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The action of the bacterial effector protein harpin on Arabidopsis thaliana

The presented thesis is concerned with plant defense responses to harpin from Pseudomonas syringae pv. syringae that can elicit the hypersensitive response (HR) in plants. In nature, harpin protein secreted by Erwinia spp., and Pseudomonas spp. via type III secretion system causes fire blight in apples and pears as well as soft-rot that affect a wide range of plants including several economically important crops. The aim of this work was to identify and characterize molecular components of the activation of natural defense mechanisms induced by harpin in Arabidopsis thaliana, and to gain insights in immune responses that are analogous to that in animals. Given the importance of mitochondria in the mediation and regulation of programmed cell death (PCD) in animal systems, the effect of harpin from Pseudomonas syringae on mitochondrial functions in Arabidopsis suspension cells was investigated in detail. Fluorescence microscopy studies suggested a co-localization of mitochondria and generation of reactive oxygen species (ROS). Among the early responses a decrease of the mitochondrial membrane potential $\Delta m$ and as a direct consequence a decline of ATP pool size, were observed. Moreover, treatment of Arabidopsis cells with harpin induced a rapid cytochrome c release from mitochondria into the cytosol and a partially nuclear translocation of the cytochrome c, which is regarded as a hallmark of PCD or apoptosis in animals. Northern and DNA array analyses showed strong induction of protecting and/or scavenging systems such as alternative oxidase (AOX) and small heat shock proteins, components that are known to be associated with cytochrome c decay. Transcriptional profiling of Arabidopsis genes behind harpin and LPS induced defense responses revealed some interesting parallels, such as high similar pattern of induced genes associated with cell rescue and general stress responses. Harpin and LPS induced an overlapping array of genes involved in cell wall biogenesis and strengthening, cellular communication and signaling. In contrast, a remarkably difference was observed regarding some of the most prominent, central components of plant defense such as WRKY transcription factors, receptor kinases, and oxidative burst-associated genes, whose expression became apparent only after treatment with harpin. The influence of harpin on transcript abundance of genes encoding mitochondrial proteins revealed a total of 199 transcripts that changed significantly during harpin treatment. It mainly concerned genes encoding mitochondrial proteins associated with metabolism, with transport mechanisms such as mitochondrial protein import apparatus, and with energy budget affecting processes like electron transport chain and citric acid cycle. The effect of harpin on all citric acid cycle related enzyme complexes was partially recovered at proteomic and metabolomic level, and confirmed by studying the activities of appropriate enzymes. In sum, the presented work demonstrates that Arabidopsis thaliana possess a cell death pathway that is activated by the bacterial effector protein harpin and mediated dependently on mitochondria. There is strong evidence that, despite the differences, plants and animals share conserved mechanisms during the PCD process.