

Fungal Response to Plant Defense Compounds : A Two-hybrid Approach to Dissect the Signal Transduction Cascade

Vijayakala Vydeeswaran, Dept of Cell Biology and Molecular Genetics, 2221, H J Patterson Bldg, Univ. of Maryland, College park, MD.

Email: vykala@wam.umd.edu

One of the ways by which plants defend themselves against pathogens is by producing a variety of fungistatic compounds like phytoalexins and/or phytoanticipins. For example, pisatin is a anti-fungal defense compound uniquely produced by the garden pea, *Pisum sativum*. However, pisatin can also trigger a counter defense pathway in the fungus *Fusarium solani*, a virulent pea pathogen. Pisatin is detoxified in *F. solani* by a cytochromeP450 monooxygenase, pisatin demethylase. The gene encoding the monooxygenase, PDA1, has been found to be strongly induced by pisatin. A 40 bp pisatin-reponsive element has been identified within the promoter sequence of PDA1. This element serves as a binding site for the binuclear zinc transcription factor PRF (**P**isatin **R**esponse **F**actor) also identified in the lab. PRF appears to mediate a signal transduction pathway in the fungus, that responds to the stress induced by pisatin. What sort of signal transduction pathway does pisatin initiate in the fungus? Where and how does PRF act in this pathway? To answer these questions, we are currently identifying proteins that interact with PRF. Yeast two hybrid analysis is being performed with PRF as the prey and the cDNA library of *F. solani* expressed as bait. Interacting proteins could include: a) coactivators and proteins which facilitate DNA binding or transcriptional regulation, b) proteins that function upstream of PRF in well known or unknown stress pathways. Binding studies in bacteria, *Escherichia coli* and yeast, *Saccharomyces cerevisiae* showed a decreased binding of PRF to DNA in response to pisatin. But, similar studies in *F. solani* and related fungus, *Neurospora crassa* showed an increased binding. This discrepancy in the binding results suggests the presence of a hetrodimeric partner to PRF absent in simpler hetrologous systems. The two-hybrid analysis has revealed two promising proteins showing high levels of interaction with PRF – V25 and V27. V25 is a novel protein, similar to the Mn/Fe superoxide dismutase but lacking all the expected domains. The protein is predicted to possess a mitochondrial targeting sequence and a nuclear localization signal. Predominantly predicted to be localized in the mitochondria, this protein is also likely to be present in the nucleus. V27 was found to have a nuclear localization signal. Currently, binding studies are underway to establish an invitro interaction between V25 and PRF. Characterization of this pathway will thus enable a better understanding of the fungal infection process and the consequent root rot caused in pea plant cultivations.