Journal of Plant Biology, December 2003, 46(4) : 215-222

<REVIEW>

Cytosolic Ca^{2+} Pulses and Protein Kinase Activation in the Signal Transduction Pathways Leading to the Plant Oxidative Burst

Stephen G. Cessna[1], Jitae Kim,[2] and Ann T.S. Taylor[3]*

[1]Department of Biology and Chemistry, Eastern Mennonite University, Harrisonburg, VA 22802, USA
[2]Chemistry Department, 1327 Brown Building, Purdue University, West Lafayette, IN 47907, USA; current address, Boyce Thompson Institute for Plant Research at Cornell University, Tower Road, Ithaca, NY 14853, USA
[3]Chemistry Department, Wabash College, PO Box 327, Crawfordsville, IN 47933, USA

The oxidative burst, the rapid production of O_2^- and H_2O_2 by plant cells in response to pathogens and stressors, is a critical step in plant disease resistance and is controlled by several different elicitor-initiated signaling pathways. While different defense elicitors appear to activate disparate initial steps in signaling the oxidative burst, all of the elicitors tested thus far appear to stimulate pathways that converge on the same three core signaling intermediates: 1) the Ca^{2+}-independent activation of a mitogen-activated protein kinase (MAPK) family member, 2) the influx of Ca^{2+} into the cytosol, deriving most critically from an internal compartment, and 3) the Ca^{2+}-dependent activation of additional protein kinases including a second MAPK homologue and possibly calcium dependent protein kinases (CDPKs). Data from several recent reports are summarized to place these signaling events into a complete and updated model of signaling to the plant oxidative burst.

Key words: calcium, kinase, oxidative burst, phosphorylation

The oxidative burst is the rapid generation of superoxide and hydrogen peroxide that is a defense strategy employed by plants during disease resistance (for reviews, see Baker and Orlandi, 1995; Blumwald et al., 1998; Bolwell et al., 1999; Grant and Loake, 2000; Wojtaszec, 1997). These free-radical species may serve many functions in plant defense, including a direct role in oxidative microbial toxicity (Baker and Orlandi, 1995), and signaling roles in the initiation of several local and systemic downstream defense responses (Levine et al., 1994; Baker and Orlandi, 1995; Wojtaszec, 1997; Orozco-Cardenas and Ryan, 1999). Because of the central place of the oxidative burst in plant defense mechanisms and the obvious deleterious consequences of unchecked oxidant production, the oxidative burst is tightly controlled by multi-step signal transduction pathways that are integrated into other defense and stress signaling pathways. This review focuses on recent advances in the understanding of the interplay between cytosolic Ca^{2+} fluxes and the activation of several different protein kinases in the initiation of H_2O_2 biosynthesis. Evidence from recent reports is compiled to present a coherent and updated signaling model for the events leading from cellular recognition of elicitor stimuli to both Ca^{2+}-dependent and independent protein kinase activation and to cytoplasmic Ca^{2+} entry, leading to the activation of oxidant synthesis.

*Corresponding author; fax +1-765-361-6340
e-mail taylora@wabash.edu

Overview of the Oxidative Burst Signal Transduction Pathway

The first step in signaling an oxidative burst response undoubtedly involves receptor recognition of different elicitor molecules followed by receptor-dependent reactions. The initial signaling intermediates induced by various oxidative burst elicitors are frequently very different. For example, the pathway initiated by oligogalacturonides appears to require G proteins (Legendre et al., 1992) and phospholipase C (Legendre et al., 1993) but not phospholipase A_2 (Chandra et al., 1996a), while an elicitor from the cell wall of *Verticillium dahliae* utilizes phospholipase A but not phospholipase C (Legendre et al., 1993; Chandra et al., 1996b). Even the initial sites of action may differ, as some elicitors appear to bind to sites on the plant cell surface (Reymond et al., 1995; Ligterink et al., 1997; Bourque et al., 1999; Lee et al., 2001), while others, including the

Abbreviations: CDPK, Calcium Dependent Protein Kinase; GMK1, *Glycine max* Mitogen Activated Protein Kinase 1; GMK2, *Glycine max* Mitogen Activated Protein Kinase 2; MAPK, Mitogen Activated Protein Kinase; SIPK, Salicylic Acid-Induced Protein Kinase; WIPK, Wounding-Induced Protein Kinase.

avrPto elicitor, may have to penetrate the plant cell to interact with its receptor (Scofield et al., 1996; Tang et al., 1996).

However, while it is apparent that these initial steps in the burst pathway vary dramatically by elicitor, it is also clear that they must eventually converge on common intermediates. One of the first common required signaling events, and thus a good candidate for a convergence point among the varied pathways, is the activation of protein kinases. A second common point in many pathways is ion fluxes. Evidence will be described later in this text that suggests that cytosolic calcium fluxes regulate some of the kinases involved in the oxidative burst. The activated kinases and calcium influx then modulate other cellular events, which presumably culminate in the activation of a Rac-dependent plasma membrane oxidase (Dwyer et al., 1996; Jabs et al., 1997; Bolwell et al., 2001; Romeis et al., 1999; Sagi and Fluhr, 1998; Torres et al., 2002; Olmos et al., 2003).

Calcium Fluxes in the Oxidative Burst Pathways

Defense elicitor-activated Ca^{2+} pulses were first recorded in 1991 in aequorin-transformed tobacco plants (Knight et al., 1991), and were then directly connected to the oxidative burst in 1997 (Chandra et al., 1997). Since the publication of those studies, we and others have found a firm causal relationship between the expression of cytosolic Ca^{2+} fluxes and the stimulation of the oxidative burst. Every oxidative burst elicitor thus far tested stimulates a Ca^{2+} flux (in *Nicotiana tabacum;* Chandra et al., 1997, Cessna and Low, 2001a; and in *Glycine max*, Navazio et al., 2002), and several different Ca^{2+} channel blockers invariably inhibit H_2O_2 production (Tavernier et al., 1995; Bolwell et al., 1999). Therefore, it appears that the requirement for cytosolic Ca^{2+} fluxes in the stimulation of the burst is quite strict.

Biochemical characterization of the cytosolic Ca^{2+} increases activated in response to oxidative burst elicitation in tobacco cells has provided substantial gains in answering two important questions. 1) What signaling events precede the Ca^{2+} pulses? 2) Does the cellular source of an elicited Ca^{2+} influx determine its efficacy in mediating the oxidative burst signal?

Events Upstream of Ca^{2+} Influx

The Ca^{2+} pulses induced by four different oxidative burst-initiating stimuli have been thoroughly studied with a large selection of pharmacological modulators of signal transduction (Cessna and Low, 2001a). The most potent inhibitors of the elicitor-induced Ca^{2+} signals were serine/threonine protein kinase inhibitors and anion channel blockers. Indeed, several reports point clearly to the involvement of protein kinases and anion channels in the burst pathway upstream of the requisite Ca^{2+} pulses (Cessna and Low, 2001a; Lecouriex et al., 2002; Navazio et al., 2002). The relationship between calcium fluxes and kinase activity will be discussed in further detail later in this article.

There are two possible explanations for the ability of anion channel blockers to inhibit a Ca^{2+} flux. First, movement of anions across a membrane will generally lead to membrane depolarization, and thus blocking an anion flux could therefore prevent the depolarization-dependent activation of voltage-regulated Ca^{2+} channels and thereby inhibit Ca^{2+} influx to the cytosol (Ward et al., 1995). Alternatively, Ca^{2+} movement across a membrane will quickly generate a reduced membrane potential, resulting in the rapid termination of Ca^{2+} flow. Simultaneous movement of anions across the same membrane would alleviate this electrical blockade, and obstruction of this flow would then inhibit Ca^{2+} movement also. Regardless of the mechanism, inhibition of Ca^{2+} movements by anion channel blockers is well established in the plant literature, especially in processes involving signal transduction (Ward et al., 1995; Xing et al., 1997; Lecourieux et al., 2002).

Localization of the Required Ca^{2+} Pulses to Release from Intracellular Compartments

Ca^{2+} does not readily diffuse within the cytosol, but rather, due to the large number of Ca^{2+} binding proteins present, Ca^{2+} remains localized near its sites of entry (Clapham, 1995). Thus, the compartment from which the Ca^{2+} influx derives partially determines the specificity encoded in the Ca^{2+} signal (Sanders et al., 1999). By manipulating the content of both the internal and external pools of signaling-ready Ca^{2+} with chelators or exogenous Ca^{2+}, and by the judicious use of selective Ca^{2+} signaling inhibitors, we have determined that the release of Ca^{2+} from an internal store, as opposed to the influx of Ca^{2+} across the plasma membrane, is required for the activation of the oxidative burst (Cessna et al., 1998; Cessna and Low, 2001b). This conclusion appears to be generally true of the activation of the plant oxidative burst, because several different elicitors in more than one plant species appear to behave in the same manner. This identification that organellar Ca^{2+} is the critical Ca^{2+} pool for oxidative burst activation does not necessarily preclude the

involvement of externally-derived influx in all oxidative burst signaling pathways. In fact, several reports have monitored Ca^{2+} pulses derived from the apoplast that are apparently required for the stimulation of H_2O_2 production (Blumwald et al., 1998). For example, using electro-physiological techniques, Gelli et al., 1997) and Zimmermann et al. (1997) have identified defense elicitor-stimulated activation of plasma membrane Ca^{2+} channels. Furthermore, inhibitors that block these channels (Gd^{3+}, La^{3+}) also blocked the resulting defense responses, suggesting that Ca^{2+} influx across the plasma membrane is required for the activation of the oxidative burst (Gelli et al., 1997; Zimmermann et al., 1997; Orozco-Cardenas and Ryan, 1999). While at first reading it may seem difficult to reconcile all of the above data, some of which point toward the requirement of an externally-derived Ca^{2+} influx, and some of which point to a requirement for internally-derived Ca^{2+} release, the following explanation for the apparent discrepancies can be offered. First of all, cellular Ca^{2+} fluxes rarely stem from a single source, but rather, entry through the plasma membrane can regulate organellar Ca^{2+} release, and the converse may also be true (Clapham, 1995; Sanders et al., 1999; Cessna and Low, 2001b). Thus, inhibition of Ca^{2+} influx through a plasma membrane channel can alter flow from an internal Ca^{2+} channel (for example, by blocking Ca^{2+}-induced Ca^{2+} release, Clapham, 1995). In light of these considerations, externally-derived Ca^{2+} fluxes may indirectly effect the activation of the oxidative burst, by altering the flow of Ca^{2+} from the internal stores (Cessna and Low, 2001b). In conclusion, our data support a hypothesis that internal Ca^{2+} release is required for the stimulation of the oxidative burst. However, more investigation in this area, including subcellular imaging of the pools tapped during elicitor stimulation and genetic identification of the Ca^{2+} channels involved would provide much more conclusive information.

Evidence for Kinase Involvement in Oxidative Burst Signaling

Preliminary evidence for kinase involvement in the oxidative burst was first noted in radiolabeling and pharmacologic studies prior to identification of kinase activation. Elicitation of plant cells treated with ^{32}P-phosphate leads to labeling of a number of proteins (Farmer et al., 1989; Felix et al., 1991; Chandra and Low, 1995), suggesting that kinases are somehow activated during the process. Assuming an analogy with signaling pathways of the neutrophil oxidative burst, these novel phosphoproteins could conceivably be other kinases, members of the superoxide-generating oxidase complex itself, or proteins not involved in the burst. Phosphorylated proteins that have thus far been identified in elicitor-stimulated Arabidopsis are AtMPK6 (Nuhse et al., 2000), AtMPK4 (Huang et al., 2000; Petersen et al., 2000), AtMPK3 (Kovtun et al., 2000) and AtPhos43 (Peck et al., 2001) and Pti in tomato (Zhou et al., 1995); however, the role of these proteins in the oxidative burst is unknown at this point.

Additional evidence for kinase participation has come from pharmacologic studies, where serine/threonine kinase inhibitors such as K-252a and staurosporine block oxidative burst stimulation in a dose-dependent manner (Schwake and Hager, 1992; Levine et al., 1994; Chandra and Low, 1995; Matthieu et al., 1996). Because addition of these same inhibitors also causes rapid termination of a previously initiated burst, it can be further suggested that continuous phosphorylation is essential for maintenance of burst activity. Interestingly, protein phosphatase inhibitors can autologously activate the burst, even in the absence of elicitors (Felix et al., 1994; Chandra and Low, 1995).

More conclusive evidence for the role of phosphorylation changes in the oxidative burst is provided by several reports that have monitored the *in vivo* activation of specific protein kinases. The first kinase demonstrated to participate in the oxidative burst was the resistance gene product, Pto. Tomato cell cultures transformed with Pto kinase displayed a prolonged two phase production of the oxidative burst in response to bacteria expressing the avrPto avirulence gene, whereas control cells lacking the Pto kinase expressed only the transient first phase of the burst (Chandra et al., 1996a and b). However, even the cells that lacked Pto kinase were able to generate substantial quantities of oxidants in response to non-race-specific elicitors, suggesting that the Pto kinase communicates the burst signal only when activated by avrPto, leaving the task to other kinases to respond to non-host-specific pathogens.

Mitogen-Activated Protein Kinase Participation in Oxidative Burst Signaling

MAP kinases have been associated with a number of plant defense responses, including salicylic acid production, wounding, and the hypersensitive response (for reviews, see Zhang and Klessig, 2000 and 2001; and Jonak et al., 2002). Several different groups have measured two different MAPK-like protein kinase activities that are both likely required for oxidant production in soybean and tobacco (Zhang and Klessig,

1997; Cazale et al., 1999; Grant and Loake, 2000; Taylor et al., 2001). The two kinases are both activated in elicitor-treated cells with the same kinetic profile as H_2O_2 production, and their activation *in vivo* was sensitive to the same protein kinase modulators that abolish burst activity. Based on their substrate specificities, their immunoprecipitation by MAPK selective antibodies, and their phosphorylation on threonine and tyrosine residues, it has been concluded that the two kinases are members of the MAP kinase family with molecular weights of around 44 and 47 kDa in soybean (Cazale et al., 1999; Taylor et al., 2001). Biochemical and genetic evidence indicates that the two soybean MAPK family members correspond to the tobacco Salicylic acid-Induced Protein Kinase (SIPK) or *Glycine max* Mitogen activated protein Kinase 1 (GMK1) (Zhang and Klessig 1997; Taylor et al., 2001; Kim, 2002) and the Wounding-Induced Protein Kinase (WIPK), or the *Glycine max* Mitogen activated protein Kinase 2 (GMK2) (Seo et al., 1995; Kim, 2002).

Like all MAPK family members, SIPKGMK1 and WIPK-GMK2 are believed to be activated after dual phosphorylation on tyrosine and threonine residues residing in an auto-inhibitory loop that guards the mouth of the active site in the non-phosphorylated form of the enzyme. This activation reaction is catalyzed by MAPK kinases (MAPKKs), which themselves are activated by phosphorylation catalyzed by MAPKK kinases (MAPKKKs). A tobacco and a soybean MAPKK homologue which uses SIPK-GMK1 as a substrate have been identified (NtMEK2 in tobacco, and GMKK1 in soybean; Yang et al., 2001; Kim, 2002). However, Yang et al. (2001) reported that the NtMEK2-SIPK/WIPK cascade is not involved in H_2O_2 production based on the data that transiently expressed NtMEK2 does not induce an oxidative burst. Furthermore, pharmacological studies suggest MAP kinases are not involved (Romeis et al., 1999), but two other groups manifested the opposite behavior (Cazale et al., 1999; Taylor et al., 2001). At this point, it is unclear whether the difference arises from elicitor-specific pathways, species differences, experimental conditions or perhaps the activation of SIPK-GMK1 and WIPK-GMK2 occurring downstream of hydrogen peroxide production. Obviously, complete resolution of this issue will require evaluation of knockout mutants of the NtMEK2-SIPK/WIPK (GMKK1-GMK1/GMK2) cascade in whole plant studies of the oxidative burst.

Interestingly, MAP kinases appear to be both upstream and downstream of oxidant production. A number of kinases show increased expression and/or activity in presence of hydrogen peroxide, including AtNDPK2 (Moon et al., 2003). Both over-expression and suppression of SIPK yield cells susceptible to oxidative stress (Samuel and Ellis, 2002), and over-expression of SIPK also leads to the hypersensitive response in the absence of a stimulus. Furthermore, protein tyrosine phosphatases are inhibited by oxidant production, and this inhibition is sufficient to activate AtMPK6, a MAP kinase that may be involved in oxidant production (Gupta and Luan, 2003; Taylor et al., 2003), leading to a feed-forward mechanism. These results suggest that the kinases that induce the burst would normally maintain the oxidase in a constitutively active state if their substrates (or themselves) were not continuously dephosphorylated by more dominant phosphatases.

There is little current information regarding SIPK-GMK1 substrates important to the burst pathway. Two primary candidates would include anion or Ca^{2+} channels or their protein regulators, and/or the oxidant generating enzyme or enzyme complex or its regulators. Alternatively, additional effector proteins that are one or more signaling steps removed from trans-membrane ion movement or oxidant production may be the immediate substrates of SIPK-GMK1. Based on pharmacological considerations further discussed below, we have placed SIPK-GMK1 and WIPK-GMK2 on an independent pathway, one branch leading to the burst by way of Ca^{2+} influx and WIPK-GMK2 activation, and the other by way of as yet unidentified signaling intermediates. In this working model, both of the branches in the pathway are required for the induction of H_2O_2 biosynthesis. While the details concerning its placement in this pathway await further experimentation, it can be determined now that SIPK-GMK1 activation is a required step in the burst pathway, residing independent from (or upstream of) Ca^{2+} influx.

Intgration of Calcium and Kinase Signals: Ca^{2+}-Dependent Protein Kinase Activation

The integration of calcium and kinase signaling appears to occur in the middle of the kinase cascade. In studies where elicitor-stimulated calcium signaling was blocked, elicitor-induced activation of WIPK-GMK2 was inhibited, but activation of SIPK-GMK1 was insensitive to the same treatments (Hoyos and Zhang, 2000; Taylor et al., 2001). It was therefore concluded that SIPK-GMK1 activation is independent of, or precedes Ca^{2+} influx in signaling the burst response, and that WIPK-GMK2 activation necessarily follows Ca^{2+} fluxes (Cessna and Low, 2001a; Taylor et al., 2001). Also consistent with the placement of SIPK-GMK1 independent of the Ca^{2+} pulse (Fig. 1) is the finding that SIPK-GMK1

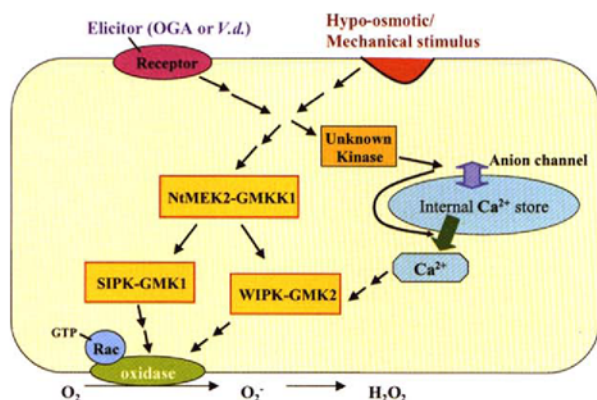

Figure 1. Activation of the oxidative burst includes activation of a kinase upstream of calcium release from an internal store, activation of SIPK-GMK1 through a calcium-independent pathway, activation of WIPK-GMK2 through a calcium dependent pathway, and activation of the oxidase and other downstream affectors.

activity is directly sensitive to both k252a and staurosporine (Zhang et al., 2000; Taylor et al., 2001). In contrast, SIPK-GMK1 activity is not affected by prior treatments of the cells with the anion channel blockers or Ca^{2+} channel blockers and modulators (Hoyos and Zhang, 2000; Taylor et al., 2001). Together, these data indicate that SIPK-GMK1 is activated independent of or upstream of both anion and calcium ion movement across a membrane (Fig. 1).

There is currently little information on the nature of the cytosolic Ca^{2+}-sensing protein that relays the Ca^{2+} signal to the oxidant producing apparatus. Candidates include one or more of the many calmodulin isoforms expressed in plant cells, a CDPK, an EF-hand containing NADPH oxidase-like protein (Keller et al., 1998), a calcineurin-type protein such as SOS3 (Liu and Zhu, 1998) or another Ca^{2+}-binding enzyme or regulatory protein. While it is possible that a CDPK may be involved in the stimulation of the oxidative burst (Romeis et al., 2000), direct evidence for CDPK involvement is based on the coincident activation of a CDPK and H_2O_2 biosynthesis, and the sensitivity of both CDPK activation and H_2O_2 production to non-specific inhibitors such as W-7 (Romeis et al., 2001). Because W-7 is unsuccessful in inhibiting the oxidative burst in many plants (Taylor et al., 2001), it can be concluded that some other Ca^{2+}-sensing protein may more commonly relay the signal to the burst machinery.

Regardless of the means by which the calcium signal is recognized, it is apparent that protein kinase activation not only precedes but also follows Ca^{2+} influx in the pathway leading to oxidant production. The most carefully studied protein kinase that can conclusively be placed in the latter portion of the pathway is the above mentioned WIPK-GMK2. Like SIPK-GMK1, WIPK-GMK2 is activated coincident with H_2O_2 production. However, in contrast to SIPK-GMK1, WIPK/GMK2 activation is blocked by agents that inhibit elicitor-stimulated Ca^{2+} signaling. A second critical difference between the two MAPK homologues is their direct sensitivity to the kinase inhibitors k252a and staurosporine. While both kinases are completely inhibited in k252a/staurosporine treated cells, SIPK/GMK1 but not WIPK/GMK2 is directly sensitive to the modulators when kinase activity is measured *in vitro*. This difference further supports the possibility that SIPK/GMK1 lies independent of (or upstream of) Ca^{2+} influx and WIPK-GMK2 activation (Fig. 1). While Ca^{2+} influxes are placed prior to GMK2 activation, there is at least one signaling event between the calcium flux and GMK2 activation, as calcium is neither required for GMK2 activation nor does it directly stimulate GMK2 activity. Little information is yet available on the nature of the substrates utilized by WIPK-GMK2 in the burst pathway. Presumably other regulatory proteins, or the oxidant-producing enzyme itself is activated by WIPK-GMK2-dependent phosphorylation.

Model of the Integration of Ca^{2+} and Kinase Activity

The simplest summary of the most current data is presented in Figure 1. Elicitors (or eliciting physical stimuli) are first perceived. Several different immediate responses to receptor activation are possible, including the direct activation of various protein kinases, and/or the activation receptor associated G-proteins. We hypothesize that while the pathways leading to the oxidative burst are initially varied, they eventually converge on the activation of SIPK-GMK1 and WIPK-GMK2, i.e., Ca^{2+}-independent and Ca^{2+}-dependent branches. Simultaneous signaling through both of these branches is required for initiation of the burst response.

Evidence for such a signaling-junction is twofold. First, activation of H_2O_2 production in plant cells cannot reproducibly be achieved with Ca^{2+}-selective ionophores (e.g., ionomycin and A23187; Chandra et al., 1997; Cessna and Low, 2001a), even though substantial Ca^{2+} influx after ionophore treatment can be measured (Chandra et al., 1997). Thus, while cytosolic Ca^{2+} influx is most definitely required for stimulation of the burst, as evidenced by the potent and universal inhibition of H_2O_2 production by Ca^{2+} influx modulators, it does not appear to be sufficient for its activation.

This indicates that a parallel pathway must be activated simultaneously. Furthermore, unlike what occurs after Ca^{2+} channel or anion channel blocker treatments, inhibition by the protein kinase inhibitors k252a and staurosporine cannot be overcome by ionophore-mediated Ca^{2+} entry (Cessna and Low, 2001a). It therefore seems likely that a kinase not only facilitates H_2O_2 production by its activation of Ca^{2+} entry, but must also be required for the activation of the Ca^{2+}-independent parallel pathway.

While this model accurately represents the current information available, it is quite possible that it is over simplified. For example, there may be additional links between SIPK/GMK1 and calcium fluxes or calcium fluxes and WIPK/GMK2 activation. Furthermore, the activation of the oxidase may require multiple inputs, e.g., calcium as well as kinase activation. Timing, localization, and exact concentration are probably all important in the integration and outcome of calcium/kinase signaling pathways. Finally, it is likely that this pathway is intricately connected to other stress-response signal transduction pathways, as is obvious from cross-talk studies (reviewed in Bowler and Fluhr, 2000). Further studies combining genetic, proteomic, and imaging techniques are required to fully comprehend the mechanism of calcium and kinase signal integration in the oxidative burst.

Received September 30, 2003; accepted October 1, 2003.

LITERATURE CITED

Baker CJ, Orlandi EW (1995) Active oxygen in plant pathogenesis. Ann Rev Phytopath 33: 299-321

Blumwald E, Aharon GS, Lam BCH (1998) Early signal transduction pathways in plantpathogen interactions. Trends Plant Sci 3: 342-346

Bolwell GP, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, Minibayeva F, Rowntree EG, Wojtaszek P (1999) Recent advances in understanding the origin of the apoplastic oxidative burst in plant cells. Free Radic Res Suppl: S137-145

Bolwell GP, Daview DR, Gerrish C, Auh C-Y, Murphy TM (1998) Comparative biochemistry of the oxidative burst produced by rose and french bean cells reveals two distinct mechanisms. Plant Physiol 116: 1379-1385.

Bourque S, Binet MN, Ponchet M, Pugin A, Lebrun-Garcia A (1999) Characterization of the cryptogein binding sites on plant plasma membranes. J Biol Chem 274: 34699-34705

Bowler C, Fluhr R (2000) The role of calcium and activated oxygens as signals for controlling cross-tolerance. Trends Plant Sci 5: 241-246

Cazale A-C, Droillard M-J, Wilson C, Heberle-Bors H, Barbier-Brygoo H, Lauriere C (1999) MAP kinase activation by hypoosmotic stress of tobacco cell suspensions: Towards the oxidative burst response. Plant J 19: 297-307

Cessna SG, Chandra S, Low PS (1998) The hypo-osmotic shock activated Ca^{2+} transient in suspension cultured tobacco cells is the product of external and internal cellular Ca^{+2} sources. J Biol Chem 273: 27286-27291.

Cessna SG, Low PS (2001a) Activation of the oxidative burst in aequorin-transformed Nicotiana tabacum cells is mediated by protein kinase- and anion channel-dependent release of Ca^{2+} from internal stores. Planta 214: 126-134

Cessna SG, Low PS (2001b) An apoplastic Ca^{2+} sensor regulates internal Ca^{2+} release in aequorin-transformed tobacco cells. J Biol Chem 276: 10655-10662

Chandra S, Low PS (1995) Role of phosphorylation in elicitation of the oxidative burst in cultured soybean cells. Proc Natl Acad Sci USA 92: 4120-4123

Chandra S, Heinstein PF, and Low PS (1996a) Activation of phospholipase A by plant defense elicitors. Plant Physiol 110: 979-986

Chandra S, Martin GB, Low PS (1996b) The Pto kinase mediates a signaling pathway leading to the oxidative burst in tomato. Proc Natl Acad Sci USA 93: 13393-13397

Chandra S, Stennis M, Low PS (1997) Measurement of Ca^{2+} fluxes during elicitation of the oxidative burst in aequorin-transformed tobacco cells. J Biol Chem 272: 28274-28280

Clapham DE (1995) Calcium signaling. Cell 80: 259-268

Dwyer SC, Legendre L, Low PS, Leto TL (1996) Plant and human neutrophil oxidative burst complexes contain immunologically related proteins. Biochim Biophys Acta 1289: 231-237

Farmer EE, Pearce G, Ryan CA (1989) In vitro phosphorylation of plant plasma membrane proteins in response to the proteinase inhibitor inducing factor. Proc Natl Acad Sci USA 86: 539-542

Felix G, Grosskopf DG, Regenass M, Boller T (1991) Rapid changes of protein phosphorylation are involved in transduction of the elicitor signal in plant cells. Proc Natl Acad Sci USA 88: 8831-8834

Felix G, Regenass M, Spanu P, Boller T (1994) The protein phosphatase inhibitor calyculin A mimics elicitor action in plant cells and induces rapid hyperphosphorylation of specific proteins as revealed by pulse labeling with [^{32}P] phosphate. Proc Natl Acad Sci USA 91: 952-956

Gelli A, Blumwald E (1997) Hyperpolarization-activated Ca^{2+}-permeable channels in the plasma membrane of tomato cells. J Membr Biol 155: 35-45

Grant JJ, Loake GJ (2000) Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol 124: 21-30

Gupta R, Luan S (2003) Redox control of protein tyrosine phosphatases and mitogen-activated protein kinases in plants. Plant Physiol 132: 1149-1152

Hoyos ME, Zhang S (2000) Calcium-independent activation of salicylic acid-induced protein kinase and a 40-kilodalton protein kinase by hyperosmotic stress. Plant

Physiol 122:1355-1363
Huang Y, Li H, Gupta R, Morris PC, Luan S, Kieber JJ (2000) ATMPK4, an Arabidopsis homolog of mitogen-activated protein kinase, is activated *in vitro* by AtMEK1 through threonine phopshorylation. Plant Physiol 122: 1301-1310
Jabs T, Schope M, Collig C, Hahlbrock K, Scheel D (1997) Elicitor stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. Proc Natl Acad Sci USA 94: 4800-4805
Jonak C, Okresz L, Bogre L, Hirt H (2002) Complexity, cross talk and integration of plant MAP kinase signalling. Curr Opin Plant Biol 5: 415-424
Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C (1998) A plant homolog of the neutrophil NADPH oxidase gp91phox subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. Plant Cell 10: 255-266
Kim J (2002) Ph. D. thesis, Dept. of Chemistry, Purdue University, IN, USA
Knight MR, Campbell AK, Smith SM, Trewavas AJ (1991) Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. Nature 352: 524-526
Kovtun Y, Chiu W-L, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci USA 97: 29402945
Lecourieux D, Mazars C, Pauly N, Ranjeva R, Pugin A (2002) Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. Plant Cell 14: 2627-2641
Lee J, Klessig DF, Nurberger T (2001) A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene HIN1 independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. Plant Cell 13: 1079-1093
Legendre L, Heinstein PF, Low PS (1992) Evidence for participation of GTP-binding proteins in the elicitation of the rapid oxidative burst in cultured soybean cells. J Biol Chem 267: 20140-20147
Legendre L, Yueh YG, Crain R, Haddock N, Heinstein PG, Low PS (1993) Phospholipase C activation during elicitation of the oxidative burst in cultured plant cells. J Biol Chem 268: 24559-24563
Levine A, Tenhaken R, Dixon R, Lamb C (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79: 583-593
Ligterink W, Kroj T, zur Nieden U, Hirt H, Scheel D (1997) Receptor-mediated activation of a MAP kinase in pathogen defense of plants. Science 276: 2054-2057
Liu J, Zhu JK (1998) A calcineurin sensor homolog required for plant salt tolerance. Science 280: 1943-1945
Matthieu Y, Sanchez RJ, Droillard MJ, Lapous D, Lauriere C, Guern J (1996) Involvement of protein phosphorylation in the early steps of transduction of the oligogalacturonide signal in tobacco cells. Plant Physiol Biochem 34: 399-408
Moon H, Lee B, Choi G, Shin D, Prasad DT, Lee O, Kwak S-S, Kim DH, Nam J, Bahk J, Hong JC, Lee SY, Cho MJ, Lim CO, Yun D-J (2003) NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulat cellular redox state and enhances multiple stress tolerance in transgenic plants. Proc Natl Acad Sci USA 100: 358-363
Navazio L, Moscatiello R, Bellincampi D, Baldan B, Meggio F, Brini M, Bowler C, Mariani P (2002) The role of calcium in oligogalacturonide-activated signaling in soybean cells. Planta 215: 596-605
Nuhse TS, Peck SC, Hirt H, Boller T (2000) Microbial elicitors induce activation and dual phosphorylation of the *Arabidopsis thaliana* MAPK 6. J Biol Chem 275: 7521-7526
Olmos E, Martinez-Solano JR, Piqueras A, Hellin E (2003) Early steps in the oxidative burst induced by cadmium in cultured tobacco cells (BY-2 line). J Exp Bot 54: 291-301
Orozco-Cardenas M, Ryan CA (1999) Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. Proc Natl Acad Sci USA 96: 6553-6557
Peck SC, Nuhse TS, Hess D, Iglesias A, Meins F, Boller T (2001) Directed proteomics identifies a plant-specific protein rapidly phosphorylated in response to bacterial and fungal elicitors. Plant Cell 13: 1467-1475
Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, Johanson B, Nielsen HB, Lacy M, Austin J Parker JE, Sharma SB, Klessig DF, Martienssen R, Mattsson O, Jensen AB, Mundy J (2000) *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. Cell 103: 1111-1120
Reymond P, Grunberger S, Paul K, Muller M, Farmer EE (1995) Oligogalacturonide defense signals in plants: large fragments interact with the plasma membrane in vitro. Proc Natl Acad Sci USA 92: 4145-4149
Romeis T, Ludwig AA, Martin R, Jones JDG (2001) Calcium-dependent protein kinases play and essential role in a plant defence response. EMBO J 20: 5556-5567
Romeis T, Piedras P, Jones JD (2000) Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response. Plant Cell 12: 803-816.
Romeis T, Piedras P, Zhang S, Klessig DF, Hirt H, Jones JDG (1999) Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound and salicylate responses. Plant Cell 11: 273-287
Sagi M, Fluhr R (2001) Superoxide production by plant homologues of the gp91(phox) NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. Plant Physiol 126: 1281-1290
Samuel MA, Ellis BE (2002) Double jeopardy: both overexpression and suppression of a redox-activated plant mitogen-activated protein kinase render tobacco plants ozone sensitive. Plant Cell 14: 2059-2069

Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. Plant Cell 11: 691-706

Schwake R, Hager A (1992) Fungal elicitors induce a transient release of active oxygen species from cultured spruce cells that is dependent on Ca^{2+} and protein kinase activity. Planta 187: 136-141

Scofield SR, Tobias CM, Rathjen JP, Chang JH, Lavelle DT, Michelmore RW, Staskawicz BJ (1996) Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. Science 274: 2063-2065

Seo S, Okamoto M, Seto H, Ishizuka K, Sano H, Ohashi Y (1995) Tobacco MAP kinase: a possible mediator in wound signal transduction pathways. Science 270: 1988-1992

Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB (1996) Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. Science 274: 2060-2063

Tavernier E, Wendehenne D, Blein JP, Pugin A (1995) Involvement of free calcium in action of cryptogein, a proteinaceous elicitor of hypersensitive reaction in tobacco cells. Plant Physiol 109: 1025-1031

Taylor AT, Kim J, Low PS (2001) Involvement of mitogen-activated protein kinase activation in the signal-transduction pathways of the soya bean oxidative burst. Biochem J 355(Pt 3): 795-803

Taylor ATS, Steffen N, Weitzel CS, Rheinhardt SJ, Johnson EG (2003) Characterization of the oxidative regulation of a phosphatase from *Arabidopsis*. Plant Journal (submitted)

Torres MA, Dangl JL, Jones JD (2002) Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci USA 99: 517-522

Ward JM, Pei ZM, Schroeder JI (1995) Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell 7: 833-844

Wojtaszek B (1997) Oxidative burst: an early plant response to pathogen infection. Biochem J 322: 681-692

Xing T, Higgins VJ, Blumwald E (1997) Race-specific elicitors of *Cladosporium fulvum* promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells. Plant Cell 9: 249-259

Yang K-Y, Liu Y, Zhang S (2001) Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. Proc Natl Acad Sci USA 98:741-746

Zhang S, Klessig DF (1997) Salicylic acid activates a 48 kDa MAP kinase in tobacco. Plant Cell 9: 809-824

Zhang S, Klessig DF (2000) Pathogen-induced MAP kinases in tobacco. Results Probl Cell Differ 27: 65-84

Zhang S, Klessig DF (2001) MAPK cascades in plant defense signaling. Trends Plant Sci 6: 520527

Zhang S, Liu Y, Klessig DF (2000) Multiple levels of tobacco WIPK activation during the induction of cell death by fungal elicitins. Plant J 23: 339-347

Zhou J, Loh YT, Bressan RA, Martin GB (1995) The tomato gene Pti1 encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. Cell 83: 925-935

Zimmermann S, Nurnberger T, Frachisse JM, Wirtz W, Guern J, Hedrich R, Scheel D (1997) Receptor-mediated activation of a plant Ca^{2+}-permeable ion channel involved in pathogen defense. Proc Natl Acad Sci USA 94: 2751-2755