β-Catenin is a multifunctional protein that is known to participate in two well defined cellular processes, cell-cell adhesion and Wnt-stimulated transcriptional activation. Here we report that β-catenin participates in a third cellular process, the establishment of a bipolar mitotic spindle. During mitosis, β-catenin relocalizes to mitotic spindle poles and to the midbody. Furthermore, biochemical fractionation demonstrates the presence of β-catenin in purified centrosome preparations. Reduction of cellular β-catenin by RNA interference leads to the failure of centrosomes to fully separate, resulting in a marked increase in the frequency of monoastral mitotic spindles. Our results define a new and important function for β-catenin in mitosis and demonstrate that β-catenin is involved in vital biological processes beyond cell adhesion and Wnt signaling.

The faithful distribution of the genetic material between daughter cells during cell division requires the precise segregation of duplicated chromosomes. This crucial duty is performed by a microtubule-based machine known as the mitotic spindle (1). The dynamic interactions of numerous structural and motor proteins underlie the formation of a bipolar mitotic spindle (1, 2). However, many of the molecules that regulate spindle assembly remain elusive (3).

β-Catenin is a multifunctional protein with critical roles in both cell-cell adhesion and transcriptional regulation (4, 5). In cooperation with α-catenin, β-catenin links cadherins to the actin cytoskeleton, thereby establishing a functional complex that is required for strong cell-cell adhesion (4, 6, 7). β-Catenin also functions in signal transduction as a component of the Wnt/Wingless pathway that is crucial for development (5, 8, 9). β-Catenin has a cellular role in addition to cell adhesion and Wnt signaling. We demonstrate that β-catenin is a component of the mammalian mitotic spindle and that it functions to ensure proper centrosome separation and the subsequent establishment of a bipolar spindle.

**EXPERIMENTAL PROCEDURES**

**Materials**—The cDNAs for Xenopus β-catenin-myc and β-catenin-GFP (green fluorescent protein) were a gift from Randall Moon (University of Washington) and that for Myc-ΔN-TCF was a gift from Bert Vogelstein (Johns Hopkins University). Anti-β-catenin mouse monoclonal antibodies were from Zymed Laboratories Inc. and BD Transduction Laboratories; anti-β-tubulin-Cy3 directly conjugated mouse monoclonal antibody and anti-γ-tubulin mouse monoclonal antibody were from Sigma; anti-c-myc rabbit polyclonal antibody was from Santa Cruz Biotechnology; and anti-c-myc mouse monoclonal antibody was from Zymed Laboratories Inc.

**Cell Culture and Transfection**—L and HEK 293 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum; NIH 3T3 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% calf serum; and HeLa cells were grown in MEM Dulbecco’s modification containing 10% fetal bovine serum, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate. For Fig. S1, L or NIH 3T3 cells were transfected using LipofectAMINE (Invitrogen) with an expression vector encoding Xenopus β-catenin fused to GFP (14).

**Generation of Stable HeLa Cell Lines**—Xenopus β-catenin with six myc epitopes appended to the C terminus was subcloned into the retroviral vector pLPCX (BD Biosciences). Control or Xenopus β-catenin retrovirus was produced by transfecting a HEK 293 virus-packaging cell line with empty pLPCX vector or pLPCX encoding Xenopus β-catenin, respectively. Recombinant retroviruses were harvested from the media of the transfected HEK 293 cells and used to infect HeLa cells. HeLa cells expressing the viruses were selected and maintained in normal HeLa media additionally containing puromycin (2 μg/ml).

**Production of Wnt-3A-conditioned Media**—Media were harvested from confluent L cells expressing Wnt-3A or a control vector (courtesy of Karl Willert, Stanford University), cleared by centrifugation, and filtered.

**Immunofluorescence and Microscopy**—Cells shown in Figs. 1 and 3C were grown on coverslips and fixed rapidly according to established protocols (15, 16) in 4% paraformaldehyde, pH 11, for 10 min at room temperature. The same staining pattern was observed with an alternate protocol (17) involving permeabilization in 0.5% Triton X-100 and fixation at pH 7 (Figs. 3D and S1, A and B). Antibody incubations were as follows: Figs. 1, 3C, and S1, A and B, Zymed Laboratories Inc. anti-β-catenin (1:100), fluorescein isothiocyanate-conjugated anti-mouse (1:100), and Cy3-conjugated anti-β-tubulin (1:100); Fig. 3D, Sigma anti-γ-tubulin (1:1000), fluorescein isothiocyanate-conjugated anti-mouse (1:100), and Cy3-conjugated anti-β-tubulin (1:100). In all cases, DNA was stained using Hoechst 33342 (1:1000) in phosphate-buffered saline. For Fig. 3D, centrosome number was assessed by γ-tubulin staining as described previously (18). Images were obtained using an LSM-410 laser scanning confocal microscope with a 40× or 63× oil immersion objective (Carl Zeiss). All images are single confocal sections with the exception of those in Fig. S1C, which were taken using a Nikon TE300 fluorescence light microscope.

**Centrosome Isolation**—Ten 15-cm plates of mouse L fibroblasts or HeLa cells were grown to confluence and then treated with 10 μg/ml

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[ ] The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

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* The abbreviations used are: TCF, T cell factor; GFP, green fluorescent protein; siRNA, small interfering RNA.
noodazole and 5 μM -cytochalasin B for 90 min. Centrosomes were isolated according to the method of Mitchison and Kirschner (19). Briefly, cells were subjected to a sequence of rapid washes and then lysed, and the lysate was cleared and filtered. This lysate was centrifuged on a cushion of 20% Ficoll, to concentrate centrosomes. Crude centrosomal material was collected from the surface of the Ficoll cushion using a Pasteur pipette, assayed by refractometry (Milton Roy Co.) to ensure that the Ficoll concentration was ~10%, and then loaded onto a continuous sucrose gradient, ranging from 20 to 62.5% sucrose. The gradient was centrifuged at 27,000 rpm in a Beckman SW28 ultracentrifuge rotor at 4 °C.

RNA Interference—Double-stranded 21-mer RNA oligonucleotides homologous to sequences in human β-catenin (20) and the luciferase plasmid pGLO2 (21) were purchased as desalted, deprotected, purified duplexes (Dharmacon Research). HeLa cells were transfected with the RNA duplexes using Oligofectamine as described previously (20) and harvested 64 h post-transfection for analysis.

Immunoblotting—Equal amounts of each fraction (for analysis of sucrose fractions from the centrosome preparation) or equal amounts of protein from each small interfering RNA (siRNA)-transfected cell lysate (for analysis of siRNA-mediated reduction of protein levels) were heated in SDS sample buffer, separated by SDS-PAGE, and transferred to nitrocellulose membranes. Immunoblot analyses were performed using anti-β-catenin (BD Transduction Laboratories), anti-γ-tubulin (Berkley Antibody Co.), anti-plakoglobin (Zymed Laboratories Inc.), and anti-actin, followed by the addition of horseradish peroxidase-conjugated secondary antibodies and subsequent chemiluminescence detection.

Analysis of Spindle Morphology—HeLa cells were fixed 64 h after transfection with siRNA, and immunofluorescence staining was performed as described above. Mitotic cells were scored as having normal bipolar spindles or monoastral spindles, and the percentage of mitotic cells with monostral spindles were represented as (%No. of monostral spindles/total no. of mitotic cells) × 100. Data represent the mean ± S.E. of the mean of three independent experiments in which >2000 cells were analyzed per experiment.

RESULTS AND DISCUSSION

We were prompted to investigate the possibility that β-catenin has an important cellular role in addition to cell adhesion and Wnt signaling while examining the fate of β-catenin released from the cadherin cytoplasmic domain (22, 23). In the process of these studies, we noticed an unusual staining pattern of β-catenin in mitotic cells and decided to examine its distribution more carefully during the cell cycle. During interphase, endogenous β-catenin was seen to localize diffusely throughout the cytoplasm and nucleus and at cell-cell junctions (Fig. S1A). However, during M phase, β-catenin localized strikingly to the centrosomal region at the poles of the mitotic spindle and to the spindle midzone during midbody formation (Fig. 1). This staining pattern was observed in mitotic L, NIH 3T3, PtK1, and HeLa cells. β-Catenin first became concentrated at centrosomes during metaphase (Fig. 1A) and remained at the spindle poles during anaphase and early telophase (Fig. 1, B and C). As the cleavage furrow constricted between the two daughter cells, β-catenin also appeared at the midbody (Fig. 1C). By late telophase, β-catenin was no longer seen at the centrosomes, but it remained concentrated at the midbody (Fig. 1D). This staining pattern was not observed when the primary antibody was pre-incubated with purified β-catenin, nor was it observed when the primary antibody or secondary antibody was omitted, nor when a control IgG primary antibody was used (data not shown). Furthermore, when a fusion of β-catenin to GFP (14) was expressed in L cells and NIH 3T3 cells, the protein localized to the spindle poles and the midbody in live, mitotic cells (Fig. S1C), just as was observed for endogenous β-catenin in fixed cells. Additionally, we were unable to detect any concentration of the β-catenin homolog plakoglobin at mitotic spindle poles or the midbody, indicating that the observed localization is specific for β-catenin (data not shown). This dramatic redistribution of β-catenin during mitosis is suggestive of a function for this protein distinct from its interphase roles.

![Fig. 1. β-Catenin localization in mitotic cells.](http://www.jbc.org/)

As an independent approach to confirm the association of β-catenin with centrosomes, we isolated centrosomes from L cells and HeLa cells using an established method (19). Immunoblot analysis of fractions from the final step in the purification procedure revealed that β-catenin was indeed present in this centrosomal preparation, where it co-fractionated with γ-tubulin (Fig. 2, top panel), a core component of the centrosome (24). The two proteins were concentrated at the position of the sucrose gradient corresponding to a sucrose concentration of 49–51% (Fig. 2, bottom panel), the position where cen-
trosomes have been reported to sediment (19).

To examine the functional consequence of the surprising localization of β-catenin, we reduced the level of β-catenin in HeLa cells using siRNA (Fig. 3A). Examination of mitotic cells in which β-catenin levels were reduced by ~90% (Fig. 3A) revealed a marked increase in the frequency of monoastral spindles (Fig. 3, B and C). This finding suggests a role for β-catenin in establishing spindle bipolarity. To determine whether this effect was direct or mediated via a transcriptional target of β-catenin, we overexpressed a dominant-negative mutant of TCF (25) to block β-catenin-mediated transcriptional activation. This treatment completely abrogated Wnt-3A-stimulated activation of a β-catenin-responsive transcriptional reporter but did not induce formation of monoastral spindles (data not shown). Thus, the effect on spindle morphology appears to result directly from the depletion of β-catenin from centrosomes. However, we cannot rule out the possibility that spindle formation might involve cortical or cytoplasmic β-catenin acting in a hitherto undescribed role.

Monastral spindles can result from defects in centrosome duplication or from defects in centrosome separation (26). To begin to explore the mechanism through which depletion of β-catenin disrupts bipolar spindle formation, we assessed the number of centrosomes in the monoastral spindles of β-catenin-depleted cells. All of the monoastral spindles examined exhibited two puncta of γ-tubulin staining (Fig. 3D, arrowheads), indicating that the centrosomes had duplicated but were not properly separated.

To verify that the observed increase in monoastral mitotic spindles was specifically due to loss of β-catenin, we performed a rescue experiment by reintroducing a form of β-catenin that would be refractory to siRNA. We accomplished this using Xenopus β-catenin, the amino acid sequence of which is 95% identical to human β-catenin but which has two nucleotides in the siRNA target sequence that differ from those in human β-catenin. Because even a single mismatch in the target sequence has been shown to abolish siRNA-mediated gene silencing (27), we reasoned that Xenopus β-catenin should be highly refractory to siRNA directed against human β-catenin. We generated stable HeLa cell lines expressing either myc-tagged Xenopus β-catenin or a control vector (Fig. 4A) and then used siRNA to knock down the endogenous β-catenin without disrupting the ectopically expressed Xenopus β-catenin (Fig. 4A). Examination of the spindle morphology of these cells revealed that treatment with β-catenin siRNA resulted in a clear increase in monoastral mitotic spindles in those cells expressing the control retrovirus but no increase in those cells expressing the Xenopus β-catenin retrovirus (Fig. 4B). These data indicate that introduction of Xenopus β-catenin can rescue the mitotic defect caused by β-catenin siRNA treatment and provide verification that the observed increase in monoastral mitotic spindles is due specifically to the loss of β-catenin. The specificity of this phenotype is further demonstrated by our observations that reduction of plakoglobin levels using siRNA does not result in an increase in monoastral mitotic spindles and that plakoglobin overexpression cannot effectively rescue the monoastral spindle phenotype.
caused by reduction of cellular β-catenin.2

The identification herein of a novel function for β-catenin in centrosome separation suggests that β-catenin plays a more general role as a molecular scaffold than previously thought. This notion is supported by some interesting observations that have come from genetic studies in Drosophila and Caenorhabditis elegans suggesting a possible role for homologs of β-catenin in orienting or anchoring the mitotic spindle (28, 29), as well as by the finding that β-catenin may anchor microtubules at adherens junctions via its interaction with dynein (30). In both cell adhesion and Wnt signaling, β-catenin acts as an adaptor to direct the local formation of functional protein complexes (31). It seems likely that β-catenin is employed in a similar capacity in mitotic spindle formation. A complex assembly of many structural and motor proteins is required to establish a bipolar mitotic spindle (1, 2). β-Catenin may regulate this dynamic process to ensure that components of the mitotic machinery are assembled in the correct place and at the correct time for cell division to proceed efficiently. Clearly, the identification of the binding partners of β-catenin at the centrosomes and midbody will be essential to a complete mechanistic understanding of its role in mitosis.

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Identification of a Role for β-Catenin in the Establishment of a Bipolar Mitotic Spindle
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