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REVIEW

Scaffold proteins of MAP-kinase modules

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Mitogen-activated protein kinases (MAPKs) regulate critical signaling pathways involved in cell proliferation, differentiation and apoptosis. Recent studies have shown that a novel class of scaffold proteins mediates the structural and functional organization of the three-tier MAPK module. By linking the MAP3K, MAP2K and MAPK into a multienzyme complex, these MAPK-specific scaffold proteins provide an insulated physical conduit through which signals from the respective MAPK can be transmitted to the appropriate spatiotemporal cellular loci. Scaffold proteins play a determinant role in modulating the signaling strength of their cognate MAPK module by regulating the signal amplitude and duration. The scaffold proteins themselves are finely regulated resulting in dynamic intra- and inter-molecular interactions that can modulate the signaling outputs of MAPK modules. This review focuses on defining the diverse mechanisms by which these scaffold proteins interact with their respective MAPK modules and the role of such interactions in the spatiotemporal organization as well as context-specific signaling of the different MAPK modules.
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Introduction

Cells respond to various intracellular and extracellular cues by eliciting specific responses that are mediated by a complex array of signaling networks. These signaling networks, consisting of distinct as well as overlapping signaling pathways, involve distinct ligands, receptors, G proteins, small guanosines triphosphatases, kinases and transcription factors. In many instances, the same or similar signaling molecules are used to elicit distinctly different functional responses by these signaling pathways (Gutkind, 1998; Dhanasekaran and Reddy, 1998; Hunter, 2000). In spite of such shared use of signaling molecules, the signaling networks are precisely regulated with minimal or no crossover with adjacent signaling pathways. Defining the mechanism(s) by which an individual pathway is insulated from the 'noise' or the 'signal drifting' from the adjacent signaling pathways is, thus, of crucial interest. Especially, this is critical in signaling pathways leading to the activation of mitogen-activated protein kinase (MAPK) modules that are involved in different aspects of cell growth including oncogenesis (Faux and Scott, 1996; Fanger *et al*., 1997; Minden and Karin, 1997; Pawson and Scott, 1997; Dhanasekaran and Reddy, 1998; Gutkind, 1998; Whitmarsh and Davis, 1998; Garrington and Johnson, 1999; Davis, 2000; Chang and Karin, 2001; Morrison and Davis, 2003; Park *et al*., 2003; Qi and Elion, 2005; Whitmarsh, 2006).

MAPK modules consist of three distinct kinases, namely an upstream MAP kinase kinase kinase (MAP3K), a MAP kinase kinase (MAP2K), and a downstream MAPK (Faux and Scott, 1996; Fanger *et al*., 1997; Minden and Karin, 1997; Pawson and Scott, 1997; Dhanasekaran and Reddy, 1998; Gutkind, 1998; Whitmarsh and Davis, 1998; Garrington and Johnson, 1999; Davis, 2000; Chang and Karin, 2001; Morrison and Davis, 2003; Park *et al*., 2003; Raman and Cobb, 2003; Qi and Elion, 2005; Whitmarsh, 2006). In a typical signaling pathway activated by growth or stress stimuli, Ras- or Rho-family of small GTPases stimulate the activity of an upstream MAP kinase kinase kinase kinase (MAP4K), which in turn stimulates a specific three-tier MAPK module via the sequential phosphorylation of constituent MAP3K, MAP2K and MAPK (Minden and Karin, 1997; Dhanasekaran and Reddy, 1998; Davis, 2000; Chang and Karin, 2001; Morrison and Davis, 2003; Raman and Cobb, 2003). The MAPKs, thus activated, translocate from the cytoplasm to the nucleus where they regulate target transcription factor activity through phosphorylation (Su and Karin, 1996; Dhanasekaran and Reddy, 1998; Khokhlatchev *et al*., 1998; Brunet *et al*., 1999; Garrington and Johnson, 1999; Davis, 2000; Chang and Karin, 2001; Pearson *et al*., 2001; Huang *et al*., 2004). Thus, the phosphorelay system defined by the three-tier MAP3K–MAP2K–MAPK modules regulates diverse aspects of cell growth, differentiation and apoptosis. Of the different MAPK modules that have been identified, the ones involving extracellular signal-regulated kinase (ERK)-, c-Jun N-terminal kinase (JNK)- and p38MAPK are well characterized (Fanger *et al*., 1997; Dhanasekaran and Reddy, 1998; Whitmarsh and Davis, 1999; Chang and Karin, 2001; Pearson *et al*., 2001;

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Volmat and Pouyssegur, 2001). In many instances, these MAPK modules have been shown to regulate distinctly different cellular responses in a cell type and/or context-specific manner (Whitmarsh and Davis, 1998; Garrington and Johnson, 1999; Karandikar and Cobb, 1999; Burack and Shaw, 2000). Only recently, the unique role of scaffold proteins in providing a molecular mechanism for such context-specific signaling by MAPKs is being understood (Whitmarsh and Davis, 1998; Garrington and Johnson, 1999; Karandikar and Cobb, 1999; Burack and Shaw, 2000). In many instances, the insulation as well as context-specific signaling of MAPK-signaling modules is facilitated by specific scaffold proteins that provide a signaling conduit by assembling a discrete set of signaling proteins into a complex (Faux and Scott, 1996; Pawson and Scott, 1997; Garrington and Johnson, 1999; Davis, 2000; Ferrell, 2000; Morrison and Davis, 2003; Park *et al*., 2003; Kolch, 2005; Qi and Elion, 2005; Whitmarsh, 2006). By bringing the signaling partners in close proximity, these proteins enable the rapid transmission of signals within the MAPK module. In addition to an anchoring role, emerging studies from yeast as well as mammalian cells indicate that these proteins also play a catalytic role in the respective signaling pathways. They play a major role in insulating kinase modules from each other so that crosstalk between them can be minimized. More interestingly, studies from the scaffold proteins of *Saccharomyces cerevisiae* indicate that different scaffold proteins can recruit a specific kinase module so that it can be localized to different regions of the cells to carry out different functional responses (Elion, 2001; Elion *et al*., 2005; Qi and Elion, 2005). In this review, we analyse our present understanding of the scaffold proteins that are associated with MAPKs and their possible 'catalytic', 'anchoring' and/or insulating roles in different signaling pathways.

Scaffold proteins in yeast MAP-kinase signaling modules

The earliest evidence for scaffold proteins in MAPK modules came from studies analysing the signaling pathways in the budding yeast *S. cerevisiae* (Gartner *et al*., 1992; Zhou *et al*., 1993; Choi *et al*., 1994; Kranz *et al*., 1994; Marcus *et al*., 1994; Printen and Sprague, 1994). In *S. cerevisiae*, the three-tier kinase module involved in pheromone signaling consists of a MAP3K Ste11, a MAP2K Ste7 and a MAPK, Fus3 (Herskowitz, 1995; Elion, 2001; Elion *et al*., 2005). As shown in Figure 1, in the haploid cells of *S. cerevisiae*, the mating pheromones stimulate the pheromone receptors. A pheromone-activated receptor stimulates a heterotrimeric G protein by catalyzing the exchange of guanine nucleotides in the α-subunit, Gpa1. The GTP-bound

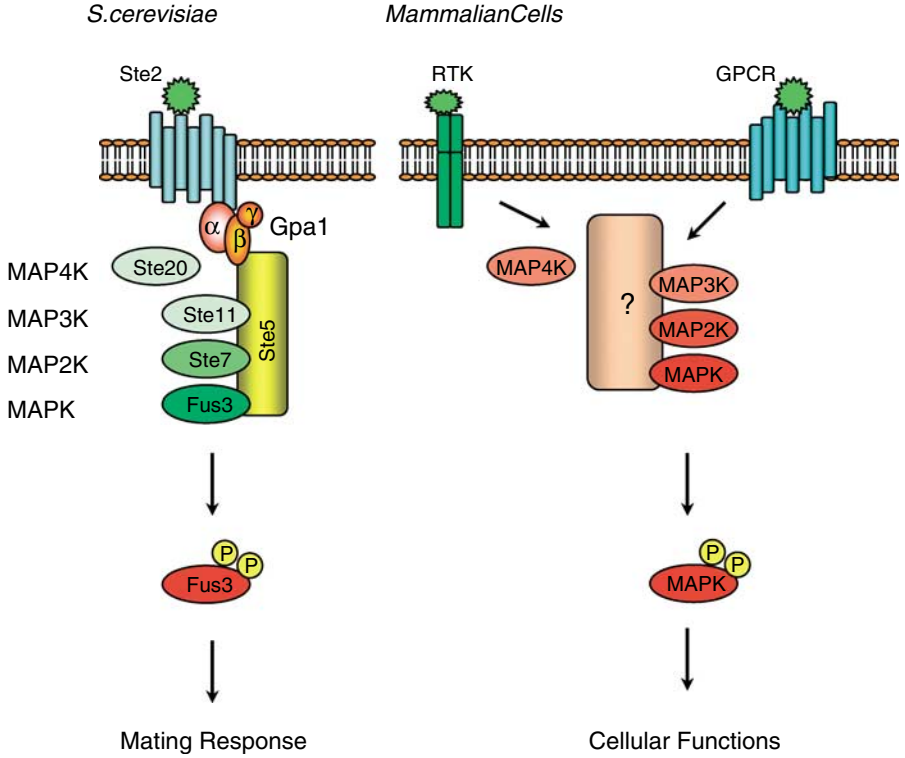

Figure 1 An archetypal MAPK scaffold involving Ste5 in *Scerevisiae*. Scaffolding proteins such Ste5 of *S. cerevisiae* is involved in assembling the three-tier kinases consisting of MAP3K, MAP2K and MAPK into a signaling module. In this canonical MAPK scaffold, Ste5 links the heterotrimeric G protein, Gpa, specifically the $G\beta\gamma$ subunit (Ste4/Ste18), to the Ste11-Ste7-and Fus3 kinase module in response to the activation of the GPCR Ste2/3 by the mating pheromones. Although such a module that can tether a GPCR to MAPK module has not been identified to data, it is likely that the organization of 'nested scaffolds' (Kolch, 2005) or modular arrangement of co-operating scaffolds (Vomastek *et al*., 2004) can provide more versatile adaptable scaffolding in mammalian cells (see text for details).

α-subunit and the βγ-subunit (Ste4 and Ste18, respectively) disassociate and the disassociated βγ-subunit activates Ste20 kinase, a MAP4K analogous to the mammalian p21-associated kinase (PAK). Ste20 kinase activates the three-tier MAPK module defined by Ste11, Ste7 and Fus3. Initial studies indicated that a scaffold protein encoded by *Ste5* is critically required for this pathway and mutation of Ste5 completely abolished the mating response of *S. cerevisiae* (Choi *et al*., 1994; Marcus *et al*., 1994; Printen and Sprague 1994). Further analyses of Ste5 using yeast two-hybrid assays and copurification studies indicated that different domains of Ste5 specifically interact with Ste11, Ste7 and Fus3. It has also been shown that Ste5 interacts with the βγ-subunit defined by Ste4 and Ste18 through the cystein-rich N-terminal Ring-H2 domain (Sette *et al*., 2000). Interestingly, the N-terminal Ring-H2 domain is also involved in the pheromone-dependent dimerization of Ste5, which has been shown to be essential for the signaling pathway (Feng *et al.,* 1998). In conclusion, pheromones through the pheromone receptor stimulate the association of βγ-subunit to Ste5 with the subsequent Ste5-dependent phosphorylation of βγ (Feng *et al*., 1998). The binding of the βγ-subunit induces conformational changes in Ste5 that promotes the binding of Ste11, Ste7 and Fus3 as well as optimizing the orientation of Ste11 to be phosphorylated by Ste20 so that the phosphorylation cascade can be initiated (Feng *et al*., 1998; Qi and Elion, 2005). The phosphorylated Fus3 translocates to the nucleus where it stimulates the transcriptional activation of Ste12-dependent genes (Elion, 2001; Qi and Elion, 2005).

However, the MAP4K, MAP3K and MAP2K of this kinase module, namely Ste20, Ste11 and Ste7, have also been identified to be involved in the regulation of other signaling pathways such as vegetative growth, invasive growth, starvation and osmolarity responses (Qi and Elion, 2005). It has been noted that in the absence of pheromone signal, a MAP-kinase module involving STE20–Ste11–Ste7–KSS1 is activated during vegetative growth as well as during starvation (Elion, 2001). Analyses of the signaling events involved in these responses indicated that Ste5 plays a crucial role in coordinating pheromone-dependent assembly of STE20–Ste11–Ste7–Fus3 module. Although both Fus3 and Kss1 kinases share the upstream kinases STE20, Ste11 and Ste7, the activation of one pathway does not crosstalk with the other, primarily owing to the insulatory role of Ste5. In addition, it appears that there are other scaffold proteins such as Spa2p and Sph1p that can provide scaffolding function in these alternate MAP kinase pathways (Roemer *et al*., 1998). Thus, these studies provide evidence to the critical role of scaffold proteins in facilitating specific signaling conduits through which the signals can be transmitted to the intended target specifically and rapidly with little or no crosstalk (Elion, 2001; Ptashne and Gann, 2003: Flatauer *et al*., 2005). Ste5 is not recruited.

In addition to the distinct scaffold proteins, in some instances, the kinases themselves may fulfill a role of scaffold protein. For example, during the osmoadaptive pathway of *S. cerevisiae*, the osmosensor Sho1 or SLN1 is activated and the activated osmosensor stimulates the Hog1 MAP kinase module through PBS2, a MAP2K (Posas *et al*., 1998). Interestingly, in this pathway, PBS2 appears to provide a scaffolding role by binding to both the upstream MAP3K, Ste11 and the downstream MAPK, Hog1 (Posas and Saito, 1997). It should be noted here that the osmosensors Sho1 and SLN1 activate PBS2-Hog1 through two different upstream kinases. Although SLN1 activates PBS2-Hog1 through SSK2/SSK22 MAP3K, SHO1 activates PBS2-Hog1 through the MKKK, Ste11. It appears that the differential docking by PBS2 determines its coupling to the upstream SSK2/SSK22 or STE11. Thus, PBS2 can assemble the MAP3K, SSK2/SSK22 and Hog1 during SLN1 activation, whereas it can link STE11 and Hog2 upon SHO1 activation (Posas and Saito, 1997; Tatebayashi *et al*., 2003). Moreover, other regulatory proteins such as YPD1, SSK1 (in the case of SLN1 pathway) and STE50 (in the case of SHO1 pathway) may play a predominant role in signal insulation between these closely related pathways (Tatebayashi *et al*., 2003).

Scaffold proteins in mammalian MAPK signaling modules

From the observation that a disproportionately large number of MKKKs are present to regulate a limited number of MAPKs in mammalian cells (Uhlik *et al*., 2004), it has been inferred that the scaffold proteins facilitate the context-specific assembly of different MAP3Ks, MAP2Ks and MAPKs. To date, nine distinct MAPK modules have been identified. They are ERK1/2, ERK3, ERK4, ERK5, ERK6/p38MAPKγ, ERK7, ERK8, JNK1/2/3 and p38MAPKα/β/δ (Bogoyevitch and Court, 2004; Zarubin and Han, 2005; Bogoyevitch, 2006; Coulombe and Meloche 2006). Of these different MAPK modules, ERK1/2-, JNK1/2/3- and p38MAPKα/β/γ/δ-modules have been well characterized (Faux and Scott, 1996; Pawson and Scott, 1997; Garrington and Johnson, 1999; Davis, 2000; Ferrell, 2000; Morrison and Davis, 2003; Park *et al*., 2003; Kolch, 2005; Qi and Elion, 2005; Whitmarsh, 2006). Consistent with the major interest in characterizing these three groups of MAPK modules, many of the scaffold proteins identified to date are involved in the regulation of these modules.

Scaffold proteins in ERK signaling modules

ERK1/2-mediated signaling pathways are involved in several aspects cell growth and homeostasis including cell proliferation, survival and motility, in a context-dependent manner. Presumably, the context- or celltype-specific signaling by ERK1/2 signaling pathways involves specific scaffold proteins. Several such scaffold proteins that can modulate the assembly and the

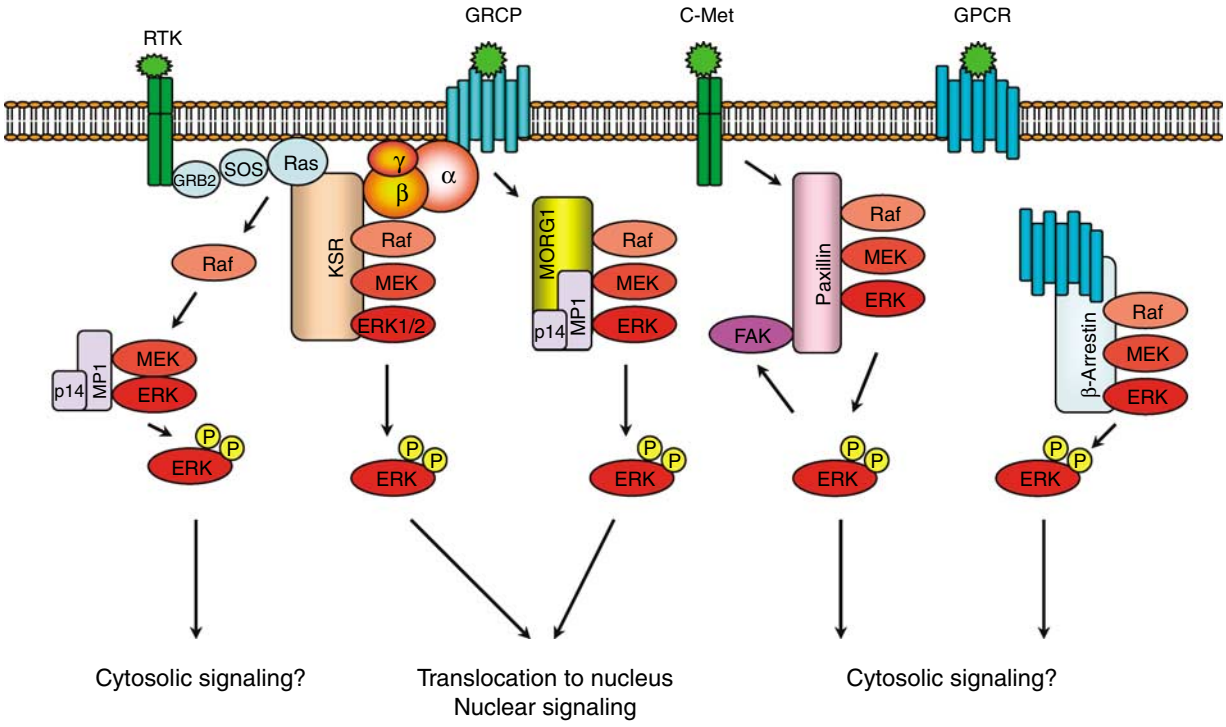

Figure 2 Scaffold proteins involved in ERK-signaling pathway. Scaffold protein KSR links signaling from RTKs and GPCRs to ERK-signaling modules. MORG1 specifically involved in linking GPCRs to ERK1/2 module. MP1 and p14 complex can interact with MORG 1 or RTK-signaling to direct the signal to MEK1-ERK1 signaling module. The scaffolding roles of paxillin and *β*-arrestin appears to be involved in directing the signaling outputs from ERK-module to specific cytosolic compartments (see text for details).

activation of ERK module have been identified. They are kinase suppressor of Ras (KSR) signaling (Kornfeld *et al*., 1995; Sundaram and Han, 1995; Therrien *et al*., 1995), MEK partner 1, MP1 (Schaeffer *et al*., 1998), MAPK organizer 1, MORG1 (Vomastek *et al*., 2004), Paxillin (Ishibe *et al*., 2003) *β*-arrestin (Lefkowitz and Shenoy 2005), and MEKK1 (Karandikar *et al*., 2000) (Figure 2). In addition to these scaffold proteins, proteins such as CNK1 (Ziogas *et al*., 2005), CNK2 (Bumeister *et al*., 2004), IQGAP1 (Roy *et al*., 2004, 2005), PEA-15 (Renganathan *et al*., 2005) and 14-3-3 (Van der Hoeven *et al*., 2000; Bridges and Moorhead, 2005) that have been shown to interact with different components of the ERK module, can also play modulatory role in ERK signaling (Morrison and Davis, 2003; Kolch, 2005).

KSR

KSR was originally identified as a regulator of RAS signaling in *Drosophila* (Therrien *et al*. 1995) and *Caenorhabditis elegans* (Kornfeld *et al*. 1995; Sundaram and Han, 1995). Mammalian KSR is a 105 kDa protein with several distinct domains through which it interacts with Raf, MEK and ERK (Kornfeld *et al*., 1995; Sundaram and Han, 1995; Therrien *et al*., 1995; Morrison, 2001; Roy and Therrien, 2002; Morrison and Davis, 2003; Kolch, 2005) (Figure 2). Although KSR contains a kinase domain (Kornfeld *et al*., 1995; Sundaram and Han, 1995; Therrien *et al*., 1995), it is not clear whether the kinase domain is functional. In the case of mammalian KSR, the kinase domain does not appear to be functional as a critical lysine residue required for kinase activity in kinase subdomain II is replaced by arginine. Nevertheless, the scaffolding function of KSR appears to be independent of its kinase activity (Therrien *et al*., 1996; Michaud *et al*., 1997). Two isoforms of KSR, named KSR1 and KSR2, have been identified (Muller *et al*., 2000; Ohmachi *et al*., 2002; Channavajhala *et al*., 2003) Although most of the findings defining the role of mammalian KSRs have been derived from KSR1, it is possible that KSR1 and KSR2 are involved in distinctly different scaffolding functions. In pathways leading to the activation of the ERK module by growth factors, KSR1 is mapped downstream of Ras and upstream of Raf (Kornfeld *et al*., 1995; Sundaram and Han, 1995; Therrien *et al*., 1995). Coexpression studies have indicated that KSR1 forms a high-molecular weight signaling complex consisting of Raf, MEK1/2, ERK1/2 (Morrison, 2001; Roy and Therrien, 2002), 14-3-3 (Xing *et al*., 1997; Cacace *et al*., 1999), G*βγ*-subunits (Bell *et al*., 1999), along with other molecular chaperones such as heat shock proteins HSP68, HSP70 and HSP90 (Stewart *et al*., 1999).

The scaffolding function of KSR1 is dynamically regulated through its interaction with many different signaling proteins. Recent studies have unraveled the sequence of events by which the scaffolding function of KSR1 is finely regulated (Xing *et al*., 1997; Cacace *et al*., 1999; Muller *et al*., 2001; Ory *et al*., 2003; Ory and Morrison, 2004). In quiescent cells, KSR1 interacts

constitutively with MEK1/2 and the core domain of PP2A (Ory *et al*., 2003), and the KSR1–MEK–PP2A complex is retained in the cytosol through its interactions with 14-3-3 and the Ras-sensitive E3–ubiquitin ligase impedes mitogenic signal propagation (IMP) (Raabe and Rapp, 2003; Matheny *et al*., 2004; Ory and Morrison, 2004; Kolch, 2005; Matheny and White, 2005). Growth factor stimulation leads to the activation of Ras, which stimulates the degradation of IMP thereby relieving its hold on KSR1. In addition, Ras stimulates the dephosphorylation of KSR1 and Raf at specific Ser residues involved in 14-3-3 binding via PP2A (Sieburth *et al*., 1999; Muller *et al*., 2001; Ory *et al*., 2003). The resultant disassociated KSR1–MEK1/2 complex translocates to the plasma membrane where it completes its scaffolding function by assembling the upstream Raf and downstream ERK1/2 kinases (Morrison and Davis, 2003; Ory and Morrison, 2004; Kolch, 2005).

Although these studies have firmly established the molecular interactions of KSR1, the functional consequences of such interactions were derived from studies using two different strategies (Nguyen *et al*., 2002; Roy *et al*., 2002). By ectopically expressing KSR in Schneider cells, it has been shown that KSR strongly promotes Ras–Raf mediated phosphorylation of MEK (Roy *et al*., 2002). Interestingly, KSR mutants that do not interact with MEK failed to promote MEK phosphorylation and the formation of higher order complexes involving Raf, MEK and ERK (Roy *et al*., 2002), thus attesting to the scaffolding role of KSR. Studies with KSR1-deficient mice further substantiated such scaffolding role for KSR (Nguyen *et al*., 2002). Although KSR-deficient mice were normal, the MEFs generated from $KSR^{-/-}$ mice showed at least a 50% reduction in EGF- or PMA-responsive ERK activation. In addition, T-cell activation and proliferation that proceed via the ERK pathway were found to be affected in these animals. Furthermore, middle T antigen-induced tumor development was attenuated in $KSR^{-/-}$ animals. Similar to the results observed with KSR mutants in Schneider cells (Roy *et al*., 2002), high-molecular weight complexes containing KSR, MEK and ERK were absent in lysates from $KSR^{-/-}$ brain tissue, whereas they could be seen in the lysates from the wild-type animals (Nguyen *et al*., 2002). Although these studies have established KSR as a scaffold protein that can potentiate the activation of ERK, it has also been observed that the overexpression of KSR1 strongly inhibited ERK signaling (Denouel-Galy *et al*., 1998; Joneson *et al*., 1998; Sugimoto *et al*., 1998). However, it should be noted here that the overexpression of a putative scaffolding protein can lead to the sequestration of individual components, resulting in a series of non-functional monovalent scaffolds as opposed to multivalent functional scaffolds, thereby exerting an apparent inhibitory effect (Cacace *et al*., 1999; Ferrell, 2000; Levchenko *et al*., 2000). Therefore, it is possible that the previously observed inhibitory effect of KSR1 is due to such 'combinatorial inhibition' (Levchenko *et al*., 2000). Since such 'combinatorial inhibition' is often considered as a hallmark of a 'true' scaffold protein, KSR appears to qualify as a valid scaffold protein of the ERK module.

MP1

MP1 or MEK Partner 1 is a small protein of 13.5 kDa molecular weight and was identified in a yeast two-hybrid screen for MEK1-interacting proteins (Schaeffer *et al*., 1998). MP1 was found to interact with MEK1 and ERK1 but not with Raf. Overexpression of MP1 was found to potentiate the activation of ERK1, suggesting the possibility that by tethering MEK1 and ERK1 in close proximity to one another, MP1 enhances the signaling through this pathway. An interesting aspect of MP1 is that it specifically interacts with MEK1 and ERK1 but not to MEK2 and ERK2 (Schaeffer *et al*., 1998). Thus, it appears that MP1 directs the signals exclusively to the MEK1–ERK1 pathway. In addition, MP1 is involved in spatiotemporal organization of the ERK signaling complex through its interacting partners. MP1 interacts with p14, a highly conserved protein of 14 kDa protein associated with late endosomes (Wunderlich *et al*., 2001). Overexpression studies have indicated that MP1 exits as a complex with MEK1–ERK1 module along with p14. Silencing either MP1 or p14 has been observed to attenuate MEK1–ERK1 signaling (Teis *et al*., 2002). However, the scaffolding role of MP1–p14 appears to be restricted to late endosomes as the forced mislocalization of MP1 abrogates the ability of MP1–p14 to enhance ERK1 signaling. In addition, such mislocalization drastically reduced the duration of ERK1 signaling, suggesting that the MP1–p14-mediated signaling conduit is required for the sustained activation of ERK-1 (Teis *et al*., 2002; Kolch, 2005).

Recently, it has also been observed that the MP1–p14 scaffold is involved in PAK1-dependent ERK activation during adhesion and cell spreading (Pullikuth *et al*., 2005). During fibronectin-stimulated spreading of REF52 fibroblasts, MP1 has been shown to be absolutely required for the phosphorylation of MEK1 by PAK1 and subsequent MEK1 and ERK activation during adhesion. In addition, MP1–p14-mediated ERK activation has been shown to be required for the suppression of the Rho-Rho-associated coiled coil–containing protein kinase (ROCK) signaling axis, which is an additional requisite for cell adhesion and spreading. In contrast, the MP1 scaffold has been shown to be dispensable for platelet-derived growth factor (PDGF)-activated ERK response in these cells (Pullikuth *et al*., 2005). Thus, these findings exemplify a spatiotemporal scaffolding mechanism in which fibronectin signaling to ERK module is insulated from PDGF signaling and vice versa through MP1–P14 scaffold.

MORG1

MORG1, a 35 kDa molecular weight protein belonging to the WD-40 protein family, was isolated as a binding partner of MP1 in a yeast two-hybrid screen using MP1 as the bait (Vomastek *et al*., 2004). Although the MP1–p14 heterodimer does not interact with Raf, the MAP3K

of the module, MORG1 can associate with Raf-1, B-Raf, MEK1, MEK2, ERK1 and ERK2. Expression of MORG1 has been shown to enhance the FBS-stimulated ERK response, whereas silencing MORG1 has been shown to attenuate such a response (Vomastek *et al*., 2004). More interestingly, the scaffolding role of MORG1 in ERK signaling appears to be ligand/receptor-specific. Although MORG1 enhanced lysophosphatidic acid (LPA)- and phorbol ester-mediated ERK response, it failed to enhance EGF-mediated ERK response (Vomastek *et al*., 2004). Likewise, silencing MORG1 did not have any effect on EGF-stimulated ERK response. Thus, it appears MORG1 is involved in facilitating signal transmission from G-protein coupled receptors such as those of LPA but not from receptor tyrosine kinases (RTKs). It is not clear at present how the scaffolding function of MORG1 is coordinated with that of MP1. As both MORG1 and MP1 can be colocalized at vesicle-like structures, it is possible that they cooperate with each other in stabilizing the ERK module at specific cellular locations (Vomastek *et al*., 2004; Kolch, 2005). It is likely that such cooperativity and/or complementation among scaffold proteins is involved in the fine-tuning the signaling outputs from ERK module.

Paxillin

Context-specific scaffolding for the ERK module is provided by the cytoskeletal protein paxillin during hepatocyte growth factor (HGF)-mediated epithelial cell morphogenesis (Ishibe *et al*., 2003). Paxillin is a 68 kDa protein located at focal adhesions in association with other focal adhesion-specific proteins such as vinculin, FAK, actopaxin and PAK (Brown and Turner, 2004). An analysis of HGF-stimulated cell spreading in mIMCD-3 cells indicated a scaffolding role for paxillin in recruiting the ERK module to the focal adhesion complex to transmit signals to other signaling proteins such as focal adhesion kinase (FAK) (Ishibe *et al*., 2003). In quiescent cells, paxillin is constitutively associated with MEK. However, in cells stimulated with HGF, Src-mediated phosphorylation of paxillin at Y118 leads to its recruitment of inactive ERK. Subsequently, activated Raf associates with paxillin thereby activating the tethered MEK and ERK (Ishibe *et al*., 2003). Activated ERK phosphorylates other focal complex proteins including paxillin to facilitate the recruitment of FAK to the focal adhesion complexes for rapid turnover. Thus, paxillin provides an efficient scaffold for the ERK module to organize a signaling hub in the vicinity of focal adhesions to promote cell spreading via ERK signaling (Ishibe *et al*., 2003).

β-*arrestin*

Arrestins were identified as proteins involved in desensitizing GPCR signaling, primarily through receptor internalization (Lefkowitz and Shenoy 2005). However, through their ability to recruit different signaling proteins, β-arrestin 1 and 2 appear to play a significant scaffolding role in ERK signaling. β-arrestin 2 has been shown to interact with all the components of the ERK1-as well as ERK2-modules. β-arrestins interact with phosphorylated GPCRs to promote their endocytosis. During this receptor endocytic internalization process, β-arrestin-scaffolded ERK modules are also internalized along with the GPCRs to end up in endocytic vesicles in the cytosol. Presumably, such close proximity of the receptors and the ERK module results in the activation of ERK pathway. Interestingly, the ERK signaling facilitated by β-arrestin does not propagate to nucleus. In addition, β-arrestin-mediated ERK signaling has a delayed kinetics of activation but with longer duration of activity. Thus, it appears that β-arrestin provides a spatiotemporal signaling conduit for GPCR mediated, G protein-independent ERK signaling.

MEKK1

The 195 kDa MEKK1 is a MAP3K involved in the activation of both the ERK and JNK modules (Lange-Carter *et al*., 1993; Minden *et al*., 1994; Xu *et al*., 1996). Analysis of molecular interactions of MEKK1 unraveled that it can physically associate with the components of the three-tier ERK module, namely Raf-1, MEK1 and ERK2 in addition to the upstream Ras (Russell *et al*., 1995; Karandikar *et al*., 2000). Mapping the binding sites of MEKK1 involved in each of these interactions has indicated that these kinases are closely aligned on the scaffold surface provided by MEKK1 (Karandikar *et al*., 2000). The observation that MEKK1 immunoprecipitated from cells treated with sorbitol, coprecipitates with activated MEK1 and ERK2 indicates that the kinase module assembled by MEKK1 is functional (Karandikar *et al*., 2000). Consistent with this observation, coexpression of a constitutively active mutant of MEKK1 has been shown to stimulate the phosphorylation of MEK1 and MEK2 (Xu *et al*., 1995). Interestingly, MEKK1 has also been shown to phosphorylate MEK1 and MEK2 *in vitro* (Xu *et al*., 1995). Thus, it appears that MEKK1 can play dual role in the ERK1/2 signaling pathway. Although it can act as a scaffold protein mediating the assembly of a signaling complex involving Ras, Raf, MEKs and ERKs, it can also play a catalytic role by phosphorylating and activating MEKs. However, it is not clear at present whether these two functions of MEKK1 are mutually exclusive or context-specific.

In addition to these proteins, it has also been observed that there are several other proteins such as CNK1, CNK2, IQGAP1 and 14-3-3 that can interact with different components of the ERK module to elicit context-specific response (Van der Hoeven *et al*., 2000; Bumeister *et al*., 2004; Roy *et al*., 2004, 2005; Ziogas *et al*., 2005). For instance, CNK1 appears to be involved in Src-mediated activation of Raf-1, a requisite for the full activation of Raf-1 (Ziogas *et al*., 2005). In the case of CNK2, it has been shown that it provides a specific signaling conduit for NGF-mediated activation of the ERK module (Bumeister *et al*., 2004). In contrast, IQGAP1, a putative CDC42/Rac-GAP, interacts with both MEK1 and ERK1 to promote EGF stimulated ERK signaling pathway (Roy *et al*., 2004, 2005).

Likewise, the 14-3-3 protein has been shown to tether protein kinase C (PKC) signaling to Raf (Van der Hoeven *et al*., 2000) in addition to its modulatory role in KSR scaffolds (Cacace *et al*., 1999). PEA-15 appears to mediate the cytoplasmic sequesteration of ERKs (Formstecher *et al*., 2001). As these scaffold proteins do not interact with all of the components of the three-tier kinase module, it can be argued that they do not form a 'true' functional scaffolds by themselves. Nevertheless, it is possible that they can act as 'nested scaffolds' (Kolch, 2005) in tandem or in cooperation with other scaffold proteins in ERK signaling. A growing list of such 'nested scaffolds' in mammalian cells may be indicative that such modular scaffold organization is more adaptable and versatile for the dynamic signaling needs of the mammalian cells.

Scaffold proteins in JNK signaling modules

In line with the dynamic role of JNK signaling in several signaling pathways including cell proliferation, differentiation and apoptosis, several scaffold proteins involved in JNK-signaling module have been identified. The mammalian JNK scaffold proteins that have been identified thus far include JNK-interacting protein (JIP)1, JIP2, JSAP1/JIP3, JLP and POSH along with the different splice variants of JIP1 (JIP1a/Islet-brain 1 (IB1), JIP1b), JIP2 (JIP2 and IB2), JSAP1 (JSAP1a, b, c, d and JIP3) and JLP (JLP, JIP4 and Spag9) (Tapon *et al*., 1998; Ito *et al*., 1999; ; Whitmarsh and Davis, 1998, 1999; Yasuda *et al*., 1999; Lee *et al*., 2002; Kelkar *et al*., 2005; Jagadish *et al*., 2005a, b). In addition, multiple upstream MAP3Ks and MAP2Ks have also been demonstrated to play the dual role of a scaffolding protein in addition to containing intrinsic kinase activity (Garrington and Johnson, 1999; Elion, 2001; Morrison and Davis, 2003). The JIP family forms a major class of scaffold proteins containing multiple protein-interacting domains (Figure 3). These JNK-interacting proteins have been shown to coordinate the signaling of JNK as well as p38MAPK-modules (Figure 4).

JIP1

JIP1 was identified in a screen for JNK-interacting proteins using a yeast two-hybrid assay (Dickens *et al*., 1997). A splice variant of JIP1 was also identified as IB1, a nuclear protein involved in enhancing the function of the *Glut2* gene promoter in pancreatic cells (Bonny *et al*., 1998). Two isoforms of JIP1, namely JIP1a and JIP1b (IB1), have been identified. Although both the isoforms contain an N-terminal JNK binding domain (JBD), a SRC homology (SH3) domain and phosphotyrosine-binding (PTB) domain, JIP1b contains an additional c-terminal PTB domain (Dickens *et al*., 1997). As most of the functional studies have been characterized using JIP1a, it is commonly referred as JIP1. JIP1 interacts with both JNK1 and JNK2

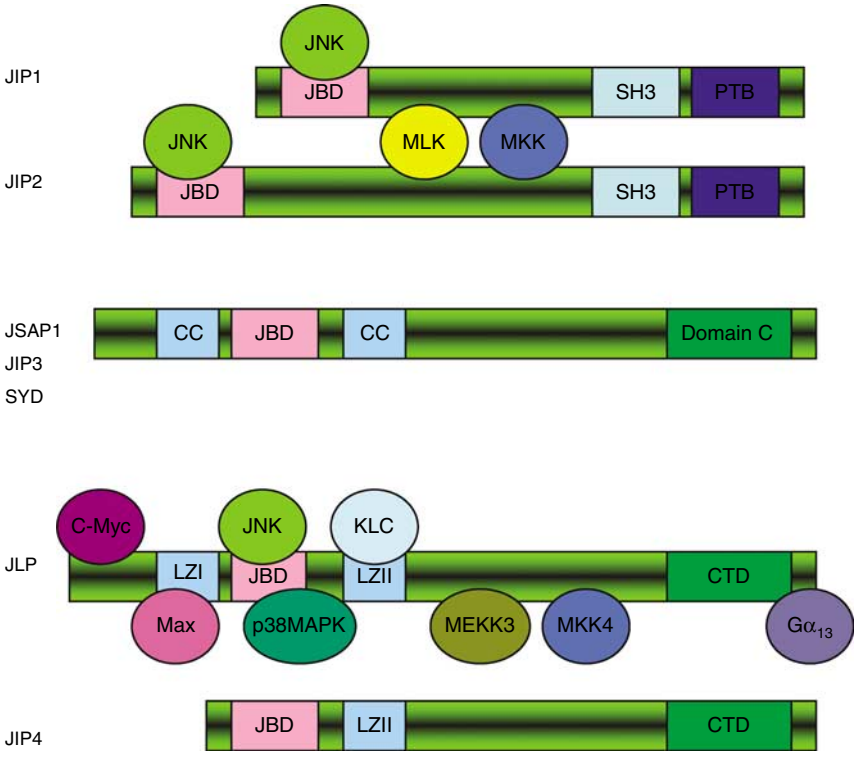

Figure 3 Structure of the JIP family of scaffolding proteins. JIP scaffolding proteins contain multiple protein binding sites to facilitate the formation of signaling cascades. JBD, JNK-binding domain; SH3, Src homology 3 domain; PTB, phosphotyrosine-binding domain; CC, coiled coil; LZI and LZII, the first and second leucine zipper domain; CTD, the carboxyl terminal domain.

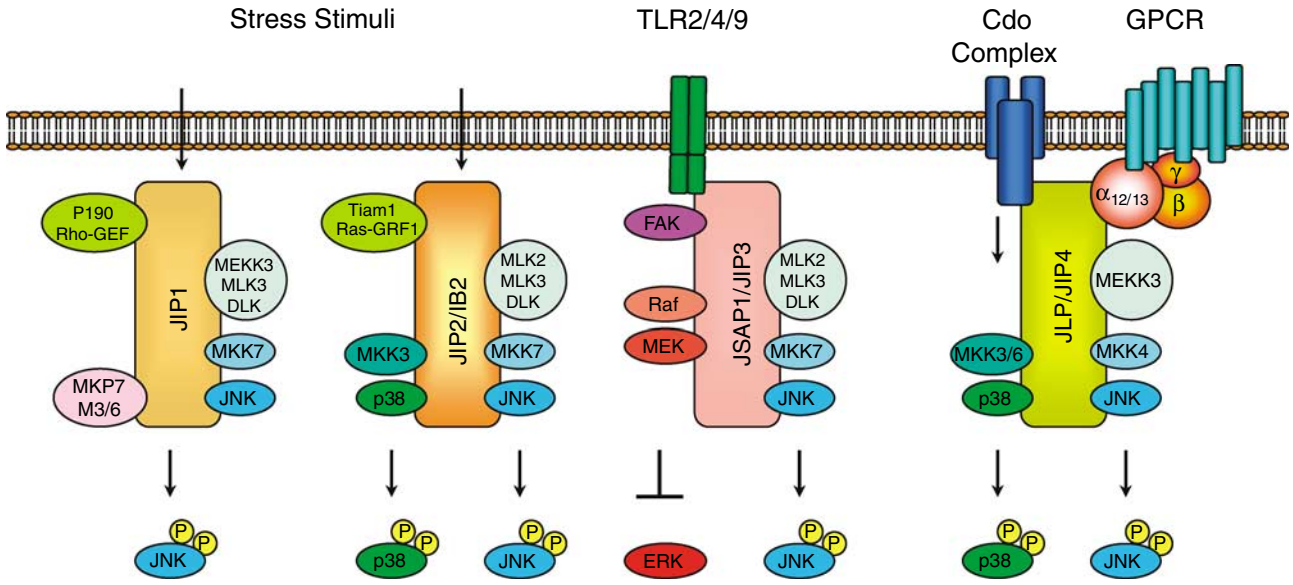

Figure 4 Scaffolding roles of JNK-interacting proteins. Four distinct JNK-scaffolding proteins, namely JIP 1, 2, JSAP1 and JLP (JNK-associated leucine zipper protein/ JIP4) have been identified to provide scaffolding functions for JNK as well as p38MAPK-signaling modules. These scaffold proteins play anchoring as well as catalytic role in JNK- and p38MAPK-mediated signaling pathways in different cellular contexts (see the text for details). In addition to their role in assembling the three-tier kinase module, these proteins also interact with the upstream signaling components such as cell surface receptors, receptor-like proteins, upstream G proteins and/or GEFs that can activate the respective kinase module (see text for details).

(Whitmarsh *et al*., 1998; Yasuda *et al*., 1999; Nihalani *et al*., 2001). The MAP3Ks that have been demonstrated to associate with JIP1 are MEKK3, mixed-lineage protein kinase (MLK3), dual leucine zipper-bearing kinase (DLK) and histidine protein kinase (HPK1) (Whitmarsh *et al*., 1998; Yasuda *et al*., 1999; Nihalani *et al*., 2001). Interestingly, MKK7 is the only JNK-specific MAP2K that interacts with JIP1 (Yasuda *et al*., 1999; Whitmarsh *et al*., 1998). In accordance with the scaffolding role of JIP1 in the JNK-signaling pathway (Figure 4), the coexpression of JIP1 has been shown to enhance the activation of JNK via MLK3 and MKK7 (Whitmarsh *et al*., 1998; Nihalani *et al*., 2001).

It has also been shown that JIP1 can interact with the dual-specificity phosphatases MKP7 and M3/6 (Willoughby *et al*., 2003). As the association of MKP7 with JIP1 led to a decrease in JNK activation, it has been suggested that JIP-1 regulates JNK activity in a dynamic fashion by assembling a signaling complex consisting of both the stimulatory upstream kinases and attenuating phosphatases (Willoughby *et al*., 2003). Another interesting observation is the finding that JIP1 interacts with a guanine nucleotide exchange factors (GEF) involved in Rho signaling (Meyer *et al*., 1999). It has been shown that JIP1 physically associates with p190-RhoGEF (Meyer *et al*., 1999). As it has been shown that Rho can activate the JNK module (Marinissen *et al*., 2004), this finding suggests the possibility that JIP-1 tethers Rho–GEF to JNK module for such Rho-mediated activation of JNK (Figure 4). However, such a linear sequence of events linking JIP1–RhoGEF interaction and Rho-activation of JNK remains to be established.

Although most of these studies were focused on JIP1a, the functional role of JIP1b or IB1 has also been investigated. It has been observed that interleukin-1β (IL1β) induces apoptosis of pancreatic β-cells via the activation of JNK (Haefliger *et al*., 2003. Interestingly, the activation of JNK correlated with a concomitant decrease in the levels of JIP1b/IB1 (Haefliger *et al*., 2003; Nikulina *et al*., 2003). Consistent with this negative correlation, the overexpression of JIP1b has been shown to inhibit IL1β-mediated apoptosis by 55% (Nikulina *et al*., 2003). In addition, small interfering RNA (siRNA) studies as well as studies in mice have shown that JIP1/IB1 inhibition increases JNK levels and subsequent apoptosis by twofold (Bonny *et al*., 2000; Haefliger *et al*., 2003). Thus, in pancreatic β-cells, JIP1b acts as an inhibitor of JNK, presumably by sequestering the upstream kinases. In contrast, using adenoviral expression of JIP1b/IB1, it has been shown that the overexpression of JIP1/IB1 can potentiate tumor necrosis factor-α and interferon-γ induced apoptosis of these cells (Ling *et al*., 2003). Thus, whether JIP1b/IB1 activates or inhibits JNK-module in pancreatic β-cells appears to be critically dependent on the expression levels of JIP1b/IB1 (Ling *et al*., 2003).

In addition, JIP1b plays a critical role in Alzheimer's β-amyloid precursor protein (APP) signaling and Alzheimer's disease progression (Matsuda *et al*., 2001; Scheinfeld *et al*., 2002; Taru *et al*., 2002). APP is the precursor protein of the β-amyloid peptides, which are the key factors in β-amyloid plaques found in Alzheimer's patients (Scheinfeld *et al*., 2002). JIP1b, and not JIP1a, has been shown to be required for the interaction of this precursor protein with JNK (Scheinfeld *et al*.,

2002). As the expression and/or the activation of stress-activated kinases including JNK has been associated with the pathophysiology of Alzheimer's disease (Ferrer, 2004), it is possible that JIP1b is critically involved in providing the scaffold that links APP with the JNK module. The observation that JIP1-deficiency suppresses JNK-mediated, excitotoxic-induced neuronal apoptosis in the hippocampus and postischemic brain, identifies JIP1 as the primary JNK-scaffolding protein associated with neuronal functions (Whitmarsh *et al*., 2001).

JIP2

JIP2 and its splice variant known as IB2 were the second group JIPs to be identified (Yasuda *et al*., 1999, Negri *et al*., 2000). JIP2, similar to JIP1, contains a JNK-binding domain, a PTB domain as well as an SH3 domain (Yasuda *et al*., 1999, Negri *et al*., 2000). Northern blot analysis indicates that aside from the brain, JIP2 is also expressed in several other human tissues including prostate, ovary and pancreas (Yasuda *et al*., 1999). Although identified as a JNK-interacting protein, JIP2 has also been shown to interact with p38MAPK (Buchsbaum *et al*., 2002, Schoorlemmer and Goldfarb, 2002). Thus, JIP2 has been shown to interact with JNK1, JNK2 and p38MAPK. The MAP3Ks that interact with JIP2 includes DLK, MLK2 and MLK3. Similar to JIP1, JIP2 specifically interacts with MKK7 and not to MKK4. JIP2, therefore, appears to assemble a JNK-signaling complex involving MKK7 as the MAP2K. (Yasuda *et al*., 1999; Negri *et al*., 2000; Schoorlemmer and Goldfarb, 2002).

JSAP1

JSAP1 was initially identified as JNK/stress-activated protein kinase-associated protein 1 (JSAP1) by a yeast two-hybrid screen using JNK3 as the bait (Ito *et al*., 1999). It was also identified as a binding partner for JNK-1 in a yeast two-hybrid screen and named JIP3 (Kelkar *et al*., 2000). Interestingly, JSAP1/JIP3 lacks the SH3 domain of other JIPs and instead contains a leucine zipper domain (Kelkar *et al*., 2000). Coimmunoprecipitation studies have indicated that JSAP1 interacts with the MAP3Ks MEKK1 (Ito *et al*., 1999), MLK3 (Kelkar *et al*., 2000), or activator of S phase kinase (ASK1) (Matsuura *et al*., 2002). The MAP2Ks that have been shown to interact with JSAP1/JIP3 are MKK4 and 7 (Ito *et al*., 1999, Kelkar *et al*., 2000). Although all of the members of JNK family of MAPKs have been shown to interact with JSAP1/JIP3, JNK3 shows more affinity for JSAP1/JIP3 than JNK1 or JNK2 (Kelkar *et al*., 2000). To date, four alternatively spliced isoforms of JSAP1/JIP3 have been identified and named JSAP1a, JSAP1b, JSAP1c and JSAP1d (Ito *et al*., 2000). The expression profiles of these isoforms have indicated that while all of them show equal expression in brain, they do exhibit isoform-specific expression or lack of expression in some tissues (Ito *et al*., 2000). Thus, JSAP1a is not expressed in heart tissues. However, it is the only isoform expressed in the spleen and lung. JSAP1b is not expressed in spleen, heart and lung, whereas JSAP1c as well as JSAP1d are not expressed in spleen, lung, liver, kidney and testes. Nevertheless, JSAP1c or JSAP1d appears to be the only isoform(s) expressed in heart (Ito *et al*., 2000). Together with the findings that JSAP1c and JSPA1d are relatively weaker in their interactions with JNK3, it is likely that JSAP1 isoforms play a critical role in assembling distinct JNK modules (such as JNK1, 2 and 3) in a tissue-specific manner. Confirming the scaffolding role of JSAP1/JIP3 in the JNK-signaling module, ectopic expression studies have established the stimulatory role of JSAP1/JIP3-scaffold in the JNK3-signaling module (Kuboki *et al*., 2000; Matsuura *et al*., 2002).

An interesting aspect of the scaffolding role of JSAP1 is that it can also interact with Raf-1 and MEK1 of the ERK-signaling module (Ito *et al*., 1999). In contrast to its stimulatory role in JNK-signaling module, JSAP1 appears to inhibit the ERK-module through its interactions with Raf-1 and MEK1 (Ito *et al*., 1999; Kuboki *et al*., 2000). Coexpression studies in COS-7 cells have indicated that the expression of JSAP1 greatly enhances the activation of JNKs but potently attenuate the activation of the ERK-signaling module (Kuboki *et al*., 2000). It has been suggested that by sequestering Raf-1 and MEK1 from ERK-modules, JSAP1 inhibits ERK-activity. This ability of JSAP1 to regulate two different kinase modules in an opposing fashion may have an important physiological significance. In cellular contexts where an increase in JNK activity along with a decreased ERK activity is required as in the case of growth arrest, differentiation or apoptotic pathways, JSAP1 may play a critical role (Kuboki *et al*., 2000).

In addition to the signaling components of the JNK- and ERK-signaling modules, JSAP1 has been shown to interact with several other critical signaling proteins such as FAK, kinesin light chain and toll-like receptors (Bowman *et al*., 2000; Byrd *et al*., 2001; Verhey *et al*., 2001; Matsuguchi *et al*., 2003; Takino *et al*., 2002, 2005). Through these associations, JSAP1 appears to link the JNK module to specific signaling pathways in a context-specific manner. It has been demonstrated that JSAP1 can also associate with FAK (Takino *et al*., 2002) and JSAP-1 mediated scaffold involving the JNK-signaling module and the components of the FAK-signaling pathway – including FAK- is involved in fibronectin-mediated cell migration (Takino *et al*., 2005). The molecular paradigms emerging from these studies suggest that the stimulation of integrin receptors by fibronectin recruits the JSAP1-scaffold via FAK. The resultant JSAP1–FAK association promotes the activation of JNK via a pathway involving Src-mediated activation of p130cas, recruitment of CrkII, CrkII-mediated activation of Rac-GEF, Rac-mediated activation of Pak1, and Pak1 mediated activation of the JNK module consisting of MEKK1–MKK4–JNK1 (Takino *et al*., 2005). As FAK-activation is required for cell motility, JSAP1-medaited enhanced JNK activation somehow regulates the autophosphorylation as well as kinase activity of FAK, and as JSAP1 associates with microtubules through its interaction with kinesin light chain, it appears that JSAP1 plays a critical role in cell

motility. The findings that JSAP1-deficient mice show several abnormalities in brain development including axon guidance defects of the corpus callosum (Kelkar *et al*., 2003; Ha *et al*., 2005), and JSAP1-null embryonic stem cells are deficient in the formation of lamellipodial protrusions (Takino *et al*., 2005), attests to the critical role played by the JSAP1 scaffold in cell motility in organismal development and homeostasis. Furthermore, the observation that the increased expression of JSAP1 and associated activation of JNK and FAK can be associated with advanced malignancy of brain tumors identifies a role for JSAP1 in the pathophysiology of malignant cancers (Takino *et al*., 2005).

Another novel aspect of JSAP1 is that it has been shown to be involved in toll-like receptor (TLR) signaling (Matsuguchi *et al*., 2003). Searching for proteins associated with the cytoplasmic domain of mouse TLR4 by yeast two-hybrid screening identified JSAP1/JIP3 as a TLR4-associated protein. Further analysis has shown that JSAP1 can physically associate with TLR4 that can be activated by lipopolysaccharides (LPS) in stimulating JNK (Matsuguchi *et al*., 2003). Of the different TLRs, JSAP1 has been shown to associate with TLR2, TLR4 and TLR9. In the case of TLR4, it appears that JSAP1/JIP3 constitutively associates with TLR4 through its N-terminal region. It has also been shown that the coexpression of JSAP1/JIP3 increases the complex formation between TLR4 and JNK and enhances LPS-mediated activation of JNK. Thus, the scaffold provided by JIP3 appears to link TLR4 to the JNK-signaling module. This is further substantiated by the observation that RNA interference (RNAi)-mediated interference of JIP3 inhibited LPS-mediated activation of JNK (Matsuguchi *et al*., 2003).

JLP

JLP is the most recently identified member of the JIP-family (Lee *et al*., 2002). JLP was identified in a screen for Myc/Max-interacting proteins using Max as a probe in a λgt11 expression library (Lee *et al*., 2002). JLP contains leucine zipper domains as well as a C-terminal domain highly conserved with respect to that of JSAP1/JIP3 (Lee *et al*., 2002. JLP also contains three SH2- and SH3-binding sites. JLP has been shown to interact with Myc as well as Max through distinct non-overlapping regions. Although JLP interacts with Max through the first leucine zipper domain, its interaction with Myc has been shown to involve a domain at the proximal N-terminus and a domain flanked by the two leucine zipper domains (Lee *et al*., 2002). In addition to these transcription factors, JLP interacts with specific MAP3K, MAP2K and MAPK components of JNK/p38MAPK module. The MAP3K and the MAP2K that interact with JLP have been identified as MEKK3 and MKK4 respectively (Lee *et al*., 2002). Both JNK1 and p38MAPKα have been identified to be the downstream MAPKs that can interact with JLP. The interacting sites of JNK1 as well as p38MAPKα have been mapped to two distinct domains of JLP, spanning amino acids 1–110 and 210–398. However, the findings that either of these domains is sufficient for JNK- or p38MAPK interaction suggest the interesting possibility that in cellular contexts that warrant the co-regulation of both the kinases, a single molecule of JLP can tether both JNK and p38MAPK so that signals can be transmitted from the upstream MAP3K and MAP2Ks. The observation that MKK4, the MAP2K recruited by JLP, can stimulate both JNK and p38MAPK, supports such an interesting JLP-mediated co-regulation of JNK and p38MAPK (Figure 4). Although such co-regulation of JNK and p38MAPK by JLP remains to be established, JLP has been shown to enhance the activation of JNK in several different cell types (Lee *et al*., 2002; Nguyen *et al*., 2005; Kashef *et al*., 2005, 2006).

Recently, it has been shown that JLP physically interacts with the α-subunits belonging to G12 family of heterotrimeric G proteins that are potently involved in the activation of the JNK module (Kashef *et al*., 2005, 2006). It has been determined that JLP forms a multiprotein complex involving the α-subunit of G12 ($G\alpha_{12}$) or G13 ($G\alpha_{13}$) along with the components of JNK-signaling module and potentiates the activation of JNK-signaling pathway by these α-subunits (Kashef *et al*., 2005, 2006). It has also been identified that the C-terminal region of JLP, spanning amino acids 1165–1307, is the critical domain involved in the interactions with $G\alpha_{12}$ or $G\alpha_{13}$. Overexpressing this $G\alpha_{12/13}$-interacting domain, it has been established that JLP is required for the activation of JNK by $G\alpha_{12/13}$ coupled LPA receptors. Likewise, a critical role for JLP has been established in retinoic acid-mediated endodermal differentiation of P19 embryonic carcinoma cells (Kashef *et al*., 2006), which requires the stimulation JNK module by $G\alpha_{13}$ (Jho *et al*., 1997). It is significant to note here that JLP is the first mammalian scaffold protein that has been demonstrated to link a heterotrimeric G protein to a MAPK module. In this respect, JLP is very much analogous to analogous to Ste5 of *S. cerevisiae*, which can link Gpa1 signaling to Fus3 module. In addition to these interactions, JLP has also been shown to interact with kinesin motor proteins through kinesin light chain 1 (KLC1) (Nguyen *et al*., 2005). Through these interactions, JLP is likely to play a critical role in the spatiotemporal regulation of JNK-signaling.

Two different splice variants of JLP, namely JNK – interacting protein-4 or JIP4 (Kelkar *et al*., 2005) and sperm associated antigen 9 or SPAG9 (Jagadish *et al*., 2005a, b), that share common 3′ exons, but differ in the 5′ exon region have also been identified. Unlike the ubiquitous expression of JLP, the JIP4-splice variant shows a strong expression in testis, brain, kidney and liver, with weaker expression in heart (Kelkar *et al*., 2005). Although JIP4 has been shown to interact with MEKK3, MKK4 and JNK, it appears to be more involved in the activation of p38MAPK module involving the MAP2Ks MKK3 and MKK6 (Kelkar *et al*., 2005). The expression of the 84 kDa splice variant SPAG9 appears to be restricted to the haploid spermatid cells during spermatogenesis in the macaque, baboon and human species (Shankar *et al*., 2004).

Although the MAP3Ks and MAP2Ks that interact with SPAG9 are yet to be identified, the ability of JNKs to interact with SPAG9 has been well characterized. It has been shown that SPAG9 interacts with JNK1, JNK2 and JNK3 through a specific N-terminal JNK-binding domain (Jagadish *et al*., 2005a, b). These studies have also indicated that SPAG9 interacts with higher binding affinity to JNK2 as well as JNK3 compared to JNK1. Interestingly, these studies also point out that SPAG9 does not interact with any other MAPKs including p38MAPK. Despite these intriguing observations, the functional significance of SPAG9 and its associated JNK module in spermatid development and/or function remains to be established.

POSH

Plenty of SH3 (POSH) protein was identified as a Rac1-interacting protein in a yeast two-hybrid screen (Tapon *et al*., 1998). POSH interacts with GTP-bound active Rac1 via a specific Rac-binding region. In addition, POSH has been shown to associate with different MAP3Ks, MAP2Ks and MAPKs of the JNK-signaling module such as MLKs, MKK4, MKK7, JNK1 and JNK2 (Xu *et al*., 2003). The POSH-mediated signaling complex has been shown to be involved in nerve growth factor (NGF)-withdrawal mediated activation of JNK and apoptosis of PC12 cells (Xu *et al*., 2003). Expression of dominant-negative POSH or silencing POSH expression using siRNA abrogated the apoptotic response. Thus, POSH functions as a scaffold protein in linking Rac1 and downstream JNK module in an apoptotic pathway. Recently, it has been observed that POSH associates with JIP1 and that this POSH–JIP1 complex is involved in inducing apoptosis (Kukekov *et al*., 2006). Although both POSH and JIP1 can independently interact with different MAP3Ks, MAP2Ks and JNKs, it has been reasoned that for the efficient activation of JNK module, the cooperation between POSH and JIP1 may be required (Kukekov *et al*., 2006). It remains to be seen whether such synergy exists among other JNK-scaffold proteins.

In addition to the JIPs, MEKK1 has been shown to play a scaffolding role in JNK signaling pathways (Xu and Cobb, 1997; Garrington and Johnson, 1999). It has been observed that the 195 kDa MEKK1 binds to MKK4 and to JNK1, thus assembling a three-tier module without any additional scaffold proteins (Xu and Cobb, 1997; Uhlik *et al*., 2004; Takekawa *et al*., 2005). It has also been documented that β-arrestin 2 plays a scaffolding role in JNK-signaling pathway by sequestering the components of JNK along with the internalized GPCRs in the endosomes (Lefkowitz and Shenoy, 2005). In this context, it is worth noting that these scaffold proteins themselves are regulated by the constituent MAP3Ks, MAP2Ks and MAPKs. Although, the precise role of these phosphorylation events are not fully understood, it is possible that the anchoring as well as catalytic activities of the scaffold proteins are finely regulated by the constituent kinases through these phosphorylations.

Scaffold proteins in p38MAPK signaling modules

To date, only three scaffold proteins have been identified to be involved in p38MAPK-signaling pathway. Although several proteins have been shown to interact with p38MAPK and MKK3, the upstream MAP2K of the p38MAPK module (Schoorlemmer and Goldfarb, 2002; Barr *et al*., 2002; Buchsbaum *et al*., 2002; Uhlik *et al*., 2003; Kelkar *et al*., 2005; Krauss *et al*., 2005; Sabio *et al*., 2005), the true scaffolding role in p38MAPK-signaling has been established only for a protein known as Osmosensing Scaffold for MEKK1 (OSM), JIP2, and JLP (Uhlik *et al*., 2003; Kelkar *et al*., 2005; Takaesu *et al*., 2006).

OSM

OSM was identified as a MEKK3 interacting protein in a yeast two-hybrid screen (Uhlik *et al*., 2003). Further analysis of this protein has indicated that it can interact with both MEKK3 and MKK3. Overexpression of OSM has been shown to enhance MEKK3-mediated phosphorylation of MKK3. As MEKK3-MKK3-p38MAPK module is involved hyper-osmotic stress signaling pathway, the role of OSM in osmotic stress signaling was examined. These studies have shown that OSM and MEKK3 could be colocalized in actin ruffles following hyper-osmotic stress. In addition, these studies have also demonstrated that OSM is required for hyper-osmotic stress induced activation of p38MAPK, as the silencing of OSM completely inhibited hyper-osmolarity induced activation of p38MAPK. In contrast, RNAi-mediated knockdown of OSM had only a moderate inhibition on anisomycin-induced activation of p38MAPK. Thus, these studies point to a context-specific scaffolding role for OSM. Furthermore, OSM has been shown to interact with Rac, a GTPase that can activate MEKK3 in addition to being critically involved in cell shape changes associated with osmotic-stress. The scaffolding function unraveled by these studies depict a model in which OSM exists in a stable complex with MEKK3 in the cytosol and recruits MEKK3 along with the downstream MKK3-p38MAPK to Rac-enriched, actin-mediated membrane ruffles, where it facilitate the activation of p38MAPK in response to hyperosmolarity (Uhlik *et al*., 2003).

JIP2

Initial evidence that JIP2 can act as a scaffold protein for p38MAPK module came from the studies that identified JIP2 as a protein that interacts with Tiam1, a Rac-specific GEF (Buchsbaum *et al*., 2002). Analyses of JIP2-interacting MAP2Ks, and MAPKs, using co-immunoprecipitation studies indicated that JIP2 could physically associate with MKK3 and p38MAPKα (Buchsbaum *et al*., 2002; Robidoux *et al*., 2005). Furthermore, the overexpression of JIP2 along with Tiam-1 enhanced the complex formation and activation of the p38MAPK signaling pathway (Buchsbaum *et al*., 2002). Although, the MAP3K recruited by JIP2 in the activation of p38MAPK was not identified in this study, the findings that JIP2 can associate with MLK3 and

MLK3 can activate p38MAPK via MKK3 points to MLK3 as the candidate MAP3K in JIP2-mediated p38MAPK scaffold (Figure 4). In this context, it is interesting to note that IB2, a splice variant of JIP2 has been shown to associate with an upstream intracellular signaling molecule known as fibroblast growth factor homologous factor-2 (FHF2) in order to link and activate a p38MAPK-signaling module consisting of MLK3, MKK3 and p38MAPKδ (Schoorlemmer and Goldfarb, 2002). Thus, it appears that JIP2 can provide a functional scaffold for p38MAPK signaling module starting with the activation of Rac (Figure 4).

JLP

The observations that JLP can interact with p38MAPK provided an early indication that JLP can provide a scaffold for p38MAPK. In addition, the findings that JLP interacts with MKK4, the MAP2K that can activate p38MAPK and MEKK3, the MAP3K that can activate p38MAPK pointed out the possible role of JLP in the regulation of the p38MAPK-signaling module (Lee *et al*., 2002). Subsequently, it was shown that JIP4, the splice variant of JLP, could associate with both MKK3 and MKK6 and enhance, albeit weakly, the activation of p38MAPK (Kelkar *et al*., 2005). Consistent with these observations, it has been observed that JLP plays a critical scaffolding role for p38MAPK in Cdo-mediated myogenic differentiation.

Cdo is a receptor-like transmembrane protein belonging to cell surface Ig superfamily that promotes myogenic differentiation (Kang *et al*., 1998; Krauss *et al*., 2005). Although Cdo has been shown to be critically involved in promoting myogenic differentiation, mice lacking Cdo have been shown to exhibit delayed skeletal muscle development (Krauss *et al*., 2005). However, the mechanism through which Cdo transmits signals to cytosol has remained largely unknown. A yeast two-hybrid screen for Cdo-interacting proteins identified JLP as the interacting protein of Cdo-intracellular domain (Takaesu *et al*., 2006). Using mouse myoblast cell lines C2C12, it has been shown that JLP and p38MAPK coimmunoprecipitate with Cdo only when the cells are actively undergoing differentiation. In addition, C2C12 cells in which the expression of JLP was downregulated using RNAi, differentiated less efficiently than the control transfectants. Furthermore, primary myoblasts from *Cdo*$^{-/-}$ mice, that show a defective differentiation program, are deficient in their ability to activate p38MAPK. However, the identities of the MAP3K, MAP2K and the upstream small GTPase(s) involved in this pathway are yet to be resolved. Nevertheless, these studies illustrate a novel role for JLP in which it provides a scaffolding role linking a membrane receptor to p38MAPK module (Figure 4). It is quite significant that this is one of the few instances in which a mammalian scaffold protein links a receptor-like protein to a specific MAPK module (Matsuguchi *et al*., 2003; Takaesu *et al*., 2006).

Scaffold proteins in ERK5 signaling module

Growth factors, oxidative stress and osmotic stress have been shown to stimulate the three-tier ERK-5 kinase module consisting of MEKK2/3, MEK5 and ERK5 (Nakamura and Johnson, 2003; Nakamura *et al*., 2006). The MAP3Ks (MEKK2 and MEKK3) as well as the MAP2K (MEK5) of this module contain a Phox/Bem1P (PB1) domain, which is involved in protein–protein interactions (Lamark *et al*., 2003; Nakamura *et al*., 2006). As PB1 domain-containing proteins can associate with each other (Ponting *et al*., 2002), MEKK2/3 and MEK5 associate with each other and form a signaling complex (Nakamura *et al*., 2006). More interestingly, it has been shown that MEK5 uses part of its PB1 domain to tether ERK5 in addition to MEKK2 and MEK5. Thus, the PB1 domain of MEK5 acts as a scaffold to tether the upstream MEKK2/3 as well as downstream ERK5. Although the C-terminal region of the MEK5-PB1 domain is required for MEKK2/3 binding, N-terminal amino acids 18 to 25 of the PB1 domain and amino acids 117–131, C-terminal to the PB1 domain, are required for ERK5 binding (Nakamura *et al*., 2006). Thus, it has been demonstrated that the N-terminal as well as C-terminal regions of the MEK5-PB1 domain are involved in ERK5 binding, whereas the C-terminal region is involved in MEKK2/3 binding (Nakamura *et al*., 2006). In light of the observation that ERK5 is the only MAP2K that contain PB1 domain, the fidelity in signal coupling appears to be mediated by the MEK5 scaffold. The ability of the MAP2K to provide scaffolding function in ERK5 signaling is quite analogous to the scaffolding role of the PBS2, the MAP2K of Hog-1 kinase module in *S. cerevisiae* (Figure 5).

Structural aspects of scaffolding function

Identifying the mechanism by which the scaffolding proteins accelerate the spatiotemporal kinetics of signaling events, and hence their structure and functional relationship, has been of great interest. In the case of Ste5 of *S. cerevisiae*, it has been proposed that the non-contiguous specific protein interacting sites fold together in the tertiary structure of the protein to form a functional domain (Elion, 2001). Therefore, it is possible that the multiple sites of specific kinase-interaction sites seen in scaffold proteins constitute a single domain in the tertiary structure. These potential structure and functional correlates can be established once the three-dimensional structures of the scaffold proteins are resolved.

On the basis of homodimerization property of Ste5, another functional aspect of scaffolding protein has been speculated. It has been proposed that scaffold homodimerization can bring molecules in close proximity, irrespective of their interacting sites, by two mechanisms (Elion, 2001). In one instance, the scaffolding monomer, such as Ste5, can tether all of the signaling components and facilitate the linear transmission of a signal in an accelerated manner. Alternatively, each Ste5 monomer can bind to a different set of signaling proteins, and through homodimerization,

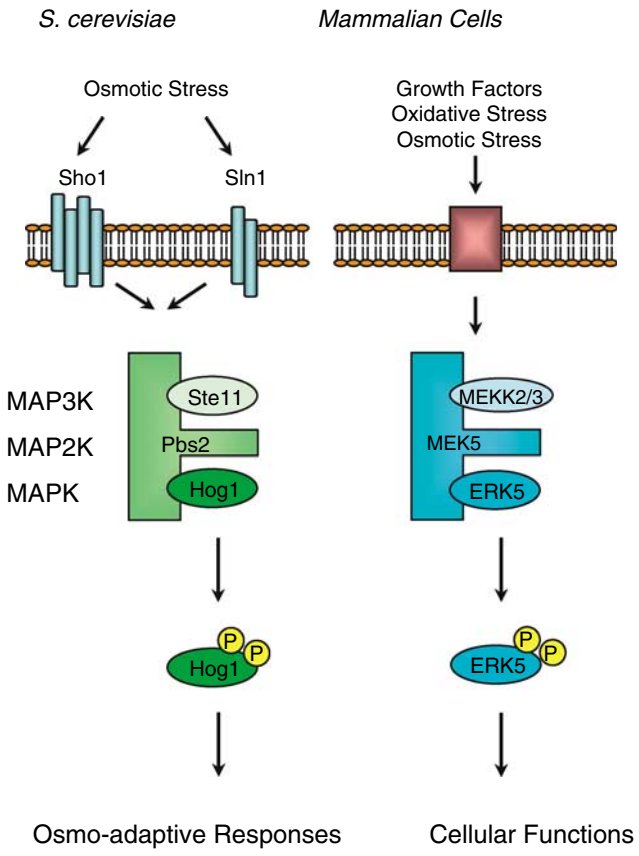

Figure 5 Scaffolding role of MAP2Ks. In *S. cerevisiae*, during osmotic stress, the osmosensor Sho1 or SLN1 stimulates the Hog1 MAP kinase module. In this pathway, Pbs2, the MAP2K of the Hog-1 kinase module, provides a scaffolding role by binding to the upstream MAP3Ks Ste11, Ssk2 or Ssk22 and the downstream MAPK, Hog1 through distinct docking domains (Posas and Saito, 1997). Although Sln1 activates PBS2-Hog1 through Ssk2/Ssk22 MAP3K, Sho1 activates PBS2-Hog1 through the MKKK, Ste11. Thus, PBS2 can assemble the MAP3K, SSK2/SSK22 and Hog1 during SLN1 activation whereas it can link STE11 and Hog2 upon SHO1-activation (Posas and Saito, 1997; Tatebayashi *et al*., 2003). In mammalian cells growth factors, oxidative stress and hyperosmotic stress activate the three-tier ERK-5-kinase module consisting of MEKK2/3, MEK5 and ERK5. In this module, MEK5, the MAP2K acts as a scaffolds by binding to the MAP3Ks (MEKK2/3) and the MAPK (ERK5) through distinct PB1-domains (Nakamura and Johnson, 2003; Nakamura *et al*., 2006). See text for details.

these molecules are brought together in close proximity and subsequently lead transactivation of these molecules in an accelerated manner. These two possibilities need not be mutually exclusive. As several mammalian scaffold proteins including JLP have been shown to homodimerize (Lee *et al*., 2002), it is likely that the homodimers of these proteins play an essential role in MAPK signaling. Likewise, heterodimerization of scaffold proteins can bring together different sets of signaling proteins. The observed interactions between MP1 and MORG-1 (Vomastek *et al*., 2004; Kolch, 2005) or POSH and JIP1 (Kukekov *et al*., 2006) may be indicative of such heterodimerization-mediated synergy among the scaffold proteins.

Aside from their scaffolding role, the scaffold proteins, such as the JIPs and JLP are known to interact with kinesin motor proteins (Kelkar *et al*., 2005; Nguyen *et al*., 2005) that are involved in cargo movement along microtubules (Vale *et al*., 1985; Brady, 1995). In the case of JLP, it has been shown that it interacts with KLC and that this interaction is required for the proper cytosolic localization of JLP (Nguyen *et al*., 2005). The observations that these scaffold proteins can interact with both signaling- and motor-proteins have many implications. It can be envisaged that in response to a signal, the scaffold protein such as JLP binds to specific signaling module(s) and migrates along with the critical signaling components towards the nucleus to facilitate the transactivation of the expression of specific genes.

Conclusions

Studies carried out thus far in defining the structural and functional roles of scaffold proteins have identified the different mechanisms through which these proteins accelerate the spatiotemporal kinetics of signaling by the respective MAPK-signaling modules. In addition to their ability to accelerate the kinetics of signaling by different MAPK modules, they are also involved in downregulating the activities of MAPKs in a context specific manner employing different mechanisms (Lu *et al*., 2002; Willoughby *et al*., 2003; Witowsky and Johnson, 2003; Matheny and White, 2005; Willoughby and Collins, 2005; Xia *et al*., 2006). Thus, using both positive and negative signaling inputs, scaffold proteins precisely regulate the signaling outputs from their cognate MAPK-signaling modules. Further analyses of this novel class of proteins should elucidate the mechanisms through which different MAPK-signaling pathways are insulated from each other so that the signal networking in the cell is stringently regulated. Considering the cardinal roles played by the MAPK modules in different aspects of cell growth and oncogenesis, defining the spatiotemporal mechanisms by which these scaffold proteins finely regulate the signaling amplitude or duration of the MAPK-signaling modules should unravel novel therapeutic targets.

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