

NSF-0343866 Using a functional genomics approach to study ROI metabolism in plants

Summary of the Proposed Project.

Reactive oxygen intermediates (ROI) control many different processes in plants. However, they are also toxic molecules capable of injuring plant cells. How this conflict is resolved in cells is largely unknown. Nonetheless, it is clear that the steady-state level of ROI in cells needs to be tightly regulated. **Our goal is to identify and characterize regulatory transcripts directly involved in controlling ROI metabolism in plants.** We propose a unique approach of using knockout lines, deficient in ROI-scavenging enzymes, to induce the accumulation of ROI in specific cellular compartments, in the absence of external stimuli or stress. **We hypothesize that knockout plants with suppressed capability to scavenge ROI will accumulate ROI and activate their ROI signal transduction pathway.** The study of gene expression in these plants will therefore enable us to identify and characterize some of the key regulatory genes that control ROI accumulation in plants. Moreover, our approach will enable us to simplify the complex interactions that exist between ROI and many different biotic and/or abiotic signaling pathways, and to isolate and characterize signal transduction transcripts directly involved in regulating ROI metabolism in plants. **Based on our preliminary results we hypothesize that zinc-finger proteins play a central role in the ROI signal transduction pathway of plants.** We will determine how they control ROI metabolism and how they fit into the hierarchy of ROI-response genes.

Knockout *Arabidopsis* lines with suppressed capability to scavenge ROI will be used to induce an internal oxidative stress in plants under controlled conditions (using a time-course design). Microarray analysis coupled with physiological and biochemical measurements will be used to identify regulatory transcripts induced/suppressed in cells upon ROI accumulation. Seedlings of transgenic plants constitutively expressing zinc-finger proteins and other transcription factors, induced/suppressed in cells upon ROI accumulation, will be screened for tolerance/sensitivity to oxidative stress on agar plates. The function of specific transcripts, identified by our screen as capable of altering the ROI-tolerance of plants, will be determined in transgenic plants and knockout lines. A genetic screen to identify ROI-sensing components will be developed.

Broader Impacts of the Proposed Project.

Data, methods, and biological material will be made available to the public as soon as they are confirmed. Up to 20 undergraduate students, including minorities, will be recruited into the project and introduced to modern research techniques in plant biology, including microarray analysis and mutant characterization. An online course for undergraduate students will be developed to share knowledge and enhance awareness to ROI and the important role they play in plants and other organisms. A high school teaching kit that includes a video presentation and several basic experiments demonstrating the importance of ROI to plant protection will be developed. Public access to the project will be ensured through a website specifically developed for the project.

ROI are implicated in a diverse array of biological processes. The ROI-sensitive/tolerant lines, the database of ROI-associated transcripts and the flow-chart of ROI response in plants (developed during the course of this study), could be used by many different research groups and scientists to determine to what extent and in what manner ROI are involved in the biological process/pathway they are studying. They would therefore serve as a valuable resource and enhance our overall understanding of ROI function in plants during normal metabolism, as well as during periods of environmental stress.

Arabidopsis 2010: Integrating the “Unknown-eome” with Abiotic Stress Response Networks in Arabidopsis

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Long-Term Goal and Background:

The long-term goal of this research project is to facilitate the assignment of function to every gene in *Arabidopsis thaliana*. To date, the function of the proteins encoded by more than 20% of plant genes is completely unknown, with the function of up to 43% of these proteins poorly characterized. The specific focus of this research is genes of unknown function and the networks that respond to environmental stress, such as chilling, drought, salt, flooding, high light, and oxidative environments. Environmental stress is the primary cause of crop loss world-wide. A plant's survival and growth is dependent on its ability to cope with environmental stresses. It is clear that a plant's stress response results from a complex set of changes in gene networks and metabolism. While 1000s of genes have been linked to stress networks, in only a few cases have their biochemical and genetic functions been elucidated. In fact, most of the stress-regulated genes identified to date have completely unknown function(s).

Specific Aims and Expected Outcome:

- 1. Determine the change in stress tolerance for > 1,600 plant lines with a disrupted expression of genes of unknown function.** The approach is to test plant lines for resistance or susceptibility to stresses including water deficit, salt, high light, heat, chilling, freezing, anaerobiosis, heavy metal and oxidative stress. The primary goal is to begin a systematic approach to evaluate the contribution of all *Arabidopsis* genes to stress tolerance. Expected outcome: Assigning a putative function to > 1,600 genes with an unknown function.
- 2. Determine the relationship of genes of unknown function within a global protein-protein interaction network.** The approach is to determine > 20,000 examples of protein-protein interactions using a random yeast two-hybrid screening strategy. The primary goal is to uncover links between genes of known and unknown function. Expected outcome: A protein-protein map for *Arabidopsis*.
- 3. Over-express > 150 selected genes of unknown function in plants, test transgenic plants for changes in stress tolerance, and search for interacting protein partners.** The hypothesis to be tested for each gene is that its over-expression can alter a particular stress response. Expected outcome: Identifying proteins of unknown function with a central importance in stress response and their protein partners.
- 4. Profile changes in gene expression in > 20 stress-response mutants grown under at least two different stress conditions.** The primary goal is to begin creating a network of gene expression changes that can help better focus in-depth experiments on enzymes and regulators of potential importance to environmental stress. Expected outcome: Identifying gene networks involved in environmental stress.
- 5. Coordinate a centralized website for information on genes of unknown function (“unknowns”) from Arabidopsis and provide outreach and training activities for those under privileged and underrepresented in science, on the role of science in agriculture, the environment, and human health.** Web-based resources will be established to disseminate unpublished information and link annotation and data to all *Arabidopsis* “unknowns”. All plant lines and results of the project will be released to the academic community on a biannual basis. Expected outcome: A centralized website to enhance research on genes with unknown function.

Broader Impacts:

The two key "Broader Impacts" of the proposed research are: 1) **development and maintenance of a centralized website** for information on *Arabidopsis* genes of unknown function. This site will track and disseminate information on homozygous T-DNA insertion lines for “unknown” genes and their

phenotypes, and provide gene structure, gene annotation, protein interaction and mRNA profiling data. 2) **Educational outreach** for K-12 and **interdisciplinary training** to postdoctoral, graduate and undergraduates trainees. A summer outreach workshop will teach high school students about Mendelian inheritance in Arabidopsis, the role of science in agriculture, the environment, and human health. This laboratory-based internship program will provide self-confidence building experiences and expose the students to laboratories and scientists. Both undergraduate and K-12 outreach and training activities will target the under privileged and underrepresented in science.