The role of scaffold proteins in MEK/ERK signalling

D.B. Sacks
Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Thorn 530, 75 Francis Street, Boston, MA 02115, U.S.A.

Abstract
Signal transduction networks allow cells to recognize and respond to changes in the extracellular environment. All eukaryotic cells have MAPK (mitogen-activated protein kinase) pathways that participate in diverse cellular functions, including differentiation, survival, transformation and movement. Five distinct groups of MAPKs have been characterized in mammals, the most extensively studied of which is the Ras/Raf/MEK/ERK (extracellular-signal-regulated kinase)/ERK cascade. Numerous stimuli, including growth factors and phorbol esters, activate MEK/ERK signalling. How disparate extracellular signals are translated by MEK/ERK into different cellular functions remains obscure. Originally identified in yeast, scaffold proteins are now recognized to contribute to the specificity of MEK/ERK pathways in mammalian cells. These scaffolds include KSR (kinase suppressor of Ras), β-arrestin, MEK partner-1, Sef and IQGAP1. Scaffolds organize multiprotein signalling complexes. This targets MEK/ERK to specific substrates and facilitates communication with other pathways, thereby mediating diverse functions. The adapter proteins regulate the kinetics, amplitude and localization of MEK/ERK signalling, providing an efficient mechanism that enables an individual extracellular stimulus to promote a specific biological response.

Introduction
Signal transduction networks enable alterations in the extracellular environment to be transmitted to the inside of the cell. The ubiquitous MAPK (mitogen-activated protein kinase) pathways participate in diverse cellular functions, including cell differentiation, cell division, cell movement and apoptosis [1,2]. Five distinct groups of MAPKs have been characterized in mammals: (i) ERK1 (extracellular-signal-regulated kinase 1) and ERK2, (ii) JNK1 (c-Jun N-terminal kinase 1), JNK2 and JNK3, (iii) p38 isoforms α, β, γ and δ, (iv) ERK3 and ERK4, and (v) ERK5 [2]. ERK1/2, JNKs and p38 kinases are the most extensively studied.

The archetypal MAPK pathway consists of a three-kinase cascade, a MEKK (MAPK kinase kinase; or MAPKKK) that activates the downstream MEK (MAPK/ERK kinase; or MAPKK) by phosphorylation, which in turn elicits a phosphorylation-dependent increase in the activity of the MAPK (Figure 1) [2,3]. The MAPK then induces phosphorylation of a variety of cytosolic or nuclear targets, which mediate the cellular response to the original stimulus. MAPKs can be activated by a variety of stimuli, but ERK1/2 are preferentially activated by growth factors and phorbol esters, while JNK and p38 kinase respond to stress stimuli such as osmotic shock, ionizing radiation and cytokines [2]. The Ras/Raf/MEK/ERK cascade is conserved in all eukaryotes and plays a vital role in several biological processes. The pathway is triggered by a diverse range of stimuli acting through cell-surface receptors and is under the control of the small G-protein Ras [4]. Stimuli include EGF (epidermal growth factor), IGF-1 (insulin-like growth factor 1), PDGF (platelet-derived growth factor), NGF (nerve growth factor) and phorbol esters. Binding of growth factor to its cognate receptor recruits SOS (son of sevenless), which induces conversion of membrane-bound Ras-GDP into Ras-GTP (Figure 1) [5]. When GTP-bound, Ras binds to Raf and recruits it to the cell membrane where Raf is activated [6,7]. Raf binds to and phosphorylates the dual-specificity protein kinases MEK1 and MEK2, which in turn stimulate ERK1 and ERK2 by catalysing their phosphorylation on threonine and tyrosine residues [3,5]. ERK modulates the function of numerous substrates in all cellular compartments, including the nucleus, cytoplasm, membranes and the cytoskeleton [2]. Targets range from transcription factors and membrane proteins to the cytoskeleton and other protein kinases. The complexity of the MEK/ERK cascade is enhanced by the presence of more than one of each of the kinases, namely three Rafs (A-Raf, B-Raf and C-Raf) and two distinct MEKs and ERKs (MEK1 and MEK2, and ERK1 and ERK2) (Figure 1). Accumulating evidence reveals that Ras/MEK/ERK signalling is not a simple linear pathway. It is a complex array with cross-talk, feedback loops, branch points and multicomponent signalling complexes [5].

Specificity in MEK/ERK signalling
Multiple extracellular stimuli converge on the Ras/Raf/MEK/ERK pathway and elicit diverse responses. For example, activation of MEK/ERK by NGF induces differentiation of

Key words: growth factor, IQGAP1, kinase suppressor of Ras (KSR); mitogen-activated protein kinase (MAPK); mitogen-activated protein kinase/extracellular-signal-regulated kinase kinase (MEK); scaffold.
Abbreviations used: EGF, epidermal growth factor; ERK, extracellular-signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MEKK, MAPK kinase kinase; MP1, MEK partner-1; NGF, nerve growth factor; SOS, son of sevenless.

1Email dsacks@rics.bwh.harvard.edu.
Growth factors bind to and activate specific receptors on the cell surface. The receptors activate SOS, a guanine nucleotide-exchange factor (GEF) that converts Ras from the inactive (GDP-bound) form into the active (GTP-bound) form. The active Ras initiates a cascade that proceeds via sequential phosphorylation and activation of protein kinases, culminating in modulation of the function of multiple substrates in the cell. P, phosphate.

PC12 (pheochromocytoma) neuronal cells, while activation of the same MEK/ERK pathway by EGF causes PC12 cells to proliferate [8]. A longstanding question in the signal transduction field is how a specific signal at the membrane induces a specific cellular response, despite activating a signalling pathway that is shared by multiple receptors. Some progress has been made in our understanding of the mechanisms over the last decade and a number of hypotheses have been proposed to explain signalling specificity. Specificity can arise (i) at the level of the receptor via distinct receptor-activated pathways; (ii) through the modulation of signalling kinetics where different cellular responses are induced by altering the duration or strength of activation; (iii) through the integration of different signalling pathways; and (iv) at the level of downstream signalling components where different tissues might express distinct MAPK-responsive transcription factors or other targets [9].

Scaffold proteins in MEK/ERK signalling

Recent evidence has validated an important role for scaffold proteins in regulating MAPK signalling (reviewed in [10,11]). Originally identified in yeast [12], scaffolds also contribute to the regulation of MAPK pathways in mammalian cells. Several functions have been proposed for scaffold proteins [13,14]: (i) binding to the scaffold may be necessary for activation of the kinase; (ii) scaffolds may allow faster signalling by preventing phosphatases from acting on the kinases; (iii) the scaffold may recruit a complex of consecutive kinases to an activated receptor at the plasma membrane or to another location; (iv) scaffolds can bring together proteins for their interaction or sequester them so that they do not interact with other proteins; and (v) scaffolds can control subcellular location. These functions are not mutually exclusive and a single scaffold may regulate signalling pathways by more than one of these mechanisms. An additional level of complexity is provided by the observation that these interactions may be controlled by phosphorylation or proteolysis of scaffolds or their binding partners. A number of scaffolds that regulate MEK/ERK signalling have been identified. These include KSR (kinase suppressor of Ras 1), MP1 (MEK partner-1), β-arrestins, MEK kinase 1 and IQGAP1 [10,11]. These scaffolds have been the subject of extensive reviews [10,11]. Here, I briefly address KSR, then focus on the most recently identified scaffold, IQGAP1.

KSR

KSR is one of the best-characterized scaffolds in the Ras/MEK/ERK pathway. Evidence reveals that KSR binds directly to C-Raf, MEK and ERK [10]. Interestingly, MEK is constitutively associated with KSR, while binding of ERK and probably C-Raf is mediated by activation of Ras [15]. KSR is believed to produce a docking platform at the plasma membrane for ERK [10]. In resting cells, KSR is retained in the cytoplasm by 14-3-3 proteins [16] and in a Triton
X-100-insoluble compartment by IMP (impedes mitogenic signal propagation), a Ras-responsive E3 ubiquitin ligase [17]. Cell stimulation induces dephosphorylation of KSR and destruction of IMP, resulting in movement of KSR to the plasma membrane where it facilitates signalling from C-Raf to MEK to ERK [15]. Recent studies using cells obtained from KSR-null mice reveal that KSR modulates the intensity and duration of Raf/MEK/ERK signalling, altering cell fate [18,19].

**IQGAP1**

IQGAP1 is a widely expressed protein that participates in many different aspects of cell physiology [20–22]. Binding to a diverse spectrum of target proteins enables IQGAP1 to influence multiple functions, including those of the actin cytoskeleton, cell–cell adhesion and transcriptional activation [20,21]. Evidence suggests that IQGAP1 is a scaffolding protein that links components of signalling cascades [21]. For example, by forming multiprotein complexes, IQGAP1 couples Ca\(^{2+}\)/calmodulin signalling with cell structure and the cytoskeleton [via actin and the Rho family GTPases, Cdc42 (cell division cycle 42) and Rac1] and cell–cell adhesion (via the E-cadherin–\(\beta\)-catenin complex) [20]. In addition, IQGAP1 was recently identified as a scaffold in MEK/ERK signalling [23,24]. IQGAP1 binds directly to MEK1, MEK2, ERK1 and ERK2 in vitro and in intact cells. This interaction modulates activation of MEK and ERK by EGF. Both knockdown and overexpression of IQGAP1 attenuate EGF-stimulated activation of MEK and ERK, suggesting that IQGAP1 functions as a scaffold by facilitating coupling of kinases in the Ras/MEK/ERK pathway [23,24]. Further intriguing observations were made by direct analysis of the effects of EGF. EGF has no effect on the binding of ERK to IQGAP1. In contrast, inoculation of human epithelial cells with EGF promoted the interaction of IQGAP1 with MEK1, with a concomitant reduction in MEK2 binding to IQGAP1 [24]. These observations raise the possibility that IQGAP1 may preferentially activate the MEK1 pathway. It has been proposed that MEK1 stimulates proliferation, while MEK2 promotes differentiation [25], and it is tempting to speculate that IQGAP1 influences the cellular outcome of MEK/ERK signalling.

The cellular response to growth factors and extracellular signals requires the transmission of signals from the cell surface to the cytoskeleton, nucleus and other regions in the cell. ERKs are capable of both cytoskeletal and mitogenic regulation [26]. Regulation of the cytoskeleton is an important aspect of the mitogenic response and a substantial proportion of MAPK activity is directed to microtubule-associated substrates outside the nucleus. The mechanism by which MEK/ERK regulates the cytoskeleton is not understood and it is not unreasonable to hypothesize that IQGAP1 has a role. This premise is supported by evidence. Both ERK2 [27] and IQGAP1 [28] co-localize with microtubule-associated protein 2 in neuronal cells. Moreover, both MAPK [29] and IQGAP1 [30,31] participate in actin dynamics and cell migration. Thus IQGAP1 may assemble an activated ERK module at the site of actin polymerization [11] and link MAPK signalling to the cytoskeleton [21]. Further work is necessary to validate this premise.

**Scaffold stoichiometry**

It has been recognized that the relative amounts of the scaffold and kinases in a cell have a large impact on MAPK signalling. For example, both increasing and decreasing intracellular IQGAPI1 concentrations markedly impair stimulation of MEK and ERK by EGF [24]. Maximal activation of MEK and ERK by EGF is observed only when cellular IQGAPI1 concentrations are close to normal levels. Deviation by \(\geq 50\%\) from these values reduces active MEK and ERK by \(>50\%\). Although these findings initially seem counter-intuitive, they actually support the notion that IQGAPI1 is a scaffold that links MEK to ERK. The scaffold function of IQGAPI1 is optimum only when all components are present in an appropriate stoichiometric ratio. The low concentration of IQGAPI1 relative to MEK and ERK (1:9:20) implies that a small change in the amount of IQGAPI1 will produce a large change in its relative stoichiometry to the kinases. When IQGAPI1 is in excess, non-functional binary complexes of IQGAPI1 with only one of the components (i.e. MEK or ERK) of the kinase cascade are formed [24]. When the concentration of the scaffold is low, functional complexes of IQGAPI1 with both MEK and ERK are unable to form. Analogous observations have been made with other scaffolds. Overexpression of KSR inhibited ERK-dependent biological effects [15] and activation of ERKs by EGF was reduced by 50% in KSR-deficient mouse fibroblasts [32]. Similarly, high concentrations of MP1 decrease MEK1–ERK1 binding [33]. Additional verification of the concept is provided by computer modelling of the MAPK cascade, which suggests that increasing or reducing the scaffold concentration decreases maximal activation of the MAPK cascade [34]. In vivo scaffold levels can be regulated by modulating gene expression or protein stability, or via sequestration in specific subcellular compartments [35].

**Spatiotemporal regulation of MEK/ERK signalling**

Spatiotemporal regulation of MAPK signalling has an important role in MAPK function [36]. Transient stimulation of MEK/ERK in PC12 cells leads to proliferation, while sustained stimulation leads to differentiation [8]. Moreover, the compartmentalization of Ras has a major influence on the specific effectors it binds and the intensity of signalling, thus modifying biological outcomes [37]. The subcellular localization of MEK and ERK changes in response to growth factors [10]. Many scaffolds are not uniformly distributed in the cell and regulate the activity and spatial distribution of ERK signalling. For example, a change in localization accounts for the effect of p14 on MAPK signalling; reducing p14 induces translocation of MP1 from endosomes to the cytoplasm, resulting in reduced phosphorylation of MEK and ERK [38]. The protein Sef binds to activated MEK at the
Golgi and inhibits the dissociation of the MEK–ERK complex [39]. By this mechanism, Sef blocks nuclear translocation of activated ERK and prevents ERK from activating nuclear substrates. ERK signalling to cytoplasmic targets is not altered, revealing that Sef is a spatial regulator for ERK signalling. Similarly, increased levels of IQGAP1 are found in discrete subcellular locations [40,41]. Moreover, several stimuli, including manipulation of intracellular Ca2+ concentrations [42,43], alteration of calmodulin function [40] and activation of E-cadherin [40], induce the movement of IQGAP1 between different subcellular regions. It is plausible therefore that changes in IQGAP1 concentrations in microdomains of the cell can influence MAPK in distinct signalling compartments.

Summary
Considerable progress has been made in the last decade in our comprehension of how specificity is achieved in MAPK signalling and the contribution of scaffold proteins. Nevertheless, there remain several gaps in our knowledge. For example, the specific contribution of individual scaffolds is incompletely understood. Despite the presence of a number of scaffolds, elimination of any one adaptor protein impairs activation of the MAPK pathway. Why are there multiple MEK/ERK scaffolds? What, if any, is the communication between different scaffolds? Do the scaffolds facilitate cross-talk between the MAPK pathway and other signalling pathways? What is the mechanism underlaying the selectivity of ERK1 and ERK2, and do scaffolds participate? We look forward to future studies that will further elucidate the roles of these important regulatory molecules.

I thank Monideepa Roy for helping with experiments published previously, Rob Krikorian for expertly preparing this paper and Matthew Brown for the diagram. Work in our laboratory is supported, previously, Rob Krikorian for expertly preparing this paper and Matthew Brown for the diagram. Work in our laboratory is supported, in part, by grants from the National Institutes of Health.

References

Received 29 June 2006