A review of redox signaling and the control of MAP kinase pathway in plants

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Mitogen-activated protein kinase (MAPK) cascades are evolutionarily conserved modules among eukaryotic species that range from yeast, plants, flies to mammals. In eukaryotic cells, reactive oxygen species (ROS) has both physiological and toxic effects. Both MAPK cascades and ROS signaling are involved in plant response to various biotic and abiotic stresses. It has been observed that not only can ROS induce MAPK activation, but also that disturbing MAPK cascades can modulate ROS production and responses. This review will discuss the potential mechanisms by which ROS may activate and/or regulate MAPK cascades in plants. The role of MAPK cascades and ROS signaling in regulating gene expression, stomatal function, and programmed cell death (PCD) is also discussed. In addition, the relationship between Rboh-dependent ROS production and MAPK activation in PAMP-triggered immunity will be reviewed.

1. Introduction

Reactive oxygen species (ROS), including hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), hydroxyl radical (HO\textsuperscript{•}), singlet oxygen (1O\textsubscript{2}), and superoxide anion (O\textsubscript{2}\textsuperscript{−}), are formed upon partial reduction of oxygen (O\textsubscript{2}). In plants, ROS are inevitably generated as the by-products of cellular respiration, photosynthesis, protein folding, and a number of metabolic reactions [1]. In mitochondria, O\textsubscript{2}\textsuperscript{−} is formed by the mitochondrial electron transport chain at the respiratory complexes I, II, and III. O\textsubscript{2}\textsuperscript{−} can then dismutate to form H\textsubscript{2}O\textsubscript{2} either spontaneously or catalytically by mitochondrial superoxide dismutase (SOD) [2]. During photosynthesis, various types of ROS are generated by different pathways in chloroplasts [1]. For instance, H\textsubscript{2}O\textsubscript{2} is produced constitutively in light via chlorophyll [3]. Photoreduction of O\textsubscript{2} by the reducing side of photosystem II (PSII) produces O\textsubscript{2}\textsuperscript{•−} which dismutates to form H\textsubscript{2}O\textsubscript{2}[4]. On the PSII electron donor side, H\textsubscript{2}O\textsubscript{2} can be produced by inhibition of enzymatic activity of the water-splitting manganese complex coupled with incomplete oxidation of water. PSI produces O\textsubscript{2}\textsuperscript{•−} by donating an electron to O\textsubscript{2} instead of ferredoxin [3]. In peroxisomes, H\textsubscript{2}O\textsubscript{2} is generated as an end product by oxidation pathways such as the glycolate oxidation and β-oxidation of long-chain fatty acids [5]. In the apoplastic, O\textsubscript{2}\textsuperscript{•−} can be produced by plasma membrane-located respiratory burst oxidase homolog (Rboh) proteins (also known as plant NADPH oxides). The O\textsubscript{2}\textsuperscript{•−} dismutates to form H\textsubscript{2}O\textsubscript{2}, spontaneously or catalytically by apoplastic SOD [6]. H\textsubscript{2}O\textsubscript{2} can also be generated by class III peroxidases in the cell wall, e.g. in response to pathogen attack [7]. Ozone (O\textsubscript{3}) can be degraded into secondary ROS, including H\textsubscript{2}O\textsubscript{2}, O\textsubscript{2}\textsuperscript{•−}, and HO\textsuperscript{•} by reacting with components of the cell walls, cellular membranes, and apoplastic fluids [8]. ROS formation occurs under normal growth conditions but can be accelerated when plants are challenged by biotic or abiotic stresses, e.g. plant-pathogen interactions, high light, salinity, and drought [9,10]. Furthermore, ROS production in these subcellular compartments is not isolated [11]. ROS generation in specific subcellular compartments may lead to ROS accumulation in other compartments.

High levels of ROS can lead to detrimental effects (ROS stress). ROS stress causes direct or indirect ROS-mediated damage of a variety of molecules including lipid peroxidation in cellular membranes, protein denaturation, carbohydrate oxidation, and pigment breakdown [12]. ROS stress can eventually lead to changes in gene expression and even cell death. However, ROS stress can be controlled by ROS-scavenging systems. Generally, the ROS-scavenging systems are divided into enzymatic and nonenzymatic mechanisms [1]. Enzymatic ROS-scavenging mechanisms include a battery of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (PRX), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and dehydroascorbatreductase (DHR) [1]. Antioxidant enzymes exist in multiple isoforms. For instance, SODs are classified into three
groups based on the metal cofactors: iron SOD (Fe-SOD) is predominantly localized in the chloroplast, manganese SOD (Mn-SOD) is localized in the mitochondria and peroxisomes, and copper/zinc SOD (Cu/Zn-SOD) is localized in the cytosol and chloroplast [13–15]. Non-enzymatic ROS scavenging mechanisms consist of soluble molecules which can be oxidized by ROS. Glutathione (GSH) and ascorbate (ASC) are the major non-enzymatic systems by which H₂O₂ can be reduced to H₂O and O₂ [16]. Maintenance of the reduced and oxidized glutathione ratio (GSH/GSSG) is critical for ROS homeostasis. In contrast to their potentially harmful effects, ROS play an important role in regulating plant growth and development, and response to biotic or abiotic stresses. It has long been observed that a virulent pathogens, successfully recognized by plants via R proteins, induce a biphasic ROS production with a low-amplitude and transient first phase (ROS burst) followed by a sustained phase of much higher magnitude [17,18]. In abiotic stress, the biphasic increases of ROS production can also be observed, for instance, during ozone or wounding stress [18,19]. It has been demonstrated that, in particular tissues of Arabidopsis thaliana, cold, heat, high light, wounding, or salinity triggers AtRbohD-dependent ROS production. This local production of ROS can initiate rapid and long-distance signals traveled from the affected tissue to the entire plant, which has been shown to be accompanied by apoplastic ROS production and depends on AtRbohD [20]. Further research indicates a model that ROS induced ROS release (termed the ROS wave [21]), coupled with calcium signaling and other systemic signals (e.g. abscisic acid (ABA), hydraulic waves, and electric signals), plays an important role in abiotic stresses [22–24]. Furthermore, in response to Pseudomonas syringae pathovar tomato (Pst) DC3000, it was demonstrated that AtRbohD could also facilitate a long-distance signal for activation of defense response at distal sites [25].

The mitogen-activated protein kinase (MAPK or MPK) cascade is an intracellular pathway conserved among eukaryotes. A MAPK cascade is composed of three kinases, MAPK, MAPK kinase (MKK), and MAPKK kinases (MAPKKK or MEKK) [26]. The three kinases form the basic module of a MAPK cascade, MAPKKK-MKK-MAPK, in which MAPKK phosphorylates and activates MKK, and the activated MKK phosphorylates and activates MAPK [27]. MAPK cascades are involved in a many aspects of plant physiology, including cell division [28], plant growth and development [29], plant resistance to pathogens [30] and insect herbivores [31], and plant response to abiotic stresses [32,33].

It has been observed that, on one hand, exogenous application of H₂O₂ or ozone activates components of MAPK cascades, and initiation of ROS signaling can lead to changes in MAPK cascades (Table 1; [34,35]), on the other hand, manipulating MAPK cascade results in initiation of ROS responses (Table 2; [34,35]). These observations indicate that MAPK cascades are complicated in both ROS signaling and responses.

2. Activation and regulation of MAPK cascades by ROS

Experimental results indicate that O₂ deprivation leads to mitochondria-dependent ROS production in A. thaliana [36]. Under O₂ deprivation, as well as application of cell respiration inhibitors anti-mycin A (AA) or potassium cyanide (KCN), resulted in ROS production and transient activation of AtMPK6 and AtMPK3 [36]. The authors suggested that low O₂ stimulates mitochondrial ROS that moves to the cytoplasm to activate MAPKs, leading to retrograde signaling between mitochondria and nucleus. As the authors point out, however, the O₂ deprivation treatment also removes carbon dioxide. In addition, the inhibitors AA and KCN may affect photosynthesis. Therefore, it cannot be excluded that, in green leaves, signaling from the chloroplast may also participate in activating AtMPK6 and AtMPK3. Recently, it has been shown that, when A. thaliana plants shifted from low-light to high-light 8–800 μmol quanta m⁻² s⁻¹), AtMPK6 can be activated in retrograde signaling between chloroplast and nucleus. In this signaling, AtMPK6 regulates gene expression of several key transcriptional factors, e.g. ERF6 and ERF104, possibly prior to H₂O₂ accumulation [37].

Exogenous application of H₂O₂ has been shown to activate components of MAPK cascade (Table 1). In A. thaliana, AtMPK6 and AtMPK3 can be activated by various concentration of H₂O₂. Experiments in protoplasts showed that activation of AtMPK6 and AtMPK3 requires ANP1 (a MAPKKK) that itself can be activated by H₂O₂ [38], and AtMKK4-AtMPk6 and AtMKK3-AtMPK7 may be two pathways involved in H₂O₂ responses [39]. H₂O₂ also activates an oxidative signal inducible1 (OX1) kinase that is required for full activation of AtMPK6 and AtMPK3 [40]. Exposure of acute ozone (high dose within a short time frame, e.g. 250–700 nL⁻¹ within 24 h) activates AtMPK3/AtMPK6 in A. thaliana [41] and SIPK/WIPK in tobacco [42], which has been shown to be important for ozone sensitivity.

One way that changes in apoplastic redox status trigger intracellular signaling is likely by H₂O₂-H₂O₂ has the longest lifetime among the various ROS and can be transported across the plasma membrane [43]. Although H₂O₂ could simply diffuse across the plasma membrane, it is suggested that H₂O₂ may preferentially enter the cell by aquaporin channels [44–46]. This regulated entry may provide another way of adjusting local concentrations of H₂O₂. If H₂O₂ can be channeled to an intended intracellular compartment, it is speculated that this could provide a layer of specificity in ROS signaling. Inside the cell, while it is possible that H₂O₂ may initiate MAPK cascades directly, it is indicated that the prominent role of H₂O₂ is to disturb ROS homeostasis via a redox relay mechanism [47]. As a result of these disturbances, intracellular levels of ROS may rapidly rise, transferring the oxidation to target MAPK cascades. Indeed, this mechanism has been demonstrated in mammalian cells-H₂O₂-induced oxidation of thioredoxin (Trx) leads to the conformational change of Trx and disruption of interaction between Trx and apoptosis signal-regulating kinase 1 (ASK1), resulting in ASK1 activation and subsequent phosphorylation of its substrate p38 MAPK [48]. In plants, activation of MAPKKks, e.g. ANP1, OMTK, or MEKK1, by H₂O₂ has been observed (Table 1). It remains to be seen if a redox sensor could activate the MAPKK in a redox-dependent manner. In Nicotiana tabacum (tobacco), recent study showed that infiltration of either reduced glutathione (GSH) or the oxidized form (GSSG) could activate SIPK and WIPK [49]. Transgenic tobacco expressing the bifunctional glutathione biosynthetic enzyme from Streptococcus thermophilus (StGCl-GS) showed increased levels of glutathione accumulation and displayed activation of SIPK and to a lesser extent of WIPK [49]. These suggest that the oxidative shift in cytosolic redox potential could activate MAPK signaling (Fig. 1).

H₂O₂ may also activate MAPK by inactivating the MAPK repressors. Arabidopsis genome encodes five dual-specificity MAPK phosphatases (MKPs), dual-specificity protein tyrosine phosphatase 1 (AtDpsPtp1), MAP kinase phosphatase 2 (AtMKP2), indole-3-butyric acid response 5 (IBR5), propyzamide hypersensitive 1 (PHS1), and MAP kinase phosphatase 1 (AtMKP1) [50,51]. Among these, DsPtp1 [52], AtMKP1 [53,54], and AtMKP2 [55] have been implicated in ROS signaling and response. In the Wassilewskija (WS) ecotype of A. thaliana, atmkp1 mutants produce higher ROS in response to etiolation (synthetic polypeptide that corresponds to the bacterial PAMP elongation factor-Tu, EF-Tu; [53]). AtMKP2-RNAi plants are hypersensitive to ozone treatment and display significantly prolonged AtMPK3 and AtMPK6 activation [55]. Over-accumulation of H₂O₂ or O₂⁻ was observed in response to methyl viologen (MV) in atmkp2 mutants [56]. Furthermore, it has been shown that both AtDpsPtp1 and AtPtp1 (one of the Tyr phosphatases) could dephosphorylate and inactivate MAPKs [52,57]. The activity of AtPtps and AtDpsPtps requires a highly conserved Cys residue: Cys265 in AtPtp1 [57] and Cys135 in AtDsPtp1 [52]. Particularly, the inactivation of AtMPK6 by AtPtp1 in vitro and in vivo is negatively influenced by H₂O₂ treatment, suggesting that AtPtp1 may act as a redox sensor and H₂O₂ may...
shown to be activated by H2O2 and to undergo H2O2-dependent sulfenylation [59]. Reduced glutathione; GST Reduced glutathione; GSSG Oxidized glutathione; StGCL-GS Bifunctional enzyme for glutathione synthesis in Streptococcus thermophiles; VIGS Virus-induced gene silencing.

There are also experiments that indicate a mechanism that ROS may directly act on the MAPK protein. A recent study of proteins with cysteine thiol oxidized to a sulfenic acid showed that AtMPK2, AtMPK3, and AtMPK7 undergo H2O2-dependent sulfenylation [59]. Recent studies of proteins with oxidized cysteine [64]. Therefore, it is possible that MAPKs can be modified by Cys oxidation, resulting in changes to their aggregation, stability, or interaction with other proteins; but activation solely depends on phosphorylation by upstream MKK.

In addition, apoplastic ROS are involved in cell wall integrity (CWI) signaling [65]. Potential CWI sensors include the members of wall-associated kinases (WAKs) and Catharanthus roseus RLK1-like kinases (CrRLK1Ls). It is possible that apoplastic ROS may activate MAPK signaling by CWI sensors, e.g. in cell wall metabolism and development [65,66]. Apoplastic ROS may also directly change the activity of extracellular proteins by redox-dependent modifications [67]. In response to ozone, it is hypothesized that cysteine-rich receptor-like kinases (CRKs) are good candidates for relaying extracellular ROS signaling to intracellular MAPK activation [8] (Fig. 1).

### 3. Regulation of ROS-related genes by MAPK cascades

In *A. thaliana*, MEKK1–AtMKK1/AtMKK2–AtMPK4 cascade is involved in ROS homeostasis. Transcriptional analysis showed that some redox-related genes are misexpressed in *mekk1* or *atmpk4* mutants [62,63]. The microarray analysis concerning MEKK1–AtMKK1/AtMKK2–AtMPK4 cascade revealed that, on one hand, there is a great deal of overlap among ROS-related genes in *mekk1*, *atmpk4*, *atmpk6*, and *atmpk4* mutants. On the other hand, some ROS-related genes, e.g. *CSD1* (encoding Cu/Zn-SOD1), *CSD2* (encoding Fe-SODs), and *APX3* (encoding APX1), are differentially expressed between *mekk1*, *atmpk1/atmpk2*, and *atmpk4* mutants [68]. Among the genes encoding ROS scavenging enzymes that are crucial for redox homeostasis, *CAT1* expression is down-regulated in *mekk1* and *atmpk4* mutants, but unaffected in *atmpk1/atmpk2* double mutants. Furthermore, the steady-state transcripts of *CAT1* and *CAT3* are up-regulated in *mekk1* mutants and in *atmpk1/atmpk2* double mutants, but not in *atmpk4* mutants [68]. These results suggest that, in regulating ROS scavenging genes, MEKK1, AtMPK4, or AtMKK1/AtMKK2 may function independently of the MEKK1–AtMKK1/AtMKK2–AtMPK4 cascade in response to specific ROS signaling or in different cell-compartments. Furthermore, in *atmpk4* mutants or *atmpk4/ics1* double mutants, it was shown that most of the analyzed genes encoding ROS scavenging enzymes, e.g. *GSR2* (encoding glutathione reductase-2), *FSDs* (encoding Fe-SODs), and *APXs* are down-regulated, whereas *CSD2* can be up-regulated [69]. The enzyme analysis demonstrated Cu/Zn-SOD and APX activities were increased whereas Fe-SOD activity was decreased in *atmpk4* mutants or *atmpk4/ics1* double mutants [69] (Fig. 2).

### Table 1

| Plant species          | H2O2 Application or ROS Alteration | Activation or Responses of MAPK Signaling | Reference(s) |
|------------------------|-----------------------------------|------------------------------------------|--------------|
| *Arabidopsis thaliana* | 2 mM H2O2                         | MEKK1                                    | Nakagami et al. [61] |
| *Arabidopsis thaliana* | 200 μM H2O2                       | ANP1 (MAPKKK)                            | Kotturi et al. [38] |
| *Arabidopsis thaliana* | 4 mM H2O2                         | AtMKK4-AtMPK6                            | Doczi et al. [39] |
| *Arabidopsis thaliana* | 5 mM H2O2                         | AtMPK1/AtMPK2                            | Ortiz-Masia et al. [61] |
| *Arabidopsis thaliana* | 200 μM, 1 mM, 2 mM, 4 mM, 10 mM, or 20 mM H2O2 | AtMPK3/AtMPK6 | Desikan et al. [60]; Gupta and Luan [58]; Kim et al. [172]; Kotturi et al. [38]; Moon et al. [173]; Nakagami et al. [63]; Rentel et al. [40]; Wang et al. [167]; Yuasa et al. [174] |

*GSH* Reduced glutathione; *GSSG* Oxidized glutathione; *StGCL-GS* Bifunctional enzyme for glutathione synthesis in *Streptococcus thermophilus*; *VIGS* Virus-induced gene silencing.
| Plant species | Alteration of MAPK signaling | ROS responses | Reference(s) |
|---------------|-----------------------------|---------------|--------------|
| Arabidopsis thaliana | amp2/amp3 mutant (WS) | Increasing O$_2^•−$ production; Inhibiting H$_2$O$_2$ production in response to OGs or elf18; Over-accumulating Fe-SOD1, Mn-SOD, DHAR1, and CPN20 | Savatin et al. [151]; Takac et al. [182] |
| Arabidopsis thaliana | amp1/amp2 or amp1/amp3 mutant (WS); amp1/amp2/amp3 (conditional) mutant | Inhibiting H$_2$O$_2$ production in response to OGs or elf18 | Savatin et al. [151] |
| Arabidopsis thaliana | atmkk1 | Inhibiting CAT1 expression and H$_2$O$_2$ accumulation in response to ABA, drought, and salt stresses | Xing et al. [70] |
| Arabidopsis thaliana | AtMKK1-or AtMPK6-overexpressing | Promoting CAT1 expression and H$_2$O$_2$ accumulation in response to ABA | Xing et al. [70,71] |
| Arabidopsis thaliana | AtMKK5-overexpressing | Promoting CSD1/CSD2 expression under high light stress | Xing et al. [14] |
| Arabidopsis thaliana | AtMKK5-RNAi | Hypersensitive to ozone; Inhibiting CSD1/CSD2 expression under high light stress; Inhibiting salt-induced FSD2/FSD3 expression | Miles et al. [183]; Xing et al. [14]; Xing et al. [15] |
| Arabidopsis thaliana | atmpk1 mutant (WS) | Enhancing ROS production in response to elf26 | Anderson et al. [53] |
| Arabidopsis thaliana | atmpk2 mutant | Over-accumulating H$_2$O$_2$ or O$_2^•−$ in response to MV | Lumbrañas et al. [56] |
| Arabidopsis thaliana | AtMPK2-RNAi | Hypersensitive to ozone | Lee and Ellis [55] |
| Arabidopsis thaliana | AtMPK6-RNAi | Hypersensitive to ozone; Over-accumulating antioxidant proteins in response to ozone | Miles et al. [184]; Miles et al. [119] |
| Arabidopsis thaliana | atmPK43 mutant | Suppressing H$_2$O$_2$ accumulation in SIT1-overexpressing seedlings | Li et al. [185] |
| Arabidopsis thaliana | atmPK6 mutant | Inhibiting H$_2$O$_2$-induced NO generation; Inhibiting CAT1 expression and H$_2$O$_2$ accumulation in response to ABA; Promoting primary root elongation in response to H$_2$O$_2$; Suppressing H$_2$O$_2$ accumulation in SIT1-overexpressing seedlings | Han et al. [186]; Li et al. [185]; Wang et al. [167]; Xing et al. [71] |
| Arabidopsis thaliana | atmPK8 mutant | Over-expression of AtRbohD and OXI1, and over-accumulating H$_2$O$_2$ in response to wounding | Takahashi et al. [84] |
| Arabidopsis thaliana | AtMPK9-GFP-overexpressing | Decreasing H$_2$O$_2$ accumulation in response to wounding | Takahashi et al. [84] |
| Arabidopsis thaliana | atmPK9/atmPK12 mutant | Blocking H$_2$O$_2$-induced stomatal closure | Jammes et al. [159] |
| Arabidopsis thaliana | DEX-inducible AtMPK2-RNAi | Hypersensitive to ozone | Lee and Ellis [55] |
| Arabidopsis thaliana | Guard cell-specific AtMKP3-RNAi | Inhibiting stomatal response to H$_2$O$_2$ | Godesblat et al. [153] |
| Arabidopsis thaliana | mekk1, atmkk1/atmkk2, or atmkk4 mutant | Accumulating H$_2$O$_2$; Alteration of ROS-related genes | Björnsson et al. [73]; Gao et al. [105]; Gasromski et al. [69]; Ichimura et al. [62]; Nakagami et al. [63]; Pitzschke et al. [68] |
| Arabidopsis thaliana | mkkk20 mutant | Over-accumulating H$_2$O$_2$ in response to salt stress | Kim et al. [172] |
| Arabidopsis thaliana | Overexpressing DEX-inducible NmMEK2/DD, AtMKK4/DD, or AtMKK5/DD | Over-accumulating H$_2$O$_2$ and HR-like cell death | Ren et al. [98] |
| Arabidopsis thaliana | MKK2/DD-overexpressing | Inhibiting ROS production in response to fgl22 | Mithoe et al. [165] |
| Arabidopsis thaliana | Pd98059 treatment | Inhibiting G/GO-induced gtl1 and pdl1 expression | Grant et al. [18,187] |
| Glycine max | GmMPK4-VIGS | Accumulating H$_2$O$_2$ | Liu et al. [188] |
| Nicotiana attenuata | NaMPK4-RNAi | Inhibiting H$_2$O$_2$-induced stomatal closure | Hettenhausen et al. [157] |
| Nicotiana benthamiana | Transient expression of NbMEK1/DD or SMEK2/DD | NbRbohB expression and NbRbohB-dependent ROS generation in response to INF1 or R3a/AVR3a | Adachi et al. [88]; Asai et al. [87]; Yoshioka et al. [86]; Asai et al. [87] |
| Nicotiana benthamiana | SIPK-VIGS; NTF6-VIGS | Reducing NbRbohB expression and NbRbohB-dependent ROS production in response to INF1 | Zhang et al. [64] |
| Nicotiana tabacum | Over-accumulating H$_2$O$_2$ and cell death | | |
| Nicotiana tabacum | NtMPK4-RNAi | Hypersensitive to ozone | Gomi et al. [156] |
| Nicotiana tabacum | OsIRRI-overexpressing | Hypersensitive to H$_2$O$_2$ stress (100 mM) | Li et al. [189] |
| Nicotiana tabacum | Overexpressing DEX-inducible NmMEK2/DD | Promoting chloroplastic H$_2$O$_2$ production under light and HR-like cell death | Liu et al. [103] |
| Nicotiana tabacum | SIPK-overexpressing | Reducing H$_2$O$_2$ accumulation in response to harpin | Samuel et al. [99] |
| Nicotiana tabacum | SIPK-overexpressing or SIPK-RNAi | Hypersensitive to ozone | Samuel and Ellis [118] |
| Nicotiana tabacum | ZnMPK7-overexpressing | Reducing H$_2$O$_2$ accumulation under osmotic stress | Zong et al. [190] |
| Nicotiana tabacum | SIPK-RNAi | Over-accumulating H$_2$O$_2$ in response to harpin | Samuel et al. [99] |
| Solanum | Smpk1/Smpk2-VIGS; Smpk2-VIGS | Reducing H$_2$O$_2$ accumulation in response to BR | Nie et al. [179] |

(continued on next page)
MEKK1 inhibits the rapid expression of stress-responsive genes possessing CGCGTT in wounding [73]. Pharmacologically, PD98059 (a broad-spectrum inhibitor of MEKK) can inhibit the activity peak and can also delay the peak time of RSRE response to flg22 (a 22-amino acid synthetic polypeptide that corresponds to a highly conserved epitope of the Pseudomonas PAMP flagellin), indicating MAPK cascades may regulate the rapid transcription of genes possessing CGCGTT regulatory elements during plant immune responses [73]. The reports also support the idea that RSRE activation is likely regulated by ROS, as (1) the earlier peak time of RSRE is consistent with the over-accumulation of ROS in mekk1-5 mutants; and (2) MV-induced 1O2 could activate RSRE [73].

In A. thaliana, several transcription factors have been identified that act downstream of MAPKs in ROS responses. These include the MYB-transcription factor 44 (MYBB4; [74,75]), the heat shock transcription factor A4a (HSFA4A; [76]), and the ethylene response factor 6 (ERF6; [77,78]). Particularly, AtMPK6-ERF6 have been demonstrated to regulate ROS-responsive gene via GCC box (AGCGGCC) in response to biotic and abiotic stresses [77,78]. Interestingly, AtMPK6-ERF6 can also bind the GCC box to regulate Jasmonic acid/ethylene (JA/ET)-responsive genes in response to necrotrophic fungal pathogen Botrytis cinerea [79–82]. It is unclear whether JA/ET and ROS signaling can partly become merged in regulating genes with GCC box in the promoters, e.g. in response to B. cinerea. In rice, salt-responsive ERF1 (SERF1) is a transcription factor. Expression of SERF1 gene can be induced by salt and H2O2 treatment. Analysis of SERF1 loss-of-function plants showed that SERF1-dependent genes are H2O2 responsive. Further analysis indicated that SERF1 is a substrate of MAPK5 (OsMPK5). SERF1 regulates H2O2-responsive genes, e.g. MAP3K6, MAPK5, DREB2A, ZFP179, and SERF1, by DREB-specific cis-element GCCGC or ACCGCC [83] (Fig. 2).

Besides regulating H2O2-responsive genes, MAPK signaling has also been implicated in regulating genes encoding Rboh in ROS production. In wounding response, AtMPK8 negatively regulates AtRbohD expression. In atmpk8 mutants, AtRbohD expression was higher compared with wild type. Further analysis showed that AtMPK8 negatively regulates ROS accumulation via repressing AtRbohD expression [84]. In response to flg22, AtRbohD is up-regulated in ERP104-overexpressing plants and ERP104 has been identified to be a substrate of AtMPK6 [85]. In erf6 mutants, it seems that AtRbohD expression can be up-regulated [77]. However, although AtMPK6-ERF6 and AtMPK6-ERP104 have been demonstrated to regulate stress-responsive genes via GCC box, it is unlikely that they directly regulate AtRbohD expression via GCC box, since no GCC box was found in the upstream 2500 bp region of AtRbohD promoter. The precise role of MAPK signaling in regulating AtRbohD expression needs to be elucidated in future studies. In N. benthamiana, NbrbshB gene, the orthologue of Arabidopsis AtRbohD, was shown to be important for ROS production in immunity. It has been shown that NbrbshB expression was induced by MAPK signaling (possibly by NbrMKK2-SIPK and NbrMKK1-NTF6 cascades) in response to INF1, the elicitor produced by the oomycete Phytophthora species [86,87]. The recent discovery demonstrated that NbrMKK2-mediated MAPK signaling regulates cis-element (TGGGTCAACAC) of the NbrbshB promoter by phosphorylating WRKYs (e.g. WRK9/WRK10/WRK11) in response to INF1 or R3a/AVR3a (the potato R protein/oomycete effector pair) but not flg22 [88] (Fig. 2). In addition, maize MAPK5 (ZmMPK6-2) is one of the orthologues of Arabidopsis AtMPK6 [89,90]. Based on pharmacological evidence, it is proposed that the expression of ZmRboh genes could be regulated by MAPK5 in maize leaves in response to ABA, H2O2, and brassinosteroids [91,92].

The mechanisms by which MAPK signaling controls gene expression remain to be elucidated. Experimental data showed that components of MAPK cascade can be detected in the nucleus [93]. AtMPK4 [94], AtMPK6 [85], and SIPK [95] have been implicated in ROS signaling and responses. In addition, in the early stages of O3 treatment, it is indicated that activated AtMPK3 and AtMPK6 are translocated to the nucleus where they are thought to phosphorylate substrates and regulate gene expression [41]. Furthermore, AtMPK3/AtMPK6 can phosphorylate ERF6. The stabilization and accumulation of ERF6 were reported to initiate transcriptions of stress genes [82]. It is possible that the mechanisms underlying ROS-related gene expression by MAPK cascades might be different in response to different types of stimuli or depending on the physiological context. ROS may directly regulate transcription factors, e.g. H2O2 can regulate transcription.
Fig. 2. Putative regulation of ROS-related genes by MAPK cascades. Under salt stress, rice MAPK5 (OsMPK6) regulates H₂O₂-responsive genes by DREB-specific cis-element GCCGAC or ACCGAC. ERF6 and ERF104 are two substrates of AtMPK6 and can regulate stress-responsive genes via GCC box. Gene expression of ROS scavenging enzymes are changed in atrmpk4 (under high light or salt stress) or atrmpk4 mutants. Defective MEKK1-AtMKK1/AtMKK2-AtMPK4 cascade affects ROS homeostasis. MEKK1 pathway may regulate stress-responsive genes possessing cis-element CCGGTG in wounding by ROS signaling. In N. benthamiana, NtMKK2-mediated MAPK signaling regulate cis-element (TTTGGTCAAAC) of the NbRbohB promoter by phosphorylating WRKYs (e.g. WRKY7/WRKY8/WRKY9/WRKY11) in response to INFI or Rla/AVR3a.

Factors with redox-sensitive Cys residue [96]. The regulation of transcription factors by ROS may influence the conformational changes, nucleocytoplasmic shuttling, proteolysis, or interaction with other regulators [96]. Although it has not been demonstrated, it is possible that some MAPK substrates may be regulated by ROS directly. In this scenario, ROS may lead to simultaneous multisite modifications of the same pathway, which may guarantee efficiency or specificity of signaling transduction.

4. Induction of cell death by ROS and MAPK cascades

4.1. ROS and MAPK cascades in HR-like cell death

The incompatible plant–pathogen interaction can induce plant programmed cell death (PCD) at the infection sites known as hypersensitive response (HR) [97]. HR is often characterized by discrete cellular lesions and preceded by an ROS burst. HR and the accompanying ROS is thought to act against hemi-biologic and biotic pathogens that obtain their nutrition from living host cells. Initiation of HR may further lead to activation of systemic acquired resistance to avoid the death of the entire plant.

Previous studies revealed that transgenic A. thaliana with inducible AtMKK4DD (the constitutively active mutants of AtMKK4 protein) or AtMKK5DD exhibit cell death when AtMKK4DD or AtMKK5DD are induced. The cell death is similar when an HR is induced by infection by the bacterial pathogen Pst DC3000 [98]. The HR-like cell death is preceded by H₂O₂ generation and AtMPK3/AtMPK6 activation. In tobacco leaves, transient expression and induction of AtMKK4DD, AtMKK5DD, or NtMKK2DD also leads to PCD with prolonged activation of SIPK/WIPK, the orthologues of AtMPK6/AtMPK3. There are also reports that in N. tabacum and N. benthamiana, SIPK-mediated MAPK cascade regulate harpin-induced cell death [99] and INFI-induced cell death [100]. Furthermore, Zhang et al., 2012a showed that many bacterial, fungal, and oomycete factors secrete necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLP) that trigger PCD. Interestingly, the MAPKKKα-NhMKK2-WIPK cascade might regulate Nep1Mo-activated H₂O₂ accumulation and contribute to Nep1Mo-triggered cell death, as virus-induced gene silencing WIPK but not SIPK affects Nep1Mo-induced H₂O₂ accumulation [101].

The relationship between ROS and AtMPK6/AtMPK3 (SIPK/WIPK) remains elusive. It is suggested that harpin-induced ROS accumulation is required for activation of SIPK and WIPK [99]. In another study, however, it was shown that activation of the SIPK/Net4/ WIPK cascade by pathogens Phytophthora cryptogea promotes the ROS generation in chloroplasts, which plays an important role in executing HR-like cell death [102]. Further studies indicated a rapid inhibition of photosynthetic CO₂ fixation by AtMKK2DD-induced SIPK/Nt4/WIPK activation, leading to the generation of ROS in chloroplasts under illumination [103]. In the dark, cell death is only delayed but not blocked [103]. Therefore, it seems that the chloroplast is not the sole organelle to execute HR-like cell death. It has been shown that SIPK/WIPK function downstream of HSP90 to transduce the cell death signal to mitochondria in TMV resistance gene N-mediated HR-like cell death [104].

4.2. ROS and MAPK cascades in spontaneous cell death

Genetically, MEKK1-AtMKK1/AtMKK2-AtMPK4 cascade has been demonstrated to negatively regulate H₂O₂ accumulation and spontaneous cell death [62,63,105]. A nucleotide binding leucine-rich repeat (NB-LRR) protein, suppressor of mkkl mkk2 2 (SUMM2), acts as genetic repressor of MEKK1-AtMKK1/AtMKK2-AtMPK4 cascade [106]. It is assumed that another repressor of MEKK1-AtMKK1/AtMKK2-AtMPK4 cascade, SUMM1 (encoding MEKK2), triggers SUMM2-mediated responses, although it is unclear whether SUMM1 could initiate a MAPK cascade [107]. Therefore, H₂O₂ accumulation and spontaneous cell death in a defective MEKK1-AtMKK1/AtMKK2-AtMPK4 cascade may result from the disinhibition of SUMM1 and SUMM2. Shih-Heng Su et al. 2013 further showed that upregulation of MEKK2 RNA abundance is required for the response of defect in MEKK1-AtMKK1/AtMKK2-AtMPK4 cascade [108]. Recently, two substrates, protein associated with topoisomerase II 1 (PAT1; [109]) and Arabidopsis SH4-related 3 (ASR3; [110]), were demonstrated to act downstream of AtMPK4 to negatively regulate gene expression in response to flg22. However, linking AtMPK4 to MEKK2 expression in spontaneous cell death remains elusive.

L-myoinositol 1-phosphate synthase 1 (MIPS1) is the rate-limiting enzyme in myo-inositol biosynthesis. Mutation in MIPS1 results in spontaneous cell death [111,112]. It is possible that MIPS1 controls its own transcription by the AtMPK4 pathway, as MIPS1 expression is down-regulated in atrmpk4 mutant [113]. ROS have been involved in the spontaneous cell death in mips1 mutants. For instance, mips1 mutants show increased sensitivity to ROS stress and reduced ascorbic acid levels [112], and a decrease in myo-inositol accumulation is necessary for the spontaneous cell death in catalase-deficient cat2 mutants [114,115]. Furthermore, the cleavage and polyadenylation
specificity factor 30 (CPSF30), encoding a polyadenylation factor subunit homolog, is required for spontaneous cell death in mips1 or atm6 mutants [116]. It was shown that CPSF30 is a general controller of the salicylic acid (SA) pathway rather than a direct regulator of ROS production or scavenging in the spontaneous cell death [116]. However, in the process of spontaneous cell death, CPSF30 may be connected with ROS signaling and responses by unknown mechanisms, as CPSF30 mutant is more tolerant than wild type in ROS stress [117].

4.3. ROS and MAPK cascades in acute ozone-induced PCD

Exposure to acute ozone can induce ROS burst leading to cell death that shares similarities with the HR [19,118,119]. Acute ozone induces MAPK cascades (Table 1). Genetic manipulation of MAPK cascades affects the cell death phenotype observed in O₃ responses (Table 2). Mutations of radical-induced cell death1 (rcd1) show more ozone-sensitive and prolonged activation of AtMPK3/AtMPK6 compared with the wild-type plants [120], indicating that prolonged activation of MAPK signaling may participate in the induction of cell death process in response to acute ozone.

It is possible that regulation of Rboh-dependent ROS production is the initial step in ozone signaling. It was reported that, in A. thaliana, exposure of acute ozone induced biphasic ROS generation that is associated with the pathogen response [19]. Recent reports showed that in the JA receptor mutant coil-16, ozone-induced cell death was suppressed by impairment of AtRbohF [121]. This observation is reminiscent of reports showing that AtRbohD/AtRbohF-dependent ROS can suppress SA-dependent HR in cells around infection sites in response to Pst DC3000, with AtRbohD having a prominent role [122].

4.4. ROS and MAPK cascades in fumonisin B1- and sphingolipid-induced PCD

Fumonisin B1 (FB1) is a specific inhibitor of ceramide synthase. FB1 application can generate an accumulation of LCBS and it has been used to investigate the functions of sphingolipid metabolism in plants [123,124]. FB1-induced cell death in A. thaliana is associated with ROS production, callose deposition, and PR gene expression, which is similar to HR [125]. The mutant fumonisin B1 resistant1-1 (br1-1) is defective in a long-chain base I subunit of serine palmitoyltransferase and is compromised in ROS generation and subsequent induction of PCD when challenged by FB1 [123]. Direct feeding experiments showed that dihydrospingosine (d18:0), phytosphingosine (t18:0), and sphingosine (d18:1) can induce ROS generation followed by cell death, whereas the phosphorylated form of d18:0 (d18:0-P, dihydro-sphingosine-1-phosphate) could block the ROS generation and PCD, indicating that homeostasis of free LCBS and its phosphorylated form may determine the cell fate [123]. AtMPK6 plays a regulatory role in PCD induced by LCBS and FB1 [124]. In the atmpk6 seedling, exogenous application of d18:0 did not lead to severe PCD. The PCD phenotype result from the increase of endogenous LCBS induced by FB1 was attenuated in atmpk6 seedlings [124]. In another study, it is reported that t18:0-P rapidly and transiently formed in A. thaliana upon chilling (4 °C). The activity of AtMPK6 (0–30 min) was stimulated by exogenous t18:0-P [126]. The authors showed that d18:0-P or t18:0 cannot induce AtMPK6 activity at 22 °C or 4 °C, in contrast with the observations that unphosphorylated LCBI t18:0 can trigger AtMPK6 activation [124], possibly reflecting differences in the biological material used and in the treatment procedure. It is speculated that AtMPK6 acts downstream of homeostasis between free LCBS and the phosphorylated derivatives. Activation of AtMPK6 may lead to response to chilling stress in the presence of elevated t18:0-P. However, the prolonged activation of AtMPK6 may promote ROS accumulation and PCD in the presence of elevated d18:0.

4.5. ROS and MAPK cascades in self-incompatibility-induced PCD

Self-incompatibility (SI) is a genetic mechanism used by many angiosperm species to prevent inbreeding. Studies in Pseudomonas aeruginosa showed that recognition of incompatible pollen can trigger signaling to initiate PCD in pollen tubes. This signaling includes activation of a 56 kDa MAPK [127,128]. The activation of 56 kDa MAPK was shown at 5 min, peaking at 10 min, and lasting up to 30 min after SI induction [127]. Because around 10 min after SI induction may play a pivotal role in determining irreversible PCD of incompatible pollen [127–129], it seems that a MAPK cascade is involved in initiating SI-induced PCD. Increases in ROS, however, can be detected at 2 min and can last up to 15 min, indicating that ROS signaling acts upstream of the 56 kDa MAPK activation [128,129]. The possible role of the 56 kDa MAPK cascade is to stimulate nitric oxide (NO) signaling to activate caspase-3-like activity, as increases in NO occurred at 15–40 min after SI induction and pharmacological application of U0126 (the specific MKK inhibitor) can prevent activation of caspase-3-like activity [128,129].

5. Rboh-dependent ROS production and MAPK cascades in PAMP-triggered immunity

Plants use two layers of immune systems to defend against pathogens. The first layer is based on perception of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) in the cell surface, leading to the PAMP triggered immunity (PTI). The second layer is based on recognition of microbial effectors secreted in cells by resistance (R) proteins, leading to the effector triggered immunity (ETI; [130]). In A. thaliana, perception of bacterial PAMPs flagellin and EF-Tu by the corresponding PRRs, flagellin-sensing 2 (FLS2) and EF-Tu receptor (EFR), activates early common responses such as ROS production and MAPK activation [131] and references therein). In response to flg22 or Pst DC3000, the rapid and transient ROS production (about 0–30 min) is dependent on AtRbohD [132–135], one of the ten Rboh proteins in A. thaliana [9]. Quantitative phosphoproteomic analysis showed that phosphorylation of AtRbohD plays an important role in AtRbohD-dependent ROS production [132,136]. Recent reports demonstrated that the membrane-anchored Botrytis-induced kinase 1 (BKI1), a multifunctional receptor-like cytoplasmic kinase, directly regulates AtRbohD-dependent ROS production in response to flg22 or elf18 [137,138].

In response to flg22 or elf18, the kinetics of AtMPK3/AtMPK6 activation is similar to that of AtRBOHD-dependent ROS production (peaks within 5–10 min and diminishes after 30 min; as illustrated in Fig. 3 and references therein). However, accumulating evidence indicates that PAMP-activated AtMPK3/AtMPK6 may occur independently of the PAMP-triggered AtRbohD-dependent ROS production. Genomic screen and genome-wide gene expression profiling indicated that calcium-dependent protein kinases (CPKs) and MAPKs are probably activated independently downstream of the FLS2 receptor. CPKs, but not MPKs, may play a role in regulating ROS production in response to flg22 [139]. AtMPK3/AtMPK6 activation by flg22 (within 15 min) in seedlings of atrbohD mutants is comparable with wild-type [140]. Reciprocally, neither atmpk3 nor atmpk6 mutants show short flg22-triggered ROS production (0–40 min; [141]). Overexpression of the phosphatase AP2C1 could dephosphorylate and inactivate AtMPK3 and AtMPK6, but no alternation of ROS (0–48 min) was detected in two independent lines in response to flg22 [142]. Using conditional loss-of-function atmpk3/atmpk6 double mutants, Xu et al., 2014 showed that the flg22-triggered ROS production (0–30 min) in wild-type plants and atmpk3/atmpk6 double mutants is similar [143]. Furthermore, in L-type lectin receptor kinase-VL2 (lecrk-VL2–1) mutant, activation of AtMPK3/AtMPK6 was reduced, but BIK1 phosphorylation and ROS production were not affected [144]. In detiolated 3 (det3) mutants, flg22-induced ROS was reduced, whereas flg22-induced AtMPK3/AtMPK6 activation was enhanced [145].
addition, results from *N. benthamiana* also showed that SIPK and WIPK are dispensable for ROS production in response to flg22 [146]. It seems that AtMPK3/AtMPK6 activation by elf18 is also dispensable for elf18-induced ROS production. Normal function of EFR requires endoplasmic reticulum (ER) quality control systems. Signaling defects in ER quality control of EFR biogenesis affect the full ROS production in response to elf18 [147–150]. In *psl1-1* (encoding calreticulin3 in ER quality control signaling) mutants, elf18-induced ROS production (0–40 min) is retained at intermediate levels, whereas MAPK activation is much reduced [147,150]. In *psl5–1* (encoding α-subunits of endoplasmic reticulum resident glucosidase II) mutants, elf18-induced ROS production (0–30 min) is strongly reduced below detectable levels, whereas an increase of MAPK activity is observed [148]. In the *Wassilewskija* (WS) ecotype *A. thaliana*, elf26-triggered ROS production (30 min) is not reduced in both *atmpk6* mutants and *atmpk1/atmpk6* (WS) double mutants [53]. Recently, it has been shown that in *anp2anp3* (WS) double mutants or β-estradiol-inducible *anp1anp2anp3* (WS) conditional triple mutants, elf18-induced production of ROS was compromised but activation of AtMPK3/AtMPK6 was normal [151]. Collectively, these data demonstrate that, in the early stage of PTI (about the first 30 min), PAMP-activated AtMPK3/AtMPK6 may occur independent of the PAMP-triggered ROS production.

In PTI, AtMPK4 can be activated by flg22, and it is assumed that AtMPK4 pathway positively regulates PTI [106]. The role of AtMPK4 in regulating ROS production in PTI is unclear. Experimental results suggest that flg22-induced ROS production was enhanced in *atmpk4* mutants [143], and *A. thaliana* expressing a constitutively active AtMPK4 showed a reduced ROS production in response to flg22 [152]. These results indicate that AtMPK4 negatively regulates ROS production in PTI. In another study, Kong et al. identified that the function of AtMPK4 is dependent on MEKK2. ROS production induced by flg22 was comparable in wild type and *mekk2* mutants [107], indicating that disruption of AtMPK4-MEKK2 signaling pathway may not change AtRbohD-dependent ROS production in PTI. It is possible that mutations in the MEKK2 gene and disinhibition of AtMEKK2 activation (in *atmpk4* mutants) can lead to different conclusions about the role of AtMPK4 in regulating ROS production in PTI.

6. Regulation of stomatal function by MAPK cascades in ROS signaling

MAPK cascades have been shown to play a role in stomatal function in response to H2O2. AtMPK3 antisense plants are less sensitive to exogenous H2O2, both in inhibition of stomatal opening and in promotion of stomatal closure, suggesting a positive role of AtMPK3 in H2O2 signaling in stomata [153] (Fig. 4). AtMPK3 also regulates stomatal closure in response to *Pst* DC3000 [154] or *Xanthomonas campestris* [153,155], likely acting downstream of H2O2 but indepen-
dent of ABA. AtMPK6 is a positive regulator of flg22-triggered stomatal closure [152], although it is not clear if this function is related to H₂O₂.

In the atrbohD mutant, flg22-induced AtMPK3 and AtMPK6 activation is not affected [106,140], suggesting that AtMPK3 and AtMPK6 may not function downstream of AtRbohD-dependent ROS production in stomata. It has been shown that AtMPK6 is not involved in ABA-mediated stomatal closure [154]. In ABA inhibition of stomatal opening, AtMPK6 seems to participate in ABA-activated H₂O₂ production downstream of AtMKK1 [71].

In *N. tabacum*, NtMPK4-silenced plants showed enhanced sensitivity to ozone with impaired regulation of stomatal closure [156]. In *N. attenuate*, silencing of *NaMPK4* gene impaired stomatal closure induced by exogenous H₂O₂ [157]. In seedlings of *A. thaliana*, *AtMPK4* is predominantly expressed in stomata [27,158]. In immunity, AtMPK4 seems does not regulate stomatal closure in response to pathogens, as stomata in *AtMPK4*-overexpressing plants responded normally to *Pst* DC3000 or flg22 [152]. Loss-of-function of *MEKK1* resulted in H₂O₂ accumulation in stomata [62,63], suggesting a role of *MEKK1* in regulating ROS homeostasis in stomata (Fig. 4).

It is reported that the AtMPK9 and AtMPK12 act redundantly as positive regulators of ROS-mediated stomatal closure in response to ABA [159], yeast elicitor [160], or methyl jasmonate [161]. However, AtMPK9 and AtMPK12 may act via a different mechanism in stomata. It has been shown that AtMPK12 but not AtMPK9 can interact with MAPK phosphatase IBR5 [162]. Recent research indicates that AtMPK9 can be activated by autophosphorylation that may be independent of any upstream MAPKKKs or MKKs [163]. Furthermore, *AtMPK12* allele with an amino acid substitution alone could impair the ABA inhibition of opening [164].

### 7. Concluding remarks and perspectives

 ROS production can be either harmful or beneficial to plants. Different types of ROS (H₂O₂, HO·, O₂⁻, or O₂⁻⁻) and subcellular production sites (plastidic, cytosolic, peroxisomal, or apoplastic) may determine the physiological, biochemical, and molecular responses. The concentration of ROS and its interaction with other signaling components may also determine the specificity of ROS response. The complex role of MAPK cascades in ROS signaling and responses has been revealed in regulation of ROS-related genes, executing plant cell death, and modulating stomatal function. How ROS activates MAPK cascades still remains unclear. It is possible that plants not only use MAPK cascades to transduce ROS signaling to gene expression and sometimes cell death, but also initiate the negative feedback regulation by MAPK cascades to maintain ROS homeostasis. The different combinations of the three tiers of kinases, distribution, timepoint-dependent activation, strength, duration, and availability of substrates of MAPK cascades may determine the feed-forward or feed-back outcomes (Fig. 5).

Accumulating evidence indicates that, in *A. thaliana*, *AtMPK3/AtMPK6* activation and AtRbohD-dependent ROS production are two independent pathways in the early stage of PTI (Fig. 3). Most recently, however, it was found that MKKK7, a MAPKKK, attenuates AtMPK6 activity and suppresses ROS production in response to flg22, through direct modulation of the FLS2 complex [165]. In response to wound- ing, AtMPK8 negatively regulates ROS production by repressing AtRbohD expression [84]. These results suggest a complicated relationship between MAPK signaling and AtRboh-dependent ROS production. Furthermore, both MAPK signaling and AtRboh-dependent ROS production have been implicated in O₃ [121]. Further studies are needed to unravel the whole picture about the role of MAPK signaling in Rboh-dependent apoplastic production of ROS.

A large body of research demonstrates that exogenous H₂O₂ can activate MAPK signaling (Table 1). It seems that exogenous application of H₂O₂ alone is not enough to induce the long-distance signaling manifested by AtRbohD-dependent ROS-triggered ROS waves [20,22,24]. Therefore, it is likely that activation of MAPK signaling by exogenous application of H₂O₂ is a relatively localized event. The molecular mechanism by which apoplastic ROS activate intracellular MAPK pathway needs further to be investigated.

Whereas ROS have been implicated in PCD in plant developmental processes and stress responses, the sub-cellular location and the mechanism of ROS generation during PCD are not clear. Compartment-specific ROS signals and communication between compartments may determine the outcome of ROS responses [11]. For instance, disturbance of the ROS homeostasis in chloroplasts, mitochondria, or peroxisomes tends to switch signaling to result in PCD [103,104]. However, Rboh-dependent ROS appear to play a protection role in limiting the spread of PCD, e.g. in response to O₃ or *Pst* DC3000 [121,122]. It is speculated that low concentrations and restricted distribution of ROS mediates the role as signaling components to enhance cell survival and proliferation, whereas high concentrations of ROS (over a certain threshold) throughout the cell mediate the role as a death signal. MAPK cascades may act upstream of chloroplasts, mitochondria, or peroxisomes to regulate ROS homeostasis, or act downstream of these organelles to signal ROS response. In resistance to biotrophic or hemibiotrophic pathogens, MAPK cascades and ROS may elicit the escalated signaling at the interface of plant-pathogen interactions to induce HR that restricts pathogen access to water and nutrients. In response to necrotrophic pathogens or abiotic stresses, however, it is not yet known weather activation of MAPK cascades is simply to amplify the death signal. Furthermore, it remains to be seen how plants can discriminate between pathogens and beneficial microorganisms to initiate different MAPK activation and ROS responses.

PCD is a complex process that involves many signaling pathways. A strong interplay of ROS and MAPK cascades with other signaling molecules exists during plant PCD. It has been suggested that the HR is triggered only by balanced production of NO and ROS [166]. MAPK cascades are also involved in regulating NO signaling [87,167]. ROS and SA act synergistically to drive HR [168], and the phenotype of
spontaneous cell death in defect of MEKK-AtMKK1/2-AtMPK4 cascade result from accumulation of both ROS and SA [105,158]. Ethylene signaling plays an important role in cell death that involves MAPK cascades [169–171]. Furthermore, crosstalk of ROS and MAPK cascades with calcium and lipid signaling fine-tunes the biological responses. The hierarchy of these events remains to be further elucidated. Although many questions still remain unanswered, further research will increase our understanding of the role of MAPK cascades in ROS signaling and responses.

Conflict of interest

No conflicts of interest to declare.

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