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MOLECULAR BIOLOGY AND GENETICS OF PROGRAMMED CELL DEATH IN *ARABIDOPSIS THALIANA*

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SUMMARY

Programmed cell death (PCD) is essential for plant development and stress responses. In order to gain insights into PCD in plants, Molecular biology and Genetics approaches were applied to discover genes involved in the regulation and execution of the cell death in *Arabidopsis thaliana*. Using the fungal AAL-toxin and hydrogen peroxide (H_2O_2) as cell death triggers, a comprehensive transcriptional analysis revealed a set of genes regulated in a common fashion by the two PCD stimuli. These included an oxoglutarate-dependent dioxygenase and various oxidoreductases, the transcription factors Zat11, WRKY75 and NAM, proteasomal components, a heterologous group of genes with diverse functions, and genes encoding proteins with unknown functions. A number of those genes are being functionally studied by gene silencing or isolating homozygous knockout lines. In addition, our group has isolated mutants that are more tolerant to AT as well as to the fungal AAL toxin, thus indicating a new link between redox and sphingolipid signaling. These genetic studies were further substantiated by molecular and biochemical data bringing new insights into the interplay between H_2O_2 and sphingolipids during PCD.

INTRODUCTION

Programmed cell death (PCD) is a genetically controlled suicidal process in which severely damaged or unwanted cells self-destruct upon developmental or environmental cell death stimuli. Examples include embryo formation, aleurone degeneration, maturation of tracheal elements (TE) and epidermal trichomes,

formation of lace leaf shape, leaf senescence and the hypersensitive response (HR) against pathogens (Gunawardena et al., 2004;Pennell and Lamb, 1997). Thus, PCD is essential for the normal plant growth and survival under stress conditions (for example, defense against pathogens – biotic stress). On the other hand, PCD can be very dangerous unwanted process for the plant cell: some necrotrofic pathogens like the fungal phytopathogen *Alternaria alternata f.sp. lycopersici* can secrete toxins (AAL-toxin) that trigger PCD in healthy tissues, so that the pathogens can feed on the death tissues (Wang et al., 1996). In addition, extreme abiotic stress factors can also lead to PCD.

While the process is well studied in animals, little is known about the mechanisms and genes involved in plant PCD. The main aim of our research is to unravel the mechanisms behind plant PCD by discovering genes involved in the regulation and execution of PCD in *Arabidopsis thaliana*.

To realize that goal two experimental approaches are used: (1) Expression profiling of PCD by microarrays followed by functional studies of the identified genes and (2) Genetic screening for mutations in genes that render plants altered in PCD responses.

An essential requirement for successful outcome of the proposed research is the availability of reliable system(s) to study PCD. We have developed two effective systems to investigate PCD *in planta*. The first one is based on the fungal toxin AAL produced by the necrotrophic phytopathogen *Alternaria alternata f.sp. lycopersici* (Gechev et al., 2004). The AAL-toxin interferes with sphingolipid metabolism by inhibiting ceramide synthase, a key enzyme for biosynthesis of complex ceramides, which leads to reduced ceramide levels, accumulation of precursors, and eventually PCD (Spassieva et al., 2002). The second one is based on H_2O_2 as a cell death trigger. Elevated levels of H_2O_2 can be accomplished by inhibiting catalase activity the catalase inhibitor AT (3-aminotriazol), which in combination with sufficient light intensities leads to endogenous accumulation of H_2O_2 and PCD. (Gechev et al., 2005). This paper describes the thranscriptonal analysis during AAL-toxin- and H_2O_2-induced cell death as well as isolation of mutants blocked in AAL-toxin- and/or H_2O_2-induced cell death.

RESULTS AND DISCUSSION

Transcriptional profiling of PCD in *Arabidopsis thaliana*. We have used either the AAL-toxin (Gechev et al., 2004) or H_2O_2 (Gechev et al. 2005) to analyze the transcriptome of Arabidopsis during the early stages of PCD, when there are no visible signs of cell death. Despite the different phenotypes caused by the two cell death triggers, there were many similarities in the expression patterns of the 21500 genes studied. The similarities included induction of common transcription factors (Zat11, WRKY75 and NAM), ethylene and oxidative burst-related genes, proteasomal components, a heterologous group of genes with diverse functions, and genes encoding proteins with unknown functions. The data collected by the microarray analysis was verified by Northern blots with selected number of genes

(Gechev et al. 2004; Figure 1). Twenty of the overlapping genes were chosen for further functional analysis by gene silencing and isolating knockout lines.

Genetics of PCD in *Arabidopsis thaliana*. Reactive oxygen species do not cause simply necrosis; rather, they can trigger a genetically controlled program leading to cell death, as evidenced by the abolishment of the cell death after mutation of a single gene (Wagner et al., 2004). In this line, we conducted a large-scale genetic screening in order to find mutants perturbed in AAL-toxin- and H_2O_2-induced cell death. Several mutants were successfully recovered from chemically (EMS) as well as T-DNA mutagenized collections. Figure 2 shows how this screening looks like, a single survivor among hundreds of dead plants. Amazingly, some of the mutants show enhanced tolerance to oxidative stress generated by H_2O_2 and paraquat, others show developmental efects (for example, altered leaf shape). The existence of mutants that are more tolerant to AT as well as to the fungal AAL toxin indicate a new link between redox and sphingolipid signaling (Gechev and Hille 2005). Cloning of the respective genes would reveal more of the complex plant PCD network.

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FIGURE

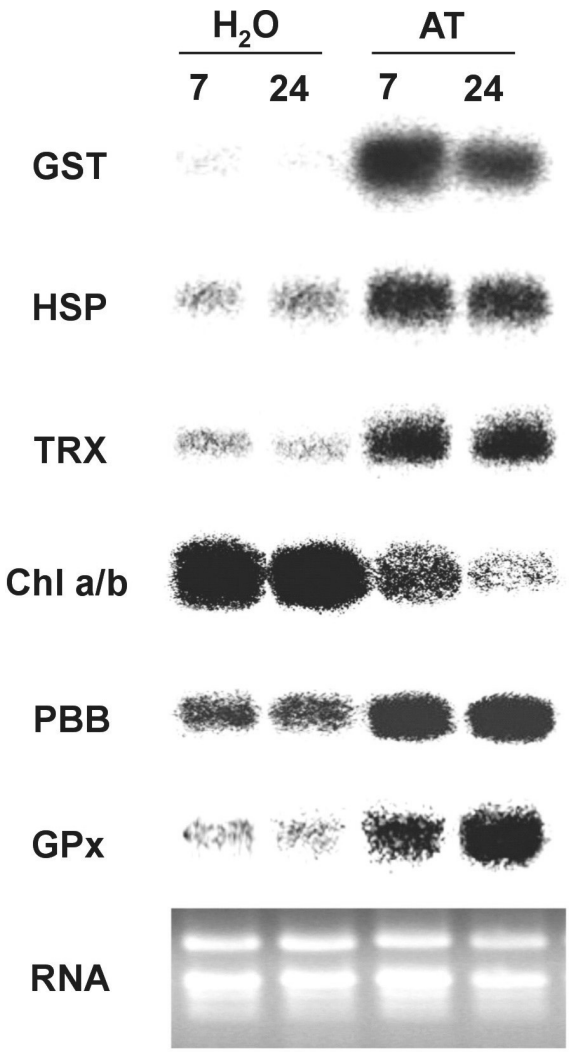

Fig. 1. *Expression profiling during H_2O_2-induced cell death. The expression pattern of selected genes from the microarray experiments was verified by Northern analysis. Leaves were treated either with water (controls) or AT and samples taken at 7 and 24 hours. Genes are as follows: GST, glutathione-S-transferase (At2G29470.1); HSP, heat shock protein HSP70 (At3G12580); TRX, thioredoxin (At1G45145.1); Chl a/b, light-harvesting chlorophyll a/b binding protein (At2G05070.1); PBB, 20S proteasome β subunit (At5G40580.1); GPx, glutathione peroxidase (At2G31570.1).*

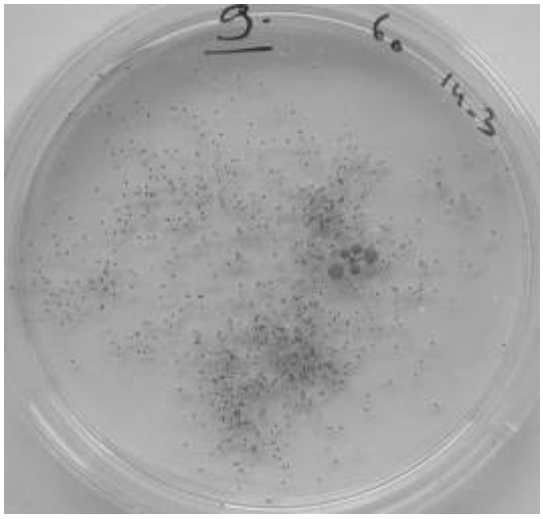

Fig. 2. *Mutant screening for plants tolerant to AAL-toxin-induced cell death. Seeds from 40000 EMS-mutagenized and self-pollinated plants were plated on MS plant growth media supplemented with 60 nM AAL-toxin. A single survivor is well-visible among the dead plants.*