A Transcriptomic Footprint of Reactive Oxygen Species

Reactive oxygen species (ROS) are a paradox for plants. ROS can be deleterious to cellular functions yet also are important signaling molecules. They are an unavoidable consequence of life in an oxygen-rich environment, and plants have devised numerous ways to deal with them, including changes in gene expression upon exposure. An article in the June 2006 Focus Issue on ROS, "Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis" by Gadjev et al. (2006), looked into changes in the transcriptome due to ROS.

BACKGROUND

ROS are by-products of photosynthetic and metabolic activity and, as such, are generated in different subcellular locations. This raises an important question of whether the subcellular site of ROS generation is important for the specificity and selectivity of ROS signals. ROS are important regulators of many processes, including stress responses, programmed cell death, and plant development (for review, see Gapper and Dolan, 2006; Halliwell, 2006; Dietz, 2008).

ROS can induce singlet and triplet oxygen, superoxide, nitric oxide, and hydroxyl radicals; these species can cause damage to proteins, nucleic acids, and lipids by oxidation. Antioxidant systems keep the levels of ROS low but never completely eliminate them, and repair systems are necessary to repair the damage that does occur. This continual cellular damage by ROS is believed to contribute to the aging process.

The perception and propagation of ROS signals and how these signals then induce specific cellular responses are not well known. The study by Gadjev et al. (2006) sought to address these questions by the use of transcriptome data generated from ROS-related microarray experiments.

WHAT WAS SHOWN

To determine the specificity of ROS-driven transcript expression, Gadjev et al. (2006) collected transcriptome data from in-house or publicly available datasets generated from ROS-related microarray experiments. The datasets were selected to allow examination not only of the effect of different ROS, but also of how the accumulation ROS in a variety of subcellular compartments altered gene expression. This included experiments with transgenic plants with disruptions in the activity of an antioxidant enzyme (catalase [CAT], cytosolic ascorbate peroxidase, or copper/zinc superoxide dismutase), the mutant fluorescent (flu), and exogenous application of oxidative stress-causing agents (methyl viologen, Alternaria alternata toxin, 3-aminotriazol, and ozone) to plants. The disruptions in antioxidant enzymes included experiments in which the enzyme activity was reduced or completely abolished. The flu mutants accumulate the photosensitizer protochlorophyllide in the dark, leading to the production of singlet oxygen in the chloroplast upon exposure to light (Meskauskiene et al., 2001).

This analysis showed that a majority of the transcripts responding to the stress were altered only in one experiment, i.e. by one species of ROS. The authors considered these transcripts to be "hallmarks for a specific oxidative stress characterized by the chemical identity of the produced ROS and/or the subcellular site of its production" (Gadjev et al., 2006, p. 441). The difference in the time it took for a transcript to respond to a stress—up- or down-regulated—also varied by experiment. For example, although there was some overlap in the change in gene expression between the flu mutants and plants exposed to methyl viologen, the flu plants had a more rapid change in gene expression. The authors interpreted the time difference to be due to the surface application of methyl viologen that then needs to diffuse to the chloroplast for the generation of singlet oxygen in the chloroplast to alter gene expression.

Most interestingly, a set of general oxidative stress markers was identified. This set of five transcripts was up-regulated at least 5-fold in at least seven out of the eight experiments. Three of the five are of unknown function, while the other two are a defensin-like protein (At2g43510) and a disease resistance protein (At1g57630). The defensin-like protein had greater than 5-fold increase in all experiments except APX1-1.5h. Defensins are small, Cys-rich proteins structurally related to a protein present in vertebrate and invertebrate systems (for review, see Thomma et al., 2002). As the name implies, defensins are involved in pathogen defense, specifically part of the innate immune response system, and are a large, diverse family in plants, with the majority having antifungal activity. Previous studies have demonstrated that these proteins are induced by ROS, especially H₂O₂. The Toll-interleukin-1 (TIR) class disease resistance protein had a greater than 5-fold increase in all eight repeats (Leu-rich repeats [LRRs], the other being the non-TIR class (for review, see DeYoung and Innes, 2006). NBS-LRR proteins are involved in the pathogen recognition as well as response and thus are expected to respond to ROS stimuli.

A bulk of the genes that had a change in expression level responded only in one experiment, highlighting that the type of ROS and/or the subcellular location of its generation determines the gene response. The flu
mutants had the largest number of “unique” genes up- or down-regulated. The genes that had the largest change in expression were three ethylene-responsive element-binding proteins, supporting a connection between ethylene and singlet oxygen as was previously observed by Danon et al. (2005) who observed that by blocking ethylene production, the cell death that normally occurs in flu mutants upon moving from dark to light was partially blocked.

THE IMPACT

CPR5 has been shown to be involved in a variety of processes, including both developmental and defense responses such as enhanced pathogen defense responses (Bowling et al., 1997), abnormal trichome development (Kirik et al., 2001), and dark-induced leaf senescence (Yoshida et al., 2002). A recent study by Jing et al. (2008) suggests that the early onset of senescence observed in cpr5 mutants is caused by a “deregulation of the cellular redox balance” (Jing et al., 2008, p. 85). They took a bioinformatics approach and examined publicly available datasets of presymptomatic cpr5. They found an up-regulation of ROS-associated genes and concluded that the cpr5 mutants, even before they display characteristic phenotype, are in a state of high cellular redox stress. A proteomics study confirmed that there is indeed an increase in expression of three of the five “hallmark” ROS genes identified by Gadjev et al. (2006), including a defensin-like protein (At2g43510) and a TIR class disease resistance protein (At1g57630). An increase in one-third of transcription factors identified by Gadjev et al. (2006) to be up-regulated by ROS were also found to be increased in presymptomatic cpr5 mutants.

Due to the ability of the ROS H2O2 to “cross” membranes, it has been increasingly shown to play an important role in cell signaling. CAT, a small multi-gene family in Arabidopsis, is the main enzyme that controls H2O2 levels. CAT2 and CAT3 have previously been demonstrated to have circadian regulation, while CAT1 has not. A recent paper by Xing et al. (2007) reported that CAT1 expression is regulated by abscisic acid, drought, and salt stress. CAT1 gene expression was shown to be mediated by AtMEK1, an Arabidopsis MAPK kinase, by triggering H2O2 signal production.

CONCLUSION

One goal of the Gadjev et al. (2006) article was to “provide a framework” (Gadjev et al., 2006, p. 436) for future studies on ROS signals and to facilitate studies of oxidative stress response in plants. As demonstrated by the studies by Jing et al. (2008) and Xing et al. (2007), as well as many of the other citing papers, this work has achieved this goal and continues to provide scaffolding for numerous “wet lab” studies.

LITERATURE CITED


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