

C2.1-5

HOW PROTEOSTASIS SHAPES PLANT-BACTERIA INTERACTIONS

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Text

Protein homeostasis is epitomized by a tight equilibrium of protein biosynthesis and degradation; the 'life and death' of proteins. Approximately one-third of newly synthesized proteins are degraded. As such, regulated protein turnover is required to maintain cellular integrity and survival. Autophagy and the ubiquitin-proteasome system (UPS) are the two principal intracellular degradation pathways in eukaryotes. Both degradation pathways orchestrate many cellular processes during plant development and upon environmental stimuli. As such, both pathways play a major role during plant-microbe interactions. We have recently identified that autophagy and the proteasome system are exploited by bacterial pathogens to reprogram host cellular pathways. By studying this intimate interplay, we can utilize plant pathogenic bacteria as tools to understand host cellular degradation machineries and to decipher novel components and functions. In my presentation, I will not only cover our recent work on the role of autophagy and the proteasome in plant-microbe interactions but will report on our attempts to identify new autophagy regulators and new functions of known UPS components. I will highlight different examples and discuss our recent advances.

C2.1-6

CRISPRI AS A TOOL FOR THE FUNCTIONAL STUDY OF GENE FAMILIES IN XANTHOMONAS

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Text

The *Xanthomonas* genus includes several plant pathogens responsible for significant crop losses worldwide. Most of the functional studies of virulence in this genus have been carried out by directed mutagenesis through homologous recombination. However, this strategy is

cumbersome for the study of gene families. CRISPR interference (CRISPRi) allows precise silencing of target genes by using a catalytically dead Cas9 (dCas9) which interferes with gene expression. Because of its RNA-directed nature, this technology can be used for silencing several genes in a single experiment in bacteria and other organisms. We implemented a CRISPRi strategy to silence several members of the Transcriptional activator-like effectors (TALE) gene family at once in four different species of *Xanthomonas*. Our results underscore the importance of the activation of the *SWEET* gene family in cassava upon infection by *Xanthomonas phaseoli* pv. *manihotis*. Remarkably, we report the importance of this gene family in the infection of this host by the non-vascular pathogen *X. cassavae*. In addition, we successfully silenced several TALE genes in a total of five species, including *X. oryzae* pv. *oryzae*, *X. citri* pv. *citri* and *X. campestris* pv. *campestris* using CRISPRi, confirming the importance of this gene family in these pathosystems. The CRISPRi tool can be further modulated to silence sets of genes within a gene family for functional studies in *Xanthomonas* and other plant pathogenic bacteria.

F2.1-1

PLANT-ENCODED ARTIFICIAL SMALL RNAs DIRECT GENE SILENCING IN PSEUDOMONAS SYRINGAE AS WELL AS DISEASE PROTECTION

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Text

Plant small RNAs (sRNAs) can trigger non-cell autonomous RNA interference (RNAi) in interacting eukaryotic pathogens or parasites possessing canonical RNAi factors. However, it is currently unknown whether a similar process could operate against a phytopathogenic bacterium, which lacks a eukaryotic-like RNAi machinery. We recently demonstrated that *Arabidopsis*-encoded artificial sRNAs can trigger the sequence-specific silencing of a virulence factor from *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto* DC3000). However, the sRNA species that are implicated in this phenomenon remain elusive. In the present study, we identified and characterized two populations of apoplasmic sRNAs that orchestrate antibacterial gene silencing. The first one involves sRNAs that are associated with extracellular vesicles (EVs), and presumably incorporated in ribonucleoprotein complexes. Intriguingly, the second one involves sRNA duplexes that are in a free form, and thus referred to here as extracellular free small RNAs or efsRNAs. Here, I will present the experimental data supporting these findings. I will also discuss the relevance of these findings in the understanding of how plants regulate transcriptome, community composition and genome evolution of associated bacteria.

F2.1-2