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Protein Phosphorylation and Cellular Information Transfer: Signaling by MAP Kinase Cascades

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Summary. Living cells, unicellular organisms as well as cells of multicellular organisms, are permanently exposed to a multitude of signals. Cells have to transform these external stimuli into physiological intelligible signals that are transduced from outside of the cell into the cell to induce a proper cellular response. Extracellular stimuli are perceived and internalised by various cellular receptors. Subsequently, signals are transduced by one of many protein kinase signaling cascades. Mitogen-activated protein kinases (MAPKs) belong to the evolutionary most conserved class of such molecular switches. MAPKs can change the activity of target proteins and thereby bring about physiological responses to external signals. This review discusses the basic principles of MAPK pathways in the context of cellular information processing: Cellular bioinformatics is an increasingly important interdisciplinary field with important implications for basic and applied sciences.

Keywords. Protein phosphorylation; Signal transduction; Mitogen-activated protein kinase.

Introduction

Signal Transduction and Protein Phosphorylation

Reversible protein phosphorylation constitutes a major mechanism of intracellular information transfer. Protein phosphorylation involves the enzyme-catalysed transfer of a phosphate group of *ATP* (adenosine triphosphate) to the hydroxyl group of a serine, threonine, or tyrosine residue of a protein. This reaction is catalysed by protein kinases while the reverse reaction, the hydrolytical cleavage of phosphate groups from proteins (dephosphorylation), is catalysed by protein phosphatases. During intracellular signaling a great many proteins are modified by the covalent

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In memoriam of *H. Ruis*

addition or removal of a phosphate group which affects the properties of a protein including protein activity, protein stability and/or localization within a cell. The changes induced by protein phosphorylation are very fast and thus allow an immediate cellular response to a sudden external stimulus.

Mitogen Activated Protein Kinases (MAPKs)

Various protein kinases participate in cellular signal transduction. Mitogen-activated protein kinases (MAPKs) are protagonists of protein kinases that mediate signal transmission. MAPKs constitute a family of serine/threonine protein kinases with a two-lobed structure, where the active site is located at the domain interface. The amino-terminal domains are predominantly composed of β-strands whereas the carboxy-terminal domains are mainly α-helical. The activity of MAPKs is tightly regulated by phosphorylation. In its inactive, unphosphorylated form, the substrate-binding pocket is blocked and some catalytic residues are misaligned [1]. MAPKs are activated by dual phosphorylation on a threonine and tyrosine residue in the activation loop, which induces a conformational change enabling binding of the substrate to the substrate-binding pocket [2]. In addition to the transient enzyme-substrate interaction at the active center, docking interactions are involved in regulating the efficiency and specificity of the enzymatic reaction.

MAPKs are evolutionary conserved in both unicellular and multicellular organisms. They constitute an integral part of diverse signal transduction pathways that convey different extracellular signals to various cellular targets (Fig. 1). In *Saccharomyces cerevisiae*, MAPK pathways control mating, sporulation, pseudohyphal growth, invasive growth, and cell wall integrity as well as osmoregulation. In plants, MAPKs have been implicated in abiotic and biotic stress response, hormone signaling, and cell division. In mammals, MAPKs regulate cell growth, differentiation, and various stress responses (for reviews see for example Refs. [3–5]).

MAPK Signal Transduction Cascades

MAPKs are part of a phosphorylation cascade that is composed of three sequentially activated protein kinases: MAPKKK (MAPK kinase kinase), MAPKK (MAPK kinase) and MAPK (Fig. 1). Upon receptor stimulation at the cellular surface MAPKKKs are activated. MAPKKKs are serine/threonine protein kinases that phosphorylate MAPKKs on serine and/or threonine residues within the conserved $S/T\text{-}X_{3-5}\text{-}S/T$ motif (single letter code) and thereby activate MAPKKs. MAPKKs in turn are dual-specific kinases that phosphorylate MAPKs on a threonine and tyrosine residue at the signature sequence *T-X-Y*. As mentioned above this dual phosphorylation of MAPKs renders the enzyme active. MAPKs are proline directed serine/threonine kinases phosphorylating numerous substrates on serine and/or threonine residues in different cellular compartments. Thus the signal is transduced in the form of a phosphorylation cascade from upstream kinases to downstream targets.

Within a MAPK module (MAPKKK-MAPKK-MAPK) direct enzyme substrate interactions play a critical role: a MAPKKK directly binds to a MAPKK and a MAPKK binds to a MAPK, which itself interacts with distinct protein substrates.

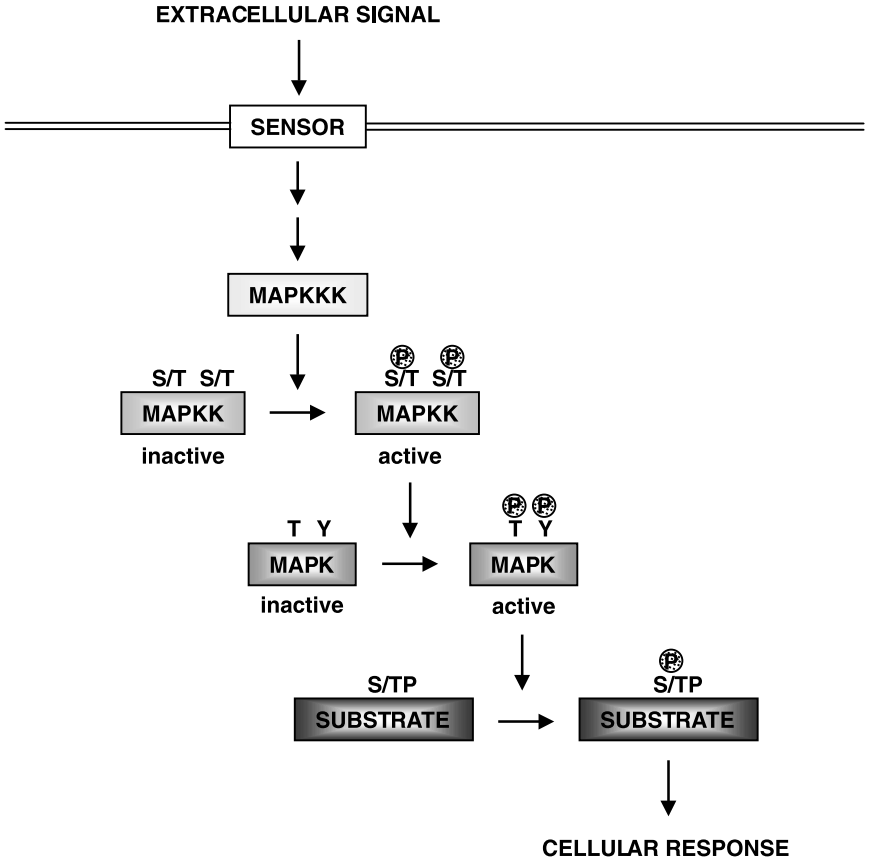

Fig. 1. Simplified representation of a mitogen-activated protein kinase (MAPK) signal transduction cascade. An extracellular signal is perceived by a membrane-located sensor. Subsequent activation of the MAPK cascade can occur via several intermediate steps and different routes. Within the MAPK module the signal is transmitted by sequential phosphorylation events: MAPKKK (MAPK kinase kinase) phosphorylates and thereby activates MAPKK (MAPK kinase), which in turn phosphorylates and activates MAPK. Active MAPK phosphorylates various substrates including transcription factors, cytoskeletal proteins and other kinases that finally bring about cellular response

Additionally, specific docking sites on MAPKs serve for the binding of substrates, activators, and regulators and thus increase the fidelity and efficiency of enzymatic reactions.

Complexity and Specificity of MAPK Cascades

In a single cell several three-component MAPK signaling modules exist in parallel mediating different responses. A cell usually contains numerous members of each component of the MAPK cascade which are organized in distinct MAPK modules. A particular extracellular stimulus activates a specific MAPK module that signals independently to initiate a unique cellular program. However, some components of a MAPK module are shared by different signaling pathways. In budding yeast *S. cerevisiae* five distinct MAPK pathways have been described (for reviews see

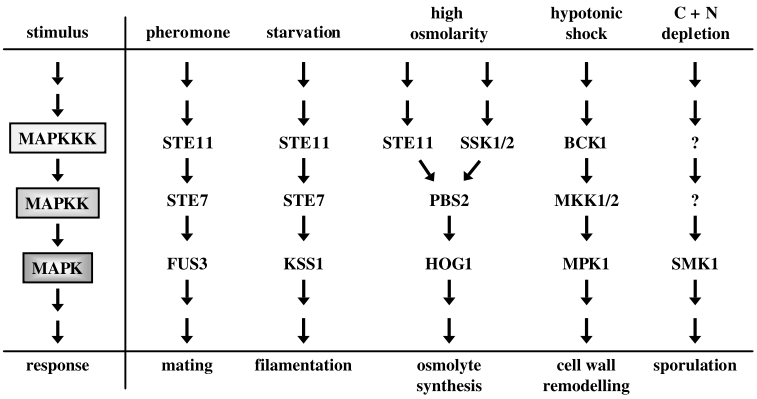

Fig. 2. Schematic overview of yeast MAPK cascades. Multiple MAPK cascades regulate growth and differentiation of *Saccharomyces cerevisiae*. The pheromone response pathway is necessary for haploid yeast cells to mate and is initiated by a peptide pheromone. The signal activates a MAPK cascade: STE11 (MAPKKK) phosphorylates and thereby activates STE7 (MAPKK). Subsequently, STE7 activates FUS3 (MAPK) by phosphorylation leading to induction of mating-specific genes, cell cycle arrest and morphological changes. Starvation of haploid cells induces invasive growth that is, like the mating response, mediated by STE11 and STE7. However, in the filamentation MAPK module, KSS1 (MAPK) is activated to induce pseudohyphal differentiation. High extracellular osmolarity can activate two distinct MAPKKKs, STE11 and SSK1/2. The pathways converge at the level of PBS2 (MAPKK) that phosphorylates and thus activates HOG1 resulting in osmolyte synthesis. In response to hypotonic shock, the maintenance of the integrity of the cell wall is regulated by a the BCK1 (MAPKKK) – MKK1/2 (MAPKK) – MPK1 (MAPK) module. Depletion of carbon and nitrogen induces the activation of SMK1 (MAPK) leading to sporulation of diploid cells

for example Refs. [3, 6, 7]) (Fig. 2). Stimulation of haploid cells with mating pheromone leads to the activation of the STE11 (MAPKKK) – STE7 (MAPKK) – FUS3 (MAPK) phosphorylation cascade, which ultimately results in cell cycle arrest and transcriptional activation of mating-specific genes. Two components of the mating MAPK module, STE11 and STE7, are also involved in the filamentation pathway activating KSS1 (MAPK). Moreover, STE11 is activated by high osmolarity conditions in the PBS2 (MAPKK) – HOG1 (MAPK) cascade. Whereas activation of STE11 by pheromone induces mating-specific genes, genes of the filamentation pathway or the high-osmolarity response are not activated.

How is the specificity of distinct pathways maintained even though individual components participate in more than one signaling pathway? The formation of multi-protein signaling complexes is probably of central importance for insulation of individual MAPK cascades. Signaling molecules can directly bind to each other by protein–protein interaction domains or are tethered by scaffold proteins. Scaffold proteins organize groups of interacting proteins into signaling complexes to facilitate optimal information flow. They specifically bind particular isoforms of MAPK module elements thereby assembling MAPK components appropriately. Accessory molecules as well as specific components of MAPK cascades can serve as scaffolds. In *S. cerevisiae*, a scaffolding function has been identified for STE5 that binds constituents of the mating-response MAPK module [8–10]. Distinct

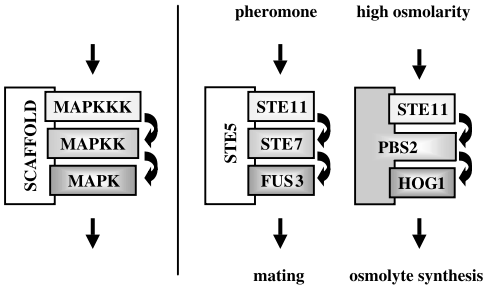

Fig. 3. Organization of MAPK modules by scaffold proteins. In *Saccharomyces cerevisiae*, STE11 (MAPKKK) can function in both the mating and the high osmolarity pathway. During the mating response, the scaffold protein STE5 tethers STE11, STE7 (MAPKK), and FUS3 (MAPK) into a complex. In the high osmolarity pathway, PBS2 (MAPKK) binds STE11 and HOG1 (MAPK) to form a signaling complex

regions of STE5 interact with STE11, STE7, and FUS3 and link the kinase cascade to upstream activators. Similar to STE5, PBS2 organizes a multi-component signaling complex [11]. PBS2 coordinates the osmoregulatory MAPK module binding STE11, HOG1, and the upstream osmosensor SHO1. In contrast to STE5, which is an accessory protein, PBS2 is both a component of the MAPK cascade transducing the high-osmolarity signal and a scaffold protein binding the different components of the MAPK module into a multi-protein complex (Fig. 3). The colocalization of successive members of a MAPK cascade by scaffold proteins does not only increase the local concentration of the assembled components and thereby enhances the efficiency of enzyme-substrate interactions but also limits illegitimate cross-interaction with related MAPK modules. The latter is illustrated by STE11 that is shared by distinct MAPK pathways (Fig. 1). In response to pheromone, STE5 restricts STE11 to phosphorylate STE7 resulting in FUS3 activation and subsequently in mating. However, in response to high extracellular osmolarity, PBS2 binds STE11 and HOG1 leading to synthesis of osmolytes.

MAPK Pathways in Plants

In multicellular organisms, like plants, MAPK signaling is highly complex. A particular extracellular stimulus can activate several different MAPK pathways. On the other hand, a specific MAPK cascade can be activated by a variety of signals and cross-talk between different pathways exists (Fig. 4).

More than one hundred distinct genes encode MAPKs, MAPKKs, and MAPKKs in plants [12], providing the building blocks for a vast number of potential MAPK modules. MAPKs are activated by environmental stress situations as well as by hormones, developmental decisions, and during the cell cycle [5]. In plants, stress-responsive MAPK pathways are best understood and will be presented as an example. Experimental evidence from different plant species indicates that several MAPK pathways are activated by various types of stress. The MAPKs SIMK and SAMK from *Medicago sativa* and their orthologs from tobacco and *Arabidopsis* are activated in response to pathogen-associated stimuli as well as by

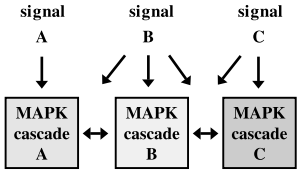

Fig. 4. Schematic depiction of signal convergence, divergence, and cross-talk. A particular stimulus can activate a single or several MAPK pathways. A specific MAPK module can be activated by different signals. Additionally, communication between MAPK pathways is common and can happen at different levels

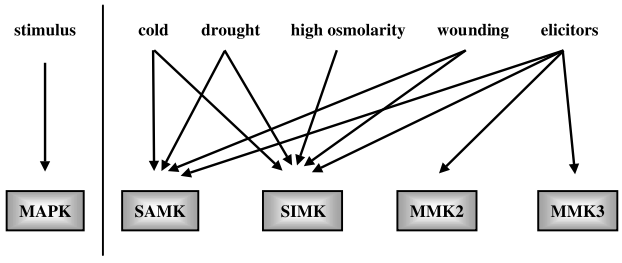

Fig. 5. Stress-activated MAPKs in *Medicago sativa*. Adverse environmental conditions like cold, drought, high osmolarity, wounding, and elicitors differentially activate the MAPKs SAMK, SIMK, MMK2, and MMK3

abiotic stresses including drought, cold, mechanical stimulation, and wounding [13–17]. SIMK and SAMK are both involved in mediating these diverse stresses, but only SIMK is induced by hyperosmotic stress conditions [18] (Fig. 5).

Pathogens constitute a continuous threat for plants. Plants use sophisticated mechanisms for pathogen recognition and induction of defence responses. Thus it is not surprising that two additional alfalfa MAPKs, MMK2 and MMK3, that are insensitve to aboitic stimuli, respond to pathogen-derived elicitors [19] (Fig. 5). The involvement of several MAPK pathways in pathogen response might contribute to the flexibility of plants towards the multitude of pathogens to induce the appropriate response by regulation of gene expression and cytoplasmatic targets. Intriguingly, MMK3 not only plays a role in pathogen-induced signal transduction but also in cell division [20] representing another example for activation of a specific MAPK by distinct signals.

Conclusions

MAPK cascades are part of complex cellular signaling networks. Intracellular signal transduction is not linear but comprises various signaling pathways that are interconnected. In higher organisms, induction of a biological response often requires more than one input signal. Moreover, cells are exposed to different stimuli at the same time. Thus coordination of pathways and integration of signals is necessary for generating an appropriate cellular response. Upon stimulation by a specific or a combination of different signals, MAPK cascades communicate with

other pathways thereby affecting information flow within these pathways. These properties of cellular signaling pathways strongly resemble other complex systems found in nature such as the highly interconnected neuronal networks of the human brain or public transport systems of big cities. Consequently, an understanding of cellular bioinformatics should not only further basic biology or medicine but could also be of value for many other problems of complex systems.

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