Current Biology *19*, 816–826, May 26, 2009 DOI 10.1016/j.cub.2009.04.033

Article

HAUS, the 8-Subunit Human Augmin Complex, Regulates Centrosome and Spindle Integrity

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Summary

Background: The assembly of a robust microtubule-based mitotic spindle is a prerequisite for the accurate segregation of chromosomes to progeny. Spindle assembly relies on the concerted action of centrosomes, spindle microtubules, molecular motors, and nonmotor spindle proteins.

Results: Here we use an RNA-interference screen of the human centrosome proteome to identify novel regulators of spindle assembly. One such regulator is HAUS, an 8-subunit protein complex that shares homology to *Drosophila* Augmin. HAUS localizes to interphase centrosomes and to mitotic spindle microtubules, and its disruption induces microtubule-dependent fragmentation of centrosomes along with an increase in centrosome size. HAUS disruption results in the destabilization of kinetochore microtubules and the eventual formation of multipolar spindles. These severe mitotic defects are alleviated by codepletion of NuMA, indicating that both factors regulate opposing activities. HAUS disruption alters NuMA localization, suggesting that mislocalized NuMA activity contributes to the spindle and centrosome defects observed.

Conclusion: The human Augmin complex (HAUS) is a critical and evolutionary conserved multisubunit protein complex that regulates centrosome and spindle integrity.

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Introduction

The mitotic spindle is a dynamic microtubule-based structure that mediates the accurate segregation of chromosomes during division. To assemble a bipolar mitotic spindle, the cell relies on the coordinated action of centrosomes, molecular motors, and nonmotor spindle proteins that modulate the behavior of microtubules. Microtubule nucleation can be initiated from various cellular locations including centrosomes, chromosomes, and from within the mitotic spindle itself [1]. Centrosomes anchor and stabilize the minus ends of microtubules and represent the major microtubule-organizing centers (MTOCs) in mammalian cells. Centrosomes are composed of a pair of 9-fold symmetric barrel-shaped centrioles that mark the site where a proteinaceous matrix of microtubule-nucleating pericentriolar material (PCM) is assembled. In order to establish a bipolar mitotic spindle, the centrosome first needs to duplicate so that two centrosomes are present during mitosis, each organizing one mitotic spindle pole [2]. Microtubules are nucleated from centrosomes by multisubunit complexes containing γ-tubulin, and their recruitment to the vicinity of centrioles is mediated at least in part by NEDD1 and CEP192 [3–6]. How this process is coupled to bipolar spindle assembly and how large complexes akin to γ-TuRCs are regulated during centrosome maturation and spindle assembly remains poorly understood.

Microtubule nucleation from chromosomes and from within the mitotic spindle plays a prominent role in its assembly process. Indeed, mitotic spindles can readily form in the absence of centrosomes, emphasizing the importance of such nucleation pathways [1, 7–9]. Microtubule nucleation from chromatin is regulated by a Ran-GTP gradient in the vicinity of chromosomes [10, 11]. Within the mitotic spindle, microtubule nucleation is mediated by docking of γ-tubulin and associated proteins onto preexisting microtubules, leading to amplification of microtubule polymer mass [12–14]. The *Drosophila* Augmin complex and one of its subunits, Wac, regulate γ-tubulin recruitment within the spindle [12, 15]. FAM29A, the human homolog of the *Drosophila* Augmin subunit Dgt6, is also required for the recruitment of γ-tubulin to spindle microtubules [12, 13]. Phenotypes analogous to those observed in FAM29A RNAi-treated cells also occur upon the depletion of the microtubule binding protein HICE1 [16]. Kinesin family members and cytoplasmic dynein also participate in spindle assembly, the fine balance of their activities necessary for robust assembly [17, 18]. Nonmotor spindle proteins, like NuMA, play prominent roles in mitotic spindle assembly by focusing mitotic spindle poles and bundling spindle microtubules [19–25]. The coordinated action of both motor and nonmotor spindle proteins is thus necessary for robust bipolar spindle formation.

Here we identify an 8-subunit complex with homology to *Drosophila* Augmin [12, 13] that we have named HAUS, for homologous to Augmin subunits. The HAUS complex is required for mitotic spindle assembly and for maintenance of centrosome integrity. NuMA codepletion suppresses the spindle assembly defects associated with HAUS depletion, suggesting that HAUS regulates mitotic spindle assembly by opposing NuMA activity.

Results

Identification of Novel Regulators of Mitotic Spindle Assembly

In 2003, Andersen and colleagues defined the complement of proteins biochemically enriched in centrosome preparations [26]. We hypothesized that these 113 proteins, henceforth referred to as the centrosome proteome, might contain uncharacterized regulators of mitotic spindle assembly. We generated endoribonuclease-prepared siRNAs (esiRNAs) targeting the 113 members of the centrosome proteome (Figure S1 and Table S1 available online). HeLa cells were seeded in 96-well plates and transfected with the centrosome proteome esiRNA minilibrary, or with an esiRNA against firefly luciferase as negative control. Cells were fixed 72 hr after transfection and stained to visualize chromosomes (DAPI), centrosomes (Pericentrin), microtubules (α-tubulin), and mitotic cells (Phospho-Histone H3) (Figure S1A). Our analysis revealed that 44/113 (39%) of the esiRNAs caused an increase in mitotic index greater than two standard deviations above control luciferase-transfected cells (Z score > 2; Figure S1B). Importantly, esiRNA against four proteins with previously uncharacterized roles in mitosis scored highly (Z score > 5) including C4ORF15, C14ORF94, KIAA0841, and CEP70 (Figure S1B). To determine whether the high mitotic index observed was a consequence of underlying defects in spindle assembly, we analyzed mitotic profiles (Figure S1A). This analysis revealed that 40/113 (35%) of the esiRNA tested caused a significant increase in abnormal spindle percentage (Z score > 2; Figure S1C). The complete data sets (mitotic indices, percent abnormal spindles, statistics, and correlation scores) are included in Table S1 and Figure S2.

C4ORF15 and C14ORF94 Are Required for Mitotic Centrosome and Spindle Integrity

To further scrutinize the spindle assembly defect observed upon C4ORF15 and C14ORF94 knockdown, HeLa cells were transfected with esiRNA targeting luciferase (as a negative control), C4ORF15, and C14ORF94 and analyzed at high resolution via deconvolution microscopy. Control transfected cells exhibited bipolar mitotic spindles with morphologically normal centrosomes (Figure 1A). In contrast, we frequently observed disorganized bipolar mitotic spindles and multipolar spindles in C4ORF15 or C14ORF94 RNAi-treated cells (Figure 1A). Morphologically aberrant mitotic centrosomes were also frequently detected (Figure 1A, insets). Consistent with their enrichment in centrosome preparations, C4ORF15 and C14ORF94 localized to interphase centrosomes and to spindle microtubules during mitosis (Figure 1B) [4, 5]. Our results thus far suggest that C4ORF15 and C14ORF94 are two novel centrosome proteins that relocalize to spindle microtubules at the onset of mitosis and are implicated in the regulation of mitotic spindle assembly and centrosome integrity.

A Novel Multisubunit Protein Complex Required for Mitotic Spindle Assembly

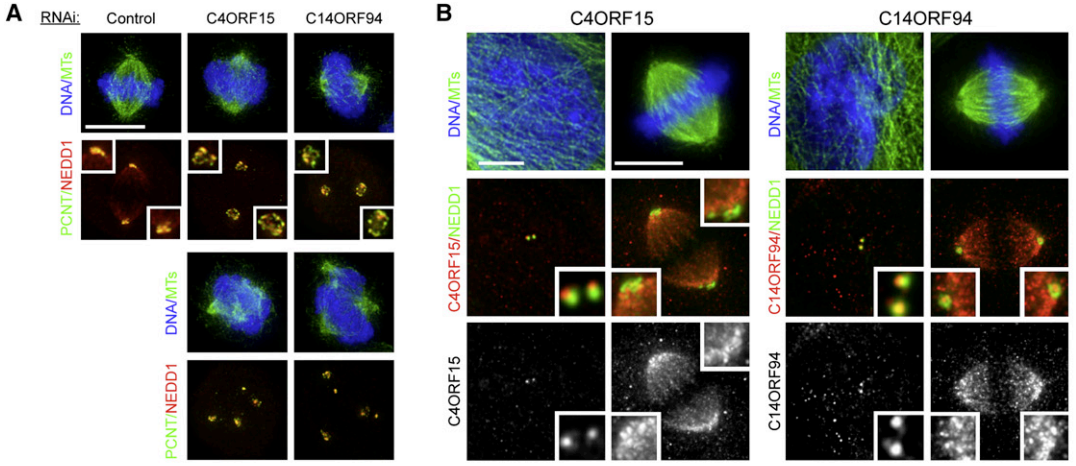
In collaboration with the MitoCheck consortium, we participated in a large-scale proteomic analysis of mitotic regulators where several genes identified in our RNAi screen were used as baits to identify interacting proteins (J.R.A.H., B.H., and J.M.P., unpublished data) [27]. Their initial experiments revealed that FAM29A interacted with several proteins, including the aforementioned C4ORF15 and C14ORF94, suggesting that they operate in a larger protein complex. To validate and further characterize associated proteins, C4ORF15 and C14ORF94 were FLAG tagged and subjected to FLAG immunopurification followed by mass spectrometry [28]. The number of peptides detected across two biological replicates are indicated in Figure 1C. C14ORF94 and C4ORF15 interacted with each other, FAM29A, two other hits from our RNAi screen (CEP27 and KIAA0841), and three other proteins (HICE1, CCDC5, and UCHL5IP) not part of the centrosome proteome (Figure 1C; Figure S3A). These six putative interactors were FLAG tagged and analyzed as above and in every case the seven other proteins were recovered (Figure 1C; Figure S3A). The MitoCheck consortium identified this 8-subunit complex independently.

To confirm these interactions, we performed coimmunoprecipitation experiments with FLAG-tagged C14ORF94 and FAM29A in combination with GFP variants of the other members of the complex, or an unrelated GFP-tagged negative control. FLAG-tagged C14ORF94 was recovered together with varying amounts of the GFP-tagged proteins in C14ORF94 immunoprecipitates (Figure 1D). Equivalent results were obtained with FLAG-tagged FAM29A as bait (data not shown). A schematic representation of the detected interactions between complex members is shown in Figure S3B. To estimate the molecular weight of this protein complex, HeLa cell extracts were separated on a Superose 6 column and the fractions blotted for CEP27, C4ORF15, and FAM29A. The three proteins peaked in fractions 11 and 12, which based on globular protein standards correspond to a Stokes radius between 12.3 and 15.5 (Figure 1E). Taken together, these results strongly suggest that we identified a high molecular weight 8-subunit complex required for mitotic spindle assembly in human cells.

The FAM29A subunit is homologous to Dgt6, a member of the 8-subunit Augmin complex in *Drosophila* [12, 13]. Three other subunits of the complex, CEP27, CCDC5, and HICE1, have been implicated in mitotic progression [16, 26, 29, 30]. The four remaining proteins (C14ORF94, C4ORF15, KIAA0841, and UCHL5IP) were not previously associated with a role in mitosis. Bioinformatic analyses of these eight proteins revealed that C4ORF15 and KIAA0841 share homology to Dgt3 and Dgt5 of *Drosophila* Augmin (Figures S4 and S5 and Supplemental Experimental Procedures). To reflect this homology, we henceforth refer to this protein complex as HAUS, for homologous to Augmin subunits and to individual subunits as HAUS1 to HAUS8 as indicated in Figure 1F.

The HAUS Complex Is Required for Mitotic Centrosome and Spindle Integrity

We investigated the spindle assembly defect induced by the depletion of the remaining six subunits. In control-transfected cells, spindles were bipolar with prominent NEDD1 labeling and centrosomes of normal morphology (Figure 2A) [4, 5]. In HAUS-depleted cells, disorganized bipolar mitotic spindles and multipolar spindles with fragmented centrosomes were detected (Figure 2A). This correlated with an increase in mitotic index and abnormal spindle percentage relative to control-transfected cells (Figure 2B). The amount of NEDD1 and γ-tubulin associated with spindle microtubules was reduced to 32% and 25% of control levels in HAUS2 and HAUS6 RNAi-treated cells (Figure 2C and data not shown). Microtubule density inside the spindle in HAUS2 and HAUS6 transfected cells was decreased to 66% and 57% of control levels, respectively (Figure 2D), consistent with previous results [12, 13]. Microtubule density at mitotic spindle poles was respectively increased 23% and 20% relative to control



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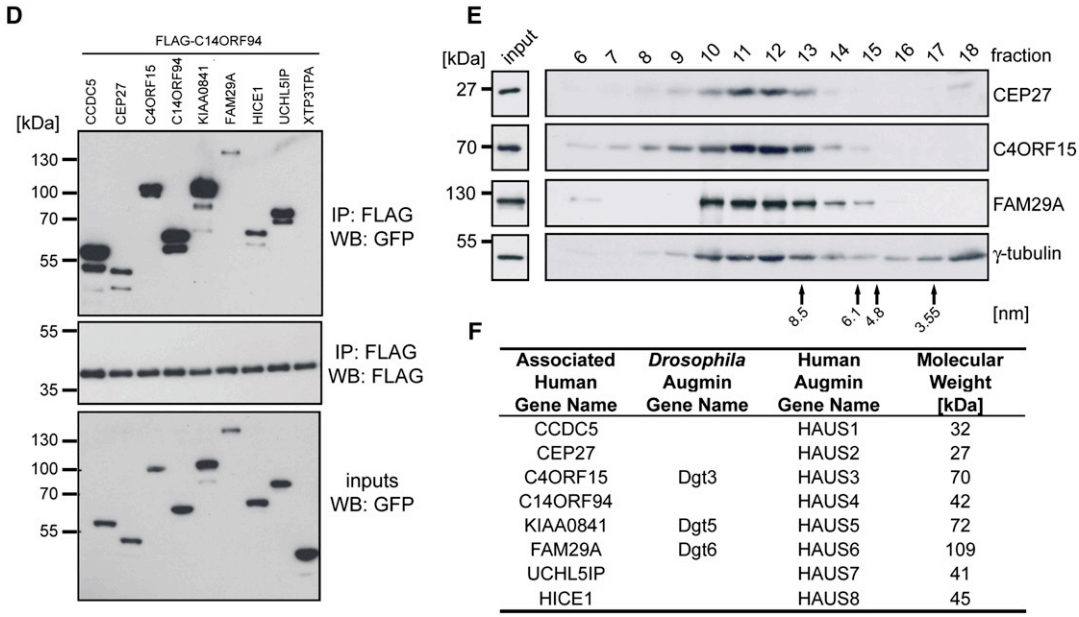
Baits / Hits	C14ORF94	C4ORF15	CCDC5	CEP27	FAM29A	KIAA0841	HICE1	UCHL5IP
C14ORF94	407	41	108	54	18	31	58	34
C4ORF15	94	89	96	75	9	30	78	26
CCDC5	135	51	254	68	9	23	55	25
CEP27	36	14	37	40	56	7	28	6
FAM29A	144	65	94	118	27	25	92	43
KIAA0841	135	62	97	93	17	172	78	42
HICE1	67	33	47	54	8	12	205	45
UCHL5IP	38	15	46	32	5	9	23	93



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Associated Human Gene Name	*Drosophila* Augmin Gene Name	Human Augmin Gene Name	Molecular Weight [kDa]
CCDC5		HAUS1	32
CEP27		HAUS2	27
C4ORF15	Dgt3	HAUS3	70
C14ORF94		HAUS4	42
KIAA0841	Dgt5	HAUS5	72
FAM29A	Dgt6	HAUS6	109
UCHL5IP		HAUS7	41
HICE1		HAUS8	45

Figure 1. A Novel Multisubunit Protein Complex Required for Mitotic Spindle Assembly

(A) Control, C4ORF15, and C14ORF94 esiRNA transfected HeLa cells were stained for DNA (blue) and microtubules (green) and shown as merged images in top panels. Pericentrin (PCNT, green) and NEDD1 (red) are also shown as merged images in the bottom panels. Representative cells with centrosome fragmentation (top) or multipolar spindle (bottom) phenotypes are shown. Insets are 2-fold magnifications of centrosomal regions. Scale bar represents 10 μm.

(B) Interphase (left) and mitotic (right) HeLa cells were labeled for DNA (blue) and microtubules (green) and shown as merged images in top panels. NEDD1 (green) and C4ORF15 (red) or C14ORF94 (red) are shown as merged images in middle panels. The bottom panels show the indicated proteins in grayscale for better visualization. Insets are 4-fold (interphase cells) and 2-fold (mitotic cells) magnifications of boxed regions. Scale bars represent 10 μm.

(C) Summary of peptides detected by mass spectrometry. FLAG-tagged baits are shown in columns whereas interactors are shown in rows. The total number of peptides across the two biological replicates analyzed for each bait is indicated; gray shading indicates bait peptides. See Figure S3A for complete mass spectrometry data.

(D) HEK293 cells were cotransfected with plasmids expressing FLAG-tagged C14ORF94 and each of the eight putative interactors N-terminally fused to GFP. The unrelated protein XTP3TPA tagged with GFP was used as negative control. Baits were recovered with anti-FLAG resin and bound fraction blotted with GFP (top) and FLAG (middle) antibodies. Inputs blotted with GFP are also shown (bottom).

(E) Gel filtration chromatography of CEP27, C4ORF15, FAM29A, and γ-tubulin. The three proteins CEP27, C4ORF15, and FAM29A peak around fraction 11 and 12. Inputs (30 μg) are shown on the right. The position and Stokes radii of protein standards used to calibrate the gel filtration column are indicated below.

(F) Proposed nomenclature for HAUS and associated subunits. HAUS for homologous to Augmin subunits 1 to 8. Current gene names and homologs detected in *Drosophila* Augmin are indicated. Predicted molecular weights are also indicated.

(Figure 2D). The total fluorescence intensity of Pericentrin-positive structures and the total volume they occupied were significantly increased after HAUS2 and HAUS6 RNAi treatment (Figures 2E–2G).

Each of the HAUS subunits localized to interphase centrosomes and to spindle microtubules (Figure 1B; Figure S6). These results are consistent with previous localization data [13, 16, 26, 30] but importantly show for the first time that HAUS3, HAUS4, HAUS5, and HAUS7 localize to interphase centrosomes and to spindle microtubules. In cells depleted of individual proteins by RNAi, spindle microtubule labeling was decreased, showing specificity of the antibodies (Figure S7A). Western blot analyses of cells transfected with esiRNA targeting HAUS2, HAUS3, and HAUS6 (Figure S7B) revealed a sharp decrease in bands of ~27 kDa, ~70 kDa, and ~110 kDa corresponding to the respective predicted molecular weight of the three proteins (Figure S7B). To determine whether the localization of the HAUS complex to spindle microtubules is dependent on the other subunits of the complex, we examined whether depleting other HAUS subunits would perturb HAUS6 localization. In all cases, we found that HAUS6 levels associated with spindle microtubules were reduced (Figure 2H). Similar results were obtained for HAUS2 (Figure S8A). Taken together, these results suggest that HAUS is a centrosomal complex that relocalizes to spindle microtubules.

HeLa cells expressing α-tubulin and NEDD1 tagged with GFP were used to monitor spindle and centrosome behavior by time-lapse microscopy. Under HAUS2 and HAUS6 RNAi conditions, bipolar mitotic spindles initially formed followed by centrosome fragmentation, which ultimately led to spindle multipolarity (Figure 3A; Movies S1 and S2). Identical results were observed with all HAUS subunits (data not shown). Mitotic cells did not contain more than four centrioles, suggesting that supernumerary centrioles are not the cause of spindle multipolarity (Figure 3B; Figure S8B). Analysis of GFP::α-tubulin time-lapse movies revealed that mitotic progression was drastically delayed in HAUS-depleted cells, ~400 min upon depletion of HAUS2 and HAUS6, relative to ~60 min in controls (Figure 3C; Movies S1 and S2). To rule out off-target effects, we generated HeLa cells expressing murine HAUS2, HAUS4, and HAUS6 fused to GFP [6, 31]. The murine transgenes are resistant against esiRNA targeting their human counterpart because of sufficient divergence in their nucleotide sequence. The three murine GFP fusions localized to mitotic spindles (Figure S8C) and rescued the spindle assembly and centrosome fragmentation defects caused by the depletion of the endogenous proteins (Figure S8D and data not shown).

The observed decrease in microtubule density within the spindle (Figures 2A and 2D) could be indicative of chromosome capture and/or kinetochore microtubule stability defects. To investigate these possibilities, we subjected cells transfected with control, HAUS2, or HAUS6 esiRNA to cold treatment for 10 min, a condition that depolymerizes the bulk of microtubules but leaves kinetochore microtubules largely intact [32]. Approximately 75% of HAUS2- and HAUS6-depleted cells had defective kinetochore microtubule attachments compared to only 11% in control cells (Figure 4A). Consistent with this observation, after HAUS depletion, cells were arrested in mitosis through the activation of the spindle assembly checkpoint, as judged by persistent labeling of Bub1 and BubR1 at kinetochores (Figure S8E and data not shown). Taken together, these results strongly suggest that the HAUS complex is required for kinetochore fiber formation and/or stability.

Centrosome Fragmentation Is a Microtubule-Dependent Process

Time-lapse imaging indicated that although seemingly normal bipolar spindles can initially form in the absence of HAUS, centrosomes eventually fragment, culminating in the formation of multipolar spindles (Figure 3A; Movies S1 and S2). We envisaged two possibilities that could explain these observations: HAUS could be required to maintain centrosome or PCM integrity or its depletion could generate an imbalance of forces within the spindle that centrosomes cannot sustain, ultimately leading to their fragmentation. To discriminate between these two possibilities, we investigated the effect of microtubule depolymerizing agents on the fragmentation of mitotic centrosomes induced by the disruption of the HAUS complex. Strikingly, we observed that nocodazole treatment reduced the number of cells with fragmented centrosomes to control levels (Figures 4B and 4C). Next, we imaged NEDD1::GFP cells transfected with HAUS2 esiRNA and found that shortly after nocodazole addition, initially fragmented centrosomes rapidly coalesced into two foci indistinguishable from centrosomes observed in control luciferase transfected cells (Figure 4D; Movie S3). Together, these results indicate that microtubules are required for centrosomal fragmentation to occur and that the fragmentation process is reversible.

Considering that microtubules are required for centrosome fragmentation upon disruption of the HAUS complex, we reasoned that motor proteins or regulators like dynactin and nonmotor spindle proteins might be working in concert with microtubules to drive centrosome fragmentation upon HAUS depletion [24, 33–35]. We therefore sought to identify putative HAUS-opposing activities by executing a small-scale RNAi suppressor screen with esiRNAs targeting 59 known motor and nonmotor spindle proteins (Table S2). HeLa cells were transfected with esiRNA targeting HAUS2 and esiRNA targeting each of the individual motor and nonmotor spindle proteins. Cells were stained for microtubules, DNA, Pericentrin, and Phospho-Histone H3, and the percentage of abnormal spindles was assayed essentially as described in Figure S1A. Statistical analysis of our data set revealed that only the esiRNA targeting NuMA was capable of suppressing spindle abnormalities with high confidence (Z score < -2; Figure 5A; Table S2). Spindle assembly defects associated with HAUS4, HAUS5, and HAUS6 depletion could also be rescued by NuMA codepletion (Figure 5B). We observed a clear decrease in the amounts of both NuMA and HAUS6 proteins upon their depletion in our double-RNAi conditions (Figures 5C and 5D). Together, these results strongly suggest that HAUS and NuMA regulate opposing activities necessary for the assembly and maintenance of robust bipolar spindles.

NuMA localizes to spindle poles and extends onto spindle microtubules (Figure 6A). NuMA labeling on spindle microtubules in the vicinity of centrosomes was decreased to 33% and 28% of control levels in HAUS2 and HAUS6 RNAi-treated cells (Figure 6B). The decrease in NuMA labeling on spindle microtubules coincided with higher NuMA levels in the cytoplasm (Figures 5D and 6A). Upon HAUS disruption, NuMA no longer extended onto spindle microtubules but remained present in the periphery of centrosomes (Figures 5D and 6A). HAUS2, HAUS6, and NEDD1 levels on spindle microtubules were not altered upon NuMA depletion (Figures 6C–6E; Figure S9A). We also observed that HAUS depletion had little effect on the localization of TPX2 and Eg5 to spindle microtubules, suggesting that HAUS depletion affects only a certain subset of proteins that localize to spindle microtubules (Figures S9B and S9C). Under

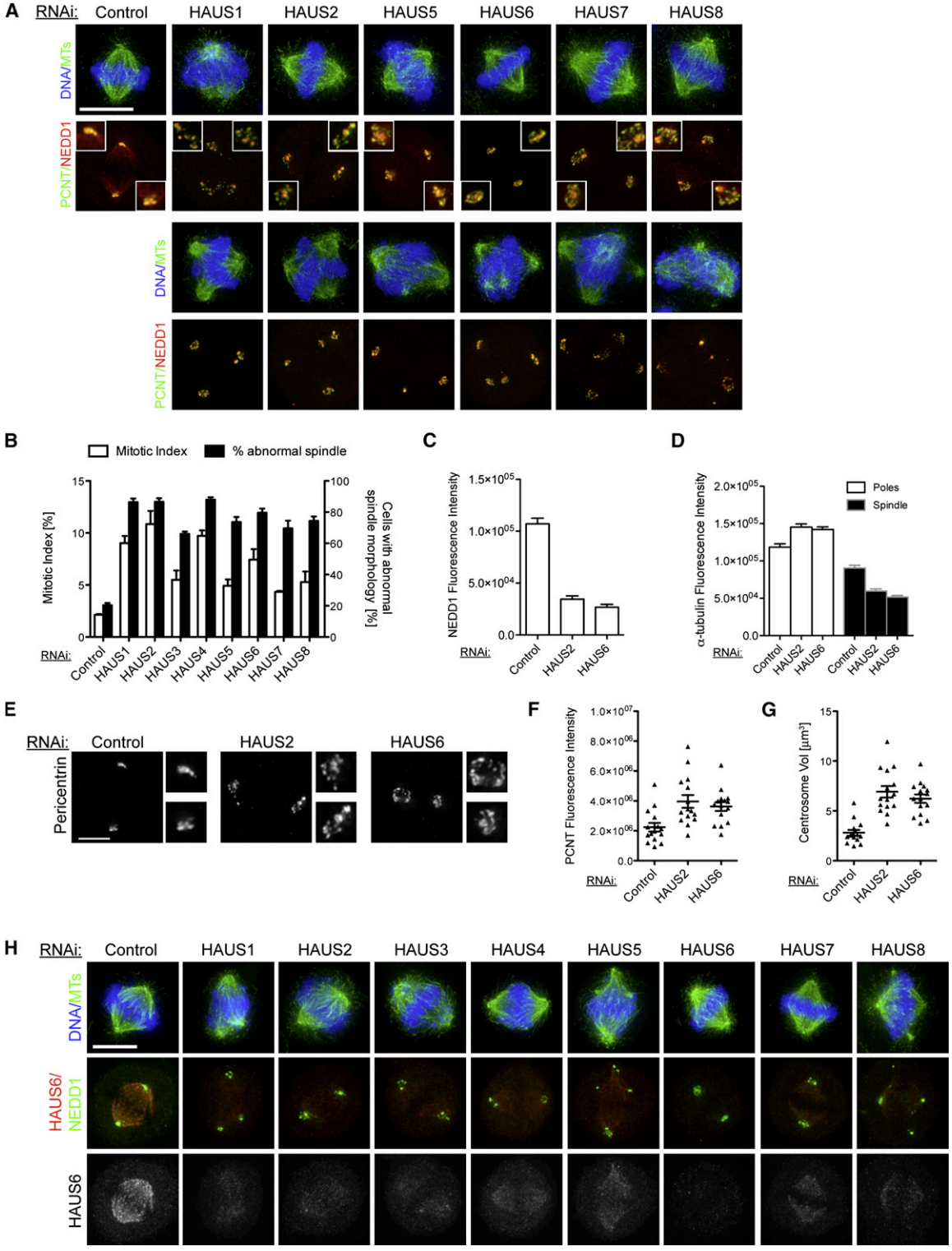

Figure 2. The HAUS Complex Is Required for Mitotic Centrosome and Spindle Integrity

(A) HeLa cells transfected with control or indicated HAUS esiRNA were stained for DNA (blue) and microtubules (green) and shown as merged images in top panels. Pericentrin (PCNT, green) and NEDD1 (red) are also shown as merged images in the bottom panels. Representative cells with centrosome fragmentation (top) or multipolar spindle (bottom) phenotypes are shown. Insets are 2-fold magnifications of centrosomal regions. Scale bar represents 10 μm.
(B) HeLa cells were treated with control or HAUS1-8 esiRNA. The mitotic index and number of cells with abnormal spindle morphology or centrosome fragmentation was quantified 72 hr after transfection. Data are shown as mean of three independent experiments ± SEM with >700 cells counted for mitotic index and 100 cells for abnormal spindle quantification. The increase in mitotic index as well as the increase in the number of cells with abnormal spindle morphology is highly statistically significant ($p < 0.0001$) for all conditions in comparison to control.

NuMA and HAUS6 codepletion conditions, NEDD1 levels on spindle microtubules remained low, suggesting that the suppression observed is not caused by more efficient localization of NEDD1 to spindle microtubules (Figure S9D). Taken together, our results suggest that HAUS and NuMA exert opposing activities necessary for robust bipolar spindle formation.

Discussion

The HAUS Complex as a Molecular Machine

We identified an 8-subunit protein complex that regulates mitotic spindle assembly in mammalian cells. This complex contains HAUS6 (FAM29A) and HAUS8 (HICE1), two microtubule binding proteins required for mitotic spindle assembly [13, 16]. The HAUS1 subunit (CCDC5) is also associated with a role in mitotic progression [30]. Three subunits share homology with *Drosophila* Augmin—HAUS3 (Dgt3), HAUS5 (Dgt5), and HAUS6 (Dgt6)—suggesting that we identified the human counterpart of *Drosophila* Augmin [12] (Figures S4A–S4C and Supplemental Experimental Procedures) [12, 13, 15, 16]. The depletion of any HAUS subunit yields indistinguishable phenotypes (Figures 1A and 2A), and individual subunits are codependent on each other for spindle localization (Figure 2H; Figure S8A). The HAUS complex is therefore a functional unit and arguably a molecular machine critical for spindle and centrosome integrity.

The depletion of the HAUS complex results in severe kinetochore microtubule defects with an accompanying decrease in the density of microtubules within the spindle (Figures 2D and 4A) [12, 13]. Recent work has shown that HAUS6 and HAUS8 have the capacity to bind and bundle microtubules [13, 16], raising the possibility that the HAUS complex participates in the formation or stabilization of kinetochore microtubules through its bundling activity. At the G2/M transition, HAUS6 transiently associates with NEDD1 and is required for loading NEDD1 and γ-tubulin containing protein complex onto spindle microtubules which, in turn, is required for microtubule amplification within the mitotic spindle necessary for the formation and stabilization of kinetochore microtubules [13]. Our results suggest that the 8-subunit human Augmin complex (HAUS) is a large molecular machine responsible for microtubule amplification within the mitotic spindle. We made the observation that HAUS disruption leads to centrosomes of larger size, much of it not necessarily associated with centrioles (Figures 2E–2G). It has been demonstrated that centriole size dictates the amount of PCM that can be recruited to the vicinity of centrioles [36–38]. These observations suggest that the control of PCM assembly might not be regulated by the availability of PCM components, so what then restricts centrosome size? Our favored hypothesis is that HAUS depletion induces the dissociation of PCM from centrioles, in a microtubule-dependent fashion, leaving room for further PCM nucleation around centrioles.

Imbalance of Forces within the Spindle

An intricate tug of war between chromosomes and spindle poles occurs during mitosis. Indeed, kinetochores exert a significant amount of force on spindle microtubules that must be counterbalanced by motor-driven cross-linking of microtubules at poles, to ensure bipolar spindle formation. One consequence of HAUS depletion is the loss of kinetochore microtubules, which would be expected to result in a net decrease in force exerted on spindle poles. Interestingly, loss of kinetochore microtubules is concomitant with centrosome fragmentation, which suggests that the counterbalancing force at spindle poles (in the absence of kinetochore pulling forces) causes centrosome fragmentation in a microtubule-dependent fashion. Wu and colleagues had previously observed split poles and centrosome defects upon the depletion of HICE1, the HAUS8 subunit of the human Augmin complex [16]. Our observation that centrosome fragmentation occurs upon the depletion of each of the eight HAUS subunits strongly suggests that loss of centrosome integrity is caused by the loss of HAUS activity as a whole, not only of one of its subunits. Centrosome integrity defects have not been observed in *Drosophila* upon Augmin depletion alone, suggesting that the mechanisms that govern mitotic spindle assembly are robust enough to tolerate loss of Augmin activity in flies [12, 15]. Interestingly, more severe defects are observed upon Augmin depletion in S2 cells in combination with defects in centrosome integrity induced by the codepletion of Centrosomin (Cnn), a major regulator of centrosome and spindle assembly in *Drosophila* [12, 39, 40].

We have shown that depletion of NuMA can compensate for loss of HAUS activity, leading to morphologically normal mitotic spindles and centrosomes (Figure 5). We observed that although HAUS depletion impairs NuMA localization to spindle microtubules, NuMA depletion has little impact on the localization of the HAUS complex, suggesting that in a purely localization sense, HAUS activity lies upstream of NuMA (Figure 6; Figure S9A). This also suggests that in absence of HAUS, mislocalized NuMA activity may be responsible for the spindle and centrosome defects observed upon HAUS RNAi. Together our results raise the interesting possibility that HAUS and NuMA exert opposing activities, the proper balance of which is necessary to maintain spindle and centrosome integrity. With the available data, we would like to suggest that HAUS is necessary for the stabilization of

(C) NEDD1 fluorescence intensity (arbitrary units) was quantified in cells treated with control, HAUS2, or HAUS6 esiRNA. NEDD1 fluorescence was measured within a defined circular area inside the spindle. One data set representative of three independent experiments is shown as mean ± SEM with a minimum of 15 cells quantified. The decrease in fluorescence intensity is highly statistically significant ($p < 0.0001$) for HAUS2/6 knockdown conditions in comparison to control.

(D) Microtubule fluorescence intensity (arbitrary units) was quantified in cells treated with control, HAUS2, or HAUS6 esiRNA. The α-tubulin fluorescence was measured within a defined circular area of the spindle pole (white bars) and inside the spindle (black bars). One data set representative of three independent experiments is shown as mean ± SEM with a minimum of 15 cells quantified. The increase in fluorescence intensity at spindle poles is statistically significant ($p = 0.0002$), and the decrease in fluorescence intensity within the spindle is highly statistically significant ($p < 0.0001$) for HAUS2/6 knockdown conditions in comparison to control.

(E) HeLa cells were treated with control, HAUS2, or HAUS6 esiRNA and stained with PCNT. Insets are 2-fold magnification views of representative centrosomes. Scale bar represents 5 μm.

(F) Fluorescence intensity (arbitrary units) of centrosome-associated PCNT per cell was quantified in cells that had been transfected with control, HAUS2, or HAUS6 esiRNA. Fifteen cells were analyzed for each condition. One data set representative of three independent experiments is shown as a scatter plot with the mean value ± SEM ($p < 0.005$).

(G) Centrosome volume (μm^3) per cell was measured after control, HAUS2, or HAUS6 RNAi. One data set representative of three independent experiments is shown as a scatter plot with the mean value ± SEM ($p < 0.0001$).

(H) Control and HAUS1-8 esiRNA transfected HeLa cells were stained for DNA (blue) and microtubules (green) and shown as merged images (top). NEDD1 (green) and HAUS6 (red) are shown as merged images (middle). HAUS6 labeling is also shown in grayscale for better visualization (bottom). Scale bar represents 10 μm.

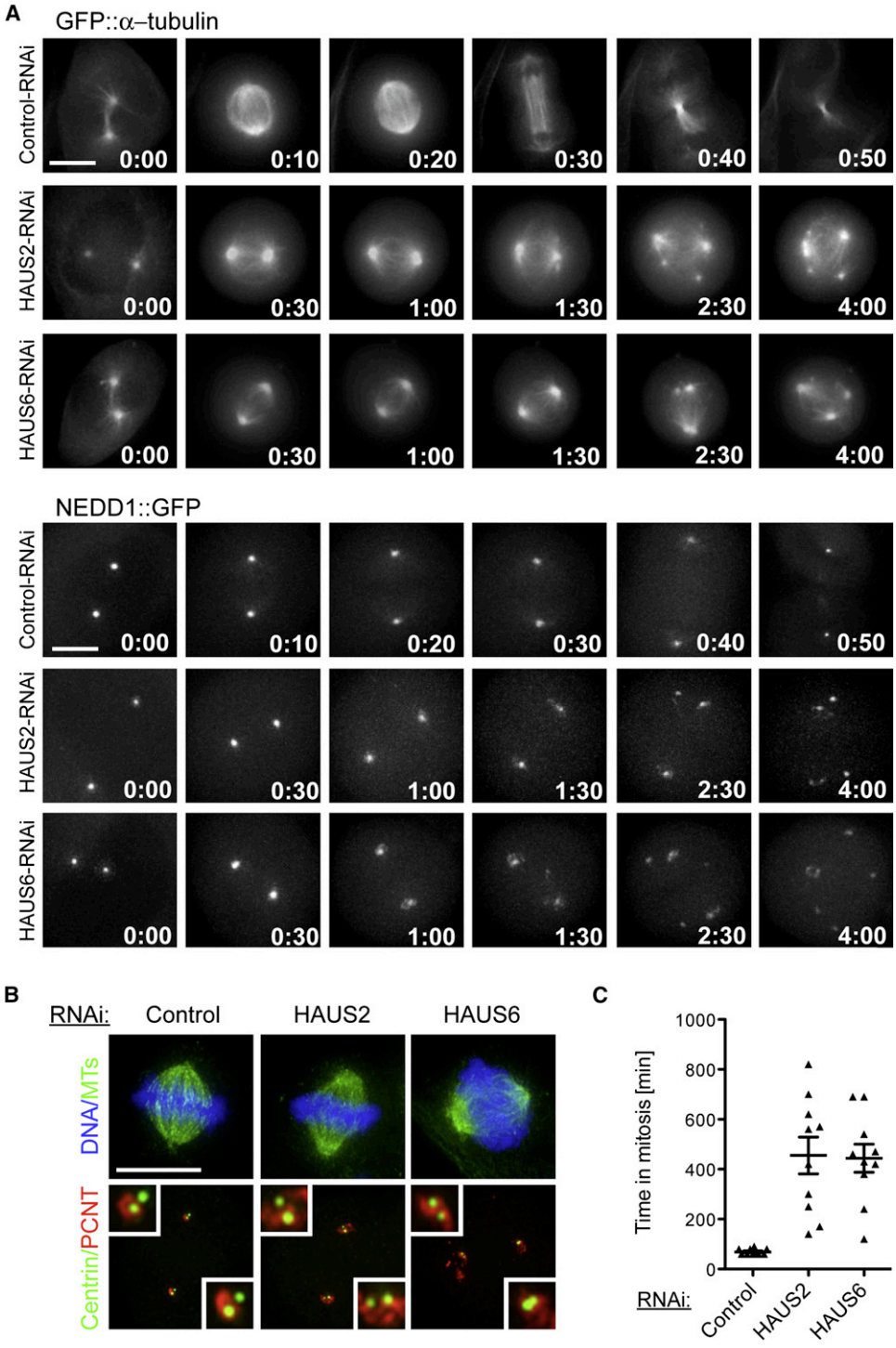

Figure 3. HAUS Depletion Results in Centrosome Fragmentation followed by the Formation of Multipolar Spindles

(A) HeLa cells expressing GFP::α-tubulin (top) from a CMV promoter or murine NEDD1::GFP from a BAC were treated with control, HAUS2, or HAUS6 esiRNA for 72 hr and then imaged during mitosis. Frames taken at the indicated time points (hr:min) relative to entry into mitosis are shown. Corresponding movies are available online (Movies S1 and S2). Scale bars represent 10 μm.

(B) Control, HAUS2, and HAUS6 esiRNA transfected HeLa cells were labeled for DNA (blue) and microtubules (green) and shown as merged images in top panels. PCNT (red) and Centrin (green) are shown as merged images in bottom panels. Insets are 4-fold magnifications of centrosomal regions. Scale bar represents 10 μm.

(C) Quantification of the duration of mitosis in control, HAUS2, or HAUS6 esiRNA transfected cells. 10 cells were quantified for each condition and data are shown as a scatter plot with the mean time ± SEM ($p < 0.0001$).

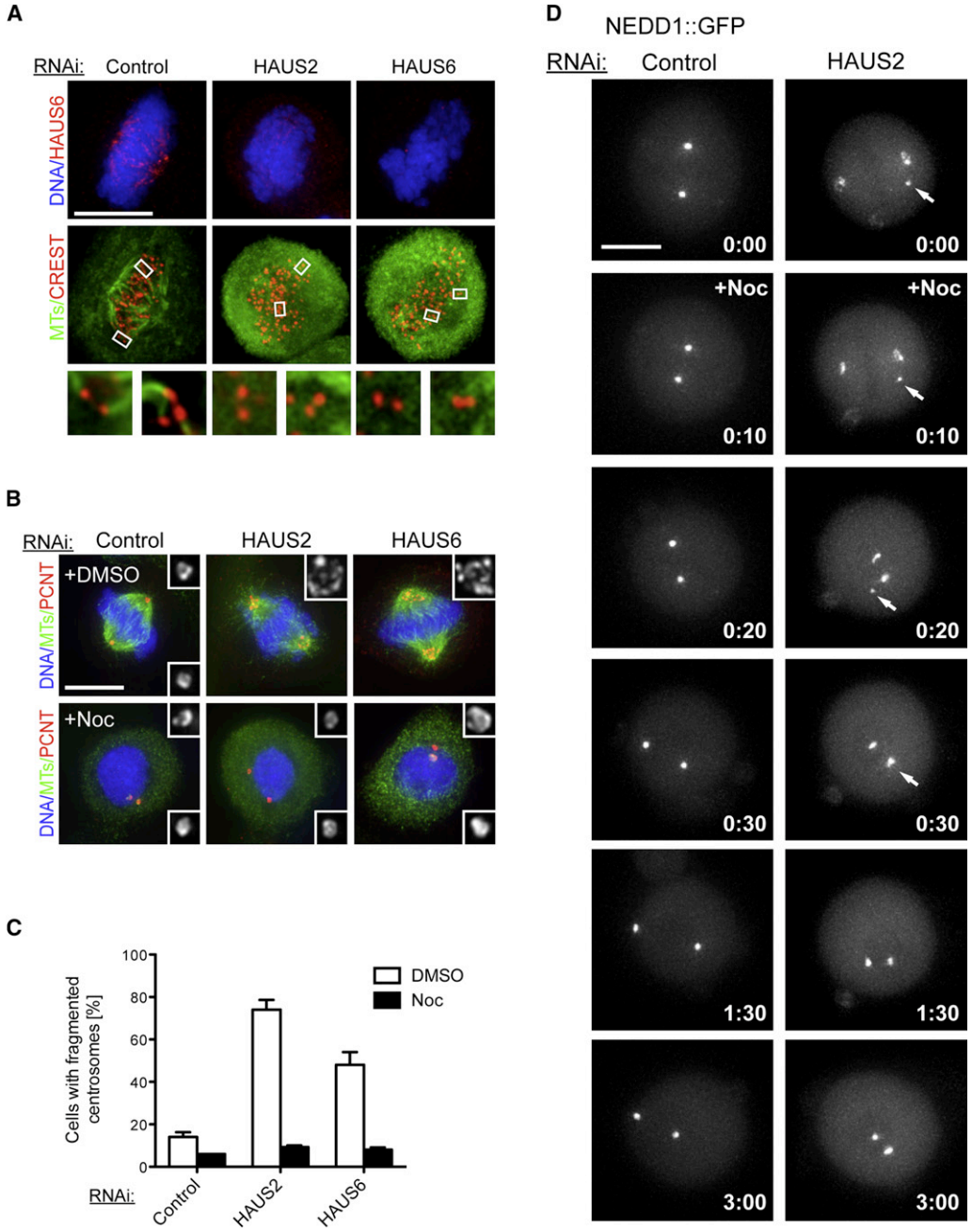

Figure 4. Centrosome Fragmentation upon HAUS Disruption Is a Microtubule-Dependent Process

(A) 72 hr after transfection with control esiRNA or esiRNA against HAUS2 or HAUS6, HeLa cells were incubated at 4°C for 10 min to depolymerize microtubules, then stained for DNA (blue) and HAUS6 (red); merged images shown in top panels. Microtubules (green) and CREST (red) are also shown as merged images in bottom panels. Insets are 3.5-fold magnifications of kinetochores in boxed regions. Scale bar represents 10 μm. 76% of HAUS2 and 74% of HAUS6 knockdown cells showed lack of kinetochore microtubules after cold treatment, in comparison to 11% in control condition (n = 100 cells for each condition).

(B) HeLa cells were treated with control, HAUS2, or HAUS6 esiRNA for 72 hr and then incubated for 6 hr in medium containing DMSO (top) or 2 μg/ml nocodazole (bottom). Cells were then stained with DNA (blue), microtubules (green), and PCNT (red) and shown as merged images. The insets show 3-fold magnifications of the centrosomal regions with Pericentrin (PCNT) staining in grayscale. Scale bar represents 10 μm.

(C) Quantification of cells with fragmented centrosomes after nocodazole or DMSO treatment. 50 cells were counted for each condition and data are shown as a mean of three independent experiments ± SEM. Differences between HAUS esiRNA transfected cells treated with DMSO or nocodazole were statistically significant ($p < 0.01$).

(D) HeLa cells expressing murine NEDD1 tagged with GFP from a BAC were treated with control or HAUS2 esiRNA for 72 hr. The time at which nocodazole was added (2 μg/ml) is indicated. Cells were serially sectioned every 10 min, and frames taken at the indicated time points (hr:min) were deconvolved and shown as maximal projections. Movie corresponding to the cells shown is available online (Movie S3). Scale bar represents 10 μm.

kinetochore microtubules, potentially through crosslinking of kinetochore and centrosomal microtubules, analogous to the function of NuMA at spindle poles, which counterbalances HAUS activity. NuMA functions through Dynein and it is interesting that none of the Dynein subunits and regulators of Dynein activity tested in our screen were capable of

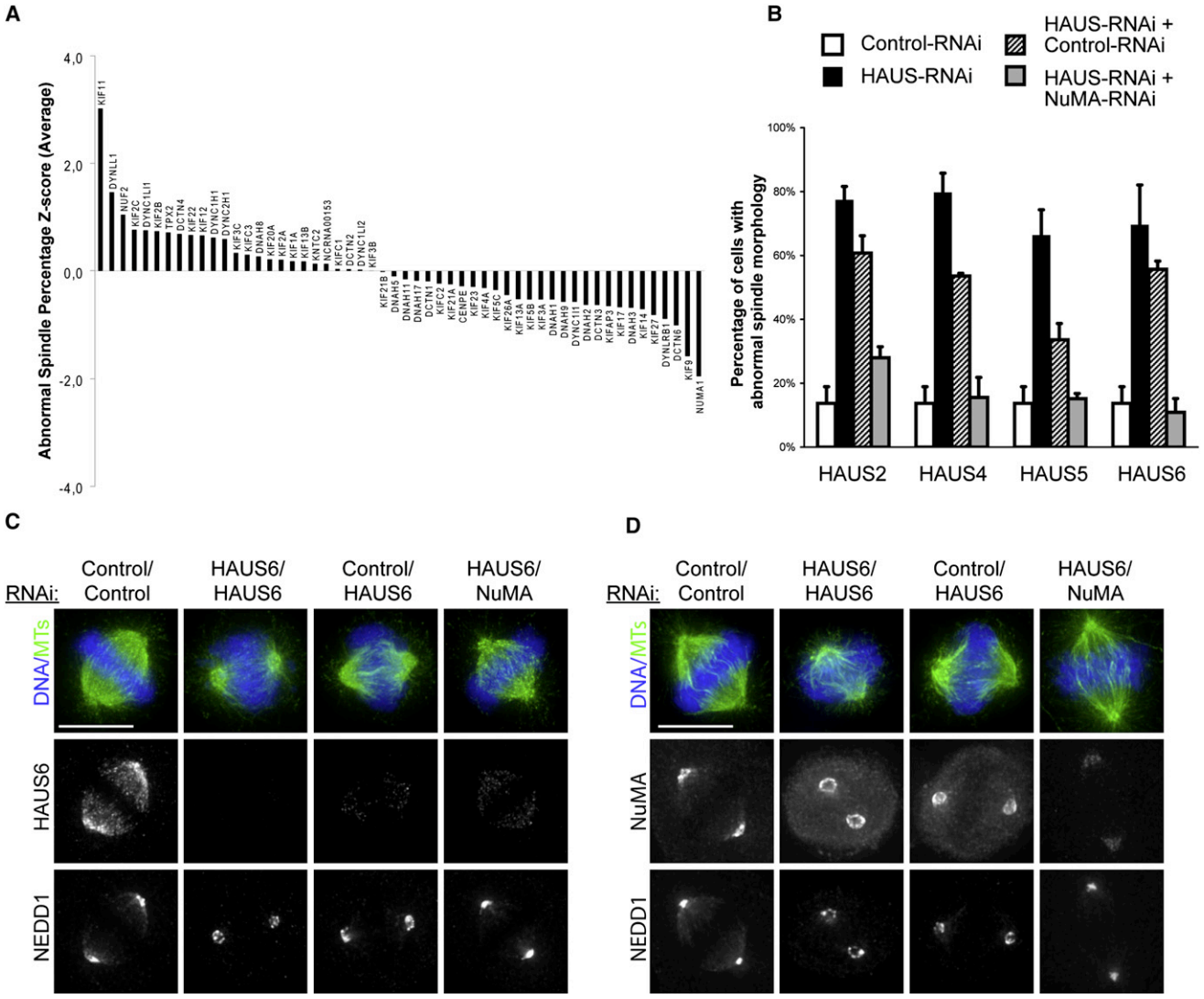

Figure 5. NuMA Opposes HAUS Activity during Mitotic Spindle Assembly

(A) An RNAi suppressor screen to identify modulators of HAUS activity. HeLa cells were transfected with HAUS2 esiRNA in combination with esiRNA targeting the majority of known molecular motors and nonmotor spindle factors. The percentage of cells with abnormal mitotic spindle morphology was quantified and shown as average Z score values relative to the plate average obtained in two independent experiments.

(B) HeLa cells were cotransfected with HAUS2, HAUS4, HAUS5, and HAUS6 esiRNA together with control, respective HAUS subunit, or NuMA esiRNA. Average abnormal mitotic spindle percentages from two independent experiments were quantified. Error bars indicate SEM. At least 40 cells were counted for each RNAi combination. Differences between HAUS2, HAUS4, HAUS5, and HAUS6 in combination with control and NuMA esiRNA were statistically significant ($p < 0.05$).

(C) HeLa cells were cotransfected with the following esiRNA combinations at equimolar ratios: control/control, HAUS6/HAUS6, control/HAUS6, and HAUS6/NuMA. After 72 hr of transfection, cells were stained for DNA (blue) and microtubules (green) and shown as merged images in the top panels. HAUS6 labeling is also shown in grayscale (middle) to appreciate its depletion. NEDD1 is shown as a measure of centrosome fragmentation (bottom). Scale bar represents 10 μm.

(D) HeLa cells were cotransfected with the same esiRNA combinations as in (C) and after 72 hr of transfection stained for DNA (blue) and microtubules (green) and shown as merged images in the top panels. NuMA labeling is also shown in grayscale (middle) to appreciate its depletion. NEDD1 is shown to visualize the centrosome fragmentation phenotype (bottom). Scale bar represents 10 μm.

compensating for loss of HAUS activity. Several reasons can explain this observation, the most likely being that Dynein activity during mitotic spindle assembly is not solely restricted to its interplay with NuMA.

HAUS6 and HAUS8 have microtubule binding and/or bundling activity, much akin to *Drosophila* Augmin, suggesting that this may be a general property of Augmin-like complexes in different organisms [12, 13, 16]. Augmin depletion in S2 cells leads to a decrease in γ-tubulin levels on spindle microtubules, which we also observed when depleting HAUS subunits [12, 13]. HAUS6 interacts in a cell cycle-dependent fashion with NEDD1 and targets it to spindle microtubules and promotes microtubule amplification within the mitotic spindle [13]. It will be interesting to further comprehend how codepletion of NuMA and HAUS bypasses this apparently key function of HAUS in spindle assembly. In conclusion, our discovery of HAUS has important mechanistic implications because we now have to consider it along with Augmin as a large,

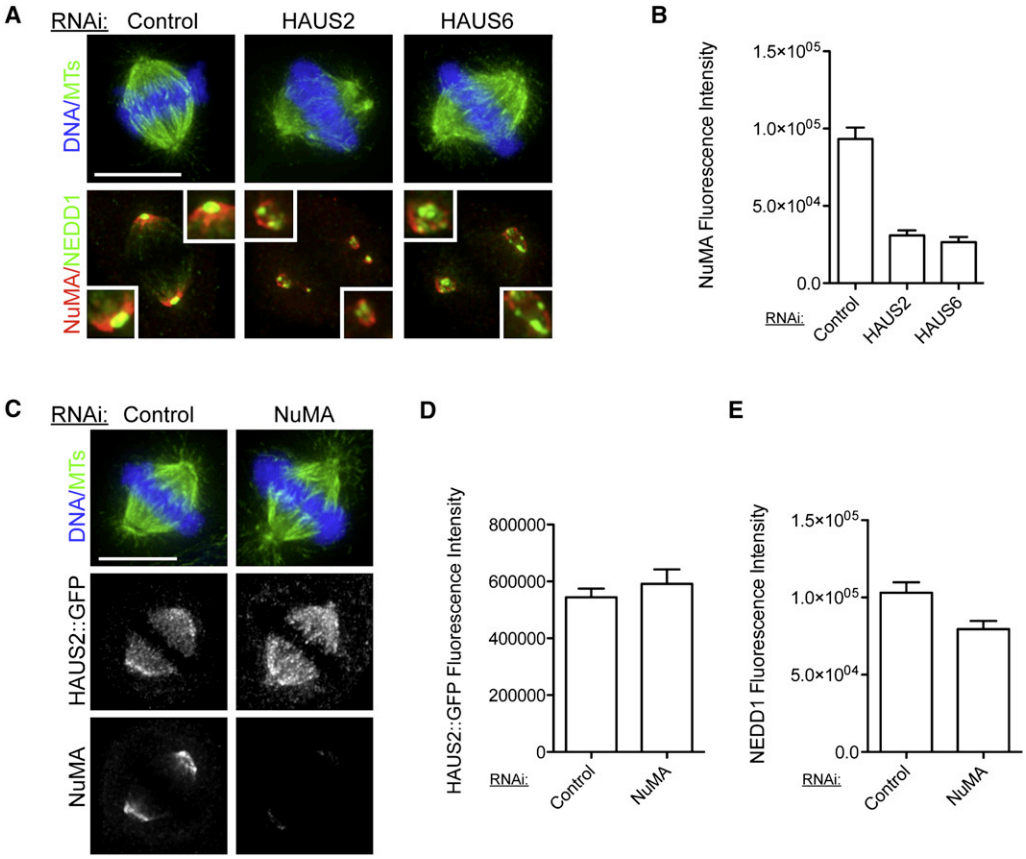

Figure 6. The HAUS Complex Is Required for NuMA Localization to Spindle Microtubules

(A) HeLa cells were treated with control, HAUS2, or HAUS6 esiRNA and then labeled for DNA (blue) and microtubules (green) and are shown as merged images (top). NEDD1 (green) and NuMA (red) labeling are also shown as merged images in the bottom panels. Insets are 2-fold magnifications of the centrosomal regions. Scale bar represents 10 μm.

(B) Fluorescence intensity (arbitrary units) of NuMA was quantified in HeLa cells transfected with control, HAUS2, or HAUS6 esiRNA. NuMA fluorescence was measured within a defined circular area inside the spindle. One data set representative of three independent experiments is presented as mean ± SEM. A minimum of 15 cells was analyzed for each condition. The decrease in fluorescence intensity is highly statistically significant ($p < 0.0001$) for HAUS2/6 knockdown conditions in comparison to control.

(C) HeLa cells expressing murine HAUS2 tagged with GFP from a BAC were transfected with control or NuMA esiRNA. 72 hr after transfection, the cells were stained for DNA (blue) and microtubules (green) and shown as merged images in the top panels, as well as GFP (middle) and NuMA (bottom). Scale bar represents 10 μm.

(D) Quantification of HAUS2::GFP fluorescence intensity (arbitrary units) in HAUS2::GFP BAC cells that were treated with control or NuMA esiRNA. Fluorescence was measured within a defined circular area inside the spindle. One data set representative of three independent experiments is shown as mean ± SEM with a minimum of 15 cells quantified.

(E) NEDD1 fluorescence intensity (arbitrary units) was quantified in HAUS2::GFP BAC cells treated with control or NuMA esiRNA. NEDD1 fluorescence was measured within a defined circular area inside the spindle. One data set representative of three independent experiments is shown as mean ± SEM with a minimum of 15 cells quantified.

evolutionary conserved molecular machine when assessing its function, regulation, and potential mechanism of action.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, nine figures, two tables, and three movies and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01032-X.

Acknowledgments

We would like to thank members of the Pelletier laboratory for stimulating discussions during the course of this work, Mario Sanches for help with chromatography, Andreas Merdes, David Drechsel, and Jeffrey Salisbury for their kind gift of NEDD1, GFP, and Centrin antibodies, and Daniel Durocher, Alex Bird, Christina Yeh, and Friederike Quittnat Pelletier for critical comments on the manuscript. The EU MitoCheck consortium is acknowledged, in particular Karl Mechtler and Martina Sykora at the Vienna mass spectrometry unit and Martina Augsburg at the BAC transgenesis unit in Dresden, for their assistance and sharing of unpublished results. Research in the laboratory of J.-M.P. was supported by Boehringer Ingelheim and the European Commision via MitoCheck. During the course of this work, M.G.F. and R.K. were supported by postdoctoral fellowships from the Fundacion Caja Madrid and the Human Frontier Science Program, respectively. M.M. is supported by an Early Researcher Award to A.-C.G., and A.-C.G. holds the Canada Research Chair in Functional Proteomics and the Lea Reichmann Chair in Cancer Proteomics and is supported by operating grants from the Canadian Institute for Health Research (CIHR MOP-84314). S.L., M.G.F., D.P., and M.B. are supported by operating grants to L.P. from the Natural Science and Engineering Research Council of Canada (RGPIN-355644-2008), National Cancer Institute of Canada (019562), and the HFSP (CDA0044/200). L.P. holds a Canada Research Chair in Centrosome Biogenesis and Function.

Received: January 6, 2009
Revised: April 15, 2009
Accepted: April 16, 2009
Published online: May 7, 2009

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Note Added in Proof

While this paper was under review, another paper describing this protein complex was published (Uehara, R., Nozawa, R.S., Tomioka, A., Petry, S., Vale, R.D., Obuse, C., and Goshima, G. (2009). The augmin complex plays a critical role in spindle microtubule generation for mitotic progression and cytokinesis in human cells. Proc. Natl. Acad. Sci. USA, in press. Published online April 14, 2009. 10.1073/pnas.0901587106).