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ARABIDOPSIS NUDIX HYDROLASE ENZYMES AS MODULATORS OF PLANT IMMUNITY AND CELL DEATH

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Nudix hydrolases belong to a large family of enzymes that are present in organisms from viruses to humans and cleave a variety of nucleoside diphosphate substrates. The *Arabidopsis thaliana* Nudix hydrolase NUDT7 acts as a negative regulator of plant immunity and programmed cell death. *Nudt7* mutants display enhanced basal resistance, exhibit retarded growth and spontaneous initiation but not spread of leaf cell death. Also, the mutants have elevated levels of salicylic acid (SA), a key molecule of plant resistance. Genetic epistasis analysis shows that all of these defects in *nudt7* plants require *ENHANCED DISEASE SUSCEPTIBILITY 1* (*EDS1*), an intracellular regulator of plant basal and systemic immunity. The expression of *NUDT7* is dependent on *EDS1* and this enzyme acts on a SA-independent branch downstream of EDS1 that we intend to characterize. NUDT7 protein shows *in vitro* activity on ADP-ribose which becomes highly toxic when it accumulates within the cell and has been shown to stimulate Ca^{2+} signalling in mammalian cells. *In vivo* localization studies revealed an exclusively cytosolic distribution of NUDT7 in healthy and pathogen-infected tissue. After pathogen infection with avirulent *Pseudomonas syringae* expressing *avrRpm1*, NUDT7 was strongly upregulated and appeared both as monomeric and oligomeric forms. Using NUDT7 epitope-tagged transgenic lines expressed under its own or the constitutive 35S promoter we are investigating potential interactors, post-translational modifications and its molecular relationship to EDS1. We will present progress on this and on our genetic positioning of *NUDT7* in the plant signalling network.

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HR SIGNALLING IN TOMATO IS REGULATED AT THE LEVEL OF THE PROTEOME, THE PHOSPHOPROTEOME AND THE TRANSCRIPTOME

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The interaction between *Cladosporium fulvum* and its only host tomato serves as a model to study the features of plant resistance which is associated with the hypersensitive response (HR). Recognition of a secreted avirulence protein (Avr4) of *C. fulvum* by the resistance protein (Cf-4) of tomato initiates the HR in the cells around the site of penetration, thereby preventing further growth of the fungus. To study the HR in intact plants, we induce a synchronous systemic HR in transgenic tomato seedlings expressing both Cf-4 and Avr4. HR-induced Cf-4/Avr4 seedlings were analysed by 2DE-DIGE analysis after 0, 3 and 24 hours to reveal proteome changes during HR signalling. Significant changes were observed at 24 hours when morphological changes in the seedlings were observed as well, whereas only marginal changes were observed at 3 hours after HR-induction. These proteome changes are preceded by transcriptional changes at earlier time-points (Gabriëls *et al*., 2006) and micro-array analysis revealed changes in the abundance of certain transcripts in Cf-4/Avr4 seedlings within 3 hours after HR induction. In addition, we found that MAP kinases are activated upon phosphorylation and function as important signalling components in the Cf-4/Avr4-induced HR (Stulemeijer *et al*., submitted). These data indicate that phosphorylation cascades play an important role in the early stages of Cf-4/Avr4 signalling. Therefore, phosphopeptides isolated from the trypsin-digested Cf-4/Avr4 proteome isolated after 1, 3 and 5 hours were identified using a Q-TOF mass spectrometer. Previously unidentified phosphoproteins were identified and analysis of these data in a quantitative manner should reveal phosphoproteins that play a role in signalling in the early stages of HR induction.

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MASTOPARAN ACTIVATES CALCIUM SPIKING ANALOGOUS TO NOD FACTOR-INDUCED RESPONSES IN *MEDICAGO TRUNCATULA* ROOT HAIR CELLS

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The rhizobial derived signalling molecule Nod factor is essential for the establishment of the legume/rhizobial symbiosis. Nod factor perception and signal transduction in the plant involves calcium spiking and leads to the induction of nodulation gene expression. It has previously been shown that the heterotrimeric G-protein agonist mastoparan can activate nodulation gene expression in a manner analogous to Nod factor activation of these genes and this requires DMI3, a calcium and calmodulin dependent protein kinase (CCaMK) that is required for Nod factor signalling. Here we show that Mastoparan activates oscillations in cytosolic calcium, similar but not identical to Nod factor induced calcium spiking. Mastoparan induced calcium changes occur throughout the cell, whereas Nod factor induced changes are restricted to the region associated with the nucleus. Mastoparan induced calcium spiking occurs in plants mutated in the receptor-like kinases NFP and DMI2 and in the putative cation channel DMI1, that are all required for Nod factor induction of calcium spiking, indicating either that Mastoparan functions downstream of these components or that it uses an alternative mechanism to Nod factor for activation of calcium spiking. However, both Mastoparan and Nod factor induced calcium spiking are inhibited by cyclopiazonic acid and n-butanol, suggesting some common mechanisms underpinning these two calcium agonists. The fact that Mastoparan and Nod factor both activate calcium spiking and can induce nodulation gene expression in a DMI3 dependent manner strongly implicates CCaMK in the perception and transduction of the calcium signal.