Current Biology, Vol. 14, R277–R278, April 6, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.03.023

Signal Transduction: IMPlications for Ras-Dependent ERK Signaling

Dispatch

Stéphane Ory and Deborah K. Morrison

Ras interacts with numerous downstream effectors to transmit a diverse array of cellular signals. A new study shows that a protein known as Impedes Mitogenic signal Propagation, IMP, is an E3 ubiquitin ligase that binds Ras and modulates MAP kinase signaling by regulating the scaffolding activity of KSR.

The Ras pathway is an essential signal transduction cascade that controls cell survival, growth, differentiation and transformation. Regulation of the plasma-membrane-bound Ras GTPase is conceptually simple — Ras acts as a molecular switch that is 'on' when bound to GTP and 'off' when bound to GDP [1]. The signaling responses induced by Ras activation are highly varied, however, due largely to the myriad effector molecules that bind Ras in its GTP-bound state, such as the MAP kinase kinase kinase Raf-1, the Ral guanine nucleotide dissociation stimulator (RalGDS), phosphatidylinositol 3-kinase, phospholipase Cε, the Ras inhibitor RIN1, and the polarity protein AF6/Canoe [2,3]. Now, with the discovery of Impedes Mitogenic signal Propagation (IMP), another layer of regulatory complexity for this important signaling cascade has been revealed. In a recent study published in *Nature* by Matheny and colleagues [4], IMP is identified as a Ras-binding protein that modulates the signaling capacity of a key Ras effector cascade, namely the Raf–MEK–ERK kinase cascade.

IMP was isolated in a yeast two-hybrid screen for proteins that interact with activated Ras, where binding was specific for Ras and not observed with other Ras family members. IMP is also known as BRAP2 and was originally discovered as a protein that associates with the nuclear localization motifs of the breast cancer tumor suppressor protein BRCA1 [5]; however, the functional significance of this interaction is unknown. IMP is a ubiquitously expressed, highly conserved protein, with one ortholog present in all eukaryotes examined [4,5]. All IMP proteins contain three conserved structural motifs — a RING-H2 domain followed by a ubiquitin-protease-like zinc finger (UBP-ZnF; also known as a PAZ domain) and leucine heptad repeats that are predicted to form a coiled coil. As would be expected for a Ras effector, Matheny *et al.* [4] found that IMP binds to Ras in a GTP-dependent and stimulus-dependent manner. Interestingly, however, IMP does not possess a classical Ras-binding domain or Ras-association domain found in many Ras-binding partners and instead is reported to interact with Ras through a region encompassing the UBP-ZnF domain.

Laboratory of Protein Dynamics and Signaling, NCI-Frederick, Frederick, Maryland 21702, USA. E-mail: dmorrison@ncifcrf.gov

When Matheny and coworkers [4] examined the effect of IMP on Ras signaling, they found that instead of cooperating with other effectors to promote Ras signaling, IMP appeared to inhibit signal transmission through the Raf–MEK–ERK cascade. Overexpression of IMP blocked Raf-dependent activation of MEK and ERK without preventing activation of Raf itself; this inhibition could be circumvented by constitutively active MEK. IMP overexpression also prevented the mitogen-induced association of Raf and MEK, indicating that high IMP levels had uncoupled Raf from its downstream substrate. Providing further evidence that IMP was an antagonist of Raf-1 signaling, depletion of IMP in mammalian cells increased the amplitude of stimulus-induced ERK activation. Thus, IMP can be added to the growing list of molecules that negatively regulate Raf–MEK–ERK signaling, a list that already includes the Raf kinase inhibitor protein RKIP [6], RIN1 [7] and Sprouty/SPRED [8,9].

Surprisingly, the inhibitory effect of IMP was not mediated by a direct interaction with Raf, MEK or ERK. Matheny and coworkers [4] found no evidence that IMP associated with or ubiquitinated Raf, MEK or ERK, and the cellular levels of these potential targets were unaltered by IMP overexpression. Instead, IMP appears to uncouple signal transmission from Raf to MEK by inactivating an important scaffolding protein of the Ras pathway, namely the kinase suppressor of Ras (KSR) [10–12]. In normal unstimulated cells, KSR constitutively associates with MEK and is retained in the cytoplasm through the binding of a dimer of 14-3-3 proteins to two phosphoserine sites [13]. In response to stimulus, the protein phosphatase PP2A dephosphorylates one of these sites, resulting in the release of 14-3-3, the rapid translocation of KSR to the plasma membrane, and exposure of KSR's MAP kinase binding site [14]. The end result is that KSR delivers MEK to activated Raf at the plasma membrane, provides a docking platform for ERK, and facilitates the sequential phosphorylation events required for ERK activation.

Evidence that KSR is the target of IMP's inhibitory effect was obtained using fibroblasts from KSR-deficient mice [15]. Reintroduction of exogenous KSR into these cells leads to enhanced levels of stimulus-induced ERK activation, which is completely abolished by IMP overexpression. IMP associates with KSR and, when overexpressed, causes hyperphosphorylation and mislocalization of KSR into a detergent-insoluble fraction. These effects resemble those observed previously for an inactive KSR mutant that is unable to bind MEK [16]. Further investigation is needed to determine how IMP binding causes the hyperphosphorylation and mislocalization of KSR and, in particular, whether IMP influences MEK binding to KSR. Nonetheless, these findings suggest that IMP may inactivate KSR by sequestering it in a cellular compartment inaccessible to activators of KSR function (Figure 1).

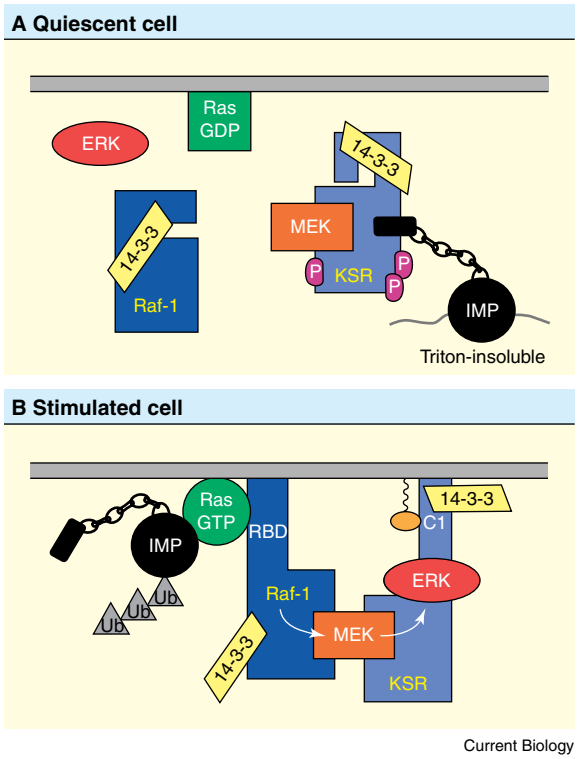

Figure 1. Model for the regulation of ERK signaling by IMP.

(A) In quiescent cells, KSR constitutively interacts with dimeric 14-3-3 and inactive MEK. KSR also associates with IMP, and when IMP is overexpressed, KSR is hyperphosphorylated and localized to a triton-insoluble cellular fraction, apparently inactivating the scaffolding activity of KSR. (B) In stimulated cells, Ras activation has dual effector inputs on the ERK cascade. First, by recruiting cytoplasmic Raf to the plasma membrane, Ras initiates the Raf activation process. Second, by recruiting IMP, Ras relieves the inhibition on KSR, allowing KSR to translocate to the plasma membrane and mediate complex formation between Raf, MEK and ERK. Ras binding also stimulates the autoubiquitination of IMP, which may target IMP for degradation or interfere with the IMP–KSR interaction.

Importantly, the inhibitory effect of IMP is relieved by activated Ras, which restores ERK signaling and correct localization of KSR. The E3 ligase activity of IMP has been implicated in this step, because activated Ras stimulates the autoubiquitination of IMP and an IMP mutant lacking E3 ligase activity acts as a constitutive 'super-inhibitor'. One caveat is that constitutively activated Ras, and not stimulus-induced normal Ras, was required for overcoming the effects of overexpressed IMP in these studies. The ability of physiological levels of Ras activation to overcome any inhibitory effects of IMP therefore remains to be confirmed in normal cells. Additional characterization might also reveal whether Ras activation targets IMP for degradation or otherwise interferes with the IMP–KSR interaction.

The overall conclusion from this new study is that Ras activation has dual effector inputs on the ERK cascade: initiating Raf activation while derepressing KSR-dependent Raf–MEK complex formation (Figure 1). In this sense, IMP is likely to serve as a negative regulator that keeps Ras signaling in check until cells receive an appropriate stimulus. IMP might thus perform an important role in fine-tuning ERK cascade activity, allowing cells to react to diverse stimuli with greater sensitivity and fidelity. Yet to be resolved is whether IMP has other functions and whether it influences signaling through other Ras effector cascades. On a more global level, because IMP depletion allowed cells to respond to suboptimal doses of an extracellular stimulus, IMP might also modulate the threshold sensitivity of cells to stimulus. If so, then IMP may play a critical role in allowing cells to adapt their response to chronic or complex signaling cues, a real-life property of cell signaling not easily explained by the simple model of a GTPase 'on/off' switch.

References

1. Bourne, H.R., Sanders, D.A., and McCormick, F. (1990). The GTPase superfamily: a conserved switch for diverse cell functions. Nature *348*, 125–132.
2. Vojtek, A.B., and Der, C.J. (1998). Increasing complexity of the Ras signaling pathway. J. Biol. Chem. *273*, 19925–19928.
3. Downward, J. (2003). Targeting RAS signalling pathways in cancer therapy. Nat. Rev. Cancer *3*, 11–22.
4. Matheny, S.A., Chen, C., Kortum, R.L., Razidlo, G.L., Lewis, R.E., and White, M.A. (2004). Ras regulates assembly of mitogenic signalling complexes through the effector protein IMP. Nature *427*, 256–260.
5. Li, S., Ku, C.Y., Farmer, A.A., Cong, Y.S., Chen, C.F., and Lee, W.H. (1998). Identification of a novel cytoplasmic protein that specifically binds to nuclear localization signal motifs. J. Biol. Chem. *273*, 6183–6189.
6. Yeung, K., Janosch, P., McFerran, B., Rose, D.W., Mischak, H., Sedivy, J.M., and Kolch, W. (2000). Mechanism of suppression of the Raf/MEK/extracellular signal-regulated kinase pathway by the raf kinase inhibitor protein. Mol. Cell. Biol. *20*, 3079–3085.
7. Wang, Y., Waldron, R.T., Dhaka, A., Patel, A., Riley, M.M., Rozengurt, E., and Colicelli, J. (2002). The RAS effector RIN1 directly competes with RAF and is regulated by 14–3-3 proteins. Mol. Cell. Biol. *22*, 916–926.
8. Sasaki, A., Taketomi, T., Kato, R., Saeki, K., Nonami, A., Sasaki, M., Kuriyama, M., Saito, N., Shibuya, M., and Yoshimura, A. (2003). Mammalian Sprouty4 suppresses Ras-independent ERK activation by binding to Raf1. Nat. Cell Biol. *5*, 427–432.
9. Wakioka, T., Sasaki, A., Kato, R., Shouda, T., Matsumoto, A., Miyoshi, K., Tsuneoka, M., Komiya, S., Baron, R., and Yoshimura, A. (2001). Spred is a Sprouty-related suppressor of Ras signalling. Nature *412*, 647–651.
10. Roy, F., Laberge, G., Douziech, M., Ferland-McCollough, D., and Therrien, M. (2002). KSR is a scaffold required for activation of the ERK/MAPK module. Genes Dev. *16*, 427–438.
11. Ohmachi, M., Rocheleau, C.E., Church, D., Lambie, E., Schedl, T., and Sundaram, M.V. (2002). C. elegans ksr-1 and ksr-2 have both unique and redundant functions and are required for MPK-1 ERK phosphorylation. Curr. Biol. *12*, 427–433.
12. Morrison, D.K., and Davis, R.J. (2003). Regulation of MAP kinase signaling modules by scaffold proteins in mammals. Annu. Rev. Cell Dev. Biol. *19*, 91–118.
13. Muller, J., Ory, S., Copeland, T., Piwnica-Worms, H., and Morrison, D.K. (2001). C-TAK1 regulates Ras signaling by phosphorylating the MAPK scaffold, KSR1. Mol. Cell *8*, 983–993.
14. Ory, S., Zhou, M., Conrads, T.P., Veenstra, T.D., and Morrison, D.K. (2003). Protein phosphatase 2A positively regulates Ras signaling by dephosphorylating KSR1 and Raf-1 on critical 14–3-3 binding sites. Curr. Biol. *13*, 1356–1364.
15. Nguyen, A., Burack, W.R., Stock, J.L., Kortum, R., Chaika, O.V., Afkarian, M., Muller, W.J., Murphy, K.M., Morrison, D.K., Lewis, R.E., et al. (2002). Kinase suppressor of Ras (KSR) is a scaffold which facilitates mitogen-activated protein kinase activation in vivo. Mol. Cell. Biol. *22*, 3035–3045.
16. Stewart, S., Sundaram, M., Zhang, Y., Lee, J., Han, M., and Guan, K.L. (1999). Kinase suppressor of Ras forms a multiprotein signaling complex and modulates MEK localization. Mol. Cell. Biol. *19*, 5523–5534.