Regulated Binding of Adenomatous Polyposis Coli Protein to Actin*

Regulated Binding of Adenomatous Polyposis Coli Protein to Actin*

Adenomatous polyposis coli (APC) protein is a large tumor suppressor that is truncated in most colorectal cancers. The carboxyl-terminal third of APC protein mediates direct interactions with microtubules and the microtubule plus-end tracking protein EB1. In addition, APC has been localized to actin-rich regions of cells, but the mechanism and functional significance of this localization has remained unclear. Here we show that purified carboxyl-terminal basic domain of human APC protein (APC-basic) bound directly to and bundled actin filaments and associated with actin stress fibers in microinjected cells. Actin filaments and microtubules competed for binding to APC-basic, but APC-basic also could cross-link actin filaments and microtubules at specific concentrations, suggesting a possible role in cytoskeletal cross-talk. APC interactions with actin in vitro were inhibited by its ligand EB1, and co-microinjection of EB1 prevented APC association with stress fibers. Point mutations in EB1 that disrupted APC binding relieved the inhibition in vitro and restored APC localization to stress fibers in vivo, demonstrating that EB1-APC regulation is direct. Because tumor formation and metastasis involve coordinated changes in the actin and microtubule cytoskeletons, this novel function for APC and its regulation by EB1 may have direct implications for understanding the molecular basis of tumor suppression.

APC3 is a large multidomain (310 kDa) protein implicated in colorectal cancer (1, 2). Mutations associated with tumorigenesis almost invariably delete the carboxyl terminus of APC, suggesting that key functions reside in this region of the protein. Whereas truncations of APC result in deregulated Wnt signaling and cell proliferation, the earliest detectable defect in the intestinal epithelium from Apc mutant mice is not increased cell proliferation but rather changes in cell architecture and cell migration (3, 4). This suggests that loss of cytoskeletal regulation by the carboxyl terminus of APC may represent a key step in tumor formation.

APC protein displays a well established localization at clusters of microtubule plus ends in cells (5–8). In addition, APC has been detected at actin-rich plasma membrane surfaces and intercellular junctions by both immunofluorescence in highly polarized cells in culture (9–11) and immunoelectron microscopy in mouse intestinal cells (12). APC also has been observed to associate with IQGAP at the actin-rich leading edge of migrating cells (13), and loss of Drosophila APC function disrupts formation of the transient actin furrows in the syncitial blastoderm (14). However, for a variety of reasons, APC association with the actin cytoskeleton has remained controversial. First, difficulties with antibody specificity have led to skepticism about non-microtubule-associated APC localization patterns (1, 8, 9). Second, molecular mechanisms connecting APC protein to the actin cytoskeleton have not been clearly defined, and no functional data have yet linked APC protein directly to the regulation of actin dynamics and/or organization.

In this study, we used purified proteins to demonstrate that APC protein directly binds to and bundles actin filaments and can physically cross-link actin filaments and microtubules in vitro. Consistent with these biochemical activities, we show that the same APC fragments localize to actin and microtubule structures in microinjected cells. In addition, these interactions are regulated by APC association with EB1, a microtubule plus-end tracking protein that binds to and partially co-localizes with APC in vivo (15, 16). Specifically, EB1 inhibited APC binding to actin filaments (F-actin) in vitro and association with actin structures in vivo. These data provide a mechanistic and functional explanation for previous observations of APC association with the actin cytoskeleton, further suggesting that APC protein may coordinate the functions of the actin and microtubule cytoskeletons in vivo.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—GST-APC-basic, GST-APC-C1, GST-APC-C, GST-EB1-C, and GST-EB1-C(KR) were expressed and purified as described previously (6). For expression of APC-C in yeast, DNA encoding human APC residues 2130–2843 was PCR-amplified and subcloned into the BamHI-NotI sites of a LRA3 2µ vector that contains an insertion of the GAL promoter and an amino-terminal His6 tag (17). The resulting plasmid (pBG680) was transformed into the Saccharomyces cerevisiae strain BJ2168 (18), and His6-APC-C was...
expressed, isolated onto nickel-nitrilotriacetic acid beads (Ni-NTA, Qiagen, Valencia, CA), and eluted as described. Eluted His$_6$-APC-C was further purified on a Superose-6 gel filtration column (GE Healthcare/Amersham Biosciences) equilibrated in IPD buffer (50 mM imidazole (pH 8.0), 1× PBS (20 mM sodium phosphate buffer, 150 mM NaCl, pH 7.4), 1 mM DTT). Peak fractions were aliquoted, snap-frozen in liquid N$_2$, and stored at −80 °C.

For bundling assays in the presence of microtubules, GST-APC-basic-His$_6$ and GST-APC-C-His$_6$ were generated. DNA encoding human APC residues 2134–2843 for APC-C and 2167–2674 for APC-basic was PCR-amplified with 3’-primer harboring His$_6$ tag and replaced the same fragment in pBG690 using PpuMI and HindIII sites for APC-C and in pBG693 using EcoRI-HindIII sites for APC-basic. The resulting plasmids (pBG812 for APC-C and pBG813 for APC-basic) were transformed into BL21pLysS, and protein expression was induced with 0.4 mM isopropyl-1-thio-D-galactopyranoside at 37 °C for 4 h. Both GST-APC-C-His$_6$ and GST-APC-basic-His$_6$ were purified first onto nickel-nitrilotriacetic acid beads, eluted with 100 mM imidazole, 0.6 M NaCl, 5% glycerol, pH 8.0, and dialyzed against 20 mM Tris-HCl, 100 mM NaCl, 5% glycerol, 0.5 mM DTT. Proteins were further purified onto glutathione-agarose beads (GE Healthcare/Amersham Biosciences) and eluted with 50 mM glutathione. Eluted proteins were concentrated with centricon-YM10 (Millipore, Bedford, MA), and the buffer was exchanged to 20 mM Tris-HCl, 100 mM NaCl, 5% glycerol, 0.2 mM DTT, pH 8.0, and snap-frozen in liquid N$_2$ to be stored at −80 °C.

Rabbit skeletal muscle actin was purified as described (19). Unlabeled tubulin was purified from bovine brain as described (20), and fluorescein isothiocyanate-labeled tubulin was obtained from Cytoskeleton (Denver, CO).

**Actin Filament Bundling Assays**—In all cases, actin filaments were manipulated using precut pipette tips to prevent filament shearing. Low speed centrifugation assays were performed as described (21). Briefly, 18 μl of preassembled F-actin and 12 μl of IPD buffer or proteins in IPD buffer were mixed and incubated for 10 min at room temperature. Reactions were centrifuged for 3 min at 16,000 × g, and the supernatant and pellet fractions were analyzed by SDS-PAGE and Coomassie staining.

For light microscopy (in Fig. 1D), 5 μl of preassembled 6 μM F-actin was mixed with 5 μl of IPD buffer or APC-C in IPD buffer. After incubation at room temperature for 10 min, 3 μl was removed and diluted in 15 μl of rhodamine-conjugated phalloidin (1.5 μM) and incubated for 5 min at room temperature. Aliquots were subsequently diluted 25-fold in fluorescence buffer containing 50 mM KCI, 1 mM MgCl$_2$, 100 mM DTT, 20 μg/ml catalase, 100 μg/ml glucose oxidase, 3 mg/ml glucose, 0.5% methylcellulose, 10 mM imidazole, pH 7.0, and 3 μl was added to a coverslip coated with poly-l-lysine for observation.

To image F-actin and microtubules cross-linked by APC-C (Fig. 5C), 2 μM preassembled F-actin was incubated with 0.1 μM APC-C and Taxol-stabilized microtubules (17% fluorescein isothiocyanate-labeled) in F-buffer (20 mM HEPES-KOH, 50 mM KCI, 2 mM MgCl$_2$, 0.2 mM CaCl$_2$, 1 mM EGTA, 0.7 mM ATP, and 0.2 mM DTT, pH 7.5) for 10 min at room temperature. F-actin was then decorated with Alexa568-conjugated phalloidin for 2 min, diluted 50-fold in fluorescence buffer, and applied to a nitrocellulose-coated coverslip for observation.

Fluorescent images were examined by fluorescence microscopy using a Zeiss Axioskop-2 mot plus microscope (Carl Zeiss, Thornwood, NY) equipped with a Hamamatsu C4742-95 charge-coupled device camera (Orca-ER, Hamamatsu Photonics, Bridgewater, NJ) running OpenLab software (Improvision, Lexington, MA).

For electron microscopy, 2 μM preassembled F-actin was incubated with 0.5 μM APC-C for 10 min at room temperature. 3 μl of this reaction was spotted onto a carbon film grid glow-discharged for 45 s in air with current of 20 mA. Samples were then negatively stained with 2% aqueous uranyl acetate and examined in a Morgagni 268 transmission electron microscope (FEI, Hillsboro, OR). Images were acquired using an AMT charge-coupled device camera (Advanced Microscopy Techniques Corp., Danvers, MA) at 80,000 kV accelerating voltage.

**Cell Culture and Microinjection**—Cell culture was performed as described previously (6, 22–24). NIH3T3 cells were grown in 10% calf serum and serum-starved at confluency for 48 h where indicated. APC proteins for microinjection were diluted to 2 mg/ml in HKCL buffer (10 mM Hepes, pH 7.2, 140 mM KCl), and we estimate that ~10% of the cell volume was routinely injected. In experiments including both APC and EB1, EB1 proteins were microinjected at 1.5-fold molar excess to APC. Following microinjection of GST-APC-basic (Fig. 4), cells were incubated for 1 h and then fixed in either 4% paraformaldehyde or Cytofix reagent (BD Biosciences). Cells microinjected with APC-C and EB1 in the absence of nocodazole (Fig. 8) were incubated for 1 h and then fixed in Cytofix. To observe APC-C localization to the actin cytoskeleton (Fig. 9), cells were microinjected with GST-APC-C (or GST-APC-C and GST-EB1-C). 20 min after incubation, cells were treated with 20 μM nocodazole, incubated for 2 h, and subsequently fixed in Cytofix.

**Immunofluorescence**—For detection of microinjected GST-APC-basic and GST-APC-C in the absence of other GST-fused proteins, cells were stained with monoclonal antibody to GST (1:100, 26H1, Cell Signaling Technology, Beverly, MA). Depending on the experiment, cells were also stained with rat monoclonal antibody YLI1/2 to detect dynamic Tyr-microtubules (1:10, European Collection of Animal Cell Cultures, Salisbury, UK) or with Alexa Fluor 647-conjugated phalloidin (Invitrogen) to detect F-actin. To detect GST-APC-C co-microinjected with GST-EB1-C, cells were stained with anti-APC rabbit polyclonal antibody C-20 (1:50, Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies with minimal cross-species reactivity (Jackson ImmunoResearch Laboratories, West Grove, PA) were used as described (22, 23). Epifluorescence microscopy was performed as described (6, 23).

**RESULTS AND DISCUSSION**

The APC Basic Domain Bundles Actin Filaments—The carboxyl-terminal third of human APC protein (APC-C; residues 2130–2843; Fig. 1, A and B) contains its microtubule-binding basic domain followed by a short EB1-binding domain. This region of the APC protein is sufficient for localization to microtubule plus ends in vivo (7). Further, tumorigenic mutations
that truncate this region of APC disrupt its localization to the actin-rich lateral membrane of cultured cells (9). To investigate possible APC-actin interactions, we purified the human APC-C polypeptide using a yeast expression system and tested its ability to bind directly to F-actin in vitro. In low speed centrifugation (16,000 × g) assays, F-actin pellets specifically when filaments are organized into higher order structures by actin-binding proteins, e.g., the bundling protein coronin/Cm1 (Fig. 1C). The addition of purified APC-C to 3 μM F-actin induced pelleting of F-actin at low speeds, an effect that was concentration-dependent (Fig. 1C). To visualize the F-actin structures formed, reactions containing 3 μM F-actin mixed with 0.5 μM APC-C or control buffer were fixed, stained with Alexa488-conjugated phallolidin, and examined by light microscopy. Single actin filaments were observed for reactions containing control buffer, whereas tight bundles of filaments were formed by APC-C (Fig. 1D) and by a known bundling protein, Cm1/coronin (25).4 We also observed APC-C co-pelleting with F-actin. Thus, APC protein both binds directly to F-actin and bundles filaments.

We next used electron microscopy to examine F-actin bundles by APC-C. In control reactions lacking APC-C, single actin filaments were observed (Fig. 2A). By contrast, F-actin mixed with APC-C was organized into tight, ordered bundles (Fig. 2B and C). The ordered nature of these bundles demonstrates that the APC-actin interaction is highly specific. This specificity is further supported by the concentration-dependent nature of binding and by the ability of two different APC ligands to competitively displace its binding to actin (see below). Additionally, these effects were not due to protein aggregation, because APC-C (and all other APC constructs used in this study) was soluble and monodispersed by gel filtration analysis (see Fig. 6).4

APC protein functions can be regulated by phosphorylation (e.g. GSK3β decreases its interactions with microtubules (26)). Because the APC-C used for the experiments above was expressed and purified from S. cerevisiae, we investigated the possibility that post-translational modification might contribute to its interactions with actin. Two assays showed that APC-C was phosphorylated (Fig. 3, A and B). First, treatment with λ-phosphatase altered APC-C migration on SDS-PAGE. Second, a fluorescent phosphoprotein dye detected APC-C in protein gels, and the signal was abolished by treatment with λ-phosphatase. Therefore, we compared the ability of 1 μM yeast-expressed APC-C to bundle actin filaments after dephosphorylation with λ-phosphatase or mock treatment. Dephosphorylated APC-C bundled actin more efficiently than mock-treated APC-C (100 versus 50% F-actin in the pellet; Fig. 3B), suggesting that phosphorylation of APC-C diminishes its abil-

![Image](https://example.com/image1.png)

**FIGURE 1. The APC basic domain bundles F-actin in vitro.** A, schematic of APC fragments used in this study. Basic, basic domain; EB1, EB1 binding site. B, Coomassie-stained SDS-polyacrylamide gel of purified proteins. APC-C was isolated as a His6 fusion protein; APC-basic and APC-C1 were isolated as GST fusion proteins. C, low speed pelleting assay for F-actin bundling.Preassembled F-actin (3 μM) was incubated for 10 min at 25 °C with Cm1 (yeast coronin), control buffer (actin), or the indicated concentrations of APC-C. Reactions were centrifuged for 3 min at 16,000 × g, and the pellets (P) and supernatants (S) were analyzed by SDS-PAGE and Coomassie staining. The arrowhead marks APC-C, and the asterisk marks contaminant that does not co-pellet with bundled F-actin. D, fluorescence micrographs of actin filaments bundled by APC-C. Preassembled F-actin (3 μM) was incubated for 10 min at 25 °C with 0.5 μM APC-C or control buffer. Reactions were chemically fixed, stained with Alexa488-phallolidin, and visualized by fluorescence microscopy.

![Image](https://example.com/image2.png)

**FIGURE 2. APC-C organizes F-actin into ordered bundles.** Electron micrographs of 2 μM preassembled F-actin incubated for 10 min at 25 °C with control buffer (A) or 0.5 μM APC-C (B and C). Reaction samples were negatively stained and visualized by electron microscopy. The boxed area in B is enlarged in C. Scale bars, 100 nm.

![Image](https://example.com/image3.png)

**FIGURE 3. Effects of dephosphorylation on APC-C bundling of F-actin.** A, APC-C purified from yeast was treated with λ-phosphatase or mock-treated and then fractionated by SDS-PAGE and visualized by Coomassie staining (left) and phospho-protein gel stain (right). B, low speed pelleting assay for F-actin bundling. APC-C (1 μM) was treated with λ-phosphatase or mock-treated and then incubated with preassembled F-actin (3 μM) for 10 min at 25 °C. Reactions were centrifuged for 3 min at 16,000 × g, and the pellets (P) and supernatants (S) were analyzed by SDS-PAGE and Coomassie staining. C and D, low speed pelleting assay, as in B, for F-actin bundling by GST-APC-basic (C) or GST-APC-C1 (D).
ity to bundle F-actin. This conclusion was supported further by a close examination of the gels analyzing low speed pelleting reactions (Fig. 1C), which revealed that the fraction of APC-C that co-pelleted with bundled F-actin had a distinct migration from the fraction that remains in the supernatant.

To avoid potential complications from using post-translationally modified APC protein, we next isolated APC polypeptides as GST fusion proteins from *Escherichia coli*. APC-C purified from *E. coli* bundled F-actin efficiently (also see Fig. 7C). Similar results obtained for APC-C purified with His6 or GST affinity tags suggested that neither tag affected APC function.

To better understand what region of APC-C contains the F-actin bundling activity, we purified two different subfragments of APC-C from *E. coli*, the basic domain (APC-basic) and the EB1-binding domain (APC-C1) (Fig. 1B). In low speed centrifugation assays, APC-basic but not APC-C1 bundled F-actin in a concentration-dependent manner (Fig. 3, C and D). These results demonstrate that the APC basic domain, which also binds to microtubules (27), is sufficient to bind to and bundle actin filaments *in vitro*. It is intriguing that APC interactions with both microtubules and F-actin are mediated by the basic domain, as polybasic oligomers are sufficient to interact with both microtubules (28–31) and actin (32, 33).

**APC Basic Domain Associates with the Actin Cytoskeleton in Cells**—To address APC-basic association with the actin cytoskeleton in cells, we microinjected NIH3T3 fibroblast cells with purified GST-APC-basic and examined localization by immunofluorescence using anti-GST antibodies. In most cells, GST-H18528 APC-basic associated with both microtubules and actin stress fibers (Fig. 4, A–D). However, in a small percentage of cells, GST-H18528 APC-basic localized almost exclusively to actin stress fibers (Fig. 4, E–H). In serum-starved cells, which have few actin stress fibers, GST-H18528 APC-basic was mostly localized on microtubules (Fig. 4, I–L). GST alone did not localize to actin stress fibers or microtubules (Fig. 4, M–P). These *in vivo* results correlate with APC binding to and bundling F-actin *in vitro* and provide a direct molecular mechanism and function for APC association with the actin cytoskeleton. Key to this interaction

![FIGURE 4. The APC basic domain associates with F-actin and microtubules in cells.](image-url)
that APC-basic bundles the microtubules. Similar results were obtained using APC-C.4

We also used light microscopy to visualize the effects of APC-C on the organization of fluorescently labeled F-actin and/or microtubules (Fig. 5C). Consistent with the low speed pelleting results, APC-C induced formation of visible F-actin bundles in the absence of microtubules and induced formation of microtubule bundles in the absence of F-actin. Interestingly, at equal concentrations of F-actin and microtubules (2 μM each), APC-C organized bundles of microtubules that were not associated with F-actin. This suggests that APC-C may have a higher affinity for microtubules than F-actin and confirms our conclusion from low speed pelleting assays that microtubules inhibit APC-C interactions with F-actin. Intriguingly, including a lower concentration of microtubules (0.5 μM) led to visible cross-linking of F-actin and microtubules, demonstrating that APC protein has the capacity under some conditions to physically link actin and microtubule polymer systems. It is presently unclear what mechanism allows APC to bundle actin filaments and microtubules and cross-link the two systems. This may be accomplished either by APC having multiple binding sites (for actin and microtubules) in one polypeptide chain or, alternatively, by APC oligomerization. Analytical gel filtration analysis of purified APC-C is consistent with the latter possibility (Fig. 6), but it is also possible that this reflects APC-C having a highly extended conformation. Clarification of this mechanism by additional biophysical tests will require isolating larger quantities of APC-C than we were unable to obtain using the methods described here. Thus, it will be important to develop high yield APC-C purification schemes in the future.

EB1 Directly Inhibits F-actin Bundling by APC—We next investigated whether the interactions of APC-C with F-actin might be regulated by EB1, an in vivo binding partner of APC. EB1 is a microtubule plus-end tracking protein that binds directly to the carboxyl terminus of APC (15, 35), adjacent to
the APC basic domain (Fig. 1A). This interaction contributes to the formation of stable microtubules in cells (6) and has been suggested to promote microtubule formation in vitro (36). The carboxyl terminus of EB1 (EB1-C) binds to APC (see Fig. 7A) and to the p150\textsuperscript{Rh} subunit of dynactin (15, 37–39), whereas the amino terminus associates with microtubules and the formin Dia2 (6). Although APC protein and EB1 clearly interact and function together in vitro, they display only partial co-localization in cells, and APC-EB1 interactions are not required for APC protein localization to and stabilization of microtubules (16, 26, 40, 41). Thus, EB1 and APC protein may share some cellular functions (e.g. on the ends of some microtubules), but they also appear to have independent functions.

In low speed centrifugation assays, the addition of EB1-C abolished APC-C bundling of F-actin (Fig. 7C). Two complimentary experiments showed that this inhibition requires direct interactions between EB1-C and APC-C. First, the EB1 construct EB1-C(KR), which contains point mutations that disrupt APC-binding interactions (K220A, R222A) (6, 42), had no effect on APC-C bundling of F-actin (Fig. 7C). In control reactions lacking APC-C, wild type and mutant EB1-C proteins had no effect on F-actin pelleting. Second, EB1-C had no effects on actin bundling by APC-basic (Fig. 7D), which lacks the EB1 binding site (see Fig. 1A). Thus, EB1 directly inhibits APC protein bundling of F-actin.

**EB1 Directly Inhibits APC Association with the Actin Cytoskeleton in Cells**—Finally, we examined the ability of EB1 to regulate APC interactions with the actin cytoskeleton in cells. In microinjection experiments, APC-C associated with microtubules in NIH3T3 fibroblast cells (Fig. 8, A–C). However, upon disruption of the microtubule cytoskeleton with nocodazole, APC-C co-localized with the actin cytoskeleton and predominantly decorated stress fibers, similar to APC-basic (Fig. 9, A–C). Next, we co-microinjected EB1-C and APC-C into nocodazole-treated cells and detected APC-C localization by immunofluorescence using the C-20 antibody, which recognizes the APC carboxyl terminus. Co-microinjection of EB1-C led to a diffuse cytoplasmic localization of APC-C (Fig. 9, D–F). In contrast, co-microinjection of EB1-C and APC-C into cells with an intact microtubule cytoskeleton (not treated with nocodazole) did not affect APC-C localization to microtubules (Fig. 8, D–F). These results suggest that binding of EB1 specifically disrupts APC protein association with F-actin and not microtubules in cells, similar to the effects we observed in vitro. Further,
these effects in cells similarly require direct EB1–APC interactions, as co-microinjection of APC-C and EB1-C(KR) did not alter APC-C localization to the actin cytoskeleton (Fig. 9, G–I). We note that the effect of EB1-C in this experiment may not be absolute, as a minor degree of co-localization between APC-C and F-actin may remain. However, the robust distinction between APC-C localization in the presence of wild type EB1-C (Fig. 9D) versus mutant EB1-C(KR) (Fig. 9G) shows conclusively that EB1 directly inhibits APC association with the actin cytoskeleton in cells.

Taken together, these results suggest that EB1 directly inhibits APC protein association with F-actin and may thereby promote association with microtubules. In this capacity, EB1 may function as a regulatory switch, altering APC protein between microtubule plus ends and the actin cytoskeleton. Transient APC–EB1 interactions may promote APC functions at microtubule plus ends, for which APC protein has high affinity. Other molecular cues, including localized microtubule disassembly at the cortex, may promote APC protein interactions with the actin cytoskeleton. Consistent with this view, EB1 and APC only partial overlap in their cellular localizations (16), and EB1 is required for APC function at only a subset of microtubules in cells (6, 16). We have also shown that APC protein has the ability to cross-link actin and microtubule polymers in vitro. Thus, APC may be involved in linking functions of the microtubule and actin cytoskeletons, such as at cortical sites of actin-microtubule overlap.

Conclusions—Our results identify a new activity for APC protein in binding directly to and bundling actin filaments, providing a functional explanation for APC protein localization to actin-rich cellular regions. Further, we have shown that EB1 directly controls APC protein associations with microtubules versus F-actin. This work raises a number of questions about the APC mechanism of interaction with F-actin and its role in regulating actin dynamics and organization in cells. Future experiments will be directed at mapping APC residues that interact with actin and identifying sites of direct APC-actin association in vivo. Coordination of the actin and microtubule cytoskeletons by APC protein is likely to be regulated on many additional levels, including the APC ligands Dia (6) and IQGAP (13), α-catenin/β-catenin (43, 44), and possible intramolecular interactions between the amino and carboxyl termini of APC (45). Because the APC protein carboxyl terminus is deleted in most tumorigenic APC mutations, it is tempting to speculate that this cytoskeletal function is involved in APC tumor suppressor function. In particular, we note that APC protein has been localized to actin-rich intercellular junctions (10, 11), which can be found in the intestinal epithelium. The ability of APC protein to bundle F-actin and to link the actin and microtubule cytoskeletons may be required for maintenance of cell-cell adhesion, and disruption of these activities may contribute to uncontrolled cell migration and/or Wnt signaling, with implications for tumor formation. Alternatively, loss of APC interactions with actin and/or microtubules may impair cell migration from the proliferative zone of the intestinal epithelium, leading to the accumulation of cells and tumor formation.

Acknowledgements—We thank Chen Xu and Alexandra Deaconescu for technical assistance in electron microscopy.

REFERENCES
1. Bienz, M. (2002) Nat. Rev. Mol. Cell. Biol. 3, 328–338
2. Nathke, I. S. (2004) Annu. Rev. Cell Dev. Biol. 20, 337–366
3. Oshima, H., Oshima, M., Kobayashi, M., Tsutsumi, M., and Taketo, M. M. (1997) Cancer Res. 57, 1644–1649
4. Oshima, M., Oshima, H., Kitagawa, K., Kobayashi, M., Itakura, C., and Taketo, M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4482–4486
5. Nathke, I. S., Adams, C. L., Polakis, P., Sellin, J. H., and Nelson, W. J. (1996) J. Cell Biol. 134, 165–179
6. Wen, Y., Eng, C. H., Schmoranzer, J., Cabrera-Poch, N., Morris, E. J., Chen, M., Wallar, B. J., Alberts, A. S., and Gundersen, G. G. (2004) Nat. Cell Biol. 6, 820–830
7. Mimori-Kiyosue, Y., Shiba, N., and Tsukita, S. (2000) J. Cell Biol. 148, 505–518
8. Mogensen, M. M., Tucker, J. B., Mackie, J. B., Prescott, A. R., and Nathke, I. S. (2002) J. Cell Biol. 157, 1041–1048
9. Rosin-Arbesfeld, R., Ihre, G., and Bienen, M. (2001) EMBO J. 20, 5929–5939
10. Langford, K. J., Ashkam, J. M., Lee, T., Adams, M., and Morrison, E. E. (2006) BMC Cell Biol. 7, 3
11. Langford, K. J., Lee, T., Ashkam, J. M., and Morrison, E. E. (2006) Cell Motil. Cytoskeleton 63, 483–492
12. Miyashiro, I., Senda, T., Matsumine, A., Baeg, G. H., Kuroda, T., Shimano, T., Miura, S., Noda, T., Kobayashi, S., Monden, M., Toyoshima, K., and Akiyama, T. (1995) Oncogene 11, 89–96
13. Watanabe, T., Wang, S., Noritake, J., Sato, K., Fukata, M., Takefuji, M., Nakagawa, M., Iizumi, N., Akiyama, T., and Kaibuchi, K. (2004) Dev. Cell 7, 871–883
14. McCartney, B. M., Price, M. H., Webb, R. L., Hayden, M. A., Holot, L. M., Zhou, M., Bejsovec, A., and Peifer, M. (2006) Development (Camb.) 133, 2407–2418
15. Su, L. K., Burrell, M., Hill, D. E., Gyuris, J., Brent, R., Wiltshire, R., Trent, J., Vogelstein, B., and Kinzler, K. W. (1995) Cancer Res. 55, 2972–2977
16. Kita, K., Wittmann, T., Nathke, I. S., and Waterman-Storer, C. M. (2006) Mol. Biol. Cell 17, 2331–2345
17. Moseley, J. B., Maiti, S., and Goode, B. L. (2006) Methods Enzymol. 406, 215–234
18. Jones, E. W. (2002) Methods Enzymol. 351, 127–150
19. Spudich, J. A., and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871
20. Goode, B. L., and Feinstein, S. C. (1994) J. Cell Biol. 124, 769–782
21. Moseley, J. B. (2005) J. Biol. Chem. 280, 28023–28033
22. Cook, T. A., Nagasaki, T., and Gundersen, G. G. (1998) J. Cell Biol. 141, 175–185
23. Palazzo, A. F., Cook, T. A., Alberts, A. S., and Gundersen, G. G. (1999) Nat. Cell Biol. 3, 723–729
24. Kreitzer, G., Liao, G., and Gundersen, G. G. (1999) Mol. Biol. Cell 10, 1105–1118
25. Goode, B. L., Wong, J. J., Butty, A.-C., Peter, M., McCormack, A. L., Yates, J. R., Drubin, D. G., and Barnes, G. (1999) J. Cell Biol. 144, 83–98
26. Zumbrohn, J., Kinoshita, K., Hyman, A. A., and Nathke, I. S. (2001) Curr. Biol. 11, 44–49
27. Munemitsu, S., Souza, B., Muller, O., Albert, I., Rubinfeld, B., and Polakis, P. (1994) Cancer Res. 54, 3676–3681
28. Jacobs, M., Bennett, P. M., and Dickens, M. J. (1975) Nature 257, 707–709
29. Erickson, H. P., and Voter, W. A. (1976) J. Biol. Chem. 251, 1041–1048
30. Askham, J. M., Moncur, P., Markham, A. F., and Morrison, E. E. (2000) Oncogene 19, 1950–1958
31. Nakamura, M., Zhou, X. Z., and Lu, K. P. (2001) Curr. Biol. 11, 1062–1067
32. Askham, J. M., Vaughan, K. T., Goodson, H. V., and Morrison, E. E. (2002) Curr. Biol. 12, 2003–2008
APC Bundles Actin Filaments

Mol. Biol. Cell 13, 3627–3645
38. Berrueta, L., Kraeft, S. K., Tirnauer, J. S., Shuyler, S. C., Chen, L. B., Hill, D. E., and Pellman, D. (1998) Proc. Natl. Acad. Sci. 95, 10596–10601
39. Ligon, L. A., Shelly, S. S., Tokito, M., and Holzbaur, E. L. (2003) Mol. Biol. Cell 14, 1405–1417
40. Barth, A. I., Siemers, K. A., and Nelson, W. J. (2002) J. Cell Sci. 115, 1583–1590
41. Mimori-Kiyosue, Y., Shiina, N., and Tsukita, S. (2000) Curr. Biol. 10, 865–868
42. Slep, K. C., Rogers, S. L., Elliott, S. L., Ohkura, H., Kołodziej, P. A., and Vale, R. D. (2005) J. Cell Biol. 168, 587–598
43. Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S. H., Masiarz, F. R., Munemitsu, S., and Polakis, P. (1993) Science 252, 1731–1734
44. Su, L. K., Vogelstein, B., and Kinzler, K. W. (1993) Science 262, 1734–1737
45. Li, Z., and Nathke, I. S. (2005) Cancer Res. 65, 5195–5204