The International Journal of Biochemistry & Cell Biology 40 (2008) 2013–2017

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Signalling networks in focus

ERK phosphorylation: Spatial and temporal regulation by G protein-coupled receptors

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ARTICLE INFO

Article history:
Received 12 December 2007
Received in revised form 28 March 2008
Accepted 2 April 2008
Available online 6 April 2008

Keywords:
ERK
Scaffold
FRET
GPCR

ABSTRACT

G protein-coupled receptors (GPCRs) are a major target in the drug discovery process. One important response that results from activation of a wide range of GPCRs is activation of the ERK signalling cascade. Given the abundance of both upstream activators and downstream targets of ERK1/2, the precise spatiotemporal control of ERK1/2 phosphorylation is crucial for maintaining the specificity of the physiological outcome. ERK activity is regulated via a number of mechanisms including compartmentalisation and scaffolding proteins. These scaffolding proteins can enhance the transduction of a specific signalling pathway by targeting pathway components to particular intracellular locations or signalling complexes. Recently, a number of fluorescent indicators of ERK1/2 phosphorylation have been developed that allow the regulation of this pathway to be investigated with greater spatiotemporal resolution than was previously possible. These fluorescent probes in conjunction with those for other signalling cascades should help unravel the spatiotemporal organisation of this pathway.

1. Signalling network facts

- The mitogen-activated protein kinase (MAPK) cascade involves the sequential phosphorylation of Raf, MEK and ERK.
- The Raf family of serine/threonine kinases (Raf-1, A-Raf and B-Raf) are recruited to the cell membrane by the small G protein Ras.
- In quiescent cells, the cytosolic distribution of ERK1/2 is maintained by constitutive association with MEK1/2 proteins which possess a nuclear export signal.
- A plethora of extracellular stimuli can module the ERK cascade including receptor tyrosine kinases and GPCRs.

2. Introduction

Mitogen-activated protein kinase (MAPK) cascades comprise three kinase modules that regulate a number of key intracellular signalling events including cell proliferation, differentiation, survival and apoptosis. The extracellular signal-regulated kinase (ERK) pathway is one of the five MAPK cascades identified in mammals; the general paradigm for which involves the sequential phosphorylation of Raf, MAPK/ERK kinase (MEK) and ERK.

The Raf family of serine/threonine kinases (Raf-1, A-Raf and B-Raf) are typically recruited to the cell membrane by

Abbreviations: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; GPCR, G protein-coupled receptor; PLCβ, phospholipase Cβ; IP_3, inositol trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; CaM, calmodulin; PLD, phospholipase D; PLA_2, phospholipase A_2; PKA, protein kinase A; EPAC, exchange protein directly activated by cAMP; Rap1GAP, Rap-1 GTPase activating protein; PI3K, phosphatidylinositol-kinase; SH2, Src-homology 2; Shc, Src homologous and collagen; Grb, growth factor receptor-bound; GEF, guanine nucleotide exchange factor; Sos, son of sevenless; KSR, kinase suppressor of Ras; MP1, MEK-partner 1; MEKK1, MEK kinase 1; CNK, connector enhancer of KSR; SUR-8, suppressor of Ras-8; C-TAK1, Cdc25C-associated kinase 1; NGF, nerve growth factor; EGF, epidermal growth factor; FRAP, Fluorescence Recovery After Photobleaching; ERK2-GPF, ERK2 tagged with GFP; FRET, Fluorescence Resonance Energy Transfer; CFP, cyan fluorescent protein; YFP, yellow fluorescent protein.

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doi:10.1016/j.biocel.2008.04.001

the small G protein, Ras. Once at the plasma membrane, B-Raf is directly activated through interactions with GTP-bound Ras, whereas A-Raf and Raf-1 require additional tyrosine phosphorylation by membrane-bound tyrosine kinases, including c-Src (Marais et al., 1997). Activated Raf phosphorylates MEK1 and MEK2 on two serine residues which in turn dually phosphorylate ERK1 and ERK2 (generating p44 and p42 MAPK, respectively) on the tyrosine and threonine residues of the TEY motif found within the activation loop (Chen et al., 2001).

MEK1 and MEK2 are highly homologous protein kinases that to date, have not been shown to phosphorylate any physiologically relevant downstream targets apart from ERK1 and ERK2, respectively (Chen et al., 2001). In quiescent cells, the cytosolic distribution of ERK1/2 is maintained by constitutive association with MEK1/2 proteins which, unlike ERK1/2, possess a nuclear export sequence. Upon ERK1/2 phosphorylation, ERK-MEK complexes dissociate, allowing ERK1/2 to translocate into the nucleus via both passive diffusion and active transport (Adachi et al., 1999).

ERK1 and ERK2 are highly conserved proteins for which distinct roles have not yet been fully elucidated. However, knockout studies have suggested that these proteins are not functionally redundant, since, although ERK2 can maintain most physiological functions in the absence of ERK1, the deletion of ERK2 is embryonically lethal (Saba-El-Leil et al., 2003). Once activated, ERK1/2 mediates the phosphorylation of (S/T)P sites found within membrane proteins, cytoskeletal proteins, cytoplasmic proteins and nuclear substrates (Chen et al., 2001).

A plethora of extracellular stimuli can modulate the ERK cascade; often acting through cell surface receptors, such as receptor tyrosine kinases and G protein-coupled receptors (GPCRs). Despite this, considerable signal specificity can be conferred via stringent regulation of the subcellular location of ERK pathway components and their accessory proteins. This review will discuss the signalling pathways involved in GPCR-mediated ERK1/2 phosphorylation, key scaffolding proteins involved in the regulation of the ERK cascade and the recent development of fluorescent probes that allow this signalling to be investigated in both a spatial and temporal manner in living cells.

3. G protein-coupled receptor-mediated ERK1/2 phosphorylation

GPCRs are a large family of cell surface proteins that are commonly utilised by extracellular stimuli to mediate ERK1/2 phosphorylation (Fig. 1). Intracellular effectors that stimulate GPCR-mediated ERK1/2 phosphorylation include the well-characterised heterotrimeric G proteins and β-arrestins. Heterotrimeric G proteins are made up of three subunits, α, β, and γ. Upon activation, the α subunit of each of the four families of heterotrimeric G proteins, $G\alpha_{i/o}$, $G\alpha_s$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$, as well as the tightly associated βγ complex modulate ERK1/2 activation via numerous mechanisms.

$G\alpha_{q/11}$ complexes stimulate ERK1/2 phosphorylation through phospholipase Cβ (PLCβ)-mediated inositol trisphosphate (IP_3) and diacylglycerol (DAG) generation and the subsequent activation of downstream effectors such as protein kinase C (PKC) and calmodulin (CaM). $G\alpha_{12/13}$ proteins also stimulate the generation of DAG via phospholipase D (PLD) and phospholipase A_2 (PLA_2), therefore promoting ERK1/2 phosphorylation through PKC.

$G\alpha_s$ modulation of ERK1/2 phosphorylation generally involves cAMP-dependent activation of protein kinase A (PKA) or exchange protein directly activated by cAMP (EPAC). This in turn can result in the activation of a GTP-binding protein, Rap1, which causes an enhancement of B-Raf activity, but inhibition of Raf-1. Therefore, $G\alpha_s$-mediated modulation of ERK1/2 phosphorylation can be highly dependent on cellular background (Goldsmith and Dhanasekaran, 2007). An alternative mechanism of ERK1/2 phosphorylation, involving PKA-mediated receptor phosphorylation and the switching of receptor coupling from G_s to $G_{i/o}$, has also been proposed for some G_s-coupled receptors (Daaka et al., 1997).

$G\alpha_{i/o}$-mediated inhibition of cAMP accumulation and recruitment of the Rap1 GTPase activating protein (Rap1GAP) can modulate ERK1/2 phosphorylation through the suppression of Rap1 (Goldsmith and Dhanasekaran, 2007). However, βγ dimers liberated from $G_{i/o}$ proteins can also modulate ERK1/2 phosphorylation through activation of PLCβ or phosphatidylinositol-kinase (PI3K). Activation of PI3K leads to Src-mediated receptor tyrosine kinase phosphorylation and subsequent recruitment of Src-homology 2 (SH2) domain containing adaptor proteins, such as Src homologous and collagen (Shc) and growth factor receptor-bound (Grb2) proteins. These adaptor proteins then recruit guanine nucleotide exchange factor (GEF), son of sevenless (Sos), from the cell membrane which causes stimulation of Ras activity through the facilitation of GDP/GTP exchange (Goldsmith and Dhanasekaran, 2007; Selbie and Hill, 1998).

In addition to the pathways described above, both α and βγ subunits of G proteins can stimulate ERK1/2 phosphorylation through transactivation of receptor tyrosine kinases. Transactivation leads to receptor tyrosine kinase dimerisation, autophosphorylation and, as such, modulation ERK1/2 phosphorylation via the pathway described above (Goldsmith and Dhanasekaran, 2007; Luttrell et al., 1997).

A number of GPCRs have also been shown to mediate ERK1/2 activation in a G protein-independent, but β-arrestin dependent manner. Signalling through this pathway involves receptor recruitment of β-arrestin and direct interactions of β-arrestin with Raf and ERK (DeFea et al., 2000). Both spatial and temporal disparities have been detected between G protein and β-arrestin-mediated ERK1/2 phosphorylation. While G protein-mediated ERK signalling is generally relatively transient and involve nuclear translocation, β-arrestin-mediated ERK1/2 phosphorylation appears more sustained and signalling complexes are retained within the cytosol (Ahn et al., 2004; DeFea et al., 2000).

4. Scaffolding proteins involved in the regulation of the ERK cascade

Scaffolding proteins provide a mechanism of increasing the efficiency of ERK signalling by bringing successive kinases into close proximity, preventing interactions with extraneous proteins through spatial segregation and tar-