Spatial Dependence for Hydrogen Peroxide-Directed Signaling in Light-Stressed Plants

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In this article, the role of the reactive oxygen species (ROS) hydrogen peroxide (H$_2$O$_2$) as a signaling molecule involved in plant response to a sudden change in light intensity will be considered in a spatial context. The relatively low reactivity of H$_2$O$_2$ compared with other ROS (Halliwell and Gutteridge, 1985) might suggest that it could diffuse from a site of production to initiate intracellular and systemic signaling (Mullineaux and Karpinski, 2002). However, another view is promulgated here that allows for compartment-specific H$_2$O$_2$-mediated signaling associated with acclimation to high light. It is proposed that a spatial component to signaling is maintained by limiting the accumulation of H$_2$O$_2$ to its sites of production. This would allow H$_2$O$_2$ to initiate distinct signals depending on its subcellular origin (Mullineaux and Karpinski, 2002). Conversely, breakdown in the integrity of this spatial component, such that H$_2$O$_2$ diffuses into other subcellular compartments, would promote oxidative stress and trigger signaling associated with cell death.

EXCESS EXCITATION ENERGY AND ITS DISSIPATION

Plants in their natural environments are very often exposed to sudden increases in light intensity, which results in the absorption of excitation energy in excess of that required for metabolism (Asada, 1999). Plants have developed many mechanisms to dissipate excess excitation energy (EEE; Asada, 1999; Niyogi, 2000; Ort and Baker, 2002). An important means of dissipating EEE is to increase the sink capacity of metabolism for molecular oxygen (O$_2$) can be reduced by at least two reactions that would effectively compete for the consumption of reductants (Asada, 1999; Ort and Baker, 2002). Molecular oxygen (O$_2$) can be reduced by at least two reactions that would effectively compete for the consumption of reducing equivalents by CO$_2$ assimilation (Asada, 1999; Ort and Baker, 2002). These are photorespiration and the reduction of O$_2$ by PSI (Mehler reaction) coupled to the water-water cycle (Asada, 1999; Ort and Baker, 2002). The relative importance of these individual processes for the dissipation of EEE is controversial (Ort and Baker, 2002), but is not the subject of this article.

Directly or indirectly, the Mehler reaction and photorespiration cause increased production of H$_2$O$_2$, which is removed by an extensive scavenging/antioxidant network (Asada, 1999; Mullineaux and Karpinski, 2002; Ort and Baker, 2002; Mittler et al., 2004). Logically, the capacity of these reactions that photoreduce O$_2$ to contribute to the dissipation of EEE must be dependent upon the effectiveness of the ROS scavenging/antioxidant network; otherwise, ROS production would result in oxidative stress leading to cell death (Asada, 1999; Mittler et al., 2004).

ROS DERIVED FROM PHOTOREDUCTION OF O$_2$ AND SIGNALING

The configuration of processes that dissipate EEE alters as the plant develops and as adjustments are made to acclimate to sustained changes in the plant’s external environment (Mullineaux and Karpinski, 2002; Murchie et al., 2002). This acclimation process includes changes in the number and activity of genes expressing the ROS scavenging/antioxidant network and also those involved in many other metabolic processes (Mullineaux and Karpinski, 2002; Rossel et al., 2002; Ball et al., 2004; Mateo et al., 2004; Mittler et al., 2004). Therefore, the question arises of how regulatory signals are able to modulate the expression of many genes simultaneously. One possibility is that H$_2$O$_2$, arising directly from the Mehler reaction or indirectly from photorespiration could initiate signaling in response to high light stress, regardless of its significance in the dissipation of EEE (Mullineaux and Karpinski, 2002).

PHOTOACCLIMATION OR CELL DEATH?

Arabidopsis (Arabidopsis thaliana) has proved a good subject for studies linking physiological and molecular genetic responses to high light stress (Bechtold et al., 2005). A few such studies have applied a high light stress that only elicits mild photoinhibition of photosynthesis from which the plant rapidly recovers (Russell et al., 1995; Karpinski et al., 1997; Rossel et al., 2002; Fryer et al., 2003; Ball et al., 2004). Such physiological responses are quite different from those occurring in plants exposed to high light that causes severe
photoinhibition (Kimura et al., 2003) or when plants are compromised in ROS scavenging (Mateo et al., 2004; Vandenabeele et al., 2004; Davletova et al., 2005), where the genes altered in expression are associated with oxidative stress. Indeed, the induced expression of the high light-responsive gene *ASCORBATE Peroxidase2* (*APX2*) disappears in leaf tissue that suffers prolonged exposure to extreme light stress and precedes the development of oxidative damage in such tissues (Karpinski et al., 1999).

TISSUE-SPECIFIC AND SUBCELLULAR SOURCES OF ROS FOR SIGNALING IN STRESSED LEAVES

One of the most striking features of the response of an Arabidopsis leaf to a light stress is the accumulation of H$_2$O$_2$ specifically in the vascular bundles (Fig. 1; Fryer et al., 2003). Other stresses, such as wounding of a leaf or infection with an incompatible hypersensitive response-inducing pathogen under ambient light conditions, produce a similar localized response (Fig. 1; Orozco-Cárdenas et al., 2001; Chang et al., 2004; Mateo et al., 2004). In light-stressed leaves, the bundle sheath cell chloroplasts were the major source of this H$_2$O$_2$ and, in contrast, surrounding mesophyll cells did not accumulate detectable amounts of H$_2$O$_2$ (Fig. 1; Fryer et al., 2003). In all these stresses, photosynthetic electron transport is required for the accumulation of H$_2$O$_2$ (Fryer et al., 2003; Chang et al., 2004; Mateo et al., 2004). In the case of high light, it has been suggested that the accumulation of H$_2$O$_2$ in the chloroplasts of the bundle sheath results from the operation of the water-water cycle and parallels the induction of *APX2* expression (Ört and Baker, 2002; Fryer et al., 2003; Mateo et al., 2004). In light-stressed leaves, the bundle sheath cell chloroplasts were the major source of this H$_2$O$_2$ and, in contrast, surrounding mesophyll cells did not accumulate detectable amounts of H$_2$O$_2$ (Fig. 1; Fryer et al., 2003). Other stresses, such as wounding of a leaf or infection with an incompatible hypersensitive response-inducing pathogen under ambient light conditions, produce a similar localized response (Fig. 1; Orozco-Cárdenas et al., 2001; Chang et al., 2004; Mateo et al., 2004). In light-stressed leaves, the bundle sheath cell chloroplasts were the major source of this H$_2$O$_2$ and, in contrast, surrounding mesophyll cells did not accumulate detectable amounts of H$_2$O$_2$ (Fig. 1; Fryer et al., 2003). In all these stresses, photosynthetic electron transport is required for the accumulation of H$_2$O$_2$ (Fryer et al., 2003; Chang et al., 2004; Mateo et al., 2004). In the case of high light, it has been suggested that the accumulation of H$_2$O$_2$ in the chloroplasts of the bundle sheath results from the operation of the water-water cycle and parallels the induction of *APX2* expression (Ört and Baker, 2002; Fryer et al., 2003; Mateo et al., 2004).
DURING HIGH LIGHT STRESS

It has been suggested that the H$_2$O$_2$ from bundle sheath cell chloroplasts may be secreted into the transpiration stream and engage in systemic signaling (Karpinski et al., 1999; Fryer et al., 2003; Ball et al., 2004). However, this hypothesis would require H$_2$O$_2$ to move from the chloroplast through the cytosol of light-stressed leaves. Using a mutant partially defective in chloroplast Cu/Zn superoxide dismutase (csd2-1; Rizhsky et al., 2003), the expression of a number of genes coding for parts of the antioxidant network have been shown to be dependent on a fully functioning water-water cycle in both nonstress and high light-stress conditions (Rizhsky et al., 2003; A. Zamboni and P.M. Mullineaux, unpublished data).

H$_2$O$_2$ METABOLISM IN BUNDLE SHEATH CELLS

It is clear that bundle sheath cells differ from neighboring leaf tissues in H$_2$O$_2$ metabolism (Fryer et al., 2003). The expression of APX2 specifically in bundle sheath cells (Fryer et al., 2003; Ball et al., 2004) hints at the possibility of a different antioxidant network in this tissue compared with that found in mesophyll tissue. A further possibility is that bundle sheath tissue is capable of making more H$_2$O$_2$ than mesophyll tissues. Under high light, photosynthetic electron transport operates at similar quantum efficiencies in both tissues (Fryer et al., 2003). However, it may be that bundle sheath cells cannot increase rates of CO$_2$ assimilation to dissipate EEE (Fryer et al., 2003). This notion comes from observations that CO$_2$ for photosynthesis in bundle sheath cells may be produced from malate transported from the roots (Hibberd and Quick, 2002) and not from the atmospheric CO$_2$, which is unlikely to exhibit significant rates of diffusion from stomatal cavities to vascular tissues in photosynthesizing leaves (Morison et al., 2005). The combination of high rates of electron transport combined with a limited capacity for CO$_2$ fixation would favor the photoreduction of O$_2$ and increased production of H$_2$O$_2$ by the water-water cycle (Fryer et al., 2003).

PEROXISOME-SOURCED H$_2$O$_2$ FOR SIGNALING DURING HIGH LIGHT STRESS

Peroxisome glycollate oxidase, part of the photosynthetic electron transport chain, is a source of considerable amounts of H$_2$O$_2$ in C$_3$ plants under high light conditions (Asada, 1999), which is effectively scavenged by catalase and other components of a peroxisome-located ROS/antioxidant network (Mittler et al., 2004). Plants with suppressed peroxisome catalase activity exposed to high light develop a range of oxidative stress symptoms (Vandenabeele et al., 2004). Such plants have been used to study the expression of several hundred genes responsive to oxidative stress (Vanderauwera et al., 2005). However, for wild-type plants, it is not clear that peroxisome-sourced H$_2$O$_2$ would be implicated in initiating acclimation to a changed light environment. Sustained exposure of wild-type plants to high light promotes oxidative stress (≥10-fold; Karpinski et al., 1999; Mateo et al., 2004) and this may be linked to the progressive inhibition of catalase (Shang and Feierabend, 1999) such that H$_2$O$_2$, from the peroxisome could trigger signaling responses. A problem for the plants in such conditions may be that full recovery cannot be achieved because the stress is too severe and instead triggers cell death.

PLASMA MEMBRANE/APOPLASTIC SOURCES OF H$_2$O$_2$ DURING HIGH LIGHT STRESS

Recent evidence also suggests a role for plasma membrane/apoplast sources of H$_2$O$_2$ in regulating gene expression under these conditions (Davletova et al., 2005). This suggestion partly comes from studies on high light-stressed plasma membrane NADPH oxidase (Atrboh) knockout (KO) mutants. Induction of APX1 expression in KO-AtrbohD plants subjected to prolonged high light stress (up to 6 h) was partly suppressed compared with wild-type plants (Davletova et al., 2005). Similarly, in double KO-AtrbohD/F plants exposed to a 5-fold increase in light for 1 h, or in plants under ambient light with constitutively enhanced apoplastic H$_2$O$_2$, APX2 expression was shown to be partly blocked or strongly induced, respectively (U. Bechtold and P.M. Mullineaux, unpublished data). In contrast, 27 other high light-responsive genes showed little or no effect in these mutants (U. Bechtold and P.M. Mullineaux, unpublished data). Taken together these data suggest that high light-mediated H$_2$O$_2$-induced signaling could be routed via the plasma membrane in some tissues (e.g. bundle sheath).

INTEGRITY OF THE ANTIOXIDANT CAPACITY OF THE CYTOSOL IN MAINTAINING DISCRETE SUBCELLULAR LOCATIONS FOR H$_2$O$_2$

Under high light, the cytosol may increase its antioxidant/ROS scavenging capacity at a time when several other compartments of the cell increase H$_2$O$_2$ turnover or accumulation. Exposure to high light causes a sustained increase in the foliar concentration of the thiol antioxidant reduced glutathione (GSH; Karpinski et al., 1997; Müller-Moulé et al., 2003). In Arabidopsis, the GSH biosynthetic pathway is partitioned between plastids and the cytosol such that increased GSH levels would occur predominantly in the cytosol (Mullineaux and Rausch, 2005). An increased rate of photosynthesis and increasingly alkaline and oxidizing conditions in the chloroplast stroma may stimulate GSH biosynthesis (Meyer and Hell, 2005; Mullineaux and Rausch, 2005). In addition, high light stress induces genes coding for key components of the cytosolic antioxidant/ROS scavenging network, thus adding to cytosol H$_2$O$_2$. 


scavenging capacity (Karpinski et al., 1997; Ball et al., 2004; Davletova et al., 2005). Under these conditions of mild or moderate light stress, H$_2$O$_2$ is unlikely to be able to accumulate in the cytosol. Therefore, to fulfill a signaling role, H$_2$O$_2$ would have to initiate this process at or very near its site of production, such as in the chloroplast stroma (Mullineaux and Karpinski, 2002). Conversely, when the integrity of H$_2$O$_2$ compartmentation breaks down, then oxidative stress ensues, perhaps triggering different cell death-associated signaling pathways.

COORDINATION OF CHLOROPLAST AND PLASMA MEMBRANE H$_2$O$_2$ SYNTHESIS

The above arguments contend that one signaling route in light-stressed cells involves chloroplast-sourced H$_2$O$_2$, which is transduced to another (non-ROS) factor to exit the chloroplast, traverses the reducing cytosolic compartment, and initiates a second burst of ROS catalyzed by NADPH oxidases (AtRbohD/F) at the plasma membrane. This would subsequently initiate downstream signaling responses in the same and neighboring cells. Such a biphasic accumulation of H$_2$O$_2$ has been suggested to explain some of the observations made in a recent study (Davletova et al., 2005), but direct evidence of such a pattern of H$_2$O$_2$ production in light-stressed leaves is not yet available. However, in guard cells and neighboring epidermal cells of ozone-fumigated Arabidopsis leaves, a biphasic burst of H$_2$O$_2$ has been observed, the first originating from the chloroplast of guard cells and the second mediated by one or more NADPH oxidases that subsequently trigger further rounds of ROS production at the plasma membrane of adjacent epidermal cells (Joo et al., 2005). The coordination of this ozone-induced biphasic ROS signal involves the heterotrimeric G protein $\alpha$-, $\beta$-, and G$\alpha$-subunits (Joo et al., 2005). The early component of this oxidative burst, sourced from the chloroplasts, may require the heterotrimer for signaling, whereas the Ga-subunit is required to activate the NADPH oxidase-catalyzed production of ROS (Joo et al., 2005).

A LINKING ROLE FOR ABSCISIC ACID?

The same G protein-protein has also been implicated in abscisic acid (ABA) signaling (Himmelbach et al., 2003; Pandey and Assmann, 2004). Further, ABA-mediated closure of stomata involves a downstream production of H$_2$O$_2$ produced by AtRbohD and AtRbohF (Kwak et al., 2003). This is important because ABA signaling has become increasingly implicated in the regulation of high light-responsive gene expression (Fryer et al., 2003; Rossel et al., 2006). A mutant, altered expression of APX2 8-1 (alx8-1), selected for constitutive expression of APX2, has double the foliar ABA content of wild-type plants (Davletova et al., 2005). Under these conditions of mild or moderate light stress, H$_2$O$_2$ is unlikely to be able to accumulate in the cytosol. Therefore, to fulfill a signaling role, H$_2$O$_2$ would have to initiate this process at or very near its site of production, such as in the chloroplast stroma (Mullineaux and Karpinski, 2002). Conversely, when the integrity of H$_2$O$_2$ compartmentation breaks down, then oxidative stress ensues, perhaps triggering different cell death-associated signaling pathways.

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