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MAPK machinery in plants

Recognition and response to different stresses through multiple signal transduction pathways

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The mitogen-activated protein kinase (MAPK) cascades play diverse roles in intra- and extra-cellular signaling in plants. MAP kinases are the component of kinase modules that transfer information from sensors to responses in eukaryotes including plants. They play a pivotal role in transduction of diverse extracellular stimuli such as biotic and abiotic stresses as well as a range of developmental responses including differentiation, proliferation and death. Several cascades are induced by different biotic and abiotic stress stimuli such as pathogen infections, heavy metal, wounding, high and low temperatures, high salinity, UV radiation, ozone, reactive oxygen species, drought and high or low osmolarity. MAPK signaling has been implicated in biotic stresses and has also been associated with hormonal responses. The cascade is regulated by various mechanisms, including not only transcriptional and translational regulation but through post-transcriptional regulation such as protein-protein interactions. Recent detailed analysis of certain specific MAP kinase pathways have revealed the specificity of the kinases in the cascade, signal transduction patterns, identity of pathway targets and the complexity of the cascade. The latest insights and finding are discussed in this paper in relation to the role of MAPK pathway modules in plant stress signaling.

Introduction

Protein kinases and phosphatases play a central role in signal transduction through the phosphorylation and de-phosphorylation of proteins. This not only leads to the activation of defense responses, but also to the activation of developmental processes like cell growth and differentiation. One of the most commonly studied mechanisms is the mitogen activated protein kinase (MAPK) cascade, comprising a class of protein kinases that plays a crucial role in eukaryotic systems often linking perception of external stimuli with changes in cellular organization or gene expression. MAPK cascades are ancient and conserved signaling cassettes that are found in unicellular and multicellular eukaryotes. Phosphorylation plays a central role in the progression of the signal through the MAP kinase cascade.

MAP Kinase was first identified by Sturgill and Ray[1] as microtubule-associated protein kinase and thus, was christened microtubule-associated protein kinases. MAPK comprise a family of serine/threonine protein kinases involved in the transduction of a variety of extracellular signals and the regulation of different developmental processes. MAPK pathways transfer information from sensors to activate cellular responses in all eukaryotes. A surprisingly large number of genes encoding MAPK pathway components have been uncovered by analyzing model plant genomes, highlighting their important role in signal transduction.[2] Recent investigations have confirmed major roles of defined MAPK pathways in development, cell proliferation and hormone physiology, as well as in biotic and abiotic stress signaling.

MAP Kinase cascades comprise of a series of sub-families, i.e., MAP4K, MAP3K, MAP2K, MAPK, that are sequentially activated.[3,4] The sequential activation of the MAPK cascade eventually results in the activation of transcription factors, phospholipases or cytoskeletal proteins, microtubule-associated proteins and the expression of specific sets of genes in response to environmental stimuli.[5-7] Plant homolog of all components of this cascade have been identified.[8-14] MAPK also activates protein kinases that serve as a MAPK substrate named as MAPK activated protein kinases (MAPKAP-Kinase) found in mammalian system.[15] Four classes of MAPK in *Arabidopsis thaliana* have been found.[16,17] A generally accepted pathway of the MAPK cascade is shown in **Figure 1**. Animal MAPK comprises of three large families, i.e., ERK, JNK, p38 family. While plant MAPKs also constitute a large family—for example the Arabidopsis genome consists of 23 MAPKs, twelve of which are ERK type and the other are plant specific[18,19]—no obvious JNK or p38 MAPK homolog have been identified.

Different MAPK cascades are present in a single cell and often share common components. There is some crosstalk between pathways, but MAPK cassettes appear to be insulated from each other by the intrinsic specificity of the MAP2Ks and MAP3Ks and by binding interactions that are thought to organize the cassette into multi-enzyme complexes.[20] Analysis of plant defense signaling cascades demonstrate the convergence of resistance gene recognition, elicitor, salicylate and also wound responses at the

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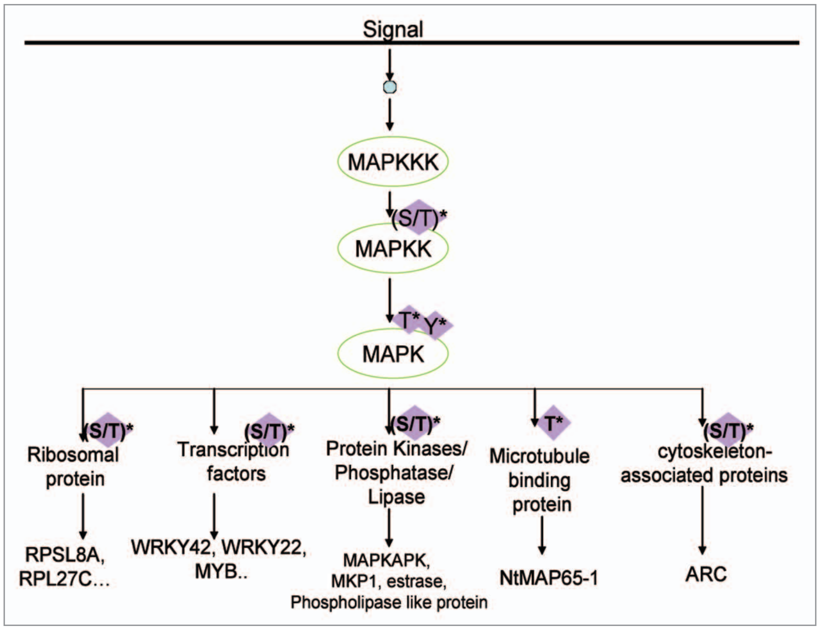

Figure 1. A signal transduction cascade navigates the signal from MAPKKKK to MAPK by triggering a series of Threonine/Tyrosine and Serine/Threonine phosphorylation events, finally culminating in activated MAPK being transported to the nucleus where it is involved in the phosphorylation of transcription factors and reconfiguring a specific response related transcriptional reprogramming.

level of MAPK activation[21] leading to changes in gene expression, cell division, cell growth and cell differentiation.[22] Currently it is unclear whether these signaling pathways merge at the MAPK, upstream of MAPK or whether the same MAPK can mediate disparate responses by interacting with other proteins.[23] This review attempts to address these complexities of MAPK signaling and highlight their role in diverse biological processes.

Classification of MAP Kinases

All MAPKs in plants are classified on the basis of two methods. One is phylogenetic based on analysis of the Arabidopsis genome and EST sequence data generated through sequencing projects.[19,24] The other approach is based on functional analysis involving specific features and sequence signature motifs.[4] The classification based on signature motif comprises of six families representing six functional groups.[25] However, as phylogenetic relationships may not represent functional relationships the classification remains to be experimentally validated based on the biochemical, molecular and in silico studies.

The Arabidopsis genome contains 23 MAPKs, 10 MAP2Ks, 80 MAP3Ks and 10 MAP4K.[16,19,26] Compared with MAPKs and MAP2Ks, MAP3Ks have more complex and variable primary structures and domain compositions. Average molecular mass of MAPKs ranges between 42–52 KDa.

Structure and Conformation Studies of MAPK

Crystallographic studies suggest that almost all members of MAPK family share similar three dimensional structures and function that are highly conserved.[27-30] All MAPK are either two lobed structures or have two different structural subtypes comprising those containing a TEY phosphorylation motif in the activation loop (TEY subtype) and those containing a TDY motif (TDY subtype) within its active site buried at domain interface.[31,32] The N-terminal domain (135 residues) is composed largely of beta-sheets and a glycine-rich loop that is termed the phosphate anchor ribbon, which acts as ATP binding pocket.[33] The C-terminal domain (225 residues) contains a catalytic base, magnesium binding sites and the phosphorylation lip (activation loop).[28,29] A common structural feature of MAPKs is the presence of a phosphorylation site in the activation loop between kinases domains.[34]

This 12 amino acid activation loop is a poorly conserved element at the C-terminal domain[33,34] and forms the mouth of the active site i.e., ATP binding sites. The ATP binds between the two domains and the protein substrate is believed to be bound on the surface of the C-terminal domain. The N and C-terminals are predominantly composed of beta-strand and alpha-helices respectively. The sequence of phosphorylation and activation loop influences the substrate specificity. The TXY(X-D/E/P) motif is a dual phosphorylation site and phosphorylation of both residues is a prerequisite for the activation of this cascade.[37,38]

Alignment of the amino acid sequences of many protein kinases reveals a common catalytic domain of 250–300 residues encoding the "two domain" structure.[39] Protein kinases possess 12 conserved stretches of amino acids within their catalytic domains known as sub-domains.[33,39,40] MAPKs have divergent region between sub-domain VII and VIII, located just before the TEY sequence, representing the phosphorylation site for activation by MAP2K. This domain also participates in the interaction of MAPK with its direct upstream activator MAP2K.[41]

MAPK Substrate Specificity

MAPKs are proline-directed serine/threonine kinases which phosphorylate the serine/threonine in the dipeptide motif S/T-P. However, ~80% of all proteins have S/T-P sequences so it is unlikely that all these proteins are MAPK substrates.[42] Notably, leucine at -1 and proline at -2 and -3 positions appear to influence whether the dipeptide acts as a phospho-acceptor.[43]

An important point to note is that the MAPK cascade is highly specific for its substrate i.e., MAPKK can specifically and selectively interact with a specific MAPK. Among various MAPK modules the recognition of an appropriate specific substrate by MAPK depends upon the differential interaction of two sites i.e., catalytic site and docking site. A typical MAPK consist of an active site and a common docking groove which are closely located and are implicated in recognition and binding of target proteins. MAPK signaling location, specificity and duration are regulated by scaffolding proteins and MAPK phosphatases.[44]

Scaffolding helps in holding the components of MAPK cascade via a single adapter together. Additionally, this arrangement also facilitate the interaction with the upstream factors that activates them, thus facilitating substrate recognition by the MAPK module.[44-46]

The specificity of different MAPK cascades functioning within the same cell is generated through the presence of docking domains that occur between sequence motifs in the substrate distal from the phosphorylation site and regions of the kinase outside the active site[47] and that are found in various components of MAPK modules and scaffold proteins. Docking interactions enhance substrate affinity and specificity. Interaction of MAPK with partner proteins has been mapped by mutagenesis, hydrogen exchange-mass spectrometry and x-ray crystallography.[48,49]

Downstream phosphorylation events of MAPK cascades occur which may influence the regulation of different genes. Since, identification of first plant MAPK substrate in vivo, 1-aminoacyclopropane-1-carboxylic acid synthase, the rate-limiting enzyme in ethylene biosynthesis[50]—48 substrates of MAPK3 and 39 substrates of MAPK6 have been identified in Arabidopsis through the application of high-throughput proteomic screening.[51,52] Additionally, MKS1 (MAP kinase substrate 1), which is required for full salicylic acid-dependent resistance,[53] has been reported to be phosphorylated by MAPK4 in Arabidopsis. Consistent with this, it has been reported that plant-specific WRKY transcription factors that contain the WRKYGQK core sequence and zinc-finger motif are phosphorylated by MAPKs, like MAPK4. This signaling cascade appears to be mediated through interaction with MKS and the target WRKY transcription factors such (WRKY25 and WRKY33).[53,54] WRKY factors are associated with MAPK in the nucleus and the majority of terminal MAPKs appear to be within nucleus, associated with transcriptional complexes at target genes.[55] A tobacco MAPK, NRK1/NTF6, positively regulates expansion of the phragmoplast. Phosphorylation of NtMAP65-1, a microtubule bundling protein by NRK1/NTF6 reduces its microtubule bundling activity, but not its binding ability and any single or point mutation in MAPKs phosphorylation site delays cytokinesis.[7] MAP Kinase is also required to control of MAP65-1 in metaphase and telophase.[56]

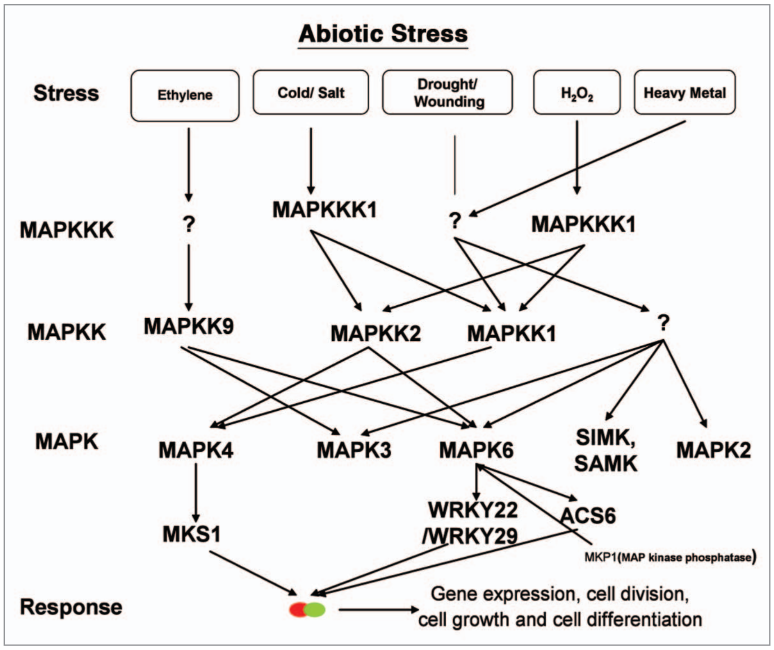

Figure 2. Model describing the interconnectivity between different members of kinases cascades involve in abiotic stress responses.

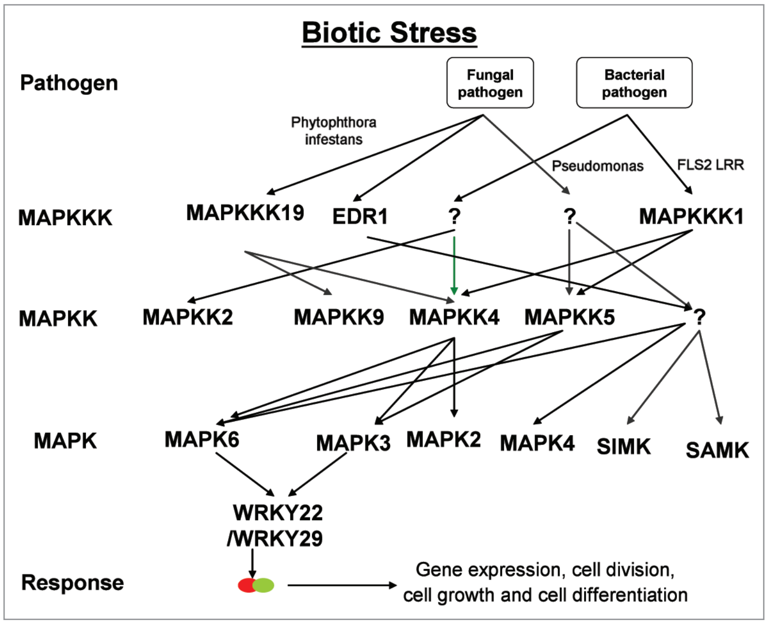

Figure 3. Model describing the interconnectivity between different members of kinases cascades involved in biotic stress responses.

The MAPK Cascade and its Regulation

A MAPK cascade minimally consists of a MAP3K-MAP2K-MAPK module that is linked in various configurations to upstream receptors and downstream targets. Among all components, MAP2Ks acts as a point of intersection and integration between converging signals from upstream MAP3Ks and divergent outputs through downstream MAPKs.[32] Activation of a MAP3K can occur through physical interaction or phosphorylation by the receptor itself, intermediate bridging factors or interlinking MAP4Ks.[16] MAP3Ks are serine/threonine kinases that activate MAP2Ks through phosphorylation on two serine/threonine residues in a conserved S/T-X_{3-5}-S/T motif.[57] Likewise, MAP2Ks are dual-specificity kinases that phosphorylate MAPKs on threonine and tyrosine residues in the T-X-Y motif.

Based on the number of MAP4K, MAP3K, MAP2K and MAPK, there are potentially thousands of combinations (10 x

80 x 10 x 23 = 184,000) that can be integrated into signaling pathways.[19] The 20 MAPKs can be classified into four groups according to their sequences (TEY subtype) and structures.[17] The related A, B and C groups and TDY subtype forming a more distant group D that lacks the CD domain, that is, serves as a docking interaction with MAP2K.[17,58] However, remaining 3MAPKs (MAPK21–23) form a separate group-MHK group, related to both MAPKs and cyclin-dependent kinases (CDKs), but it is not clear whether they are also part of a MAPK signaling pathways[32] or belong to other signaling pathways.

MAPK3 and MAPK6 of Group A and MAPK4 of Group B, have been extensively studied.[59-67] MAPK4 is found to be an important negative regulator of systemic acquired resistance (SAR). These MAPKs are predominately involved in orchestrating response to stress and can be activated by a diverse set of stresses, including pathogens, osmotic stress, cold stress and oxidative stress.[59,60,68-70] Downstream substrates of MAP2K3 i.e., MAPK1, MAPK2, MAPK7 and MAPK14 of Group D have been identified by yeast two-hybrid analysis.[71] MAPK18, a member of group D, might target the microtubule cytoskeleton, which is proposed to be involved in integrating signaling pathways.[72]

The ten MAP2K members are classified into four groups. Group A includes MAP2K1, MAP2K2 and MAP2K6; Group B comprises a single member, MAP2K3; Group C MAP2K4 and MAP2K5; and Group D has MAP2K7, MAP2K8, MAP2K9 and MAP2K10. Classification of MAP2Ks is based upon protein alignment. Different members of same group are activated in the presence of different stimuli.[73,74]

The MAP3K family forms the largest and most complex group compared to the MAPK and MAP2K classes. 80 MAP3K are divided into three sub-families, i.e., MEKK family (21 members), ZIK like family (11 members) and the Raf-like family (48 members).[58]

MAP2K1 is involved in defense responses including flg22-induced activation of MAPK4.[60,75] MAP2K2 is involved in cold and stress signaling. MAP2K6[59] is involved in cell division. MAP2K3 participates in jasmonate-mediated developmental signaling and in pathogen defense responses through activation of MAPK6.[76] MAP2K4 and MAP2K5 are implicated in stomatal developmental responses.[59,60,71,77] Function of the Group D MAP2Ks is not well understood. MAP2K7 has shown to be a negative regulator of polar auxin transport[78] and a positive regulator of SAR.[79] MAP2K9 may act as a negative regulator of abiotic stress responses.[80] It was hypothesized that MAP2K7/MAP2K9 regulate cell death during pathogen defense.[81] It has been reported recently that the MAP2K9-MAPK3/MAPK6 cascades promote ethylene-insensitive3 (EIN3)-mediated transcription in ethylene signaling.[82] By contrast to this MAP3K8 (MEKK1) was also shown to act upstream of MAPK3,[60] therefore it may be concluded that components involved in different functions might also form the same cascade.

The first complete MAPK cascade in regulating plant defense against bacterial pathogen, MAP3K1-MAP2K4/MAP2K5-MAPK3/MAPK6-WRKY22/WRKY29, was proposed as being downstream of the flagellin receptor kinase (FLS2 LRR), which potentially activates the MAP3K1 by phosphorylating the Ser/Thr residues.[60] Beside this, the MAPK cascade comprising YODA-MAP2K4/MAP2K5-MAPK3/MAPK6, plays an important role in regulating stomatal development.[83-86] Another MAPK cascade, MAP3K1-MAPKK1/MAPKK2-MAPK4-MKS1 mediate jasmonate- and salicylate-dependent defense responses.[66] MAP3K1 acts as a negative regulator of plant immune responses and along with MAPK4 it interacts with two MAP2Ks i.e., MAP2K1 and MAP2K2.[87]

The MAP3K1-MAP2K1/2-MAPK4/6 module was activated by various stress treatments.[59,68,75,88] The MAP2K3-MAPK6 module was activated by JA.[76] Similarly, tobacco stress-induced protein kinase (SIPK) and wound-induced protein kinase (WIPK) share a common upstream MAP2K, NtMEK2.[14] One of the best studied MAP kinases, MAPK 6, was found to be activated after ethylene treatment. MAPK6 is also activated in the constitutive triple response1 (*ctr1*) mutant and is implicated in stomatal development, biotic and abiotic stress responses and embryo and floral organ development. The MAPK6 along with MAPK3, are required for complete "priming" of plants. The pre-stress deposition of these kinases is a critical step for full induction of defense responses during induced resistance.[89]

When the transcriptional activation of MAPK cascades are studied on the basis of Pearson correlation coefficients for all MAPK components of Arabidopsis genes, different co-regulation of MAPK components were evident. MAP2K1, MAP2K2, MAP2K4, MAP2K5 and MAPK3 are all strongly co-regulated with a number of WRKY transcription factors. MAP2K9, EDR1 and MAP3K8 (MEKK1) and MAPK3 show global correlations in their expression with each other. Similarly, a strong correlation was also seen in between MAP2K6 and MAP3K12, which are implicated in the regulation of cell proliferation or cytokinesis.[90] Slightly weaker, but still significant correlations were seen between MAP2K6 and MAPK13, components that have been shown to interact and constitute a signaling pathway together.[91] Likewise, MAP3K12, MAP2K6, MAPK13 all have a clear mitosis-specific expression pattern in cell synchronization experiments.[32]

MAPK Function

In plants, MAPK signaling appears to involve cross-talk with a variety of stress responses and developmental processes forming complex interconnected networks within cells.[92] Traditional genetic and biochemical methods have identified MAP3K/MAP2K/MAPK signaling modules with overlapping roles in controlling diverse cellular functions. These include cell division, development, hormone signaling (abscisic acid-ABA, auxin and ethylene) and synthesis and response to abiotic stress (wounding, high and low temperature, high salinity, UV radiation, ozone, ROS, drought and high and low osmolarity, heavy metals) and pathogens.[4,93-98] The responses to pathogen attack may include changes in redox status, the hypersensitive response-HR, cell death, generation of reactive oxygen species (ROS), systemic acquired resistance (SAR), activation of pathogenesis related-PR genes and other protective genes.[25,99]

Role of MAPK Under Abiotic Stress

Environmental stresses, such as cold, drought, salinity and heavy metals are important factors that affect growth and metabolism of plants. The MAP kinase pathways are intracellular signal modules that mediate signal transduction from the cell surface to the nucleus. Several cascades are induced by different abiotic stresses.[4,100]

Heavy metal toxicity is one of the current environmental health problems which result in the potentially dangerous bioaccumulation of toxic levels of heavy metals in the ecosystem. It has been reported that As_3^+, V_4^+, Cr_3^+, Cu_6^+ and Zn_2^+ activate MAP kinases in mammalian systems.[101,102] Recently it was shown that heavy metals can activate MAPKs in higher plants.[4,103] $CdCl_2$ application results in the transcriptional activation of Arabidopsis MAP3K Arabidopsis MAP3K.[103,104] Likewise, exposure of Medicago seedlings to excess copper or cadmium ions results in the activation of four distinct MAPKs: SIMK (stress-induced MAPK), MMK2 (MAPK2), MMK3 (MAPK3) and SAMK (stress-activated MAPK).[4,105] MAPK3 and MAPK6 are activated in response to cadmium through the accumulation of ROS level produced by oxidative stress in Arabidopsis.[93]

In plants cold, drought and salt stresses all stimulate the accumulation of compatible osmolytes and antioxidants.[106] Different MAPKs are activated by salt stress at different times after the onset of stress and the activities of these MAPKs are activated within different time periods.[107] MAPK4 and MAPK6 are activated by cold, salt and drought.[68,108] MAP2K1 is transcriptionally induced by salt stress, drought and cold,[8] but it also mediates flagellin signaling via activation of MAP2K4 and MAP2K5.[60] In contrast, a MAPK module consisting of MAP3K1-MAP2K1/MAP2K2-MAPK4/MAPK6 has now been confirmed in cold and salt stress by yeast two hybrid analyses and yeast complementation.[59] Yeast two-hybrid analysis has recently been used to screen protein-protein interaction between all the Arabidopsis MAPKs. The outcome of these studies will generate an extensive pattern of interactions.[109] Besides this, MAP2K1 is also get activated by wounding and drought stress and can phosphorylate MAPK4.[59,77]

Many stress-responsive genes have been identified and altered gene expression plays an important role in plant drought resistance.[110-112] Nine genes have been identified that play an important role in mediating drought tolerance. Recently a novel nuclear protein kinase i.e., Drought-sensitive mutant 1 (DSM1) have been identified with sequence similarity to Raf-like MAP3Ks that play critical roles in drought and oxidative stress resistance in rice either by direct or indirect regulation.[113]

MAPKs are known to be activated by osmotic stresses in Medicago and Tobacco.[4] During osmolarity signaling MAP kinases module seem to be widely involved.[114] Understanding the in vivo function of Arabidopsis histidine kinase1 (AtHK) and other putative histidine kinases that function as osmosensors and show relation between osmotic stress-activated MAPK pathways will provide insight into osmotic stress signal transduction.[115] To survive under high osmotic stress condition the two component signal regulates *High Osmolarity Glycerol* (*HOG*), which result in production of high glycerol.[116] Yeast cells also respond to this hypertonic shock by activation of a MAP kinase cascade i.e., the HOG response pathway.[117] Likewise, in alfalfa SIMKK-SIMK and in tobacco NtMEK2 (MAP2K)-SIPK/WIPK (SA-Induced Protein Kinase/Wounding-Induced Protein Kinase) are reported to be involved in osmotic stress.[14,118] MAPK3 can also be activated by osmotic stress[119] whereas; MAPK4 and MAPK6 are activated by wounding and touch.[68] An interlink between osmotic stress and oxidative stress has been seen in Arabidopsis where it has been shown that many MAPK components have been activated or their gene expression is induced by salt and other stresses.[120,121]

Hydrogen peroxide (H_2O_2) is produced by various environmental and developmental stimulants and can act as a signaling molecule that regulates plant development and stress tolerance and programmed cell death (PCD) including aleurone cell death, the hypersensitive response to pathogens and allelopathic plant-plant interactions.[122] H_2O_2-induced PCD itself is essential for a number of developmental processes and environmental responses. H_2O_2 elicits the activation of a MAPK in Arabidopsis[123] and it activates two MAPKs in Arabidopsis plants, one of which is also activated independently via SA, JA and ethylene signaling pathways.[124] H_2O_2 activates, through the MAP3K ANP1, AtMPK6 and the related AtMPK3.[125-127] As alluded to above, AtMPK6 is activated in response to elicitor challenge and cold stress.[68,127,128] In addition, ozone and H_2O_2 treatment induced the activation of the tobacco ortholog of AtMPK, SIPK1.[129] It was shown that transgenic tobacco plants overexpressing a tobacco MAP3K orthologue of ANP1 had increased tolerance to heat shock, freezing and salt stress, thus indicating that manipulation of a key signaling component responsive to H_2O_2 can protect plants against various environmental stresses.[130] MAP2K is an important regulator of stomatal movement, which is believed to mediate the H_2O_2 generation induced by ABA in guard cells of *Vicia faba*.[131] H_2O_2 may have an autocatalytic function to facilitate its own generation, e.g., once an H_2O_2-generation system was triggered, a small amount of H_2O_2 could accelerate MAP2K or CDPK activation due to the formation of an H_2O_2 feedback-loop. This crosstalk of H_2O_2 and MAPKKs or CDPKs may lead to the formation of a self-amplification loop.[132] A rapid and very complex response are seen in nitric oxide (NO) and H_2O_2 in hypersensitive cell death.[133] NO is involved in the activation of MAPK activity during the plant defense responses against pathogen infections in tobacco (*Nicotiana tabacum*)[134] and *Arabidopsis thaliana*,[135] and the adventitious root formation induced by indole acetic acid in cucumber (*Cucumis sativus*).[136] An NO-activated protein kinase in tobacco with characteristics of a MAPK was identified as SIPK, for which SA is necessary for activation.[135] NO synthesis and signaling also are implicated in the regulation of protein phosphates.[131]

Role of MAPK under Biotic Stress

The plants respond to pathogen attack by activating multi-step defense responses, including rapid production of reactive oxygen species (ROS), strengthening of cell walls and induction of the

HR leading to localized cell death at the sites of infection. Plant defense responses also include synthesis of pathogen-related proteins and phytoalexins.[60,110] It has been firmly established that MAPKs play a central role in pathogen defense in Arabidopsis, tobacco, tomato, parsley, brassica and rice.

The role of MAPKs has been seen during symbiotic association between plant and pathogenic organism. A putative role of the MAPKs i.e., SIMK and SAMK in rhizobia-legume symbiosis has been suggested in *Lupinus albus*. MAPKs are involved in the infection and nodulation of *L. albus* by Bradyrhizobium sp. (Lupinus). Inoculation of *L. albus* with *B. lupinus* leads to activation of SIMK and SAMK while inoculation with dead *B. lupinus* along with *Sinorhizobium meliloti* did not induce SIMK and SAMK.[137] This suggests that activation of these MAPK pathways is a specific response of the host cells to live bacteria which may lead to a successful symbiotic interaction, suggesting that MAPKs may take part in the recognition of compatible partners. Beside this symbiotic association, host MAPK cascades are also activated by fungal pathogens.[138] Infection by plant fungal pathogen *Phytophthora infestans* led to the rapid transcriptional induction of *MAP3K19*, *MAP2K9* and *MAP2K4*, while with that of *Botrytis cinerea* infection led to the rapid transcriptional induction of *MAP3K18, MAP3K19* and *MAP3K20, Raf43, ZIK2, ZIK8*, suggesting that signaling to bacterial and fungal pathogen attack is distinct.[32] The alfalfa MAPKs, SIMK and SAMK, which were originally identified as inducible by osmotic stress[139] and wounding,[140] respectively, were later also found to be activated by various fungal elicitors.[141] Two other alfalfa MAPKs, MMK2 and MMK3 (Medicago MAPK2 and MAPK3, respectively), are involved in cell growth and division[142] but are also activated by elicitors. Despite all this, a fungal biocontrol agent *Trichoderma asperellum* has recently been shown to induce systemic resistance in plants through a mechanism that employs jasmonic acid and ethylene signal-transduction pathways leading to induction of a Trichoderma-induced MAPK (*TIPK*) gene in cucumber (*Cucumis sativus*).[143]

Role of MAPKs in Stress-Induced Plant Growth and Development

MAPKs signaling act as a positive regulators for environmental stress-induced, stomatal closure and negative regulators for stomatal development.[83,84,144] Stomatal development in Arabidopsis is regulated by a MAPK cascade involving the MAP3K-YODA, the MAP2Ks-MAP2K4 and MAP2K5, the MAPKs MAPK3 and MAPK6 and the bHLH transcription factor SPEECHLESS (SPCH).[62,145] This cascade regulates asymmetric cell division that provides cell fate specification in stomata. *Arabidopsis thaliana* MAP3K-YODA plays an important role in early embryo development and its mutant loses the property to differentiate an extra embryonic suspensor. Absence of this protein compromises stomatal cell specification.[83,86] It has been demonstrated that MAP2K7 and MAP2K9 also play role as a positive and negative regulators respectively, at different stages of stomatal development.[147] A role for MAPK6 in early root development in *Arabidopsis thaliana* has been reported. *MAPK6* expression is high in most apical parts of the root meristem and in the root transition zone.[146]

Crosstalk of MAPKs in Different Stress-Induced Pathways

Emerging evidence suggests that hormone signaling pathways (abscisic acid, salicylic acid, jasmonic acid, auxin and ethylene) as well as ROS signalling pathways, play key roles in the crosstalk between biotic and abiotic stress signaling.[148] These hormones may interact with one another in regulating stress signaling and plant stress tolerance. MAPKs may have role in ABA signaling, which mediates responses to environmental stress, chiefly water stress. MAPK9 and MAPK12, which are preferentially activated in guard cells, share functional redundancy and serve as positive regulators acting downstream of reactive oxygen species and calcium signaling, but upstream of anion channels in guard cell ABA signaling.[149] Besides this, MAPK are activated by low concentration application of ABA to barley aleurone protoplasts.[150] The role of ABA in osmotic stress signal transduction was previously addressed by studying the stress induction of several genes in Arabidopsis.[151] A calcium-independent protein kinase in faba bean (*Vicia faba*) has been isolated, and which was activated upon ABA treatment but unexpectedly could not be immunoprecipitated with an anti-phosphotyrosine antibody. Thus, the role of MAPK in ABA signaling has not yet been directly established but cannot yet be ruled out.

The role of MAPK signaling cascades and protein phosphorylation in auxin signaling has been documented in numerous studies. For example, auxin was shown to activate an unknown MAPK in Arabidopsis seedling roots.[152] A tobacco MAP3K activated in response to oxidative stress was found to negatively regulate auxin induced gene expression.[153] Recently, the Arabidopsis MAP2K7 was shown to control polar auxin transport.[78] However, there are no MAPK substrates known to affect gene expression in response to auxin. Some evidence shows that H_2O_2 induced MAPK cascade leads to specific stress-responsive gene regulation but it inhibits the action of auxin,[154] revealing a molecular link between oxidative stress response and auxin signal transduction.

Conclusion

Significant progress on the description of MAPK signaling in plants has been made in recent years using a combination of physiological, biochemical and genetic approaches. Over the past few years researchers have identified a multitude of signaling responses that involve MAPKs. These studies are now focused on some of the complete MAPK cascades that have been identified for signaling in biotic and abiotic stresses but a few are still missing. So a need still exists to design novel approaches and strategies to define specific functions and elucidate the underlying mechanisms of signal transduction through identification and validation of functional plant MAPK components. Potentially useful approaches to dissect the stress stimulated MAP kinase pathways in response to various stimuli will come from integrating publically available transcriptomics datasets and development of targeted phospho/proteomics approaches in combination with knockout and knock in transgenic approaches to perturb MAPK signaling networks. Substrates and signaling components in different stress induced signaling pathways will be identified through the application of protein-protein interaction, approaches (yeast two hybrid system, SPR, Immuno-chips).

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