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Poster Abstract - C.35

PROTEOMIC ANALISYS OF S-NITROSYLATED PROTEINS IN *ARABIDOPSIS* DURING THE DISEASE RESISTANCE RESPONSE

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The free radical gas nitric oxide (NO), first characterised as an endothelium-derived relaxation factor, is a pleiotropic signaling molecule in animal cells, involved in a myriad of cellular processes including neuronal signaling, blood pressure homeostasis and immune response. In plants, NO is an emerging regulator implicated in several processes and acts as a key signal in plant resistance to incompatible pathogens by triggering resistance-associated cell death. Recent evidences indicate that in animal tissues NO regulates these diverse biologic processes by directly modifying proteins. NO and related species can oxidize, nitrate or nitrosylate proteins (Stamler et al., 1998). Nitrosylation refers to the binding of a NO group to a transition metal or cysteine residue, is a reversible modification and plays a central role in NO-mediated signalling (Stamler et al., 2001). Accumulating data suggest that many proteins are nitrosylated by NO (for over 100 representative examples are available on line at http://www.cell.com/cgi/content/full/106/6/675/DC1) and this suggests nitrosylation as an ubiquitous post-translational modification regulating protein function. Indeed, nitrosylation shares many features in common with phosphorylation, the prototypic posttranslational modification involved in signal transduction regulation. Both modifications are reversible and specific, allowing cells to flexibly and precisely modify protein function in response to environmental signals (Mannick and Schonhoff, 2002).

Proteomic studies represent a powerful complement to transcriptomic studies because they allow evaluation of the expressed proteins and potential post-transcriptional modifications as S-nitrosylation. To detect and characterize genes encoding proteins which are S-nitrosylated in *Arabidopsis thaliana* undergoing hypersensitive disease resistance response, a proteomic analysis has been undertaken. Proteins were extracted from Arabidopsis leaves 4h and 8h after challenge with an avirulent pathogen (*Pseudomonas syringae* pv. *tomato* carrying the avirulence gene *avrB*). Unchallenged leaves were used as control. The so-called “biotin switch” method (Jaffrey et al., 2001) has been used to detect and purify the pool of proteins S-nitrosylated in response to pathogen infection. After 2-Dimensional electrophoresis, standard maps of infected and non-infected leaves at two different time points have been generated and analysed with the PDQuest software. S-nitrosylated proteins differentially expressed were extracted from the gels, and identification by mass spectrometry is in progress. Proteins will then be identified by searching the protein database as the National Center for Biotechnology

Information (NCBI). We expect to characterize the function of the corresponding genes by comparing post-translational modification profiles and gene expression data deriving from genome-wide microarray analysis.