APC binds intermediate filaments and is required for their reorganization during cell migration

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Intermediate filaments (IFs) are components of the cytoskeleton involved in most cellular functions, including cell migration. Primary astrocytes mainly express glial fibrillary acidic protein, vimentin, and nestin, which are essential for migration. In a wound-induced migration assay, IFs reorganized to form a polarized network that was coextensive with microtubules in cell protrusions. We found that the tumor suppressor adenomatous polyposis coli (APC) was required for microtubule interaction with IFs and for microtubule-dependent rearrangements of IFs during astrocyte migration. We also show that loss or truncation of APC correlated with the disorganization of the IF network in glioma and carcinoma cells. In migrating astrocytes, vimentin-associated APC colocalized with microtubules. APC directly bound polymerized vimentin via its armadillo repeats. This binding domain promoted vimentin polymerization in vitro and contributed to the elongation of IFs along microtubules. These results point to APC as a crucial regulator of IF organization and confirm its fundamental role in the coordinated regulation of cytoskeletons.

Introduction

The cytoskeleton, composed of actin microfilaments, microtubules, and intermediate filaments (IFs), is a fundamental element of eukaryotic cells. The regulation of microfilaments and microtubules has received a great deal of attention (Bugyi and Carlier, 2010; Etienne-Manneville, 2010; Lee and Dominguez, 2010). In contrast, the regulatory mechanisms of cytoplasmic IF rearrangements are still poorly characterized. Vimentin IF organization has been shown to depend mainly on microtubules (Goldman, 1971; Prahlad et al., 1998). Depolymerization of microtubules leads to a retraction of IFs close to the nucleus. This retraction requires actin dynamics and is likely to be caused by the retrograde flow of actin filaments emerging from motile cell edges (Bershadsky et al., 1991; Dupin et al., 2011). Several proteins have been involved in the connection between IFs and microtubules. Plectin, for instance, contains interaction domains for both IFs and microtubules (Wiche et al., 1982; Herrmann and Wiche, 1987). Moreover, microtubule-associated motors promote the transport of IFs along microtubules in fibroblasts and neuronal cells and contribute to axon elongation (Liao and Gundersen, 1998; Yabe et al., 1999; Helfand et al., 2002, 2003).

Astrocytes express the astrocyte-specific glial fibrillary acidic protein (GFAP), vimentin, nestin, and in some circumstances, synemin (Sultana et al., 2000). The expression levels of these proteins vary during astrocyte differentiation, astrogliosis, and also in astroglomas, suggesting that IFs may contribute to astrocyte motility (Dahlstrand et al., 1992; Ehrmann et al., 2005; Jing et al., 2005). In fact, GFAP and vimentin knockout mice have revealed that these IF proteins are essential for astrocyte motility both in vivo and in vitro (Lepekhin et al., 2001). Here, we use an in vitro wound-healing assay to characterize IF rearrangements during astrocyte migration and determine the role of microtubules and associated proteins in these events.

Adenomatous polyposis coli (APC) is a tumor suppressor regulating cell differentiation via the Wnt pathway (Näthke, 2004; Segditsas and Tomlinson, 2006) and cell polarity and motility in several cell types, including astrocytes (Etienne-Manneville et al., 2005; Barth et al., 2008). APC contributes to cell migration through the regulation of the actin and microtubule cytoskeletons (Etienne-Manneville, 2009). Although two APC isoforms...
Figure 1. APC is required for IF rearrangements during cell migration. (A, B, and C) Expression and localization of IFs. Primary rat astrocytes were cultured and submitted to a wound-healing assay. Cells were lysed and analyzed by immunoblotting (A) or fixed and stained with the indicated antibodies (B and C). The large view of the wounded monolayer (C, left) has been reconstituted from adjacent images. (D) Migrating astrocytes were fixed and stained with phalloidin for F-actin or anti–α-tubulin for microtubules and antivimentin. A zoom of the boxed regions is shown on the right images.
exist (APC and APC2), only APC is expressed in astrocytes (Cahoy et al., 2008; Shintani et al., 2012). We show here that, in addition to its connection with microtubules and microfilaments (Watanabe et al., 2004), APC directly interacts with IFs and controls their organization during cell migration.

**Results and discussion**

**APC is required for IF rearrangements during astrocyte migration**

Rat primary astrocytes express GFAP, vimentin, and nestin (Fig. 1 A; Yang et al., 2010) in a dense filamentous network in which these three proteins were indistinguishable by immunofluorescence (Fig. 1 B). In confluent, nonmigrating astrocytes, IFs mainly accumulated around the nucleus (Fig. 1 C). During wound-induced migration, IFs reorganized along the polarized microtubule network and extended in the forming protrusion (Fig. 1, C and F). IFs aligned along microtubules, whereas they did not seem to follow actin fibers (Fig. 1 D). Astrocyte treatment with the microtubule-depolymerizing drug nocodazole disorganized the IF network that retracted around the nucleus (Fig. 1 F), confirming the crucial role of microtubules in the regulation of IFs (Goldman, 1971; Prahlad et al., 1998). This prompted us to search for a microtubule-associated protein that may be involved in IF reorganization during migration.

Prime candidates were microtubule-associated motor proteins that bind vimentin and neurofilaments and steer the bidirectional transport of IFs on microtubules (Liao and Gundersen, 1998; Helfand et al., 2002; Wagner et al., 2004). However, treatment with the kinesin inhibitor aurintricarboxylic acid (ATA) or siRNA-induced depletion of dynein heavy chain (DHC) had no obvious effect on IF organization (Fig. S1, A and B). These results indicate that none of these proteins is absolutely required for IF rearrangements during astrocyte migration, although they may contribute to a small extent, and suggest that the relative contribution of IF-binding proteins may depend on the set of IF proteins expressed in a given cell type as well as on the cell responses to external stimuli.

Other candidates are APC and EB1 (end binding 1), which associate with microtubules and control their organization in migrating astrocytes (Etienne-Manneville and Hall, 2003; Etienne-Manneville et al., 2005). EB1, a protein associated with the plus ends of growing microtubules, regulates microtubule assembly (Etienne-Manneville, 2010). EB1 depletion by siRNA (Fig. 1 E) slightly altered microtubule organization at the leading edge (Fig. 1 F) as expected (Etienne-Manneville et al., 2005) but did not significantly perturb IFs, which still aligned with microtubules and filled the protrusion (Fig. 1 F). In contrast, APC knockdown by siRNA had a strong effect on IFs. In APC-depleted cells, the expression levels of tubulin, GFAP, vimentin, and nestin were unchanged (Fig. 1 E), but IFs separated from microtubules and retracted near the nucleus, as in nocodazole-treated cells (Fig. 1 F). This retraction was also observed using both GFAP and vimentin staining and was also confirmed by the use of another siRNA sequence directed against APC (Fig. S1, C and D; Etienne-Manneville et al., 2005).

APC form clusters at the plus ends of leading edge microtubules in an EB1-dependent manner to control microtubule anchoring and astrocyte polarization (Etienne-Manneville and Hall, 2003; Etienne-Manneville et al., 2005). EB1 depletion or expression of the EB1 carboxy-terminal domain, which prevents the interaction of APC, other EBs, and p150Glued with microtubule plus ends (Su et al., 1995), did not affect IF polarized organization (Fig. 1 F and Fig. S3 A). Collectively, our results show that APC controls the polarized rearrangement of IFs during astrocyte migration. They also strongly suggest that this new role of APC is independent of APC functions at microtubule plus ends.

**Loss or truncation of APC dramatically affects the IF network in cancer cells**

Loss of APC or mutations of APC are frequently found in cancers such as colon cancers and brain tumors (Qualman et al., 2003; Pecina-Slaus et al., 2006; Segditsas and Tomlinson, 2006), leading us to investigate the organization of the IF network in cancer cells. In the U138 glioblastoma cell line, expression of full-length APC was barely detectable, IF spreading was strongly altered, and the IF network that remained was retracted and encircled the nucleus (Fig. 2, B and C).

We also studied IF organization in two adenocarcinoma cell lines, which express keratins and vimentin. HCT116 cells express full-length APC, and SW480 cells express a truncated APC (Rowan et al., 2000) as confirmed by Western blotting with two different APC antibodies directed against the amino-terminal or the carboxy-terminal part of APC (Fig. 2, A and D). APC truncation associated with a retraction of the vimentin network in SW480 cells, whereas in HCT116 cells expressing full-length APC, the vimentin network spread throughout the cytoplasm (Fig. 2 E). In contrast, the keratin network was well spread in both cell types (Fig. 2 E). We conclude that loss or truncation of APC is associated with a disorganization of the vimentin network but does not affect the organization of keratin filaments. This result suggests that loss of APC may contribute to the abnormal organization of the vimentin network in epithelial cancer cells that have undergone epithelial mesenchymal transition.

**APC colocalizes and interacts with IFs**

In migrating astrocytes, IFs did not extend up to the leading edge and did not seem to interact with EB1-dependent APC clusters at microtubule plus ends (Fig. 3 A and Fig. S2, A–C).

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(E) Astrocytes were nucleofected with the indicated siRNAs. 3 d later, cell lysates were analyzed by immunoblotting using the indicated antibodies. The histogram on the right shows the means ± SD of the relative protein levels in APC-depleted cells compared with control cells from three independent experiments. (F) Astrocytes were nucleofected with the indicated siRNA and cultured for 3 d or treated with 20 µM nocodazole (1 h) before wounding. 6 h after wounding, cells were fixed and stained with the indicated antibodies. A zoom of the boxed regions is shown on the right images. The dotted lines show the direction of the wound. Images are representative of at least three independent experiments. IB, immunoblot. Bars: (B, D, and F) 10 µm; (C) 20 µm.
Although much less concentrated than at the plus ends, APC was also visible along microtubules (Fig. 3A), where it colocalized with IFs (Fig. 3A and Fig. S2, A–C).

We used biochemical approaches to study the interaction between APC and IFs. Coimmunoprecipitation confirmed that APC interacts with vimentin, GFAP, and nestin in astrocytes (Fig. 3B). Although a proteomic approach recently showed that APC coprecipitates with a subset of keratins, keratin 81 and keratin 82 (Wang et al., 2009), experiments performed in SW480 showed that vimentin, but not keratin, interacted with APC (Fig. 3C). Our result may explain why, in contrast to the vimentin network, the keratin network is not affected by APC truncation (Fig. 2E) or microtubule depolymerization (Herrmann and Aebi, 2000).

We then performed a cosedimentation assay with purified recombinant IF proteins. In vitro polymerization of vimentin and GFAP was induced by the addition of salt (150 mM NaCl; Zackroff and Goldman, 1979). Polymerized IFs were incubated with ice-cold cell lysates and sedimented by centrifugation. Under these conditions, Plectin, a well-known partner of IFs (Wiche, 1998), was found in the pellet fraction, whereas tubulin, EB1, and actin were not (Fig. 3D). In astrocytes, 18.5% of total endogenous APC cosedimented with vimentin (Fig. 3D), showing that, in vitro, APC association with IFs does not involve other cytoskeletal elements.

APC is a multidomain protein bearing an amino-terminal oligomerization domain followed by armadillo repeats, a central region composed of amino acid repeats that are important for Wnt signaling, and a carboxy-terminal region, which itself includes several protein interaction domains (Fig. 3E; Etienne-Manneville, 2009). To map the potential IF-binding domain of APC, HEK293 cell lysates expressing various APC fragments (amino-terminal part [nAPC], medium part [mAPC], or the carboxy-terminal part [cAPC]) were subjected to the cosedimentation assay. nAPC, but not mAPC or cAPC, sedimented with polymerized vimentin (Fig. 3F), suggesting that the amino-terminal region of APC was solely involved in APC interaction with IFs. In the absence of vimentin, nAPC was not detected in the pellet fraction, indicating that it cannot sediment on its own. The interaction of nAPC with IF is also consistent with the result in Fig. 3C showing the interaction between vimentin and a truncated form of APC corresponding to the amino-terminal half of the protein (Fig. 2A).

The APC armadillo domains directly bind IFs

We then examined whether APC binding to IFs was direct. Purified recombinant GST-APC fragments, corresponding anti-APC antibody. The black line indicates that intervening lanes have been spliced out. [C] Cells were plated on coverslips. After 1 h, cells were fixed and stained with the antivimentin antibody. Quantification of the area covered by the vimentin network in astrocytes and U138 cells was plotted as a function of time. Data are given as means ± SD of three independent experiments. [E] HCT116 cells or SW480 cells were fixed and stained with anti-vimentin or anti-pankeratin antibodies. Images are representative of at least three independent experiments. Dotted lines show cell contours obtained from α-tubulin staining (not depicted). IB, immunoblot; mut, mutant; wt, wild type. Bars, 10 µm.
to three different regions of the amino-terminal portion of APC (Fig. 4 A), were subjected to the cosedimentation assay with polymerized vimentin. APCn2, but not APCn1 or APCn3, directly bound to polymerized vimentin (Fig. 4 B). The specificity of the interaction was confirmed using the armadillo repeats of p120catenin, which did not cosediment with vimentin (Fig. S2 D). The binding of APCn2 to GFAP was reproducibly detectable but was weaker than the binding of APCn2 to vimentin (Fig. S2 E). The interaction of the APC armadillo repeats with vimentin was also confirmed by coprecipitation in HEK293 cells (Fig. 4 C). In the same conditions, the APCn3 fragment was not coprecipitated with vimentin. We tested whether truncation suppressing one to six of the seven armadillo repeats affected vimentin binding in cosedimentation and coimmunoprecipitation assays. Deletion of one carboxy-terminal or the amino-terminal armadillo repeat was sufficient to totally prevent the interaction of APC armadillo repeats with vimentin (Fig. S2, F and G). We conclude that APC directly binds, via its armadillo repeats (APCn2), to polymerized vimentin and that the affinity of APC for IFs varies with the IF composition.

We then asked whether APC binding could affect IF assembly. In vitro vimentin polymerization was monitored by absorbance at 320 nm after addition of NaCl to vimentin (Fig. 4 D; Zackroff and Goldman, 1979). Addition of the GST-APCn2 fragment induced a dose-dependent increase of vimentin assembly. In contrast, GST alone or GST-APCn1 and GST-APCn3 had no effect. Immunofluorescence analysis of the polymerized filaments confirmed that in the presence of APCn2, polymers were significantly more abundant (Fig. 4 E). These results confirm that APC armadillo repeats interact with vimentin. They also suggest that APC, like Nudel with neurofilaments (Nguyen et al., 2004), may regulate vimentin polymerization and may thereby promote elongation of IF along microtubules.

**APC acts as a major molecular bridge between IFs and microtubules**

To substantiate a direct interaction of APC with IFs in vivo, we used the recently developed proximity ligation assay (DuoLink; Olink Bioscience). Based on the detection of a coupling between secondary antibodies bound to target proteins, this technique allows visualization and localization of individual protein–protein interactions. Fluorescent dots indicating the localization of interacting vimentin and APC were clearly detectable in the APC + vimentin staining (Fig. 5 A). This fluorescent signal was more than three times stronger in migrating cells than in nonmigrating cells, suggesting that APC interaction with IF is increased during cell migration (Fig. 5 A, right). N-cadherin + vimentin staining, used as a negative control, produced no visible signal (Fig. 5 A). In addition, siRNA-induced knockdown of APC strongly reduced the fluorescence signal, demonstrating the specificity of the staining (Fig. 5 A, right). Altogether, these data confirm that the APC–vimentin direct interaction observed in the cosedimentation assay also occurs in astrocytes and is increased during cell migration. APC colocalized with vimentin at a discrete position of the network, suggesting that APC binding to IF may be regulated by local factors. Most of the APC–vimentin complexes localized along microtubules, indicating that APC may simultaneously interact with IFs and microtubules and that microtubule association with APC may favor its binding to IFs (Fig. 5 B).

We thus performed cosedimentation experiments at RT to prevent cold-induced depolymerization of microtubules. In these conditions, microtubules cosedimented with IFs (Fig. 5 C). At RT, like at 4°C, full-length APC cosedimented with IFs (Fig. 3 D and Fig. 5 C). When cosedimentation was performed from APC-depleted cell lysates, the sedimentation of tubulin was considerably reduced (Fig. 5 C), demonstrating that APC is a major mediator of the IF–microtubule interaction in migrating astrocytes.

We first asked whether APC may indirectly affect IF organization by regulating gene transcription via Wnt signaling. We thus expressed a nonphosphorylatable mutant of β-catenin in astrocytes, which accumulated in the cell nucleus. Expression of S37A β-catenin did not perturb IF elongation in the protrusion of migrating astrocytes (Fig. S3 A). The mechanism by which APC couples IFs to microtubules likely relies on the interaction of APC armadillo repeats with IFs (Fig. 4). In agreement with this hypothesis, expression of APC constructs containing the armadillo repeats (nAPC, APCn2, and APCn12) induced the retraction of IFs that appeared uncoupled from microtubules. Expression of GFP, mAPC, and APCn3 had no effect on IF organization (Fig. 5 D). The amino-terminal part of APC does not interfere with microtubule polymerization during astrocyte migration (Etienne-Manneville and Hall, 2003), and none of the amino-terminal constructs noticeably affected microtubule stability (Fig. S3, B and C). These results show that APC armadillo repeats, which directly interact with IFs, are involved IF rearrangements during astrocyte migration. To investigate whether APC-mediated regulation of IFs was important for cell polarization and migration, we analyzed the polarization and migration of wound edge astrocytes microinjected with the aforementioned APC constructs. Loss of IF polymerized organization induced by expression of nAPC and APCn2 correlated with a strong inhibition of astrocyte migration but not of centrosome reorientation (Fig. 5, E and F). Inhibition of astrocyte migration was similarly observed when cells were transfected with a GFAP carboxy-terminal deletion mutant (GFAP dn; Fig. 5 E), which, like a vimentin carboxy-terminal deletion mutant (Whipple et al., 2008), totally disrupts the IF network. These results suggest that APC-dependent reorganization of IFs is required for cell migration but not for cell polarization. The role of IFs in astrocyte migration may reflect the role of IFs in the overall cell architecture or in vesicle trafficking (Potokar et al., 2010).

The effects of APC constructs on IF organization were also confirmed in HeLa cells, which express vimentin and nestin but not GFAP (Fig. 5 G). After cell spreading on the coverslip, the vimentin network covered >80% of the surface of HeLa cells. IF spreading was inhibited by overexpression of nAPC and APCn2 but not APCn3 (Fig. 5 G).

We conclude that APC is a key regulator of vimentin organization in several cell types, including astrocytes, HeLa cells, and transformed epithelial cells. Together with its functions...
Figure 3. **APC colocalizes and interacts with IFs.** (A) Immunofluorescence images showing vimentin, APC, and α-tubulin (microtubule) costaining in the protrusion of migrating astrocytes. Higher magnifications of the boxed area are shown on the right. APC is visible along microtubules (arrowheads). Arrows indicate the colocalization of vimentin and APC on microtubules. The graphs show intensity profiles of vimentin, APC, and tubulin stainings along the dotted lines (i) and (ii). Data are representative from three independent experiments. Bars, 10 µm. (B) Immunoprecipitations (IP) were performed with anti-APC (APC) or control rabbit IgG (IgG) antibodies using astrocyte lysates and were analyzed by immunoblotting using the indicated antibodies.
in the regulation of actin and microtubule dynamics (Etienne-Manneville, 2009; Okada et al., 2010), this novel role of APC in the regulation of IFs should provide a better understanding of how cellular cytoskeletons are coordinated.

**Materials and methods**

**Materials**

We used the following reagents: anti–α-tubulin YL1/2 (AbD Serotec); anti–GFP-HRP ab6663 (obtained from Abcam); anti-EB1 #610534 and anti–mouse IgG (IgG) antibodies using SW480 cell lysates and were analyzed by immunoblotting with the indicated antibodies. (D and E) In vitro polymerization of vimentin in the presence or absence of various APC fragments. (D) The absorbance of the solution during vimentin polymerization was monitored at 320 nm. Data are representative of three independent experiments. (E) Assembled filaments obtained after 5 min of polymerization were stained with the anti-His antibody. Bars, 20 µm. The quantification of the fluorescence after washing of the soluble proteins is shown on the right. Data (n = 3) are given as means ± SD. *, P < 0.05. IB, immunoblot; IP, immunoprecipitation.
Figure 5. APC interacts with IFs and microtubules to control the coupling between these two cytoskeletal components. (A) Migrating astrocytes were fixed, and direct interaction between APC and vimentin was analyzed with the DuoLink technique using anti-APC and antivimentin antibodies. The same experiment with N-cadherin and vimentin is shown as a negative control. Direct interactions appear in red on the image. Nuclei are shown in blue. The histogram shows the relative fluorescence intensity of the DuoLink staining obtained with anti-APC and antivimentin antibodies in migrating and nonmigrating cells that have been nucleofected with the indicated siRNA. (B) After DuoLink staining using APC and vimentin antibodies, cells were further stained with the anti-α-tubulin antibody (microtubules). Images are representative of at least three independent experiments. Bars, 0.1 µm. (left) 16 h after injection, cells were fixed and stained with the indicated antibodies. (right) The percentage of cells with an elongated IF network that filled the protrusion together with microtubules was determined. (E) Wound edge astrocytes were microinjected with the indicated constructs. (left) After 16 h, cells were fixed and stained with the indicated antibodies. (right) The percentage of cell area covered by vimentin was determined. Data are given as means ± SD of three independent experiments, with a total of ≥100 cells. (G) HeLa cells were transfected with the indicated constructs. (left) After 16 h, cells were fixed and stained with the indicated antibodies. (right) The percentage of cell area covered by vimentin was determined. Data are given as means ± SD of three independent experiments, with a total of ≥100 cells. *, P < 0.05; **, P < 0.01; ***, P < 0.005. Higher magnifications of the boxed area in the images are shown on the right.

anti-N-cadherin #610920 (obtained from BD); anti-Pericentrin PRB-432C (obtained from Covance); anti-APC c-20, anti-HA Y11, antivimentin C-20, anti-GFAP C-19, anti-GST B-14, and anti-His SC-803 (all obtained from Santa Cruz Biotechnology, Inc.); anti-α-tubulin AC-40, anti-GFAP G9269, antivimentin V6630, anti-FLAG F7425, anti-β-tubulin T4026, antiacetylated tubulin T6793, anti-Plectin P9318, and anti-Myc M4439 (all obtained from Sigma-Aldrich); anti-APC Ab-1 (EMD Millipore); antinestin Rat-401 (EMD Millipore); antipan-Keratin C11 (Cell Signaling Technology); anti-HA 3F10 and anti-GFP clones 7.1 and 13.1 (Roche); goat anti–human IgG Cy2, Cy5, and TRITC secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.); anti-mAPC (I. Nathke, University of Dundee, Dundee, Scotland, UK); nocodazole and taxol (Sigma-Aldrich); and trichostatin A (Merck).
All siRNA duplexes were obtained from Privalgo (Table S1). siRNA constructs were introduced into rat astrocytes by nucleofection technology (Amasia Biosystems; Etienne-Manneville, 2006). Duolink was used according to the vendor’s instructions. The transfections of HEK293 and Hela cells were performed with the calcium phosphate method.

**Plasmids**

We used the following plasmids: pEFGP-C1-APC, pEFGP-C3-nAPC, pEGFP-C3-nAPC, and pEGFP-C3-APC (I. Nafthke); pEGFP-C1-EB1 axon terminal (185–268 aa; R.D. Vale, University of California, San Francisco, San Francisco, CA); pEGFP-N3-GFAP and pEGFP-N3-vimentin (D. Pham-Dinh, Institut National de la Santé et de la Recherche Médicale, Paris, France); and pMT23–β-catenin S37A (R. Kypri, Imperial College London, London, England, UK). Vimentin, GFAP, and GFAP dn (1–198 aa) were generated by PCR and cloned into pMW-His or pFLAG-CMV5. nAPC (1–1,018 aa), mAPC (1,014–2,039 aa), cAPC (2,038–2,884 aa), APCn1 (1–334 aa), APCn2 (334–740 aa), APCn3 (740–1,018 aa), APCn12 (1–740 aa), APC armadillo 1–6 (334–694 aa), APC armadillo 2–7 (453–740 aa), APC armadillo 3–7 (506–740 aa), and p120catenin armadillo (363–830 aa) were generated by PCR and cloned into pCMV-HA, pCMV-GFP, pGEX-4T, or pGEX-4T-His6. The GST-APCn3-His6 construct was generated by inserting a His tag in the carboxy-terminal part of the APCn3 fragment cloned in pGEX-4T1. Mouse p120 armadillo repeats (368–825 aa) were subcloned in pCMV-GFP.

**Protein purification**

Vimentin and GFAP were produced in Escherichia coli strain BL21 transformed with pMW-His-GFAP and vimentin, respectively. The purification procedure was previously described (Herrmann et al., 2004). GST–APCn1, -APCn2, -APCn3, and -APCn3-His6 were produced in BL21 transformed with pGEX-4T1-APCn1, APCn2, APCn3, and pGEX-4T1-APCn3-His6, respectively. Proteins were affinity purified with glutathione–Sepharose 4B (GE Healthcare) and Nitrilotriacetic acid agarose (Invitrogen) and then dialyzed with buffer A (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 0.1 mM EGTA, pH 8.0, and 1 mM DTT). Additional His tagging in the carboxy-terminal part of the APCn3 fragment was required for efficient protein production and purification. The protein was purified using Ni-nitrilotriacetic acid agarose (Qiagen) and dialyzed.

**IF cosedimentation assay**

1 µg of purified IFs was centrifuged before use. Filament assembly was initiated by the addition of NaCl (final concentration of 150 mM). Preassembled filaments were incubated with cell lysates in buffer B [25 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0, 150 mM NaCl, 1 mM MgCl2, 0.5% Triton X-100, and protease inhibitor cocktail] at 4°C or RT. Assembled filaments were collected by centrifugation at 15,000 g for 10 min at 4°C or RT and extensively washed with buffer B. Samples were analyzed by immunoblotting. For direct binding assays, 30 µM IFs were incubated with 15 µM of purified GST, GST–APCn1, -APCn2, or -APCn3 in buffer C [10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 0.1 mM EGTA, pH 8.0, 1 mM DTT, 0.1% Triton X-100, and 150 mM NaCl] at RT. Reactants were then centrifuged at 15,000 g for 10 min at RT, and the pellets were extensively washed with buffer C. Samples were analyzed by immunoblotting.

**Immunoprecipitation**

Cells were washed with PBS and lysed with buffer B (see previous paragraph). Cell lysates were centrifuged at 15,000 g for 10 min at 4°C. The supernatant was incubated with antibody and protein G-Sepharose beads for 2 h at 4°C. Beads were extensively washed with buffer B and analyzed by immunoblotting.

**Vimentin assembly assay**

The vimentin assembly assay was described before (Herrmann et al., 2004). 9 µg vimentin was clarified by centrifugation before use. Filament assembly was initiated by the addition of 10X buffer C. Polymerization was monitored by a spectrophotometer (Ultrospec 2100 pro; GE Healthcare) at 320 nm. For visualization of assembled IFs, filaments were fixed with 0.5% glutaraldehyde on coverslips and stained with the anti-His antibody for fluorescence microscopy.

**Image acquisition and statistical analysis**

Fixed cells were imaged on a microscope (DM6000 B; Leica) using an HCX Plan Apochromat 40×/1.25 NA oil confocal scanning objective (Leica). Microscopes were equipped with a camera (DFC350FX; Leica), and images were collected with LAS software (Leica). Colocalization of IFs, APC, and microtubules was quantified using the ImageJ software [National Institutes of Health]. Statistical analysis was performed using a Student’s t test.

**IF organization in astrocytes.** The astrocyte monolayer was scratched, and front cells were immediately microinjected. Cells were fixed after 16 h and stained with antibulin and anti-vimentin (or GFAP or nestin) antibodies. Cells at the wound edge were scored as “cells with IF coaligned with microtubules” when the IF network was reorganized along with the polarized microtubule network and filled the protrusion.

**IF organization in Hela cells, SW480 cells, HCT116 cells, and U138 cells.** Vimentin spreading in Hela cells was quantified as the ratio of the vimentin spreading area and the total cell area. The vimentin spreading area and the total cell area were manually determined using images of vimentin staining and GFP, HA, or tubulin staining. Statistical analysis was performed using a Student’s t test.

**Centrosome orientation**

Centrosome reorientation in response to the scratch has already been characterized (Etienne-Manneville and Hall, 2001). This assay was performed 8 h after wounding, and only the migrating astrocytes of the wounded edge were quantified. Centrosomes were located in front of the nucleus, within the quadrant facing the wound, were scored as correctly oriented. A score of 25% represents the absolute minimum corresponding to random centrosome positioning.

**Online supplemental material**

Fig. S1 shows that the inhibition of IF reorganization is specifically caused by siRNA-mediated depletion of APC but not by the depletion of DHC1, p150Glued by siRNA, or by the kinase inhibitor AT1. Fig. S2 shows that microtubule-associated APC colocalizes with GFAP and nestin and that the armadillo repeats of APC, but not of those p120catenin, interact with vimentin and GFAP. Fig. S3 shows that the role of APC in IF organization does not involve β-catenin-mediated transcription or APC function at microtubule plus ends and is not correlated with APC-mediated regulation of microtubule stability. Table S1 shows the sequences of the various siRNA used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201206010/DC1. Additional data are available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201206010.dv.

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