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Determining Cellular Responses: Phytoglobins May Direct the Traffic

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How stem cells retain their undifferentiated state or how differentiated cells are capable of having dissimilar responses to perturbations are major open questions in plant biology. Cell-specific phytoglobin expression may be one mechanism determining cell fate by the modulation of nitric oxide (NO), affecting cellular hormonal responses and processes such as cell differentiation.

Plant Cell Fate Determination

Plant tissues comprise cells that have acquired a diverse fate depending on internal or external stimuli. However, the ontogeny of these cells can be traced back to a limited number of stem cells, which, by retaining an undifferentiated and uncommitted state, generate derivatives that embark on different differentiation processes. Mechanisms by which stem cells acquire and maintain their state and factors triggering cell-specific differentiation processes remain unknown and constitute a fundamental question in plant biology.

One possible mechanism determining plant cell fate involves the role of NO, as a central signal transduction component in many plant processes, and phytoglobins, previously termed non-symbiotic hemoglobins, which scavenge NO and modulate cellular events. NO is involved in hormonal regulation [1,2], programmed cell death (PCD) [3], and cell division and differentiation [3,4]. Phytoglobins bind and retain oxygen strongly, allowing capture of oxygen at very low cellular oxygen concentrations. In their oxygenated states, phytoglobins are capable of metabolizing NO to nitrate. Phytoglobins are encoded by three to five genes. Cell specificity and response to growth or environmental events are defining characteristics of phytoglobin gene expression [3]. An example of the effect of altering phytoglobin expression on cell fate is during somatic embryogenesis [5]. Suppression of one phytoglobin gene causes massive PCD of embryonic cells leading to abortion while suppression of a different phytoglobin gene results in suspensor cell PCD, increasing somatic embryo production; that is, one gene protects tissue from events leading to abortion while the second is capable of regulating embryo production.

NO as a Key Component in Regulating Cellular Processes

NO generation is associated with hormonal responses [2,6] and actions that result in, or are a consequence of, the production of reactive oxygen species (ROS) or reactive nitrogen species [4] (Figure 1). NO has been implicated in environmental stress responses [2,7], such as its effect on ethylene formation in the hyponastic response accompanying plant submergence [8] and during root hypoxic responses [3]. Furthermore, ethylene response factors (ERFs) are

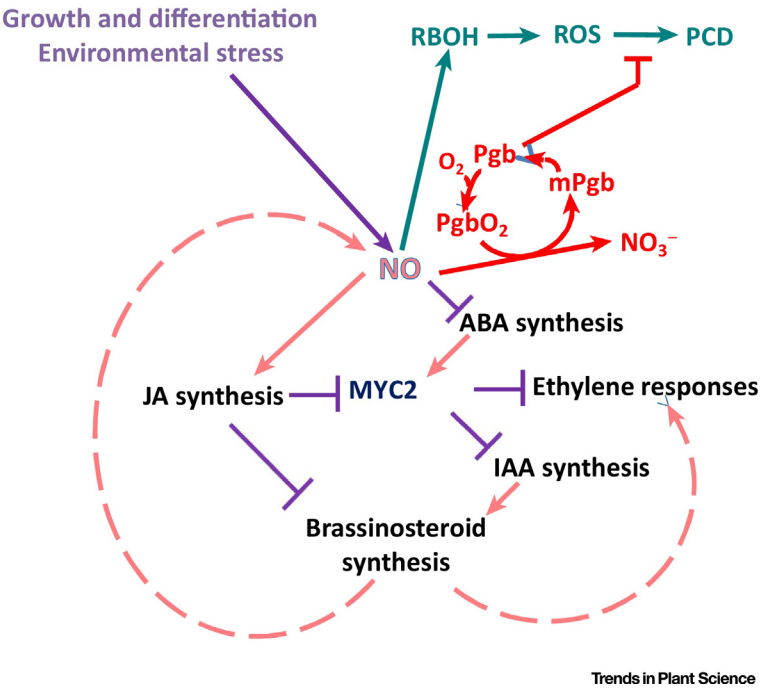

Figure 1. Nitric Oxide (NO) and Phytoglobin Involvement in Growth and Stress Responses. The production of NO is a characteristic of many plant responses during development and to environmental stress. Depending on the cell and the circumstances, it may be either a component of the signal transduction pathway of some hormone or an elicitor of programmed cell death (PCD). NO upregulates the respiratory burst oxidase homologs (RBOHs) involved in the production of reactive oxygen species (ROS), which are considered major inducers of PCD. In developmental or environmental responses involving jasmonic acid (JA), NO stimulates its synthesis resulting in the inhibition of MYC2, a bHLH transcription factor that suppresses indoleacetic acid (IAA) synthesis and the ethylene response. JA has been shown to inhibit brassinosteroid synthesis independently of MYC2. NO also blocks the synthesis of abscisic acid (ABA), a hormone known to upregulate MYC2 formation. Phytoglobin (Pgb), in the oxygenated form, can alter these responses by scavenging NO either in the nucleus, where it would interrupt signal transduction requiring NO, or in the cytoplasm, where it would reduce the concentration of ROS by direct scavenging or through the induction of antioxidant enzymes. The ramifications of varying NO levels can affect cell expansion, cell division, and the metabolism associated with these events.

determinants in the avoidance or escape from low-oxygen environments and ERF turnover via the N-end rule pathway, requiring NO as a substrate, may be a major regulatory event in the hypoxic response [8]. NO is also a significant factor in abscisic acid (ABA)-stimulated developmental and environmental responses [6,9]. Plants exposed to biotic stress also produce NO that affects hormonal responses, protein nitrosylation, and the induction of PCD in plant tissues in response to pathogen invasion [7,9].

NO also influences developmental processes. Cell wall modification [4,6] and embryogenesis in both monocots [5] and dicots [1,3] are influenced by the level of NO. There are also numerous examples of NO affecting various aspects of root development, from responses to nitrate and tropic responses to stem cell maintenance and lateral root development. In addition, NO has been implicated in leaf senescence and in fruit abscission. Hormonal crosstalk is frequently observed in physiological studies and there are suggestions that NO is centered at the interface of the various hormonal signal transduction pathways [2].

Phytoglobin Action in the Cell

One of the predominant features of plant environmental stress is increased cellular production of ROS [4] (Figure 1). This generally occurs as a result of disruption of mitochondrial processes leading to increased cytoplasmic NO production, via reactions occurring in the electron transport chain or elsewhere in the cell, and the induction of NADPH oxidases. The action of phytoglobin in removing NO both downregulates NADPH oxidase gene expression [3,5,10] and promotes expression of genes associated with antioxidant removal [3]. During monocot somatic embryogenesis [5] and hypoxic stress [11], alterations in the expression of phytoglobins strongly affect PCD by modifying NO levels, which influences the expression of NADPH oxidase genes that are responsible for the production of ROS. Phytoglobins are also capable of modulating many hormonal signal transduction pathways through their metabolism of NO [2]. In dicot somatic embryogenesis, phytoglobin affects the expression of auxin [1] and jasmonic acid [3] genes through modulation of cellular NO. It also affects arabidopsis (*Arabidopsis thaliana*) shoot organogenesis by altering the expression of genes encoding cytokinin perception and signalling and there is evidence of cytokinin, ABA, and jasmonic acid

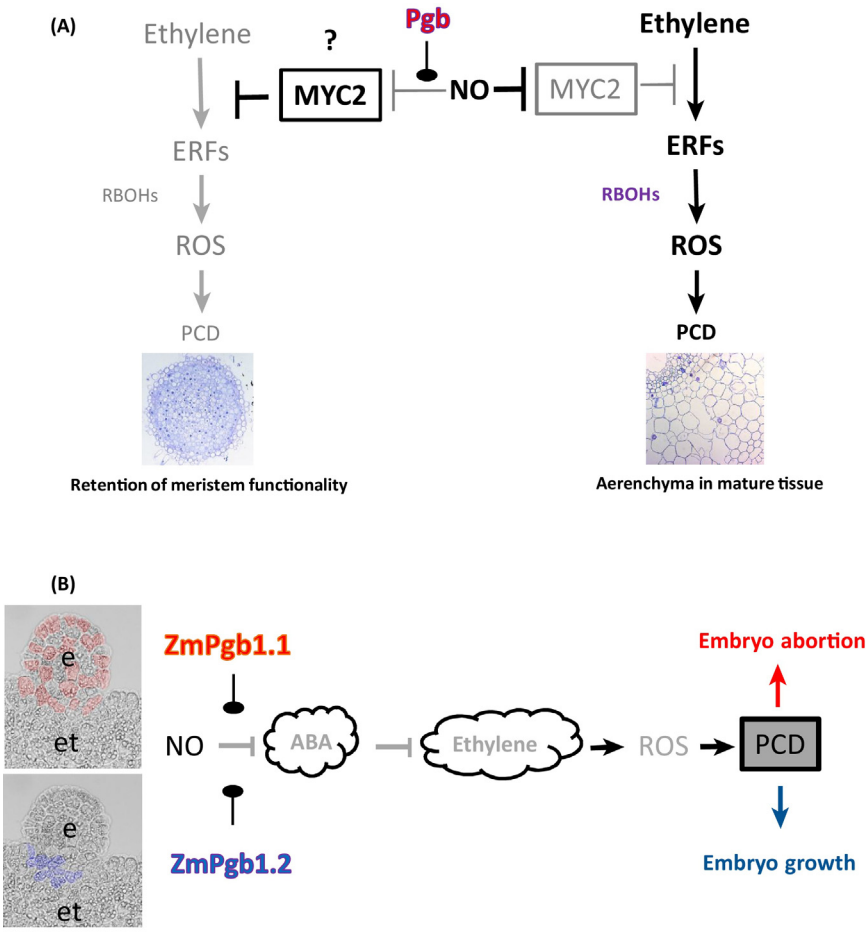

Figure 2. Cell-Specific Phytoglobin Expression Influences Cell Fate in Relation to Programmed Cell Death (PCD). (A) PCD during hypoxic stress. Nitric oxide (NO) is produced under hypoxic stress. In cells lacking phytoglobins (Pgb), NO inhibits MYC2 expression, relieving the inhibition of this transcription factor on genes associated with ethylene production and ethylene response factors (ERFs). These conditions favor the production of reactive oxygen species (ROS) that induce PCD. In mature root tissue, this leads to the formation of aerenchyma, allowing passage of oxygen from the shoot to the root to avoid hypoxic stress. In meristematic root cells where phytoglobin is present at high levels, there would be no inhibition of MYC2 formation by NO since it is scavenged by phytoglobin. Here we propose that it is MYC2 that blocks ethylene synthesis and sensitivity to ethylene, limiting ROS formation and resulting in the retention of functional root meristematic cells. (B) PCD during maize somatic embryogenesis. Immature embryos (e) are connected to the subtending embryogenic tissue (et). Maize class 1 phytoglobin s (*ZmPgb1.1* and *ZmPgb1.2*) regulate the levels of cellular NO during somatic embryogenesis. NO, in turn, regulates the expression of abscisic acid (ABA) genes, the product of which can inhibit ethylene expression and sensitivity, influencing ROS production and PCD. Of the two Pgb genes, *ZmPgb1.1* is expressed in embryonic cells (highlighted in red). When this gene is suppressed, PCD occurs in these cells, aborting embryogenesis. *ZmPgb1.2* is expressed primarily in basal cells (highlighted in blue) anchoring the embryo to the subtending tissue. Suppression of this gene induces PCD in the 'anchor' cells, releasing the immature embryos and allowing them to develop further. RBOH, respiratory burst oxidase homolog.

involvement in phytoglobin expression. During flooding or drought, phytoglobins, via effects on NO, influence ethylene formation and signal transduction pathways that maintain the integrity of root meristematic cells by preventing their precocious differentiation [1,10]. The addition of brassinolide to arabidopsis cell suspension cultures induces differentiation of the cells into tracheary elements. Unpublished work in our laboratory has shown that suppression of either class 1 or class 2 arabidopsis phytoglobins enhances this process and that the effect involves NO and ethylene. Many of these actions require the presence of phytoglobin within the nucleus of the cell [12]. While it is unknown how phytoglobin is transported into the nucleus, it is found in the nucleus and has been demonstrated to effectively act within the nucleus [12]. Thus, by modulating NO phytoglobin influences cell behavior and fate during development and under stress conditions.

Evidence for Cell-Specific Expression of Phytoglobins

A typical consequence of ROS production in the cell, as a result of environmental stress or plant development, is the induction of PCD [3]. Three to five types of phytoglobin genes encode the protein. Because of this diversity, there is the potential to direct gene expression to specific sets of cells in accordance with the perturbation initiating the process. Evidence of cell-specific phytoglobin expression associated with PCD has been found with hypoxic stress [10,11] (Figure 2A) and with the developmental program associated with somatic embryogenesis [3] (Figure 2B).

In dicotyledonous plants such as arabidopsis, *Pgb* gene expression directed to specific cells affects the cell's response during development or environmental stress. Suppression of the expression of *AtPgb2* enhances somatic embryogenesis in arabidopsis [1] by increasing auxin production, with polarization of PIN1 to yield auxin maxima particularly at the base of the cotyledons, the site of embryogenic tissue formation. This effect is due to the accumulation of NO in these cells as a result of the absence of phytoglobin, inhibiting *MYC2* expression, which relieves the inhibition of auxin synthesis (Figure 1).

These examples demonstrate the potential of cell-specific phytoglobin expression as one method by which plants control the fate of cells in both developmental processes and response to stress.

Concluding Remarks

Factors influencing cell fate and differentiation, the external morphology of organs and organisms based on selective PCD, and the response of cells to their external environment are fundamental topics in plant biology. The ideas described here provide one thread to pull that may unravel the process by which these events are controlled in often specific plant domains. The distinctive, simple chemical action of phytoglobin would seem to provide an uncomplicated means of regulating plant cellular responses. From the perspective of the phytoglobins, there are many unanswered questions before satisfying conclusions can be drawn. What determines which phytoglobin is expressed in which cell in response to the many different effectors to which the cell may be exposed? Why do more mature cells have differing capacities to respond with phytoglobin expression than stem cells or partially differentiated cells? Is phytoglobin expression a defining characteristic of plant cells in their capacity for totipotency?

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