonstrates that XopJ displays protease activity. Transient co-expression of effector and target protein in *N. benthamiana* suggests that XopJ proteolytically degrades RPT6 to inhibit the proteasome assembly and hence its activity. XopJ-mediated suppression of the proteasome function interferes with the proteasomal turnover of NPR1, the master regulator of SA responses and thus leads to the accumulation of ubiquitinated NPR1. These data suggest that XopJ acts as a protease to degrade RPT6 leading to malfunction of the proteasome and hence resulting into a reduced proteasome-mediated turnover of NPR1.