Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization

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Direct Interaction of Pericentrin with Cytoplasmic Dynein Light Intermediate Chain Contributes to Mitotic Spindle Organization

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Abstract. Pericentrin is a conserved protein of the centrosome involved in microtubule organization. To better understand pericentrin function, we overexpressed the protein in somatic cells and assayed for changes in the composition and function of mitotic spindles and spindle poles. Spindles in pericentrin-overexpressing cells were disorganized and mispositioned, and chromosomes were misaligned and missegregated during cell division, giving rise to aneuploid cells. We unexpectedly found that levels of the molecular motor cytoplasmic dynein were dramatically reduced at spindle poles. Cytoplasmic dynein was diminished at kinetochores also, and the dynein-mediated organization of the Golgi complex was disrupted. Dynein coimmunoprecipitated with overexpressed pericentrin, suggesting that the motor was sequestered in the cytoplasm and was prevented from associating with its cellular targets.

Immunoprecipitation of endogenous pericentrin also pulled down cytoplasmic dynein in untransfected cells. To define the basis for this interaction, pericentrin was coexpressed with cytoplasmic dynein heavy (DHCs), intermediate (DICs), and light intermediate (LICs) chains, and the dynamitin and p150

Key words: pericentrin • centrosomes • mitotic spindle • cytoplasmic dynein light intermediate chains • aneuploidy

The centrosome is the major microtubule nucleating organelle in animal cells (Kellogg et al., 1994; Zimmerman et al., 1999). It is usually composed of a pair of centrioles surrounded by a protein matrix from which microtubules are nucleated (Szollosi et al., 1972; Gould and Borisy, 1990). The centrosome proteins, pericentrin and γ-tubulin, are localized to the matrix material where they form a unique lattice-like network (Dictenberg et al., 1998). The lattice appears to represent the higher order organization of γ-tubulin rings, structures comprised of γ-tubulin and several other proteins that appear to provide the templates for nucleation of microtubules at the centrosome (Moritz et al., 1995; Zheng et al., 1995; Schnackenberg et al., 1998). γ-Tubulin and pericentrin are also part of a large cytoplasmic protein complex that may represent the fundamental subunit of microtubule nucleation before its assembly at the centrosome (Dictenberg et al., 1998). In addition, the Drosophila melanogaster protein, A sp (abnormal spindle protein), has been shown to play a role in the centrosomal recruitment of γ-tubulin (Avide and Glover, 1999). However, the precise role of this protein and others in the assembly, organization, and activity of centrosomes is unknown (see Zimmermann et al., 1999).

The assembly and molecular organization of the centrosome is important for bipolar spindle assembly during mitosis (for review see Waters and Salmon, 1997). Functional abrogation or depletion of pericentrin or γ-tubulin disrupts centrosome assembly and organization, and creates structural defects in microtubule asters and spindles (Doxsey et al., 1994; Felix et al., 1994; Stearns and Kirschner, 1994). Alternative pathways for assembly of microtubule asters and spindles in the absence of centrosomes have been described (Gaglio et al., 1997; Merdes and Cleveland, 1997; Waters and Salmon, 1997; Hyman and Karsenti, 1998). In these acentrosomal spindle assembly systems, the molecular motor cytoplasmic dynein and the nuclear mitotic apparatus protein (NuMA) play key roles in the assembly of functional microtubule asters and spindles.
roles in the organization and focusing of the spindle poles (Hald et al., 1996; Merdes et al., 1996; Gaglio et al., 1997). These proteins are also involved in the organization of spindle poles in the presence of centrosomes (Merdes and Cleveland, 1997; Karki and Holzba ur, 1999).

The precise role of pericentrin in spindle function is currently unknown. The protein has been shown to contribute to the organization of microtubule arrays in both interphase and mitosis. Pericentrin antibodies introduced into mouse oocytes and Xenopus laevis embryos disrupt the organization of centrosomes and meiotic and mitotic spindles (Doxsey et al., 1994). Moreover, when added to Xenopus extracts, the antibodies inhibit assembly of microtubule asters. Recently, it has been shown that pericentrin levels are elevated in human tumor cells that exhibit defects in centrosome structure, spindle organization, and chromosome segregation (Pihan et al., 1998; Pihan, G., and S. Doxsey, unpublished observations). This suggests that pericentrin may contribute to tumorigenesis through the organization of dysfunctional spindles that misaggregate chromosomes and generate aneuploid cells (see Doxsey, 1998; Pihan and Doxsey, 1999).

To further examine the role of pericentrin in spindle organization, we overexpressed the protein in somatic cells. Cells with excess pericentrin formed aberrant mitotic spindles, misaggregated chromosomes, and became aneuploid. We found that cytoplasmic dynein was displaced from centrosomes and kinetochores, and the dynein-mediated organization of the Golgi complex was impaired. An interaction between cytoplasmic dynein and pericentrin was identified and shown to be mediated specifically by light chain (LIC) subunits (Gil1 et al., 1994; Hughes et al., 1995) of the motor protein. These results indicate that pericentrin and dynein act together to ensure proper organization and function of centrosomes and spindles.

Materials and Methods

cDNA Constructs

A full-length mouse pericentrin was constructed using a three-clone strategy. Pericentrin clone pc1.2 (Doxsey et al., 1994) was excised with restriction enzymes Pvu I and EcoRV. The 5' end of the final clone was amplified by PCR using VENT polymerase from clone PCR 1 (Doxsey et al., 1994) using a 5' primer (5'-CCGATATCAGAATTCAGAGC-3') with an EcoRV restriction enzyme site and a 3' primer (5'-GAGGTAGAGGCAGATGGAAGACG-3') with a PvuI restriction enzyme site. The amplified PCR product was digested with EcoRV and PvuI. Plasmid pcDNA1(Amp) (Invitrogen Corp.) was used to construct a vector with 13 amino acids of hemagglutinin (HA) protein (MAYPDYPCYASL, pHAI; Wilson et al., 1984) inserted at the HindIII site in the polylinker (a gift of Michael Green, Glaxo Wellcome, Oncor). The vector was linearized with EcoRV and ligated to form the full-length pericentrin, as described (Sambrook et al., 1989). The correct orientation of the fragments was confirmed by PCR using the T7 vector primer and the 5'-GTTTGAAAGCCGGAGGTT-3' primer (5'-AGGGAAGCCAGATGGAAGACG-3'). The amplified PCR product was digested with EcoRV and PvuI. The 5'-GTTTGGCTGAGAGGCT-3' primer was used to confirm the 3' end of the final clone.

Cell Culture, DNA Transfection, Cell Viability, and Growth

COS-7 cells were cultured as described (American Type Culture Collection) with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma Chemical Co.). Cells were grown on 12-mm round glass overlips in 35-mm culture dishes (Falcon Plastics) and transfected with 2 μg of plasmid DNA (HA-tagged pericentrin [HA-Pc], β-galactosidase, pHAI, or no DNA) using lipofectamine (GIBCO BRL); transfection efficiency was -15%. Cells were fixed 35–42 h after transfection and processed for immunofluorescence staining, immunoprecipitation, metabolic labeling, or Western blotting. Cell viability was determined using mitotracker (Sigma Chemical Co.), which measures energy-dependent electron transport in mitochondria. Cell growth was determined by measuring the ratio of transfected cells to the total cell population; there was little change in this ratio over a 50-h time period.

Antibodies

Afinity-purified rabbit IgG was prepared from sera raised against the COOH terminus of pericentrin (Doxsey et al., 1994) and used at 1:1,000 for immunofluorescence microscopy and Western blotting. A anti-HA mAbbs (12CA5) were obtained from Berkeley Antibody Co., Inc., and anti-HA polyclonal antibodies were a gift from Joanne Buxton (UMass Medical School, Worcester, MA; Meisser et al., 1997). Antibodies to α and γ tubulin, mouse IgG, and rabbit IgG were obtained from Sigma Chemical Co. A antibodies to β-galactosidase were from Boehringer Mannheim Corp. A antibodies to the following proteins were also used in these studies under conditions described in the accompanying references: dynein heavy chain (DHC; J.R. Buxton, 1984), DIC L5 (Vaughan and Vale, 1995), 71.2 (Dillman and Pfister, 1994), dynamitin (Cheverev et al., 1996), p150(γl) (Vaughan and Vale, 1995; Vaughan et al., 1999), anti-p58 Golgi protein (Bloom and Brashear, 1989), and CENP-E (Lombillo et al., 1995). Fluorescein (FITC) and cyanine (cy3)-conjugated IgGs were obtained from Jackson Immunoresearch Laboratories, Inc. HRP-conjugated IgGs were from Nycomed Amersham Inc. A antibodies were used alone or in combination as described in the text.

Immunofluorescence Microscopy and Quantification of Protein and DNA

Immunofluorescence microscopy was performed essentially as described (Doxsey et al., 1994; Dittberner et al., 1998). Unless otherwise stated, COS-7 cells expressing HA-Pc, β-galactosidase, pHAI, or mock transfected were fixed in 100% methanol at −20°C. Where indicated, cells were detergent-extracted to remove cytoplasmic protein before fixation (0.5% TX-100 in 80 mM Pipes, pH 6.8, 5 mM EGTA, 1 mM MgCl 2, for 1 min). In most cases, monoclonal or polyclonal HA antibody was detected with FITC-labeled secondary antibody, and antibodies used in colabeling experiments were detected with cy3 secondary antibodies. In all cases, cells were stained with 4',6-diamidino-2-phenylindole (DAPI) to detect chromatin. Cells were observed using an Axios phot of fluorescence microscope with a 100X objective (Carl Zeiss Inc.).

Quantification of centrosomal staining in mitotic cells and DNA (DA PI) was performed as described (Dittenberg et al., 1998). In brief, the total fluorescence from centrosomes and nuclei in individual cells was determined. Background values from three positions in the cytoplasm and camera noise (dark current) were subtracted (≤10% of total). For centrosome staining, fluorescence signals were obtained from only one centrosome per mitotic cell, to avoid photobleaching. Cells with low, intermediate, and high expression levels were included in all analyses.

For coexpression studies, see FIG. 8. A HA-Pc and dynein, or dynactin cDNAs were cotransfected into COS-7 cells and processed 38–46 h later. Cells were washed in PBS, lysed in modified RIPA buffer at 4°C for 20 min (150 mM NaCl, 50 mM Tris, pH 8.0, 1 mM EDTA, 1% IGEPA L) with leupeptin, aprotinin, and A EB5F (Boehringer Mannheim Corp.), and protease inhibitors. Mammalian HA bound to glutathione G beads (Pharmacia Biotech) was added to lysates for 4°C for 12 h, and beads were collected and washed five times with modified RIPA buffer. Proteins were exposed to SDS-PAGE and transferred to PVDF membranes (Millipore Corp.). The presence of dynein/dynactin subunits was assayed by Western blot with anti-myc, anti-p50, and anti-p150 antibodies.
Results

Previously, we demonstrated that functional abrogation of pericentrin disrupts centrosome and spindle organization in several systems (Doxsey et al., 1994). Based on these observations, we reasoned that an artificial elevation of pericentrin levels would provide additional information on protein function and interaction. To this end, we constructed and expressed an HA-Pc in COS-7 cells, and examined centrosome and spindle composition and function.

As expected, HA-Pc had an electrophoretic mobility of ~220 kD and was found in both Triton X-100 soluble and insoluble fractions (Fig. 1 A). Immunofluorescence analysis demonstrated that the more abundant detergent soluble fraction was distributed throughout the cytoplasm (Fig. 1 D, inset), whereas the detergent insoluble fraction colocalized with γ tubulin at centrosomes (Fig. 1, B and C). Centrosome localization of HA-Pc was unaltered when microtubules were depolymerized, suggesting that the protein was an integral component of centrosomes and not simply bound there by microtubules (data not shown).

Mitotic Spindles Are Structurally and Functionally Disrupted in Pericentrin-overexpressing Cells

The organization of microtubules in interphase HA-Pc expressing cells was indistinguishable from control cells (Fig. 1 D). Moreover, there was no detectable difference in microtubule nucleation from centrosomes (Fig. 1, E–H). The most dramatic consequence of HA-Pc expression was disruption of mitotic spindle organization (Figs. 2 and 3). A significant fraction of mitotic COS-7 cells at all expression levels exhibited spindle defects (75.7 ± 6.1%, n = 423), compared with nontransfected cells (2.5 ± 1.5%, n = 598) and vector DNA transfected cells (3.0 ± 1.0, n = 201). Three categories of spindle defects were observed. Spindles with structural defects were detected in 36.2% of transfected cells and included multipolar, monopolar, and distorted spindles (Fig. 2, D–I). Mispositioned spindles were observed in 22.0% of the cells, and were often positioned far from the cell center (Fig. 2, J–L). Spindles with misaligned, missegregated, and mono-oriented chromosomes were commonly observed (42.5%; Fig. 3, also see Fig. 5, L, M, Q, and R). Spindle defects occurred alone or in combination.

Despite the presence of improperly attached chromosomes, HA-Pc cells progressed through mitosis and were

[Image 308x317 to 547x750]

Figure 1. HA-Pc overexpression has no detectable effect on microtubule nucleation or organization. A, Triton X-100 soluble (lane 1) and insoluble fractions (lane 2) of HA-Pc-expressing COS-7 cells immunoblotted with anti-HA antibodies. Detergent extracted COS-7 cell showing centrosome-associated HA-Pc (B), which colocalizes with γ tubulin (C). Microtubule organization in a pericentrin-expressing interphase cell (D, inset) is similar to surrounding control cells. The extent of microtubule regrowth from prometaphase centrosomes after nocodazole-induced depolymerization is similar in an HA-Pc-expressing cell (G and H) and a control cell (E and F). Inset in G, HA stain. DAPI staining shows prometaphase chromosomes (F and H). Note that individual microtubules are not easily observed (E and G) after short periods of microtubule regrowth. Bars: (C, for B and C) 1 μm; (D) 5 μm; (H, for E–H) 10 μm.
frequently observed in later stages of mitosis with misseg-
regated chromosomes (Fig. 3, C and D). The percentage of
mitotic figures in the population of HA-Pc–expressing
cells (3.1 ± 0.9%, n = 3,490) was not significantly different
from control cells transfected with other constructs or
mock transfected cells (2.9 ± 1.0 to 4.4 ± 2.1%, n = 5,002),
and the cell viability and growth rate appeared unchanged.
Nuclei exhibited a remarkably wide variation in DNA
content. Values ranged from zero to five times those of
controls (Fig. 3, E and F), demonstrating that the cells
were becoming aneuploid. From this analysis, we conclude
that pericentrin overexpression causes multiple mitotic
spindle defects leading to chromosome missegregation
and aneuploidy.

**Cytoplasmic Dynein Is Dissociated from Multiple
Cellular Sites in HA-Pc–expressing Cells**

The spindle defects in pericentrin-overexpressing cells
were similar to those previously observed in cells overex-
pressing the dynamitin subunit of dynactin (Echeverri et
al., 1996; Burkhardt et al., 1997). Dynactin is a protein
complex which regulates the function of cytoplasmic dy-
nein, a minus end microtubule motor protein involved in
numerous physiological processes (reviewed in Valette and Sheetz, 1996; Karki and Holzbaur, 1999). Dynein and dynactin have been localized to prometaphase kinetochores, centrosomes, spindle poles, and the plasma membrane (Pfarr et al., 1990; Steuer et al., 1990; Clark and Meyer, 1992; Echeverri et al., 1996; Busson et al., 1998). Overexpression of dynamitin disrupts the dynactin complex, releases cytoplasmic dynein from mitotic kinetochores, disrupts mitosis, and alters the distribution of membranous organelles, including the Golgi complex (Echeverri et al., 1996; Burkhardt et al., 1997).

To test the possibility that cytoplasmic dynein or dynactin contributed to the pericentrin overexpression phenotype, we examined the distribution of these protein complexes in pericentrin-overexpressing cells. The level of cytoplasmic dynein immunoreactivity in mitotic cells was dramatically reduced at spindle poles (8–12-fold; Fig. 4, G–J). The motor appeared to be specifically displaced from spindle poles and not simply masked from antibody access for several reasons. First, diminished dynein staining was detected with two independent antibody preparations raised against the DIC (L5, polyclonal and 74.1, monoclonal). Second, control cells expressing β-galactosidase (Fig. 4, A–C) or untransfected cells (Fig. 4, D–F) had normal levels of dynein at their poles (Fig. 4 J). Third, there was no detectable change in the distribution and abundance of several other centrosome and spindle pole components. The centrosome localization and levels of the dynactin subunits, dynamin (Fig. 5, A–D) and p150<sub>glued</sub>, did not appear to be altered, although there was some variability in p150<sub>glued</sub> levels in prometaphase (Fig. 5 E).

Consistent with defects in the Golgi complex induced by overexpression of the dynamitin subunit of dynactin (Burkhardt et al., 1997), HA-Pc overexpression caused dispersal of Golgi elements. This was observed by immunostaining with antibodies to the Golgi protein, p58 (Bloom and Brashear, 1989; Fig. 6; 77.6%, n = 251). In adjacent nontransfected control cells, Golgi complexes had the characteristic tightly focused appearance and were found in the perinuclear region of the cells (Fig. 6 B, arrowheads; 95.6%, n = 497). Disruption of the Golgi complex was also observed using a green fluorescent protein (GFP)-tagged N-acetylglucosamine transferase in cotransfection experiments with pericentrin (data not shown). Golgi complex dispersal did not appear to result from impaired microtubule integrity, as no detectable changes in the microtubule network were observed (see Fig. 1 D).

**Pericentrin Interacts Directly with Cytoplasmic Dynein through the Light Intermediate Chain**

The loss of cytoplasmic dynein from spindle poles and kinetochores, and the abrogation of cellular functions mediated by dynein (spindle positioning, Golgi complex organization) suggested that overexpressed pericentrin sequestered the motor in the cytoplasm. This was tested directly by coimmunoprecipitation assays. Antibodies to
both DIC and DHC precipitated HA-Pc (Fig. 7A, lanes 5 and 6), whereas a control IgG preparation did not (Fig. 7A, lane 7). Conversely, antibodies to HA, but not to control IgGs, precipitated DIC (Fig. 7A, lanes 1–3). Under the same conditions, antibodies to dynactin components (dynamitin and p150
\textsuperscript{glued}) did not precipitate detectable amounts of HA-Pc (Fig. 7A, lanes 8 and 9), although they immunoprecipitated other proteins of the dynactin complex (Fig. 7A, lanes 11, 12). In cells metabolically labeled with \textsuperscript{35}S methionine, HA-Pc was specifically immunoprecipitated with antibodies to DHC, but not to preimmune sera (Fig. 7B). Moreover, despite very low levels of endogenous pericentrin in nontransfected control cells (Doxsey et al., 1994), we were able to specifically detect DHC after immunoprecipitation of pericentrin from lysates prepared from large numbers of cells (Fig. 7C). These results suggest that overexpressed pericentrin binds to and sequesters dynein in the cytoplasm, and prevents it from associating with its cellular targets.

To determine whether the dynemin-pericentrin interaction was direct or indirect, we cotransfected cells with HA-Pc and individual dynein and dynactin subunits, and performed a series of immunoprecipitation and immunoblot analyses. Immunoprecipitation of HA-pericentrin failed to
pull down the DHCs and DICs or the dynactin subunits p150Glued and dynamitin (Fig. 8 A). However, a myc-tagged rat cytoplasmic dynein light intermediate chain (Hughes, S., A. Purohit, S. Doxsey, and R. Vallee, manuscript in preparation) and its COOH-terminal fragment N174 clearly coimmunoprecipitated with HA-pericentrin (Fig. 8 A). When cells cotransfected with the LIC N174 fragment and HA-Pc were labeled with $^{35}$S-methionine, the only bands specifically immunoprecipitated with anti-HA antibodies were HA-Pc and N174 (Fig. 8 B). Dynamitin did not appear to be required for the pericentrin–LIC interaction since overexpression of dynamitin had no effect on the ability of the proteins to coimmunoprecipitate (data not shown). These results provide strong evidence for a direct interaction between HA-Pc and the light intermediate chain of cytoplasmic dynein.

**Discussion**

We have found that pericentrin overexpression has profound effects on the organization, positioning, and function of mitotic spindles, and on the organization of the Golgi complex. Several studies show that cytoplasmic dynein is involved in processes affected by pericentrin overexpression (for reviews see Holzbaur and Vallee, 1994; V allee and Sheetz, 1996; Karki and Holzbaur, 1999). Con-
sistent with a role for cytoplasmic dynein in mediating the pericentrin overexpression phenotype is the reduction of dynein staining intensity at the prometaphase kinetochore and the centrosome/spindle pole. Our immunoprecipitation data further support an interaction between pericentrin and cytoplasmic dynein. Our data indicate that the interaction is direct and specifically mediated by the light intermediate chains of the motor protein complex. Thus, this study provides the first evidence for a dynein–pericentrin interaction, and identifies the first functional role for LICs.

Mechanism of Dynein–Pericentrin Interaction

The function of the light intermediate chains has been obscure. They have only been identified in cytoplasmic forms of dynein and contain well-conserved P-loop elements of unknown function near their NH$_2$ termini (Paschal et al., 1987; Gill et al., 1994; Hughes et al., 1995). Previous studies have implicated a different class of dynein subunit, the intermediate chains, in subcellular targeting. The intermediate chains reside at the base of the dynein complex and interact with the p150$^{Glu}$ subunit of the dynactin complex (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995). Dissociation of the dynactin complex by dynamitin overexpression was found to release dynein from prometaphase kinetochores. Together, these data supported a role for dynactin in anchoring dynein to at least one form of subcellular cargo through the intermediate chains (Echeverri et al., 1996). This mechanism has received further support from evidence that mutations in zw10, a dynactin-anchoring kinetochore component, also release dynein from the kinetochore (Starr et al., 1998).

The current studies identify an additional and previously unsuspected mechanism for linking dynein to its cargo. The presence of cytoplasmic dynein, but not dynactin, in pericentrin immunoprecipitates, strongly suggests that dynactin is not necessary for the pericentrin/dynein interaction. Coexpression of recombinant dynein and dynactin subunits with pericentrin reveal a direct interaction with the light intermediate chains, further supporting a dynactin-independent mechanism. Thus, these results identify the light intermediate chains as an additional class of dynein-anchoring or -targeting subunit. Whether these polypeptides serve in a subset of dynein-mediated processes, such as interactions with soluble protein complexes versus membranous organelles or kinetochores, remains to be determined.

Whether light intermediate chain-mediated dynein in-

![Figure 6](image1.png)

Figure 6. Golgi complexes are disrupted in pericentrin-overexpressing cells. An HA-Pc COS-7 cell (A) showing dispersal of the Golgi complex as revealed by staining with anti-p58 antibodies (B, center). In adjacent nontransfected cells, the Golgi complexes are well organized (B, arrowheads) and found in the typical juxtanuclear region (C, DAPI). Bar, 10 μm.

![Figure 7](image2.png)

Figure 7. Pericentrin and cytoplasmic dynein coimmunoprecipitate. A, Detergent lysates of HA-Pc-expressing COS-7 cells were used for immunoprecipitations with antibodies to DIC, DHC (polyclonal anti-rat heavy chain1, Mikami, A., and R. Vallee, unpublished results), p50, p150, HA, or nonspecific IgG, as indicated. Immunoprecipitates were immunoblotted for DIC (lanes 1–3), HA (lanes 4–9), or p150 (lanes 10–12) as indicated. Dynein coimmunoprecipitates with HA-Pc (lane 2), but not with control IgG (lane 3). Conversely, HA-Pc coimmunoprecipitates with dynein components (lanes 5 and 6), but not with control IgG or dynactin components (lanes 7–9). HA-Pc was partially degraded in some cases (lower band in lanes 4–6), but not others (see Fig. 1 A and 7 B). An ∼60-kD band is nonspecifically precipitated by IgG (lanes 4, 5, and 7). B, (35)S)methionine-labeled cells expressing HA-Pc (lanes 1 and 3) or mock transfected (lane 2) immunoprecipitated with antibodies to DHC or preimmune sera (Preim) as indicated. HA-Pc is only detected in HA-Pc-expressing cells after DHC immunoprecipitation (lane 3). C, Lysates from nontransfected control cells were used for immunoprecipitation with antipericentrin antibodies (lane 1) or no antibody (beads, lane 2), and proteins were immunoblotted with anti-DHC antibody. Molecular mass markers are indicated (in kD $\times 10^{-3}$).
Pericentrin binds dynein light intermediate chain functions.

One distinction between the pericentrin and dynamitin overexpression effects is that there is no detectable change in the mitotic index of pericentrin-overexpressing cells. This result is puzzling in view of the similarity in mitotic defects observed in the two cases, including the production of multipolar mitotic spindles. The latter structures are suggestive of mitotic failure (i.e., cytokinesis failure) which typically occurs after a delay in mitosis. A through pericentrin-overexpressing cells do not exhibit a mitotic delay, they appear to grow and divide normally. This suggests a defect in the checkpoint that regulates the transition from metaphase to anaphase (Rudner and Murray, 1996), an idea we are currently testing.

Pericentrin previously has been shown to be part of a large protein complex that includes γ tubulin (Dichtenberg et al., 1998). Thus, it is possible that disruption of γ tubulin in pericentrin-overexpressing cells contributes to the spindle defects. However, we believe this is unlikely because recruitment of γ tubulin to spindle poles is not noticeably different than in control cells. Moreover, the ability of individual mitotic spindle poles to nucleate microtubules, a function thought to be mediated by γ tubulin, appears unchanged in pericentrin-overexpressing cells. Some pericentrin-overexpressing cells have multiple γ tubulin staining structures that seem to contribute to the formation of multipolar spindles. Since each of the multiple poles has...
approximately the same amount of γ tubulin as normal spindle poles, we believe that they represent bona fide centrosomes (with centrioles). We are currently investigating how these multiple foci of γ tubulin are generated and whether they contribute to aneuploidy in pericentrin-overexpressing cells.

It is unclear why recruitment of γ tubulin and NuMA to spindle poles appear to be unaffected by the HA-PC-induced dynein disruption since the evidence suggests that both proteins may also interact with, or be under the control of, cytoplasmic dynein. One possibility is that cell cycle variability in the localization and levels of these proteins (Compton and Cleveland, 1993; Dictenberg et al., 1998), together with variability in the level of pericentrin overexpression, make it difficult to detect significant differences. A further possibility is that the proposed HA-PC-induced sequestration of dynein may be less than complete, allowing some dynein-mediated transport to occur. This may be sufficient to localize the spindle pole proteins examined in this study, but insufficient to maintain Golgi complex organization or localize dynein to spindle poles and kinetochores. Alternatively, dynein may interact with many different cargos (e.g. vesicles, protein complexes) whose localization is differentially affected by pericentrin overexpression. This could explain why NuMA and dynactin, which form a discrete complex with dynein in X enucleus extracts (Méredes et al., 1996), appear to accumulate at normal levels at spindle poles.

A final interesting feature of the pericentrin-overexpressing phenotype is the generation of aneuploid cells. In fact, pericentrin-overexpressing cells have chromatin levels both below and above diploid, suggesting that they undergo persistent chromosome missegregation as described (Lengauer et al., 1997). Since little is known about how aneuploid cells are generated, this cell system provides a powerful model to study this phenomenon. This system may also prove useful in understanding human tumorigenesis since pericentrin levels are elevated in most aneuploid tumors (Doxsey, 1998; Pihan et al., 1998; Pihan, G., and S. Doyxey, unpublished observations). For these reasons, it is important to determine the precise contributions of dynein and other pericentrin-interacting molecules in the generation of aneuploidy and spindle defects in pericentrin-overexpressing cells.

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