Knockdown of dynactin's p150^{Glued} subunit abrogates microtubule organization

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Nicholas Quintyne, and has been approved by the members of the supervisory committee. It was submitted to the faculty of The Honors College and was accepted in partial fulfillment of the Requirements for the Degree of Bachelor of Arts in Liberal Arts and Sciences.

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ABSTRACT

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Dynactin is a multifunctional protein complex composed of at least 11 different subunits. Dynactin functions as a cofactor for cytoplasmic dynein facilitating long-range vesicle movements, microtubule anchoring, endomembrane localization, and mitotic progression. Previous studies have shown that dynactin binds to microtubules at the centrosome maintaining a radial array in interphase. The p150^{Glued} subunit contains two distinct microtubule-binding sequences named CAP-Gly and Basic. While both domains can interact with microtubule, CAP-Gly has a much greater affinity for binding to microtubules, suggesting that the two domains may be active for different dynactin-based functions within the cell. Using siRNA, we found that knockdown of p150^{Glued} was sufficient to alter the maintenance of radial microtubule arrays, cause an increase in centrosome number and mitotic index. In the future we will replace the endogenous protein with versions lacking the CAP-Gly or Basic domains to investigate the contribution of each to microtubule anchoring and cytoskeletal architecture.

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INTRODUCTION

Dynactin was first identified as a cytosolic activity that increased cytoplasmic dynein processivity along microtubules (Schroer & Sheetz, 1991). Further work determined that dynactin was a multisubunit protein consisting of at least 11 domains (Gill et al., 1991; for a review see Schroer, 2004). It is important in many cellular processes. Dynactin is required for mitosis in multicellular organisms and plays a role in subcellular movement and cargo binding (Echeverri et al., 1996; Burkhardt et al., 1997; Valetti et al., 1999). Dynactin is a required factor for microtubule anchoring at the centrosome (Quintyne et al., 1999). Disruption of dynactin leads to a breakdown of plus-end directed and minus-end directed organelle transport (Deacon et al., 2003). Research into the function and regulation of dynactin has lead to insights in the mechanics of degenerate neurons (Lane et al., 2001; Puls et al., 2003). A subunit of dynactin, $p150^{Glued}$, may be involved in muscular dystrophy (Tokito & Holzbaur, 1998).

Cytoplasmic Dynein

Cytoplasmic dynein is a minus-end directed microtubule motor (Paschal & Vallee, 1987) thought to be involved in vesicle movement, spindle pole retention, organelle retention, and chromosome movement (for a review see Holzbaur & Vallee, 1994). Cytoplasmic dynein was first identified in sea urchin eggs, (Hisanaga & Sakai, 1983) then in *Caenorhabditis elegans* (Lye et al., 1987) and bovine brain (Paschal et al., 1987). Antibodies to dynein subunits have been shown to localize at the kinetochores during mitosis (Pfarr et al., 1990). Dynein has also been found to associate with various membrane organelles such as endocytic vesicles, lysosomes, microsomes, and parts of the

Goli apparatus (Lin and Collins, 1992; Corthezy-Theulaz et al., 1992). As shown in figure 1, dynein is a multisubunit protein that consists of two catalytic heavy chains (HC, 532 kDa), several intermediate chains (IC, 74kDa), light intermediate chains (LIC 53-59kDa), and light chains (LC) (Paschal et al., 1987; Vaughan & Vallee, 1995; King & Schroer, 2000). By using energy from ATP, the heavy chains generate force to move the protein and cargo along microtubules. The LC are involved in cargo binding, the LIC binds directly to the dynein heavy chain and may mediate the attachment of dynein to centrosomes and other cargoes, and IC are the location of dynactin binding.

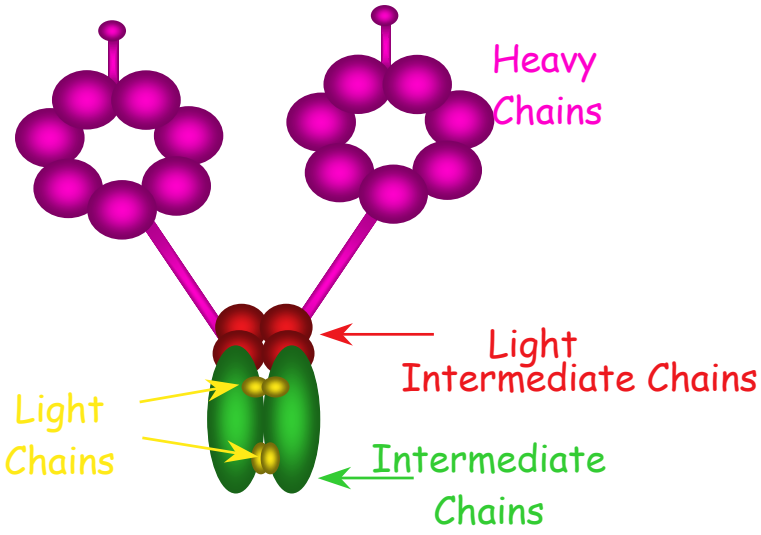

Figure 1. Structural model of cytoplasmic dynein. (Figure courtesy of SJ King).

Dynactin

Dynactin (shown in Figure 2) is multisubunit protein complex (Gill et al., 1991) that allows cytoplasmic dynein to carry out long-range vesicle movements on microtubules *in vitro* (Schroer & Sheetz, 1991). Studies in yeast, *Drosophila*, and filamentous fungi firmly established dynactin as a necessary cofactor to cytoplasmic dynein (for a review

see Schroer, 1994). Because dynactin is required for mitosis in multicellular organisms, it is essential for viability. It has been shown that mutations to dynactin subunits can cause defects in axonal transport (Puls et al., 2003). The largest subunit of dynactin, $p150^{Glued}$, participates in binding to dynein and increased motor processivity (Karki & Holzbaur, 1995; Vaughan & Vallee, 1995; King & Schroer, 2000). The subunit $p150^{Glued}$ is also a target of apoptotic cleavage and therefore is important for normal cell function (Lane et al., 2001).

Structure and Subunits

Protein isolation from animal brains has allowed for the study and characterization of dynactin (Bingham et al., 1998). Dynactin is complex molecule composed of 11 distinct peptide units that make up a projection arm and rod-like domain. Since there are multiple copies of certain subunits, each dynactin molecule is made of over 20 individual peptides, has a mass of approximately 1.2 MDa (Schroer, 2004) and is divided into two distinct domains. Most of the mass is contained in the projecting arm (also called the shoulder/sidearm), which includes $p150^{Glued}$, dynamitin, and p24/p22 (Schroer, 2004). The shoulder/sidearm allows for microtubule attachment. The rod-like domain includes CapZ, Arp1, Arp11 and other proteins, and serves to allow dynactin to bind to various membranes and intracellular cargos.

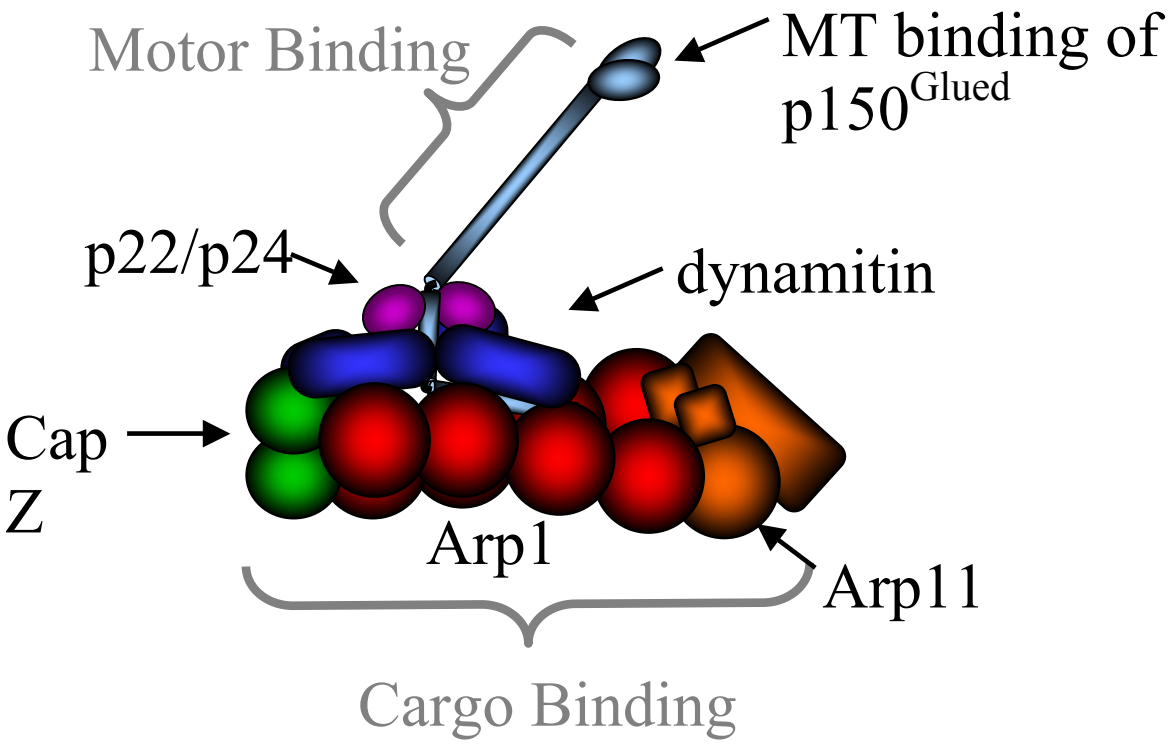

Figure 2. **Dynactin Ultrastructural Model.** Dynactin is a multisubunit protein complex that has been shown to be a required cofactor of dynein and is also required for MT anchoring at the centrosome. The two globular heads of the long arm, p150glued, have MT binding sites. (Figure adapted from Quintyne et al., 1999).

Arp1 Rod

The Arp1 rod is approximately 10 x 40nm, resembles an actin filament, and is composed of Arp1, Arp11, Actin, CapZ, p62, p27, and p25. Arp1 can hydrolyze ATP (Bingham & Schroer, 1999) and bind directly to Golgi structures via spectrin family proteins (Holleran et al., 2001). The end of Arp1 terminates with CapZ, a plus end actin-capping protein (Schafer et al., 1994). Arp11 caps the minus end of Arp1. The p62 subunit is thought to be involved in protein-protein binding because it has a zinc-binding motif (Eckley et al., 1999). p25 and p27 may serve as adapter proteins allowing the Arp1 rod to bind to various subcellular structures (Eckley et al., 1999).

The Shoulder/sidearm

The shoulder/sidearm extends from the Arp1 rod and contains three subunits, $p150^{Glued}$, dynamitin, and p24/p22. Dynactin molecules have two copies of $p150^{Glued}$, two copies of p24/p22, and four copies of dynamitin which self-associate by forming α-helical structures called coiled coils (Melkonian et al., 2007). As shown in Figure 3, $p150^{Glued}$ contains two coiled coils and two globular heads at the extreme N terminal. The CC2 domain in Figure 3 is contained within the shoulder the sits on Arp 1. $p150^{Glued}$ can take various isoforms via alternate splicing (Tokito & Holzbaur, 1998). Each globular head contains a conserved CAP-Gly (Cytoskeleton associated protein, Glycine-rich) motif and a basic motif (Culver-Hanlon et al., 2006). Interactions with microtubule based motors such as dynein take place within the middle portion of $p150^{Glued}$. The C-terminal end of $p150^{Glued}$ contains a second CC domain suggested to interact with the Arp1 rod (Waterman-Storer et al., 1995). While there are likely additional phosphorylation sites, it has been shown that phosphorylation of $p150^{Glued}$ at Ser19 reduces its affinity for microtubules (Vaughan et al., 2002).

The subunit dynamitin is involved in linking $p150^{Glued}$ and p24/p22 via a series of coiled coil motifs (Melkonian et al., 2007). Dynamitin may allow for flexibility in the in the area where projecting arm and Arp1 rod connect (Eckley et al., 1999). Dynamitin has been shown to bind to the kinetochore protein Zw10 (Starr et al., 1998). p24/p22 is dynactin's smallest subunit (21kDa) and can interact with $p150^{Glued}$ and dynamitin (Karki et al. 1998; Pfister et al., 1998).

Figure 3. **Schematic of dynactin p150^{Glued}.**
Both the "CAP-Gly" (cytoskeleton-associated protein, Glycine-rich) domain and the "basic" domain can bind microtubules. Two copies of p150^{glued} form an α-helical coiled-coil structure. (Figure courtesy of SJ King).

CAP-Gly and Basic Motifs

The N terminal fragments of p150^{Glued} have been shown to bind microtubules *in vitro* (Waterman-Storer et al., 1995). Both the CAP-Gly (a.a. 1-110) and the basic domains (a.a. 115-208) have microtubule binding motifs. CAP-Gly is a conserved microtubule-binding motif. These motifs are key to dynactin's function because microtubule binding is needed in order to increase dynein processivity (King & Schroer, 2000). These domains may also play a role in microtubule anchoring at the centrosome and mitotic spindle poles (Quintyne et al., 1999; Quintyne & Schroer, 2002). Using microtubule co-pelleting assays and serial deletion constructs of p150^{Glued}, it was discovered that a domain with a high percentage of basic amino acids (the basic domain) could bind microtubules in the absence of the CAP-Gly domain and its sequence was highly conserved in other vertebrate species (Culver-Hanlon et al., 2006). It is currently thought that the CAP-Gly domain has a higher affinity for microtubules than the basic domain. The basic domain can "skate" along microtubules in the absence of dynein (Culver-Hanlon et al., 2006).

Dynein-Dynactin Interactions

The interaction of dynein and dynactin is fairly well understood. Dynein is thought to bind to dynactin at dynactin's shoulder/sidearm complex. $p150^{Glued}$ binds directly with the intermediate chain (IC) of dynein (Karki & Holzbaur, 1995; Vaughan & Vallee, 1995; King et al., 2003). As mentioned earlier, dynactin increases the processivity of dynein by binding to both the microtubule and dynein *in vitro* (King & Schroer, 2000). According to the current dynactin tether model, dynein binds to $p150^{Glued}$, allowing dynein to transport the cargo carried by the Arp1 rod. The basic domain prevents the complete dissociation of the dynein-dynactin-cargo complex when dynein prematurely slips off the microtubule (Culver-Hanlon et al., 2006). This increases the processivity of the dynein motor.

Centrosomal Anchoring

Dynactin works independently of dynein in many cellular processes such as anchoring microtubules at the centrosome. The centrosome serves as the microtubule-organizing center (MTOC) in most cells. Cells in G_0 or G_1 usually have two centrioles that are surrounded by the pericentriolar matrix (PCM). These are duplicated during S phase and migrate to opposite poles during mitosis. γ tubulin and other proteins are involved in forming the γ–TuRC, which nucleates microtubules (for review see Wiese & Zheng, 1999). Microtubule elongation occurs at the plus-ends by the addition of α and β tubulin sunbunits. Although γ tubulin is needed for microtubule nucleation, it has may not be necessary for microtubule retention (Quintyne et al., 1999). The centrosome is a dynamic structure that changes composition throughout the cell cycle and full understanding of its

structure and function is far from complete. Various proteins are associated with the MTOC. PCM-1, pericentrin, and NuMA are brought to the centrosome via dynein-mediated transport (Balczon et al., 1999; Zimmerman & Doxsey, 2000).

Antibodies to dynactin showed that it accumulates at the centrosomes throughout interphase (Quintyne & Schroer, 2002) and during mitosis at the spindle poles (Echeverri et al., 1996). In vitro experiments have shown that inhibition of dynein or dynactin results in unfocused microtubule arrays. More specifically, loss of $p150^{Glued}$ leads to loss of a focused radial microtubule array in interphase suggesting that this subunit plays a key role in retaining microtubules at the centrosome (Quintyne et al., 1999). Currently, the microtubule anchoring model involves dynactin, nudel (Guo et al., 2006), Cep135 (Ohta et al., 2002), and Ninein (Mogensen et al., 2000). Of these proteins, dynactin is the only one with a known MT binding motif.

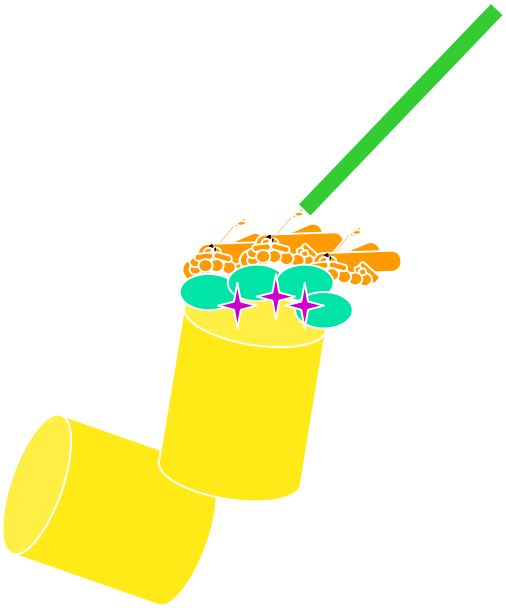

Figure 4. Proposed model of centrosomal anchoring. Orange- dynactin, blue- ninein, purple- nudel/Cep135. These proteins are involved in microtubule retention at the centriole (yellow). (Figure courtesy of NJ Quintyne).

The objective of this study is to further elucidate the role $p150^{Glued}$ in centrosomal anchoring. By transfecting cells with mutant versions of the $p150^{Glued}$ microtubule binding domains we hope to gain a better understanding in the role of the domains in microtubule retention at the centrosome. We predict that loss of the CAP-Gly domain will be involved in microtubule retention to a greater degree than the basic domain.

METHODS

Cell Culture

Cos-7 cells, an African green monkey kidney cell line, was obtained from American Type Cell Culture (Manassas, VA). Cos-7 cells were cultured in Dulbecco's Modified Eagle Medium (Sigma Chemical Corp.; St. Louis, MO) supplemented with 10% Fetal Bovine Serum (Hyclone; Logan, UT) and 1% penicillin-streptomycin (Quality Biological, Inc.; Gathersburg, MD). Cells were plated in $10cm^2$ tissue culture petri dishes and maintained in a 37°C, 5% CO_2, and 19.5% O_2 environment.

Cells were separated from the Petri dish using 0.05% tryspin-EDTA (MP Biomedical, Inc.; Solon, OH). For experiments, cells were plated on $22mm^2$ cover slips at a density of 1.5×10^5 cells/slip. Cells were incubated overnight prior to transfection or immunofluorescence.

Plasmid Preparation

We received four $p150^{Glued}$ knockdown plasmids courtesy of the lab of Stephen King (University of Missouri Kansas City). Each plasmid was transformed into TurboCells competent *E. coli* , (Genlantis; San Diego, CA) plated onto Luria plates (BD Bacto; Franklin Lakes, NJ) supplemented with ampicillin (1:1000 ratio), (Sigma) and grown overnight at 37°C. Single colonies were isolated and grown in liquid LB with Ampicillin (1:1000) as directed by the Qiagen MaxiPrep protocol. Plasmids were isolated following the MaxiPrep directions (Qiagen; Valencia, CA). Plasmid DNA was digested with the restriction enzyme PvuI and the plasmid identities were confirmed using gel

electrophoresis using a 1% agarose gel and 1x TAE buffer. Plasmid concentration was determined using spectrophotometry (λ= 260-280 nm).

Transfection

Cells were transfected using polyethylenimine (PEI) reagent (Polysciences, Inc.; Warrington, PA) or Lipofectamine (Invitrogen; Carlsbad, CA) following the manufactures instructions. For $p150^{glued}$ knockdown, cells were transfected with a cocktail of plasmids (1A, 3B, 4A, 5A). Once transfected, cells were incubated for 48 hours.

Immunofluorescence

Immunofluorescence was performed as previously described (Quintyne et al., 1999). In brief, cells on coverslips were rinsed with PBS then fixed for 10 minutes with -20°C MeOH. Cells were blocked for 15 minutes with PBST/1% BSA, incubated with the primary antibody solution for 30 minutes, rinsed 3X with PBS, incubated in secondary antibody for 15 minutes, and rinsed 3X with PBS. DAPI (4,6-diamidino-2-pheylindole; Sigma) was used to stain chromatin. Coverslips were mounted using 3:1 Mowiol:n-propyl gallate (Fluka; Germany and MP Biomedical) and secured with nail polish. Slides were stored at -20°C.

The following primary antibodies were used in this study: α-tubulin-DM1A (Sigma); γ-tubulin-GTU88 (Sigma); $p150^{glued}$ (BD Transduction Laboratories). Secondary antibodies used were: Alexa488 anti-mouse and anti-rabbit (Molecular Probes), Texas Red X anti-mouse and anti-rabbit (Molecular Probes).

Slides were viewed using an Olympus IX-81 inverted fluorescence microscope (100x objective/NA 1.65; Olympus America, Inc.; Center Valley, PA). Images were

captured using a Hamamatsu C4742-95 high-resolution cooled-CCD camera (Hamamatsu; Bridgewater, NJ) and recorded using Slidebook software (version 4.1; Intelligent Imaging Innovation, Inc.; Denver, CO). All images were imported into Adobe Photoshop® (Adobe Systems) as TIFFs for contrast manipulation and figure assembly.

Immunoblotting

Cells were grown to 80-90% confluence in 10mm Petri dishes and transfected following the protocol outlined above. Trypsin-EDTA was used to separate the cells from the plate. This was followed by a 5 minute spin at 1000RPM. Cells were resuspended in 300μL Tris HCL + 1% triton X-100 lysis buffer, spun for 5 minutes on fast. Proteins were loaded into a 12% SDS-PAGE gel and run at 150V for 1:15 minutes. The proteins were transferred to a nylon membrane. Membranes were blocked in 5% non-fat dried milk with TBS-tween-20. Primary antibodies were added and allowed to incubate overnight at 4°C. Membranes were incubated with Western Blue at RT for 1 hour.

RESULTS

Purification of plasmid

The p150^{Glued} knockdown plasmids were transformed into *E. coli* and then isolated. The electrophoresis gel indicated that the plasmids isolated after transformation matched the original plasmids.

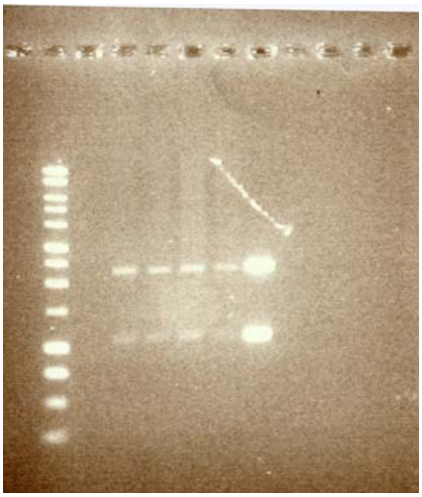

Figure 5. DNA gel. Plasmids 1A, 3B, 4A, and 5A were isolated from Turbo cells, digested with PvuI, and compared to the standard (rightmost lane).

Western blotting suggests partial p150^{Glued} knockdown

To test if the p150^{Glued} knockdown plasmids worked we conducted a Western blot. COS-7 cells were transfected and lysed after 48 hours. Protein extracts were run on a gel and transferred to a nylon membrane where they were probed with p150^{Glued} and actin antibodies. The actin antibody did not appear on the Western blot regardless of the ratio of antibody used making it difficult to draw conclusions as to the evenness of the amount of sample loaded. It appears that some degree of p150^{Glued} knockdown occurred when the PEI reagent was used. Thus, we decided to use PEI in future transfections. FuGENE shows no knockdown.

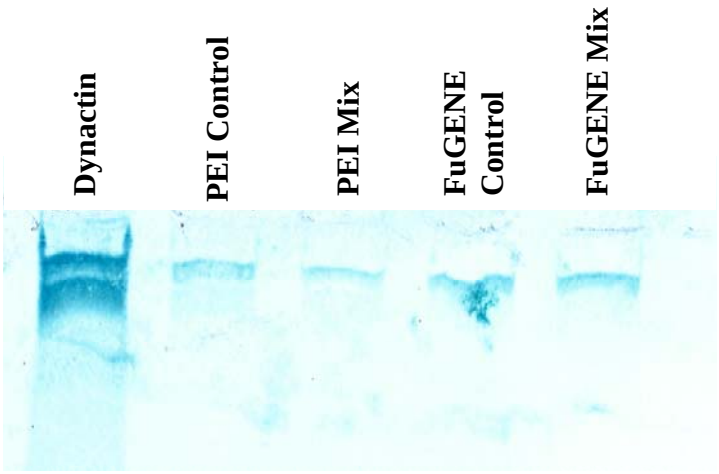

Figure 6. Expression of p150^{Glued}. Western blot suggests that PEI transfection slightly decreased p150^{Glued} expression. The FuGENE method does not indicate that the transfection worked. Mix indicates that cells were transfected with a mix of plasmids 1A, 3B, 4A, and 5A.

Determination of transfected cells

Only cells which accept the p150^{Glued} plasmid should show a change in protein expression. To test the efficiency of the transfection, co-transfection experiments were conducted. In these experiments the plasmid to knockdown p150^{Glued} and a plasmid to express GFP-centrin were transfected simultaneously into COS-7 cells. Cells were fixed after 48 hours. The experiment showed that the transfection efficiency was low and that it was difficult to determine which cells were transfected as cells showed varying background levels of FITC after being exposed to various light intensities from the microscope. Entire slides were treated as transfected in the following experiments because of these difficulties.

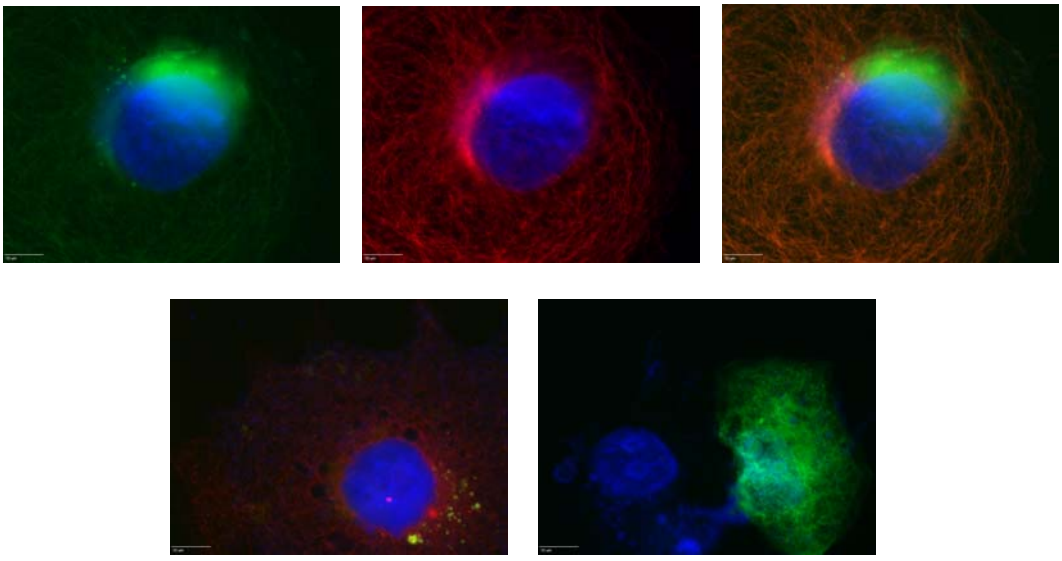

Figure 7. Representative images of co-transfected cells. COS-7 cells transfected with GFP-centrin and $p150^{Glued}$ knockdown plasmid. Cells fixed after 48 hours and stained as follows: microtubules (red), nucleus (blue), and GFP-centrin (green).

Loss of radial array

In interphase the microtubules of COS-7 cells are usually arranged in a radial array. It has been shown that loss of dynactin perturbs microtubule organization. To test this control and transfected COS-7 cells were stained for α tubulin and scored for the presence or absence of a radial microtubule array. In control cells 92.50% of cells were scored as having a radial array. After transfection the percentage of cells with a radial array decreased to 41.82%.

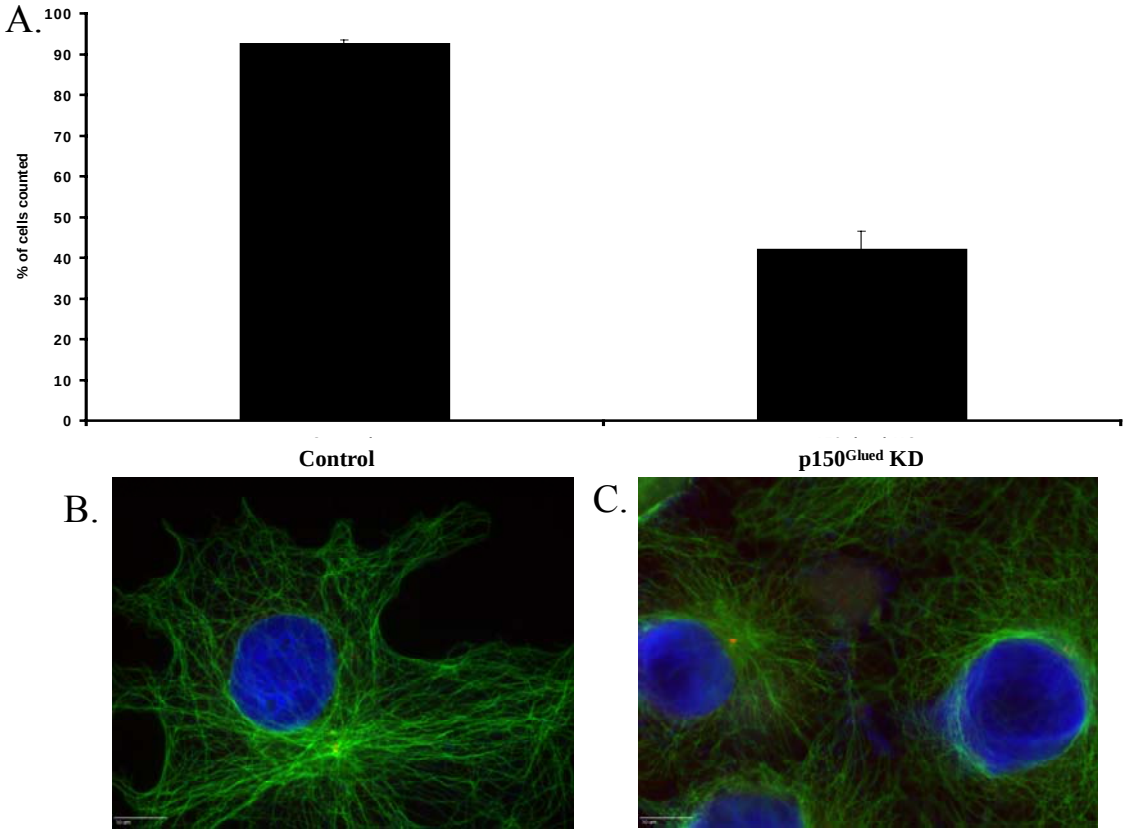

Figure 8. Radial Array. (A)The graphs shows the percentage of COS-7 with a radial array. Knockdown of p150^{Glued} significantly decreased the percentage of cells with a radial microtubule array. B and C are representative images of Cos-7 cells stained with antibodies to a-tubulin (green), g-tubulin (red), and DAPI (blue). (B) radial microtubule array in untreated cells and (C) Transient p150^{Glued} knockout showing a radial MT array (left) and a non-radial MT array (right).

Loss of p150^{Glued} increases centrosome number

COS-7 cells were transfected with a mix of p150^{Glued} knockdown plasmid and fixed after 48 hours. Centrosome number was determined by counting γ tubulin foci. Cells were scored normal if 1-4 γ tubulin foci were present. Cells exhibiting 5 or more centrosomes were scored as abnormal. In control cells, 99.01% of counted cells were scored as normal. After treatment this number decreased to 91.10%. Numbers are an average of three independent experiments.

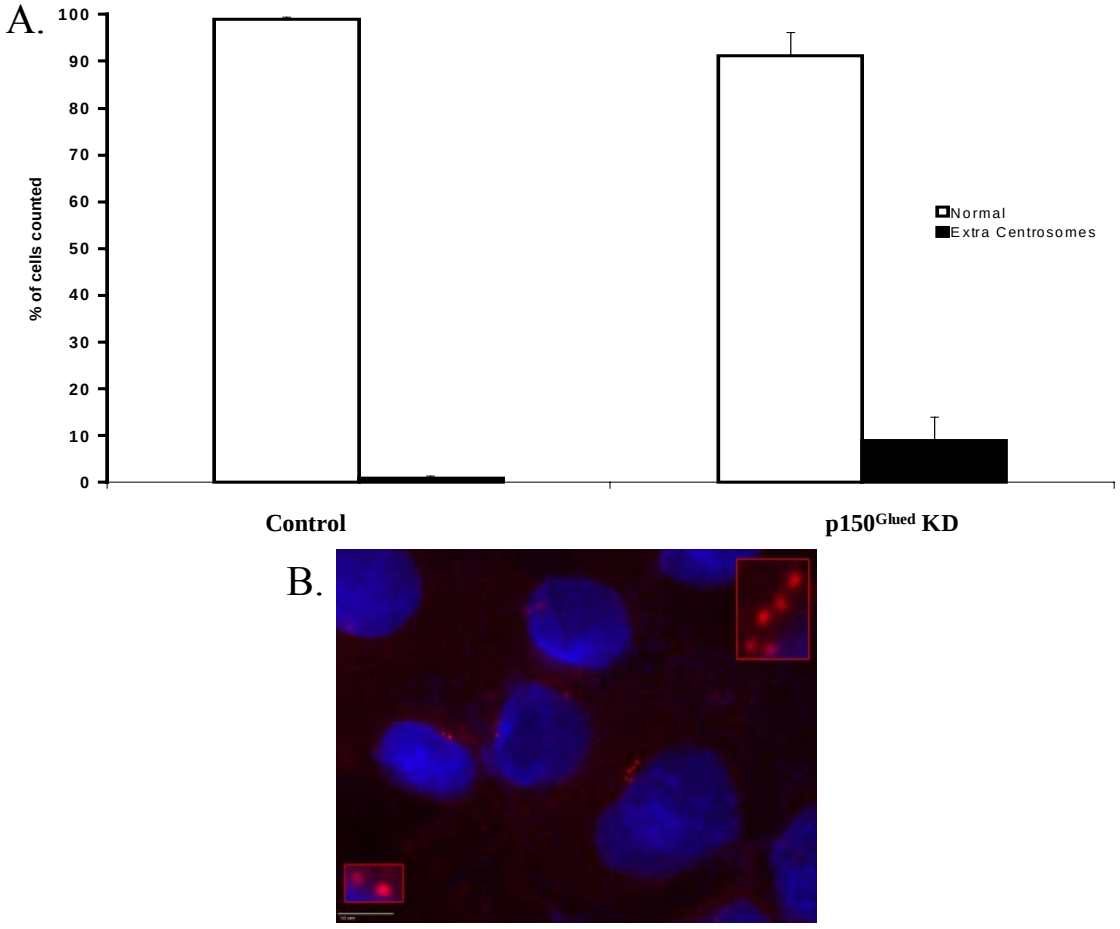

Figure 9. Increase in supernumerary centrosomes. (A)The percentage of COS-7 cells with a normal number of centrosome decreased after $p150^{Glued}$ knockdown. (B) Effects of p150glued knockout on centrosome number and organization. Representative image of Cos-7 cells stained with antibodies to a-tubulin (green), γ-tubulin (red), and DAPI (blue). Red boxes have a 4X magnification.

Loss of $p150^{Glued}$ increases mitotic index

Mitotic index measures the rate at which cells are dividing. To test if loss of $p150^{Glued}$ altered the rate of mitosis, mitotic index was determined by viewing cells stained with DAPI. The control COS-7 cells had a mitotic index of 5.06%. After knocking down $p150^{Glued}$, the mitotic index increased to 13.90%. Interestingly, a 3-fold increase in the number of binucleate cells was also observed.

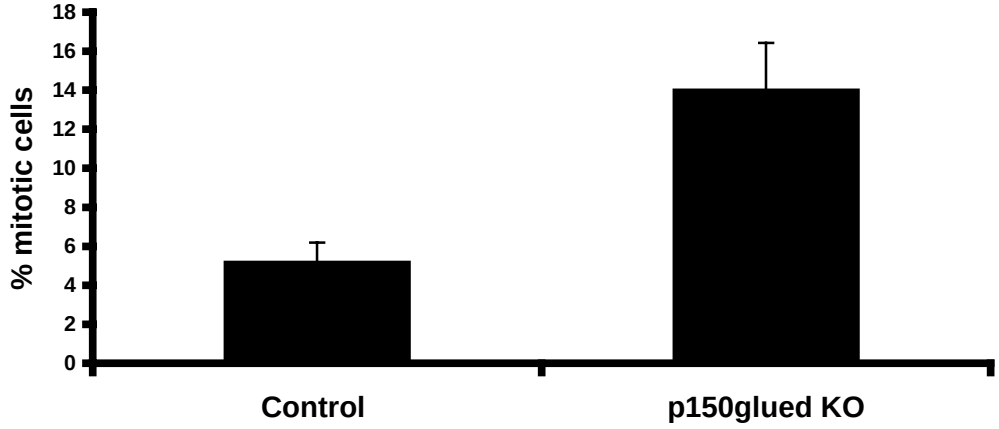

Figure 10. Increase in mitotic index. COS-7 cells were fixed and stained with DAPI 48 hours after being transfected to knockdown $p150^{Glued}$ expression. Graph is an average of three independent experiments.

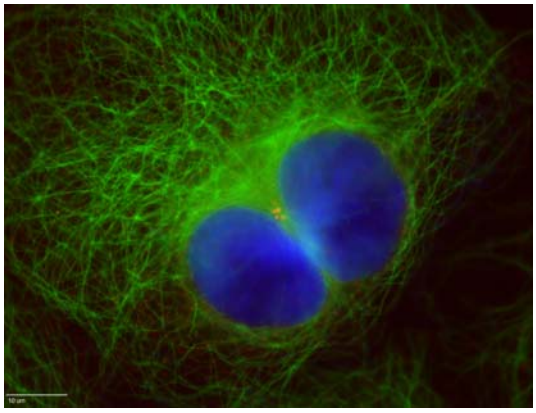

Figure 11. Representative image of a binucleate cell. Cell was transfected to knockdown $p150^{Glued}$ expression and stained. Nucleus (blue), microtubules (green), and centrosomes (red).

DISCUSSION

The present study has increased understanding of the role of the $p150^{Glued}$ subunit of dynactin in interphase fibroblasts. While the interactions between dynactin, microtubules, and the centrosome are complex, our data allows us to draw some simple conclusions. First, our experiments confirm that $p150^{Glued}$ is required for microtubule anchoring at the centrosome. The knockdown of $p150^{Glued}$ resulted in an increase in microtubule disorganization. The $p150^{Glued}$ subunit must play a role in tethering microtubules to the centrosome or holding other key retention proteins in place. This conclusion is supported by other studies. Both underexpression and overespression of the $p150^{Glued}$ subunit can abrogate microtubule organization. For example, Quintyne and coworkers (1999) found that overexpression of $p150^{Glued}$ caused a 75% decrease in the number of cells with a radial array and proposed that dynein-mediated transport is needed for dynactin and $p150^{Glued}$ to be delivered to the centrosome. Since $p150^{Glued}$ crucial in increasing dynactin processivity, loss of the $p150^{Glued}$ subunit would decrease processivity and the motor's ability to localize dynactin at the centrosome.

Centrosomes duplication is highly regulated usually occurring during S phase and extra centrosomes have been linked to an increase in the formation of multipolar spindles and certain types of cancer. Our data supports a connection between loss of $p150^{Glued}$ and the presence of supernumerary centrosomes. Extra γ tubulin foci were observed in transfected cells. We observed an increase in mitotic index and an increase in the number of binucleate cells when $p150^{Glued}$ was knocked down. An increase in binucleate cells has been observed in other studies that perturbed dynactin expression (Quintyne et al., 1999). Loss of $p150^{Glued}$ could result in a disruption of chromosome segregation increasing the

length of mitosis. This would explain the increase in mitotic index. Overexpression of dynamitin or Arp1 has been shown to similarly increase mitotic index, as the cells arrest in prometaphase (Echeverri et al., 1996; Clark and Meyer, 1999)). These arrested cells are capable of eventually completing mitosis, albeit taking a much longer time (Clark and Meyer, 1999). A failure of cytokinesis could explain the extra centrosomes in transfected cells. Multiple γ tubulin foci could also accumulate because of centrosome fragmentation.

In the future other thesis students will investigate the role of the CAP-Gly and basic microtubule binding domains $p150^{Glued}$ in microtubule anchoring at the centrosome. The constructs for these experiments were unavailable of the time of my research. We were unable to restore $p150^{Glued}$ expression using the current $p150^{Glued}$ plasmid. Experiments aimed at restoring function were unsuccessful because the plasmid had a high copy promoter (data not shown). Other studies have indicated that overexpression of $p150^{Glued}$ can abrogate microtubule arrays and lead to supernumerary centrosomes (Quintyne et al., 1999), therefore not allowing us to differentiate between cells that had compromised microtubule arrays due to knockdown of $p150^{Glued}$ expression, and those that had compromised microtubule arrays from $p150^{Glued}$ overexpression. A low-copy plasmid, with a different promoter, is currently being made to solve this problem.

REFERENCES

Balczon R, Varden CE, Schroer TA. 1999. Role for microtubules in centrosome doubling in Chinese hamster ovary cells**.** *Cell Motil Cytoskeleton* 42:60-72.

Bingham JB, King SJ, Schroer TA. 1998. Purification of dynein and dynactin from brain tissue. *Methods Enzymol* 298: 171-84.

Bingham JB, Schroer TA. 1999. Self-regulated polymerization of the actin-related protein Arp1.*Curr Biol* 9(4):223-6.

Burkhardt JK, Echeverri CJ, Nilsson T, Vallee RB. 1997. Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. *J Cell Biol* 139(2):469-84.

Clark IB, Meyer DI. 1999. Overexpression of normal and mutant Arp1alpha (centractin) differentially affects microtubule organization during mitosis and interphase. *J Cell Sci* 1123507-18.

Corthrsy-Theulaz I, Pauloin A, Pfeffer SR. 1992. Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. *J. Cell Biol* 118:1333-1345.

Culver-Hanlon TL, Lex SA, Stephens AD, Quintyne NJ, King SJ. 2006. A microtubule-binding domain in dynactin increases dynein processivity by skating along microtubules. *Nature Cell Biology* 8:3: 264-270.

Deacon SW, Serpinskaya AS, Vaughan PS, Fannarraga ML, Vernos I, Vaughan KT, Gelfand VI. 2003. Dynactin is required for bi-directional organelle transport. *J Cell Biol* 160(3): 297-301.

Echeverii CJ, Paschal BM, Vaughan KT, Vallee RB. 1996. Molecular characterization of

the 50kD subunit of dynactin reveals function for complex in chromosome alignment and spindle organization during mitosis. *J. Cell Biol* 132:617-19.

Eckley DM, Gill SR, Kelkonian KA, Bingham JB, Goodson HV, et al. 1999. Analysis of dynactin subcomplexes reveals a novel actin-related protein associated with the Arp1 filament pointed end. *J.Cell Biol* 147:307-19.

Gill SR, Schroer TA, Szilak I, Steuer ER, Sheetz MP, Cleveland DW. 1991. Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by dynein transport. *J Cell Biol* 115 (6): 1639-1650.

Guo J, Yang Z, Song W, Chen Q, Wang F, Zhang Q, Zhu X. 2006. Nudel Contributes to Microtubule Anchoring at the Mother Centriole and Is Involved in Both Dynein-dependent and -independent Centrosomal Protein Assembly. *Mol. Biol. Cell* 17:680-89.

Hisanaga S, Sakai H. 1983. Cytoplasmic dynein of the sea urchin egg. II. Purification, characterization and interactions with microtubules and Ca-calmodulin. *J Biochem* 93(1):87-98.

Holleran EA, Ligon L., Tokito M, Stankewhich MC, Morrow JS, et al. 2001. beta III spectrin binds to the Arp1 subunit of dynactin. *J. Biol. Chem* 276:36598-605.

Holzbaur ELF, and Vallee RB. 1994. Dynein's: molecular structure and cellular function. *Anna. Rev. Cell Biol* 10:339-372.

Karki S, Holzbaur EL. 1995. Affinity chromatography demonstrates a direct binding between cytoplasmic dynein and the dynactin complex. *J Biol Chem* 270(48):28806-11.

Karki S, LaMonte B, Holzbaur EL. 1998. Characterization of the p22 subunit of

dynactin reveals the localization of cytoplasmic dynein and dynactin to the midbody of dividing cells. *J. Cell Biol* 142:1023-34.

King SJ, Brown CL, Maier KC, Quintyne NJ, Schroer TA. 2003. Analysis of the dynein-dynactin interaction in vitro and in vivo. *Mol Biol Cell* 14(12):5089-97.

King SJ, Schroer TA. 2000. Dynactin increases the processivity of the cytoplasmic dynein motor. *Nat Cell Biol* 2(1):20-4.

Lane JD, Vergnolle MA, Woodman PG, Allan VJ. 2001. Apoptotic cleavage of cytoplasmic dynein intermediate chain and p150(Glued) stops dynein-dependent membrane motility. *J Cell Biol* 2001 Jun 25;153(7):1415-26.

Lin SX, Collins CA. 1992. Immunolocalization of cytoplasmic dynein to lysosomes in culture cells. Z *Cell Sci* 101:125-137.

Lye RJ, Porter ME, Scholey JM, McIntosh JR. 1987. Identification of a microtubule-based motor in the nematode C. elegans. *Cell* 51:309-318.

Melkonian KA, Maier KC, Godfrey JE, Rodgers M, Schroer TA. 2007. Mechanism of dynamitin-mediated disruption of dynactin. *J Biol Chem* 282(27):19355-64.

Mogensen MM, Malik A, Piel M, Bouckson-Castaing V, Bornens M. 2000. Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. *J. Cell Sci* 113:3013–3023.

Ohta T, Essner R, Ryu J, Palazzo RE, Uetake Y, Kuriyama R. 2002. Characterization Cep135, a novel coiled-coil centrosomal protein involved in microtubule organization in mammalian cells. *J. Cell Biol* 156:87-99.

Paschal BM, Vallee RB. 1987. Retrograde transport by the microtubule associated protein MAP 1C. *Nature (Lond.)* 330:181-183.

Paschal BM, Shpetner HS, Vallee RB. 1987. MAP 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties. *J. Cell Biol* 105:1273-1282.

Pfarr CM, Coue M, Grisson PM, Hays TS, Porter ME, McIntosh JR. 1990. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature (Lond.)* 345:263-265.

Pfister KK, Benashski SE, Dillman JF, Patel-King RS, King SM. 1998. Identification and molecular characterization of the p24 dynactin light chain. *Cell Motil Cytoskeleton* 41(2):154-67.

Puls I, Jonnakuty C, LaMonte BH, Holzbaur EL, Tokito M, et al. 2003. Mutant dynactin in motor neuron disease. *Nat.Genet* 159:245-54.

Quintyne NJ, Gill SR, Eckley DM, Crego CL, Compton DA, Schroer TA. 1999. Dynactin is required for microtubule anchoring at fibroblast centrosomes. *J. Cell Biol* 147:321-334.

Quintyne NJ, Schroer TA. 2002. Distinct cell cycle-dependent roles for dynactin and dynein at centrosomes. *J. Cell Biol* 159:245-54.

Schafer DA, Gill SR, Cooper JA, Heuser JE, Schroer TA. 1994. Ultrastructural analysis of the dynactin complex: An actin-related protein is a component of a filament that resembles F actin. *J.Cell Biol* 126:403-412.

Schroer TA. 2004. Dynactin. *Annu. Rev. Cell Dev. Biol* 20:759-79.

Schroer TA. 1994. New insights into the interaction of cytoplasmic dynein with actin-related protein, Arp 1. *J. Cell Biol* 127:1-4.

Schroer TA, Sheetz MP. 1991. Two activators of microtubule-based vesicle transport. *J Cell Biol* 115(5):1309-18.

Starr DA, Williams BC, Hays TS, Goldberg ML. 1998. ZW10 helps recruit dynactin and dynein to the kinetochore. *J. Cell Biol* 142:763-74.

Valetti C, Wetzel DM, Schrader M, Hasbani MJ, Gill SR, Kreis TE, Schroer TA. 1999. Role of dynactin in endocytic traffic: effects of dynamitin overexpression and colocalization with CLIP-170. *Mol Biol Cell* 10(12):4107-20.Vaughan PS, Miura P, Henderson M, Byrne B, Vaughan KT. 2002. A role for regulated binding of p150(Glued) to microtubule plus ends in organelle transport. *J. Cell Biol* 158:305-19.

Vaughan KT, Vallee RB. 1995. Cytoplasmic dynein binds dynactin through a direct interaction between intermediate chains and $p150^{Glued}$. *J. Cell Biol* 131:1507-16.

Waterman-Storer CM, Karki S, Holzbaur EL. 1995. The p150Glued component of the dynactin complex binds to both microtubules and the actin-related protein centractin (Arp1). *Proc. Natl. Acad. Sci. USA* 92:1634-38.

Wiese C, Zheng Y. 1999. Gamma-tubulin complexes and their interaction with microtubule-organizing centers. *Curr. Opin. Struct. Biol* 9:250–259.

Zimmerman W, Doxsey SJ. 2000. Construction of centrosomes and spindle poles by molecular motor-driven assembly of protein particles. *Traffic* 1:927–34.