J. Biochem. **136**, 557–561 (2004)
DOI: 10.1093/jb/mvh159

Regulatory Mechanisms and Function of ERK MAP Kinases

Satoru Torii, Kei Nakayama, Takuya Yamamoto and Eisuke Nishida*

Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502

Received September 18, 2004; accepted September 18, 2004

Spatiotemporal control of the Ras/ERK MAP kinase signaling pathway is a key factor for determining the specificity of cellular responses including cell proliferation, cell differentiation and cell survival. The fidelity of this signaling is regulated by docking interactions as well as scaffolding. Subcellular localization of ERK is controlled by cytoplasmic ERK anchoring proteins that have a nuclear export signal (NES), such as MEK. In quiescent cells, ERK and MEK localize to the cytoplasm. In response to stimulation, dissociation of the MEK-ERK complex is induced and activated ERK translocates to the nucleus. Recently, several negative regulators for Ras/ERK signaling have been identified and their detailed molecular mechanisms have been analyzed. Among them, Sprouty and Sef act as a temporal and a spatial regulator, respectively, for Ras/ERK signaling. Thus, multiple factors are involved in control of Ras/ERK signaling.

Key words: docking interaction, negative regulator, nuclear export signal, nuclear translocation, spatiotemporal control.

Overview

The Ras/ERK MAP kinase signaling pathway is highly conserved throughout evolution, and plays an important role in various cellular responses including cell proliferation, cell differentiation and cell survival (*1–5*). Many studies about regulation of Ras/ERK signaling have been reported. Spatiotemporal control of this signaling is a key factor for determining the specificity of cellular responses (*6–8*).

The fidelity of ERK MAP kinase signaling

The MAP kinase (MAPK) cascades convey signals in the form of phosphorylation events. Therefore, MAPKs form a complex with their cognate MAPKKs, substrates and phosphatases. There are three major subgroups of the MAPK family: ERK, p38 and JNK/SAPK. These members are activated by different stimuli and are involved in signaling to different responses through different pathways. In order to achieve the specificity and efficiency of the enzymatic reaction, there are two main mechanisms in the MAP kinase cascades: the docking interaction and the scaffolding (*9*, *10*).

MAPKs utilize the common docking (CD) domain for docking interactions with MAPKKs, MAPKAPKs and phosphatases (*11*). The CD domain is featured by a cluster of negatively charged amino acids and is located in the C-terminal portion of MAPKs in the primary sequence. MAPKs have another site called the ED site, near the CD domain in the steric structure, which could determine the docking specificity towards MAPKAPKs (*12*). In addition, hydrophobic regions of MAPKs are also important for docking interactions (*13–16*). All of these docking sites locate outside the catalytic domain and determine the specificity of interacting molecules (Fig. 1, left).

Scaffolding proteins interact with several components of the MAPK cascades to tether both enzymes and substrates specifically to achieve accurate signal transduction (*10*). KSR and MP-1 are known to function as an ERK scaffold. Actually, these molecules have been reported to associate with several components of the ERK signaling pathway and to enhance ERK activation (*17*, *18*). The JNK-interacting protein (JIP) family is also a well-known scaffold protein family (*10*). The JIP proteins bind to JNK, MKK7 and members of the mixed-lineage protein kinase (MLK) group. In addition, a recent report showed that JIP1 and JIP2 also interact with MKP-7 (*19*). Thus, JIP scaffold complexes include both activating and inhibitory components of the JNK signaling pathway (Fig. 1, right).

Through these molecular mechanisms described above, MAPKs react with appropriate partners to avoid undesirable outcomes. Therefore, the docking interaction and the scaffolding may regulate not only the efficiency and specificity of the cascade but also the ordered and integrated signaling.

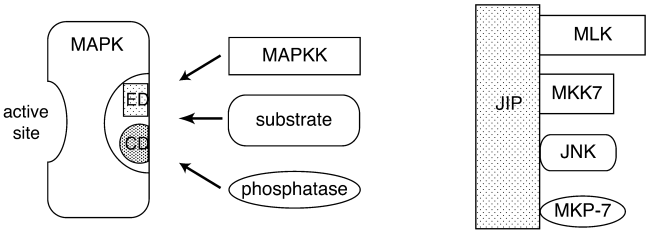

Fig. 1. **In the MAPK cascade, two kinds of mechanisms for determining the efficiency and specificity are used.** The docking interactions are achieved through the docking groove including the CD domain and the ED site (left). The scaffold protein tethers several components of the JNK signaling pathway (right).

*To whom correspondence should be addressed. Tel: +81-75-753-4230, Fax: +81-75-753-4235, E-mail: L50174@sakura.kudpc.kyoto-u.ac.jp

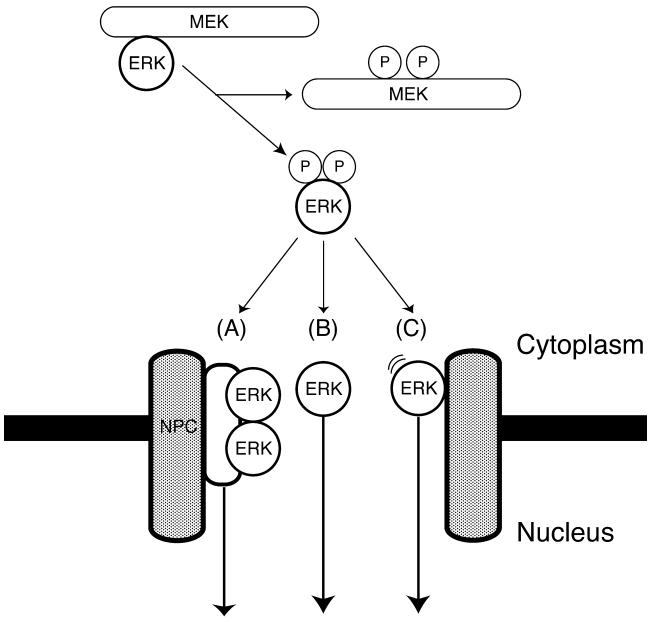

Fig. 2. **A model for nuclear translocation of ERK.** The activation of the ERK pathway results in the phosphorylation of ERK and the subsequent dissociation of ERK from MEK. Dissociated ERK translocates from the cytoplasm to the nucleus by three pathways; (A) active transport of a dimer, (B) passive diffusion of a monomer and (C) transport mediated by direct interaction of ERK with the nuclear pore complex.

Subcellular localization of ERK

MAPKs dramatically change their subcellular localization upon the extracellular stimuli. In quiescent cells, ERK localizes to the cytoplasm. This cytoplasmic localization of ERK is mediated by its specific binding to classical MAPKKs, MEK1 and MEK2, which localize to the cytoplasm. The cytoplasmic localization of MEK is achieved by its NES sequence in its amino-terminal domain (*20*). In addition to MEK, PEA-15, which also contains NES, binds ERK and retains ERK in the cytoplasm (*21*).

Phosphorylation of ERK induced by extracellular stimuli, such as growth factors, leads to dissociation of ERK from MEK. The dissociated ERK translocates from the cytoplasm to the nucleus. There are three pathways for the nuclear import of ERK; (i) passive diffusion of a monomer, (ii) active transport of a dimer, which is mediated by the low molecular weight GTPase Ran and the importin-β family protein(s), and (iii) Ran/importin-β family-independent transport, which is mediated by direct interaction of ERK with the nuclear pore complex (Fig. 2 and Refs. *22–25*). In the nucleus, ERK phosphorylates and activates several nuclear targets such as transcription factors. Nuclear localization of ERK appears to be prerequisite for proper cellular responses (*26*). The nuclear accumulation of ERK may require an unidentified nuclear anchor(s) (*27*). The nuclear anchor(s) may be a short-lived protein synthesized by the ERK pathway activation. Interestingly, in fission yeast, the nuclear import of stress-activated MAPK Spc1 is coupled with its dissociation from MAPKK Wis1, and the nuclear retention of Spc1 requires a nuclear anchor (*28*). Therefore, several aspects of the mechanism of nuclear translocation of MAPK may be evolutionarily conserved. Further studies are required to elucidate the mechanism for nuclear import of other MAPK family members.

To prepare the subsequent stimulation, ERK must relocalize to the cytoplasm. This relocalization of ERK could be achieved by an NES-dependent nuclear export. MEK, which mostly localizes to the cytoplasm due to its NES, can shuttle between the cytoplasm and the nucleus. It has been suggested that the relocalization of inactive ERK involves MEK-dependent active transport; MEK transiently enters the nucleus and binds inactive ERK to export it from the nucleus (*29*). The regulation of subcellular localization of ERK should be crucial for specific cellular responses to extracellular stimuli.

Temporal control of ERK signaling by Sprouty1/2

Sprouty and Sprouty-related protein with EVH-1 domain (Spred) have been identified as conserved inhibitors for Ras/ERK MAP kinase signaling (*30–37*). Recent studies have demonstrated detailed molecular mechanisms of action of Sprouty or Spred (*37–49*). Spred inhibits Ras/ERK signaling at the level of Raf by binding to Ras and Raf (*37*). However, the action mechanism of Sprouty has been controversial.

Sprouty proteins bind various proteins. We have reported that Sprouty1/2 becomes phosphorylated on a conserved tyrosine residue (Y53 in Sprouty1 or Y55 in Sprouty2) in their amino-terminal domain in a stimulus-dependent manner (Fig. 3 top left) and binds to Grb2 (*40*). This binding prevents Grb2 from binding to either FRS2 or Shp2, leading to inhibition of Ras/ERK signaling. Moreover, we have shown that Sprouty1/2 would be dephosphorylated by Shp2 (*47*). Other several reports have indicated that Sproury2 becomes phosphorylated on the same conserved tyrosine in a stimulus-dependent manner, and becomes bound to c-Cbl, the E3 ubiquitin ligase (*43–45*, *49*). This association results in polyubiquitylation and subsequent degradation of Sprouty2 by the proteasome. In EGF signaling, c-Cbl normally binds to the activated EGF receptor and promotes its polyubiquitylation and subsequent degradation. However, binding of Sprouty2 to c-Cbl inhibits this function of c-Cbl. As a result, Sprouty2 enhances EGF signaling in contrast with its function in FGF signaling (*41*, *43–45*). The other group reported that the carboxy-terminal cysteine-rich domain of Sprouty4 binds to Raf1 (*46*). This interaction inhibits VEGF-induced activation of Raf, but not Ras-induced one. As Sprouty4 does not become phosphorylated in response to FGF or EGF stimulation (*40*, *49*), the action mechanism of Sprouty4 could be different from that of Sprouty1/2. Nevertheless, the conserved tyrosine residue in their amino-terminal domain appears to be required for Sprouty proteins' inhibitory activity. Mutant Sprouty proteins in which the conserved tyrosine residue (Y53 in Sprouty1/4 or Y55 in Sprouty2) is mutated act as a dominant-negative form (*39*, *40*, *49*). By using this dominant-negative form of Sprouty, we have found that Sprouty1/2 could control the duration of ERK activity (*40*). Overexpression of Sprouty1 results in transient ERK activation, whereas overexpression of Sprouty1 Y53F results in sustained ERK activation (Fig. 3, top right). Correspondingly, overexpression of Sprouty1 Y53F or Sprouty2 Y55F in PC12 cells enhances FGF-

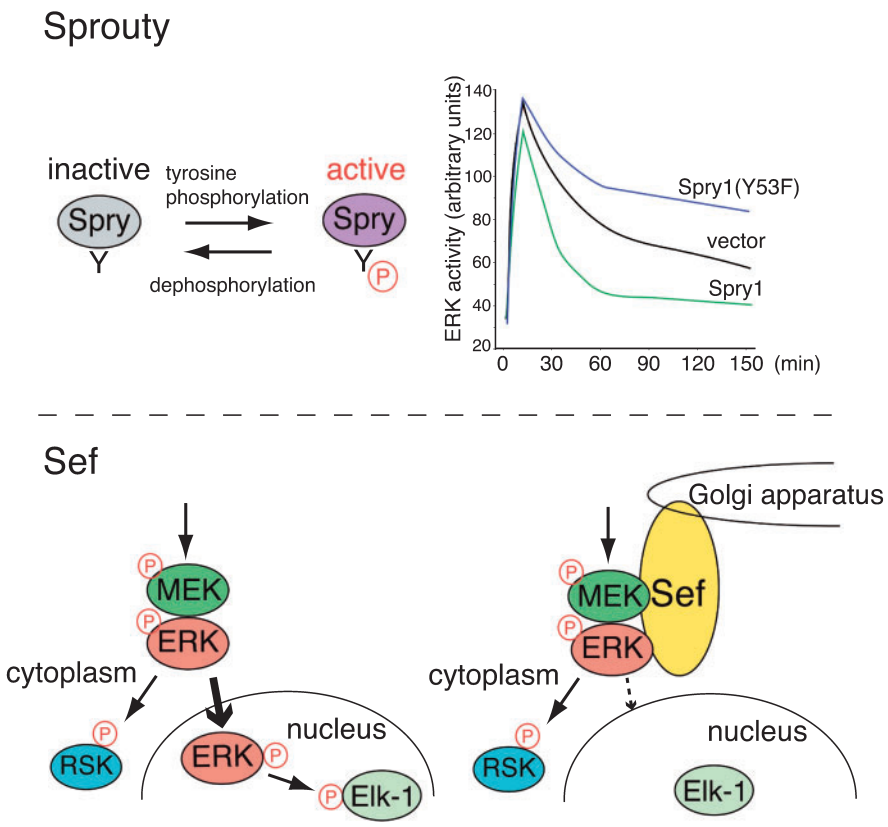

Fig. 3. **Spatiotemporal control of Ras/ERK signaling.** Sprouty (Spry) is phosphorylated and activated in a stimulus-dependent manner (top left). Sprouty controls the duration of ERK activation and provides temporal control for ERK signaling (top right). Sef provides spatial control for ERK signaling. Sef inhibits dissociation of the MEK/ERK complex and retains activated ERK on the Golgi apparatus. Thus Sef specifically inhibits ERK nuclear translocation without inhibiting its activity in the cytoplasm (bottom).

induced neurite outgrowth. Thus Sprouty provides temporal control for Ras/ERK signaling.

Spatial control of ERK signaling by Sef

Recently, Sef, a putative transmembrane protein, was identified in zebrafish as a negative feedback inhibitor for Ras/ERK signaling (*50*, *51*). Sef has been identified in other vertebrates and thus is thought to be a conserved inhibitor for Ras/ERK signaling (*50–54*). However, there are contradicting reports concerning the action point of Sef. Several reports indicate that Sef acts downstream of, or at MEK and inhibits phosphorylation of ERK (*50*, *55*, *56*). In contrast, other reports argue that Sef inhibits FGF signaling upstream of Ras by binding to FGF receptor (*57*, *58*)

Most recently, our analyses have shown that Sef acts as a spatial regulator for Ras/ERK signaling by specifically blocking ERK nuclear translocation without inhibiting its activity in the cytoplasm (*59*). Immunoprecipitation assays have shown that Sef binds to the MEK-ERK complex. Rather surprisingly, the binding of Sef to the MEK-ERK complex did not inhibit the phosphorylation or the kinase activity of ERK. Moreover, in immunofluorescence experiments, Sef colocalized with activated ERK as well as activated MEK mainly on the Golgi apparatus in stimulated cells. Recent reports suggested that part of Ras is localized and activated on the Golgi apparatus in response to EGF stimulation (*60–62*). Sef on the Golgi apparatus could bind well to the MEK-ERK complex, which is activated downstream of Ras on the Golgi. Notably, Sef blocks active MEK-induced dissociation of the MEK-ERK complex and EGF-induced ERK nuclear translocation. Furthermore, we have found that Sef inhibits phosphorylation of nuclear ERK substrates without affecting phosphorylation of cytoplasmic ERK substrates. Sef inhibits stimulus-dependent phosphorylation of Elk-1, a nuclear target of ERK, but does not inhibit phosphorylation of RSK2, a well-known ERK substrate in the cytoplasm. Downregulation of endogenous Sef by siRNA enhances stimulus-induced ERK nuclear translocation and the expression level of ERK target genes, such as *c-fos* without affecting phosphorylation of both ERK and RSK2. Thus we propose that Sef is a specific inhibitor of Ras/ERK signaling to the nucleus by targeting ERK to the cytoplasm (Fig. 3, bottom) and provides spatial control for Ras/ERK signaling.

Conclusion and future prospects

As describe above, spatiotemporal control of ERK MAP kinase signaling is finely regulated by multiple factors, such as Sprouty and Sef. Sprouty is phosphorylated in a stimulus-dependent manner and provides temporal control for ERK signaling. The next challenges may include elucidation of control mechanisms of ERK signaling by Sprouty in vivo. Sef binds to active MEK, and targets ERK to the cytoplasm. Therefore, Sef provide spatial control of ERK signaling. The next challenges may include elucidation of regulatory mechanisms of Sef.

REFERENCES

1. Sturgill, T.W. and Wu, J. (1991) Recent progress in characterization of protein kinase cascades for phosphorylation of ribosomal protein S6. *Biochim. Biophys. Acta* **1092**, 350–357

2. Nishida, E. and Gotoh, Y. (1993) The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem. Sci.* **18**, 128–131
3. Lewis, T.S., Shapiro, P.S., and Ahn, N.G. (1998) Signal transduction through MAP kinase cascades. *Adv. Cancer Res.* **74**, 49–139
4. Chang, L. and Karin, M. (2001) Mammalian MAP kinase signalling cascades. *Nature* **410**, 37–40
5. Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.E., Karandikar, M., Berman, K., and Cobb, M.H. (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr. Rev.* **22**, 153–183
6. Marshall, C.J. (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**, 179–185
7. Bivona, T.G. and Philips, M.R. (2003) Ras pathway signaling on endomembranes. *Curr. Opin. Cell Biol.* **15**, 136–142
8. Pouyssegur, J. and Lenormand, P. (2003) Fidelity and spatiotemporal control in MAP kinase (ERKs) signalling. *Eur. J. Biochem.* **270**, 3291–3299
9. Biondi, R.M. and Nebreda, A.R. (2003) Signalling specificity of Ser/Thr protein kinases through docking-site-mediated interactions. *Biochem. J.* **372**, 1–13
10. 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
11. Tanoue, T., Adachi, M., Moriguchi T., and Nishida, E. (2000) A conserved docking motif in MAP kinases common to substrate, activators and regulators. *Nat. Cell Biol.* **2**, 110–116
12. Tanoue, T., Maeda, R., Adachi, M., and Nishida, E. (2001) Identification of a docking groove on ERK and p38 MAP kinases that regulates the specificity of docking interactions. *EMBO J.* **20**, 466–479
13. Xu, B., Stippec, S., Robinson, F.L., and Cobb, M.H. (2001) Hydrophobic as well as charged residues in both MEK1 and ERK2 are important for their proper docking. *J. Biol. Chem.* **276**, 26509–26515
14. Zhou, B., Wu, L., Shen, K., Zhang, J., Lawrence, D.S., and Zhang, Z.Y. (2001) Multiple regions of MAP kinase phosphatase 3 are involved in its recognition and activation by ERK2. *J. Biol. Chem.* **276**, 6506–6515
15. Chang, C.I., Xu, B., Akella, R., Cobb, M.H., and Goldsmith, E.J. (2002) Crystal structures of MAP kinase p38 complexed to the docking sites on its nuclear substrate MEF2A and activator MKK3b. *Mol. Cell* **9**, 1241–1249
16. Seidel, J.J. and Graves, B.J. (2002) An ERK2 docking site in the pointed domain distinguishes a subset of ETS transcription factors. *Genes Dev.* **16**, 127–137
17. Schaeffer, H.J., Catling, A.D., Eblen, S.T., Collier, L.S., Krauss, A., and Weber, M.J. (1998) MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. *Science* **281**, 1668–1671
18. Morrison, D.K. (2001) KSR: a MAPK scaffold of the Ras pathway? *J. Cell Sci.* **114**, 1609–1612
19. Willoughby, E.A., Perkins, G.R., Collins, M.K., and Whitmarsh, A.J. (2003) The JNK-interacting protein-1 scaffold protein targets MAPK phosphatase-7 to dephosphorylate JNK. *J. Biol. Chem.* **278**, 10731–10736
20. Fukuda, M., Gotoh, I., Gotoh, Y., and Nishida, E. (1996) Cytoplasmic localization of mitogen-activated protein kinase kinase directed by its NH2-terminal, leucine-rich short amino acid sequence, which acts as a nuclear export signal. *J. Biol. Chem.* **271**, 20024–20028
21. Formstecher, E., Ramos, J.W., Fauquet, M., Calderwood, D.A., Hsieh, J.C., Canton, B., Nguyen, X.T., Barnier, J.V., Camonis, J., Ginsberg, M.H., and Chneiweiss, H. (2001) PEA-15 mediates cytoplasmic sequestration of ERK MAP kinase. *Dev. Cell* **1**, 239–250
22. Khokhlatchev, A.V., Canagarajah, B., Wilsbacher, J., Robinson, M., Atkinson, M., Goldsmith, E., and Cobb, M.H. (1998) Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* **93**, 605–615
23. Adachi, M., Fukuda, M., and Nishida, E. (1999) Two co-existing mechanisms for nuclear import of MAP kinase: passive diffusion of a monomer and active transport of a dimer. *EMBO J.* **18**, 5347–5358
24. Matsubayashi, Y., Fukuda, M., and Nishida, E. (2001) Evidence for existence of a nuclear pore complex-mediated, cytosol-independent pathway of nuclear translocation of ERK MAP kinase in permeabilized cells. *J. Biol. Chem.* **276**, 41755–41760
25. Whitehurst, A.W., Wilsbacher, J.L., You, Y., Luby-Phelps, K., Moore, M.S., and Cobb, M.H. (2002) ERK2 enters the nucleus by a carrier-independent mechanism *Proc. Natl Acad. Sci. USA* **99**, 7496–7501
26. Brunet, A., Roux, D., Lenormand, P., Dowd, S., Keyse, S., and Pouyssegur, J. (1999) Nuclear translocation of p42/p44 mitogen-activated protein kinase is required for growth factor-induced gene expression and cell cycle entry. *EMBO J.* **18**, 664–674
27. Lenormand, P., Brondello, J.M., Brunet, A., and Pouyssegur, J. (1998) Growth factor-induced p42/p44 MAPK nuclear translocation and retention requires both MAPK activation and neosynthesis of nuclear anchoring proteins. *J. Cell Biol.* **142**, 625–633
28. Gaits, F., Degols, G., Shiozaki, K., and Russell, P. (1998) Phosphorylation and association with the transcription factor Atf1 regulate localization of Spc1/Sty1 stress-activated kinase in fission yeast. *Genes Dev.* **12**, 1464–1473
29. Adachi, M., Fukuda, M., and Nishida, E. (2000) Nuclear export of MAP kinase (ERK) involves a MAP kinase kinase (MEK)-dependent active transport mechanism. *J. Cell Biol.* **148**, 849–856
30. Hacohen, N., Kramer, S., Sutherland, D., Hiromi, Y., and Krasnow, M.A. (1998) *sprouty* encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways. *Cell* **92**, 253–263
31. Casci, T., Vinos, J., and Freeman, M. (1999) Sprouty, an intracellular inhibitor of Ras signaling. *Cell* **96**, 655–665
32. de Maximy, A.A., Nakatake, Y., Moncada, S., Itoh, N., Thiery, J.P., and Bellusci, S. (1999) Cloning and expression pattern of a mouse homologue of *Drosophila sprouty* in the mouse embryo. *Mech. Dev.* **81**, 213–216
33. Kramer, S., Okabe, M., Hacohen, N., Krasnow, M.A., and Hiromi, Y. (1999) Sprouty: a common antagonist of FGF and EGF signaling pathways in Drosophila. *Development* **126**, 2515–2525
34. Minowada, G., Jarvis, L.A., Chi, C.L., Neubuser, A., Sun, X., Hacohen, N., Krasnow, M.A., and Martin, G.R. (1999) Vertebrate Sprouty genes are induced by FGF signaling and can cause chondrodysplasia when overexpressed. *Development* **126**, 4465–4475
35. Reich, A., Sapir, A., and Shilo, B. (1999) Sprouty is a general inhibitor of receptor tyrosine kinase signaling. *Development* **126**, 4139–4147
36. Chambers, D. and Mason, I. (2000) Expression of *sprouty2* during early development of the chick embryo is coincident with known sites of FGF signalling. *Mech. Dev.* **91**, 361–364
37. 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
38. Gross, I., Bassit, B., Benezra, M., and Licht, J.D. (2001) Mammalian Sprouty proteins inhibit cell growth and differentiation by preventing Ras activation. *J. Biol. Chem.* **276**, 46460–46468
39. Sasaki, A., Taketomi, T., Wakioka, T., Kato, R., and Yoshimura, A. (2001) Identification of a dominant negative mutant of Sprouty that potentiates fibroblast growth factor- but not epidermal growth factor-induced ERK activation. *J. Biol. Chem.* **276**, 36804–36808
40. Hanafusa, H. Torii, S., Yasunaga, T., and Nishida, E. (2002) Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nat. Cell Biol.* **4**, 850–858

41. Wong, E.S., Fong, C.W., Lim, J., Yusoff, P., Low, B.C., Langdon, W.Y., and Guy, G.R. (2002) Sprouty2 attenuates epidermal growth factor receptor ubiquitylation and endocytosis, and consequently enhances Ras/ERK signalling. *EMBO J.* **21**, 4796–4808
42. Yusoff, P., Lao, D.H., Ong, S.H., Wong, E.S., Lim, J., Lo, T.L., Leong, H.F., Fong, C.W., and Guy, G.R. (2002) Sprouty2 inhibits the Ras/MAP kinase pathway by inhibiting the activation of Raf. *J. Biol. Chem.* **277**, 3195–3201
43. Fong, C.W., Leong, H.F., Wong, E.S., Lim, J., Yusoff, P., and Guy, G.R. (2003) Tyrosine phosphorylation of Sprouty2 enhances its interaction with c-Cbl and is crucial for its function. *J. Biol. Chem.* **278**, 33456–33464
44. Hall, A.B., Jura, N., DaSilva, J., Jang, Y.J., Gong, D., and Bar-Sagi, D. (2003) hSpry2 is targeted to the ubiquitin-dependent proteasome pathway by c-Cbl. *Curr. Biol.* **13**, 308–314
45. Rubin, C., Litvak, V., Medvedovsky, H., Zwang, Y., Lev, S., and Yarden, Y. (2003) Sprouty fine-tunes EGF signaling through interlinked positive and negative feedback loops. *Curr. Biol.* **13**, 297–307
46. 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
47. Hanafusa, H., Torii, S., Yasunaga, T., Matsumoto, K., and Nishida, E. (2004) Shp2, an SH2-containing protein-tyrosine phosphatase, positively regulates receptor tyrosine kinase signaling by dephosphorylating and inactivating the inhibitor Sprouty. *J. Biol. Chem.* **279**, 22992–22995
48. Kim, H.J. and Bar-Sagi, D. (2004) Modulation of signalling by Sprouty: a developing story. *Nat. Rev. Mol. Cell. Biol.* **5**, 441–450
49. Mason, J.M., Morrison, D.J., Bassit, B., Dimri, M., Band, H., Licht, J.D., and Gross, I. (2004) Tyrosine phosphorylation of Sprouty proteins regulates their ability to inhibit growth factor signaling: a dual feedback loop. *Mol. Biol. Cell* **15**, 2176–2188
50. Furthauer, M., Lin, W., Ang, S.L., Thisse, B., and Thisse, C. (2002) Sef is a feedback-induced antagonist of Ras/MAPK-mediated FGF signalling. *Nat. Cell Biol.* **4**, 170–174
51. Tsang, M., Friesel, R., Kudoh, T., and Dawid, I.B. (2002) Identification of Sef, a novel modulator of FGF signalling. *Nat. Cell Biol.* **4**, 165–169
52. Lin, W., Furthauer, M., Thisse, B., Thisse, C., Jing, N., and Ang, S.L. (2002) Cloning of the mouse *Sef* gene and comparative analysis of its expression with *Fgf8* and *Spry2* during embryogenesis. *Mech. Dev.* **113**, 163–168
53. Niehrs, C. and Meinhardt, H. (2002) Modular feedback. *Nature* **417**, 35–36
54. Kawakami, Y., Rodriguez-Leon, J., Koth, C.M., Buscher, D., Itoh, T., Raya, A., Ng, J.K. Esteban, C.R., Takahashi, S., Henrique, D., Schwarz, M.F., Asahara, H., and Izpisua Belmonte, J.C. (2003) MKP3 mediates the cellular response to FGF8 signalling in the vertebrate limb. *Nat. Cell Biol.* **5**, 513–519
55. Yang, R.B., Ng, C.K., Wasserman, S.M., Komuves, L.G., Gerritsen, M.E., and Topper, J.N. (2003) A novel IL-17 receptor-like protein identified in human umbilical vein endothelial cells antagonizes basic fibroblast growth factor-induced signaling. *J. Biol. Chem.* **278**, 33232–33238
56. Preger, E., Ziv, I., Shabtay, A., Sher, I., Tsang, M., Dawid, I.B., Altuvia, Y., and Ron, D. (2004) Alternative splicing generates an isoform of the human Sef gene with altered subcellular localization and specificity. *Proc. Natl Acad. Sci. USA* **101**, 1229–1234
57. Kovalenko, D., Yang, X., Nadeau, R.J., Harkins, L.K., and Friesel, R. (2003) Sef inhibits fibroblast growth factor signaling by inhibiting FGFR1 tyrosine phosphorylation and subsequent ERK activation. *J. Biol. Chem.* **278**, 14087–14091
58. Xiong, S., Zhao, Q., Rong, Z., Huang, G., Huang, Y., Chen P., Zhang, S., Liu, L., and Chang, Z. (2003) hSef inhibits PC-12 cell differentiation by interfering with Ras-mitogen-activated protein kinase MAPK signaling. *J. Biol. Chem.* **278**, 50273–50282
59. Torii, S., Kusakabe, M., Yamamoto, T., Maekawa, M., and Nishida, E. (2004) Sef is a spatial regulator for Ras/MAP kinase signaling. *Dev. Cell* **7**, 33–44
60. Chiu, V.K., Bivona, T., Hach, A., Sajous, J.B., Silletti, J., Wiener, H., Johnson, R.L. 2nd, Cox, A.D., and Philips, M.R. (2002) Ras signalling on the endoplasmic reticulum and the Golgi. *Nat. Cell Biol.* **4**, 343–350
61. Bivona, T.G., Perez De Castro, I., Ahearn, I.M., Grana, T.M., Chiu, V.K., Lockyer, P.J., Cullen, P.J., Pellicer, A., Cox, A.D., and Philips, M.R. (2003) Phospholipase Cgamma activates Ras on the Golgi apparatus by means of RasGRP1. *Nature* **424**, 694–698
62. Zhang, S.Q., Yang, W., Kontaridis, M.I., Bivona, T.G., Wen, G., Araki, T., Luo, J., Thompson, J.A., Schraven, B.L., Philips, M.R., and Neel, B.G. (2004) Shp2 regulates SRC family kinase activity and Ras/Erk activation by controlling Csk recruitment. *Mol. Cell* **13**, 341–355