1. Phylogeny  
   RAF1, also known as c‑RAF, is a member of the Raf family of serine/threonine protein kinases that function as critical MAP3Ks in the Ras‑dependent MAPK/ERK cascade (an2015raf‐interactomeintuning pages 3-4). It is evolutionarily conserved across metazoans and falls into the kinase grouping described by Manning et al., which identifies the Raf family as part of an essential core of eukaryotic signaling enzymes (martinvega2023navigatingtheerk12 pages 2-4). Within the human kinome, RAF1 clusters with its homologs B‑RAF and A‑RAF, yet exhibits distinctive regulatory and catalytic features that have diverged during evolution (taylor2013pseudokinasesfroma pages 1-3). Moreover, comparative studies indicate that while all three isoforms share a common ancestral origin, RAF1 in particular has maintained its role as a regulatory switch downstream of Ras GTPases in a wide variety of organisms (martinvega2023navigatingtheerk12 pages 2-4).
2. Reaction Catalyzed  
   RAF1 catalyzes the transfer of the γ‑phosphate group from ATP to target serine or threonine residues on substrate proteins, most notably the dual‐specificity MAP2Ks MEK1 and MEK2 (an2015raf‐interactomeintuning pages 16-18). The generalized reaction is:  
     ATP + [protein]‑(L‑serine/threonine) → ADP + [protein]‑(L‑serine/threonine‑phosphate) + H⁺ (an2015raf‐interactomeintuning pages 16-18).  
   This phosphorylation event subsequently enables the activation of downstream effectors in the MAPK cascade (an2015raf‐interactomeintuning pages 16-18).
3. Cofactor Requirements  
   The kinase activity of RAF1 is dependent on the presence of ATP as the phosphate donor and requires divalent metal ions, with Mg²⁺ being the principal cofactor to aid in ATP coordination and phosphoryl transfer (an2015raf‐interactomeintuning pages 16-18). These cofactors are necessary for the correct folding of the active site and efficient catalysis of the phosphorylation reaction (martinvega2023navigatingtheerk12 pages 28-29).
4. Substrate Specificity  
   RAF1 exhibits substrate specificity primarily toward MEK1 and MEK2, which contain serine residues within their activation loops that are targeted for phosphorylation (an2015raf‐interactomeintuning pages 16-18). In addition to MEK kinases, RAF1 phosphorylates several other substrates including BAD (Bcl‑2‑antagonist of cell death) at Ser‑75, various adenylyl cyclases (ADCY2, ADCY5, ADCY6) to induce their activation, and PPP1R12A resulting in inhibition of phosphatase activity (an2015raf‐interactomeintuning pages 13-15). Though no single consensus motif has been defined for RAF1 substrates, the enzyme displays a marked preference for phosphorylating specific serine/threonine residues within functionally critical domains (martinvega2023navigatingtheerk12 pages 28-29).
5. Structure  
   RAF1 comprises a modular architecture that includes three conserved regions: CR1, CR2, and CR3 (an2015raf‐interactomeintuning pages 8-9).  
   • CR1, located at the N‑terminus, contains the Ras‑binding domain (RBD) and the cysteine‑rich domain (CRD); together they mediate interaction with activated Ras, as well as membrane localization through association with anionic phospholipids (an2015raf‐interactomeintuning pages 11-13).  
   • CR2 is a serine/threonine‑rich regulatory region that features key phosphorylation sites, notably Ser‑259, whose phosphorylation promotes the binding of inhibitory 14‑3‑3 proteins (an2015raf‐interactomeintuning pages 6-8).  
   • CR3 constitutes the C‑terminal kinase domain responsible for catalytic activity and harbors several regulatory elements such as the activation loop, the αC‑helix, and the hydrophobic spines (taylor2013pseudokinasesfroma pages 4-6).  
   Structural studies based on crystallographic and computational models reveal that RAF1’s kinase domain adopts the canonical bilobal fold with a smaller N‑terminal lobe and a larger C‑terminal lobe, and the proper alignment of the regulatory (R‑spine) and catalytic (C‑spine) elements is essential for its activity (taylor2013pseudokinasesfroma pages 1-3). The interface for dimerization, critical for optimal kinase function, is also embedded within the kinase domain, further emphasizing the importance of domain–domain interactions in RAF1’s structure (an2015raf‐interactomeintuning pages 9-10).
6. Regulation  
   RAF1 activity is tightly controlled through a multiplicity of regulatory mechanisms. Phosphorylation plays a central role, with several residues acting as molecular switches; for instance, phosphorylation at Ser‑259 creates a docking site for 14‑3‑3 proteins, which maintain RAF1 in an autoinhibited conformation (an2015raf‐interactomeintuning pages 6-8). Conversely, phosphorylation of residues in the activation loop, particularly Ser‑338 and Ser‑339—events mediated by kinases such as PAK1—promotes RAF1 activation (an2015raf‐interactomeintuning pages 13-15).  
   Additionally, RAF1 regulation is achieved through protein–protein interactions; binding to active Ras is required to relieve autoinhibition and recruit RAF1 to the plasma membrane, where further conformational changes and dimerization occur (martinvega2023navigatingtheerk12 pages 28-29). The interplay with molecular chaperones, such as HSP90, and scaffolding proteins like 14‑3‑3 ensures proper folding and stabilization of the active form (an2015raf‐interactomeintuning pages 9-10). The enzyme also functions as part of larger signaling complexes that integrate multiple upstream signals, acting as a nexus for Ras–RAF–MEK signaling (rauch2011thesecretlife pages 9-11). Finally, allosteric mechanisms involving shifts in the hydrophobic spines and repositioning of the αC‑helix further modulate catalytic activity in response to extracellular stimuli (taylor2013pseudokinasesfroma pages 4-6).
7. Function  
   RAF1 functions as a pivotal regulatory link between membrane‑associated Ras GTPases and the downstream MAPK/ERK cascade, controlling critical cell fate decisions such as proliferation, differentiation, survival, apoptosis, and oncogenic transformation (an2015raf‐interactomeintuning pages 13-15). Upon activation, RAF1 phosphorylates MEK1 and MEK2, thereby initiating a cascade that leads to ERK activation and the subsequent regulation of gene expression programs that influence cell cycle progression and survival (martinvega2023navigatingtheerk12 pages 28-29). Beyond its canonical role in the MAPK pathway, RAF1 phosphorylates additional substrates, including BAD—a key regulator of apoptosis—thereby promoting cell survival (an2015raf‐interactomeintuning pages 13-15). RAF1 also modulates the activity of adenylyl cyclases (ADCY2, ADCY5 and ADCY6), PPP1R12A, and cardiac troponin T, reflecting its involvement in diverse cellular processes ranging from metabolic regulation to myocardial function (an2015raf‐interactomeintuning pages 13-15). Moreover, RAF1 has been implicated in the activation of NF‑κB and in the regulation of cytoskeletal elements that influence cell motility (rauch2011thesecretlife pages 9-11).
8. Other Comments  
   RAF1 is a significant target in cancer therapy owing to its role in propagating oncogenic signals. Inhibition strategies include molecules that target the catalytic site or interfere with regulatory protein interactions; for instance, FOBISIN101 has been reported to disrupt the 14‑3‑3 binding that contributes to RAF1’s autoinhibition (an2015raf‐interactomeintuning pages 11-13). Additionally, the paradoxical activation of RAF kinases by some inhibitors poses a therapeutic challenge, highlighting the need for compounds that selectively modulate RAF1’s activity without eliciting adverse feedback activation (rauch2011thesecretlife pages 9-11). RAF1 mutations are less common than aberrations in B‑RAF; however, dysregulation of RAF1—whether by loss of normal inhibitory interactions or aberrant upstream signaling—has been associated with various malignancies, making it an important focus for targeted therapy development (an2015raf‐interactomeintuning pages 13-15, martinvega2023navigatingtheerk12 pages 28-29). Furthermore, RAF1’s dual role as both a kinase and a scaffold protein underscores its multifaceted functions in cellular signaling, warranting careful consideration in drug development and therapeutic intervention strategies (taylor2013pseudokinasesfroma pages 1-3).
9. References
10. an2015raf‐interactomeintuning pages 3-4
11. an2015raf‐interactomeintuning pages 6-8
12. an2015raf‐interactomeintuning pages 8-9
13. an2015raf‐interactomeintuning pages 9-10
14. an2015raf‐interactomeintuning pages 11-13
15. an2015raf‐interactomeintuning pages 13-15
16. an2015raf‐interactomeintuning pages 16-18
17. an2015raf‐interactomeintuning pages 19-20
18. an2015raf‐interactomeintuning pages 22-22
19. martinvega2023navigatingtheerk12 pages 1-2
20. martinvega2023navigatingtheerk12 pages 2-4
21. martinvega2023navigatingtheerk12 pages 14-16
22. martinvega2023navigatingtheerk12 pages 28-29
23. taylor2013pseudokinasesfroma pages 1-3
24. taylor2013pseudokinasesfroma pages 4-6
25. rauch2011thesecretlife pages 9-11

References

1. (an2015raf‐interactomeintuning pages 13-15): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
2. (an2015raf‐interactomeintuning pages 3-4): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
3. (an2015raf‐interactomeintuning pages 8-9): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
4. (an2015raf‐interactomeintuning pages 9-10): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
5. (martinvega2023navigatingtheerk12 pages 2-4): Ana Martin-Vega and Melanie H. Cobb. Navigating the erk1/2 mapk cascade. Biomolecules, 13:1555, Oct 2023. URL: https://doi.org/10.3390/biom13101555, doi:10.3390/biom13101555. This article has 58 citations and is from a peer-reviewed journal.
6. (rauch2011thesecretlife pages 9-11): Jens Rauch, Natalia Volinsky, David Romano, and Walter Kolch. The secret life of kinases: functions beyond catalysis. Cell Communication and Signaling : CCS, 9:23-23, Oct 2011. URL: https://doi.org/10.1186/1478-811x-9-23, doi:10.1186/1478-811x-9-23. This article has 243 citations.
7. (taylor2013pseudokinasesfroma pages 1-3): Susan S. Taylor, Andrey Shaw, Jiancheng Hu, Hiruy S. Meharena, and Alexandr Kornev. Pseudokinases from a structural perspective. Biochemical Society Transactions, 41:981-986, Jul 2013. URL: https://doi.org/10.1042/bst20130120, doi:10.1042/bst20130120. This article has 56 citations and is from a peer-reviewed journal.
8. (an2015raf‐interactomeintuning pages 11-13): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
9. (an2015raf‐interactomeintuning pages 16-18): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
10. (an2015raf‐interactomeintuning pages 19-20): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
11. (an2015raf‐interactomeintuning pages 6-8): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
12. (martinvega2023navigatingtheerk12 pages 1-2): Ana Martin-Vega and Melanie H. Cobb. Navigating the erk1/2 mapk cascade. Biomolecules, 13:1555, Oct 2023. URL: https://doi.org/10.3390/biom13101555, doi:10.3390/biom13101555. This article has 58 citations and is from a peer-reviewed journal.
13. (martinvega2023navigatingtheerk12 pages 28-29): Ana Martin-Vega and Melanie H. Cobb. Navigating the erk1/2 mapk cascade. Biomolecules, 13:1555, Oct 2023. URL: https://doi.org/10.3390/biom13101555, doi:10.3390/biom13101555. This article has 58 citations and is from a peer-reviewed journal.
14. (taylor2013pseudokinasesfroma pages 4-6): Susan S. Taylor, Andrey Shaw, Jiancheng Hu, Hiruy S. Meharena, and Alexandr Kornev. Pseudokinases from a structural perspective. Biochemical Society Transactions, 41:981-986, Jul 2013. URL: https://doi.org/10.1042/bst20130120, doi:10.1042/bst20130120. This article has 56 citations and is from a peer-reviewed journal.
15. (an2015raf‐interactomeintuning pages 22-22): Su An, Yang Yang, Richard Ward, Ying Liu, Xiao‐Xi Guo, and Tian‐Rui Xu. Raf‐interactome in tuning the complexity and diversity of raf function. The FEBS Journal, Jan 2015. URL: https://doi.org/10.1111/febs.13113, doi:10.1111/febs.13113. This article has 25 citations.
16. (martinvega2023navigatingtheerk12 pages 14-16): Ana Martin-Vega and Melanie H. Cobb. Navigating the erk1/2 mapk cascade. Biomolecules, 13:1555, Oct 2023. URL: https://doi.org/10.3390/biom13101555, doi:10.3390/biom13101555. This article has 58 citations and is from a peer-reviewed journal.