Regulation of Cdc42-mediated morphological effects: a novel function for p53

Gilles Gadéa, Laure Lapasset,
Cécile Gauthier-Rouvière and Pierre Roux¹

Centre de Recherche en Biochimie Macromoléculaire,
CNRS UPR1086, IFR 24, 1919 route de Mende,
F-34293 Montpellier cedex 5, France

¹Corresponding author
e-mail: roux@crbm.cnrs-mop.fr

The tumour suppressor functions of p53 that are important for its activity depend on its role as a cell cycle arrest mediator and apoptosis inducer. Here we identify a novel function for p53 in regulating cell morphology and movement. We investigated the overall effect of p53 on morphological changes induced by RhoA, Rac1 and Cdc42 GTPases in mouse embryonic fibroblasts (MEFs). Interestingly, p53 exerted a selective effect on Cdc42-mediated cell functions. (i) Both overexpression of wild-type p53 and activation of endogenous p53 counteracted Cdc42-induced filopodia formation. Conversely, p53-deficient MEFs exhibited constitutive membrane filopodia. Mechanistic studies indicate that p53 prevents the initiating steps of filopodia formation downstream of Cdc42. (ii) Overexpression of p53 modulates cell spreading of MEFs on fibronectin. (iii) During cell migration, the reorientation of the Golgi apparatus in the direction of movement is abolished by wild-type p53 expression, thus preventing cell polarity. Our data demonstrate a previously uncharacterized role for p53 in regulating Cdc42-dependent cell effects that control actin cytoskeletal dynamics and cell movement. This novel function may contribute to p53 anti-tumour activity.

Keywords: Cdc42/filopodia/p53/polarization/spreading

Introduction

The p53 tumour suppressor protein is among the most frequent targets for inactivation in human cancers (Hollstein *et al.*, 1994; Levine, 1997). Functional inactivation of wild-type p53 is generally considered to favour the emergence of abnormal cells with deregulated growth that give rise to malignant transformation. p53 maintains genomic stability by regulating responses to DNA damage and other forms of genotoxic insult. Both protein accumulation and post-translational modifications appear to be essential to support the full tumour suppressor function of p53 (Giaccia and Kastan, 1998). The predominant biochemical activity of p53 relies on the transactivation and transcriptional repression of specific target genes. This appears to be the major mechanism by which p53 implements its anti-proliferative activities: cell cycle arrest in G₁/S and G₂/M phases and induction of apoptotic

cell death, mechanisms that underpin its role as suppressor of oncogenic transformation.

Oncogenic transformation is characterized by the alteration of cell growth properties associated with morphological changes characteristic of primary tumours and invasive metastatic cells. Surprisingly, although the role of the tumour suppressor p53 as cell cycle regulator and apoptosis inducer has been widely studied (for reviews see Hansen and Oren, 1997; Levine, 1997; Agarwal *et al.*, 1998; Giaccia and Kastan, 1998; Prives, 1998), its influence on cell morphology received less attention. In addition to its interaction with the microtubule network, p53 is likely to influence the actin cytoskeleton since actin genes are transcriptional targets of p53 (Guenal *et al.*, 1997; Comer *et al.*, 1998). A recent report demonstrates a direct interaction between F-actin and p53 (Metcalf *et al.*, 1999). Moreover, p53 is able to revert different aspects of the oncogene-transformed cell phenotype. In particular, expression of p53 modifies the cell shape of Ras-transformed fibroblasts (Gloushankova *et al.*, 1997). These observations suggest that p53 may contribute to regulate actin cytoskeleton rearrangements upon oncogenic transformation.

Morphological alterations of Ras-transformed cells, i.e. loss of stress fibres, decrease of adhesion to the substratum and increases in membrane ruffling and motility (Bar-Sagi and Feramisco, 1986), are mediated largely by Rho family members. These small GTPases regulate multiple actin cytoskeleton-dependent cell functions, such as cell shape (Tapon and Hall, 1997), cell motility (Aepfelbacher *et al.*, 1994, 1996), cell polarity (Adams *et al.*, 1990), cell adhesion (Nobes and Hall, 1995a,b; Braga *et al.*, 1997) and cell spreading (Price *et al.*, 1998). Several clues regarding Rho family functions have been provided by extensive studies of three proteins: RhoA, Rac1 and Cdc42. All three GTPases have been shown to connect extracellular signals to the actin cytoskeleton. In fibroblasts, lysophosphatidic acid-stimulated stress fibre formation and focal adhesions require RhoA. Rac1 mediates formation of epidermal growth factor-, platelet-derived growth factor- and insulin-induced ruffles and lamellipodia. Cdc42 mediates formation of bradykinin-stimulated thin membrane protrusions, namely microvilli and filopodia (reviewed in Hall, 1998). Further evidence for the involvement of Rho family members in mediating oncogenic Ras functions was provided by the observation that dominant-negative mutants of RhoA, Rac1, Cdc42 and RhoG significantly reduce oncogenic Ras focus-forming activity (reviewed in Zohn *et al.*, 1998). Conversely, expression of activated forms of RhoA, Rac1, Cdc42 and RhoG enhances the number of Ras-induced foci. Rho GTPases are not interchangeable in mediating Ras transformation, but rather each protein contributes to a distinct aspect of the transformed

phenotype: coordinated activation of different combinations of Rac1, RhoG and Cdc42 elicits a cooperative focus-forming activity, suggesting that they activate distinct pathways (Roux *et al.*, 1997). It was proposed that Rac1 modulates cell contact inhibition, whereas Cdc42 regulates anchorage-independent growth (Qiu *et al.*, 1997; Philips *et al.*, 2000). The role of RhoA is less clear, since Ras-transformed cells show severe disruption of actin stress fibres and focal adhesion, more consistent with a RhoA pathway inactivation (Izawa *et al.*, 1998).

Similarly to dominant-negative Rho GTPases, wild-type p53 also strongly reduces the number of Ras-transformed foci (Eliyahu *et al.*, 1989). To clarify further the functional links between these two signalling pathways regulating Ras transformation, we studied the effects of p53 on morphological changes induced by expression of activated forms of Rac1, RhoA and Cdc42. We report here that p53 selectively inhibits formation of Cdc42-induced filopodia, but has no effect on either Rac1-induced lamellipodia and ruffles or RhoA-induced stress fibre formation. Loss of p53 functions in embryonic fibroblasts derived from mice harbouring a targeted disruption of the p53 gene results in numerous and persistent membrane filopodia. Analysis of p53 effects on Cdc42-mediated cell functions revealed that p53 affects cell movement by reducing cell spreading and polarization rates upon migration. Thus, p53 modulates cytoskeleton organization and consequently may prevent aberrant cell movement during malignant transformation.

Results

p53 selectively inhibits morphological changes induced by Cdc42, but not those induced by Rac1 and RhoA

To examine the overall effect of p53 on Rho GTPase-dependent F-actin structures, mouse embryonic fibroblasts (MEFs) were transiently co-transfected with plasmids encoding wild-type p53 and green fluorescent protein (GFP)-tagged constitutively active forms of Cdc42 (Cdc42-V12), Rac1 (Rac1-V12) or RhoA (RhoA-V14). After 20–24 h, the transfected cells were detected by fluorescence and analysed for morphological changes associated with actin polymerization using rhodamine-labelled phalloidin. As shown in Figure 1A, expression of GFP-tagged Cdc42-V12 alone led to the appearance of numerous long F-actin-rich membrane extensions, called filopodia (panels a, b and c, arrows). In contrast, cells co-expressing p53 and GFP-Cdc42-V12 did not present any filopodia (panels a, b and c, arrowheads), suggesting that p53 inhibited Cdc42-V12-induced filopodia formation. Conversely, p53 expression had no effect on RhoA-dependent stress fibre formation (panels d, e and f) or on Rac1-dependent ruffles and lamellipodia (panels g, h and i). To confirm that the inhibition of Cdc42-induced filopodia was due to p53 activity, we performed co-transfection of the GFP-tagged Cdc42-V12 with two naturally occurring p53 mutants, namely p53 H273 and p53 H175 (Figure 1B). Both mutations map to the DNA-binding domain of p53 and result in the loss of the sequence-specific DNA-binding activity, therefore acting as dominant-negative mutants of the endogenous p53 (Ory

Fig. 1. Effects of p53 on Cdc42-, Rac1- and RhoA-induced F-actin changes. (A) Proliferating MEFs were co-transfected with plasmids encoding wild-type p53 and GFP-tagged Cdc42-V12 (panels a, b and c), RhoA-V14 (panels d, e, and f) or Rac1-V12 (g, h and i). After 20–24 h, cells were fixed and stained for F-actin (panels c, f and i) and p53 using 240 monoclonal antibody (panels b, e and h). GFP-positive cells were also detected (panels a, d and g). Scale bar: 10 μ m. (B) MEFs were transfected with GFP-tagged Cdc42-V12, in combination with dominant-negative p53 mutants (p53 H273 in panels a, b and c or p53H175 in panels d, e and f). Cells were treated as in (A): co-transfected cells were visualized for p53 (panels a and b), GFP (panels b and e) and F-actin (panels c and f). Scale bar: 10 μ m. (C) Quantification of MEFs having filopodia. MEFs, treated as in (A) and (B), were scored positive when presenting at least five filopodia. For each experiment, 300 cells were counted. The mean, average and SD were calculated from six independent experiments.

et al., 1994). Expression of either the p53 H273 (panel a) or p53 H175 (panel d) mutant had no effect on filopodia extensions (panels c and f) induced by Cdc42-V12 (panels b and e). A quantitative analysis shows that almost all Cdc42-V12-expressing cells had exaggerated filopodia

($98 \pm 2\%$, Figure 1C). This number was reduced to $22 \pm 6\%$ by the co-expression of wild-type p53, but was unaffected by the co-expression of either of the two p53 mutants.

To overcome the possibility that suppression of filopodia was due to overexpression of ectopic p53 or Cdc42, an alternative approach was used: endogenous Cdc42 and p53 activities were stimulated (Figure 2). Cdc42-dependent filopodia formation was induced by bradykinin (Kozma *et al.*, 1995; Nobes and Hall, 1995a). p53 was activated either by tumour necrosis factor- α (TNF- α) or using two cytotoxic drugs, adriamycin and etoposide, previously shown to induce accumulation of p53 in MEFs (Lowe *et al.*, 1993; Klefstrom *et al.*, 1997). First, we verified that TNF- α , adriamycin and etoposide up-regulate endogenous p53 activity in MEFs, by monitoring the p53-dependent transactivation of the firefly luciferase reporter gene linked to the p53-responsive promoter derived from the *mdm2* gene (Barak *et al.*, 1994) (Figure 2A). Expression of wild-type p53 led to an 80-fold transactivation of the p53-responsive element. In contrast, expression of p53 mutants did not activate the *mdm2* promoter. Treatment of cells with TNF- α , adriamycin or etoposide resulted, respectively, in a 28-, 19- and 24-fold activation of the p53-responsive element, reaching up to 36, 23 and 30% of the value obtained with the positive control (wild-type p53). Secondly, we controlled that bradykinin-induced filopodia formation in MEFs depends on Cdc42 function: expression of the dominant-negative Cdc42 mutant (Cdc42-N17) abolished bradykinin-induced filopodia in MEFs (data not shown), as previously reported in other cell types (Kozma *et al.*, 1995). Finally, we monitored the effect of p53 up-regulation by TNF- α , etoposide and adriamycin on Cdc42-dependent filopodia formation by bradykinin. As shown in Figure 2B, bradykinin led to the appearance of filopodia after 12 min of treatment (panel b). In contrast, pre-treatment with TNF- α , adriamycin or etoposide prior to bradykinin addition led to a dramatic decrease in filopodia formation (panels c, d and e, respectively). Quantification of these results (Figure 2C) shows that bradykinin-mediated filopodia formation was reduced from 62% to 28, 16 and 20% by TNF- α , adriamycin and etoposide, respectively. In addition, filopodia formation occurring naturally in 13% of MEFs was also reduced by endogenous p53 induction using any of these three drugs.

Taken together, these results indicate that both ectopic expression of p53 and activation of endogenous p53 lead to inhibition of Cdc42-induced filopodia formation.

p53-deficient fibroblasts accumulate filopodia

The above results demonstrate that p53 interferes with the signal transduction pathway leading from activated Cdc42 to filopodia formation. The question arises as to whether the inactivation of p53 functions might affect the actin cytoskeleton. To address this point, we analysed F-actin organization of embryonic fibroblasts derived from mice harbouring a targeted disruption of the p53 gene (p53^{-/-} MEFs). p53^{+/+} and p53^{-/-} MEFs were taken at the same low passage number (1–6) to avoid genetic abnormalities acquired during continuous passage (Harvey *et al.*, 1993). F-actin organization of these two cell types was compared using rhodamine-labelled phalloidin staining (Figure 3A,

Fig. 2. Endogenous Cdc42-induced filopodia formation is inhibited by TNF- α -stimulated p53 activity. (A) MEFs were transfected with the firefly luciferase gene under the control of the *mdm2* promoter and treated for 10 h with either TNF- α , adriamycin or etoposide. The luciferase activity was assayed 24 h after transfection. The p53-responsive element construct was transfected in combination with either wild-type p53, as a positive control, or with the dominant-negative mutants p53 H273 or p53 H175. The luciferase activity was assayed 24 h later. The results are shown as the mean $\pm$ SD of four measurements. (B) Proliferating MEFs were either untreated (panel a), treated with bradykinin alone (panel b) or pre-treated with TNF- α (panel c), adriamycin (panel d) or etoposide (panel e) for 10 h, followed by a 12 min treatment with bradykinin, then stained for F-actin. Scale bar: 10 μ m. (C) Quantification of MEFs having filopodia. MEFs, treated as in (B), were scored positively when presenting at least five filopodia. For each experiment, 100 cells were scored and results are the mean $\pm$ SD of three independent experiments.

Fig. 3. Absence of p53 activity leads to a persistent accumulation of filopodia. (A) MEFs (panels a, c and e) and p53^{-/-} MEFs (panels b, d and f) were stained for F-actin (panels a and b), processed for SEM (panels c and d) or analysed for ezrin distribution (panels e and f). Scale bar: 10 μ m. (B) Still phase-contrast images from the accompanying videos of MEFs and p53^{-/-} MEFs. Scale bar: 10 μ m. (C) Detailed phase-contrast images describe the sequential initiating steps of filopodia formation in p53^{-/-} MEFs. Scale bar: 1 μ m.

a and b). Plasma membrane organization was also analysed by scanning electron microscopy (SEM) (panels c and d). Both methods pointed out a clear difference between the two cell types: p53^{-/-} MEFs exhibit numerous F-actin-containing peripheral microspikes (arrows in panels b and d), whereas these structures are scarce in p53^{+/+} MEFs (panels a and c). In addition, globular membrane protrusions (arrowheads), only visible by SEM and not by F-actin staining, were observed exclusively in p53^{-/-} MEFs (panel d). Finally, we stained p53^{+/+} and p53^{-/-} MEFs with antibodies against ezrin, a marker of microspikes (Amieva *et al.*, 1999). In normal MEFs (panel e), ezrin was distributed throughout the cytoplasm, whereas a plasma localization both in thin membrane

extensions (arrows) and in globular membrane protrusions (arrowheads) was observed in p53^{-/-} MEFs (panel f).

To ascertain that actin microspikes observed in p53^{-/-} MEFs resulted from membrane protrusions (filopodia) and not from membrane retractions (retractions fibres), we compared p53^{+/+} and p53^{-/-} MEFs by time-lapse phase-contrast microscopy analysis. Time-lapse sequences were collected by taking one image every 3 s during 10 min. As shown in Figure 3B and the accompanying videos (see Supplementary data available at *The EMBO Journal Online*), membranes of p53^{-/-} MEFs display extensive dynamic activities and present a lot of extensions protruding out of the cell from peripheral globular membrane structures, rapidly extending and shortening. In contrast, membranes of MEFs present far fewer protrusions, eliciting only large lamellipodia. To ascertain definitively the presence of filopodia in p53^{-/-} MEFs, high resolution phase-contrast analysis was performed (Figure 3C). We identified these peripheral globular membrane structures as characteristics of the different stages of filopodia emergence as described previously (Stekette *et al.*, 2001). These structures include an engorgement (E) consisting of an influx of cytoplasm into an associated adjacent filopodium, followed by a development of a focal phase density (fd) at the leading margin or/and along the parental filopodium, and next protrusion of a convex projection with wide bases, namely nub. This nub grew up and transformed into one or several filopodia, whose size (1–15 μ m), morphology and dynamics are similar to those described previously (Kozma *et al.*, 1995; Nobes and Hall, 1995b).

Wild-type p53 activity is required to inhibit filopodia formation

In order to confirm the role of p53 in inhibition of filopodia formation, we next examined whether expression of wild-type p53 in p53^{-/-} MEFs abolishes the constitutive presence of filopodia. F-actin organization of p53^{-/-} MEFs expressing either GFP-tagged wild-type p53, p53 H273 or p53 H175 was analysed (Figure 4A). Reintroduction of wild-type p53 into p53^{-/-} MEFs (panel a) prevented filopodia formation (panel b), whereas either of the two p53 mutants p53 H273 (panel c) or p53 H175 (panel e) were ineffective (panels d and f, respectively). GFP alone had no effect on filopodia formation in p53^{-/-} MEFs (data not shown).

To examine the mechanism whereby p53 expression reverses filopodia formation in p53^{-/-} MEFs, transfected cells were observed by phase-contrast time-lapse microscopy. At 12 h after transfection, a microscope field with GFP-positive cells was selected. As shown in Figure 4B and the accompanying videos, GFP-tagged p53 expression (panel a) restored a phenotype identical to that of p53^{+/+} MEFs, i.e. protrusive structures on the cell surface were barely detectable, showing the scarcity or the absence of filopodia (panel b). In contrast, under the same conditions, expression of the either of the two p53 mutants p53 H273 (panel c) or p53 H175 (not shown) had no obvious effect (panel d). Filopodia formation in p53^{-/-} MEFs expressing only GFP was not impaired (not shown). Quantitative analysis performed on numerous p53^{-/-} MEFs shows that the number of cells harbouring filopodia was dramatically reduced by expression of wild-type p53 (from 88 ± 5 to

Fig. 4. Wild-type p53 is required to inhibit filopodia. (A) p53^{-/-} MEFs were transfected with plasmids expressing either wild-type p53 (panels a and b), p53 H273 (panels c and d) or p53 H175 (panels e and f). Cells were stained for p53 expression (panels a, c and e) and F-actin (panels b, d and f). Scale bar: 10 μ m. (B) p53^{-/-} MEFs transfected with GFP-tagged forms of either wild-type p53 (panels a and b) or p53 H273 (panels c and d) were detected for GFP (panels a and c). Still phase-contrast images from the accompanying videos show cells transfected with either wild-type p53 (panel b) or p53 H273 (panel d). Scale bar: 10 μ m. (C) Quantification of MEFs presenting filopodia. MEFs, treated as in (B), were scored positively when presenting at least five filopodia. For each experiment, 50 cells were scored and results presented are the mean $\pm$ SD of three independent experiments.

22 $\pm$ 6%), but not by the expression of the mutated p53 or GFP alone (Figure 4C).

All these experiments demonstrate that (i) wild-type p53 activity is required to inhibit filopodia, probably through its DNA-binding and transcriptional activity; and (ii) p53 inhibits initiating steps of filopodia formation, preventing the appearance of focal densities and nubs.

Fig. 5. p53 acts downstream of Cdc42 to modify the actin cytoskeleton. (A) p53^{-/-} MEFs were transfected with plasmids encoding GFP-tagged Cdc42-N17 (panels a and b) or the Myc-tagged CRIB domain of WASP (panels c and d). Transfected cells detected for GFP (panel a) or stained for Myc (panel c) were stained for F-actin (panels b and d). Scale bar: 10 μ m. (B) MEFs and p53^{-/-} MEFs transfected or not with wild-type p53 were lysed and the GTP-bound form of Cdc42 was analysed as described in Materials and methods. Cdc42-GTP precipitated with GST-PAK1 and total Cdc42 present in the lysates was analysed by immunoblotting with an anti-Cdc42 antibody. (C) p53^{-/-} MEFs were co-transfected with Myc-tagged wild-type p53 and GFP-tagged Cdc42-V12 (panels a, b and c). Fixed cells were visualized for GFP (panel b) and stained for p53 (panel a) and actin (panel c). Scale bar: 10 μ m.

p53 acts downstream of Cdc42 to inhibit filopodia formation

In order to analyse further the hierarchical organization of Cdc42 and p53 signalling in filopodia formation, we expressed GFP-tagged Cdc42-N17, the dominant-negative form of Cdc42, in p53^{-/-} MEFs and analysed the resulting F-actin modifications (Figure 5A). Expression of Cdc42-N17 in p53^{-/-} MEFs (panel a) did not impair filopodia formation (panel b). Alternatively, inhibition of endogenous Cdc42 was performed by expression of the Cdc42-interacting domain of the Wiskott-Aldrich syndrome protein (WASP), known to inhibit the endogenous Cdc42 activity through competition with its effector binding site (Aspenstrom *et al.*, 1996). Expression of the WASP fragment (panel c) in p53^{-/-} MEFs did not affect the abundance of filopodia (panel d). A quantitative

analysis of these results confirmed that neither Cdc42-N17 nor the WASP fragment affects the number of p53^{-/-} MEFs with filopodia ($88 \pm 5\%$ in control p53^{-/-} MEFs; $82 \pm 4\%$ in Cdc42-N17-expressing p53^{-/-} MEFs; and $86 \pm 6\%$ in WASP fragment-expressing p53^{-/-} MEFs). Interestingly, the level of active Cdc42 was the same in either normal MEFs, p53^{-/-} MEFs or p53^{-/-} MEFs expressing wild-type p53, as tested by measuring the level of GTP-bound Cdc42 (Figure 5B). These data demonstrate that p53-dependent inhibition of filopodia does not occur at the Cdc42 level.

In order to test whether Cdc42 can rescue p53-dependent inhibition of filopodia formation in p53^{-/-} MEFs, we co-transfected wild-type p53 with GFP-tagged Cdc42-V12 in p53^{-/-} MEFs (Figure 5C). Cells expressing both wild-type p53 (panel a) and Cdc42-V12 (panel b) still did not present any filopodia at their surface, although they featured other characteristics of Cdc42 activation, including reduction of stress fibres and an increase in diffuse and punctate actin staining (panel c). Taken together, these data strongly suggest that p53 acts downstream of Cdc42 to inhibit filopodia formation in p53^{-/-} MEFs.

The absence of p53 stimulates cell spreading

Cell spreading has been shown to be mediated largely by the Cdc42-induced filopodia process (Price *et al.*, 1998). The inhibition of Cdc42-induced filopodia by p53 prompted us to explore whether p53 interferes with cell spreading. We first compared normal and p53^{-/-} MEFs for their ability to spread on culture dishes pre-coated with fibronectin. Spreading was monitored by time-lapse microscopy, at the frequency of one image every 30 s (Figure 6A). In p53^{-/-} MEFs (panels a–f), as soon as substrate attachment occurred (2–5 min), numerous membrane peripheral filopodia were formed (arrowheads, panels b and c). Even when cell spreading was fully achieved (60 min, panel f), filopodia were still present. In contrast, observation of cell spreading in wild-type MEFs (panels g–l) revealed crucial differences. First, the time required for spreading was increased in MEFs compared with p53^{-/-} MEFs (compare at 5 and 10 min, panels b and c with panels h and i). Secondly, filopodia were no longer visible in MEFs and were replaced progressively by lamellipodia from 15 min onwards (arrows in panels j, k and l).

To determine to what extent p53 modulates spreading, MEFs and p53^{-/-} MEFs were transfected with GFP alone, or with either wild-type or mutant forms of GFP-tagged p53. A quantitative analysis of the spread cells at 10 min revealed that the absence of p53 increased spreading ($54 \pm 9\%$ in p53^{-/-} MEFs; $6.5 \pm 5\%$ in MEFs) (Figure 6B). Expression of GFP–wild-type p53 in both cell types dramatically reduced the number of spread cells ($6 \pm 2\%$ in MEFs and $2 \pm 1\%$ in p53^{-/-} MEFs). To test whether p53 activity was required to inhibit Cdc42-mediated cell spreading, we expressed Cdc42-V12 concomitantly with either wild-type or mutated forms of p53 in MEFs. Cdc42-V12 alone increased the number of spread cells 5.9-fold, demonstrating that Cdc42 contributes to cell spreading in this cell type. On the other hand, Cdc42-increased cell spreading was returned to basal level by wild-type p53 expression, whereas p53 H273 and p53 H175 were inefficient.

Fig. 6. Inhibition of cell spreading by p53. (A) Time-lapse images of freshly plated p53^{-/-} MEFs (a–f) or MEFs (g–l). Frames at selected times after plating show the rapid development of filopodia protruding from the cell (arrowhead) persisting in p53^{-/-} MEFs and transformed into lamellipodia (arrows) in MEFs. Scale bar: 10 μ m. (B) A quantitative analysis of spread cells at 10 min, transfected as follows: MEFs and p53^{-/-} MEFs were transfected with GFP alone or GFP-tagged wild-type p53. In addition, MEFs (10^5) were first infected with 10^5 colony-forming units of pLXSN wild-type retroviral particles (control) or pLXSN Myc-tagged Cdc42-V12 retroviral particles, then transfected with GFP-tagged Cdc42-V12 and GFP alone or GFP-tagged p53 forms of either the wild-type or mutants. For each experiment, 100 cells were scored and results are the mean $\pm$ SD of three independent experiments.

Fig. 7. Wild-type p53 inhibits Golgi reorientation during cell migration. (A) Control MEFs (panel a) or MEFs treated with etoposide to activate p53 (panel b and c) were stained for Golgi distribution 4 h after wounding (panels a and c). Double-headed arrows indicate the orientation of the wound, and single-headed arrows indicate the direction of cell migration. Considering the direction of cell migration as a median, a 120° sector facing the wound was drawn to measure the Golgi orientation. Scale bar: 10 μ m. (B) A quantitative analysis of control MEFs or MEFs treated with TNF- α , adriamycin or etoposide having their Golgi apparatus in the forward 120° sector was performed 4 h after wounding. In a confluent monolayer, random orientation of the Golgi corresponds to 33% in the 120° sector. For each experimental condition, at least 100 cells were scored. Results are the mean $\pm$ SD of three independent experiments. (C) p53^{-/-} MEFs transfected with either GFP alone (panels a and b), GFP-tagged p53 H273 (panels c and d), p53 H175 (panels e and f) or wild-type p53 (panels g and h) were stained for Golgi localization (panels b, d, f and h). GFP-expressing cells are shown in panels a, c, e and g. Scale bar: 10 μ m. (D) A quantitative analysis of the experimental conditions described in (C) and of p53^{-/-} MEFs expressing GFP-tagged Cdc42-N17 was performed as in (B). Approximately 100 cells were scored for each condition. Values represent the mean $\pm$ SD of three independent experiments.

These data demonstrate that p53 prevents the increase in cell spreading induced by overexpression of constitutively active Cdc42.

Expression of p53 wt inhibits cell polarization

Cdc42 contributes to the cell movement in controlling cell polarity (Nobes and Hall, 1999; Etienne-Manneville and Hall, 2001). Our results show that up-regulation of p53 affects the Cdc42-mediated signalling pathway. We therefore analysed further the role of p53 in the establishment of the polarized morphology of migrating cells, using a wound healing assay. Monolayers of confluent MEFs or p53^{-/-} MEFs scraped in order to generate a 100–150 μ m wide wound, closed the gap by 6 h. First row cells moved perpendicularly to the wound edge and adopted a polarized morphology characterized by reorientation of the microtubule-organizing centre (MTOC), the microtubule cytoskeleton and the Golgi apparatus (Nobes and Hall, 1999; Etienne-Manneville and Hall, 2001). We have investigated the role of p53 in the reorientation of the Golgi apparatus, using the anti-p115 antibody, which detects the *cis*-Golgi compartment. As expected, MEFs rearrange their Golgi

apparatus in the direction of movement, perpendicularly to the wound (Figure 7A, a). On the other hand, in etoposide-treated MEFs in which p53 was activated (panel b), no such Golgi reorientation was observed (panel c), indicating that p53 prevented Golgi reorganization during cell movement. A quantitative analysis showed that activation of endogenous p53 correlates with a decrease in the number of polarized cells (82 $\pm$ 3% in untreated MEFs to ~50% in p53-expressing MEFs pre-treated with TNF- α , adriamycin or etoposide) (Figure 7B).

To determine whether wild-type p53 activity is required for cell polarity inhibition, we transfected p53^{-/-} MEFs with GFP–wild-type p53, or either of the two mutants GFP–p53 H175 or GFP–p53 H273. GFP-positive cells in the front row were evaluated for Golgi reorientation as described above. As seen in Figure 7C and quantified in Figure 7D, whereas neither GFP alone (panels a and b) nor the two mutants p53 H273 (panels c and d) and p53 H175 (panels e and f) had any effect, reintroduction of wild-type p53 into p53^{-/-} MEFs (panel g) severely decreased Golgi polarization (panel h) (57 $\pm$ 1% in wild-type p53-expressing cells; 85 $\pm$ 2% in control cells, Figure 6C).

From these experiments, we conclude that p53 inhibits the establishment of cell polarity during *in vitro* wound healing.

Discussion

The data presented here demonstrate that activation of functional wild-type p53 prevents formation of specific actin-containing structures protruding from the cell surface and characterized as filopodia. Both overexpression of wild-type p53 and activation of endogenous p53 counteract Cdc42-induced filopodia formation. Our data suggest that transcriptional activity of p53 is required to inhibit Cdc42-induced filopodia formation, since p53 H273 and p53 H175, two mutants deficient in the DNA-binding activity, were inactive in our assay. Consistent with this observation, cells lacking p53 activity (p53^{-/-}) exhibited constitutive membrane filopodia. This behaviour has direct effects on cell functions: (i) non-adherent cells that overexpressed p53 take more time to spread fully upon reattachment onto the extracellular matrix; and (ii) during cell migration, the reorientation of the Golgi apparatus in the direction of movement is severely decreased by wild-type p53 expression, thus preventing cell polarity.

What is the mechanism that underlies p53-dependent inhibition of Cdc42-mediated functions?

Neither stress fibres nor lamellipodia were affected by p53 expression, demonstrating that p53 is not a ubiquitous inhibitor of actin polymerization. In MEFs, p53 counteracted Cdc42-induced filopodia and, in p53^{-/-} MEFs, no filopodia were observed when p53 was expressed, even when Cdc42 was activated. This indicates that p53 activity specifically targets the Cdc42-induced filopodia signalling pathway. As such, p53 might act directly on Cdc42 or, alternatively, it could interfere with a Cdc42 mediator of filopodia formation. In the absence of p53, cells exhibited constitutive membrane filopodia that were not impaired by Cdc42-N17, a dominant-negative mutant of Cdc42. This suggests that p53 can act downstream of Cdc42 to influence filopodia formation.

Previous studies have established a hierarchical link between several Rho GTPases. Cdc42 can rapidly activate Rac1, which in turn activates RhoA (Kozma *et al.*, 1995; Nobes and Hall, 1995a). p53-deficient MEFs could harbour persistent filopodia at their surface, if the Rac1 activity level was not sufficient to promote lamellipodia formation from pre-existing filopodia. As expected, the dominant-negative forms of Rac1 and RhoA do not affect filopodia in p53^{-/-} MEFs. Thus, filopodia formation can occur in the absence of Rac1 and RhoA activities, in agreement with other reports (Kozma *et al.*, 1995; Nobes and Hall, 1995a).

Filopodia are structures that are particularly dynamic. Their presence at the cell surface has already been described as transient and rapid (<1 min; Steketee *et al.*, 2001). This behaviour was confirmed by our time-lapse microscopy experiments in p53^{-/-} MEFs, which present an exaggerated abundance of filopodia. However, reintroduction of p53 into p53^{-/-} MEFs slows down membrane activities, strongly reducing the focal density spots and the

so-called nubs that precede filopodia emergence. These results support a model in which p53 acts on filopodia by preventing initiating steps of their formation.

Previous reports have established that the cell spreading process initiates with the integrin-mediated activation of Cdc42 that promotes the extension of filopodia. Activation of Cdc42 leads to the subsequent activation of Rac1 and the formation of lamellipodia (Price *et al.*, 1998). Our data demonstrate that p53 inhibits Cdc42-mediated cell spreading. Since evidence has also been presented that p53 prevents Cdc42-mediated filopodia formation, the mechanism whereby p53 inhibits cell spreading seems likely to be due to its effect on filopodia formation.

Activation of Cdc42 by integrin signalling also leads to the control of cell polarity during migration. In this case, a previous report indicates that activated Cdc42 promotes two distinct coordinated pathways, which are both required to establish cell polarization: the first one leads to the formation of protrusions through Rac1, and the second is essential for the polarization of the Golgi apparatus, MTOC and microtubule (Etienne-Manneville and Hall, 2001). Since our experiments indicate that p53 prevents filopodia formation, it is tempting to speculate that p53 affects the first pathway, but we cannot exclude the possibility that p53 plays a role in the second one, or both.

How are the known activities of p53 related to its novel inhibitory function in filopodia formation and cell movement?

The p53 function in regulating filopodia formation, cell spreading and cell migration may play an important role in carcinogenesis. In particular, cell progression to metastasis requires specific properties: cells are released from the primary tumour and invade the surrounding tissue before entering the circulation for transport towards other tissues. To colonize new tissues, cancer cells extravasate as single cells by adhesion and spreading along the vessel wall, using pseudopodal projections to migrate into the surrounding tissue. Abrogation of p53 functions might favour cell spreading and migration of cancer cells, thus accelerating the invasion of the target tissue. In favour of this hypothesis, we observed that, in our wound closure assays, the motility of cells in the wound is altered by overexpression of p53 (data not shown). Therefore, the morphological properties of p53-deficient cells would favour cell motility and metastasis during carcinogenesis.

In conclusion, we have defined p53 as a modulator of Cdc42-induced cytoskeletal reorganization. This novel function of p53 might contribute to its tumour suppressor function by controlling cell morphology.

Materials and methods

DNA constructs and reagents

Human wild-type p53 cDNA or its mutated forms, H175 and H273, were cloned into the *Bam*HI site of pCDNA3 vector (Invitrogen), then transferred to pEGFPC1 (Clontech) to give GFP-tagged proteins. Constructs expressing Myc epitope-tagged mutant Rac1, Cdc42 and RhoA proteins and their various mutants were kindly provided by P.Chavrier (Dutartre *et al.*, 1996). The GFP fusion proteins were cloned in the pEGFP-C1 vector (Roux *et al.*, 1997; Gauthier-Rouviere *et al.*, 1998; Ory *et al.*, 2000). The pcDNA3myc-NWASP containing the Cdc42-interacting domain of WASP was described previously in Philips *et al.*

(2000). The pGL2B-mdm2 plasmid in which the luciferase reporter gene is controlled by the p53-responsive element of *mdm2* (Barak *et al.*, 1994) was a kind gift of E.Yonish-Rouach. pTKRL plasmid was from Promega. TNF- α , adriamycin and etoposide (Sigma) were used in all experiments at concentrations of 100 ng/ml, 5 μ g/ml and 25 μ g/ml, respectively.

Cell culture, transfection and retroviral infections

Homozygous (p53^{-/-}) mice were generated originally by mating heterozygote (p53^{+/-}) mice of C57BL/6 and 129/Sv genetic background (Jacks *et al.*, 1994), and were obtained from CDTA (Orleans, France). They were maintained on a 129/Sv $\times$ C57BL/6 genetic background. MEFs were generated from individual fetuses isolated from pregnant C57BL/6 females mated 12 days previously with 129/Sv males to retain the genetic background. MEFs and p53^{-/-} MEFs were cultured at 37°C in the presence of 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). Control pLXSN and pLXSN-myc Cdc42-V12 retroviral particle production and infection of target cells were performed as described previously (Roux *et al.*, 1997). Infected cells were selected with 600 μ g/ml G418, and neomycin-resistant clones were pooled. For immunofluorescence experiments, cells were plated on 18 mm diameter glass coverslips 16–24 h before transfection by the lipofectamine method (0.5–1 μ g of plasmid DNA per 35 mm diameter well containing three glass coverslips), as recommended by the supplier (Gibco-BRL). At 4 h after transfection, the medium was replaced by DMEM supplemented with 10% FCS. Expressing cells were observed under a fluorescence microscope 18–24 h after transfection.

Immunofluorescence and filopodia measurements

MEFs and p53^{-/-} MEFs were transfected on coverslips at a confluence of ~30%. After 20–24 h, cells were fixed for 5 min in 3.7% formalin [in phosphate-buffered saline (PBS)] followed by a 15 min permeabilization with 0.1% Triton-X100 (in PBS) and incubation in PBS containing 0.1% bovine serum albumin (BSA). Expression of GFP-tagged proteins was visualized directly, while expression of Myc epitope-tagged proteins was visualized after a 60 min incubation with the 9E10 anti-Myc monoclonal antibody (gift from D.Mathieu, IGM) (1:2 dilution in PBS/BSA), followed by incubation with affinity-purified fluorescein-conjugated goat anti-mouse antibody (Cappel-ICN) (1:40 dilution). Cells were stained simultaneously for F-actin using rhodamine-conjugated phalloidin (0.5 U/ml; Sigma). Alternatively, cells were stained with an anti-ezrin polyclonal antibody, described previously (Andreoli *et al.*, 1994), provided by P.Mangeat, followed by incubation with affinity-purified fluorescein-conjugated goat anti-rabbit antibody (Cappel-ICN; 1:20 dilution). Cells were washed in PBS and mounted in Mowiol (Aldrich). To consider a cell as having filopodia, we evaluated the number of filopodia on its surface; F-actin-stained cells containing at least five filopodia were scored as positive.

Cell imaging

For immunofluorescence, cells were observed using a DMR B microscope (Leica, Germany) with a PL APO 40 $\times$ objective (NA 1.00), or (for specification see figure legends) a PL APO 63 $\times$ oil-immersion Immersol 518 F (Zeiss, Germany) and illumination of the preparation by a 100 W HBO 103W/2 light bulb (Osram, Germany). Images thus obtained were captured with an ORCA 100 (B/W) 10 bits cooled CCD camera (C mount 1 $\times$), a C 4742-95 controller and HIPIC controller program run by a PC-compatible microcomputer (Hamamatsu, Japan). Images were saved in TIFF format (8 bits) for processing and mounting with Microsoft PowerPoint.

SEM

MEFs and p53^{-/-} MEFs were grown on coverslips and then fixed in 0.1 M sodium cacodylate pH 7.2 containing 2% glutaraldehyde and 0.1 M sucrose for at least 1 h, and processed as described (Brunk *et al.*, 1981). Samples were observed using a Hitachi S4000 scanning microscope at 15 kV.

Time-lapse imaging

Time-lapse phase-contrast microscopy was performed on a Leica DL IRBE (Leica, Wetzlar, Germany) inverted microscope equipped with an automatic shutter and GFP filter sets, a 63 $\times$ oil-immersion objective (NA 1.3; Leica), sample heater (37°C) and a home-made CO₂ incubation chamber. Images were captured with MicroMax 1300 CCD camera (RS-Princeton Instruments, Trenton, PA) imaging software, converted to TIFF files that were edited with NIH Image and compiled into QuickTime movies. The exposure time was fixed at 50 ms.

Spreading assays

To determine cell spreading on fibronectin, non-treated 35 mm plates were coated with 10 μ g/ml fibronectin (Sigma) for 2 h at 37°C. Cells (5 $\times$ 10⁴ per plate) were pre-incubated for 24 h in low serum (0.5% FCS) to minimize the influence of growth factors on spreading, then detached and plated on the appropriate substratum. Cells adhered to the surface within 5 min after seeding and spread over different periods of time depending on the experimental conditions. Cell attachment took place at 37°C. At 5 min after seeding, the medium was changed to eliminate unattached cells. Adherent cells were photographed under phase contrast and counted as flattened or round. The number of cells in a constant unit area (0.003 mm²) was estimated. All spreading assays were performed in triplicate.

Cell polarization assay

Cell polarization was performed by measuring Golgi apparatus reorientation as described in Nobes and Hall (1999). Briefly, 70% confluent cells were transfected or not with GFP-tagged constructs using the Lipofectamine method. After 20–24 h, when cells were confluent, monolayers were scratched (100–150 μ m wide), then fixed 4 h later and the Golgi apparatus localized by immunofluorescence using anti-p115 antibody (Transduction Laboratories). First row wound cells presenting their Golgi within the forward-facing sector were scored positive. For each point, ~100 cells were examined. All polarization assays were performed in triplicate.

Cdc42 activity assay

The Cdc42 activity assay was performed as described (Ory *et al.*, 2000). Briefly, 3 $\times$ 10⁵ cells, transfected or not with wild-type p53, were lysed before incubation with GST-PAK fusion protein, the Cdc42-binding domain (CRIB) from human PAK1B (amino acids 56–272), coupled to glutathione-Sepharose beads (Pharmacia Biotech). After precipitation, complexes were washed four times with lysis buffer, eluted in SDS-PAGE sample buffer, immunoblotted and analysed with antibodies against Cdc42 (Transduction Laboratories). Aliquots taken from supernatants prior to precipitation were used to quantify total Cdc42 GTPase present in cell lysates.

p53 transactivation assay

p53 transactivation was measured using the dual-luciferase assay system from Promega. Cells (5 $\times$ 10⁴) were seeded onto 12-well plates and transfected 18 h later in OptiMEM containing 0.440 μ g of DNA (0.2 μ g of appropriate GTPase plasmids, 0.2 μ g of pGL2B-mdm2-luciferase plasmid and 0.04 μ g of pTKRL plasmid) using 0.33 μ l of Lipofectamine (Gibco-BRL) for 4 h. Cells were then left for 24 h in DMEM supplemented with 10% FCS, harvested in 250 ml of passive lysis buffer (PLB; Promega), and luciferase activity was measured following the Dual-luciferaseTM Reporter Assay Protocol as recommended by Promega, using a luminometer fitted with two injectors (Berthold).

Supplementary data

Supplementary data for this paper are available at *The EMBO Journal* Online.

Acknowledgements

We are grateful to Ph.Fort and A.Blangy for continual support and stimulating criticisms, E.Vignal and M.de Toledo for discussions, P.Travo for constructive microscopy, A.Behfar, U.Hibner, A.Gandarillas and J.Piette for critical comments on the manuscript, F.Comunale for technical support, and Drs A.Brunet, Ph.Chavrier, M.Clarke, R.Davis, B.Dérjard, P.Mangeat, M.Martin, A.Hall, S.H.Liang, L.Machesky, D.Mathieu and C.Norbury for providing useful plasmids, cells and reagents. Supported by the Ligue Nationale contre le Cancer (équipe labellisée), Association pour la Recherche contre le Cancer (contrat 5978), INSERM and CNRS.

References

- Adams,A.E., Johnson,D.I., Longnecker,R.M., Sloat,B.F. and Pringle,J.R. (1990) CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.*, **111**, 131–142.
- Aepfelbacher,M., Vauti,F., Weber,P.C. and Glomset,J.A. (1994) Spreading of differentiating human monocytes is associated with a

- major increase in membrane-bound CDC42. *Proc. Natl Acad. Sci. USA*, **91**, 4263–4267.
- Aepfelbacher, M., Essler, M., Huber, E., Czech, A. and Weber, P.C. (1996) Rho is a negative regulator of human monocyte spreading. *J. Immunol.*, **157**, 5070–5075.
- Agarwal, M.L., Taylor, W.R., Chernov, M.V., Chernova, O.B. and Stark, G.R. (1998) The p53 network. *J. Biol. Chem.*, **273**, 1–4.
- Amieva, M.R., Litman, P., Huang, L., Ichimaru, E. and Furthmayr, H. (1999) Disruption of dynamic cell surface architecture of NIH3T3 fibroblasts by the N-terminal domains of moesin and ezrin: *in vivo* imaging with GFP fusion proteins. *J. Cell Sci.*, **112**, 111–125.
- Andreoli, C., Martin, M., Le Borgne, R., Reggio, H. and Mangeat, P. (1994) Ezrin has properties to self-associate at the plasma membrane. *J. Cell Sci.*, **107**, 2509–2521.
- Aspenstrom, P., Lindberg, U. and Hall, A. (1996) Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott–Aldrich syndrome. *Curr. Biol.*, **6**, 70–75.
- Bar-Sagi, D. and Feramisco, J.R. (1986) Induction of membrane ruffling and fluid-phase pinocytosis in quiescent fibroblasts by ras proteins. *Science*, **233**, 1061–1068.
- Barak, Y., Lupo, A., Zauberman, A., Juven, T., Aloni-Grinstein, R., Gottlieb, E., Rotter, V. and Oren, M. (1994) Targets for transcriptional activation by wild-type p53: endogenous retroviral LTR, immunoglobulin-like promoter and an internal promoter of the *mdm2* gene. *Cold Spring Harbor Symp. Quant. Biol.*, **59**, 225–235.
- Braga, V.M., Machesky, L.M., Hall, A. and Hotchin, N.A. (1997) The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell–cell contacts. *J. Cell Biol.*, **137**, 1421–1431.
- Brunk, U., Collins, V.P. and Arro, E. (1981) The fixation, dehydration, drying and coating of cultured cells for SEM. *J. Microsc.*, **123**, 121–131.
- Comer, K.A., Dennis, P.A., Armstrong, L., Catino, J.J., Kastan, M.B. and Kumar, C.C. (1998) Human smooth muscle α -actin gene is a transcriptional target of the p53 tumor suppressor protein. *Oncogene*, **16**, 1299–1308.
- Dutartre, H., Davoust, J., Gorvel, J.P. and Chavrier, P. (1996) Cytokinesis arrest and redistribution of actin-cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42Hs. *J. Cell Sci.*, **109**, 367–377.
- Eliyahu, D., Michalovitz, D., Eliyahu, S., Pinhasi-Kimhi, O. and Oren, M. (1989) Wild-type p53 can inhibit oncogene-mediated focus formation. *Proc. Natl Acad. Sci. USA*, **86**, 8763–8767.
- Etienne-Manneville, S. and Hall, A. (2001) Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKC ζ . *Cell*, **106**, 489–498.
- Gauthier-Rouviere, C., Vignal, E., Meriane, M., Roux, P., Montcourier, P. and Fort, P. (1998) RhoG GTPase controls a pathway that independently activates Rac1 and Cdc42Hs. *Mol. Biol. Cell*, **9**, 1379–1394.
- Giaccia, A.J. and Kastan, M.B. (1998) The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.*, **12**, 2973–2983.
- Gloushankova, N., Ossovskaya, V., Vasiliev, J., Chumakov, P. and Kopnin, B. (1997) Changes in p53 expression can modify cell shape of ras-transformed fibroblasts and epitheliocytes. *Oncogene*, **15**, 2985–2989.
- Guenal, I., Risler, Y. and Mignotte, B. (1997) Down-regulation of actin genes precedes microfilament network disruption and actin cleavage during p53-mediated apoptosis. *J. Cell Sci.*, **110**, 489–495.
- Hall, A. (1998) Rho GTPases and the actin cytoskeleton. *Science*, **279**, 509–514.
- Hansen, R. and Oren, M. (1997) p53: from inductive signal to cellular effect. *Curr. Opin. Genet. Dev.*, **7**, 46–51.
- Harvey, M. *et al.* (1993) *In vitro* growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene*, **8**, 2457–2467.
- Hollstein, M. *et al.* (1994) Database of p53 gene somatic mutations in human tumors and cell lines. *Nucleic Acids Res.*, **22**, 3551–3555.
- Izawa, I., Amano, M., Chihara, K., Yamamoto, T. and Kaibuchi, K. (1998) Possible involvement of the inactivation of the Rho–Rho-kinase pathway in oncogenic Ras-induced transformation. *Oncogene*, **17**, 2863–2871.
- Jacks, T., Remington, L., Williams, B.O., Schmitt, E.M., Halachmi, S., Bronson, R.T. and Weinberg, R.A. (1994) Tumor spectrum analysis in p53-mutant mice. *Curr. Biol.*, **4**, 1–7.
- Klefstrom, J., Arighi, E., Littlewood, T., Jaattela, M., Saksela, E., Evan, G.I. and Alitalo, K. (1997) Induction of TNF-sensitive cellular phenotype by c-Myc involves p53 and impaired NF- κ B activation. *EMBO J.*, **16**, 7382–7392.
- Kozma, R., Ahmed, S., Best, A. and Lim, L. (1995) The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol. Cell. Biol.*, **15**, 1942–1952.
- Levine, A.J. (1997) p53, the cellular gatekeeper for growth and division. *Cell*, **88**, 323–331.
- Lowe, S.W., Ruley, H.E., Jacks, T. and Housman, D.E. (1993) p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell*, **74**, 957–967.
- Metcalfe, S., Weeds, A., Okorokov, A.L., Milner, J., Cockman, M. and Pope, B. (1999) Wild-type p53 protein shows calcium-dependent binding to F-actin. *Oncogene*, **18**, 2351–2355.
- Nobes, C.D. and Hall, A. (1995a) Rho, rac and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia and filopodia. *Cell*, **81**, 53–62.
- Nobes, C.D. and Hall, A. (1995b) Rho, rac and cdc42 GTPases: regulators of actin structures, cell adhesion and motility. *Biochem. Soc. Trans.*, **23**, 456–459.
- Nobes, C.D. and Hall, A. (1999) Rho GTPases control polarity, protrusion and adhesion during cell movement. *J. Cell Biol.*, **144**, 1235–1244.
- Ory, K., Legros, Y., Auguin, C. and Soussi, T. (1994) Analysis of the most representative tumour-derived p53 mutants reveals that changes in protein conformation are not correlated with loss of transactivation or inhibition of cell proliferation. *EMBO J.*, **13**, 3496–3504.
- Ory, S., Munari-Silem, Y., Fort, P. and Jurdic, P. (2000) Rho and rac exert antagonistic functions on spreading of macrophage-derived multinucleated cells and are not required for actin fiber formation. *J. Cell Sci.*, **113**, 1177–1188.
- Philips, A., Roux, P., Coulon, V., Bellanger, J.M., Vie, A., Vignais, M.L. and Blanchard, J.M. (2000) Differential effect of rac and cdc42 on p38 kinase activity and cell cycle progression of nonadherent primary mouse fibroblasts. *J. Biol. Chem.*, **275**, 5911–5917.
- Price, L.S., Leng, J., Schwartz, M.A. and Bokoch, G.M. (1998) Activation of Rac and Cdc42 by integrins mediates cell spreading. *Mol. Biol. Cell*, **9**, 1863–1871.
- Prives, C. (1998) Signaling to p53: breaking the MDM2–p53 circuit. *Cell*, **95**, 5–8.
- Qiu, R.G., Abo, A., McCormick, F. and Symons, M. (1997) Cdc42 regulates anchorage-independent growth and is necessary for Ras transformation. *Mol. Cell. Biol.*, **17**, 3449–3458.
- Roux, P., Gauthier-Rouviere, C., Doucet-Brutrin, S. and Fort, P. (1997) The small GTPases Cdc42Hs, Rac1 and RhoG delineate Raf-independent pathways that cooperate to transform NIH3T3 cells. *Curr. Biol.*, **7**, 629–637.
- Steketee, M., Balazovich, K. and Tosney, K.W. (2001) Filopodial initiation and a novel filament-organizing center, the focal ring. *Mol. Biol. Cell*, **12**, 2378–2395.
- Tapon, N. and Hall, A. (1997) Rho, Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton. *Curr. Opin. Cell Biol.*, **9**, 86–92.
- Zohn, I.M., Campbell, S.L., Khosravi-Far, R., Rossman, K.L. and Der, C.J. (1998) Rho family proteins and Ras transformation: the RHOad less traveled gets congested. *Oncogene*, **17**, 1415–1438.

Received July 27, 2001; revised March 18, 2002;
accepted March 22, 2002