GAPDH, NtOSAK and CDC48, a conserved chaperone-like AAA-ATPase, as nitric oxide targets in response to (a)biotic stresses

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Session: NO in plant signaling

IS-23

S-Nitrosylation in plant defense

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Nitric oxide (NO) is an important signalling molecule in animal and plant defense responses. In the latter, the redox-active molecule NO has an essential role in restriction of pathogen attack by induction of defense genes and programmed host cell death. NO can react with the thiol group of cysteine residues to form S-nitrosothiols (S-nitrosylation). A number of NO-affected proteins in plants seem to be regulated by S-nitrosylation making this type of protein modification a predominant mechanism in NO-signalling. Recently, we demonstrated that NO is a redox regulator of a central transcriptional system for systemic acquired resistance in *Arabidopsis thaliana*. A change in the cellular redox status during the salicylic acid-mediated activation of defense leads to S-nitrosylation of the regulatory protein NPR1 and to its active monomeric form. Subsequently, the NPR1 monomers are translocated into the nucleus, where they interact with defense-associated transcription factors. Additional examples and evidences for NO-dependent modification of cysteine residues, describing its chemistry/formation, specificity, and possible physiological functions in plants will be discussed. The presentation underlines the importance of NO as a redox regulator.

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IS-24

Nitric oxide produced during the hypersensitive response modulates the plant signaling network and inhibits the pathogen's virulence machinery

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Nitric oxide (NO) is a well-recognized key signaling mediator of the plant defense response to pathogens. Like in animals, NO acts directly or through mediators of its activity such as reactive NO derivatives (RNS) and second messengers like cGMP.

We aim at deciphering the ''signaling network'' mediated in plant by NO and RNS upon pathogen attack, and by using the HKGreen-2 dye we demonstrated that peroxynitrite, the RNS originated by the reaction between NO and O_2^-, accumulates in plants during the hypersensitive response (HR). Peroxynitrite is not a ''death messenger'' in plants, and its accumulation in plants correlates with an increase in tyrosine nitrated proteins, and we demonstrated that *in vitro* peroxynitrite targets specifically some MAPK kinases, inhibiting their activity and thus precisely modulating the most complex plant signal transduction network by tyrosine nitration. Furthermore, we recently found that NO can participate in plant defenses also by inhibiting pathogen effector activity. The effector HopAI1, a phosphothreonine lyase produced by many *Pseudomonas syringae* strains which suppresses plant immunity via MAPK inhibition, is a target of NO and its activity is dramatically inhibited by S-nitrosylation. Whereas HopAI1 expressed in *Arabidopsis thaliana* does not affect the HR induced by the avirulent *P. syringae* AvrRpt2, the expression of a mutated (Cys free) form of HopAI1 strongly reduces the hypersensitive cell death as well as plant resistance. This suggests that NO modulates the plant signaling network through nitration of specific MAPK and inhibits pathogen's effectors that would attenuate the plant defence response.

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IS-25

GAPDH, NtOSAK and CDC48, a conserved chaperone-like AAA-ATPase, as nitric oxide targets in response to (a)biotic stresses

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Increasing evidences support the assumption that nitric oxide (NO) acts as a physiological mediator in plants facing (a)biotic stresses [1,2]. Understanding its effects requires a deep analysis of the molecular mechanisms underlying its mode of action. In the recent years, efforts have been made in identifying and understanding the function of plant proteins regulated by NO at the post-translational level, notably by S-nitrosylation [3].

We demonstrated that the glycolytic enzyme GAPDH undergoes a fast and transient S-nitrosylation in tobacco cells exposed to a salt stress [4]. S-nitrosylation affects only a small proportion of the GAPDH population and does not affect glycolysis. Interestingly, *in vivo* GAPDH interacts with the protein kinase NtOSAK (*Nicotiana tabacum* osmotic stress-activated protein kinase), a member of the SnRK2 protein kinase family previously shown to be rapidly activated through NO in response to (a)biotic stresses [5]. Our current hypothesis is that S-nitrosylated GAPDH might acts as a phosphor-relay recruiting protein substrates for NtOSAK.

Besides GAPDH, we identified proteins undergoing S-nitrosylation in tobacco cell suspensions exposed to cryptogein, a 10 kDa protein produced by the oomycete *Phytophthora cryptogea* [6]. These proteins include CDC48, a conserved chaperone-like AAA$^-$ATPases. Using a combination of structural and biochemical analysis, we provided evidence that NO induces a local conformational change within the protein and inhibits its enzymatic activity. The physiological incidence of this process will be discussed.

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