RAF/MAP kinase cascade

Gavathiotis, E., Roskoski, R Jr., Rothfels, K.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

17/11/2022

https://reactome.org
**Introduction**

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

**Literature references**


Reactome database release: 82

This document contains 6 pathways and 8 reactions (see Table of Contents)
The RAS-RAF-MEK-ERK pathway regulates processes such as proliferation, differentiation, survival, senescence and cell motility in response to growth factors, hormones and cytokines, among others. Binding of these stimuli to receptors in the plasma membrane promotes the GEF-mediated activation of RAS at the plasma membrane and initiates the three-tiered kinase cascade of the conventional MAPK cascades. GTP-bound RAS recruits RAF (the MAPK kinase kinase), and promotes its dimerization and activation (reviewed in Cseh et al, 2014; Roskoski, 2010; McKay and Morrison, 2007; Wellbrock et al, 2004). Activated RAF phosphorylates the MAPK kinase proteins MEK1 and MEK2 (also known as MAP2K1 and MAP2K2), which in turn phosphorylate the proline-directed kinases ERK1 and 2 (also known as MAPK3 and MAPK1) (reviewed in Roskoski, 2012a, b; Kryiaklis and Avruch, 2012). Activated ERK proteins may undergo dimerization and have identified targets in both the nucleus and the cytosol; consistent with this, a proportion of activated ERK protein relocalizes to the nucleus in response to stimuli (reviewed in Roskoski 2012b; Turjanski et al, 2007; Plotnikov et al, 2010; Cargnello et al, 2011). Although initially seen as a linear cascade originating at the plasma membrane and culminating in the nucleus, the RAS/RAF MAPK cascade is now also known to be activated from various intracellular location. Temporal and spatial specificity of the cascade is achieved in part through the interaction of pathway components with numerous scaffolding proteins (reviewed in McKay and Morrison, 2007; Brown and Sacks, 2009).

The importance of the RAS/RAF MAPK cascade is highlighted by the fact that components of this pathway are mutated with high frequency in a large number of human cancers. Activating mutations in RAS are found in approximately one third of human cancers, while ~8% of tumors express an activated form of BRAF (Roberts and Der, 2007; Davies et al, 2002; Cantwell-Dorris et al, 2011).

**Literature references**


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RAS proteins undergo several processing steps during maturation including farnesylation, carboxy-terminal cleavage and carboxymethylation, among others. These steps are required for their membrane localization and function and ultimately for their ability to activate RAF (reviewed in Gysin et al, 2011; Ahearn et al, 2018).

**Literature references**


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RAS intrinsic GTPase activity hydrolyzes GTP to GDP

**Location:** RAF/MAP kinase cascade

**Stable identifier:** R-HSA-9649736

**Type:** transition

**Compartments:** plasma membrane

RAS proteins have weak intrinsic GTPase activity in the absence of other effectors (Gibbs et al, 1984; reviewed in Pylayeva-Gupta et al, 2011). Nucleotide attack is mediated by residue Q61 and facilitated by van der Waals bonds contributed by glycine residues at position 12 and 13; these three residues account for the majority of oncogenic and pathogenic mutations found in RAS proteins (reviewed in Prior et al, 2012). GAP proteins stimulate the intrinsic GTPase activity of RAS proteins by inserting an arginine residue into the active site, which contributes to proper positioning of the critical Q61 RAS residue (reviewed in King et al, 2013).

**Preceded by:** Intrinsic nucleotide exchange on RAS

**Followed by:** Intrinsic nucleotide exchange on RAS

**Literature references**


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Intrinsic nucleotide exchange on RAS

**Location:** RAF/MAP kinase cascade

**Stable identifier:** R-HSA-9649735

**Type:** transition

**Compartments:** plasma membrane, cytosol

Inactive RAS:GDP is converted at a low rate to the active GTP-bound state through release of GDP and binding of GTP. This intrinsic GEF activity is weak due to the picomolar affinity of the protein for both nucleotides, but is stimulated by the interaction of RAS proteins with guanine nucleotide exchange factors (Marshall et al, 2012; reviewed in Bourne et al, 1991; Hennig et al, 2015; Pei et al, 2018).

**Preceded by:** RAS intrinsic GTPase activity hydrolyzes GTP to GDP

**Followed by:** RAS intrinsic GTPase activity hydrolyzes GTP to GDP

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The human genome is predicted to encode 27 RAS guanine nucleotide exchange factors (GEFs) that promote the exchange of GDP for GTP on membrane-associated RAS in response to RAS-MAPK pathway activation by growth factors, hormones, cytokines and other stimuli (reviewed in Cherfils and Zeghouf, 2013; Cargnello and Roux, 2011). Nucleotide exchange stimulates a conformational change in RAS to facilitate its interaction with RAF, ultimately promoting the phosphorylation of downstream effectors MAPK3 and MAPK1 (also known as ERK1 and ERK2) (reviewed in Cseh et al, 2014; Vigil et al, 2010).

Followed by: RAS:GTP binds PI3K, RAS:GTP binds RAL GDS proteins

Literature references


Zeghouf, M., Cherfils, J. (2013). Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol. Rev.*, 93, 269-309.
GTP-bound RAS interacts with PI3K through a direct interaction with the 110 kDa catalytic subunit (Sjolander et al, 1991; Rodriguez-Viciana et al, 1994; Rodriguez-Viciana et al, 1996; Pacold et al, 2000; reviewed in Gysin et al, 2011; Castellano and Downward, 2011; Martini et al, 2014). Interaction with RAS stimulates the activity of PI3K by promoting a conformational change and/or mediating recruitment to the plasma membrane, among other possible mechanisms (Pacold et al, 2000; Denley et al, 2008; Zhang et al, 2019). The PI3K signaling pathway contributes to RAS-mediated cellular proliferation and survival and through RAC, contributes to cytoskeletal rearrangements and cell motility (reviewed in Vivanco and Sawyers, 2002; Castellano and Downward, 2011; Martini et al, 2014; Nussinov et al, 2015).

Preceded by: RAS GEFs promote RAS nucleotide exchange

Literature references


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RAL GDS and related family members RGL1, 2 and 3 are small GTPase proteins in the RAS family that act as effectors downstream of HRAS and other RAS proteins (reviewed in Ferro and Trabalzini, 2010; Gentry et al, 2014). RALGDS family members bind to HRAS in the GTP-bound state through the RALGDS Ras binding domains (RBDs) and acts as RAL A and RAL B-specific guanine nuclear exchange factors (GEFs) (Hofer et al, 1994; Spaargen et al, 1994; Kikuchi et al, 1994; Wolthius et al, 1996; Peterson et al, 1996; Shao et al, 2000; Ehrhardt et al, 2001; reviewed in Gentry et al, 2014).
Mammals have three RAF isoforms, A, B and C, that are activated downstream of RAS and stimulate the MAPK pathway. Although CRAF (also known as RAF-1) was the first identified and remains perhaps the best studied, BRAF is most similar to the RAF expressed in other organisms. Notably, MAPK (ERK) activation is more compromised in BRAF-deficient cells than in CRAF or ARAF deficient cells (Bonner et al, 1985; Mikula et al, 2001, Huser et al, 2001, Mercer et al, 2002; reviewed in Leicht et al, 2007; Matallanas et al, 2011; Cseh et al, 2014). Consistent with its important role in MAPK pathway activation, mutations in the BRAF gene, but not in those for A- or CRAF, are associated with cancer development (Davies et al, 2002; reviewed in Leicht et al, 2007). ARAF and CRAF may have arisen through gene duplication events, and may play additional roles in MAPK-independent signaling (Hindley and Kolch, 2002; Murakami and Morrison, 2001).

Despite divergences in function, all mammalian RAF proteins share three conserved regions (CRs) and each interacts with RAS and MEK proteins, although with different affinities. The N-terminal CR1 contains a RAS-binding domain (RBD) and a cysteine-rich domain (CRD) that mediate interactions with RAS and the phospholipid membrane. CR2 contains inhibitory phosphorylation sites that impact RAS binding and RAF activation, while the C-terminal CR3 contains the bi-lobed kinase domain with its activation loop, and an adjacent upstream "N-terminal acidic motif" -S(S/G)YY in C- and A-RAF, respectively, and SS-DD in B-RAF - that is required for RAF activation (Tran et al, 2005; Dhillon et al, 2002; Chong et al, 2001; Cutler et al, 1998; Chong et al, 2003; reviewed in Matallanas et al, 2011).

Regulation of RAF activity involves multiple phosphorylation and dephosphorylation events, intramolecular conformational changes, homo- and heterodimerization between RAF monomers and changes to protein binding partners, including scaffolding proteins which bring pathway members together (reviewed in Matallanas et al, 2011; Cseh et al, 2014). The details of this regulation are not completely known and differ slightly from one RAF isoform to another. Briefly, in the inactive state, RAF phosphorylation on conserved serine residues in CR2 promote an interaction with 14-3-3 dimers, maintaining the kinase in a closed conformation. Upon RAS activation, these sites are dephosphorylated, allowing the RAF CRD and RBD to bind RAS and phospholipids, facilitating membrane recruitment. RAF activation requires homo- or heterodimerization, which promotes autophosphorylation in the activation loop of the receiving monomer. Of the three isoforms, only BRAF is able to initiate this allosteric activation of other RAF monomers (Hu et al, 2013; Heidorn et al, 2010; Garnett et al, 2005). This activity depends on negative charge in the N-terminal acidic region (NtA; S(S/G)YY or SSDD) adjacent to the kinase.
domain. In BRAF, this region carries permanent negative charge due to the presence of the two aspartate residues in place of the tyrosine residues of A- and CRAF. In addition, unique to BRAF, one of the serine residues of the NtA is constitutively phosphorylated. In A- and CRAF, residues in this region are subject to phosphorylation by activated MEK downstream of RAF activation, establishing a positive feedback loop and allowing activated A- and CRAF monomers to act as transactivators in turn (Hu et al, 2013; reviewed in Cseh et al, 2014). RAF signaling is terminated through dephosphorylation of the NtA region and phosphorylation of the residues that mediate the inhibitory interaction with 14-3-3, promoting a return to the inactive state (reviewed in Matallanas et al, 2011; Cseh et al, 2014).

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MAP2K and MAPK activation

Location: RAF/MAP kinase cascade

Stable identifier: R-HSA-5674135

Activated RAF proteins are restricted substrate kinases whose primary downstream targets are the two MAP2K proteins, MAPK2K1 and MAPK2K2 (also known as MEK1 and MEK2) (reviewed in Roskoski, 2010, Roskoski, 2012a). Phosphorylation of the MAP2K activation loop primes them to phosphorylate the primary effector of the activated MAPK pathway, the two MAPK proteins MAPK3 and MAPK1 (also known as ERK1 and 2). Unlike their upstream counterparts, MAPK3 and MAPK1 catalyze the phosphorylation of hundreds of cytoplasmic and nuclear targets including transcription factors and regulatory molecules (reviewed in Roskoski, 2012b). Activation of MAP2K and MAPK proteins downstream of activated RAF generally occurs in the context of a higher order scaffolding complex that regulates the specificity and localization of the pathway (reviewed in Brown and Sacks, 2009; Matallanas et al, 2011).

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Phosphorylated MAPK monomers can dimerize - generally into MAPK1 and MAPK3 homodimers, as the heterodimer is unstable- but the physiological significance of dimerization is unclear (Khokhlatchev et al, 1998; reviewed Rosokoski, 2012b). MAPKs have both cytosolic and nuclear targets and dimerization may be particularly important for MAPK-dependent phosphorylation of cytosolic targets. Phosphorylation of cytosolic MAPK targets appears to happen predominantly in the context of larger scaffolding complexes, and since the scaffolds and cytosolic MAPK substrates contact the same hydrophobic surface of MAPK, dimerization is necessary to allow assembly of a functional complex (Casar et al, 2008; Lidke et al, 2010; reviewed in Casar et al, 2009). Consistent with this, disrupting either MAPK dimerization or the MAPK interaction with the scaffolding protein abrogated proliferation and transformation (Casar et al, 2008). Note that, for simplicity in this diagram, dimerization is shown as happening between free cytosolic monomers of activated MAPK rather than in the context of the scaffolding complex.

Although predominantly cytoplasmic in resting cells, a proportion of activated MAPK translocates to the nucleus upon stimulation where it activates nuclear targets. Despite early studies to the suggesting that dimerization was required for nuclear translocation, a few recent papers have challenged this notion (Lenormand et al, 1993; Chen et al, 1992; Khokhlatchev et al, 1998; Casar et al, 2008; Lidke et al, 2010; Burack and Shaw, 2005; reviewed in Roskoski, 2012b).

Followed by: Phosphorylated MAPKs translocate into the nucleus, PEA15 binds MAPK monomers and dimers

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After phosphorylation by MAP2Ks, a proportion of activated MAPK translocates into the nucleus where it activates nuclear targets (reviewed in Roskoski, 2012b). MAPKs, which lack a nuclear localization signal (NLS), may 'piggyback' into the nucleus in complex with other nuclear-targeted proteins or may translocate by virtue of interaction with components of the nuclear pore complex (Brunet et al, 1999; Adachi et al, 1999; Matsubayashi et al, 2001; Whitehurst et al, 2002; Khokhlatchev et al, 1998; reviewed in Roskoski, 2012b). Although dimerization of MAPKs was thought to be critical for nuclear translocation, a number of studies have now challenged the physiological relevance of MAPK dimerization and this remains an area of uncertainty (Lenormand et al, 1993; Chen et al, 1992; Casar et al, 2008; Lidke et al, 2010; Burack and Shaw, 2005; reviewed in Casar et al, 2009; Roskoski, 2012b)

**Preceded by:** p-T,Y MAPKs dimerize

**Literature references**


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**Phosphorylated MAPKs translocate into the nucleus**

**Location:** RAF/MAP kinase cascade

**Stable identifier:** R-HSA-5674387

**Type:** omitted

**Compartments:** nucleoplasm
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PEA15 binds MAPK monomers and dimers

Location: RAF/MAP kinase cascade

Stable identifier: R-HSA-5675206

Type: binding

Compartments: cytosol

PEA15 is a cytoplasmic anchor that binds directly to activated MAPKs prevents their translocation into the nucleus (Formstecher et al, 2001; Whitehurst et al, 2004; Hill et al, 2002; Chou et al, 2003). PEA15 also protects phosphorylated MAPKs in the cytoplasm from inactivating dephosphorylation (Mace et al, 2013). In this way, binding of PEA15 promotes phosphorylation of cytoplasmic MAPK targets at the expense of nuclear ones.

Preceded by: p-T,Y MAPKs dimerize

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The duration and extent of activated MAPK signaling is regulated at many levels through mechanisms that include phosphorylation and dephosphorylation, changes to protein interacting partners and subcellular localization (reviewed in Matallanas et al, 2011).

Activated RAF proteins are subject to MAPK-dependent phosphorylation that promotes the subsequent dephosphorylation of the activation loop and Nta region, terminating RAF kinase activity. This dephosphorylation, catalyzed by PP2A and PP5, primes the RAF proteins for PKA or AKT-mediated phosphorylation of residues S259 and S621, restoring the 14-3-3 binding sites and returning the RAF proteins to the inactive state (von Kriegsheim et al, 2006; Dougherty et al, 2005; reviewed in Matallanas et al, 2011). The phosphorylated RAF1 Nta is also subject to additional regulation through binding to the PEBP1 protein, which promotes its dissociation from MAP2K substrates (Shin et al, 2009).

Activated MAPK proteins also phosphorylate T292 of MAP2K1; this phosphorylation limits the activity of MAP2K1, and indirectly affects MAP2K2 activity through by modulating the activity of the MAP2K heterodimer (Catalanotti et al, 2009; reviewed in Matallanas et al, 2011).

Dephosphorylation of MAPKs by the dual specificity MAPK phosphatases (DUSPs) plays a key role in limiting the extent of pathway activation (Owens et al, 2007; reviewed in Roskoski, 2012b). Class I DUSPs are localized in the nucleus and are induced by activation of the MAPK pathway, establishing a negative feedback loop, while class II DUSPs dephosphorylate cytoplasmic MAPKs (reviewed in Rososki, 2012b).

MAPK signaling is also regulated by the RAS GAP-mediated stimulation of intrinsic RAS GTPase activity which returns RAS to the inactive, GDP bound state (reviewed in King et al, 2013).

**Literature references**


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The intrinsic GTPase activity of RAS proteins is stimulated by the GAP proteins, of which there are at least 10 in the human genome (reviewed in King et al, 2013).

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