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Review: Mitogen-Activated Protein kinases in nutritional signaling in *Arabidopsis*

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Highlights

- Mitogen-Activated Protein kinases cascades are part of many signaling pathways
- MAPKs might act as central hubs integrating multiple signals
- Expression of a subclass of MAPKs is modified in response to nutritional variation
- New evidences for the role of MAPKs in nutritional signaling are now emerging

Abstract

Mitogen-Activated Protein Kinase (MAPK) cascades are functional modules widespread among eukaryotic organisms. In plants, these modules are encoded by large multigenic families and are involved in many biological processes ranging from stress responses to cellular differentiation and organ development. Furthermore, MAPK pathways are involved in the perception of environmental and physiological modifications. Interestingly, some MAPKs play a role in several signaling networks and could have an integrative function for the response of plants to their environment. In this review, we describe the classification of MAPKs and highlight some of their biochemical actions. We performed an *in silico* analysis of *MAPK* gene expression in response to

nutrients supporting their involvement in nutritional signaling. While several MAPKs have been identified as players in sugar, nitrogen, phosphate, iron and potassium-related signaling pathways, their biochemical functions are yet mainly unknown. The integration of these regulatory cascades in the current understanding of nutrient signaling is discussed and potential new avenues for approaches toward plants with higher nutrient use efficiencies are evoked.

Keywords: MAPK; ; ; ; , nutrients, signaling, gene expression, *Arabidopsis thaliana*

Contents

1. Introduction

Plants are sessile organisms facing a large range of environmental biotic and abiotic stresses as well as important fluctuations in nutrient supplies. Plants must continually adapt their metabolism and development to environmental parameters in order to perpetuate progeny. Nutritional signaling has been intensively studied and seems to involve large and complex signaling networks. Despite the well-documented importance of Mitogen Activated Protein Kinase (MAPK) modules for biotic and abiotic stress signaling [reviewed in [1-4]], only limited information is yet available for their contribution to nutritional signaling.

Nutritional signaling, such as sugar, phosphate or nitrate signaling, has seen major breakthroughs during recent years (reviewed in [5], [6]). The regulation at the level of transcript accumulation has been studied intensively in many cases. Post-translational regulation such as activation/inactivation by phosphorylation, is less well described. MAPK modules are involved in signal transduction resulting in appropriate response in plants. MAPKs are part of signaling cascades and are post-transcriptionally regulated by biotic and abiotic stresses (reviewed in [7-9]). A common MAPK module contains 3 types of kinases encoded by distinct gene families: a MAPK (or MPK), a MAPK kinase (MAPKK or MKK) and a MAPKK kinase (MAPKKK) (Fig. 1). Animal and yeast MAPK modules are activated post-transcriptionally by various cellular mechanisms such as phosphorylation or interaction with G proteins or receptors [1]. Despite intense efforts by many plant research groups, we are far from understanding the activation mechanism of MAPKKKs as a first step of MAPK module initiation. MAPKKKs contain regulatory motifs such as proline-rich sequences, leucine zippers or binding sites for G proteins that may allow their activation [10].

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Several well studied protein kinases participate in nutritional signaling. Among the best studied ones is Target Of Rapamycin kinase (TOR kinase), which regulates many metabolic processes in response to the energy status of the cell [review in [11]]. Furthermore, Sucrose non-fermenting 1-Related protein Kinases (SnRKs) are involved in the regulation of carbon and nitrogen metabolism [reviewed in [12]]. In particular, SnRK1s and SnRK3s participate in such pathways. SnRK1s have been suggested as a key switch of sucrose signaling with substrates in carbon, but also nitrogen metabolism [13] and as an interface between metabolic and stress signaling [7]. SnRK3s, also known as CIPKs (CBL-Interacting Protein Kinases) that interact with Ca^{2+} sensors CBLs (Calcineurin B-Likes) have been identified as molecular players for sucrose signal transduction [14] and for the regulation of nitrate, magnesium and potassium transporters [15-18]. This review focuses on the role of MAPKs in nutritional signaling.

2. Current knowledge on MAPKs

More than 1000 genes encode protein kinases in *Arabidopsis thaliana* [19]. Among them, about 120 genes code for kinases potentially involved in MAPK signaling. Receptors and sensors activate one or several MAPKKKs able to phosphorylate and activate one or several MAPKKs in response to a stimulus, through yet unknown mechanisms. In turn, these phosphorylate and activate one or several MAPKs [5]. These phosphorylation cascades lead to the regulation of transcription factors and diverse targets (Fig.1). In the case of the well-studied MAPKs such as MPK3, MPK4 and MPK6, a constantly growing number of substrates has been identified [20-27], suggesting that these stress-responsive MAPKs could have up to hundred substrates.

The *Arabidopsis* genome sequence information allowed a classification of MAPK-related kinases based on sequence homology [19, 28] (Fig. 1). In *Arabidopsis*, there are 20 genes coding for

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MAPKs and 10 for MAPKKs. Additionally, *Arabidopsis* possesses at least three families of kinases, which, in animals, were shown to have a MAPKKK activity: MEKK (MAPK/ERK kinase kinase, 20 genes), ZIK (ZR1-interacting *kinase, 11 genes)* and RAF (Rapidly Accelerated Fibrosarcoma*, 48 genes*). This activity has been confirmed for several of the plant MEKK-type MAPKKKs [29-32], while no such evidence has been obtained for ZIKs. The members of the RAF family have been occasionally shown to rather act as negative regulators of MAPK modules [2, 33-35], but for example for Constitutive triple response 1 (CTR1), a key component of ethylene signaling, the direct protein targets need to be studied further [36].The small number of MAPKK encoding genes compared to the numbers of MAPKs and MAPKKKs suggests a convergence and divergence point for signaling pathways. Moreover, it is discussed that MAPKKs with homologous sequences could be involved in similar functions despite the fact that the specific mechanisms are still unknown [1]. In other plant species including crops, gene families coding for MAPK related kinases are roughly organized in the same subclades. For example, among monocots, rice genome codes for 16 MAPKs, 8 MAPKKs and 75 MAPKKKs which are organized in RAF, ZIK and MEKK families [37, 38]. Legumes such as *Lotus japonicus*, *Medicago truncatula*, and *Phaseolus vulgaris* or cucurbits such as *Cucumis sativus* possess slightly smaller kinase families but at least one member of all the subclades identified in *Arabidopsis* [39, 40]. We focus in this review on the reference species *Arabidopsis thaliana*, the only plant system allowing so far a larger picture of the various functions of MAPKs. Knowledge in other plant species, which is rapidly growing thanks to genomic data and the knowledge acquired in the model plant *Arabidopsis*, would deserve a particular attention and therefore will not be covered by this review.

By far, the most studied MAPK modules are MKK4/5-MPK3/6 and MEKK1-MKK1/2- MPK4 that are involved in responses to biotic and abiotic stresses and in important developmental processes [1-3, 41, 42]. These few MAPK modules highlight the complexity of MAPK functions in plants in which the same MAPK can be used to build different MAPK modules in distinct contexts and regulate distinct cellular responses. For example, MPK4 which belongs to a biotic stress activated module constituted of the MAPKKK MEKK1 and the 2 MAPKKs MKK1/2 [7], is also an important actor of cytokinesis, working downstream of the MAPKKKs ANP1/2/3 and the MAPKK MKK6 [43]. Beside the MAPK cascades involving these three iconic stress-activated MAPKs (MAPK3/6/4), another MAPK module has been recently identified as MAPKKK17/18-MKK3-MPK1/2/7/14, which is activated by reactive oxygen species and abscisic acid [32]. Many other MAPKs participate to abiotic and biotic stress signaling [3, 7, 44, 45] and the identification of the complete MAPK modules will clarify the interplay between these signaling cascades. Overall, the majority of *MAPK* genes have not been studied so far.

3. MAPK are involved in nutritional signaling and metabolism

Although MAPKs are known to be involved in many regulatory pathways, little is known about their role in nutritional stress signaling and in the regulation of primary metabolism in plants.

3.1. *MAPK*s are regulated at the gene expression level by nutrient status

We analyzed the expression of genes coding for protein kinases that are putatively involved in MAPK modules in response to nutrient supply in *Arabidopsis* using data from the GENEVESTIGATOR database [46]. We focused our analysis on all 10 MAPKKs and 20 MAPKs and on the 20 MEKK-like MAPKKKs, as these are more likely to be involved in MAPK cascades [19]. Among all data from nutrient perturbations (involving modification of ammonium, nitrate,

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glucose, sucrose, iron, potassium, phosphorus, sulphate supply) we only considered the treatments leading to mRNA level changes with a $\log_2$ ratio > 1.5 and with a p value < 0.05 for at least one of the kinase genes. The strongest modifications of steady state mRNA levels were found for MAPKKKs (Fig. 2).

 Glucose and sucrose supply increased the mRNA levels of *MAPKKK18* and *MAPKKK19,* respectively. Nitrogen resupply upregulated the expression of *MAPKKK14* and *MAPKKK19*. Iron availability influenced the expression of *MAPKKK1*, *MAPKKK2* and *MAPKKK12*. Gene expression of *MAPKKK14* and *MAPKKK19* was strongly induced by phosphorus deprivation (Fig.2).

Changes in the expression levels of MAPKKs and MAPKs by the perturbation treatments related to nutrient sensing and nutrient availability were less pronounced. An increase of the steady state level of mRNA is observed for MPK19 and MKK7 after sucrose supply, MPK11, and MPKK9 in response to nitrogen starvation. Taken together, our analysis shows that several MAPKKKs are strongly regulated at the gene expression level by nutrient related signals while the expression level of MAPKKs and MAPKs is less impacted by nutrient signals. Nonetheless, a change of the expression levels is only a hint that these protein kinases might be involved in the response to the nutrient status of the plant and in nutrient signaling. In addition, very rapid and transitory changes in gene expression in response to nutrients might have been missed, as most of the data analyzed correspond to longer term treatments. Indeed MAPK expression increases as rapidly as 15 minutes after touch or wounding and then decreases to initial levels [47]. The complexity of the regulation of MAPKs involving gene expression changes as for example shown here for MAPKKK in response to nutrient signals or as for MAPK3 and MAPK11 in response to biotic stress, and in addition major post-translational regulation mainly by phosphorylation may reflect the

importance of the multilayer control of the activity of these protein kinase for plant responses to their environment.

3.2. MAPKs in Nutritional Signaling

The molecular events involved in the nutritional signaling pathways are far from being fully elucidated. However, a detailed understanding has been obtained for sugar-sensing [48] and more recently for the sensing of inorganic nutrients, such as nitrate, ammonium, phosphate and magnesium and other micronutrients [49-51]. More recently, the participation of proteins belonging to the MAPK families in nutrient sensing has been revealed, however the biochemical events underlying these mechanisms are not well known yet.

Sugar signaling and carbohydrate metabolism

A putative MAPKKK belonging to the RAF family, SIS8/RAF5 (*At1g73660*), has been proposed to be a regulator of sugar response in *Arabidopsis* [52]. Indeed, a reduced sensitivity to high concentrations of sucrose was observed for at*sis8* mutant seedlings. However, only small differences (< 30% change from wild type) in expression levels of genes involved in the sugar response pathways were observed between the *sis8-3* mutant and the wild-type plants grown on normal or sugar-containing media. Yeast Two-Hybrid and BiFC (bimolecular fluorescence complementation) experiments showed that SIS8 interacts with an UDP-glucosyltransferase (UGT72E1) in the nucleus. *Arabidopsis* plants impaired in this UDP-glucosyltransferase have the same sugar-insensitive phenotype as *sis8* mutant plants, indicating that they function together in the sucrose response in *Arabidopsis*. However, the biochemical function of SIS8 is yet unknown and no protein kinase activity has been reported.

Evidence that MAPKs directly regulate enzymes of sugar uptake and assimilation have also been discovered. VIK (VH1-Interacting-Kinases; RAF17; At1g14000), a RAF-like kinase, participates in the regulation of glucose loading into vacuoles [53]. VIK interacts directly with the main vacuolar glucose transporter, TMT1 (Tonoplast Monosaccharide Transporter1). The VIK-dependent phosphorylation of TMT1 stimulates its activity. Moreover, mutants in either *VIK* or *TMT1* have the same physiological phenotypes [53]. Interestingly, both SIS8 and VIK, two RAF-type kinases, seem to regulate directly cellular enzymatic activities without the involvement of the expected downstream actors, MAPKK and MAPKs, confirming that they are not usual MAPKKKs in plants.

Nitrogen sensing and metabolism

Several findings suggest a role of MAPKs in nitrogen sensing and metabolism. The systematic identification of MAPKK/MPK substrates using the phosphorylation of high-density protein microarrays revealed 570 potential MAPK targets [21]. Interestingly, in this study, Nitrate Reductase (NR2), a key enzyme for nitrate assimilation was phosphorylated by MPK7. Furthermore, important regulatory proteins involved in nitrate signaling are among these identified MAPK targets such as the LOB domain binding proteins LDB37and LDB39, transcription factors negatively regulating anthocyanin biosynthesis in response to nitrogen supply [54], and the protein kinase CIPK23 that regulates the nitrate transceptor NRT1.1 [16]. However, these targets have only been identified *in vitro* and *in planta* confirmation is still missing in most cases. A further direct proof of the role of MAPKs in nutrient signaling comes from a report demonstrating that NR2 interacts with and is phosphorylated by MPK6 [55]. This study focused on the role of NR2 for NO production but suggests that MPK6 could directly regulate nitrate assimilation. Further dedicated studies will be necessary to confirm this suggestion.

Phosphorylation of NR2 by MAPKs was confirmed in global phospho-proteomic studies [22, 56]. However, in these studies no significant overrepresentation of nutrient response or primary metabolism related MAPK targets have been observed.

Interestingly, five *MAPKKK* genes have been identified among the direct targets of the transcription factor NLP7 (NIN Like Protein 7), a master regulator of early nitrate signaling in roots [57]. Indeed, NLP7 binds to the promoter regions of *MAPKKK13, 14, 17, 18 and 19* in response to a short (10 minutes) nitrate resupply to N-starved plantlets. In addition *MAPKKK13* and *MAPKKK14* are differentially expressed in *nlp7* mutants after nitrate resupply. The regulation of *MAPKKK14* and *MAPKKK13* expression by nitrate has been also observed in transcriptomic studies (Fig. 2). In addition *MAPKKK13* has been identified in the target genes of the closely related transcription factor NLP8 which is a master regulator of nitrate-regulated germination [58]. Thus, it is possible that these MAPKKKs act downstream of NLPs in the primary nitrate signaling pathway, or that feedback regulation circuits exist between NLPs and the mentioned MAPKKKs. Interestingly, *MAPKKK13, 14, 17, 18 and 19* belong to a subclade of MEKK-like MAPKKKs, which have been suggested to activate MKK3-modules by the increase of their protein level in response to signals [59].

Another example for MAPK involvement in nitrogen signaling is the recent discovery that MEKK1 (MAPKKK8) participates in glutamate signaling [60]. Glutamate is an amino acid directly derived from the assimilation pathway of nitrate. Although glutamate has not yet been proven to act as a signaling molecule, glutamate receptors (GLR or ionotropic glutamate receptor-related) have been found in plants [61, 62]. A chemical genetic approach using 2–(4–chloro-3-methylphenyl)-2-oxoethyl thiocyanate, a compound known to inhibit a yeast MAPKKK caused suppression of the

glutamate-triggered changes in root architecture of *Arabidopsis*. This work revealed that MEKK1 plays a role in transducing the glutamate signal to modulate root architecture changes [60]. Despite having been the focus of many studies, the involvement of MEKK1 in a complete MAPK module is still unclear [7]. On one hand, *mekk1* plants show the immunity-related dwarfism of *mpk4* and *mkk1mkk2* [63-65], but, on the other hand, MEKK1 has also been proposed as activator of the MKK4/5-MPK3/6 module [29]. The fact that MPK6 regulates NR activity could suggest a glutamate-dependent feedback mechanism for nitrogen assimilation and signaling.

Sensing and signaling of the phosphate-status

The regulatory networks involved in the sensing and signaling of the phosphate-status involved a large number of molecular player, reviewed in [66]. *MPK3* and *MPK6* expression is upregulated and MPK3 and MPK6 are activated in response to low phosphate concentrations. *Arabidopsis* plants impaired in either *MPK3* or *MPK6* take up less phosphate whereas plants in which MKK9, one of the upstream activators of MPK3/6, is constitutively activated have enhanced phosphate uptake and upregulation of genes involved in phosphate-acquisition [67]. Interestingly, genes coding for transcription factors HRS1 and WRKY75, which are involved in the interplay between nitrate and phosphate signaling [67], are regulated by this MAPK module [67]. Potential application of these findings for improving phosphate acquisition needs to be challenged in crop species and under field condition. Besides this, it is very probable that interactions with micro-organisms, which commonly activate MAPK modules, also regulate many important physiological functions such as nutrient transport and metabolism. Indeed, the interaction of *Arabidopsis* with the root endophyte *Piriformospora indica* led to MAPK activation [68].The MAPK regulation of phosphate uptake in the context of mycorrhiza interaction could be of particular interest, as the

improvement of plant phosphate uptake by mycorrhization is an important feature of this symbiosis. This question could be easily addressed, exploiting the interaction between *Arabidopsis* and the root endophyte *Colletotrichum tofieldiae* that leads to improved phosphate acquisition [69].

Iron deficiency

Iron deficiency was shown to activate MPK3/MPK6 [70]. It also stimulates ethylene production through the accumulation of *ACC SYNTHASE* (*ACS*) transcripts. Interestingly during pattern-triggered immunity (PTI), ACS2/6 were both shown to be direct targets of the module MKK4/MKK5-MPK3/6 [71, 72]. In addition *ACS* genes are transcriptionally activated by MPK3/6 through the phosphorylation of the WRKY33 transcription factor [73]. Coherently, during iron deficiency, *mpk3* and *mpk6* mutants have a reduced production of ethylene. These mutants have reduced have reduced level of *Ferric Reduction oxidase 2* (*FRO2)* and *Iron Regulated Transporter 1* (*IRT1)* transcripts, two crucial genes necessary for iron uptake [70] as ethylene is necessary for transcriptional regulation of these Fe-deficiency responsive genes.

Potassium homeostasis

The potassium transporter HAK5, a putative H+/K+ symporter that mediates high-affinity uptake under potassium deficiency [74-76] interacts with INTEGRIN-LINKED KINASE1 (ILK1) a RAF-like MAPKKK that was previously called ANKYRIN-PROTEIN KINASE 1 [77]. In plants, *ILK* expression has been associated with changes in root development [78] and with plant defense to bacterial pathogen and osmotic stress sensitivity [79]. In addition ILK1 is involved in plant responses to the bacterial-derived pathogen-associated molecular pattern (PAMP), flg22. Indeed, flg22 treatment induced a rapid potassium efflux in wild type, while mutants with impaired ILK1 or HAK5

expression showed an increased potassium loss compared to wild-type plants [79]. Interestingly, HAK5 and ILK1 promote both growth under limiting potassium supply. Thus, the MAPKKK ILK1 could be a link between plant defense pathways and potassium homeostasis.

A RAF-like MAPKKK (HCR1, At3g24715) was recently characterized as a major player in determining the resilience of plants to flooding by integrating two soil signals, potassium and oxygen availability [80]. HCR1 negatively controls root water permeability (or hydraulic conductivity) under oxygen limitation dependent on the presence of potassium. HCR1 positively regulates the protein level of RAP2.12, without modifying its mRNA abundance. RAP2.12 is an ethylene-responsive factor group VII (ERF-VII) transcription factor involved in the primary activation of hypoxia signaling. The authors hypothesized that HCR1 may phosphorylate RAP2.12 itself or one of its protein partners involved in its stabilization. Interestingly, the finding that a MAPKKK is a key player for the oxygen-mediated regulation of root water permeability opens up the field for further studies on the mechanisms by which oxygen signaling regulates nutrient uptake and the potential involvement of other MAPKKKs [80].

3.3. Potential interplay of known MAPK signaling pathways and nutrient-related signal transductions

Despite a growing literature showing that MAPK modules are involved in various aspects of plant nutrition and metabolism, a lot remains likely to be unveiled. The fact that some signals, like hormones or secondary messengers, are known to be connected to both, known MAPK modules and regulation circuits controlling metabolism/nutrition suggests that important interplay exist. For example, several MAPK modules are activated by H_2O_2 [9, 10, 81] and others regulate reactive oxygen species homeostasis [19, 65, 82-84]. Plant responses to nutrient depletion do also partly

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depend on reactive oxygen species oxygen species signaling [85-88]. It would be tempting to speculate that reactive oxygen species signaling may affect nutrient responses via the action of MAPK cascades. MAPK modules are involved also in hormones signaling. For example, MAP3K17/18-MKK3-MPK1/2/7/14 is activated by jasmonic acid [32, 89], MPK6 activity is regulated by ethylene and is a modulator in the production of ethylene [90, 91] and MPK4 activity keeps salicylic acid level low. The same hormones control several aspect of nutrient signaling. For example an ethylene and jasmonate signaling module coordinates nitrate allocation [92] and ethylene regulates high affinity nitrate transporters [93]. Ethylene signaling is involved in plant response to magnesium and sulfate starvation [94, 95]. Further interplay of hormone and nutrient signaling is summarized in excellent recent reviews [96-98]. Clear evidence of hormone-dependent regulation of nutrient responses or primary metabolism *via* MAPKs have not been obtained yet.

4. Future prospects

Here we presented an overview of the current knowledge of the role of MAPKs in nutritional signaling. MAPK modules are involved in many signaling pathways of life cycle, development and adaptation of plants to environmental stresses. However, their role in the signaling and assimilation of nutrients *in planta* needs to be further evaluated.

 Recent genome-wide analyses will certainly contribute to a better understanding of the role of MAPKs in nutritional signaling both in the identification of the molecular players of the signaling cascades and in the identification of MAPK targets. A better understanding of the regulation of nutrient uptake and assimilation in plants could then be exploited for improvement of crop productivity under changing environmental conditions. Promising results such as the modulation

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of phosphate acquisition and the adjustment of nutrient uptake by oxygen levels by different MAPK pathways (see above), suggest that it may be possible to produce crops with improved nutrient management strategies. Indeed, application of such knowledge to improve plant nutrition is often hindered by complex cross-talk between different signaling pathways. The modulation of specific and optimally placed MAPK may facilitate targeting such crosstalk and help to overcome this major pit fall for the improvement of plant nutrient efficiency. While model organisms are important for efficiently deciphering the persisting mysteries in plant nutritional biology, knowledge is also rapidly evolving in crop species. In view of the improved breeding strategies, for example, the use of CRISPR-CAS9, the engineering of complex signaling pathways using synthetic biological approaches, or the exploitation of natural variation by quantitative genetic approaches, important progress is expected in the near future.

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References

[1] J. Xu, S. Zhang, Mitogen-activated protein kinase cascades in signaling plant growth and development, Trends Plant Sci. 20 (2015) 56-64.

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[2] J. Bigeard, J. Colcombet, H. Hirt, Signaling mechanisms in pattern-triggered immunity (PTI), Mol. Plant. 8 (2015) 521-539.

[3] X. Meng, S. Zhang, MAPK cascades in plant disease resistance signaling, Annu. Rev. Phytopathol. 51 (2013) 245-266.

[4] J. Colcombet, H. Hirt, *Arabidopsis* MAPKs: a complex signalling network involved in multiple biological processes, Biochem. J. 413 (2008) 217-226.

[5] G.R. Kudoyarova, I.C. Dodd, D.S. Veselov, S.A. Rothwell, S.Y. Veselov, Common and specific responses to availability of mineral nutrients and water, J. Exp. Bot. 66 (2015) 2133-2144.

[6] A. Krapp, Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces, Curr. Opin. Plant Biol. 25 (2015) 115-122.

[7] M.C. Rodriguez, M. Petersen, J. Mundy, Mitogen-activated protein kinase signaling in plants, Annu. Rev. Plant Biol. 61 (2010) 621-649.

[8] M. Menges et al., Comprehensive gene expression atlas for the *Arabidopsis* MAP kinase signalling pathways, New Phytol. 179 (2008) 643-662.

[9] R. Doczi, L. Okresz, A.E. Romero, A. Paccanaro, L. Bogre, Exploring the evolutionary path of plant MAPK networks, Trends Plant Sci. 17 (2012) 518-525.

[10] H. Hirt, Connecting oxidative stress, auxin, and cell cycle regulation through a plant mitogen-activated protein kinase pathway, Proc. Natl. Acad. Sci. USA 97 (2000) 2405-2407.

[11] T. Dobrenel et al., TOR signaling and nutrient sensing, Annu. Rev. Plant Biol. 67 (2016) 261-285.

[12] X.F. Dong et al., The SnRK protein kinase family and the function of SnRK1 protein kinase, Int. J. Agric. Biol. 14 (2012) 575-579.

[13] N.G. Halford, S.J. Hey, Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signalling in plants, Biochem. J. 419 (2009) 247-259.

14] J. Yan et al., *Arabidopsis* CIPK14 positively regulates glucose response, Biochem. Biophys. Res. Commun. 450 (2014) 1679-1683.

[15] P. Ragel et al., The CBL-Interacting Protein Kinase CIPK23 regulates HAK5-mediated high-affinity K+ uptake in *Arabidopsis* roots, Plant Physiol. 169 (2015) 2863-2873.

[16] C.H. Ho, S.H. Lin, H.C. Hu, Y.F. Tsay, CHL1 functions as a nitrate sensor in plants, Cell 138 (2009) 1184-1194.

[17] R.J. Tang et al., Tonoplast CBL-CIPK calcium signaling network regulates magnesium homeostasis in Arabidopsis, Proc. Natl. Acad. Sci. USA 112 (2015) 3134-3139.

[18] J. Xu et al., A protein kinase, interacting with two calcineurin B-like proteins, regulates K^+ transporter AKT1 in *Arabidopsis*, Cell 125 (2006) 1347-1360.

[19] K. Ichimura and MAPK group, Mitogen-activated protein kinase cascades in plants: a new nomenclature, Trends Plant Sci. 7 (2002) 301-308.

[20] T. Feilner et al., High throughput identification of potential *Arabidopsis* mitogen-activated protein kinases substrates, Mol. Cell. Proteomics 4 (2005) 1558-1568.

[21] S.C. Popescu et al., MAPK target networks in *Arabidopsis thaliana* revealed using functional protein microarrays, Genes Dev. 23 (2009) 80-92.

[22] W. Hoehenwarter et al., Identification of novel in vivo MAP kinase substrates in *Arabidopsis thaliana* through use of tandem metal oxide affinity chromatography, Mol. Cell. Proteomics 12 (2013) 369-380.

[23] P. Pecher et al., The *Arabidopsis thaliana* mitogen-activated protein kinases MPK3 and MPK6 target a subclass of 'VQ-motif'-containing proteins to regulate immune responses, New Phytol. 203 (2014) 592-606.

[24] S. Li et al., MYB75 Phosphorylation by MPK4 Is Required for Light-Induced Anthocyanin Accumulation in *Arabidopsis*, Plant Cell 28 (2016) 2866-2883.

[25] E. Andreasson et al., The MAP kinase substrate MKS1 is a regulator of plant defense responses, EMBO J. 24 (2005) 2579-2589.

[26] S. Kang, F. Yang, L. Li, H. Chen, S. Chen, J. Zhang, The *Arabidopsis* transcription factor BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1 is a direct substrate of MITOGEN-ACTIVATED PROTEIN KINASE6 and regulates immunity, Plant Physiol. 167 (2015) 1076-1086.

[27] G.R. Lampard, C.A. Macalister, D.C. Bergmann, *Arabidopsis* stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS, Science 322 (2008) 1113-1116.

[28] N.S. Mishra, R. Tuteja, N. Tuteja, Signaling through MAP kinase networks in plants, Arch. Biochem. Biophys. 452 (2006) 55-68.

[29] T. Asai et al., MAP kinase signalling cascade in *Arabidopsis* innate immunity, Nature 415 (2002) 977-983.

[30] H. Wang, N. Ngwenyama, Y. Liu, J.C. Walker, S. Zhang, Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*, Plant Cell 19 (2007) 63-73.

[31] Y. Takahashi, T. Soyano, K. Kosetsu, M. Sasabe, Y. Machida, HINKEL kinesin, ANP MAPKKKs and MKK6/ANQ MAPKK, which phosphorylates and activates MPK4 MAPK, constitute a pathway that is required for cytokinesis in *Arabidopsis thaliana*, Plant Cell Physiol. 51 (2010) 1766-1776.

[32] A. Danquah et al., Identification and characterization of an ABA-activated MAP kinase cascade in *Arabidopsis thaliana*, Plant J. 82 (2015) 232-244.

[33] S.D. Yoo, Y.H. Cho, G. Tena, Y. Xiong, J. Sheen, Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signalling, Nature 451 (2008) 789-795.

[34] C. Zhao, H. Nie, Q. Shen, S. Zhang, W. Lukowitz, D. Tang, EDR1 physically interacts with MKK4/MKK5 and negatively regulates a MAP kinase cascade to modulate plant innate immunity, PLoS Genet. 10 (2014) e1004389.

[35] S.C. Mithoe et al., Attenuation of pattern recognition receptor signaling is mediated by a MAP kinase kinase kinase, EMBO R. 17 (2016) 441-454.

[36] C. Ju et al., CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in *Arabidopsis*, Proc. Natl Acad. Sci. USA 109 (2012) 19486-19491.

[37] L.P. Hamel et al., Ancient signals: comparative genomics of plant MAPK and MAPKK gene families, Trends Plant Sci. 11 (2006) 192-198.

[38] K.P. Rao, T. Richa, K. Kumar, B. Raghuram, A.K. Sinha, In silico analysis reveals 75 members of mitogen-activated protein kinase kinase kinase gene family in rice, DNA Res. 17 (2010) 139-153.

[39] A. Neupane, M.P. Nepal, B.V. Benson, K.J. Macarthur, S. Piya, Evolutionary history of mitogen-activated protein kinase (MAPK) genes in Lotus, Medicago, and Phaseolus, Plant Signal. Behav. 8 (2013) e27189.

[40] J. Wang et al., Genome-wide identification of MAPK, MAPKK, and MAPKKK gene families and transcriptional profiling analysis during development and stress response in cucumber, BMC Genomics. 16 (2015) 386.

[41] Q. Kong et al., The MEKK1-MKK1/MKK2-MPK4 kinase cascade negatively regulates immunity mediated by a mitogen-activated protein kinase kinase kinase in *Arabidopsis*, Plant Cell 24 (2012) 2225-2236.

[42] J.L. Qiu et al., *Arabidopsis* mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1, Plant Physiol. 148 (2008) 212-222.

[43] K. Kosetsu et al., The MAP kinase MPK4 is required for cytokinesis in *Arabidopsis thaliana*, Plant Cell 22 (2010) 3778-3790.

[44] M.A. Khokon et al., Two guard cell mitogen-activated protein kinases, MPK9 and MPK12, function in methyl jasmonate-induced stomatal closure in *Arabidopsis thaliana*, Plant Biol. 17 (2015) 946-952.

[45] G. Bethke et al., Activation of the *Arabidopsis thaliana* mitogen-activated protein kinase MPK11 by the flagellin-derived elicitor peptide, flg22, Mol. Plant Mircrobe Interact. 25 (2012) 471-480.

[46] T. Hruz et al., Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes, Adv. Bioinformatics 2008 (2008) 420-747.

[47] K. Ichimura, T. Mizoguchi, R. Yoshida, T. Yuasa, K. Shinozaki, Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6, Plant J. 24 (2000) 655-665.

[48] J. Lastdrager, J. Hanson, S. Smeekens, Sugar signals and the control of plant growth and development, J. Exp. Bot. 65 (2014) 799-807.

[49] E.A. Vidal, J.M. Alvarez, T.C. Moyano, R.A. Gutierrez, Transcriptional networks in the nitrate response of *Arabidopsis thaliana*, Curr. Opin. Plant Biol. 27 (2015) 125-132.

[50] B. Li, G. Li, H.J. Kronzucker, F. Baluska, W. Shi, Ammonium stress in *Arabidopsis*: signaling, genetic loci, and physiological targets, Trends Plant Sci. 19 (2014) 107-114.

[51] J.F. Briat, H. Rouached, N. Tissot, F. Gaymard, C. Dubos, Integration of P, S, Fe, and Zn nutrition signals in *Arabidopsis thaliana:* potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1), Front. Plant Sci. 6 (2015) 290.

[52] Y. Huang, C.Y. Li, Y. Qi, S. Park, S.I. Gibson, SIS8, a putative mitogen-activated protein kinase kinase kinase, regulates sugar-resistant seedling development in *Arabidopsis*, Plant J. 77 (2014) 577-588.

[53] K. Wingenter et al., A member of the mitogen-activated protein 3-kinase family is involved in the regulation of plant vacuolar glucose uptake, Plant J. 68 (2011) 890-900.

[54] G. Rubin, T. Tohge, F. Matsuda, K. Saito, W.R. Scheible, Members of the LBD family of transcription factors repress anthocyanin synthesis and affect additional nitrogen responses in *Arabidopsis*, Plant Cell 21 (2009) 3567-3584.

[55] P. Wang, Y. Du, Y. Li, D. Ren, C.P. Song, Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in *Arabidopsis*, Plant Cell 22 (2010) 2981-2998.

[56] I. Lassowskat, C. Bottcher, L. Eschen-Lippold, D. Scheel, J. Lee, Sustained mitogen-activated protein kinase activation reprograms defense metabolism and phosphoprotein profile in *Arabidopsis thaliana*, Front. Plant Sci. 5 (2014) article 554.

[57] C. Marchive et al., Nuclear retention of the transcription factor NLP7 orchestrates the early response to nitrate in plants, Nat. Commun. 4 (2013) 1713.

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[58] D. Yan et al., NIN-like protein 8 is a master regulator of nitrate-promoted seed germination in *Arabidopsis*, Nat. Commun. 7 (2016) 13179.

[59] J. Colcombet, C. Sözen, H. Hirt, Convergence of multiple MAP3Ks on MKK3 identifies a set of novel stress MAPK modules, Front. Plant Sci. 7 (2016) 1941.

[60] B.G. Forde, S.R. Cutler, N. Zaman, P.J. Krysan, Glutamate signalling via a MEKK1 kinase-dependent pathway induces changes in *Arabidopsis* root architecture, Plant J. 75 (2013) 1-10.

[61] J. Chiu, R. DeSalle, H.M. Lam, L. Meisel, G. Coruzzi, Molecular evolution of glutamate receptors: a primitive signaling mechanism that existed before plants and animals diverged, Mol. Biol Evol. 16 (1999) 826-838.

[62] B. Lacombe et al., The identity of plant glutamate receptors, Science 292 (2001) 1486-1487.

[63] M.C. Suarez-Rodriguez et al., MEKK1 is required for flg22-induced MPK4 activation in *Arabidopsis* plants, Plant Physiol. 143 (2007) 661-669.

[64] K. Ichimura, C. Casais, S.C. Peck, K. Shinozaki, K. Shirasu, MEKK1 is required for MPK4 activation and regulates tissue-specific and temperature-dependent cell death in *Arabidopsis*, J. Biol. Chem. 281 (2006) 36969-36976.

[65] H. Nakagami, H. Soukupova, A. Schikora, V. Zarsky, H. Hirt, A Mitogen-activated protein kinase kinase kinase mediates reactive oxygen species homeostasis in *Arabidopsis*, J. Biol. Chem. 281 (2006) 38697-38704.

[66] WR Scheible, M. Rojas-Triana, Sensing, signalling, and control of phosphate starvation in plants: molecular players and application, in Annual Plant Reviews Volume 48: Phosphorus Metabolism in Plants (eds W. C. Plaxton and H. Lambers), John Wiley & Sons, Inc., Hoboken, NJ, USA.

[67] L. Lei et al., Activation of MKK9-MPK3/MPK6 enhances phosphate acquisition in *Arabidopsis thaliana*, New Phytol. 203 (2014) 1146-1160.

[68] J. Vadassery et al., A cell wall extract from the endophytic fungus *Piriformospora indica* promotes growth of *Arabidopsis* seedlings and induces intracellular calcium elevation in roots, Plant J. 59 (2009) 193-206.

[69] K. Hiruma et al., Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent, Cell 165 (2016) 464-474.

[70] L. Ye et al., MPK3/MPK6 are involved in iron deficiency-induced ethylene production in *Arabidopsis*, Front. Plant Sci. 6 (2015) article 953.

[71] Y. Liu, S. Zhang, Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis*, Plant Cell 16 (2004) 3386-3399.

[72] L. Han et al., Mitogen-activated protein kinase 3 and 6 regulate *Botrytis cinerea*-induced ethylene production in *Arabidopsis*, Plant J. 64 (2010) 114-127.

[73] G. Li et al., Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in *Arabidopsis*, PLoS Genetics, 8 (2012) e1002767.

[74] E.P. Spalding et al., Potassium uptake supporting plant growth in the absence of AKT1 channel activity: Inhibition by ammonium and stimulation by sodium, J. Gen. Physiol. 113 (1999) 909-918.

[75] F. Aleman, M. Nieves-Cordones, V. Martinez, F. Rubio, Root K^+ acquisition in plants: the *Arabidopsis thaliana* model, Plant Cell Physiol. 52 (2011) 1603-1612.

[76] F. Rubio et al., A low K^+ signal is required for functional high-affinity K^+ uptake through HAK5 transporters, Physiol. Plant. 152 (2014) 558-570.

[77] D. Chinchilla, F. Merchan, M. Megias, A. Kondorosi, C. Sousa, M. Crespi, Ankyrin protein kinases: a novel type of plant kinase gene whose expression is induced by osmotic stress in alfalfa, Plant Mol Biol. 51 (2003) 555-566.

[78] F.F. Chinchilla et al., A mutant ankyrin protein kinase from Medicago sativa affects *Arabidopsis* adventitious roots., Funct Plant Biol, 35 (2008) 92–101.

[79] E.K. Brauer et al., The Raf-like Kinase ILK1 and the high affinity K$^+$ transporter HAK5 are required for innate immunity and abiotic stress response, Plant Physiol. 171 (2016) 1470-1484.

[80] Z. Shahzad et al., A potassium-dependent oxygen sensing pathway regulates plant root hydraulics, Cell 167 (2016) 87-98.

[81] Y. Xing, W. Jia, J. Zhang, AtMKK1 mediates ABA-induced CAT1 expression and H_2O_2 production via AtMPK6-coupled signaling in *Arabidopsis*, Plant J. 54 (2008) 440-451.

[82] F. Takahashi, T. Mizoguchi, R. Yoshida, K. Ichimura, K. Shinozaki, Calmodulin-dependent activation of MAP kinase for ROS homeostasis in *Arabidopsis*, Mol. Cell 41 (2011) 649-660.

[83] F. Jammes et al., MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling, Proc. Natl. Acad. Sci. USA 106 (2009) 20520-20525.

[84] H. Nakagami, A. Pitzschke, H. Hirt, Emerging MAP kinase pathways in plant stress signalling, Trends Plant Sci. 10 (2005) 339-346.

[85] R. Shin, D.P. Schachtman, Hydrogen peroxide mediates plant root cell response to nutrient deprivation, Proc. Natl. Acad. Sci. USA 101 (2004) 8827-8832.

[86] R. Shin, R.H. Berg, D.P. Schachtman, Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency, Plant Cell Physiol. 46 (2005) 1350-1357.

[87] D. Hopff, S. Wienkoop, S. Luthje, The plasma membrane proteome of maize roots grown under low and high iron conditions, J. Proteomics 91 (2013) 605-618.

[88] M.R. Bolouri-Moghaddam, K. Le Roy, L. Xiang, F. Rolland, W. Van den Ende, Sugar signalling and antioxidant network connections in plant cells, FEBS J. 277 (2010) 2022-2037.

[89] D. Ortiz-Masia, M.A. Perez-Amador, J. Carbonell, M.J. Marcote, Diverse stress signals activate the C1 subgroup MAP kinases of *Arabidopsis*, FEBS Lett. 581 (2007) 1834-1840.

[90] Y.H. Cho, S.D. Yoo, ETHYLENE RESPONSE 1 histidine kinase activity of *Arabidopsis* promotes plant growth, Plant Physiol. 143 (2007) 612-616.

[91] W. Wang, A.E. Hall, R. O'Malley, A.B. Bleecker, Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from *Arabidopsis* is not required for signal transmission, Proc. Natl. Acad. Sci. USA 100 (2003) 352-357.

[92] G.B. Zhang, H.Y. Yi, J.M. Gong, The *Arabidopsis* ethylene/jasmonic acid-NRT signaling module coordinates nitrate reallocation and the trade-off between growth and environmental adaptation, Plant Cell, 26 (2014) 3984-3998.

[93] D. Zheng, X. Han, Y.I. An, H. Guo, X. Xia, W. Yin, The nitrate transporter NRT2.1 functions in the ethylene response to nitrate deficiency in *Arabidopsis*, Plant Cell Environ. 36 (2013) 1328-1337.

[94] M. Liu et al ., Ethylene and nitric oxide interact to regulate the magnesium deficiency-induced root hair development in *Arabidopsis*, New Phytol. 213 (2016) 1242-1256.

[95] G. Moniuszko, Ethylene signaling pathway is not linear, however its lateral part is responsible for sensing and signaling of sulfur status in plants, Plant Signal. Behav. 10 (2015) e1067742.

[96] G. Krouk, Hormones and nitrate: a two-way connection, Plant Mol. Biol. 91 (2016) 599-606.

[97] H. Rouached, A.B. Arpat, Y. Poirier, Regulation of phosphate starvation responses in plants: signaling players and cross-talks, Mol. Plant 3 (2010) 288-299.

[98] J. Hanson, S. Smeekens, Sugar perception and signaling-an update, Curr. Opin. Plant Biol. 12 (2009) 562-567.

Figures

Fig. 1. Overview of MAPK general cascade. Classification of kinases has been established based on sequence homology. The MAPK cascade starts with the perception of a stimulus via receptor- or Receptor-Like- Kinase (G-Proteins, RLK, MAPKKKK) and activates the transduction of the signal via phosphorylation of MAPKKK, MAPKK, and MAPK (phosphorylation sites are indicated). During signal transduction several kinases could be phosphorylated at each step of the MAPK cascades to provide diversification and redundancy and to facilitate convergence and divergence of

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signaling pathways. The final substrates of the MAPK modules and in particularly for MPK3/4/6 include transcription factors, enzymes or nucleus- and cytoskeleton- proteins (summarized from [1,4,14,15])

Fig. 2. Expression profiles of *Arabidopsis* MAPK genes in response to nutritional stimuli. Data were taken from GENEVESTIGATOR database [20], analyzing datasets of wild type plants (Col-0 and WS) with at least three replicates. Expression levels are shown (A) for the 20 MEKK-like MAPKKKs, (B) for MAPKKs and (C) for MAPKs. Only differential expression with $\log_2$ ratio > 1,5 with a p value < 0,05 are taken into account. Results are presented as $\log_2$ ratios with a color code (blue= down- and red= up-regulated, respectively).

Fig 3. Overview of MAPK roles during nutrient signaling and primary metabolism. MAPKs participate in the responses to sugars, nitrate, glutamate, phosphate, iron and potassium and regulate the transport of glucose and potassium. The different pathways are described in section 3.2. MAPKKK, MAPKKs and MAPKs are indicated in red, green and brown, respectively.
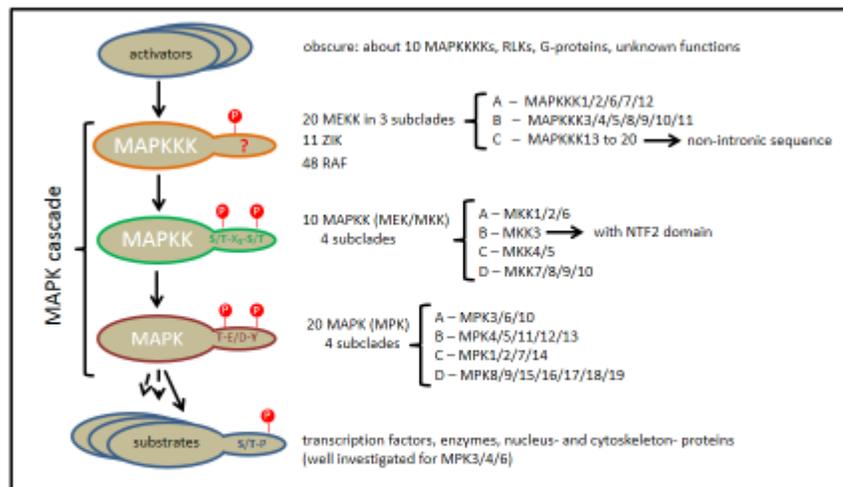
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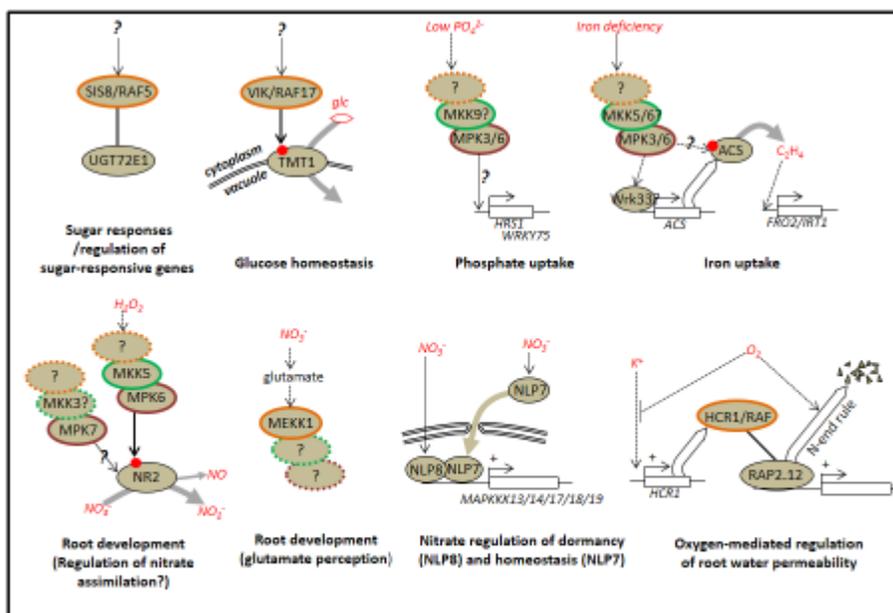obscure: about 10 MAPKKKKs, RLKs, G-proteins, unknown functions

20 MEKK in 3 subclades

A – MAPKKK1/2/6/7/12
B – MAPKKK3/4/5/8/9/10/11
C – MAPKKK13 to 20 → non-intronic sequence

11 ZIK
48 RAF

10 MAPKK (MEK/MKK)
4 subclades

A – MKK1/2/6
B – MKK3 → with NTF2 domain
C – MKK4/5
D – MKK7/8/9/10

20 MAPK (MPK)
4 subclades

A – MPK3/6/10
B – MPK4/5/11/12/13
C – MPK1/2/7/14
D – MPK8/9/15/16/17/18/19

transcription factors, enzymes, nucleus- and cytoskeleton- proteins
(well investigated for MPK3/4/6)

Fig. 1.

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Fig. 2

Figure 3.