H.F.R.I.
Hellenic Foundation for Research & Innovation

Description of the funded research project

2nd Call for H.F.R.I. Research Projects to Support Post-Doctoral Researchers

Title of the research project: **Untying the Gordian Knot of the regulation of Type III Secretion Systems from economically important pathogens**

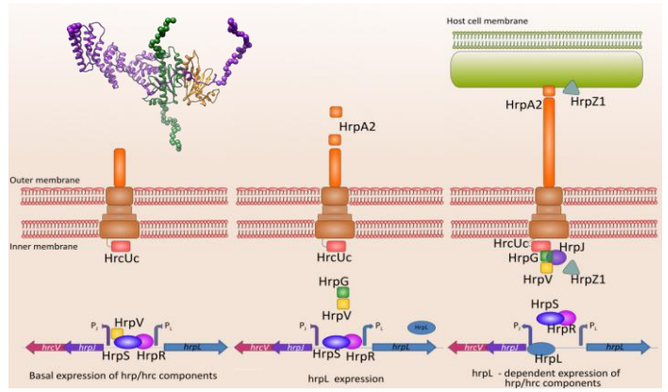
Principal Investigator: **Efstratios Mylonas**

Reader-friendly title: **RegSecSy**

Scientific Area: **Life Sciences**

Institution and Country: **Foundation for Research and Technology–Hellas, Institute of Molecular Biology and Biotechnology, GREECE**

Host Institution: **Foundation for Research and Technology–Hellas, Institute of Molecular Biology and Biotechnology**



PI **Team Post-doc**

Assistant members

Budget: 180000€

Duration: 36 months

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Research Project Synopsis

The Type III Secretion System (T3SS) is an essential tool in the arsenal of pathogenic bacteria. T3SS is a large multiprotein assembly, dedicated to the infection of a plethora of animal and plant hosts by injecting “effector” proteins inside the host cytoplasm. The main modes of regulation of the T3SS occur via transcription and secretion control, realized mostly intracellularly by protein-protein interactions, translocation, use of molecular switches etc. However, there is only an incomplete understanding of how this control occurs. We have recently uncovered a direct link between T3SS expression and secretion in the economically important plant pathogens *Pseudomonas syringae* and *Erwinia amylovora*. The HrpV and HrpG proteins act both as T3SS transcriptional regulators (negative and positive respectively) and, complexed with the “gatekeeper” protein HrpJ, prevent premature secretion of effectors.

The goal of RegSecSy is to further explore the network of intermolecular interactions that control the activation of the T3SS. On the transcription front, we aim to structurally characterize the “Enhancer Binding Protein” (EBP) complex, the effect of the negative regulator HrpV on the EBP complex and how this effect is neutralized by HrpG. Since the mechanism of the gatekeeping process in not well understood, suspected interactions with secreted harpin proteins will also be examined. A bioinformatics analysis of the T3SS “pathogenicity island” will reveal regulatory RNA structures and potential hidden open reading frames. Last, transcriptomics will examine the intersection of T3SS regulation and microbial processes crucial for host invasion, such as pH sensing, motility, biofilm formation etc.

Project originality

RegSecSy seeks to uncover, through analysis of the T3SS regulation for plant pathogens, the network of intermolecular interactions that control the activation of the T3SS and the interplay with other cascades that contribute to the bacterial infection. Our goal is to illustrate that T3SS protein expression and secretion, both equally important for bacterial pathogenicity, are not two separate but, rather, deeply intertwined processes.

The position of the T3SS gatekeeper complex proteins at the crossroads of T3SS regulation makes them most attractive targets for protein-protein interaction analysis. They are, likely, involved in the coordination of other bacterial processes during T3SS-mediated infection (such as quorum sensing, biofilm formation, motility and pH sensing) by fine-tuning the protein expression at the transcriptional and translational level.

While phenotypic observations *in vivo* and transcriptomics-bioinformatics analysis will provide insights into T3SS regulation at the cellular level, in-depth understanding requires information at the molecular and atomic level and Structural Biology is the most appropriate and accurate approach for protein function and protein-protein interactions investigation. The newly identified protein interfaces can, also, lead to applications as potential targets for novel antibacterial agents.

Expected results & Research Project Impact

Pseudomonas syringae and *Erwinia amylovora* are two of the most significant bacterial pathogens that affect a multitude of important crops including tomato, beans, maize, wheat, maples, crucifers, apple trees, pear trees etc. With the growing human population, food adequacy is an ever-present concern. Loss of crop yield because of infections, increasingly common and enriched with a wide variety of strains because of the globalization of trade, can have devastating effects. The purpose of RegSecSy is to shed light on the intracellular interactions that occur during T3SS activation. Targeting the interfaces between proteins with drugs can be a good strategy to combat the pathogens, and these aspects will be also explored when structural information becomes available (TRL 1/2).

The hosts of pathogens that utilize the T3SS are not limited to plants. Animal pathogens such as *Salmonella* and *Yersinia pestis* (causative agent of plague) can have tremendous human impact. While the T3SS of each bacterium is adapted to infect a specific set of hosts, with the appropriate changes in regulation control, host defense evasion etc, there are significant similarities and universal strategies across animal and plant pathogens. With the current study we aim to expand this knowledge to the less studied plant pathogens and decisively contribute to the understanding of T3SS regulation control.

The importance of this funding

This funding will afford me the opportunity to apply my expertise in Structural Biology in producing new knowledge and answering critical questions of the regulation of plant pathogenic bacteria at the molecular and atomic level.

The conception, execution and eventual successful completion of RegSecSy will aptly demonstrate my maturity to lead and manage a scientific team towards specific goals and to take a step towards scientific independence. It will prepare me to participate in international competitive funding programmes and research collaborations, in particular in the framework of the Horizon Europe programmes and future EU programme calls in thematic areas related to Biotechnology, Drug Design, Food Safety etc.

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