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Review

"RAF" neighborhood: Protein–protein interaction in the Raf/Mek/Erk pathway

Botond Cseh [1], Eszter Doma [1], Manuela Baccarini *

Department of Microbiology, Immunobiology and Genetics, Center of Molecular Biology, Max F. Perutz Laboratories, University of Vienna, Doktor-Bohr-Gasse 9, 1030 Vienna, Austria

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ABSTRACT

The Raf/Mek/Erk signaling pathway, activated downstream of Ras primarily to promote proliferation, represents the best studied of the evolutionary conserved MAPK cascades. The investigation of the pathway has continued unabated since its discovery roughly 30 years ago. In the last decade, however, the identification of unexpected in vivo functions of pathway components, as well as the discovery of Raf mutations in human cancer, the ensuing quest for inhibitors, and the efforts to understand their mechanism of action, have boosted interest tremendously. From this large body of work, protein–protein interaction has emerged as a recurrent, crucial theme. This review focuses on the role of protein complexes in the regulation of the Raf/Mek/Erk pathway and in its cross-talk with other signaling cascades. Mapping these interactions and finding a way of exploiting them for therapeutic purposes is one of the challenges of future molecule-targeted therapy.

1. Introduction

The Raf/Mek/Erk signal transduction pathway is the best studied of the four mitogen-activated protein kinase (MAPK) cascades present in vertebrates (Fig. 1). It is activated by growth factors, hormones and cytokines and has been shown to regulate proliferation but also differentiation, survival, senescence, and migration [1]. Typically, ligand-binding to a cell surface receptor induces a wave of tyrosine phosphorylation (autophosphorylation in the case of receptor tyrosine kinases, or phosphorylation by receptor-associated kinases if the receptor itself lacks catalytic activity) resulting in the generation of phosphotyrosine binding sites for adaptor proteins such as growth factor receptor-bound protein 2 (GRB2). GRB2 mediates the membrane translocation of the guanine nucleotide exchange factor (GEF) son of sevenless (SOS), which in turn activates the membrane bound GTPase Ras [1]. Ras functions as a binary molecular switch that cycles between inactive GDP-bound and active GTP-bound states with the help of GEFs and GTPase activating proteins (GAPs). The exchange of GTP for GDP by SOS changes the conformation of Ras, allowing its interaction with effectors such as Raf [2]. GTP-bound Ras recruits Raf to the plasma membrane and enables it to phosphorylate its only substrates, Mek1 and Mek2 [3]. These dual specificity kinases mediate the activation of Erk1 and Erk2, enabling them to phosphorylate a variety of nuclear and cytoplasmic targets [4].

Mammals express three Raf isoforms, A-, B-, and C-Raf (the latter also called Raf-1) with distinct affinities for both the activator, Ras, and the downstream target Mek. B-Raf is the isoform most similar to Rafs expressed in lower organisms [5], and can therefore be considered the archetypal mammalian Mek kinase. A-Raf and C-Raf have evolved to fulfill other, potentially Mek-independent requirements [6,7]. Accordingly, growth factor-stimulated Erk activation is decreased in B-Raf-, but not A-Raf or C-Raf -deficient cells [8–12]. Similarly, the high occurrence of B-Raf but not A-Raf or C-Raf mutations in human cancers implies a dominant role for B-Raf in signaling to the Erk pathway [13,14].

2. Homo and heterodimers in Raf activation

Homo- and heterodimerization play an important role in the Erk pathway, whether by allowing the propagation of the signal to downstream effectors, by orchestrating feedback loops within the pathway, or by enabling communication with parallel signaling circuits [15]. Dimerization of pathway components can result in their activation (Raf) or inhibition (Mek). Furthermore, binding to different scaffolds can influence the localization of the components

* Corresponding author. Fax: +43 1 4277 9546.
E-mail address: manuela.baccarini@univie.ac.at (M. Baccarini).
[1] These authors have contributed equally to the manuscript.

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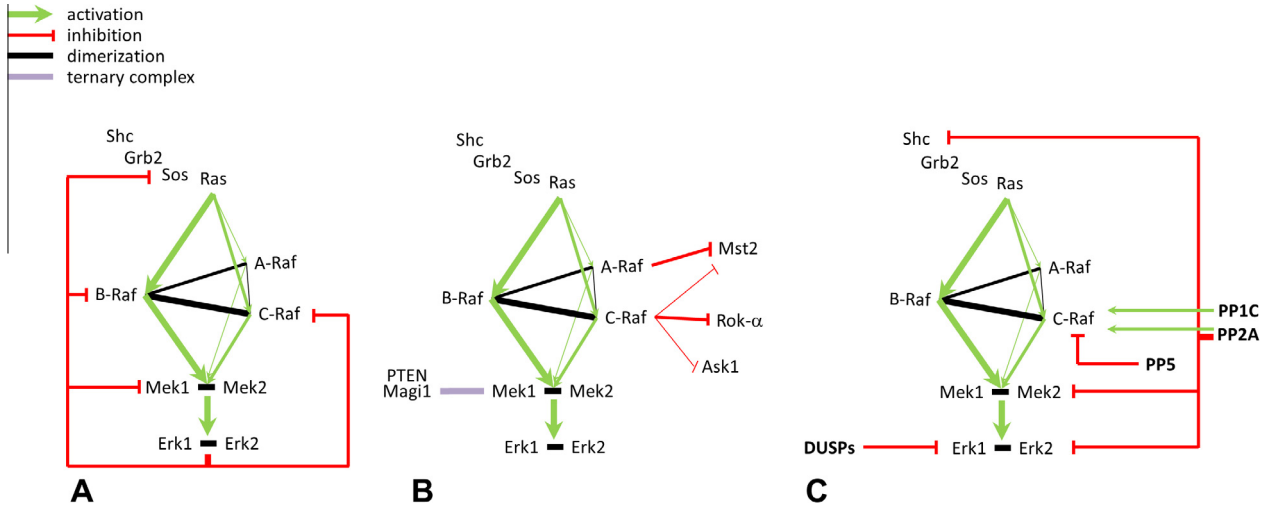

Fig. 1. The Raf/Mek/Erk Pathway. (A) *Schematic wiring of the pathway* – the Raf/Mek/Erk pathway is a three-tiered kinase cascade that operates downstream of the small GTPase Ras. The three Rafs bind Ras with different affinities, which determine their sensitivity to activated Ras. Rafs, in particular B-Raf and C-Raf, form homo- and heterodimers which phosphorylate and activate Meks, which in turn transfer the signal to Erks. Erks have many substrates whose activation leads to a variety of biological responses. Knockout studies have revealed that B-Raf is essential for Mek/Erk activation downstream of Ras; A-Raf and C-Raf can also activate Erk upon heterodimerization with B-Raf. Raf and Mek1 are the recipients of negative feedback phosphorylation by Erk, which determines the strength and duration of the Erk signal. (B) *Cross-talk with other pathways* – A-Raf and C-Raf can transmit signals in a Mek-independent manner, by communicating with parallel pathways. Both of them bind to and inhibit the proapoptotic kinase Mst2. In addition, C-Raf can bind and inhibit another proapoptotic kinase, Ask1, and the cytoskeleton-based Rok-α. An intact C-Raf:Rok-α complex is required for cell shape and motility, it impacts on angiogenesis and it is essential for preventing differentiation in Ras-driven epidermal tumors. Similar to C-Raf, Mek1 impacts a parallel pathway leading to Akt phosphorylation, by preventing PTEN-Mediated PIP3 turnover in the context of a Mek1/Magi1/PTEN ternary complex. (C) *Phosphatases interacting with Erk pathway components* – phosphatases play a dual role in Erk pathway regulation: a positive role, by facilitating C-Raf activation (PP2A, PP1C; green arrows) and a negative role (red lines) by dephosphorylating Shc, Mek and Erk (PP2A), C-Raf (PP5) or Erk (DUSPs). In Fig. 1B, line thickness is proportional to the strength and significance of the interactions.

to different cellular compartments, increasing signal fidelity and strength [16]. One of these scaffolds, the pseudokinase Ksr, interacts with Raf, Mek and Erk and localizes to the plasma membrane in a Ras-dependent manner [17].

Activation of Raf occurs via a complex, yet incompletely understood mechanism requiring membrane translocation, regulatory phosphorylation/dephosphorylation events [16] and, crucially, allosteric activation in the context of a side-to-side dimer comprising two Raf molecules or a Raf and a Ksr molecule [18–22]. Raf:Raf and Raf:Ksr dimerization depends on the dimer interface, a region located in the kinase domain, and in particular on a cluster of basic residues comprising B-RafR509, C-RafR401 and KsrR615 [21]. When these critical arginine residues are mutated to histidine (B-RafR509H, C-RafR401H, KsrR615H), activation does not take place. Conversely, the B-RafE586K mutation, which enhances dimerization and possibly allosteric transactivation, increases Erk signaling [23]. Growth factor-induced Raf dimerization can also be inhibited by an 18 amino acid peptide able to bind C-Raf and B-Raf, resulting in decreased Mek activation [23].

Of the three Raf kinases, only B-Raf is able to function as an allosteric activator in the context of the Raf heterodimers, a role independent of B-Raf kinase activity [14,19,24]. The molecular basis for this has recently been elucidated by the Shaw lab [22], who has shown that the ability of acting as an activator depends on the presence of negative charges in the Raf N-terminal acidic motif. In B-Raf, this motif is negatively charged due to the constitutive phosphorylation of Ser446 and/or 447, and to the presence of two aspartates at position 448/9 [25] (Fig. 2A). Allosteric activation by B-Raf induces cis-autophosporylation in the activation loop of the receiver kinase, i.e. C-Raf, and renders it able to phosphorylate Mek. Mek, in turn, phosphorylates the N-terminal acidic motif in C-Raf, converting it to an allosteric activator of other Rafs [22,26] (Fig. 2B and C). This model explains why C-Raf mutants devoid of kinase activity cannot function as activators, and why B-Raf can activate Mek directly as a homodimer [23]. Phosphorylated Ksr can also function as a transactivator; however, since Raf binding to Ksr induces limited kinase activity [27], in quiescent cells the constitutive association of Ksr with B-Raf may serve to prevent C-Raf binding to B-Raf, safeguarding against undue activation of the pathway [28].

Some naturally occurring mutants of B-Raf can bypass the requirement for dimerization-mediated activation. These mutations (i.e. G469A and V600E, 599insT) disrupt the interaction between the P-loop and activation-loop [14], resulting in a constitutively active B-Raf kinase largely resistant to the disruption of the dimer interface [29]. B-Raf V600E mutants require homodimerization for activation only when their binding to Ras is impaired [30]. Oncogenic Ras has also been shown to promote the binding of B-RafV600E-to wild-type C-Raf, which results in a weakening of V600E activity and of Erk activation [31]. This work implies that the B-RafV600E mutant is unable to transactivate C-Raf, which may explain why oncogenic Ras mutations and B-RafV600E appear to be mutually exclusive.

3. Signaling through Mek-Erk activation, negative feedback and pathway cross-talk

Although loss of function or conventional knockout studies have revealed distinct roles for all three kinases, embryonic- (B-Raf and C-Raf) [9,32] or post natal lethality (A-Raf) [33] has hindered the analysis of the role of Rafs in vivo. Conditional knockout models have provided an opportunity to bypass this difficulty, and to test the role of specific isoforms in the activation of the ERK pathway in different organs. In good agreement with the pivotal role of B-Raf in Raf activation, B-Raf has been identified as the essential Mek/Erk activator in the placenta during vascular development [34] and in oligodendrocyte differentiation and myelination [35]. In the context of cancer, keratinocyte-restricted B-Raf deletion reduces Ras-driven carcinogenesis, which is consistent with its importance in this type of skin tumors [36]. B-Raf is also