Journal of Experimental Botany, Vol. 55, No. 395,
Crosstalk in Plant Signal Transduction Special Issue, pp. 189–198, January 2004
DOI: 10.1093/jxb/erh012 Advanced Access publication 12 December, 2003

Journal of Experimental Botany

From signal to cell polarity: mitogen-activated protein kinases as sensors and effectors of cytoskeleton dynamicity

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Received 7 April 2003; Accepted 29 July 2003

Abstract

Mitogen-activated protein kinases (MAPKs) are ubiquitous phosphorylation enzymes involved in signal transduction, gene expression and activation of diverse cytoskeletal proteins. MAPKs participate in the regulation of a broad range of crucial cellular processes including cell survival, division, polarization, stress responses, and metabolism. Phosphorylation of cytoskeletal proteins usually results in the rearrangement of cytoskeletal arrays leading to morphological changes and cell polarization. On the other hand, some cytoskeletal motor proteins, such as kinesins, could activate MAPK members and participate in signal delivery to the proper cellular destination (e.g. during cell division). Moreover, changes in the integrity of cytoskeletal elements have direct impacts on MAPK activity. Recent evidence suggests that there is bi-directional signalling between MAPK cascades and cytoskeleton. The focus here is on this cross-talk between MAPK signalling and the cytoskeleton in various eukaryotic systems including yeast, plants, and mammals and a role is proposed for MAPKs as sensors monitoring the cytoskeleton-dependent balance of forces within the cell.

Key words: Actin filaments, cytoskeleton, kinesin, microtubules, mitogen-activated protein kinases, signalling, tip-growth.

Introduction

Multicellular organisms acquire their form by control over spatial and temporal patterns of cell division and expansion. For both cycling and differentiating plant cells, signalling to and through a dynamic cytoskeleton as well as the precise regulation of vesicular trafficking, namely exo and endocytosis, are absolutely crucial for the proper assembly and positioning of cytokinetic cell plates and for the maintenance of cell polarity, respectively. Moreover, a dynamic cytoskeleton and vesicle-based membrane traffic are essential for intra and intercellular signalling of multicellular organisms (Mathur and Hülskamp, 2002; Wasteneys and Galway, 2003). Plants are sessile organisms that had to develop strategies to adapt rapidly to changes in environmental conditions. Consequently, molecular components regulating signalling mediated via the cytoskeleton have evolved in plants in order to allow environment-dependent cell-to-cell communication and adaptation to stress.

During the last decade, it was demonstrated that sensing of the environment, via mitogen-activated protein kinase (MAPK) cascades, is involved in the regulation of cytoskeletal rearrangements. Most of these rearrangements are achieved via MAPK-mediated phosphorylation of target cytoskeleton-associated proteins. On the other hand, both stimulated and stressed cells use the cytoskeleton as a sensor for changes during cell division or differentiation resulting in the activation of MAPK

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signalling pathways (Irigoyen *et al*., 1997; Gachet *et al*., 2001). Interestingly, in this respect, cytochalasin D induces in MCF-7 cells newly formed actin aggregates which associate with endosomal marker proteins such as Rab5, paxilin, transferin, and active MAPKs (Mortensen and Larsson, 2003). Moreover, the neuronal cytoskeleton was also identified to serve as a track to deliver signalling endosomes to the proper locations, for example, from synapse towards the nucleus (McPherson *et al*., 2001). Plants and animals alike evolved proteins for calcium-mediated cell-to-cell signalling and regulated exo- and endocytosis. Animal proteins such as annexins, copins, and synaptotagmins (also present in plant but not in yeast cells) directly couple vesicle trafficking to the actin cytoskeleton (Clark *et al*., 2001; Craxton, 2001; Tomsig and Creutz, 2002).

In this review, recent major studies devoted to the cross-talk between the cytoskeleton and MAPKs in mammals and yeast are summarized and discussed. In addition, the current models are compared with recent discoveries in plants linking cell shape, cell division and morphogenesis to the cytoskeleton and MAPK cascades.

Organization of MAPK cascades

MAPKs are one of the best characterized family of signalling molecules in higher plants (Hirt, 2000*a*; Jonak *et al*., 2002). At the biochemical level, there are two possible ways how MAPKs can regulate the activity of other proteins. First, an activated MAPK can phosphorylate, and thereby regulate, the function of nuclear transcription factors or cytoplasmic cytoskeletal components and/or other kinases. Second, other regulatory proteins can influence MAPK signalling through direct physical interaction with MAP kinase components (with or without ensuing phosphorylation).

Compared with other eukaryotes, plants are equipped with much higher numbers of genes encoding MAPK signalling components. Yeast has six and mammals 13 different MAPKs (Hirt, 2000*b*; Meskiene and Hirt, 2000). In *Arabidopsis,* there are at least 20 MAPK, 10 MAPKK and 60 MAPKKK genes (MAPK group, 2002). In all eukaryotic cells, MAPKs are universal signal mediators of diverse extracellular signals. MAPKs belong to the serine/threonine class of protein kinases and are involved in a host of crucial cellular responses leading to cell survival, division or differentiation (Garrington and Johnson, 1999). MAPK signalling pathways are built up from dynamic protein complexes involving MAPK modules composed of three kinases organized in a cascade (Fig. 1). In MAPK modules, the MAPKKK, which is also a serine/threonine kinase, phosphorylates MAPKKs which, in turn, perform T and Y dual phosphorylation of MAPKs. In several cases, this basic module is held together through the scaffolding properties of some MAPKKs (e.g. Pbs2 in yeast), MAPKKKs (e.g. MEKK1 in mammals) or specific scaffold proteins (e.g. MP1 and β-arrestins in mammals) (Fig. 1). Apart from scaffolded MAPK modules, other upstream activators, including MAPKKKKs, protein kinase C, small GTP-ases (Rho, Cdc42, Rac; Rop in plants) and receptor kinases, are important for organizing signalling cascades (Fig. 1). Some of these proteins might also contribute to form signalling complexes of MAPK components with other pathways. Phosphorylation of MAPKs in many cases results in subcellular translocation and subsequent activation of divergent substrate proteins, including transcription factors, other kinases and cytoskeletal proteins. In plants, MAPKs participate substantially in transmitting biotic and abiotic stress, in the control of cell division and developmental processes regulated by hormones and other biologically active compounds, as well as in the plant response to diverse pathogens (Meskiene and Hirt, 2000; Jonak *et al*., 2002). So far, almost nothing is known about plant scaffolds, upstream regulators of MAPK modules and about molecular targets of MAPKs (Asai *et al*., 2002; Nishihama *et al*., 2002; Šamaj *et al*., 2002).

Generally, there is considerable similarity in MAPK cascades between mammalian, yeast and plant cells indicating the ubiquitous nature of this type of signalling mechanism (Fig. 1). Interestingly, all 20 plant MAPKs have highest similarity to the mammalian ERK (extracellular signal-regulated kinase) and no plant homologues of the mammalian p38 and JNK (c-Jun NH_2-terminal kinase) MAPK subfamilies have been found (Hirt, 2000*a*). Besides activation by upstream kinases, the activity and biological output of MAPK signalling pathways is regulated by direct interaction with scaffold proteins and phosphatases. Scaffold proteins are believed to bring specificity into MAPK signalling pathways. Tight control of the subcellular assembly of MAPK components into multiprotein complexes has a significant impact on signalling and is achieved by precise subcellular targeting and recruitment of MAPK modules to various membraneous compartments, for example, the plasma membrane or signalling endosomes. Phosphatases are responsible for the resetting of signalling pathways by dephosphorylation and inactivation of MAPKs (Meskiene *et al*., 1998). In addition, phosphatases can also tether MAPKs in the cytoplasm or within the nucleus (Mattison *et al*., 1999), leading to signal termination (Volmat *et al*., 2001). Importantly, MAPKs, scaffold proteins and phosphatases can shuttle between the nucleus and the cytoplasm.

MAPK signalling and the microtubular cytoskeleton in dividing cells

An association of MAPKs with the microtubular cytoskeleton was found in several mammalian cell types including neurons and oocytes. Here, MAPKs were either

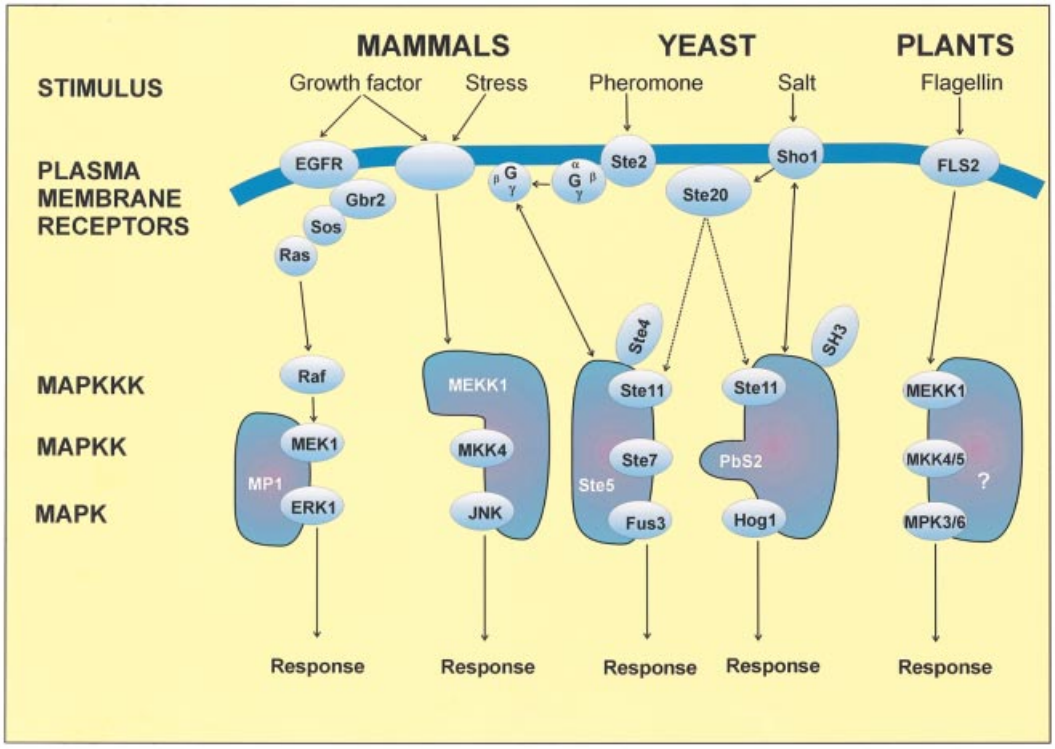

Fig. 1. Scheme of distinct MAPK signalling pathways in mammals, yeast and plants. Note the general similarity in the organization of MAPK pathways in all three eukaryotic systems. MAPKKK, mitogen activated protein kinase kinase kinase; MAPKK, mitogen activated protein kinase kinase; MAPK, mitogen activated protein kinase. Scaffolding proteins (depicted in dark blue) are integrating signalling pathways.

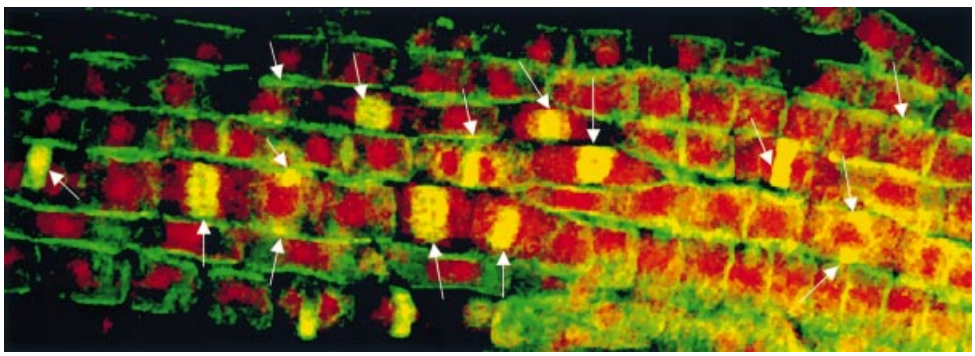

Fig. 2. Immunofluorescence co-localization of microtubules (green, labelled with FITC) and stress-induced MAP kinase SIMK (red, labelled with Texas Red) after taxol treatment of meristematic root cells of *Medicago sativa*. Note the colocalization (yellow, indicated by arrows) of mitotic microtubules (including pre-prophase bands, phragmoplasts and spindles) with SIMK. Cortical microtubules do not colocalize with SIMK.

co-localized with microtubules (Fiore *et al*., 1993; Verlhac *et al*., 1993) or associated with *in vitro* polymerized microtubules (Mandelkow *et al*., 1992). Intriguingly, in dividing fibroblast cells, one-third of the total pool of MAPK was directly associated with microtubules as revealed by immunolocalization and biochemical studies (Reszka *et al*., 1995). Microtubule drugs are widely used in cancer chemotherapy due to their cytostatic effects, and inhibitors of MAPK pathways, such as UO 126 (which specifically blocks the ERK pathway), are also under consideration. In human cancer cell lines, paclitaxel and other microtubule inhibitors including vinblastine, vincristine and colchicine induce the activation of diverse MAPKs including ERK, JNK and p38 (McDaid and Horwitz, 2001). In KB-3 cells, for example, these drugs caused significant activation of JNK with concomitant inactivation of ERK and a reduction in basal p38 MAPK activity, indicating that these three MAPK signalling pathways are co-ordinated during microtubule disruption (Stone and Chambers, 2000). In pig oocytes, activated ERK was localized to the mitotic spindle using an antibody which recognizes phosphorylated ERK. This spindle-

associated active ERK was proposed to play an important role in meiosis during spindle elongation and cleavage furrow formation (Lee *et al*., 2000).

An association of JNK and its upstream MAPKKK MLK2 with microtubules and the microtubular motor kinesin KIF3 was previously demonstrated in mammalian cells (Nagata *et al*., 1998; Zecevic *et al*., 1998). Recently, JIP scaffolding proteins, which interact with components of the JNK signalling pathway, were identified as linkers between kinesins and their vesicular cargoes (Verhey *et al*., 2001).

MAPKs also associate with microtubules in plants. In dividing plant cells of *Medicago* roots, stress-induced MAP kinase (SIMK) was localized to microtubular arrays such as pre-prophase bands (PPBs) and phragmoplasts upon salt stress (Baluška *et al*., 2000*a*). This co-localization of SIMK with mitotic microtubules (PPBs, phragmoplasts and spindles) *in planta* could be enhanced by the stabilization of microtubules by taxol (Fig. 2). These data indicate that plant mitotic microtubules can interact with SIMK in stressed cells. Moreover, both cold treatment and disruption of the microtubular cytoskeleton by oryzalin activated another stress activated MAP kinase (SAMK) in dividing alfalfa suspension cultured cells (Sangwan *et al*., 2002). In addition, other plant MAPKs including alfalfa MMK3 and tobacco NtF6 have been localized to phragmoplasts (Calderini *et al*., 1998; Bögre *et al*., 1999), a microtubule-based cytoskeletal structure driving cytokinesis of plant cells. Recently, it was shown that the tobacco MAPK kinase kinase NPK1 is essential for cytokinetic cell plate formation, which starts in the cell centre and progresses towards the cell periphery (Nishihama *et al*., 2001). This kinase binds specifically to the microtubule-associated kinesin NACK1 that is necessary for the activation and transport of NPK1 to the equatorial region of phragmoplasts (Nishihama *et al*., 2002). NPK1 possesses a functional nuclear localization sequence (NLS) within the NACK1-binding domain. In resting cells, this NLS is active and is targeting NPK1 to nuclei of non-dividing interphase cells (Ishikawa *et al*., 2002). In summary, a number of localization and functional studies in mammals and plants indicate that MAPKs can interact with components of the microtubular cytoskeleton, especially in dividing cells. Yet, only in plants was it demonstrated that kinesins in fact activate MAPKKK and subsequently transport such activated kinase to the proper cellular destination (Nishihama *et al*., 2002).

MAPK signalling and the actin cytoskeleton

MAPKs and actin in mammals

In mammalian cells, disintegration of the actin cytoskeleton by cytochalasin inhibits the activation of two MAPKs, namely ERK and p38 (Tsakiridis *et al*., 1998) indicating that the actin cytoskeleton plays a role in MAPK signalling. Upon stimulation, ERK binds actin and actin-binding proteins, such as calponin and α-actinin (Leinweber *et al*., 1999). Moreover, activated ERK co-localizes with actin bundles in stimulated cells (Khalil *et al*., 1995) and translocates from the cell cortex to actin-rich regions composed of thin actin filaments (Parker *et al*., 1998). Intact actin filaments are required for the propagation of insulin signals and the activation of ERK (Tsakiridis *et al*., 1997). In addition, cortical actin filaments are also necessary for integrin/fibronectin-mediated anchorage of fibroblasts and signalling via ERK upon growth factor stimulation. It was shown that a limited degree of adhesion-mediated cytoskeletal organization regulated by Cdc42 is required for ERK activation by a growth factor (Aplin and Juliano, 1999). Furthermore, actin bundle formation stimulated by collagen, an extracellular matrix molecule, involves ERK activation (Svoboda *et al*., 1999). ERK signalling triggered by lysophosphatidic acid (Della Rocca *et al*., 1999) also requires an intact actin cytoskeleton while cytoskeleton disruption by NO prevents stretch-induced ERK activation (Ingram *et al*., 2000). In addition, cytoskeletal reorganization caused by the actin drug, cytochalasin D, activates the ERK pathway and leads to activation of specific genes (Irigoyen *et al*., 1997).

The mammalian p38 is involved in the recovery from osmotic insult (Takekawa *et al*., 1997). p38 regulates actin turnover by mediating phosphorylation of the actin capping proteins HSP25 and HSP27 belonging to the family of small heat shock proteins (Guay *et al*., 1997). Non-phosphorylated HSP25 monomers bind to the plus ends of actin filaments and prevent actin polymerization. Upon phosphorylation, HSP25 can form oligomers which do not inhibit actin polymerization any more, leading to the stabilization of actin stress fibres (Benndorf *et al*., 1994). Overexpression of wild type and the non-phosphorylatable mutant HSP27 resulted in remarkable changes and remodelling of filamentous actin in the cell cortex which was associated with enhanced or reduced pinocytosis, respectively (Lavoie *et al*., 1993). Moreover, actin stress fibres that are regulated by the phosphorylated status of HSPs become thicker and more abundant in response to hypoxia (Kayyali *et al*., 2002). In most recent studies, it was shown that p38 phosphorylates MK2 (MAPKAP kinase 2) which, in turn, activates HSP27 resulting in a redistribution of the actin cytoskeleton in stimulated cells (Schäfer *et al*., 1998; Kayyali *et al*., 2002).

Another substrate, the regulatory light chain of myosin II, is also phosphorylated by MK2 resulting in actin-mediated Mg-ATPase activity of myosin II (Komatsu and Hosova, 1996). It was also shown that ERK regulates the myosin light chain by enhancing the activity of myosin light chain kinase (MLCK), a Ca/calmodulin-dependent enzyme (Klemke *et al*., 1997), resulting in assembly of

functional myosin motors on actin filaments during cell migration and contraction (Cheresh *et al*., 1999). An increase in myosin light chain phosphorylation by over-expressing a constitutively active form of smooth myosin light chain kinase tMK increased cytoskeletal stiffness and slowed down MAP kinase signalling (Cai *et al*., 1998). Recently, it was reported that ERK phosphorylates tropomyosin which co-localizes with actin and stress fibres upon stimulation of ERK by H_2O_2 or by expression of constitutively active MEK1 (Houle *et al*., 2003). Activated tropomyosin contributes to the formation of actin filaments, increases cellular contractility and promotes the formation of focal adhesions and membrane blebbing.

Frabin, an actin binding protein involved in microspike formation, interacts with actin and induces JNK signalling through Cdc42 activation (Umikawa *et al*., 1999). JNK activity is also required for proper actin dynamics and maturation of actin-rich structures during polarization of *Drosophila* epidermal cells (Kaltschmidt *et al*., 2002). Using Ras mutants, which are able to disrupt the actin cytoskeleton, it was shown that oncogenic Ras can specifically target the actin cytoskeleton and activate the MAPK pathway (Pawlak and Helfman, 2002).

Altogether, these findings indicate that mammalian MAPKs regulate not only the rearrangement of F-actin arrays but also the activity of myosin motors and, in this way, also acto-myosin dependent motility. Except for MAPKs themselves, other upstream members of MAPK pathways can also interact with components of the actin cytoskeleton. For example, mammalian MEKK1, an activator of ERK, p38, JNK, and NF-kB, binds to α-actinin and localizes to actin stress fibres and focal adhesions (Christerson *et al*., 1999).

MAPKs and actin in yeast

In yeast, both the cell wall integrity and the mating pathways are dependent on the actin cytoskeleton and MAPK signalling. The cell wall integrity pathway is regulated by MPK1 and is necessary for the polarization of actin filaments towards weakened cell wall domains (Mazzoni *et al*., 1993; Zarzov *et al*., 1996). MPK1 mutants show phenotypes reminiscent of actin mutants having aberrantly distributed actin cortical spots and accumulated secretory vesicles (Mazzoni *et al*., 1993).

Hog1 (high osmolarity glycerol 1) is a MAPK which regulates the osmolarity response in budding yeast. In addition, Hog1 is also required for the repolarization of the actin cytoskeleton during budding and cell growth after the recovery of yeast cells from osmotic stress (Brewster and Gustin, 1994). Hyperosmotic stress causes rapid and transient disassembly of the actin cytoskeleton (Chowdhury *et al*., 1992) and is necessary for survival after osmotic insult since mutations in actin and actin-associated proteins result in increased osmosensitivity (Botstein *et al*., 1997). Recently, the Ssk2p, one of the three MAPKK kinases of the Hog1 pathway was identified to facilitate actin cytoskeleton recovery after osmotic stress (Yuzyuk *et al*., 2002). An activated form of Hog1 (induced by osmotic insult or actin depolymerization by latrunculin A) is involved in the sensing of damage to the actin cytoskeleton and relocates from the cytoplasm to the septin-enriched bud neck forming a complex with actin. Moreover, Hog1 promotes reassembly of the polarized actin cytoskeleton and resumption of the cell cycle (Yuzyuk *et al*., 2002).

Bem1 of the yeast pheromone pathway interacts with the scaffold protein Ste5, the MAPKKKK Ste20 and actin. Mutants of Bem1 still interact with Ste5 and actin, but not with Ste20, and cause the rearrangement of the actin cytoskeleton during mating, leading to defective polarized morphogenesis and shmoo formation in yeast cells (Leeuw *et al*., 1995). PSK, a novel mammalian Ste20-like kinase is able to regulate both the actin cytoskeleton and the JNK signalling pathway (Moore *et al*., 2000). This kinase is localized to vesicles and causes reduction in abundance of actin stress fibres.

In fission yeast, a mitotic checkpoint monitors integrity of the actin cytoskeleton and proper orientation of the spindle which is dependent on stress-activated MAPK Sty1 (Gachet *et al*., 2001). The molecular target of Sty1 in this mitotic checkpoint remains unknown.

MAPKs and actin in plants

Plants possess higher numbers of genes encoding some cytoskeletal components, for example, there are eight actin genes in *Arabidopsis* (Meagher *et al*., 1999). On the other hand, plants seem to lack several actin-binding proteins known from mammals, such as tropomyosin, vinculin, talin, α-actinin, WASP, and many others (Hussey *et al*., 2002; Meagher and Fechheimer, 2003). In plant cells, disruption of the actin cytoskeleton by latrunculin B causes activation of the alfalfa MAPKs SIMK and SAMK that are involved in abiotic stress responses including osmotic, heat and cold stress (Šamaj *et al*., 2002; Sangwan *et al*., 2002). Interestingly, jasplakinolide, another actin drug which decreases actin turnover and dynamics, also activates SIMK (Šamaj *et al*., 2002) but not SAMK (Sangwan *et al*., 2002). Conversely, UO126, an inhibitor of mammalian MEK1, causes remodelling of the actin cytoskeleton in plant cells (Šamaj *et al*., 2002).

These pharmacological data indicate that MAPKs are involved in the dynamic organization of the actin cytoskeleton. In the activated form, MAPKs probably bind to and regulate components of the actin cytoskeleton. On the other hand, disturbances to the actin dynamics and organization are sensed via MAPK pathways. These mutual interactions highlight the importance of both signalling components: MAPKs and the dynamic actin cytoskeleton also in plant cells.

Exploratory and signalling nature of actin- and MAPK-based tip-growth

There is one common link connecting all the above discussed examples where MAPK cascades and the actin cytoskeleton inherently interact to drive polarity of signal-mediated cellular expansion. This link is a highly polarized cell growth mode, which is also known as a tip-growth, when cells expand strictly locally at well-defined domains (Hepler *et al*., 2001). Examples of effectively navigated tip-growing cells can be found in all eukaryotes. In yeast, filamentous tip-growth is typical for nutritionally stressed cells which start to explore their environment and for mating when mating partners approach each other via tip-growing projections known as shmoos (Gustin *et al*., 1998). In animals and humans, the best example of tip-growing cells are path-finding growth cones of neurons which navigate their growth towards relevant interacting partners (Ming *et al*., 2002). In plants, there are two distinct examples of tip-growing cells (Hepler *et al*., 2001). First, pollen tubes are able rapidly to overcome large distances by growing through female tissues in order to find and fuse with fertilization-competent ovules (Palanivelu and Preuss, 2000). Second, tip-growing root hairs search for well-watered and oxygen-rich soil portions to satisfy the high nutritional demands of higher plants (Jungk, 2001). All these cells perform signal-mediated exploratory tip-growth (Kirschner and Gerhart, 1998; West-Eberhard, 1998) which is navigated towards well-defined targets. Obviously, signals perceived at the cell periphery are transduced towards the actin cytoskeleton via MAPK cascades in tip-growing cells (Gustin *et al*., 1998; Grewal *et al*., 1999; Wu *et al*., 2001; Adams and Sweatt, 2002; Šamaj *et al*., 2002).

Sustained activity of ERK is necessary for the initiation of neurite growth (Marshall, 1995; Schmid *et al*., 2000). Both MAPK activity and a dynamic actin cytoskeleton, regulated by the Arp2/3 complex, are required for the growth of axons and dendrites and chemotactic guidance of nerve growth cones by guidance factors, such as netrin-1 or the brain-derived neurotrophic factor (Goldberg *et al*., 2000; Ming *et al*., 2002). Yeast cells respond by wall remodelling and filamentous growth when human MEK1 and ERK1 are overexpressed (Atienza *et al*., 2000). In neurons, synaptic signal transfer requires vesicular trafficking and vesicle-associated filamentous actin was shown to play a scaffolding role for regulatory molecules in the nerve terminal (Halpain, 2003; Sankaranarayanan *et al*., 2003).

In yeast, MPK1 promotes polarized cell growth during the formation of mating projections of haploid cells upon pheromone treatment (Zarzov *et al*., 1996). During mating, an example of cell-to-cell interaction in unicellular yeast, the Fus3 and its upstream kinase Ste7 are located to the tips of protruding mating projections (van Drogen *et al*., 2001) which are enriched with a fine mesh of actin filaments (Evangelista *et al*., 1997). Using fluorescence recovery after photobleaching (FRAP), it was demonstrated that Fus3 shuttles between the nucleus and the cytoplasm independently of its phosphorylation status, stimulation by pheromone, and interaction with Ste5 (van Drogen *et al*., 2001). Kss1 is another yeast MAPK that is involved in polar pseudohyphal growth and is induced by an invasive search for nutrients during nitrogen starvation (Mösch *et al*., 1996; Cook *et al*., 1997; Madhani *et al*., 1997).

In plant pathogenic fungi, MAPKs are involved in the formation and polar growth of both conidia and appressoria (Xu and Hamer, 1996). Fungi carrying mutations in MAPK genes are unable to form functional appressoria resulting in the loss of pathogenicity (Xu *et al*., 1998; Ruiz-Roldan *et al*., 2001; Kojima *et al*., 2002).

In plants, it was shown that the correct localization and activity of the stress-induced MAP kinase, SIMK, depends on the intact actin cytoskeleton in growing root hairs of *Medicago sativa* (Šamaj *et al*., 2002). Before the onset of root hair formation, most of the SIMK in trichoblasts is located in the nucleus as revealed by immunolabelling and the *in vivo* localization of GFP-tagged SIMK (J Šamaj, L Bögre, H Hirt; unpublished results). During root hair formation, SIMK becomes redistributed to growing root hair tips possessing dense meshworks of actin filaments (Baluška *et al*., 2000*b*; Šamaj *et al*., 2002). Importantly, SIMK is present in its activated form at root hair tips. Actin drugs which interfere with polymerization rates of F-actin, such as latrunculin B and jasplakinolide, cause growth inhibition and removal of both the F-actin meshwork and SIMK from tips of root hairs (Šamaj *et al*., 2002). Latrunculin B depolymerizes F-actin by sequestering G-actin monomers from the cellular actin pool (Baluška *et al*., 2000*b*; Hepler *et al*., 2001; Vidali *et al*., 2001). For jasplakinolide, both F-actin stabilization (Holzinger and Meindl, 1997; Sawitzky *et al*., 1999; Holzinger, 2001; Šamaj *et al*., 2002) and/or disruption of F-actin arrays due to abberant polymerization (Sawitzky *et al*., 1999; Ou *et al*., 2002) were reported in algal and plant cells depending most likely on the cell type and drug concentration. Upon jasplakinolide treatment of root hairs, a considerable part of SIMK co-localizes with thick actin cables. Both actin drugs also cause the activation of SIMK in dividing suspension cells. Plants overexpressing gain-of-function SIMK, which is constitutively active, show a phenotype of longer root hairs which emerge earlier than in control plants. Inhibition of MAPK activity by the inhibitor UO126 results in root hair growth inhibition accompanied by the redistribution of both F-actin and SIMK. Tip-focused activated SIMK and dynamic actin filaments seem to be essential for sustained root hair growth (Šamaj *et al*., 2002). Moreover, recent fluorescence recovery after photobleaching (FRAP) experiments

revealed that SIMK is undergoing shuttling between the nucleus and the tip region of growing root hairs (J Šamaj, L Bögre, H Hirt; unpublished results). These results suggest that SIMK might sense changes in the cytoskeleton and participate in the control of vesicular trafficking. These observations also indicate that SIMK alone, or together with other MAPKs, for example, SAMK, might be necessary for the dynamic maintenance of the balance of forces, which are disturbed during bulge initiation by the local weakening of cell walls resulting in the outgrowth of root hairs.

Are end-poles of elongating cells plant-like synaptic domains?

Neuronal synapses remotely resemble tip-growing domains via actin-dependent and calcium-regulated vesicle trafficking events. Synapses are defined as asymmetric adhesion domains specialized for rapid cell-to-cell communication (Dustin and Cooper. 2000; Dustin and Colman, 2002). Originally, this term was used exclusively for neuronal cells. Currently, the use of this term is getting wider and diverse cell types are considered to establish a synaptic-type of adhesive domains specialized for cell-to-cell communication. For instance, the term 'synapse' has been extended to other cell-to-cell contacts, including those between neurons and muscle cells, and even between non-neuronal cells of which the 'immunological synapse' is the best understood (Dustin and Cooper, 2000; Dustin and Colman, 2002).

The major difference between synapses and tip growing cells is that abundant exocytic events are fully balanced with abundant endocytic events in the case of non-growing synapses (Shupliakov *et al*., 2002). Importantly, both MAPK and the actin cytoskeleton are essential components of synapses (Wu *et al*., 2001; Adams and Sweatt, 2002). In fact, neuronal synapses represent the most advanced model for studies of the actin cytoskeleton and calcium-mediated regulation of exo-and endocytosis (Morales *et al*., 2000; Colicos *et al*., 2001; Shupliakov *et al*., 2002). An actin-based cytomatrix was found to be important for the scaffolding of regulatory signal molecules during vesicular trafficking in neuronal synapses (Halpain, 2003; Sankaranaraynan *et al*., 2003). Recently, it has been suggested that actin-enriched non-growing end-poles of elongating plant cells bear many similarities to neuronal synapses (Baluška *et al*., 2003*a*, *b*). They are enriched with both actin and unconventional myosin VIII and perform abundant recycling events of vesicles carrying putative auxin transporters and possibly also of auxin itself, implicating that auxin represents a plant neurotransmitter-like growth regulator (Baluška *et al*., 2003*b*).

Conclusions and perspectives

During the last decade, it has become obvious that cross-talk between the cytoskeleton and MAPK signalling pathways is important for controlling crucial cellular activities, such as cell division and polarized growth. MAPKs not only regulate the dynamic behaviour of the cytoskeleton via phosphorylation of cytoskeleton-associated proteins, but are also activated themselves by cytoskeletal proteins (e.g. by kinesins) and by changes in the cytoskeletal organization. However, cytoskeletal targets of activated MAPKs are unknown in plants and only little is known in other organisms. Since the cytoskeleton is the major player for controlling the cellular architecture, MAPKs should be considered as possible candidates for a surveillance apparatus, sensing the balance of forces within cells.

Other recent studies connect motor proteins, such as kinesins and myosins, to MAPK signalling pathways. While MAPKs regulate motor activity of myosins in mammals, it still remains to be determined whether MAPKs activate plant myosins and, eventually, use their motor activity for targeting of MAPK complexes to proper subcellular locations. Components of MAPK signalling pathways associate with kinesins in mammalian and plant cells, but it is not clear whether MAPKs can activate kinesins. Motor proteins are also considered to be molecular linkers between actin and microtubular cytoskeletons and, therefore, could participate in signal transfer between these two crucial cytoskeletal structures. Clearly, this is only the beginning of appreciating complex cross-talks between signalling and cytoskeletal systems and further studies will be necessary to unveil the interplay between signal transduction and the cytoskeleton in a functional context.

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