Current Biology, Vol. 14, 2033–2038, November 23, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.10.049

Adenomatous Polyposis Coli Associates with the Microtubule-Destabilizing Protein XMCAK

Jennifer D. Banks and Rebecca Heald*
Department of Molecular and Cell Biology
University of California, Berkeley
Berkeley, California 94720

Summary

During cell division, the proper formation of a bipolar spindle and its function to segregate chromosomes requires precise coordination of microtubule-stabilizing and destabilizing activities. Globally destabilized, dynamic microtubules radiating from duplicated centrosomes are locally regulated by chromosomes [1]. Proteins at the kinetochore of each sister chromatid mediate a dynamic attachment, allowing chromosome movement coupled to microtubule polymerization/depolymerization and error-correction mechanisms for improperly attached chromosomes [2]. The tumor suppressor protein adenomatous polyposis coli (APC) stabilizes microtubules both in vitro and in vivo [3–5] and is implicated in mitosis [6–9], although its mechanisms of action are not well characterized. Here, we show that in mitotic *Xenopus* egg extracts, the carboxyl-terminus of APC can associate with the amino terminus of the microtubule-destabilizing KinI, *Xenopus* mitotic centromere-associated kinesin (XMCAK) [10], in a cytoplasmic complex. We find that like XMCAK, APC can localize to the centromere as well as the kinetochore region of mitotic chromosomes and does not require microtubules for chromosomal targeting in *Xenopus* egg extracts. We propose that the presence of these proteins in a complex brings together both positive and negative microtubule effectors, whose opposing activities may be regulated by additional factors, thereby providing precise control of both global and local microtubule dynamics.

Results and Discussion

APC Interacts with XMCAK

We chose to study the role and biochemical interactions of APC in mitosis using *Xenopus* egg extracts. Sperm nuclei were added to metaphase cytostatic factor (CSF)-arrested extracts that had been depleted of APC using a combination of antibodies against the carboxyl and amino termini of the protein (Figure 1A). Instead of normal spindles, we observed large, dense microtubule structures surrounding the chromosomes. The area of these structures was, on average, at least 8 times as large as the bipolar spindles formed in mock-depleted extracts (Figures 1B and 1C). This dramatic stabilization of microtubules in APC-depleted extracts was unexpected since APC has been previously shown to be a microtubule stabilizer [3–5]. Furthermore, the large microtubule arrays were highly reminiscent of those shown to form in extracts depleted of the microtubule-destabilizing kinesin XMCAK [10], although the area of the APC-depleted spindles was approximately 25% smaller than the XMCAK-depleted spindles (Figures 1A–1C). XMCAK, or *Xenopus* kinesin catastrophe modulator-1 (XKCM1), is a member of the KinI family of kinesins that destabilizes microtubules by peeling apart protofilaments [11]. A soluble pool of XMCAK plays a major role in the global regulation of microtubules, whereas a centromere-localized pool regulates the proper attachment of kinetochore microtubules [10, 12, 13]. To determine if the APC-depletion spindle phenotype was related to misregulation of XMCAK, we tested both of our APC antibodies to ascertain if either was codepleting XMCAK from extracts. Surprisingly, the antibody against the carboxyl-terminus of APC codepleted much of the XMCAK from extracts while the amino-terminal APC antibody did not (Figure 2A). We also did not detect any significant codepletion of APC using an XMCAK antibody (Figure 2A). We speculate that the carboxyl-terminal APC antibody stabilizes a transient APC/XMCAK complex, thereby selecting for the formation and depletion of this complex. By sucrose gradient analysis, we observed cosedimentation of a pool of APC and XMCAK in fractions of crude egg extracts, thus providing additional evidence that these two microtubule-regulating proteins can exist in a complex (data not shown).

To verify the interaction and determine which domain of XMCAK interacts with APC, glutathione-S-transferase (GST) fusion proteins containing either the globular amino terminus (GST-NT), central motor (GST-CD), or α-helical carboxyl-terminus (GST-CT) of XMCAK were tested for their ability to precipitate APC from extracts [12]. GST-NT but not GST-CD, GST-CT, or GST alone was able to pull down APC (Figure 2B). Next, several domains of APC were fused to GST. GST-MT, which contains the carboxyl-terminal basic microtubule-stabilization domain [4, 14], precipitated XMCAK (Figure 2C). In contrast, GST fused to the EB1 binding domain (GST-EB1) [15] or armadillo repeat domain (GST-ARM) of APC [16] and GST alone was unable to pull down XMCAK (Figure 2C). Although GST-MT was added to extracts at a concentration in excess of endogenous XMCAK, only a small portion of the total XMCAK in extracts was precipitated by the construct (data not shown). This provides more evidence that the APC/XMCAK interaction is transient or, alternatively, that only a small pool of XMCAK may be competent for APC association under normal circumstances. GST-MT also precipitated XMCAK from extracts treated with nocodazole, a microtubule inhibitor (data not shown), indicating that microtubules are not necessary for their interaction. However, in mixtures of pure proteins, GST-MT was unable to pull down an amino-terminal XMCAK fusion protein, suggesting that APC and XMCAK do not interact directly (data not shown).

The Large Spindles Formed in APC-Depleted Extracts Can Be Rescued by the Addition of XMCAK.

Because both APC and XMCAK were depleted from extracts using the combination of carboxyl-terminal and

*Correspondence: heald@socrates.berkeley.edu

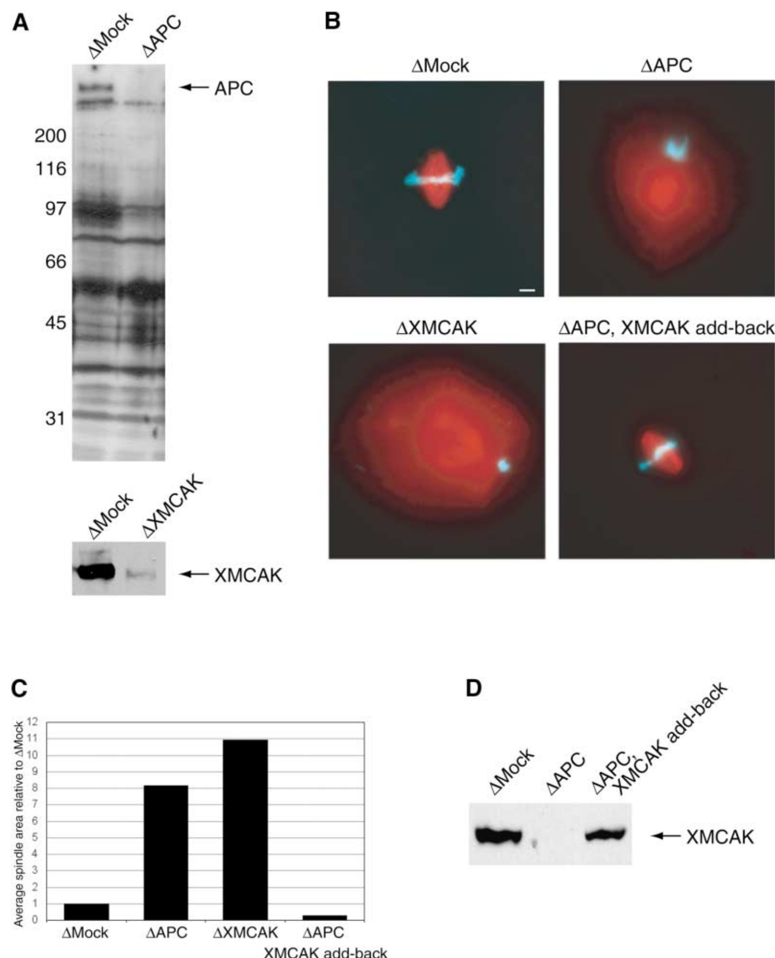

Figure 1. The Large Spindle Structures Formed in Egg Extracts Depleted of APC by a Combination of Carboxyl- and Amino-Terminal Antibodies Are Similar to Those Formed in XMCAK-Depleted Extracts

(A) Western blots of depleted extracts. Top: extract mock depleted or depleted of APC by carboxyl- and amino-terminal antibodies, probed for APC. Bottom: extract mock depleted or depleted of XMCAK, probed for XMCAK. (B) Fluorescence images of structures formed around sperm nuclei in depleted extracts. Top left: mock depleted extract. Top right: extracts depleted with carboxyl- and amino-terminal APC antibodies. Bottom left: extracts depleted of XMCAK. Bottom right: extracts depleted using carboxyl- and amino-terminal APC antibodies, rescued with recombinant XMCAK. The scale bar represents 10 μm.

(C) Bar graph quantifying the average spindle area in depleted extracts relative to those formed in mock-depleted extract. 50 spindle structures were measured for each extract type.

(D) Western blot of mock-depleted, APC-depleted, and APC-depleted rescued with recombinant XMCAK, probed for XMCAK.

amino-terminal APC antibodies, we next tested whether we could rescue the large spindle phenotype in these extracts (referred to as APC/XMCAK-depleted extracts) by the addition of recombinant XMCAK (Figures 1B–1D). A rescue was apparent, as the average area of the microtubule arrays was reduced by greater than 95%, and bipolar spindles with aligned chromosomes were observed. Although, in the add-back experiment, XMCAK was restored to approximately endogenous levels (Figure 1D), the spindles were smaller than the mock-depleted control reaction (Figure 1C). Because the microtubule arrays formed in the APC/XMCAK-depleted extracts were also smaller than those formed in extracts depleted directly with the XMCAK antibody (Figure 1C), we hypothesize that these size differences may be due to the additional loss of APC, a microtubule stabilizer [3–5], from the APC/XMCAK-depleted extracts. In line with our results, Dikovskaya et al. have recently shown that spindles formed in *Xenopus* egg extracts depleted of APC, using antibodies that do not codeplete XMCAK, are smaller than control spindles [9]. Therefore, both APC and XMCAK globally influence microtubule dynamics in egg extracts and appear to constitute opposing forces on microtubule stability.

APC Localizes to Both the Inner Centromere and the Kinetochore in Extract Spindles

To begin to address whether APC together with XMCAK could be regulating microtubule dynamics locally as well as globally, we examined its mitotic localization in cycled egg extracts. APC has been previously observed at the kinetochore and along spindle microtubules [6–9]. In extract reactions that had been cycled through interphase to allow DNA and kinetochore replication, then back into mitosis, APC antibody stained spindle microtubules and several locations on the chromosomes (Figures 3A–3G and 3L). To determine APC's precise chromosome localization, we costained for BubR1, a protein that localizes to the kinetochore of each sister chromatid. The localization of APC in relation to BubR1 could be grouped into four major categories (Figures 3A–3G and 3L). In 27% of the BubR1 pairs examined, APC was primarily observed between the two BubR1 dots, indicative of an inner-centromere localization (Figures 3B and 3L). In 34% of the chromosomes examined, APC colocalized with BubR1 at the kinetochore (Figures 3C, 3D, and 3L). Eighty percent of the chromatid pairs in this category had APC staining at only one of the kinetochores. A third category containing 19% of the BubR1 pairs examined had APC staining at both the inner centromere and the kinetochore (Figures 3E, 3F, and 3L). APC was on only one kinetochore in 60% of the chromatid pairs placed in this category. Finally, in 20% of chromosomes examined, APC could not be seen at either the kinetochore or inner centromere (Figures 3G and 3L). Similar to what has been reported previously, we found XMCAK along spindle microtubules and at the

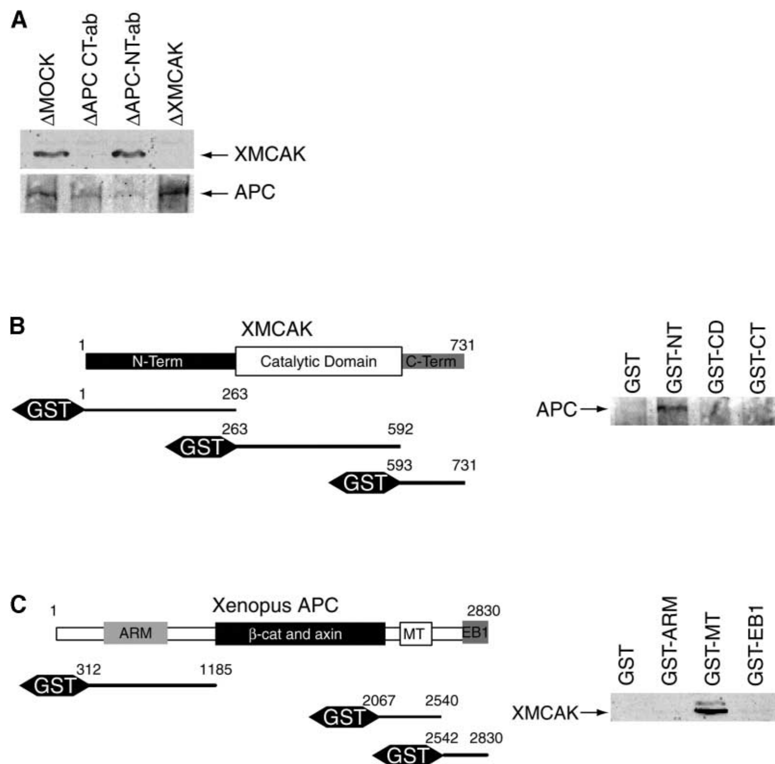

Figure 2. APC and XMCAK Associate in Extract

(A) Extracts depleted with control IgG (mock), APC carboxyl-terminal antibody, APC amino-terminal antibody, or XMCAK antibody were blotted for XMCAK (top) and APC (bottom).

(B) Left: schematic of GST fusion proteins to the amino terminus (GST-NT), central motor domain (GST-CD), and carboxyl-terminus (GST-CT) of XMCAK tested for their ability to precipitate APC from extract. Right: Western blot of precipitates probed for APC.

(C) Left: schematic of GST fusion proteins to the armadillo repeat domain (GST-ARM), basic region (GST-MT), and EB1 binding domain (GST-EB1) of APC tested for their ability to precipitate XMCAK from extract. Right: Western blot of precipitates probed for XMCAK.

inner centromere [10, 12] (Figures 3H, 3I, and 3L). We also observed a few examples of XMCAK at the kinetochore (Figures 3J, 3K, and 3L), which has recently been observed in mammalian tissue culture cells as well [17, 18]. In summary, we find that, like XMCAK, a substantial pool of APC is found at the inner centromere. The localization of MCAK to the kinetochore has been shown to correlate with changes in tension across the paired chromatids [17, 18]. Perhaps the dynamic localization of APC is also a response to such changes.

APC Localizes to the Centromere/Kinetochore in the Absence of Microtubules

In mammalian tissue-culture cells, APC has been shown to require microtubules to localize to the kinetochore [6, 7]. However, many inner-centromere proteins such as Aurora B and XMCAK do not require microtubules for their localization [19, 20]. Because we observed a significant pool of APC at the inner centromere in extract spindles, we tested whether microtubules were required for APC localization to chromosomes in this system. Extracts were cycled through interphase and back into mitosis in the presence of a nocodazole. Under these conditions, centromeric and kinetochore localization cannot be distinguished. In this situation, APC colocalized with BubR1 at the centromere/kinetochore region in the absence of microtubules (Figures 4A–4C). In agreement with previous work, we found that XMCAK also localized to the centromere/kinetochore in the absence of microtubules (Figures 4G–4I) [20]. Because the APC results are in contrast to those in mammalian cells [6, 7], it is possible that in the absence of microtubules we are specifically observing the localization of the centromere binding pool of APC that is unique to extract spindles. However, microtubules are probably not necessary for bringing APC itself to the kinetochore in mammalian tissue culture cells because the carboxyl-terminal microtubule binding domains of APC are not required for this localization [6]. Instead, microtubules may be necessary for the delivery of other kinetochore proteins, which are in turn required for APC localization. It is therefore possible that the complex that recruits APC to the centromere/kinetochore in extracts is identical to that in mammalian tissue culture cells, but in extracts, this complex is able to localize to the centromere/kinetochore in the absence of microtubules. Notably, in the presence of microtubules, 20% of the BubR1 pairs we examined had no associated APC staining at either the kinetochore or inner centromere (Figures 3G and 3L), yet in the absence of microtubules, we seldom observed BubR1 staining without corresponding APC staining (Figures 4A–4C). We propose that once the kinetochore/microtubule attachment has been established, APC can travel between the microtubule and centromere/kinetochore perhaps in response to tension.

APC Localizes to the Centromere/Kinetochore in the Absence of XMCAK

Because APC and XMCAK can interact in extracts, we next wanted to test whether APC localization to the centromere requires XMCAK. As a result of the dense microtubule network surrounding the chromosomes in the XMCAK-depleted extracts, we were unable to detect any kinetochore or centromere components by immunofluorescence (data not shown). We therefore cycled the chromosomes in XMCAK-depleted extracts in the presence of nocodazole. Under these conditions, APC was able to localize to the centromere/kinetochore in the

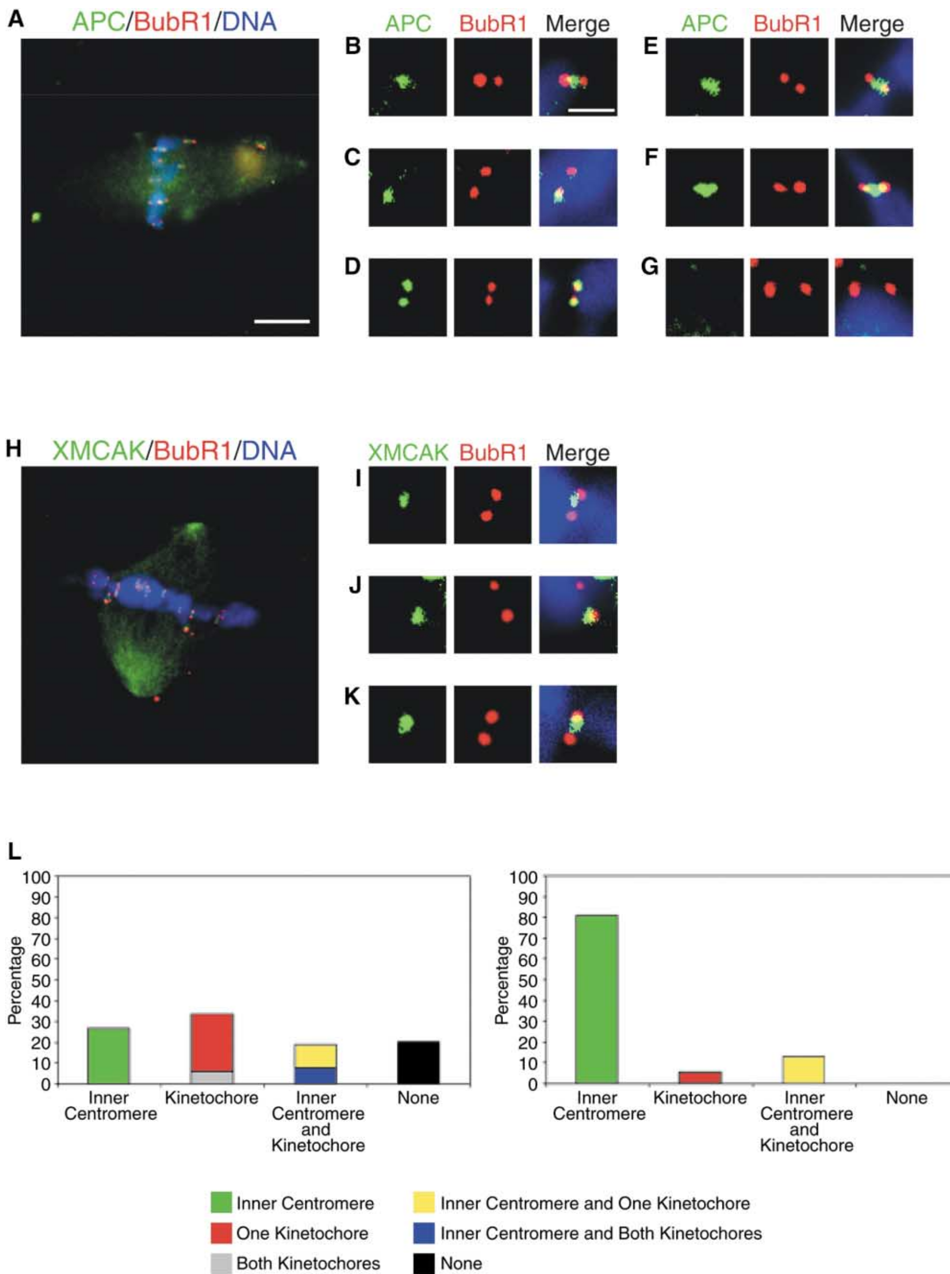

Figure 3. APC Localizes to the Kinetochore and Centromere of Metaphase Chromosomes in Egg Extracts

(A–G) Immunofluorescence image of APC (green), BubR1 (red), and DNA (blue). (A) APC is localized to the centromere, kinetochore, and microtubules on metaphase spindles. The scale bar represents 10 μm. (B–G) Examples of the categories of APC localization, relative to BubR1, observed in metaphase spindles. The scale bar represents 2.5 μm. (B) APC at the inner centromere. This image is an inset from the spindle shown in (A). (C) APC at one kinetochore. (D) APC at both kinetochores. (E) APC at the inner centromere and one kinetochore. (F) APC at the inner centromere and both kinetochores. (G) No APC at the inner centromere or the kinetochore. (H–K) Immunofluorescence image of XMCAK (green), BubR1 (red), and DNA (blue). (H) XMCAK is localized primarily to the centromere and microtubules on metaphase spindles. (I–K) Examples of the categories of XMCAK localization, relative to BubR1, observed in metaphase spindles. (I) XMCAK at the inner centromere. This image is an inset from the spindle shown in (H). (J) XMCAK at one kinetochore. (K) XMCAK at the inner centromere and one kinetochore. (L) Bar graph summarizing the localization of APC (left) or XMCAK (right), relative to BubR1. For APC, a total of 193 BubR1 pairs from 34 spindles were observed. For XMCAK, a total of 68 BubR1 pairs from 7 spindles were observed.

absence of XMCAK (Figures 4D–4F and 4J–4L). We next wanted to perform the reciprocal experiment to determine if XMCAK requires APC for centromere localization. An indirect piece of evidence for this model is the fact that the amino terminus of XMCAK that binds to the APC complex contains the centromere localization domain of XMCAK [12]. However, we were unable to completely deplete APC from the extracts. Given that XMCAK is found primarily at the inner centromere of metaphase chromosomes, yet 54% of chromosome

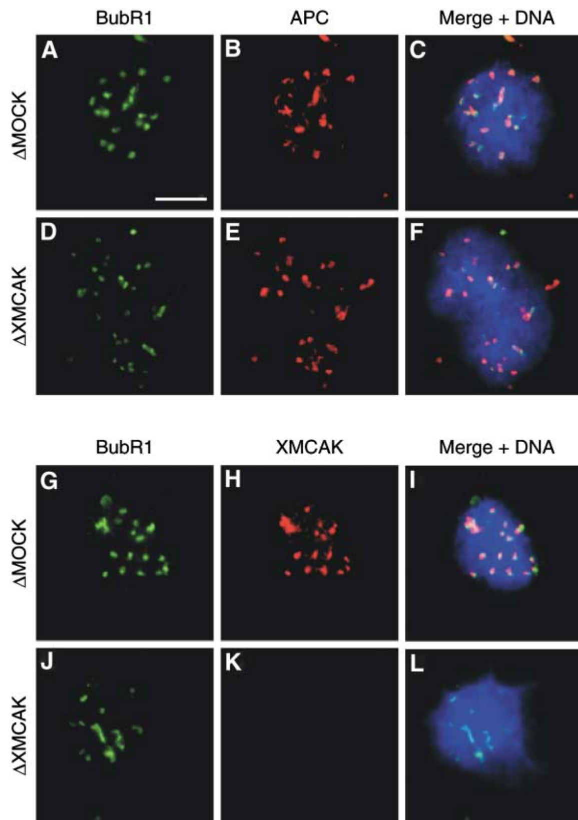

Figure 4. APC Localization to the Centromere/Kinetochore in *Xenopus* Egg Extracts Does Not Require Microtubules or XMCAK

(A–F) Immunofluorescence images of chromosomes (in blue) cycled in egg extracts in the presence of the microtubule inhibitor, nocodazole. BubR1 is in green and APC is in red. The scale bar represents 10 μm. (A–C) Mock-depleted extracts. (D–F) XMCAK-depleted extracts. (G–L) Immunofluorescence images of chromosomes (in blue) cycled in egg extracts in the presence of nocodazole. BubR1 is in green and XMCAK is in red. (G–I) Mock-depleted extracts. (J–L) XMCAK-depleted extracts.

pairs we observed had no detectable APC staining at the inner centromere, it seems unlikely that APC is required for XMCAK centromere localization. At this time, we cannot rule out the possibility that APC is required for the kinetochore localization of XMCAK.

Implications of the APC/XMCAK interaction

We hypothesize that the cytoplasmic association of APC with XMCAK could allow regulatory factors also associated with the complex to directly modulate their opposing activities, thereby providing precise control of overall microtubule length and dynamics. We are currently attempting to identify other components of the APC/XMCAK complex that may allow us to better understand its function. The activity of both proteins has been shown to be modulated by phosphorylation. Thus, kinases such as Aurora B [18, 21] and GSK3β [4] are obvious candidates for regulators of this complex. APC [6–8] and XMCAK [12] are both required for proper chromosome attachment. We have shown that both proteins can localize to the centromere and kinetochore regions of mitotic chromosomes. Although neither APC nor XMCAK appears to be necessary for the localization of the other protein to the centromere, it is possible that the soluble APC/XMCAK complex we have identified localizes to the chromosome-microtubule attachment site via other members of the complex or that the complex can reassemble on the chromosome. The activity of the two proteins could then be modulated locally to control the attachment of microtubules to chromosomes.

Conclusions

We have shown that APC and XMCAK are present in a complex in cytoplasmic extracts. Furthermore, these proteins can both localize to the centromere and kinetochore regions of mitotic chromosomes. Such a complex could allow for the direct regulation of their opposing activities by additional factors, thereby providing for the precise control of microtubule dynamics, both globally and at microtubule-chromosome attachment sites.

Supplemental Data

Supplemental Data including Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/14/22/2033/DC1/.

Acknowledgments

We thank C. Walczak and I. Näthke for invaluable advice and reagents; D. Cleveland for the BubR1 antibody; J. Miller for *Xenopus* APC cDNA plasmids; H. Niederstrasser for XMCAK; George Nads for nuclei preparation; the Heald, Weis, and Welch labs for helpful discussions; P. Kalab for technical advice; and S. Wignall for careful review of the manuscript. J.D.B. was supported by National Institutes of Health (NIH) postdoctoral fellowship grant GM20767. This research was supported by NIH grant GM057839 to R.H.

Received: July 12, 2004
Revised: September 21, 2004
Accepted: September 23, 2004
Published: November 23, 2004

References

1. Wittmann, T., Hyman, A., and Desai, A. (2001). The spindle: A dynamic assembly of microtubules and motors. Nat. Cell Biol. *3*, E28–E34.
2. Biggins, S., and Walczak, C.E. (2003). Captivating capture: How microtubules attach to kinetochores. Curr. Biol. *13*, R449–R460.
3. Deka, J., Kuhlmann, J., and Muller, O. (1998). A domain within the tumor suppressor protein APC shows very similar biochemical properties as the microtubule-associated protein tau. Eur. J. Biochem. *253*, 591–597.
4. Zumbrunn, J., Kinoshita, K., Hyman, A.A., and Nathke, I.S. (2001). Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3 beta phosphorylation. Curr. Biol. *11*, 44–49.
5. Munemitsu, S., Souza, B., Muller, O., Albert, I., Rubinfeld, B., and Polakis, P. (1994). The APC gene product associates with microtubules in vivo and promotes their assembly in vitro. Cancer Res. *54*, 3676–3681.
6. Fodde, R., Kuipers, J., Rosenberg, C., Smits, R., Kielman, M., Gaspar, C., van Es, J.H., Breukel, C., Wiegant, J., Giles, R.H., et al. (2001). Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat. Cell Biol. *3*, 433–438.
7. Kaplan, K.B., Burds, A.A., Swedlow, J.R., Bekir, S.S., Sorger, P.K., and Näthke, I.S. (2001). A role for the Adenomatous Polyposis Coli protein in chromosome segregation. Nat. Cell Biol. *3*, 429–432.
8. Green, R.A., and Kaplan, K.B. (2003). Chromosome instability in colorectal tumor cells is associated with defects in microtubule

plus-end attachments caused by a dominant mutation in APC. J. Cell Biol. *163*, 949–961.
9. Dikovskaya, D., Newton, I.P., and Näthke, I.S. (2004). The adenomatous polyposis coli protein is required for the formation of robust spindles formed in CSF Xenopus extracts. Mol. Biol. Cell *15*, 2978–2991.
10. Walczak, C.E., Mitchison, T.J., and Desai, A. (1996). XKCM1: a Xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. Cell *84*, 37–47.
11. Walczak, C.E. (2003). The Kin I kinesins are microtubule end-stimulated ATPases. Mol. Cell *11*, 286–288.
12. Walczak, C.E., Gan, E.C., Desai, A., Mitchison, T.J., and Kline-Smith, S.L. (2002). The microtubule-destabilizing kinesin XKCM1 is required for chromosome positioning during spindle assembly. Curr. Biol. *12*, 1885–1889.
13. Kline-Smith, S.L., and Walczak, C.E. (2002). The microtubule-destabilizing kinesin XKCM1 regulates microtubule dynamic instability in cells. Mol. Biol. Cell *13*, 2718–2731.
14. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., et al. (1991). Identification and characterization of the familial adenomatous polyposis coli gene. Cell *66*, 589–600.
15. Su, L.K., Burrell, M., Hill, D.E., Gyuris, J., Brent, R., Wiltshire, R., Trent, J., Vogelstein, B., and Kinzler, K.W. (1995). APC binds to the novel protein EB1. Cancer Res. *55*, 2972–2977.
16. Peifer, M., Berg, S., and Reynolds, A.B. (1994). A repeating amino acid motif shared by proteins with diverse cellular roles. Cell *76*, 789–791.
17. Kline-Smith, S.L., Khodjakov, A., Hergert, P., and Walczak, C.E. (2004). Depletion of centromeric MCAK leads to chromosome congression and segregation defects due to improper kinetochore attachments. Mol. Biol. Cell *15*, 1146–1159.
18. Andrews, P.D., Ovechkina, Y., Morrice, N., Wagenbach, M., Duncan, K., Wordeman, L., and Swedlow, J.R. (2004). Aurora B regulates MCAK at the mitotic centromere. Dev. Cell *6*, 253–268.
19. Murata-Hori, M., Tatsuka, M., and Wang, Y.L. (2002). Probing the dynamics and functions of aurora B kinase in living cells during mitosis and cytokinesis. Mol. Biol. Cell *13*, 1099–1108.
20. Desai, A., Deacon, H.W., Walczak, C.E., and Mitchison, T.J. (1997). A method that allows the assembly of kinetochore components onto chromosomes condensed in clarified Xenopus egg extracts. Proc. Natl. Acad. Sci. USA *94*, 12378–12383.
21. Lan, W., Zhang, X., Kline-Smith, S.L., Rosasco, S.E., Barrett-Wilt, G.A., Shabanowitz, J., Hunt, D.F., Walczak, C.E., and Stukenberg, P.T. (2004). Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity. Curr. Biol. *14*, 273–286.