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Hydrogen peroxide as a signal controlling plant programmed cell death

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Hydrogen peroxide (H$_2$O$_2$) has established itself as a key player in stress and programmed cell death responses, but little is known about the signaling pathways leading from H$_2$O$_2$ to programmed cell death in plants. Recently, identification of key regulatory mutants and near-full genome coverage microarray analysis of H$_2$O$_2$-induced cell death have begun to unravel the complexity of the H$_2$O$_2$ network. This review also describes a novel link between H$_2$O$_2$ and sphingolipids, two signals that can interplay and regulate plant cell death.

Introduction

Hydrogen peroxide (H$_2$O$_2$), generated by various environmental and developmental stimuli, can act as a signaling molecule that regulates plant development, stress adaptation, and programmed cell death (PCD) (Apel and Hirt, 2004). H$_2$O$_2$-induced PCD itself is essential for a number of developmental processes and environmental responses, including aleurone cell death, the hypersensitive response to pathogens, and allelopathic plant–plant interactions (Bethke and Jones, 2001; Bais et al., 2003; Apel and Hirt, 2004). The mechanisms of H$_2$O$_2$ generation and detoxification are well studied, but little is known as to how the H$_2$O$_2$ signal is perceived and then channeled downstream the signaling network in order to achieve the regulation of these processes (Apel and Hirt, 2004). Recently, mutants in the H$_2$O$_2$ signaling pathway were identified, providing a breakthrough in our understanding of how the signaling network functions (Nakagami et al., 2004; Rentel et al., 2004). In addition, mutants that are more tolerant to both reactive oxygen species and to perturbations in sphingolipid metabolism were obtained, thus revealing a new link between redox and sphingolipid signaling leading to plant PCD (Danon, A., and K. Apel, personal communication; unpublished data). These genetic studies were further substantiated by molecular and biochemical data bringing new insights into the interplay between H$_2$O$_2$ and sphingolipids during PCD (Gechev et al., 2004). Complementing these findings, recently published transcriptional analyses highlighted biochemical pathways and discovered new H$_2$O$_2$-responsive genes (Vandenabeele et al., 2003, 2004).

The H$_2$O$_2$ signaling network is emerging: mutants in the H$_2$O$_2$ pathway

As H$_2$O$_2$ is both an important signaling molecule and a toxic byproduct of cell metabolism, its cellular levels are under tight control, and their maintenance has hallmarks of homeostatic regulation. The cell can sense sublethal doses of H$_2$O$_2$ and activate peroxide-detoxifying mechanisms; alternatively, upon different cell death stimuli various H$_2$O$_2$-producing mechanisms can be activated, and as a result of this deliberate H$_2$O$_2$ production a self-destructive PCD is triggered (Gechev et al., 2002; Bais et al., 2003; Apel and Hirt, 2004). H$_2$O$_2$ produced by NADPH oxidases, for example, has multiple effects ranging from growth promotion or ABA signaling to cell death (Torres et al., 2002; Foreman et al., 2003; Kwak et al., 2003). Studies with exogenously applied H$_2$O$_2$ confirm the role of H$_2$O$_2$ as a cell death trigger and show that high concentrations can cause necrosis instead of PCD (Yao et al., 2001). In agreement with these observations, overexpression of the H$_2$O$_2$-detoxifying enzyme ascorbate peroxidase can suppress the cell death induced by H$_2$O$_2$ or nitric oxide (Murgia et al., 2004). Biochemical evidence indicated that a plant MAPK cascade is responsible for relaying the H$_2$O$_2$ signal, much alike in other eukaryotes (Kovtun et al., 2000). However, plants possess an unusually high number of MAPKs, and the kinase network can be a convergence as well as a divergence point for different stress factors (Ichimura, 2002). The recent identification of the serine/threonine kinase, oxidative signal-inducible1 (OXII), as an essential component in H$_2$O$_2$ signaling in Arabidopsis provided new insights into the complexity and specificity of the H$_2$O$_2$-relaying kinase network (Rentel et al., 2004). The Arabidopsis oxi1-null mutant showed enhanced susceptibility to pathogen infection, data consistent with a role of H$_2$O$_2$ in PCD during pathogen responses, and abnormal root hair growth, a process that is also mediated by H$_2$O$_2$ (Rentel et al., 2004). The pleiotropic role of OXII in plant stress response(s) and development is consistent with its activation not only by H$_2$O$_2$, but also by other signals, including cellulose and various abiotic stresses. OXII is needed for full activation of two stress MAPKs, AtMPK3 and AtMPK6 (Rentel et al., 2004). Interestingly, the two MAPKs are involved in both abiotic and biotic stress responses and they are also activated by the H$_2$O$_2$-regulated...
AtNDK1 interacts with the three NDK. This notion is suggested by the observations that activity may also be modulated by nucleoside diphosphate kinase (NDK). The H$_2$O$_2$ signal is mediated through alterations in Ca$^{2+}$ fluxes, redox changes, activation of MAPK cascades, and interactions with other signaling molecules like salicylic acid and nitric oxide.

**Other components of the H$_2$O$_2$ signaling network**

In addition to the MAPK cascade network, the H$_2$O$_2$ signal can also be transmitted through alterations in calcium ion fluxes and cellular redox state (Fig. 1). Both Ca$^{2+}$ and redox alterations are very early events that follow the rises in H$_2$O$_2$ levels (Rentel and Knight, 2004). A specific calcium signature in turn can lead to various downstream effects, including cell death, through the numerous Ca$^{2+}$-interacting proteins, including calmodulins and the big family of calcium-dependent protein kinases (Harper et al., 2004). Plants possess a unique set of Ca$^{2+}$/calmodulin-regulated proteins with different biological functions. Although some of these proteins like NAD kinase aid to the production of H$_2$O$_2$ and enhance cell death (Harding et al., 1997), others like catalase have the opposite effect (Yang and Poovaiah, 2002). Catalase is of paramount importance for regulating H$_2$O$_2$ homeostasis, as it can function as a cellular sink for H$_2$O$_2$. Catalase deficiency leads to elevation of H$_2$O$_2$ levels and triggering PCD (Gechev et al., 2004; Vandenabeele et al., 2004). Calcium is therefore not only essential for PCD, but also for maintaining H$_2$O$_2$ levels that ensure cell survival (Yang and Poovaiah, 2002). In addition to Ca$^{2+}$/calmodulin, catalase activity may also be modulated by nucleoside diphosphate kinase (NDK). This notion is suggested by the observations that AtNDK1 interacts with the three *Arabidopsis* catalases in a yeast two-hybrid system and that transgenic plants overexpressing AtNDK1 exhibited enhanced ability to detoxify H$_2$O$_2$ (Fukamatsu et al., 2003).

**Novel link between redox and sphingolipid signaling during plant PCD**

The fungal toxins fumonisin and AAL toxin inhibit ceramide synthase, which causes disruptions in sphingolipid metabolism and subsequent PCD (Spassieva et al., 2002). In tomato, the PCD can be prevented by a disease resistance gene called Asc. Recently, a knockout of the *Arabidopsis* homologue of Asc rendered plants sensitive to AAL toxin–induced PCD (Gechev et al., 2004). A fine balance of sphingolipids is crucial for maintaining cell survival, as not only depletion but also accumulation of ceramides can trigger PCD (Spasieva et al., 2002; Liang et al., 2003). The connection between sphingolipid metabolism and PCD was also indicated with the earlier cloning of the accelerated cell death 11 (acd11) mutant in *Arabidopsis*. A novel link between sphingolipid and redox signaling was further substantiated by isolating mutants that are more tolerant to both the fungal toxins that cause such perturbations in sphingolipid metabolism and to reactive oxygen species (unpublished data; Danon, A., and K. Apel, personal communication). One of these mutants, called EXECUTER1 (AT4G33630), has recently been identified as a nuclear-encoded chloroplast protein with no apparent homology to any other proteins (Wagner et al., 2004). Atr1 mutant, initially isolated in our group, was more tolerant to both the fungal toxins that cause such perturbations in sphingolipid metabolism and to reactive oxygen species (unpublished data; Danon, A., and K. Apel, personal communica-}

**Comprehensive analysis of gene expression during H$_2$O$_2$-induced cell death**

H$_2$O$_2$-derived signals initiate global changes in gene expression through regulation of a specific subset of transcription factors and, as a result of those changes, different genetic programs including PCD are executed. The first noninvasive in planta sys-
the particular location of H$_2$O$_2$ and other reactive oxygen species in the cell may determine different physiological or developmental responses. Equally important, the signal transduction that regulates the action of 2-Cys peroxiredoxins as floodgates for the inactivation of the peroxiredoxins, initially thought to be irreversible, is actually reversible, thus providing a way to regulate the action of 2-Cys peroxiredoxins as floodgates for H$_2$O$_2$ (Woo et al., 2003). Other molecules like two-component histidine kinases may also serve as H$_2$O$_2$ sensors, but experimental evidence for their role in plants is still lacking (Apel and Hirt, 2004). Regardless of the way of sensing, it is still unclear how this small molecule is able to trigger such different responses as stress acclimation or PCD and to initiate distinct developmental programs.

Concluding remarks

Despite the recent progress in our understanding of the signaling role of H$_2$O$_2$ in plants, there are still many unanswered questions. The H$_2$O$_2$ sensor(s) in plants are still elusive. A recent report implicates the eukaryotic antioxidant enzymes, 2-Cys peroxiredoxins, as primary sensors for H$_2$O$_2$ (Wood et al., 2003). Plant, animal, and yeast 2-Cys peroxiredoxins, in contrast with bacterial ones, are much more sensitive to H$_2$O$_2$ inhibition (Wood et al., 2003). Their ubiquity ensures that in a resting cell, H$_2$O$_2$ is kept at constant low levels to prevent any signaling. However, upon an H$_2$O$_2$ burst the sensitive 2-Cys peroxiredoxins are rapidly inactivated and then the unscavenged H$_2$O$_2$ can react as a messenger with other components of the signaling network like the yeast Orp1-Yap1 sensor (Toledano et al., 2004). Crucial for the outcome of the initial H$_2$O$_2$ signaling depends on those interactions, which in turn are presumably acting downstream from the transcription factors were a FAD-linked oxidoreductase (AT1G26380.1), an oxoglutarate-dependent dioxygenase (AT3G13610.1), and a frnF gene (AT5G38900.1). The large number of regulated genes with unknown function in these studies provides us with novel leads to search for plant-specific PCD key regulatory molecules.
determined by the particular cell type, cell compartments, and interacting proteins present at that particular time.

Microarrays with full genome coverage may be a useful tool for identification of genes and other components of the cell death machinery. However, many of the signaling molecules, including the potential sensors, are low-abundant proteins. The best way to identify such proteins can be genetic screening for mutants compromised in H$_2$O$_2$-induced PCD. Further functional studies will then be needed to establish the precise role of each played in the cell death network.

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