Rapid Stimulation of an Oxidative Burst during Elicitation of Cultured Plant Cells: Role in Defense and Signal Transduction—Commentary

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In the mid 1980s, Philip Low was hired by Monsanto as a consultant on the properties of biological membranes. At the time, Phil's research group in the Chemistry Department at Purdue was exploring basic questions of erythrocyte and chloroplast membrane biochemistry, including exploration into a new paradigm in cell biology: signal transduction. After several discussions with Monsanto biologists, Phil was awarded a Monsanto grant to determine whether plant cells engage signal transduction pathways.

To approach this problem, Phil collaborated with Peter Heinstein of Purdue's Department of Medicinal Chemistry, who was investigating the use of cultured plant cells to produce pharmaceuticals. The biosynthesis of these natural product drug compounds was known to be induced by "elicitors" (e.g. bacterial proteins). They hypothesized that elicitor-initiated signaling pathways might communicate the presence of pathogens to the interior of the cell, thereby activating genes for phytoalexin biosynthesis. To test this hypothesis, Pete brought some suspension cultures across campus to Phil's lab, where they planned to measure signaling intermediates, focusing first on any changes in transmembrane pH using pH-sensitive fluorescent dyes. When they added elicitors to the cell suspensions, the dyes were rapidly quenched after only a 2-min lag period. This finding was exciting for two reasons: (1) the response was surprisingly quick, implying that "sedentary" plants could respond to pathogens far more rapidly than had been expected; and (2) rapid biochemical changes were occurring in response to elicitors that were completely uncharacterized yet implied signaling. This exciting finding was enough to justify National Science Foundation funding, to hire a postdoc (Izydor Apostol), and to devote significant resources to the project.

Over the following several months, three key findings were made that were included in the article. First, dye quenching was irreversible (even after pH adjustment) and was blocked by peroxidase inhibitors and antioxidant enzymes, implicating hydrogen peroxide synthesis in dye oxidation, in a manner reminiscent of the oxidative burst in human neutrophils. Second, elicitor-induced oxidative destruction of indole-3-acetic acid was also measured, implying negative cross talk between auxin and defense signaling. Finally, hydrogen peroxide was placed in a signal transduction pathway prior to and required for phytoalexin production; this was the first reported evidence of a second messenger role for reactive oxygen species in plants.

The research described in this article also opened several new areas of exploration. First, subsequent studies to discern the signaling network leading from elicitation/pathogen invasion to the oxidative burst, and then to downstream responses, would yield wonderful insights into the molecular workings of the plant cell with applications in plant protection. Moreover, investigation into the fates of elicitors led to the discovery of receptor-mediated endocytosis in plant cells, which was considered at the time to be impossible due to high turgor pressure. This led to the discovery of biotin receptor-mediated endocytosis in plants, and later to folate-targeted drug delivery to cancer cells, and then to the introduction of six folate-targeted drugs into human clinical trials.

Philip Low is now a Distinguished Professor at Purdue University and the founder of Endocyte, Inc. Peter Heinstein is retired. Izydor Apostol works for a major pharmaceutical company.

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