

P017 The role of the U-box protein, U76p in regulating the cell death signalling complex and disease resistance in plants.

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The Cf-9 resistance protein from tomato confers resistance against races of leaf mold fungus, *Cladosporium fulvum* expressing the complementary Avr-9 peptide. It has been previously demonstrated that tobacco plants and cell cultures carrying Cf-9 transgene respond to Avr-9 peptide by showing a rapid induction of cell death and associated responses (HR) in a strict gene-for-gene manner indicating that all components required for efficient execution of the HR are present in this heterologous model system. Using the Cf-9 tobacco model system we isolated a plant U-box gene (u76) whose transcript level is rapidly upregulated within 10 minutes after exposure of Cf-9 tobacco cells to plant intracellular washing fluid (IF) containing Avr-9. The full-length u76 protein (u76p) has two notable domains, N-terminal U-box domain responsible for covalently attaching ubiquitin molecules to protein substrates targeted for multi-ubiquitination mediated proteasomal degradation and C-terminal ARM repeats similar to those found in b-catenin/armadillo family of proteins. Tobacco plants with reduced levels of u76 mRNA showed a complete loss of HR in leaf tissues infiltrated with Avr-9 IF. Conversely plants overexpressing u76p show an accelerated HR. Affinity purified u76p is capable of ubiquitinating protein substrates *in vitro*. We have demonstrated that u76p is a *bona fide* E3 ligase protein involved in selectively degrading key negative regulators of cell death. This data forms the first molecular evidence for the involvement of the ubiquitin-proteasome system in Cf-9/Avr-9 mediated HR.