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Role of APC and Its Binding Partners in Regulating Microtubules in Mitosis

Shirin Bahmanyar, W. James Nelson, and Angela I.M. Barth*

Abstract

Adenomatous polyposis coli (APC) is a multifunctional protein commonly mutated in colon cancer. APC contains binding sites for multiple proteins with diverse roles in signaling and the structural and functional organization of cells. Recent evidence suggests roles for APC and some of its binding partners in regulating microtubules in mitosis. APC localizes to three key locations in mitosis: kinetochores, the cortex and centrosomes. Here, we discuss possible mechanisms for APC function at these sites and suggest new pathways by which APC mutations promote tumorigenesis.

Introduction

Mutations in *adenomatous polyposis coli* (APC) are associated with most colon cancers^{1,2} although how these mutations affect the development of cancer is not fully understood. In part, this is because APC is a multifunctional protein involved in a wide variety of cellular processes, as indicated by the number of APC interacting proteins (Fig. 1A). A subset of these interacting proteins, β -catenin and Axin, form a complex that regulates Wnt signaling.^{3–7} Other interacting proteins, such as Kinesin-Associated Protein 3 (Kap3), Mitotic Centromere Associated Kinesin (MCAK), mDia, microtubules (MT) and End-Binding 1 (EB1) appear to play a role in microtubule dynamics.^{8–12} Studies have shown involvement of APC in cellular functions related to microtubule dynamics such as migration of epithelial cells and neuronal growth cones.^{13,14} Here we focus on the role of APC and its binding partners in regulating microtubules in mitosis. APC has been reported to act at three key locations for normal progression through mitosis: the kinetochore, cortex and centrosome.^{15–19} Potential roles for APC in mitosis suggest new pathways by which APC mutations can contribute to cancer progression.

APC at the Kinetochore: Regulation of Microtubule Dynamics

APC localizes to kinetochores and forms a complex with kinetochore-bound proteins.^{15,16} Microtubule-plus end proteins at kinetochores are thought to attach microtubules to chromosomes and/or regulate the local polymerization and depolymerization of microtubules.²⁰ The opposing stabilizing and destabilizing activities of kinetochore-attached microtubules facilitate chromosome congression at the metaphase plate,²¹ which is required for equal and opposite segregation of chromosomes in anaphase.

Roles for APC in either kinetochore-microtubule capture and attachment, or regulation of kinetochore-microtubule plus-end dynamics have been proposed.²² In support of a role for APC in microtubule capture and attachment at kinetochores, colon cancer cell lines with

mutant APC have fewer kinetochores with juxtaposed microtubule ends and an overall decrease in midzone microtubules.²² However, in contrast to depletion of other microtubule plus-end binding proteins (+Tips) that function in microtubule capture at kinetochores, such as CLIP170, cells depleted of APC have normal kinetochore-attached (cold-stable) microtubules.²³ and decreased levels of Mad2 at kinetochores, which normally accumulates to unattached kinetochores.^{23,24} Thus, similar to the role of APC in regulating microtubule plus-end dynamics in migrating cells and mitotic *Xenopus* extracts,^{25–27} an alternative, but not mutually exclusive function for APC at kinetochores could be to regulate local microtubule dynamics. A role for APC in regulating microtubule dynamics at kinetochores is supported by the finding that the distances between kinetochores of sister chromatids is decreased in APC depleted cells.^{23,24} This is similar to what happens in response to taxol, which inhibits microtubule dynamics but not attachment at kinetochores.^{23,24} Thus, APC depletion results in reduced tension at kinetochores, an indicator of abnormal kinetochore-microtubule dynamics.²⁸ Interestingly, reduced tension at kinetochores in APC depleted cells correlates with abnormal chromosome congression.²⁸ Since localization of APC to kinetochores is microtubule-dependent,¹⁶ APC may regulate kinetochore microtubule plus-end dynamics of unattached microtubules and thereby promote microtubule capture at kinetochores. Once the microtubules are attached, APC may regulate local kinetochore microtubule plus-end dynamics to generate tension, which is required for chromosome alignment at the metaphase plate.

APC may stimulate microtubule growth by directly interacting with microtubules through the basic domain or through its interaction with known microtubule stabilizing proteins such as EB1.^{9,29} Consistent with the latter idea, out of a panel of +Tips analyzed, EB1 was most related to APC in that its localization to kinetochores was microtubule dependent and depletion of EB1 caused reduced tension but not loss of microtubule attachment at kinetochores.²³ The only noted difference was that EB1 depletion does not engage a spindle checkpoint arrest, whereas APC depletion results in a transient delay in metaphase.²³ Nonetheless, depleting either EB1 or APC causes similar chromosome defects that correlate with and are likely a consequence of, abnormal microtubule dynamics at kinetochores.²³ APC also binds to MCAK, which destabilizes microtubules.^{10,30} Thus, APC may coordinate microtubule polymerization and depolymerization at kinetochores allowing for proper chromosome movement and congression at the metaphase plate (Fig. 1B). Future work analyzing how kinetochore proteins that form a complex with APC, such as MCAK and EB1, are affected in APC depleted cells could test this possibility.

APC at the Cortex: Role in Spindle Positioning

A potential role for APC in orienting the mitotic spindle seems to be conserved in several organisms.^{31–33} The mechanism of spindle orientation is not well understood, but the importance for microtubule attachment at specialized cortical sites has been established.^{31,32,34} In *Saccharomyces cerevisiae*, the functional analogue for APC, Kar9, specifies the cortical site for astral microtubule attachment.^{35,36} In *Drosophila*, EAPC/dAPC2 at cell-cell adhesion sites is thought to mediate attachment of astral microtubules at the membrane for proper spindle positioning.^{17,18,37,38} Less is known about the role of APC in spindle positioning in mammalian cells although there is evidence to suggest that APC may regulate astral microtubule stability³⁹ and/or provide a cortical site for astral microtubule attachment (see below).

In the current model in *S. cerevisiae*, Kar9 binds asymmetrically to the daughter-bound spindle pole body (SPB) where it interacts with Bim 1 (the yeast homologue of EB1) and is loaded onto the plus-end of a subset of microtubules polymerizing from the SPB (Fig. 2A).^{40,41} Kar9-Bim 1 bound microtubules are guided along actin cables via Myo2 to the bud

tip.^{33,42,43} Once attached to the bud tip, microtubule movement can be generated by microtubule plus-end dynamics against the cell cortex and cortically bound microtubule motors,⁴⁴ which enables spindle positioning relative to the mother-bud axis. In budding yeast, the bud neck and later the bud tip direct spindle positioning by providing a predetermined axis of cell division (Fig. 2A). In multicellular organisms, cells often use cues from surrounding cells, specifically from cell-cell adhesion sites, to orient the spindle and thus the plane of division (Fig. 2B).³⁸

In *Drosophila* and vertebrates there are two *APC* genes: *Drosophila E-APC/dAPC2* and *dAPC1* and vertebrate *APC* and *APCL*.^{17,45–48} *EAPC/dAPC2* has been reported to localize to cell-cell junctions throughout the embryo, in the neuroepithelium and germ cells of the ovary and testis,¹⁷ and this localization is actin dependent and coincides with Armadillo and DE-cadherin.⁴⁹ Thus, *dAPC2* is in the correct location to play a role in spindle attachment at cell junctions and thereby orient the spindle and plane of division. In the syncytial blastoderm, *dAPC2* mutants lose nuclei from the cortex into the internal cytoplasm consistent with a loss of spindle attachment to the cortex.¹⁸ Similarly, RNAi of *dAPC2* in the embryonic epidermis interferes with symmetric division along the planar axis of the embryo and instead these cells divide asymmetrically.³⁷ This suggests that *dAPC2* is required for spindle orientation. Finally, in male germline stem cells (GSC), centrosomes are mispositioned in *dAPC2* mutants resulting in misoriented spindles (Fig. 2B).³⁸ *dAPC1* mutants also have defects in centrosome positioning in male GSCs, however only *dAPC2* localizes to cortical attachment sites at cell junctions, whereas *dAPC1* localizes to centrosomes in these cells.³⁸ (see below and Fig. 2B). Thus, *dAPC2* may play a key function in spindle orientation by providing cortical attachment sites at adherens junctions for microtubules (Fig. 2B), although the mechanism of attachment to adherens junctions by *dAPC2* is not understood. One possibility is that *dAPC2* functions in maintaining junctions and affects spindle positioning indirectly. However, fly embryos completely null for *dAPC1* and *dAPC2* do not display obvious defects in adhesion,⁴⁷ suggesting that *dAPC2* may have a direct role in spindle positioning at cell junctions.

In vertebrates, *APC* depletion in mitotic cells results in loss of astral microtubules and mispositioning of the spindle relative to the geometric center of the cell.³⁹ *APC* localization to adherens junctions is weak and whether *APC* functions at adherens junctions in mammalian cells remains to be shown.^{50–52} In mitotic MDCK cells, small clusters of *APC* can be found at the cell cortex, some of which align with astral microtubules (S.B., unpublished data). Thus, rather than tethering microtubules at adherens junctions, *APC* may provide cortical sites at the plasma membrane that stabilize and anchor the plus-ends of astral microtubules (Fig. 1C). Whether *APC* arrives at these sites independent of microtubules, or by traveling along microtubules and/or with microtubule plus-ends^{25,53} remains to be shown. Furthermore, it will be important to determine whether these cortical sites also provide cues to orient the axis of the spindle in relation to the plane of the epithelium.

Disruption of *APC*'s binding partner EB1 results in spindle mispositioning in *Drosophila*^{37,54} and mammalian cells,³⁹ similar to the effects of disruption of *APC*. However, *Drosophila dAPC2* lacks the vertebrate C-terminal EB1 and microtubule binding sites¹⁷ and may not bind EB1 directly.³⁷ Therefore, it remains unclear whether *dAPC2* and EB1 interact in a common pathway to regulate spindle orientation in *Drosophila*. In mammalian cells, depletion of both *APC* and EB1 does not have an additive effect, supporting the idea that *APC* and EB1 function in similar pathways governing spindle orientation.³⁹ One possibility is that, similar to yeast, *APC* capture of astral microtubule plus ends through EB1 assists spindle orientation in mammalian cells (Fig. 1C). Further

experiments are needed to confirm that interactions between APC and EB1 in mammalian cells are required for spindle orientation.

Our knowledge about the function of APC in orienting the mammalian mitotic spindle is still at the early stages. Nevertheless, it is tempting to speculate that APC's role in positioning mitotic spindles is similar to its cytoskeletal roles at the cortex of interphase cells. In migrating cells, APC puncta localize to specialized cortical regions that promote microtubule growth.^{13,25,53,55} At the basal cortex, APC puncta localize along the path of microtubules and provide points at which microtubules pause and rescue.^{56,57} Thus, APC on the plasma membrane in mitotic cells may function similarly in guiding and stabilizing astral microtubules. Live cell imaging of mitotic cells to analyze the interaction of astral microtubule plus-ends with APC puncta is required to support this idea.

APC at Centrosomes: Potential Roles

APC localizes to mammalian centrosomes and this localization is conserved in the yeast analogue, Kar9 and the *Drosophila* homologue, dAPC1 (Fig. 2).^{19,38,58,87} Centrosomes nucleate and anchor microtubules and are essential for establishment of a bipolar spindle.^{59,60} APC mutant mouse embryonic stem cells (ES cells) have multipolar spindles and other centrosome abnormalities in mitosis indicating a role for APC at mitotic centrosomes.¹⁵ Very little is known about the function of APC at mammalian centrosomes. However, data from lower eukaryotes suggest several interesting functions for APC at centrosomes including recruiting interacting proteins to this site.

In *S. cerevisiae*, Kar9 localizes asymmetrically to the daughter-bound spindle pole body (SPB), the yeast equivalent of the centrosome (Fig. 2A).^{40,41,58} It is thought that the spindle pole body acts as a 'loading dock' for Kar9 assuring that only a subset of microtubules are loaded with the complex between Kar9 and Bim1.⁴¹ As noted in the previous section, Kar9-Bim1 bound microtubules are then directed to the bud tip where Kar9 mediates attachment of microtubule plus-ends to the cortex (Fig. 2A).³⁴ In *Drosophila* testes, dAPC1 localizes to the centrosomes while dAPC2 localizes to the cortex (Fig. 2B).³⁸ Although the relationship between dAPC1 at centrosomes and dAPC2 at the cortex in *Drosophila* testes is unknown, mutants in either gene cause centrosome and spindle misorientation.³⁸ Vertebrate APC and EB1 localize specifically to the mother centriole, which is known to anchor a subset of microtubules.^{19,60} Furthermore, APC decorates a subset of microtubules in migrating cells.¹² Thus, similar to yeast, a subset of microtubules anchored to the mother centriole may be loaded with APC and guided to their cortical destination (Fig. 1D).

In addition to APC, several other regulatory components of the Wnt signaling pathway localize to centrosomes. In the absence of a Wnt signal, a core destruction complex of APC, Axin and GSK3 β control β -catenin levels by phosphorylating β -catenin^{7,61} (see Kennell and Cadigan, this volume). Recent reports show that β -catenin also localizes to centrosomes in interphase and mitosis,⁶²⁻⁶⁴ and regulation of its levels at centrosomes throughout the cell cycle may be important for proper centrosome function.^{62,63} Overexpression of β -catenin results in increased centrosome number in interphase (SB unpublished results),⁶⁵ whereas β -catenin depletion inhibits centrosome separation in mitosis,^{62,63} suggesting that there may be a threshold level of β -catenin that is important in some aspect of centrosome organization and function. Consistent with a requirement for regulated levels of β -catenin at centrosomes, GSK3 β is active at centrosomes during interphase and is inactive at centrosomes in mitosis.⁶⁶ However, it is not known whether GSK3 β activity at centrosomes regulates β -catenin levels at this location.

Phosphorylated β -catenin is recognized by the SCF (Skp1-cullin-Fbox) ubiquitin ligase β -TrCP which ubiquitinates β -catenin marking it for degradation by the proteasome.^{2,7}

Components of the SCF ubiquitin ligase, Skp1 and Cul1, localize to centrosomes and are required for centriole duplication and separation in *Xenopus* egg extracts.⁶⁷ β -TrCP^{-/-} mouse embryonic fibroblasts and *Drosophila* β -TrCP/Slimb mutants have overduplicated centrosomes.⁶⁸ Furthermore, components of the proteasome machinery have been localized to the centrosome and requirements for proteolysis in centrosome duplication and separation has been suggested.⁶⁹⁻⁷¹ Thus, most components of the core destruction complex and proteasome machinery are present and functional at centrosomes. A function for APC with the other components of the destruction complex at centrosomes may be in regulating β -catenin levels, which in turn could be important for normal centrosome duplication and separation.

APC localization to centrosomes together with several APC interacting proteins suggests several interesting possibilities for APC function at this site. APC, similar to Kar9 in yeast, may load onto a subset of microtubules in mitosis destined to perform specific functions such as cortical attachment, spindle orientation and chromosome congression. On the other hand, the centrosome may act as a signaling hub where Wnt signals coordinate with the cytoskeleton to perform specific tasks (see next section). Experiments that specifically inhibit APC at centrosomes will directly test for these potential functions.

Connecting APC Functions in Regulating Wnt Signaling and Microtubules

APC has divergent roles in cells: it is a regulator of Wnt signaling and also affects cytoskeletal function. In Wnt signaling, APC forms a complex that phosphorylates β -catenin leading to its degradation. As a cytoskeletal regulator, APC binds to proteins that either stabilize or destabilize microtubules at several locations in mitotic and interphase cells. Whether there is a functional link between Wnt signaling and regulation of microtubules through APC is unknown. One possibility is that APC can translate Wnt signals into cytoskeletal changes as it has been shown recently for a noncanonical Wnt signal during cell migration.⁷² In the absence of Wnt signal, the pool of APC in the destruction complex is distinct from the pool of APC that binds to microtubules.⁷³ Moreover, GSK3 β phosphorylation of APC inhibits the interaction of APC with microtubules,⁷⁴ supporting the idea that the pool of APC in the destruction complex may be unavailable to regulate microtubules. In the presence of a Wnt signal, GSK3 β in the destruction complex is inhibited,⁷ which may release APC from the destruction complex to interact with microtubules. Determining the influence of Wnt signaling on APC's role in microtubule regulation will provide a link between the function of APC in gene expression and microtubule dynamics and will provide insight into the synergistic consequences of APC mutations that give cells the clonal advantage needed for tumor formation (see below).

Multiple Mechanisms by which APC Mutations Contribute to Cancer

Mutations in APC result in unregulated expression of Wnt-responsive genes and mitotic spindle defects. The mechanism by which APC mutations lead to unregulated gene expression and the consequences of this deregulation on cancer progression has been thoroughly examined.^{7,75,76} Here, we will focus on potential consequences of APC mutations on centrosomes and kinetochores in mitosis, which could contribute to tumorigenesis by promoting chromosomal instability (see also Caldwell and Kaplan, this volume).

Most colorectal tumors exhibit chromosomal instability (CIN), which is caused by chromosome missegregation.⁷⁷⁻⁷⁸ Abnormal centrosome number⁸⁴ and/or flawed attachment of microtubules to kinetochores⁸² can lead to chromosome segregation defects. APC mutant mouse ES cells have multipolar spindles and abnormal centrosomes indicating that APC mutations could lead to defects in centrosomes.¹⁵ Moreover, depletion of APC has

been shown to cause lagging chromosomes, which correlates highly with reduced tension caused by lack of APC at kinetochores.²³ Thus, abnormal *APC* function at centrosomes or kinetochores could cause defects in mitosis that may result in chromosomal instability. It will be necessary to determine whether mutations in *APC* correlate with centrosome or chromosome abnormalities in colon cancer tissues. Since centrosome abnormalities could arise through indirect effects of abnormal mitoses,⁸⁵ it will be important to determine whether centrosome abnormalities are present in adenomas at the earliest stages of colon cancer.

A critical consequence of *APC* dysfunction in mitosis may be that in the absence of *APC* cells are able to bypass the mitotic checkpoint.^{23,24} Depletion of *APC* results in a less efficient mitotic checkpoint.^{23,24} which would allow cells with centrosome and spindle abnormalities to progress through mitosis with chromosome segregation defects.²⁸ Checkpoint proteins sense lack of tension on kinetochores and respond by inhibiting transition into anaphase.²⁸ After prolonged culture, colon cancer cells with *APC* mutations exhibit high levels of aneuploidy, indicating that problems in chromosome segregation bypass the checkpoint machinery.⁸⁶ Furthermore, depletion of *APC* does not cause an arrest in mitosis and cells progress through mitosis with lagging chromosomes, potentially a direct cause of aneuploidy.^{23,24} In fact, *APC* depleted cells treated with low doses of nocodazole or taxol inappropriately exit from mitosis indicating a compromised mitotic checkpoint.²⁴ Reduced accumulation of Bub1 and BubR1 at kinetochores in *APC* depleted cells²⁴ might be the cause of premature mitotic exit allowing cells with kinetochore abnormalities to bypass the spindle checkpoint. Thus, *APC* function is required for two important kinetochore functions: proper microtubule attachment and regulation at kinetochores and activation of the mitotic checkpoint in response to chromosome missegregation. Mutations in *APC* may disrupt both these processes and promote CIN, thereby giving cells the clonal advantage needed for tumorigenesis.

In summary, disruption of the diverse functions of *APC* may synergistically contribute to cancer progression. Deregulated expression of Wnt-responsive genes promotes cell survival and proliferation, whereas defects in microtubule regulation at centrosomes and kinetochores could contribute to spindle abnormalities that are not properly recognized by the mitotic checkpoint and, therefore, cause CIN and, ultimately, cancer progression.

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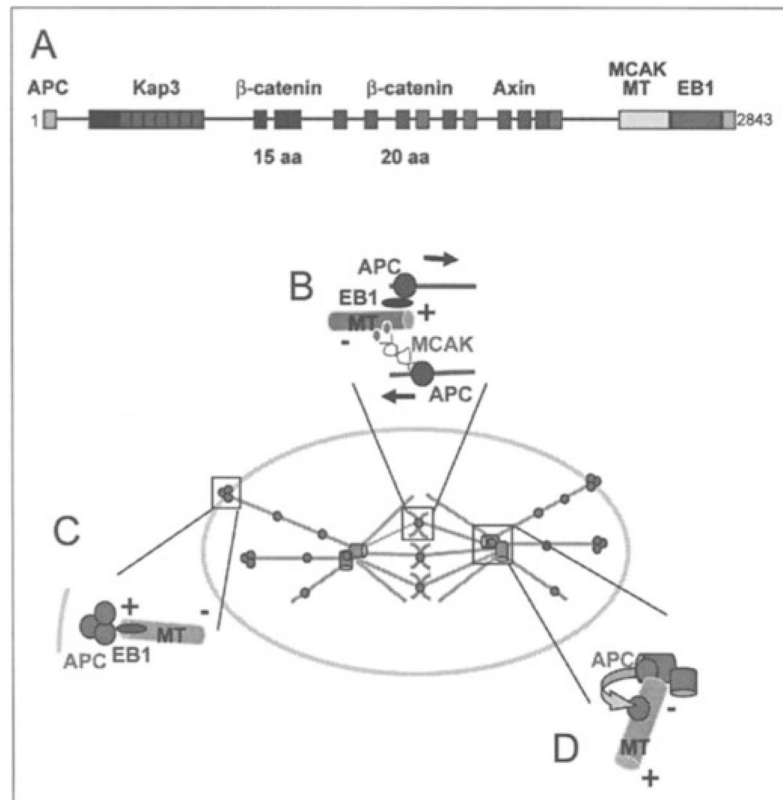


Figure 1.

APC regulates microtubules at multiple mitotic locations. a) Domains of vertebrate APC that are involved in microtubule regulation or Wnt signaling are indicated. APC can bind microtubules indirectly through its interaction with Kap3 (Kinesin associated protein 3) via the N-terminal Armadillo repeats (red), or MCAK (Mitotic Centromere Associated Kinesin) and EB1 (End-Binding 1) at the C-terminus. APC can also bind microtubules directly through its microtubule (MT) binding domain. APC has multiple binding sites for β -catenin (green and blue) and Axin (purple), which are located in the central domain. b) APC localizes to kinetochores where it may have key functions in regulating local microtubule plus-end dynamics. APC may coordinate stabilization and destabilization of microtubule plus-ends at kinetochores through its interactions with EB1 and MCAK, respectively. c) In spindle positioning, a proposed role for APC may be to stabilize and attach astral microtubules to the cortex. EB1 may target microtubule plus-ends to cortical APC clusters. Further work is required to determine whether APC localizes in clusters in mitosis. d) APC localizes to the mother centriole where it is proposed to load onto a subset of microtubules with specific functions, such as cortical attachment, spindle orientation and chromosome congression. A color version of this image is available at www.landesbioscience.com/curie.

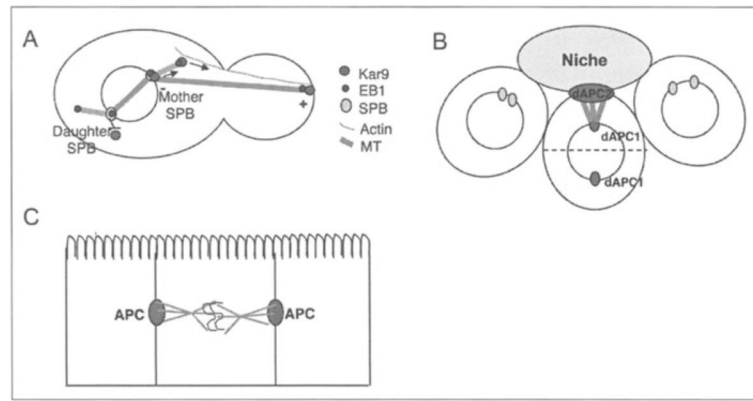


Figure 2.

Potential roles for APC in spindle positioning in budding yeast and *Drosophila*. A) Model for Kar9-Bim1 mediated spindle positioning in budding yeast. Kar9 binds asymmetrically to the daughter-bound spindle pole body (Mother SPB) where it interacts with Bim1 (the yeast homologue of EB1) and is loaded onto the plus-end of a subset of microtubules polymerizing from the SPB.^{40,41} Kar9-Bim1 bound microtubules are guided along actin cables via Myo2 (not shown) to the bud tip. Once attached to the bud tip, microtubule movement can be generated by microtubule plus-end dynamics against the cell cortex and cortically bound microtubule motors, which enables spindle positioning relative to the mother-bud axis. B) Model for dAPC2 and dAPC1 mediated spindle positioning in *Drosophila* male germline stem cells (GSC). dAPC2 localizes to the cell-cell junction between the stem cell and the hub cells where it may facilitate microtubule plus-end attachment.³⁸ The unattached centrosome migrates to the opposite pole to orient the spindle relative to the hub cells (dotted line marks plane of division). dAPC1 is also involved in spindle positioning, however dAPC1 localizes to the centrosomes, not the cell cortex.³⁸ C) In mammalian cells, tethering of astral microtubule plus-ends by APC at cell adhesion sites may orient the spindle in relation to the plane of the epithelium.