Activity and Regulation of Symbiotic Peptides: A Frontier in Chemical Ecology

Eva Kondorosi

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Balzan GPC Adviser: Charles Godfray
Deputy Principal Investigator: Gabriella Endre
Affiliated Institution: Institute of Plant Biology, Biological Research Centre, Szeged, Hungary

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Eva Kondorosi is Research Professor and Head of the Symbiosis and Functional Genomics Unit in Szeged at the Biological Research Centre, which until 2019 belonged to the Hungarian Academy of Sciences.

Legumes are particular because they can form symbiosis with nitrogen-fixing bacteria, which convert atmospheric nitrogen into ammonia and satisfy the nitrogen needs of the host plant. The symbiotic rhizobium partners are soil-dwelling alpha- or beta-proteobacteria, which are present intracellularly in the symbiotic organ, the root nodule. In recent decades the study of symbiotic nitrogen fixation has developed into a frontier research area in chemical ecology. In this symbiosis, the partners not only provide missing nutrients, but also communicate with each other and affect each other's physiology with chemical signals and effectors. The symbiosis is initiated by chemical signals, by flavonoids excreted from the root, which trigger in the bacterium partner the synthesis of lipochitooligosaccharide Nod factors required for nodule organogenesis and infection of nodule cells with rhizobia. Research conducted by Kondorosi's team has demonstrated that in *Medicago* nodules, both the infected plant cells and the intracellular bacteria undergo a coordinated multistep differentiation process. This involves astonishingly similar molecular mechanisms in the eukaryote and prokaryote partners, such as the loss of cell division ability and genome amplification by genome duplications accompanied by extreme cell enlargement. The plant controls the density of the endosymbiont population and the differentiation steps of the bacteria by 700 to 800 secreted plant peptides. Most of them are nodule-specific cysteine-rich NCR peptides, encoded by small genes and evolved specifically for symbiosis with exclusive expression in the symbiotic cells, involving different sets of genes at the early, intermediary and late stages. The mature NCR peptides have 4 or 6 conserved cysteines but otherwise highly diverse amino acid sequences, resulting in a wide variety of anionic, neutral and cationic peptides with diverse biological activities. One of the major symbiotic tasks of the NCR peptides is to inhibit and permanently abolish bacterial cell division. Several cationic peptides are involved in this process and these peptides have in vitro strong antimicrobial activities against pathogenic bacteria and fungi. The NCRs can interact with the bacterial cell envelope and with multiple intracellular bacterial targets. In addition to their role in symbiosis, NCRs represent an enormous source of pharmacologically interesting molecules.

Researchers have yet to answer questions as to why so many peptides are required for symbiosis, how many peptides are essential, what activities they represent, what the structural requirements are, how their production is regulated, and how the unexplored in vitro activity of NCR peptides can be used in medicine and agriculture.

Eva Kondorosi envisions a three-year Balzan research project with two components: i) the identification of the *in vivo* and *in vitro* activities of selected NCR peptides, and ii) the regulation of NCR genes. The project will be carried out in the Institute of Plant Biology at the Biological Research Centre, Szeged, Hungary, and involve young investigators at different levels who will work under the supervision of the Principal Investigator, Eva Kondorosi. The project will be administrated at the office of the Academia Europaea.

Research Plan

i) The first part of the project is planned to be carried out by two female postdoctoral researchers. The following research questions will be addressed:
  - What are the peptides' bacterial targets?
  - How does the formation of disulfide bridges influence the peptides' properties?
  - Which amino acids are crucial for the activity?
  - Are NCR peptides or their derivatives potent antimicrobial agents?

ii) The second part of the project will study the regulation of NCR genes. This work will involve the delimitation of promoter regions, the identification of transcription factors, cis-elements and likely signals from the bacterium provoking differential gene expression. Methodology will include *in planta* assays, yeast one-hybrid screens, DNA pull-down experiments and gel retardation assays coupled with proteomics as well as with data mining of RNA-seq results of wild-type and mutant nodules. These studies will involve a PhD student and a postdoctoral fellow hired for 18 months.

The results of the Balzan research project will be published in high-impact, peer-reviewed international scientific journals. Moreover, they will be presented in scientific conferences (such as the International Nitrogen Fixation Conference in 2019 and the European Nitrogen Fixation Conference in 2020) and disseminated to the broader scientific community and general public at various meetings like the Annual Academia Europaea Conference in 2020. The Balzan Prize fund will also be used for organizing a multidisciplinary scientific workshop in 2021 and to procure equipment and operating credit.

Progress Report 2019

The first year of the Balzan research project coincided with the confiscation of all research institutes and centers of the Hungarian Academy of Sciences, which were placed under the control of a newly created government organization, the Eötvös Lórand Research Network in September 2019. During this turbulent and uncertain time, with diminishing support for frontier research, the top-down, government-controlled grant system, and reduced academic freedom, several excellent scientists including ERC grantees left the country or did not return to Hungary. Because of these uncertainties, the postdoc positions in the Balzan research project were not advertised. Nevertheless, a young structural biologist, Salome Kyralova, joined Kondorosi's lab before the start of the Balzan research project but was forced to return to Prague. Presently one postdoc, Hilda Tiricz, is supported by the Balzan research project. She works on the NCR functions and on the identification of bacterial targets of the peptides. Studies on the regulations of NCR genes are mostly performed by a Chinese PhD student, Senlei Zhang, who will defend his thesis in 2020 and continue his studies as a postdoc with the support of the Balzan research project. Despite the difficulties in 2019, three manuscripts have been published in top scientific journals and a fourth manuscript is under review. The Balzan Prize and research project were acknowledged by Eva Kondorosi in these scientific publications and at nine international scientific conferences and events as well as in the media (TV, radio, and magazines).

Publications

Mergaert P, Kereszt A, Kondorosi É. Gene expression in nitrogen-fixing symbiotic nodule Cells in *Medicago truncatula* and other nodulating plants. The Plant Cell 32. 2019; (1), 42-68.

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Lima R M, Kylarová S, Mergaert P, Kondorosi É. Unexplored arsenals of legume peptides with potential for their applications in medicine and agriculture. Front Microbiol. 2020; (under review).