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Cdc42Hs and Rac1 GTPases Induce the Collapse of the Vimentin Intermediate Filament Network*

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In this study we show that expression of active Cdc42Hs and Rac1 GTPases, two Rho family members, leads to the reorganization of the vimentin intermediate filament (IF) network, showing a perinuclear collapse. Cdc42Hs displays a stronger effect than Rac1 as 90% versus 75% of GTPase-expressing cells show vimentin collapse. Similar vimentin IF modifications were observed when endogenous Cdc42Hs was activated by bradycinin treatment, endogenous Rac1 by platelet-derived growth factor/epidermal growth factor, or both endogenous proteins upon expression of active RhoG. This reorganization of the vimentin IF network is not associated with any significant increase in soluble vimentin. Using effector loop mutants of Cdc42Hs and Rac1, we show that the vimentin collapse is mostly independent of CRIB (Cdc42Hs or Rac-interacting binding)-mediated pathways such as JNK or PAK activation but is associated with actin reorganization. This does not result from F-actin depolymerization, because cytochalasin D treatment or Scar-WA expression have merely no effect on vimentin organization. Finally, we show that genistein treatment of Cdc42Hs and Rac1-expressing cells strongly reduces vimentin collapse, whereas staurosporin, wortmannin, LY-294002, Rp-cAMP, or RII, the regulatory subunit of protein kinase A, remain definitive. Moreover, we detected an increase in cellular tyrosine phosphorylation content after Cdc42Hs and Rac1 expression without modification of the vimentin phosphorylation status. These data indicate that Cdc42Hs and Rac1 GTPases control vimentin IF organization involving tyrosine phosphorylation events.

The Rho family of Ras-like GTPases are clustered in two distinct subgroups; the Rac/Cdc42 subgroup, including Rac1, -2, and -3, RhoG, Cdc42Hs, TC10, chp (Cdc42 homologous protein), and RhoH, and the Rho subgroup, in which RhoA, -B, and -C, RhoD, RhoL, and Rnd1, -2, and -3 are found. Many cell functions, including maintenance of morphology (1), motility (2), adhesion (3, 4), cell division (5) and proliferation (6), smooth muscle contraction (7), and vesicular transport (8, 9) are regulated by these GTPases. Cdc42Hs and Rac1, in which RhoA, Rac1, and Cdc42Hs, tightly regulate the actin filaments organization. In fibroblasts, lysophosphatidic acid-stimulated stress fiber formation requires RhoA (10, 11). Epidermal growth factor (EGF)2, platelet-derived growth factor (PDGF)-, and insulin-dependent cortical actin polymerization such as ruffles and lamellipodia requires Rac1 (4, 10), whereas bradycrin (Bdk)-stimulated filopodia formation requires Cdc42Hs (4, 12). Activation hierarchies exist among Rho GTPases. RhoG acts upstream of Rac1 and Cdc42Hs (13), and Cdc42 activation leads to subsequent activation of Rac1 (4). Evidence also suggests the existence of an antagonism between RhoA and Rac1/Cdc42Hs (14–16).

Once in a GTP-bound and -activated conformation, each of these GTPases appear to interact with specific downstream effectors proteins. More than 20 candidate targets have been identified so far, such as protein kinases PAKs, ACKs, PKN-related kinases (PRKs), and Rho kinases (ROCKs); lipid kinases phosphatidylinositol 3-kinase, PIP5K, and PLD and non-kinase proteins such as Wiskott-Aldrich syndrome protein (WASP), partner of Rac1 (POR1), plenty of SH35 (POSH) p67PHOX, IQGAPs, Rhotekin, and others (for a review see Aspenstrom (17)).

Although the role of Rho GTPases on actin cytoskeleton organization has been extensively studied, little is known on their effects on one of the other major component of the cytoskeleton of eucaryotic cells, the intermediate filaments (IFs). IFs consist of a heterogeneous tissue-specific family of proteins, which are prevalent in the perinuclear region and extend radially through the cytoplasm, eventually forming close associations with the cell surface, concentrated in regions containing desmosomes (cadherin-mediated cell-to-cell junctions), hemidesmosomes (integrin-mediated adhesive junctions), and other types of adhesion sites (18, 19). Cytoskeletal IF also interact with other cytoskeletal elements such as microtubules and microfilaments (20–23). Interaction with plasma membrane and other cytoskeletal elements involve a number of IF-associated proteins that are essential for maintaining the integrity of IF network (24). Recently, it has been shown that, at least in actively growing cells, IF are dynamic structures. IF phosphorylation appears to be one of the most predominant biochemical events in coordinating intracellular organization of the IF network (25). Cytoplasmic IF disassembled when phos-
phorylated by protein kinase A, protein kinase C, calcium calmodulin kinase II (CaMKII), and Cdc2 kinases. Interestingly, protein kinase N (PKN), a protein kinase activated by Rho, associates and phosphorylates a subunit of neuron-specific intermediate filament, NFL (26) and ROKα (RhoA-binding kinase α) phosphorylates glial fibrillary acidic protein (GFAP) (27) and vimentin (28).

In the present study we analyzed the possible role of two Rho GTPases, Rac1 and Cdc42Hs, in regulating the vimentin IF organization. We show that expression of active Rac1 and Cdc42Hs that had a differential effect on F-actin organization. Interestingly, the γ40C mutants of Cdc42Hs and Rac1, which still induced F-actin rearrangements, promoted the collapse of IF as efficiently as did the V12 or L61 mutants. The F37A mutants of Cdc42Hs and Rac1, which had lost their ability to induce filopodia and membrane ruffling, respectively, no longer induced the collapse of IF. In addition, by using various drugs known to have kinase inhibitory activity, we show that vimentin IF reorganization involved tyrosine phosphorylation events.

**EXPERIMENTAL PROCEDURES**

**Cells Culture, Treatment, and Microinjection—** Rat embryo (REF-52) or human (Ha-68) fibroblasts were cultured at 37 °C in the presence of 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Cells were plated on 18-mm-diameter glass coverslips or human (Hs-68) fibroblasts were cultured at 37 °C in the presence of fetal calf serum. Cells were plated on 18-mm diameter glass coverslips or human (Hs-68) fibroblasts were cultured at 37 °C in the presence of fetal calf serum. Cells were treated with 100 nM cytochalasin D, 0.1% bovine serum albumin, 0.1% Triton, 50 mM MES, 600 mM KCl, 10 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, and RhoA (Molecular Dynamics).

**Immunocytochemistry—** 18 h after transfection, cells were fixed with 4% paraformaldehyde (in PBS) followed by a 20-min permeabilization in 0.1% Triton X-100 (in PBS) and incubation in PBS containing 0.1% bovine serum albumin. Expression of GFP-tagged proteins was visualized directly. Cells were stained for vimentin distribution using a mouse monoclonal anti-vimentin (Sigma, France) (1:200 dilution), followed by incubation with affinity-purified tetramethyl-1-iodo-5 and 6 iso-thiocyanate-conjugated goat anti-mouse antibody (Cappel-ICN) (1:40 dilution). Cells were stained for F-actin using coumarin phenyl isothiocyanate conjugated phalloidin (Sigma, France) and for phosphotyrosine epitopes using the 4G10 monoclonal antibody. Expression of Myc epitope-tagged proteins was visualized after a 60-min incubation with 9E10 anti-Myc monoclonal antibody (gift from D. Mathieu, Montpellier, France) followed by incubation in PBS containing 0.1% bovine serum albumin, followed by incubation with affinity-purified fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Cappel, ICN). Cells were washed in PBS, mounted in Mowiol (Aldrich, Milwaukee, WI), and observed using a DMR B Leica microscope using a 40 × (NA 1.00) or 63 × (NA 1.32) planapochromatic lens. Images obtained were captured with a MicroMax 1500 VHS (B/W) cooled (−10 °C) charge-coupled device camera as 16-bit images, and using a MetaMorph (v.4.11) control program (RS-Princeton Instruments) run by a PC-compatible microcomputer. Images were saved in TIFF format (16 bit) and subsequently adapted as TIFF 8-bit format after they were opened with Adobe Photoshop for processing and mounting with Adobe Illustrator.

**Detection of Tyrosine-phosphorylated Proteins—** Cell lysates from untransfected or cells transfected with either empty pEGFPN1 vector (MOCK), Cdc42HsV12, or Rac1V12 were lysed for the addition of 1% boiling SDS, 10 mM Tris-HCl, pH 7.4. After scraping, samples (30 μg of protein) were loaded onto a 10% polyacrylamide gel and then transferred onto nitrocellulose. Initial lysates were normalized for protein content (BCA, Sigma). Membranes were saturated in 8% milk in Tris-HCl, pH 7.5, containing 0.1% Tween and subsequently incubated with a mouse monoclonal antibody directed against vimentin (clone V9, Sigma) (1/1000 dilution) followed by peroxidase-conjugated anti-mouse antibody (Amersham Pharmacia Biotech) (1/2000 dilution). After extensive washing, membranes were incubated with chemiluminescence reagent (ECL, PerkinElmer Life Sciences) and analyzed with a PhosphorImager (Molecular Dynamics).

**Detection of Tyrosine-phosphorylated Vimentin—** Untransfected cells or cells transfected with either empty pEGFPN1 vector (MOCK), Cdc42HsV12, or Rac1V12 were lysed for the addition of 1% boiling SDS, 10 mM Tris-HCl, pH 7.4. After scraping, samples (30 μg of protein) were loaded onto a 10% polyacrylamide gel and then transferred onto nitrocellulose. Membranes were treated as described above and incubated with an anti-phosphotyrosine antibody (4G10, 1/200 dilution) followed by peroxidase-conjugated anti-mouse antibody (Amersham Pharmacia Biotech) (dilution 1/2000). After extensive washing, membranes were incubated with chemiluminescence reagent (ECL, PerkinElmer Life Sciences) and analyzed with a PhosphorImager (Molecular Dynamics).

**RESULTS**

**Expression of Active Cdc42Hs and Rac1 GTPases Leads to the Reorganization of the Vimentin IF Network—** To examine the overall effect of two Rho GTPases of the Rac/Cdc subgroup on vimentin IF distribution, mammalian expression plasmids encoding GFP-tagged constitutively active (G12V or Q61L) (for review see Ref. 31), Cdc42Hs, or Rac1 were transfected into growing REF-52 cells. In addition, the activated RhoA mutant, RhoAV14, was also expressed. 18 h later, cells were fixed and immunostained with antibodies directed against vimentin and for filamentous actin (F-actin) using rhodamine-conjugated phallolidin (Fig 1A). Expression of each of these GTPases induced the formation of specific actin-driven structures. Cdc42Hs (Fig. 1A, panel a) induced filopodia (panel b), Rac1 (panel c) induced lamellipodia/taffles (panel f), and RhoA (panel e) induced stress fibers (panel j) as previously reported (4, 12). Interestingly, expression of these GTPases also led to a marked change in the organization of the vimentin network (panels c and g). Indeed, although in control nontransfected cells vimentin showed a well-spread distribution from the perinuclear region to the cell periphery (see control nontrans-
fected cells in panels c, g, and k), in Cdc42Hs- or Rac1-expressing cells, vimentin accumulated at the perinuclear area. Vimentin IF organization was also extensively modified after active RhoA expression (panel k), which is in total agreement with a previous report (32). Corresponding Normarski images were shown to precisely localize cell margins (panels d, h, and l). For each panel, cells shown are representative of five independent experiments with more than 100 observed cells. Bar, 10 μm.

B, quantitative analysis of the effect of GFP-tagged Cdc42HsV12, Cdc42HsL61, Rac1V12, Rac1L61, RhoAV14, and empty pEGFPC1 expression on vimentin IF distribution in REF-52 and Hs-68 fibroblasts. The histogram shows the percentage of Rho GTPases-expressing cells showing collapsed vimentin. The values were average for five independent experiments.

**FIG. 2.** Bradykinin or PDGF/EGF and active RhoG expression induces reorganization of vimentin IF. A, REF-52 fibroblasts were stimulated with 100 ng ml\(^{-1}\) bradykinin (panels c and d) or 5 ng ml\(^{-1}\) PDGF/EGF (panels e and f) for 15 min. REF-52 fibroblasts were transfected with GFP-tagged RhoGV12 for 18 h (panel g). Untreated cells were shown in panels a and b. Cells were fixed, permeabilized, and stained for F-actin (panels a, c, e, and h) and vimentin distribution (panels b, d, f, and i). For each panel, cells shown are representative of five independent experiments with more than 100 observed cells. Bar, 10 μm. B, pellets from nontransfected REF-52 cells or REF-52 cells transfected with GFP-Cdc42HsV12 or GFP-Rac1V12 were lysed with a mild Nonidet P-40-containing (1%) buffer, separated by SDS-polyacrylamide gel electrophoresis, and immunoblotted with vimentin antibody. The arrow indicates the position of vimentin.

**FIG. 1.** Active Cdc42Hs and Rac1 induce reorganization of vimentin IF network. A, REF-52 fibroblasts were transfected with GFP-tagged Cdc42HsV12 (panel a), Rac1V12 (panel e), and RhoAV14 (panel i). 18 h after transfection, cells were fixed and stained for vimentin (panels c, g, and k) and F-actin distribution (panels b, f, and j). Normarski images were shown in panels d, h, and l. For each panel, cells shown are representative of five independent experiments with more than 100 observed cells. Bar, 10 μm. B, pellets from nontransfected REF-52 cells or REF-52 cells transfected with GFP-Cdc42HsV12 or GFP-Rac1V12 were lysed with a mild Nonidet P-40-containing (1%) buffer, separated by SDS-polyacrylamide gel electrophoresis, and immunoblotted with vimentin antibody. The arrow indicates the position of vimentin.
vimentin IF reorganization into perinuclear caps was observed (panel d), similar to the IF redistribution observed in Cdc42HsV12-expressing cells (Fig. 1A, panel g). 15 min after stimulation with PDGF/EGF, F-actin-containing lamellipodia were observed at the cell periphery (panel e) as well as a vimentin IF perinuclear distribution (panel f) comparable to that observed in Rac1V12-expressing cells (panel k). Activation of endogenous Cdc42Hs and Rac1 was also achieved by expressing active RhoG (13, 33). In REF-52 cells transfected with GFP-tagged RhoGV12 (panel g), local actin polymerization led to the formation of lamellipodia and filopodia (panel h) as well as vimentin IF perinuclear redistribution (panel i).

Because previous work reported that IF reorganization could be associated with changes in vimentin solubility (34), we analyzed the amount of vimentin extractable using either a mild Nonidet P-40-containing lysis buffer (29) or high salt and Triton X-100-containing lysis buffer (30) after expression of active Cdc42Hs and Rac1 GTPases. The amount of vimentin present in a soluble form extractable with a mild Nonidet P-40-containing buffer was not modified after expression of these GTPases (Fig. 2B) as was the case when using the high salt and Triton X-100-containing lysis buffer (data not shown).

Taken together, these data show that expression of active Cdc42Hs and Rac1 or activation of endogenous Cdc42Hs and Rac1 all led to vimentin IF perinuclear reorganization without modification of the solubility of vimentin.

Vimentin Reorganization Is Obtained Mainly with Y40 Mutants of Cdc42Hs and Rac1—We next investigated the pathways controlled by Rac1 and Cdc42Hs responsible for vimentin IF collapse. We used effector loop mutants of GTPases previously shown to differentially bind and activate downstream effectors (35–37) (Fig. 3). The Y40C mutants of Cdc42Hs and Rac1 had lost their ability to interact with CRIB (Cdc42Hs or Rac-interacting binding) motif-containing proteins and did not activate PAK-1 and JNK activity, but they still induced cortical F-actin polymerization, filopodia, and membrane ruffling, respectively. Conversely, the F37A mutants of Cdc42Hs and Rac1 still bound the CRIB motif-containing proteins, activating PAK and JNK, but were less efficient for inducing filopodia or membrane ruffling. Cells expressing the Y40C mutants of Cdc42Hs and Rac1 showed vimentin IF reorganization comparable to the one observed in active Rho GTPases-expressing cells (V12 or L61 mutants) (compare Fig. 1B with Fig. 3). Under the same conditions, expression of F37A mutants of Cdc42Hs and Rac1 did not significantly affect vimentin IF distribution, because only 10–20% of expressing cells showed weak vimentin reorganization.

Interaction of IF with microfilaments is thought to regulate IF organization in vivo (23). Rho GTPases of the Rac/Cdc sub-group are well-known key regulators of actin microfilaments (1), suggesting that the vimentin IF collapse we observed might result from Rac1- or Cdc42-dependent F-actin reorganization. Two subpopulations of actin structures were affected by Rac1 and Cdc42Hs expression: submembranous cortical actin, which was extensively modified to produce ruffles/lamellipodia and microvilli/filopodia, and stress fibers, which were mostly depolymerized. To test the existence of a relationship between stress fibers, depolymerization, and vimentin reorganization, Rac52 cells were treated with cytochalasin D, an F-actin-depolymerizing drug, fixed, and stained for F-actin and vimentin IF distribution (Fig. 4A). 30 min after cytochalasin D addition, the level of F-actin staining became barely detectable (panel b), whereas the vimentin IF network remained unaffected (panel c). We next transfected REF-52 cells with Scar-WA, a mutant form of the Arp2/3-interacting protein Scar, which prevents assembly of F-actin structures (38). As for cytochalasin-treated cells, expression of Myc-tagged Scar-WA...
(panel d) led to a decrease of F-actin polymerization (panel e) without any significant vimentin IF modifications (panel e). At variance, as shown in Fig. 4B, coexpression of Scar-WA (panels b and e) with active Cdc42Hs (panel a) or Rac1 (panel d) as well as cytochalasin D treatment of Cdc42 and Rac1-expressing cells (data not shown) led to inhibition of Cdc42 or Rac1-dependent vimentin reorganization (panels c and f). As shown by the arrow in Fig. 4B (panel d) is a cell expressing only GFP Rac1V12 showing a collapsed vimentin. These data show that, although an overall F-actin depolymerization did not affect vimentin organization, inhibition of Cdc42Hs- and Rac1-dependent F-actin modification impaired Cdc42Hs and Rac1-induced vimentin redistribution.

Tyrosine Phosphorylation Inhibition Prevents Vimentin IF Collapse Induced by Cdc42Hs and Rac1—Phosphorylation has been shown to be a major regulatory pathway coordinating intracellular organization of the IF network, so we used various drugs having known kinase inhibitory activity to address the involvement of phosphorylation events in vimentin IF collapse in Cdc42Hs- or Rac1-expressing cells (Table I). First, we analyzed the effects of inhibiting protein kinase A activity, a protein that induced both the collapse of vimentin IF and F-actin reorganization (30) by two ways: microinjection of the RII regulatory subunit of protein kinase A with active Cdc42Hs (panels a) or Rac1 (panel a) as well as by cytochalasin D treatment of Cdc42 and Rac1-expressing cells, and tyrosine-phosphorylated vimentin was analyzed by immunoblotting with the 4G10 anti-phosphotyrosine antibody (Fig. 6B). Although in control cells a high steady-state level of tyrosine-phosphorylated proteins was observed, three additional tyrosine-phosphorylated proteins were detected in cells extracts from Cdc42Hs- and Rac1-expressing cells (marked with arrows). These data show that genistein-dependent tyrosine kinase inhibition impaired the vimentin collapse induced by Cdc42Hs and Rac1.

Increase of Tyrosine Phosphorylation after Cdc42Hs and Rac1 Expression—To further study whether Cdc42Hs or Rac1 expression leads to increased tyrosine phosphorylation, GFP-Cdc42HsV12- or GFP-Rac1V12-expressing cells were stained with 4G10 anti-phosphotyrosine antibody (Fig. 6A). As expected, focal adhesions are modified on focal contacts by Cdc42Hs and Rac1 expression. In addition, Cdc42HsV12-expressing cells (panel a) and Rac1V12-expressing cells (panel e) showed increased phosphotyrosine staining compared with nonexpressing cells (panels b and d). This was observed in 50% of Cdc42HsV12-expressing cells and in 40% of Rac1V12-expressing cells. Additionally, cell extracts from control cells, MOCK-transfected cells, or Cdc42HsV12- or Rac1V12-expressing cells were separated by gel electrophoresis, transferred to nitrocellulose, and immunoblotted with 4G10 anti-phosphotyrosine antibody (Fig. 6B). Although in control cells a high steady-state level of tyrosine-phosphorylated proteins was observed, three additional tyrosine-phosphorylated proteins were detected in cells extracts from Cdc42Hs- and Rac1-expressing cells (marked with arrows).

We finally analyzed whether vimentin might be directly phosphorylated in Cdc42Hs- and Rac1V12-expressing cells. Vimentin was immunoprecipitated from control untransfected (microinjected with the RII regulatory subunit of protein kinase A or treated with $R_{ii}$, cAMP, LY-294002, wortmannin, staurosporin, or genistein for 15–120 min. Cells were fixed and analyzed for vimentin IF. Results from three independent series of experiments are shown.

### Table I

| GTPase  | Treatment | Biological effect | Vimentin IF collapse |
|---------|-----------|-------------------|----------------------|
| Cdc42Hs | Rp-cAMP   | A-kinase inhibition | Yes                  |
| Rac1    | Rp-cAMP   | A-kinase inhibition | Yes                  |
| Cdc42HsV12 | LY294002 | PI3 inhibition     | Yes                  |
| Rac1V12 | Staurosporin | Inhibition of S/T kinases | Yes |
| Cdc42HsV12+ | Genistein | Inhibition of tyrosine kinases | No or less |

FIG. 5. Tyrosine kinase inhibition decreases vimentin IF collapse by Cdc42Hs and Rac1. REF-52 fibroblasts were transfected with GFP-tagged Cdc42Hs (panels a–c) and Rac1V12 (panels d–f). After 18 h, cells were treated with genistein for 15–120 min, fixed, and stained for vimentin (panels b and e) and F-actin (panels c and f) distribution. For each panel, cells shown are representative of three independent experiments with more than 50 observed cells. Bar, 10 μm.
phosphotyrosine antibody (panels b). After 18-h transfection, cells were fixed and stained with 4G10 anti-phosphotyrosine antibody (panels b and c). GFP-Cdc42HsV12- or GFP-Rac1V12-expressing cells were separated by gel electrophoresis, transferred to nitrocellulose, and immunoblotted with 4G10 anti-phosphotyrosine antibody. Three proteins with increased tyrosine phosphorylation level are indicated (arrows). C, vimentin was immunoprecipitated from control untransfected REF-52 cells, empty pEGFPN1 vector transfected cells (MOCK), or GFP-Cdc42HsV12- or GFP-Rac1V12-expressing cells were separated by gel electrophoresis, transferred to nitrocellulose, and immunoblotted with 4G10 anti-phosphotyrosine antibody. The arrow indicates the position of vimentin.

vimentin phosphorylation was detectable after Cdc42Hs or Rac1 expression.

Taken together these data show that, although increased tyrosine phosphorylation was observed after Cdc42Hs and Rac1 expression, no direct modification in vimentin phosphorylation was detected.

**DISCUSSION**

In this study, we showed that expression of constitutively active Cdc42Hs and Rac1 led to reorganization of the vimentin IF network. Activation of endogenous Cdc42Hs and Rac1 by bradykinin or PDGF/EGF treatment, respectively, or active RhoG expression, which activates both Cdc42Hs and Rac1 (13), also induced vimentin IF collapse. No modification on vimentin solubility was detected. By using effector loop mutants of Cdc42Hs and Rac1 we found that mainly the Y40C mutants led to the vimentin IF collapse. Cdc42Hs and Rac1 expression induced an increased tyrosine phosphorylation and addition of tyrosine kinase inhibitors led to a strong diminution of Cdc42Hs- and Rac1-induced vimentin IF collapse. Thus we suggest that Cdc42Hs and Rac1 expression leads to specific tyrosine kinase activation which in turn induces vimentin IF collapse.

Work during the past decade has established important roles of Rho GTPases in regulating the organization of the actin cytoskeleton. Using mammalian fibroblasts as model systems but also leukocytes and neuronal cells, Cdc42Hs and Rac1 have been shown to trigger the formation of filopodia and lamellipodia, respectively, whereas RhoA triggers the assembly of focal contacts and stress fibers (4). In this study we showed that Cdc42Hs and Rac1 elicit also the modification of another major cytoskeleton component, the vimentin IF network, which instead of being well-spread and distributed from the perinuclear region to the cell periphery becomes collapsed all around the nucleus. This collapse has been observed under three different experimental conditions: 1) expression of constitutive active Cdc42Hs and Rac1, 2) activation of endogenous Cdc42Hs by bradykinin (12) or Rac1 by PDGF/EGF (10), and 3) expression of active RhoG, which activates both Cdc42Hs and Rac1 (13). It has previously been reported that PDGF treatment of porcine aortic endothelial cells provokes a marked vimentin IF collapse as does expression of constitutively active Rac1 (29). Constitutively active RhoAV14-injected cells also show a collapsed IF network into irregular thick bundles within the cytoplasm (32), which differs from the Cdc42Hs- and Rac1-dependent IF collapse described above. Interestingly, blocking the RhoA signaling pathway had no effect on Cdc42Hs- and Rac1-induced vimentin collapse, suggesting that RhoA and Cdc42Hs/Rac1 act through independent pathways (data not shown).

Because the three components of the cytoskeleton, namely F-actin microfilaments, microtubules, and intermediate filaments, have been shown to be connected physically (23), the reorganization of the vimentin IF after Cdc42Hs or Rac1 expression could result from a deregulated interaction between IF and the actin cytoskeleton, because both Cdc42Hs and Rac1 induce local and peripheral actin polymerization (ruffles/lamellipodia and filopodia, respectively) and a reduction in stress fibers. This is consistent with our data showing that mainly the Y40C Cdc42Hs and Rac1 mutants, which are still able to modify the actin cytoskeleton, elicit the vimentin collapse. Microfilament disassembly upon cytochalasin D treatment or Scar-WA expression did not affect the IF network, suggesting that the IF collapse is unlikely to result from the disassembly of the stress fibers. We are unable to test only the consequence of local cortical actin polymerization on vimentin IF organization. However, in the absence of cortical F-actin polymerization upon cytochalasin D treatment or Scar-WA expression, Cdc42Hs and Rac1 do not affect vimentin reorganization, again suggesting a close correlation between actin and IF cytoskeleton.

A recent study of fibroblasts expressing a chimeric GFP-vimentin reveals that both the typical IF ends and short filamentous structures termed “vimentin squiggles” are frequently detected at the edge of the cell according to a pattern similar to focal adhesions (41). Because the expression of activated Cdc42Hs or Rac1 proteins leads to a redistribution of focal adhesions into focal contacts (4), this might therefore modify the well-spread vimentin IF distribution. Interestingly, the use of GFP-tagged vimentin showed that the collapse of IF bundles did not necessarily involve reassembly near the nucleus but rather the network being pushed back into the perinuclear region (42).

If organization has been reported to be mainly regulated by phosphorylation (25). We have thus examined whether...
Cdc42Hs- and Rac1-dependent IF vimentin collapse might be mediated by protein kinase activation. We show that, although inhibition of PKA, PI3K, and staurosporine-serine/threonine kinases did not impair vimentin collapse, a genistein-sensitive protein-tyrosine kinase is involved in the pathway leading to this IF reorganization. Among the Cdc42Hs and Rac1 effectors described so far, only one protein-tyrosine kinase family has been described (43). These Cdc42Hs-associated kinases (ACK1 and -2) might only account for Cdc42Hs-induced vimentin reorganization, because Rac1 does not bind to these proteins. Whatever the protein-tyrosine kinase activated by Cdc42Hs and Rac1, this protein is not responsible for a direct vimentin phosphorylation. In this respect, the Cdc42Hs- and Rac1-dependent vimentin reorganization again differs from the RhoA-dependent collapse, because ROK or protein kinase N (PKN), two RhoA effectors, directly phosphorylate vimentin (26, 28, 44). In addition, Rho kinase inhibition did not modify Cdc42Hs- and Rac1-dependent vimentin collapse, discounting any Rho kinase involvement in this process (data not shown). Various targets for such protein-tyrosine kinases might be proposed, such as IF-associated proteins (45, 46) or several components of focal adhesions, including vinculin, talin, tensin, and pakllin. Tyrosine kinases such as pp125FAK or p60v-src have been found associated with focal adhesions (47).

Although the absence of a clear effect of vimentin knockout mice does not help in the understanding of vimentin IF function (48), one can propose that Cdc/Rac-induced vimentin collapse contributes to the mechanisms of cell movement. Indeed, the pseudopod of crawling cells is in general devoid of filamentous IF (48), and Cdc42Hs and Rac1 GTPase are known to induce cell motility (4). An attractive function for this collapse contributes to the mechanisms of cell movement. Indeed, whatever the protein-tyrosine kinase activated by Cdc42Hs, targets for such protein-tyrosine kinases might be proposed, mediated by protein kinase activation. We show that, although inhibition of PKA, PI3K, and staurosporine-serine/threonine kinases did not impair vimentin collapse, a genistein-sensitive protein-tyrosine kinase is involved in the pathway leading to this IF reorganization.
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