

Scaffold Proteins in Mammalian MAP Kinase Cascades

Katsuji Yoshioka*

Division of Cell Cycle Regulation, Department of Molecular and Cellular Biology, Cancer Research Institute,
Kanazawa University, Takara-machi 13-1, Kanazawa 920-0934

Received February 13, 2004; accepted February 20, 2004

The mitogen-activated protein kinase (MAPK) signaling pathway, which is conserved from yeast to humans, is activated in response to a variety of extra- and intracellular stimuli, and plays key roles in multiple cellular processes, including proliferation, differentiation, and apoptosis. The MAPK pathway transmits its signal through the sequential phosphorylation of MAPK kinase kinase to MAPK kinase to MAPK. Specific and efficient activation of the MAPK cascades is crucial for proper cellular responses to stimuli. As shown in yeast, the mammalian MAPK signaling system may also employ scaffold proteins, in part, to organize the MAPK signaling components into functional MAPK modules, thereby enabling the efficient activation of specific MAPK pathways. This review article describes recent advances in the study of potential mammalian scaffold proteins that may help us understand the complex regulation, including the spatial and temporal control, of the mammalian MAPK signaling pathways.

Key words: ERK, JNK, kinesin, p38, signal transduction.

Abbreviations: ABP-280, actin-binding protein-280; APP, amyloid precursor protein; ASK, apoptosis signal-regulating kinase; DLK, dual leucine zipper kinase; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; GEF, guanine nucleotide exchange factor; GPCR, G-protein-coupled receptor; IB, islet-brain, JSAP1, JNK/stress-activated protein kinase-associated 1; JIP, JNK-interacting protein; JNK, c-Jun NH₂-terminal kinase; JLP, JNK-associated leucine zipper protein; KHC, kinesin heavy chain; KLC, kinesin light chain; KSR, kinase suppressor of Ras; MEKK, MEK kinase; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAP3K, MAPK kinase kinase; MEK, MAPK/ERK kinase; MKK, MAPK kinase; MLK, mixed-lineage protein kinase; MP1, MEK partner 1; LPS, lipopolysaccharide; OSM, osmosensing scaffold for MEKK3; POSH, plenty of SH3s; SH, src homology; SKRP1, stress-activated protein kinase pathway-regulating phosphatase 1; TLR, Toll-like receptor.

The mitogen-activated protein kinase (MAPK) signal transduction pathway is evolutionarily well conserved among eukaryotes (1). This pathway includes three kinases, MAPK kinase kinase (MAP3K), MAPK kinase (MAPKK), and MAPK. These proteins form a signaling module in which a phosphorylation relay occurs from MAP3K to MAPKK to MAPK. Extensive studies of three groups of mammalian MAPK cascades, the ERK, p38, and JNK cascades, have uncovered two qualitatively different aspects of the signaling pathways. First, the MAPK cascades play pivotal roles in multiple cellular processes, including proliferation, differentiation, and apoptosis (2, 3). Second, there exist a number of mammalian MAPK signaling components that form complex signaling networks in cells. To date, fifteen MAP3Ks, seven MAPKKs, and ten MAPKs have been reported (3, 4). Furthermore, in many cases, alternative splicing produces several isoforms of the relevant signaling components.

The mammalian MAPK cascades can be activated by a variety of stimuli, and each of the cascades preferentially responds to a distinct set of stimuli (3). Conversely, multiple distinct MAPK modules can also be activated by a common stimulus. For example, the inflammatory cytokines tumor necrosis factor α and interleukin-1 can elicit both JNK and p38 activation (3, 4). Given the large

number of signaling components involved in mammalian MAPK cascades and the complex modes of activation of the MAPK modules, it follows that cells have developed mechanisms by which the signaling components behave not as independent molecules, but as signaling modules, thereby enabling the specific and efficient activation of distinct MAPK signaling modules in parallel.

In yeast, this sequestration of MAPK signaling components into functional modules is mediated by scaffold proteins (1). The yeast scaffold protein Ste5p possesses no enzymatically functional domain, but is essential for the yeast mating pheromone pathway. Ste5p selectively binds a signaling module consisting of MAP3K Ste11p, MAPKK Ste7p, and MAPK Fus3p, and connects this module to upstream activators. Likewise, mammalian cells appear to employ, at least in part, a similar strategy to ensure signaling specificity and efficiency (Fig. 1; 3, 5, 6). Table 1 summarizes the proposed scaffold proteins in mammals. The study of these proteins may shed light on the complex regulation, including the spatial and temporal control, of the MAPK signaling pathways, and how the proper cellular responses to stimuli are elicited.

Putative Scaffold Proteins for MAPK Signaling Modules—JIP1 and its homologue. JIP1 is the founding member of a novel family of mammalian MAPK scaffold proteins. It was identified by a yeast two-hybrid system using JNK MAPK as bait (7). Transfection assays have demonstrated that JIP1 selectively binds the signaling components of all three levels of a JNK signaling module,

*To whom correspondence should be addressed. Tel/Fax: +81-76-234-4532, E-mail: katsuji@kenroku.kanazawa-u.ac.jp

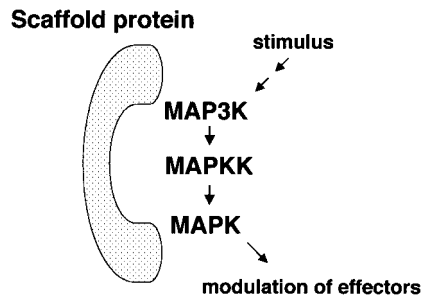


Fig. 1. **Schematic illustration of the role of scaffold proteins in MAPK signaling.** The scaffold protein organizes MAPK signaling molecules as a functional module, thereby enabling efficient activation of the specific MAPK module in response to a stimulus.

MAP3K MLK, MAPKK MKK7, and MAPK JNK, and that coexpression of JIP1 with the three kinases facilitates the activation of JNK (7). It is assumed that JIP1 organizes the MLK/MKK7/JNK module stoichiometrically and enhances the phosphorylation relay, leading to the activation of JNK. Overexpression of JIP1 alone disrupts the normal signal transduction pathway, probably by sequestering signaling molecules, thus acting as an inhibitor of JNK signaling (8). A theoretical study of MAPK scaffolds by Levchenko *et al.* (9) supports this scenario. They termed this effect, “combinatorial inhibition.” It is likely that having the correct stoichiometric amount of scaffold proteins relative to the relevant kinases is crucial for proper signal transduction. In pancreatic β -cells, JIP1 appears to suppress JNK activity, which is released during cytokine-induced apoptosis with a concomitant reduction in JIP1 (10). Although the molecular mechanism of this suppression remains unclear, it might be due in part to the “combinatorial inhibition” effect. JIP1 has recently been shown to bind the MAPK phosphatase MKP7, implying that JIP1 modulates JNK signaling through association with both protein kinases and phosphatases (11). Studies using JIP1-deficient adult mice and primary neurons have demonstrated that JIP1 is required for excitotoxic stress-induced neuronal apoptosis, which is likely to be mediated by JIP1-regulated JNK cascades (12). It has also been reported that a JIP1 deficiency in mice leads to early embryonic death (13). The reason for these different phenotypes is unknown.

JIP2, another member of the JIP group of scaffold proteins, is structurally similar to JIP1, and was initially shown to assemble JNK signaling modules in a manner similar to JIP1 (14, 15). Recent studies, however, suggest JIP2 acts as a scaffold in p38 MAPK signaling pathways (16, 17).

JSAP1 and its homologue. JSAP1 was identified as a JNK-binding protein (18, 19) that is structurally unrelated to JIP1/2. JSAP1 is expressed abundantly in the brain and nervous system (18–20). Transfection experiments suggest that JSAP1 may coordinate several distinct JNK signaling modules, containing ASK1, MEKK1, and MLK3 as the MAP3K, and enhance the MAP3K-induced activation of JNK (18, 19, 21). JSAP1 has also been identified as a TLR4-interacting protein and shown to be involved in LPS-mediated JNK activation, where it connects MEKK1-JNK cascades with TLR4 (22). In addition, JSAP1 might also function as a suppressor for ERK

Table 1. **Mammalian scaffold proteins for MAPK signaling pathways.**

Name	Alternate name	Interacting components in MAPK modules: MAP3K-MAPKK-MAPK	Reference
β -arrestin1		Raf-1-ND ^a -ERK	(35)
β -arrestin2		Raf-1-MEK1-ERK2	(36)
		ASK1-MKK4-JNK3	(37)
Filamin	ABP-280	ND ^a -MKK4-ND ^a	(38)
JIP1	IB1	MLK-MKK7-JNK	(7)
JIP2	IB2	MLK-MKK7-JNK	(14, 15)
		MLK-MKK3-p38 α	(16)
		MLK-ND ^a -p38 δ	(17)
JLP		MEKK3-MKK4-JNK, p38 α	(26)
JSAP1	JIP3	MEKK1-MKK4-JNK	(18)
		MLK-MKK7-JNK	(19)
		ASK1-MKK4/7-JNK	(21)
KSR		Raf-1-MEK-ERK	(27)
MP1		NI ^b -MEK1-ERK1	(31)
OSM		MEKK3-MKK3-p38	(40)
POSH		MLK-MKK4/7-JNK	(33)
SKRP1		ASK1-MKK7-JNK	(39)

^aND, not determined; ^bNI, no interaction.

signaling (23). A targeted gene-disruption study demonstrated that JSAP1 is required for telencephalon morphogenesis (24). The JSAP1-deficient mice die shortly after birth, most likely due to a failure to breathe (24). Although the mechanism that accounts for the breathing failure is unclear, neuronal defects associated with the JSAP1 deficiency may be implicated in this phenotype. An *in vitro* differentiation study with JSAP1-deficient mouse ES cells demonstrated that JSAP1 is important during early embryonic neurogenesis (25).

Another protein, JLP, shares considerable sequence homology with JSAP1 and was shown to bind MAP3K MEKK3, MAPKK MKK4, and the MAPKs JNK and p38 α when these molecules were coexpressed in cells (26). Thus, JLP might function as a scaffolding protein in both the JNK and p38 cascades.

KSR—KSR was originally identified in Ras-dependent genetic screens in *Drosophila* and *Caenorhabditis elegans* as a positive effector of Ras-MAPK signaling (27). Accumulating evidence indicates that KSR functions as a scaffold to coordinate the Ras-mediated Raf/MEK/ERK signaling pathway in the fly, worm, and vertebrates (27). Recent RNA interference experiments in *Drosophila* and *C. elegans* (28, 29), and studies with KSR-deficient mice (30) confirmed that KSR is required for Ras-mediated ERK pathway activation.

MP1, POSH, and β -Arrestin—Transfection assays demonstrated that MP1, a MEK-binding protein, specifically binds MAPKK MEK1 (but not MEK2) and MAPK ERK1 (but not ERK2), and enhances the activation of ERK1, suggesting a scaffold role of MP1 in ERK signaling (31). To date, no MAP3K has been reported to interact with MP1. POSH was identified as a protein that interacts with Rac, a member of the small GTPase Rho family (32), and transfection experiments suggested POSH to be a scaffold protein in the MLK-JNK apoptotic cascade (33). β -arrestins are well known as terminators in G-protein-coupled receptor (GPCR) signaling (34). Recent data sug-

gest that β -arrestins may also function as scaffold proteins in the ERK and JNK3 cascades (35–37).

Other Potential Scaffolds—Recent studies suggest there may be other mammalian MAPK signaling scaffold proteins in addition to those described above, e.g., Filamin, an actin-binding protein (38), SKRP1, a MAPK phosphatase (39), and OSM (osmosensing scaffold for MEKK3), a MEKK3-binding protein (40).

Dynamic Regulation of MAPK Signaling Modules Mediated by Scaffold Proteins—MAPK scaffold proteins may function merely to increase the local concentration of their associated signaling components, as suggested by a study using artificial scaffold proteins (41). However, more dynamic models of the regulation of the mammalian MAPK signaling modules through scaffold proteins have also been presented. Nihalani *et al.* (42) proposed the following model based on their experimental data: JIP1 maintains the MLK family member DLK in a monomeric and catalytically inactive state. Upon stimulation, the JIP1-JNK binding affinity increases and the DLK-JIP1 affinity decreases, allowing DLK dimerization, activation, and subsequent module activation. Kim *et al.* (43) found an interaction between Akt1, a member of the Akt family that regulates a diverse array of biological processes including cell survival, and JIP1, and proposed the following model: Akt1 negatively regulates JIP1's scaffolding activity. In response to an excitotoxic stimulus, the Akt1-JIP1 interaction decreases and concomitantly the JIP1-JNK association increases, leading to JNK-mediated neuronal apoptosis. A similar regulation scheme has been reported for the Akt2-POSH interaction. Figueroa *et al.* (44) identified Akt2 as a POSH-binding protein and revealed that the binding of Akt2 to POSH down-regulates POSH's scaffold activity. FGF homologous factors and two Rac-GEFs, Tiam1 and RasGRF1, have been shown to interact with JIP2, and appear to positively regulate JIP2's scaffold activity (16, 17). In addition, Matsuura *et al.* (21) proposed a dynamic regulation of the ASK1-JNK signaling pathway by the scaffold protein JSAP1 in which the stimulation-dependent phosphorylation of JSAP1 by ASK1 positively regulates JSAP1's scaffold activity.

Kinesin-Mediated Transport and Scaffold Proteins—The specific subcellular localization of signaling modules in response to stimuli is likely to be required for specific biological responses. There is evidence supporting the idea that the JIP1/2 and JSAP1 scaffold proteins are involved in the trafficking of MAPK modules. Verhey *et al.* (45) reported that these scaffold proteins interact with conventional kinesin, which consists of KHC and KLC, in a yeast two-hybrid system using KLC as bait. They also suggested that JIP1 acts as cargo for the kinesin, on which JIP1 appears to preload its associated proteins, such as MAP3K DLK and the Reelin receptor ApoER (45). In addition, amyloid precursor protein (APP) has been shown to interact with JIP1 and JIP2, and JIP1 may modulate APP metabolism to affect processing and secretion (46). JIP1 might also mediate the kinesin-dependent axonal transport of APP (47, 48). Furthermore, genetic studies in *C. elegans* and *Drosophila* established a functional relationship between JSAP1 and conventional kinesin (49, 50). The physiological relationship between JSAP1 and JNK signaling has also been clari-

fied by genetic studies in *C. elegans* (50); worms containing mutations in JSAP1 exhibit similar characteristics to those with mutations in JNK signaling components. Mutations in *C. elegans* JSAP1, JNK, and JNK kinase result in the mislocalization of synaptic vesicle markers. Recently, Setou *et al.* (51) suggested that JSAP1 is a determinant of the traffic direction of the motor protein. They showed that JSAP1 steers conventional kinesin to the axon in cultured neurons. Together, these studies indicate that some scaffold proteins such as JIP1 and JSAP1 may actually translocate MAPK modules along microtubules with kinesin to a specific destination. To date, however, it is not known whether the MAPK modules themselves are also involved in the spatial regulation.

Stimulus-Dependent Targeting of MAPK Modules by Scaffold Proteins—Morrison's group has proposed a model based on their extensive studies, which nicely accounts for mammalian KSR function: In quiescent cells, KSR is located in the cytoplasm and constitutively interacts with MAPKK MEK and 14-3-3 protein, which regulates protein function through binding to phosphoserine-containing motifs (27). In response to growth factor treatment, KSR translocates to the plasma membrane with MEK and recruits ERK; the KSR complex then provides a platform on which Raf activated by Ras can begin the phosphorylation relay from Raf to MEK to ERK. During this process, the KSR-associated proteins 14-3-3, Cdc25C-associated kinase C-TAK (52), and protein phosphatase 2A (53) function as key molecules in regulating the intracellular localization of the KSR scaffold complex and its activity.

Recent data suggest that β -arrestins facilitate GPCR-stimulated ERK and JNK3 activation and target activated MAPK modules to specific locations within cells, for example, to the leading edges of motile cells (34, 54).

p14, a protein copurifying with late endosomes/lysosomes, has been identified as an MP1-binding protein, and subsequently, Teis *et al.* (55) demonstrated that p14 is required for the targeting of the MP1 scaffold complex to endosomes and for efficient ERK signaling upon EGF-stimulation.

Future Perspectives—One of the most exciting areas in recent studies of mammalian MAPK cascades has been the identification of putative scaffold proteins. Research on the properties and roles of scaffold proteins contributes greatly to our understanding of complex intracellular signaling networks in mammalian cells. Although there has been intense research activity in this field, the following issues still need to be considered and clarified.

One key issue undoubtedly continues to be the identification of the components of each endogenous scaffold complex, in which we need to take into account the fact that mammalian MAPK modules may be dynamically regulated by scaffold proteins in response to stimuli, and furthermore, that each scaffold protein might independently organize more than one distinct MAPK module *in vivo*.

It is still nebulous how the scaffold proteins enable the efficient activation of the relevant signaling components in response to stimuli. Detailed biochemical analyses of these processes are required. It also needs to be clarified how the activated MAPK dissociates from the relevant

scaffold protein to modulate its effectors. Moreover, crystallographic studies of the scaffold proteins should provide useful information about their structure and interactions.

The possible role of scaffold proteins in the termination of MAPK signaling also needs to be explored. Because disrupting the MAPK scaffolding complexes would result in the rapid disassembly of functional MAPK modules, it is possible that scaffold proteins regulate the termination of MAPK signaling by modulating their own stability.

It is largely unknown what the physiologically relevant extracellular stimuli are that activate the MAPK scaffold complexes. Furthermore, the physiological roles of the various scaffold proteins remain to be elucidated. Thus, classical and conditional gene-disruption studies will be crucial in providing this information. In addition, a knock-in strategy might be useful whereby a mutant scaffold protein impairing the interaction with MAPK, for example, is expressed at the physiological level. This genetic approach could clarify whether the scaffold proteins possess MAPK-independent biological functions.

The author thanks Drs. M. Ito (Kitasato University) and T. Ito (BIRD, JST and Kanazawa University) for helpful comments on the manuscript.

REFERENCES

- Widmann, C., Gibson, S., Jarpe, M.B., and Johnson, G.L. (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol. Rev.* **79**, 143–180
- Chang, L. and Karin, M. (2001) Mammalian MAP kinase signaling cascades. *Nature* **410**, 37–40
- Kyriakis, J.M. and Avruch, J. (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol. Rev.* **81**, 807–869
- Ichijo, H. (1999) From receptors to stress-activated MAP kinases. *Oncogene* **18**, 6087–6093
- Schaeffer, H. and Weber, M.J. (1999) Mitogen-activated protein kinases: specific messages from ubiquitous messengers. *Mol. Cell. Biol.* **19**, 2435–2444
- Morrison, D.K. and Davis, R.J. (2003) Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell. Dev. Biol.* **19**, 91–118
- Whitmarsh, A.J., Cavanagh, J., Tournier, C., Yasuda, J., and Davis, R.J. (1998) A mammalian scaffold complex that selectively mediates MAP kinase activation. *Science* **281**, 1671–1674
- Dickens, M., Rogers, J.S., Cavanagh, J., Raitano, A., Xia, Z., Halpern, J.R., Greenberg, M.E., Sawyers, C.L., and Davis, R.J. (1997) A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* **277**, 693–696
- Levchenko, A., Bruck, J., and Sternberg, P.W. (2000) Scaffold proteins may biphasically affect the levels of mitogen-activated protein kinase signaling and reduce its threshold properties. *Proc. Natl Acad. Sci. USA* **97**, 5818–5823
- Haefliger, J.A., Tawadros, T., Meylan, L., Gurun, S.L., Roehrich, M.E., Martin, D., Thorens, B., and Waeber, G. (2003) The scaffold protein IB1/JIP-1 is crucial mediator of cytokine-induced apoptosis in pancreatic β cells. *J. Cell Sci.* **116**, 1463–1469
- Willoughby, E.A., Perkins, G.R., Collins, M.K., and Whitmarsh, A.J. (2003) The JNK-interacting protein-1 scaffold protein targets MAPK phosphatase-7 to dephosphorylate JNK. *J. Biol. Chem.* **278**, 10731–10736
- Whitmarsh, A.J., Kuan, C.Y., Kennedy, N.J., Kelkar, N., Haydar, T.F., Mordes, J.P., Apple, M., Rossini, A.A., Jones, S.N., Flavell, R.A., Rakic, P., and Davis, R.J. (2001) Requirement of the JIP1 scaffold protein for stress-induced JNK activation. *Genes Dev.* **15**, 2421–2432
- Thompson, N.A., Haefliger, J.A., Senn, A., Tawadros, T., Magara, F., Ledermann, B., Nicod, P., and Waeber, G. (2001) Islet-brain1/JNK-interacting protein-1 is required for early embryogenesis in mice. *J. Biol. Chem.* **276**, 27745–27748
- Yasuda, J., Whitmarsh, A.J., Cavanagh, J., Sharma, M., and Davis, R.J. (1999) The JIP group of mitogen-activated protein kinase scaffold proteins. *Mol. Cell. Biol.* **19**, 7245–7254
- Negri, S., Oberson, A., Steinmann, M., Sauser, C., Nicod, P., Waeber, G., Schorderet, D.F., and Bonny, C. (2000) cDNA cloning and mapping of a novel islet-brain/JNK-interacting protein. *Genomics* **64**, 324–330
- Schoorlemmer, J. and Goldfarb, M. (2002) Fibroblast growth factor homologous factors and islet brain-2 scaffold protein regulate activation of a stress-activated protein kinase. *J. Biol. Chem.* **277**, 49111–49119
- Buchsbaum, R.J., Connolly, B.A., and Feig, L.A. (2002) Interaction of Rac exchange factors Tiam1 and Ras-GRF1 with a scaffold for the p38 mitogen-activated protein kinase cascade. *Mol. Cell. Biol.* **22**, 4073–4085
- Ito, M., Yoshioka, K., Akechi, M., Yamashita, S., Takamatsu, N., Sugiyama, K., Hibi, M., Nakabeppu, Y., Shiba, T., and Yamamoto, K. (1999) JSAP1, a novel Jun N-terminal protein kinase (JNK)-binding protein that functions as a scaffold factor in the JNK signaling pathway. *Mol. Cell. Biol.* **19**, 7539–7548
- Kelkar, N., Gupta, S., Dickens, M., and Davis, R.J. (2000) Interaction of a mitogen-activated protein kinase signaling module with the neuronal protein JIP3. *Mol. Cell. Biol.* **20**, 1030–1043
- Akechi, M., Ito, M., Uemura, K., Takamatsu, N., Yamashita, S., Uchiyama, K., Yoshioka, K., and Shiba, T. (2001) Expression of JNK cascade scaffold protein JSAP1 in the mouse nervous system. *Neurosci. Res.* **39**, 391–400
- Matsuura, H., Nishitoh, H., Takeda, K., Matsuzaka, A., Amagasa, T., Ito, M., Yoshioka, K., and Ichijo, H. (2002) Phosphorylation-dependent scaffolding role of JSAP1/JIP3 in the ASK1-JNK signaling pathway. *J. Biol. Chem.* **277**, 40703–40709
- Matsuguchi, T., Matsuda, A., Sugimoto, K., Nagai, Y., and Yoshikai, Y. (2003) JNK-interacting protein 3 associates with Toll-like receptor 4 and is involved in LPS-mediated JNK activation. *EMBO J.* **22**, 4455–4464
- Kuboki, Y., Ito, M., Takamatsu, N., Yamamoto, K., Shiba, T., and Yoshioka, K. (2000) A scaffold protein in the c-Jun NH₂-terminal kinase signaling pathways suppresses the extracellular signal-regulated kinase signaling pathways. *J. Biol. Chem.* **275**, 39815–39818
- Kelkar, N., Delmotte, M.H., Weston, C.R., Barrett, T., Sheppard, B.J., Flavell, R.A., and Davis, R.J. (2003) Morphogenesis of the telencephalic commissure requires scaffold protein JNK-interacting protein 3 (JIP3). *Proc. Natl Acad. Sci. USA* **100**, 9843–9848
- Xu, P., Yoshioka, K., Yoshimura, D., Tominaga, Y., Nishioka, T., Ito, M., and Nakabeppu, Y. (2003) *In vitro* development of mouse embryonic stem cells lacking JNK/stress-activated protein kinase-associated protein 1 (JSAP1) scaffold protein revealed its requirement during early embryonic neurogenesis. *J. Biol. Chem.* **278**, 48422–48433
- Lee, C.M., Onésime, D., Reddy, C.D., Dhanasekaran, N., and Reddy, E.P. (2002) JLP: A scaffolding protein that tethers JNK/p38MAPK signaling modules and transcription factors. *Proc. Natl Acad. Sci. USA* **99**, 14189–14194
- Morrison, D.K. (2001) KSR: a MAPK scaffold of the Ras pathway? *J. Cell Sci.* **114**, 1609–1612
- Ohmachi, M., Rocheleau, C.E., Church, D., Lambie, E., Schedl, T., and Sundaram, M.V. (2002) *C. elegans ksr-1* and *ksr-2* have both unique and redundant functions and are required for MPK-1 ERK phosphorylation. *Curr. Biol.* **12**, 427–433

29. Roy, F., Laberge, G., Douziech, M., Ferland-McCollough, D., and Therrien, M. (2001) KSR is a scaffold required for activation of the ERK/MAPK module. *Genes Dev.* **16**, 427–438
30. Nguyen, A., Burack, W.R., Stock, J.L., Kortum, R., Chaika, O.V., Afkarian, M., Muller, W.J., Murphy, K.M., Morrison, D.K., Lewis, R.E., McNeish, J., and Shaw, A.S. (2002) Kinase suppressor of Ras (KSR) is a scaffold protein which facilitates mitogen-activated protein kinase activation *in vivo*. *Mol. Cell Biol.* **22**, 3035–3045
31. Schaeffer, H.J., Catling, A.D., Eblen, S.T., Collier, L.S., Krauss, A., and Weber, M.J. (1998) MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. *Science* **281**, 1668–1671
32. Tapon, N., Nagata, K., Lamarche, N., and Hall, A. (1998) A new Rac target POSH is an SH3-containing scaffold protein involved in the JNK and NK- κ B signaling pathways. *EMBO J.* **17**, 1395–1404
33. Xu, Z., Kukekov, N.V., and Greene, L.A. (2003) POSH acts as a scaffold for a multiprotein complex that mediates JNK activation in apoptosis. *EMBO J.* **22**, 252–261
34. Luttrell, L.M. and Lefkowitz, R.J. (2002) The role of β -arrestins in the termination and transduction of G-protein-coupled receptor signals. *J. Cell Sci.* **115**, 455–465
35. DeFea, K.A., Zalevsky, J., Thoma, M.S., Déry, O., Mullins, R.D., and Bunnett, N.W. (2000) β -arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *J. Cell Biol.* **148**, 1267–1281
36. Luttrell, L.M., Roudabush, F.L., Choy, E.W., Miller, W.E., Field, M.E., Pierce, K.L., and Lefkowitz, R.J. (2001) Activation and targeting of extracellular signal-regulated kinases by β -arrestin scaffolds. *Proc. Natl Acad. Sci. USA* **98**, 2449–2454
37. McDonald, P.H., Chow, C.W., Miller, W.E., Laporte, S.A., Field, M.E., Lin, F.T., Davis, R.J., and Lefkowitz, R.J. (2000) β -arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science* **290**, 1574–1577
38. Marti, A., Luo, Z., Cunningham, C., Ohta, Y., Hartwig, J., Stosel, T.P., Kyriakis, J.M., and Avruch, J. (1997) Actin-binding protein-280 binds the stress-activated protein kinase (SAPK) activator SEK-1 and is required for tumor necrosis factor- α activation of SAPK in melanoma cells. *J. Biol. Chem.* **272**, 2620–2628
39. Zama, T., Aoki, R., Kamimoto, T., Inoue, K., Ikeda, Y., and Hagiwara, M. (2002) Scaffold role of a mitogen-activated protein kinase phosphatase, SKRP1, for the JNK signaling pathway. *J. Biol. Chem.* **277**, 23919–23926
40. Uhlik, M.T., Abell, A.N., Johnson, N.L., Sun, W., Cuevas, B.D., Lobel-Rice, K.E., Horne, E.A., Dell'Acqua, M.L., and Johnson, G.L. (2003) Rac-MEKK3-MKK3 scaffolding for p38 MAPK activation during hyperosmotic shock. *Nat. Cell Biol.* **5**, 1104–1110
41. Park, S.H., Zarrinpar, A., and Lim, W.A. (2003) Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms. *Science* **299**, 1061–1064
42. Nihalani, D., Wong, H.N., and Holzman, L.B. (2003) Requirement of JNK to JIP1 and JNK-dependent JIP1 phosphorylation regulates JNK module dynamics and activation. *J. Biol. Chem.* **278**, 28694–28702
43. Kim, A.H., Yano, H., Cho, H., Meyer, D., Monks, B., Margolis, B., Birnbaum, M.J., and Chao, M.V. (2002) Akt1 regulates a JNK scaffold during excitotoxic apoptosis. *Neuron* **35**, 697–709
44. Figueroa, C., Tarras, S., Taylor, J., and Vojtek, A.B. (2003) Akt2 negatively regulates assembly of the POSH-MLK-JNK signaling complex. *J. Biol. Chem.* **278**, 47922–47927
45. Verhey, K.J., Meyer, D., Deehan, R., Blenis, J., Schnapp, B.J., Rapoport, T.A., and Margolis, B. (2001) Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J. Cell Biol.* **152**, 959–970
46. Taru, H., Kirino, Y., and Suzuki, T. (2002) Differential roles of JIP scaffold proteins in the modulation of amyloid precursor protein metabolism. *J. Biol. Chem.* **277**, 27567–27574
47. Inomata, H., Nakamura, Y., Hayakawa, A., Takata, H., Suzuki, T., Miyazawa, K., and Kitamura, N. (2003) *J. Biol. Chem.* **278**, 22946–22955
48. Matsuda, S., Matsuda, Y., and D'Adamio, L. (2003) Amyloid β protein precursor (A β PP), but not A β PP-like protein 2, is bridged to the kinesin light chain by the scaffold protein JNK-interacting protein 1. *J. Biol. Chem.* **278**, 38601–38606
49. Bowman, A.B., Kamal, A., Ritchings, B.W., Philp, A.V., McGrail, M., Gindhart, J.G., and Goldstein, L.S.B. (2000) Kinesin-dependent axonal transport is mediated by the Sunday driver (SYD) protein. *Cell* **103**, 583–594
50. Byrd, D.T., Kawasaki, M., Walcoff, M., Hisamoto, N., Matsumoto, K., and Jin, Y. (2001) UNC-16, a JNK-signaling scaffold protein, regulates vesicle transport in *C. elegans*. *Neuron* **32**, 787–800
51. Setou, M., Seog, D.H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M., and Hirokawa, N. (2002) Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature* **417**, 83–87
52. Müller, J., Ory, S., Copeland, T., Pivnicka-Worms, H., and Morrison, D.K. (2001) C-TAK regulates Ras signaling by phosphorylating the MAPK scaffold, KSR1. *Mol. Cell* **8**, 983–993
53. Ory, S., Zhou, M., Conrads, T.P., Veenstra, T.D., and Morrison, D.K. (2003) Protein phosphatase 2A positively regulates Ras signaling by dephosphorylating KSR1 and Raf-1 on critical 14-3-3 binding sites. *Curr. Biol.* **13**, 1356–1364
54. Ge, L., Ly, Y., Hollenberg, M., and DeFea, K. (2003) A β -arrestin-dependent scaffold is associated with prolonged MAPK activation in pseudopodia during protease-activated receptor-2-induced chemotaxis. *J. Biol. Chem.* **278**, 34418–34426
55. Teis, D., Wunderlich, W., and Huber, L.A. (2002) Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction. *Dev. Cell* **3**, 803–814