Activation of Rac1 by Paxillin-Crk-DOCK180 Signaling Complex Is Antagonized by Rap1 in Migrating NBT-II Cells*

Ana M. Vallés‡, Maud Beuvin, and Brigitte Boyer

From the Unité Mixte Recherche 146, Centre National de la Recherche Scientifique, Bâtiment 110, Centre Universitaire, 91405 Orsay, France

One efficient way to regulate complex cellular behaviors such as cell migration is to assemble functional networks composed of multidomain proteins (1). The focal adhesion protein paxillin is a good example of a multidomain regulator. Paxillin primary structure is composed of several protein-binding modules (proline-rich region, LD motifs, phosphorylatable tyrosine, LIM domains) that allow its association with structural and signaling molecules (2). Perturbing the formation of protein complexes has revealed the functional significance of some of these domains. This has led to the notion that paxillin is a convergence point of signaling pathways that positively modulate cell migration. For example, paxillin binding site of CrkII (R38V) has a similar inhibitory effect on cell locomotion in cells expressing Phe-31/118 paxillin mutants deficient in Rac1 GTP-loading, suggesting that formation of paxillin-Crk-DOCK180 signaling complex controls collagen-dependent migration mainly through Rac1 activation. In migrating cells, CrkII constitutive association with C3G was not sufficient to stimulate its GDP/GTP exchange activity toward the Ras family GTPase Rap1. However, when constitutively active RapV12 was overexpressed, it negatively regulated cell motility. Activation of the C3G/Rap1 signaling pathway resulted in down-regulation of the paxillin-Crk-DOCK180 complex and reduction of Rac1-GTP, suggesting that Rap1 activation could suppress the Rac1 signaling pathway in epithelial cells.

Induction of epithelial cell motility is a fundamental morphogenetic event that is recapitulated during carcinoma metastasis. Random motility of NBT-II carcinoma cells on collagen critically depends on paxillin phosphorylation at Tyr-31 and Tyr-118, the binding sites for the adapter protein CrkII. Two constitutive partners of CrkII are the exchange factors DOCK180 and C3G. CrkII bound to DOCK180 formed a signaling complex with phosphorylated paxillin that was necessary for cell migration as inferred from the inhibition caused by a DOCK180-interfering mutant. DOCK180, which acts predominantly on the Rho family GTPase Rac1, restored cell locomotion in cells expressing Phe-31/118 paxillin mutants deficient in Rac1 GTP-loading, suggesting that formation of paxillin-Crk-DOCK180 signaling complex controls collagen-dependent migration mainly through Rac1 activation. We demonstrated previously that upon collagen stimulation of NBT-II cells, the adapter protein paxillin becomes tyrosine phosphorylated. By generating interfering mutations, we showed that paxillin residues Tyr-31 and Tyr-118 are the main sites of phosphorylation by collagen (7). Phosphorylation on these residues (Phe-31/118) or a complementary mutation on the SH2 domain of CrkII (R38V) has a similar inhibitory effect on cell migration as inferred from the inhibition caused by a DOCK180-interfering mutant. DOCK180, which acts predominantly on the Rho family GTPase Rac1, restored cell locomotion in cells expressing Phe-31/118 paxillin mutants deficient in Rac1 GTP-loading, suggesting that formation of paxillin-Crk-DOCK180 signaling complex controls collagen-dependent migration mainly through Rac1 activation.

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their role in the collagen-induced random migration of NBT-II cells. We have found that DOCK180 together with paxillin-Crk constitutes the main pathway transducing collagen-mediated signals for the activation of Rac1 during NBT-II cell migration. This signaling pathway is down-regulated when cell migration is counteracted by C3G/Rap1 activation, suggesting a balance between Rac1 and Rap1 GTPases in controlling the migratory versus the stationary state of the cell.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Rat collagen type I and poly-L-lysine (PL) were from Sigma. Antibodies against paxillin, CrkII, Rap1, Rac1, (BD Transduction Laboratories), C3G, DOCK180 (Santa Cruz Technology, Inc.), phosphospecific Tyr-118-paxillin (BioSource International), glutathione S-transferase (GST; Amersham Biosciences), and GFP (Roche Applied Science) were used. Plasmids encoding full-length human DOCK180 and DOCK180-dlPS were provided by M. Matsuda (Osaka University, Osaka, Japan), myc-tagged GTPases RacV12, RacN17 vectors by A. Hall (University College, London, UK), GFP-tagged RacV12 and RacN17 by F. Sanchez-Madrid (Hospital Universitario de la Princesa, Madrid, Spain), CrkII plasmid by R. Klemke (The Scripps Research Institute, La Jolla, CA), and Rap1 plasmids by T. Kinashi (Kyoto University, Japan). GFP-tagged wild type and Phe-31/118 paxillin were described previously (7). The GST-fused SH3 domain of c-Crk was a gift of R. Birge (Rockefeller University, NY). The GST-Ral binding domain (RBD) of RalGDS was provided by F. Porteu (Institut Cochin, Paris, France).

**Cell Culture and Transfections**—Culture of NBT-II rat bladder carcinoma cells and the generation of the stable cell line Phe-31/118 cell line (clone Fx1) were described previously (7). For migration assays, transient transfections were done using the LipofectAMINE reagent (Invitrogen) following the manufacturer’s recommendations as reported previously (7). For immunoprecipitation experiments and pull-down assays, polyethyleneimine was used as transfection vehicle as described previously (21).

**Immunoprecipitations, Pull-down Assays and Western Blots**—Cells growing in complete medium were trypsinized, resuspended in complete medium, and replated on dishes precoated with PL (200 μg/ml) or collagen (10 μg/ml). Cells were lysed in Triton buffer (1% Triton X-100, 10 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 100 μM sodium orthovanadate). Lysates were processed for immunoprecipitation, or SH3-Crk pull-down, followed by immunoblotting as described before (7). Immunocomplexes were quantified using the Scion Image program (Scion Corp., Frederick, MD).

**GTPases Activity Assays**—The levels of active GTP-bound GTPases were determined as follows. For each pull-down assay, 30 μg of the GST-CRIB of PAK or GST-RBD of RalGDS were coupled to glutathione-Sepharose beads (Amersham Biosciences) at 4 °C for 30 min before assay. Cells were substrate stimulated or left in suspension for the indicated times, then lysed in Nonidet P-40 buffer (50 mM Tris, pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 5 mM MgCl2, 10% glycerol, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Lysates were centrifuged, and supernatants were incubated with the GST proteins coupled to beads for 60 min at 4 °C. Beads were washed with Nonidet P-40 buffer, and proteins were eluted in Laemmli sample buffer and separated by SDS-PAGE. The presence of active GTPases was visualized by Western blots using appropriate antibodies.

**Cell Migration Assay**—Random cell migration was assayed as described previously (7). Images were analyzed with the Metamorph software (Universal Imaging Corp.). The motility of individual cells was evaluated by tracking their movement over 12 h. The average speed of locomotion (micrometers per hour) was calculated as the total track length divided by the number of hours recorded. For each experimental condition, 90–100 cells were analyzed from at least three independent experiments. The Student’s t test was used to evaluate the data.

**RESULTS**

**CrkII-DOCK180 Signaling Complex Binds to Paxillin in NBT-II Cells**—To identify signaling partners downstream of the paxillin-Crk complex, we carried out GST pull-down assays using the N-terminal SH3 domain of Crk on protein extracts obtained from cells stimulated with collagen or PL, a control for integrin-independent cell adhesion. Both C3G and DOCK180 proteins were immunodetected in NBT-II cells and in vitro bound to the SH3 domain of Crk when stimulated with either PL or collagen, suggesting that their association with Crk was not modulated by external stimuli (Fig. 1A). Thus, at least two CrkII signaling complexes exist simultaneously in NBT-II cells in association with differentGEFs: Crk-C3G and Crk-DOCK180.

We then examined the interaction between CrkII and DOCK180 in collagen-stimulated cells in vitro. Binding of CrkII to DOCK180 was detected in co-immunoprecipitates of DOCK180 isolated from both PL and collagen-stimulated cells, confirming the presence of a constitutive Crk-DOCK180 complex in NBT-II cells. Similar results were obtained in reverse immunoprecipitation experiments using the Crk antibody (data not shown). The presence of paxillin within the Crk-DOCK180 complex was detected when cells were plated on collagen (Fig. 1B), suggesting that the formation of the trimeric complex was regulated by paxillin tyrosine phosphorylation. Examination of total lysates with phosphospecific antibodies confirmed the increase in phosphorylated Tyr-31 (data not shown) and Tyr-118 paxillin (Fig. 1B) in response to collagen stimulation compared with PL. Additional experiments using lysates from cells transiently transfected with the GFP-tagged paxillin mutant Phe-31/118, which cannot form a complex with CrkII (7), revealed no DOCK180 in the immunoprecipitates (supplemental Fig. S1). These results are consistent with our previous findings demonstrating that the formation of a paxillin-Crk complex is dependent on collagen-induced paxillin phosphorylation on Tyr-31 and Tyr-118.

**DOCK180 Is Downstream of Paxillin-Crk Complex in NBT-II Cells**—The SH3 domain of CrkII, which serves as DOCK180 binding site, clearly has an important function because a point mutation impairs migration of NBT-II cells on collagen (7). To determine whether DOCK180 was also a necessary component of the collagen-mediated random migration, we overexpressed the full-length molecule and the DOCK180 mutant deleted at amino acid 1472 (DOCK180-dlPS), which lacks Crk SH3 binding site (22) and contains a truncated “Docker” domain (11). DOCK180-dlPS can form a complex with ELMO1 but can neither bind to nor activate Rac1. Thus, its dominant-negative effect is by direct competition with endogenous DOCK180 for associated partners such as ELMO. These constructs were introduced separately into NBT-II cells together with an enhanced GFP reporter plasmid. Forty-eight hours later, cells were trypsinized and added sparsely onto collagen-coated plates, and the fluorescently labeled cells were followed by time-lapse video microscopy. Overexpression of DOCK180-dlPS significantly reduced the velocity of individual cell migration to the same extent as the complementary CrkII W169L mutation (speed of locomotion of 15.3 ± 7.3 μm/h), whereas the wild-type form of DOCK180 had an enhancing effect (Fig. 1C).

To confirm that DOCK180 was part of the paxillin-Crk signaling pathway required for cell migration, we overexpressed DOCK180 in the motility deficient NBT-II clone Fx1 stably expressing paxillin mutated on Tyr-31 and Tyr-118. Fx1 cells are unable to move on collagen because the point mutations on paxillin prevent its association with CrkII, as previously demonstrated (7). Transfection of wild-type DOCK180 but not DOCK180-dlPS (speed of locomotion of 10.6 ± 3.2 μm/h) into these cells restored the migration deficiency, as does overexpression of CrkII (7), thus placing DOCK180 downstream of paxillin (Fig. 1C). The positive effect of DOCK180 on migration required collagen stimulation because transfection with the wild-type construct had no effect when cells were plated on uncoated plastic (speed of locomotion of 5.3 ± 2.9 μm/h). All together, these results support the notion that paxillin association with CrkII and DOCK180 is an essential component of...
the random migratory behavior of NBT-II cells on collagen.

**Rac Activation Is Downstream of the Paxillin-Crk-DOCK180 Signaling Complex**—It has been reported previously that DOCK180 is a direct activator of the small GTPase Rac1 (23, 24), which is indispensable for cell migration. We therefore measured Rac1 activity of NBT-II cells under conditions of random migration on collagen. The activation state of Rac was measured in pull-down assays with a GST fusion of the CRIB domain of PAK, bearing the binding region for the GTP-bound state (25). Increased levels (up to 4.5-fold) of Rac-GTP loading were observed in lysates prepared from NBT-II cells plated on collagen relative to PL (Fig. 2A), demonstrating that Rac1 is activated in NBT-II cells induced to migrate. To determine whether Rac1 activation was contingent on the association of paxillin with CrkII and DOCK180, we carried out pull-down assays on cells overexpressing either wild-type paxillin-GFP or the mutant Phe-31/118-GFP, which cannot form a complex with CrkII and DOCK180. Kinetics studies on paxillin-GFP expressing cells demonstrated sustained activation of Rac1 after stimulation with collagen. In contrast, Rac1 activity in Phe-31/118-GFP expressing cells resulted in a 20–40% decrease in time upon collagen stimulation (Fig. 2B). Likewise, pull-down assays carried out with the Fx1 stable clone failed to reveal an increase in GTP-loaded Rac1 (Fig. 2C). Because overexpression of wild-type DOCK180 rescued the inability of Fx1 cells to move on collagen (Fig. 1C), we tested whether it could promote Rac1 GTP-loading. As shown in Fig. 2C, expression of DOCK180 in Fx1 cells led to a 2-fold increase in Rac1 activation over Fx1 control. These results demonstrate that the paxillin Phe-31/118 mutant can block the signaling pathway leading to Rac1 activation, which can be bypassed by overexpression of downstream molecules. However, Crk overexpression in Fx1 cells had a modest effect on Rac activation (1.2-fold) and a non-enhancing effect on parental cells when stimulated with collagen (supplemental Fig. S2).

That Rac1 activity is required for random migration of NBT-II cells on collagen was confirmed by transfecting cells with the dominant inhibitory mutant of Rac1 (RacN17). RacN17 expression altered the formation of lamellipodia with a 95% inhibition of cell migration induced by collagen (Fig. 2D; data not shown). The inhibitory effect of RacN17 on cell migration was partially reversed by DOCK180 (Fig. 2D), suggesting that the exchange factor sequestered the Rac-interfering mutant. Overexpression of the dominant-active mutant form of Rac1 (RacV12) enhanced cell migration on collagen by 1.5-fold over control (Fig. 2D). Expression of this constitutive active
form in Fx1 cells expressing the Phe-31/118 paxillin mutant restored cell migration (Fig. 2D). These results provide an additional element to directly implicate the paxillin-Crk-DOCK180 signaling pathway in regulating Rac1 activity required during random cell migration on collagen.

**Activated Rap1 Inhibits Collagen-mediated Migration of NBT-II Cells**—C3G is a GEF for the small GTPase Rap1 (26), which associates with the SH3 domain of CrkII in NBT-II cells (Fig. 1A). It has been reported previously that Rap1 can be activated by integrin mediated cell attachment (27). We therefore examined its activation state in NBT-II cells upon adhesion to collagen, the cell motility inducing substrate. The presence of the GTP-bound form was assessed in pull-down assays using the GST-linked RBD of RalGDS (28). GTP loading of Rap1 was detected after 30 min of plating when 90% of cells were still round and returned to basal levels as cells spread on collagen.
the substrate. In contrast, Rap1 activity was readily detectable in lysates prepared from cells maintained in suspension increasing with time as cellular aggregates formed (Fig. 3A; data not shown). Thus, in epithelial cells Rap1 activation is transient after cell-substrate adhesion and sustained when cell-cell contacts are favored, suggesting that Rap1 effect on cell adhesion could also be integrin-independent.

To address the functional significance of the decrease in Rap1 activation on collagen, we tested the consequence of expressing two mutant forms of Rap1 on random cell migration. Overexpression of the constitutively active Rap1V12 form suppressed cell migration (9.8 ± 4 μm/h), whereas the interfering mutant Rap1N17 rather enhanced cell velocity (50.8 ± 5 μm/h) (Fig. 3B). Moreover, overexpression of Rap1 exchange factor C3G or the membrane targeted C3G-F suppressed cell migration induced by collagen (Fig. 3B). Both C3G and C3G-F are able to induce GTP-loading of Rap1 of NBT-II cells by 3- and 5.5-fold, respectively, on collagen when overexpressed (Fig. 4A). Therefore, when Rap1 is activated, the random migration of NBT-II cells on collagen is suppressed.

Next, we tested the hypothesis that the inability of cells to move on collagen when Rap1 is activated results from an altered association of paxillin with CrkII. To this end, we stimulated Rap1 activation by overexpressing C3G or C3G-F and then examined the presence of the paxillin-Crk complex after collagen stimulation. Cells lysates of transiently transfected cells were immunoprecipitated with Crk antibodies and the precipitates were analyzed by Western blotting (Fig. 4B). In cells expressing either C3G or C3G-F, binding of paxillin to CrkII was diminished by an average of 30–40%, respectively, compared with control cells transfected with an empty vector. As expected, membrane-targeted C3G-F was more efficient than C3G in activating Rap1, blocking the association between CrkII and paxillin and inhibiting cell migration (Figs. 3B and 4, A and B), as estimated from the levels of overexpressed C3G protein. A similar 30% reduction in Crk-paxillin association was observed in cells transfected with the constitutive active Rap1V12 (Fig. 4C). It is noteworthy that the reduction of paxillin within the Crk immunocomplex in cells transfected with Rap1V12 was accompanied by a 60–75% decrease in Crk-Dock180 and a concomitant 1.4-fold increase in Crk-C3G complexes compared with control. Because expression of constitutively active Rap1 inhibited collagen-mediated migration, we examined its influence on the GTP-loading of Rac1 in pull-down assays. We found that the collagen-mediated Rac1 activation was significantly suppressed, up to 50%, when Rap1V12 was overexpressed (Fig. 4D). These data indicated that activation of Rap1 in NBT-II cells could down-regulate the paxillin-Crk-Dock180 molecular complex, suggesting that Rap1 could be involved in a signaling pathway that leads to Rac1 inhibition.

**DISCUSSION**

Cell migration is a complex multistep process that is under the control of many signal transduction pathways. Members of the Ras and Rho family of GTPases exhibit distinct roles in coordinating cellular responses required for cell migration (29). In this study, we describe a molecular complex composed of paxillin-Crk-Dock180 that governs one major aspect of cell migration, which is the activation of Rac1. We also report that Rac1 and the Ras-like GT Pase Rap1 can have opposing roles in regulating cell migration.

Rac1 controls actin cytoskeletal dynamics and integrin adhesions that are essential for lamellipodium extension during cell migration (30, 31). Rac1 activation is initiated by upstream signals acting upon Rac-specific GEFs that catalyze the exchange of GDP for GTP. GTP-bound Rac can then relay information to a large number of downstream effectors (32). Therefore, Rac1 is activated in NBT-II migrating cells in response to integrin-derived signals. In contrast, cells expressing the paxillin mutant Phe-31/118 are deficient in cell migration and are unable to induce Rac1 activation above basal levels. During NBT-II cell migration, phosphorylation of paxillin on Tyr-31 and Tyr-118 recruits pre-existing Crk-Dock180 molecular complexes (Ref. 7 and current study). Dock180 has been shown to regulate cell migration events by forming a signaling complex with CrkII and the SH2-binding protein p130Cas (33, 34). In NBT-II cells, however, p130Cas does not play a major role in collagen-mediated migrations, possibly reflecting cell type selectivity as discussed previously (7). Instead, we found...
that DOCK180 is an effector of paxillin signaling that is required for cell migration; it also binds to ELMO1 when the latter is overexpressed (data not shown). We could confirm that DOCK180 is downstream of phosphorylated paxillin because its overexpression rescued both the Rac1-GTP loading defect as well as the migratory impairment of cells expressing the Phe-31/118 paxillin mutant. Although Crk alone had a modest effect on Rac1 activation in Fx1 and parental cells, its overexpression promoted cell migration (7). Accordingly, expression of Crk had no effect on the GTP loading of Rac1 in fibronectin adherent COS-7 cells but enhanced their haptotactic migration (35). Therefore, paxillin association with CrkII and DOCK180 couples integrin signaling to Rac1 activation during cell migration. The translocation of paxillin to cell-substratum contact sites after collagen stimulation (7) and its adapter function may serve to recruit and stabilize signaling molecules such as CrkII and DOCK180 to these sites as previously shown for the p95PKL-PIX-PKL complex (36). These membrane-targeted complexes would then facilitate localized activation of Rac1 at the leading edge where the highest levels of Rac-GTP are preferentially found in moving cells (37, 38). In agreement, we have observed that a GFP-tagged GS-DOCK180 construct containing the CrkII binding site (39) and Rac1-GFP localized at the edge of extended lamellipodia and at cell-substratum adhesion sites when NBT-II cells were plated on collagen (data not shown).

Paxillin is a multidomain protein with scaffolding function that is linked to cell migration events by activating different signal transduction pathways. Disruption of the interaction between paxillin and p95PKL by overexpression of a paxillin LD4 domain deletion in CHO.K1 cells severely compromised directed cell migration in an in vitro wound-healing assay but did not prevent random cell migration on fibronectin (31). In response to motility signals, paxillin may associate directly with FAK, Pyk2, and Src kinases, which are key regulators of adhesion turnover in migrating cells (40). In NMuMG cells in which Rac1 activity is mainly controlled by the p130Cas-Crk-DOCK180 signaling complex, phosphorylated Tyr-31/118 paxillin positively regulates cell migration by binding to the SH2 domain of p120RasGAP and thereby suppresses RhoA activity (41). Because cells are capable of displaying diverse motile behaviors (e.g., directional versus random movement; individual versus the movement of groups of cells), the multiple signaling pathways emerging from paxillin to regulate cell migration could be engaged simultaneously or not, depending on cellular context and/or environmental conditions.

Another binding partner of CrkII in NBT-II cells is the Rap1 exchange factor C3G. Although pre-formed Crk-C3G complexes exist in NBT-II cells, Rap1 activation was not sustained after adhesion to collagen in standard pull-down assays. Paxillin-Crk-C3G complexes were never detected in paxillin immunoprecipitates (data not shown), implying that, unlike Crk-DOCK180, this signaling complex might be inaccessible to collagen-derived motility signals in migrating cells. Instead, Rap1 activity remained elevated when cell-cell contacts were favored. In addition we found that overexpression of constitutively active Rap1 or its exchange factor C3G suppressed cell migration on collagen. The inhibition of cell migration by C3G/Rap1 activation was concomitant with the disassembly of the paxillin-Crk-DOCK180 molecular complex and the reduction of Rac1-GTP loading, suggesting that Rap1 could negatively regulate Rac1 signaling and, consequently, cell migration. In this respect, we were able to switch the complex balance from Crk-DOCK180 to Crk-C3G by stimulating the C3G/Rap1 signaling pathway. One mechanism by which Rap1 could exert a negative effect on cell motility is by acting upon molecular components that modulate the assembly of the paxillin-Crk-DOCK180 signaling complex. For example, the inhibition and/or activation of specific tyrosine kinases and phosphatases could cause paxillin dephosphorylation and, consequently, a diminution of the complex. Thus, the down-regulation of the Rac1 pathway by an increase in Rap1 signaling would bring about the conversion of fibroblast-like moving of cells toward an epithelial state.

There is evidence that Rap1 proteins could participate in the formation and maintenance of adherens junctions in epithelia, as the Rho GTPases (42, 43). For example, in the developing D. melanogaster embryo, the positioning of adherens junctions...
in epithelial wing cells requires active Rap1 (44). Downstream of activated Rap1 is Canoe, the D. melanogaster ortholog of the multidomain junctional protein AF-6/afadin in vertebrates, which is required during embryonic dorsal closure, a process that relies on the movement of cohesive epithelial sheets (45). Moreover, it has been shown recently that Rap1-GFP can localize to the plasma membrane of mammalian epithelial cells, in addition to its intracellular distribution (46). Thus, in mammalian cells, Rap1 may be involved in epithelialization events like those occurring during the movement of an epithelial sheet in wound healing or the conversions of mesenchymal to epithelial cells. Therefore, our current efforts are concentrated on the identification of Rap1-dependent signals that oppose the Rac1 pathway during the reconversion process of NBT-II cells back to the epithelial phenotype that is observed when external stimuli become absent or insufficient.

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REFERENCES
1. Pawson, T., and Nash, P. (2003) Science 300, 445–452
2. Turner, C. E. (2000) Nat. Cell Biol. 2, E231–E236
3. Turner, C. E., Brown, M. C., Perrotta, J. A., Riedy, M. C., Nikolopoulos, S. N., McDonald, A. R., Bagrodia, S., Thomas, S., and Leventhal, P. S. (1999) J. Cell Biol. 145, 851–863
4. West, K. A., Zhang, H., Brown, M. C., Nikolopoulos, S. N., Riedy, C. M., Horwitz, A. F., and Turner, C. E. (2001) J. Cell Biol. 154, 161–176
5. Liu, S., Thomas, S. M., Woodside, D. G., Rose, D. M., Kiosses, W. B., Pfaff, M., and Ginsberg, M. H. (1999) Nature 402, 678–681
6. Liu, S., Kiosses, W. B., Rose, D. M., Slepak, M., Salgia, R., Griffin, J. D., Turner, C. E., Schwartz, M. A., and Ginsberg, M. H. (2002) J. Biol. Chem. 277, 20887–20894
7. Petit, V., Boyer, B., Lents, D., Turner, C. E., Thiery, J. P., and Vallés, A. M. (2000) J. Cell Biol. 148, 857–870
8. Feller, S. M. (2001) Oncogene 20, 6348–6371
9. Matsuda, M., Ota, S., Tanimura, R., Nakamura, H., Matuoka, K., Takenawa, T., Nakashima, K., and Kurata, T. (1998) J. Biol. Chem. 273, 14468–14472
10. Braga, V. M. (2002) Nat Cell Biol. 4, E188–E190
11. Brugnera, E., Haney, L., Grimsley, C., Lu, M., Walk, S. F., Tsuie-Trimpoint, A. C., Macara, I. G., Madhani, H., Fink, G. R., and Ravichandran, K. S. (2002) Nat. Cell Biol. 4, 574–582
12. Wu, Y. C., and Horvitz, H. R. (1998) Nature 392, 501–504
13. Erickson, M. R., Galletta, B. J., and Abmayr, S. M. (1997) J. Cell Biol. 138, 589–603
14. Fukui, Y., Hashimoto, O., Suino, T., Oeno, T., Koga, H., Abe, M., Inayoshi, A., Noda, M., Oike, M., Shirai, T., and Sasazuki, T. (2001) Nature 412, 826–831
15. Hasegawa, H., Kiyokawa, E., Tanaka, S., Nakashima, K., Gotoh, N., Shibuya, M., Kurata, T., and Matsuda, M. (1996) Mol. Cell. Biol. 16, 1770–1776
16. Ohba, Y., Ikuta, K., Ogura, A., Matsuda, J., Mochizuki, N., Nakashima, K., Kurokawa, K., Mayer, J., Mak, K., Miyazaki, J., and Matsuda, M. (2001) EMBO J. 20, 3333–3341
17. Uemura, N., and Griffin, J. D. (1999) J. Biol. Chem. 274, 37525–37532
18. Voss, A. K., Gruss, P., and Thomas, T. (2003) Development 130, 355–367
19. Bos, J. L., de Rooij, J., and Reedquist, K. A. (2001) Nat. Rev. Mol. Cell. Biol. 2, 369–377
20. Caron, E. (2003) J. Cell Sci. 116, 435–440
21. Edme, N., Downward, J., Thiery, J. P., and Boyer, B. (2002) J. Cell Sci. 115, 2991–2996
22. Kiyokawa, E., Hashimoto, Y., Kurata, T., Sugimura, H., and Matsuda, M. (1998) J. Biol. Chem. 273, 24479–24484
23. Côte, J. F., and Vuori, K. (2002) J. Cell Sci. 115, 4901–4913
24. Kiyokawa, E., Hashimoto, Y., Kobayashi, S., Sugimura, H., Kurata, T., and Matsuda, M. (1998) Genes Dev. 12, 3331–3336
25. Sander, E. E., ten Klooster, J. P., van Delft, S., van der Kammen, R. A., and Collard, J. G. (1999) J. Cell Biol. 147, 1099–1122
26. Gotoh, T., Hattori, S., Nakamura, S., Kitayama, H., Noda, M., Takai, Y., Kaibuchi, K., Matsu, H., Hatase, O., Takahashi, H., Kurata, T., and Matsuda, M. (1995) Mol. Cell. Biol. 15, 6746–6753
27. Feller, S. M., Rapp, U. R., and Feller, S. M. (1998) J. Biol. Chem. 273, 24297–24300
28. Franke, B., Akkerman, J. W., and Bos, J. L. (1997) EMBO J. 16, 252–259
29. Bar-Sagi, D., and Hall, A. (2000) Cell 103, 227–236
30. Eisienne-Manneville, S., and Hall, A. (2002) Nature 420, 629–635
31. Ridley, A. J. (2001) J. Cell Sci. 114, 2713–2722
32. Zheng, Y. (2001) Trends Biochem. Sci. 26, 724–732
33. Cherech, D. A., Leng, J., and Klemke, R. L. (1999) J. Cell Biol. 146, 1107–1116
34. Gu, J., Sumida, Y., Sanzen, N., and Sekiguchi, K. (2001) J. Biol. Chem. 276, 27090–27097
35. Abasst, Y. A., and Vuori, K. (2002) EMBO J. 21, 4571–4582
36. Brown, M. C., West, K. A., and Turner, C. E. (2002) Mol. Biol. Cell 13, 1550–1565
37. del Pozo, M. A., Alderson, N. B., Kiosses, W. B., Chiang, H. H., Anderson, R. G., and Schwartz, M. A. (2004) Science 303, 839–842
38. Kraynov, V. S., Chamberlain, C., Bokoch, G. M., Schwartz, M. A., Slahbaugh, S., and Hahn, K. M. (2000) Science 290, 333–337
39. Kobayashi, S., Shirai, T., Kiyokawa, E., Mochizuki, N., Matsuda, M., and Fukui, Y. (2001) Biochem. J. 354, 73–78
40. Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T., and Horwitz, A. R. (2003) Science 302, 1704–1709
41. Tsoubouchi, A., Sakakura, J., Yagi, R., Mazaki, Y., Schaefer, E., Yano, H., and Sabe, H. (2002) J. Cell Biol. 159, 673–683
42. Braga, V. M. (2002) Curr. Opin. Cell Biol. 14, 546–556
43. Van Aelst, L., and Symons, M. (2002) Genes Dev. 16, 1032–1054
44. Knox, A. L., and Brown, N. H. (2002) Science 295, 838–842
45. Boettner, B., Harjes, P., Ishimaru, S., Heke, M., Fan, H. Q., Qin, Y., Van Aelst, L., and Gaul, U. (2003) Genetics 165, 159–169
46. Bivona, T. G., Wiener, H. H., Ahern, I. M., Silletti, J., Chiu, V. K., and Philips, M. B. (2004) J. Cell Biol. 164, 461–470
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Ana M. Vallés, Maud Beuvin and Brigitte Boyer

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