The Product of the Drosophila Gene, Glued, Is the Functional Homologue of the \( \text{p150}^{\text{Glued}} \) Component of the Vertebrate Dynactin Complex*

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\( \text{p150}^{\text{Glued}} \) is the largest polypeptide in the dynactin complex, a protein heteromultimer that binds to and may mediate the microtubule-based motor cytoplasmic dynein. Cloning of a cDNA encoding \( \text{p150}^{\text{Glued}} \) from rat revealed 31% amino acid sequence identity with the product of the Drosophila gene, Glued. A dominant Glued mutation results in neuronal disruption; null mutations are lethal. However, the Glued gene product has not been characterized. To determine whether the Glued polypeptide is functionally similar to vertebrate \( \text{p150}^{\text{Glued}} \), we characterized the Glued protein in the Drosophila S-2 cell line. Antibodies raised against Glued were used to demonstrate that this protein sediments exclusively at 20 S, and associates with microtubules in a salt- and ATP-dependent manner. Immunoprecipitations from S-2 cytosol with the anti-Glued antibody resulted in the co-precipitation of subunits of both cytoplasmic dynein and the dynactin complex. An affinity column with covalently bound Glued protein retained cytoplasmic dynein from S-2 cytosol. Based on these observations, we conclude that Glued is a component of a dynactin complex in Drosophila and binds to cytoplasmic dynein, and therefore the mutant Glued phenotypes can be interpreted as resulting from a disruption in the function of the dynactin complex.

The vertebrate dynactin complex is a 20 S protein heteromultimer that is thought to be involved in mediating intracellular transport by the microtubule-based motor protein, cytoplasmic dynein (1–4, 6). The dynactin complex consists of 10 polypeptides, of molecular mass = 150, 135, 62, 50, 45, 42, 37, 32, 27, and 24 kDa (3, 5). While these polypeptides were originally identified as substoichiometric components of cytoplasmic dynein preparations (7), it was subsequently found that dynein and the dynactin complex form distinct 20 S complexes in cytosol that were separable by ion exchange chromatography (2) or immunoprecipitation (3). The demonstration of two distinct complexes made unclear the relationship between dynein and the dynactin complex in processes of intracellular motility.

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polyepitope that share particularly high sequence identity include an N-terminal domain that has recently been shown to mediate the binding of p150Glued to microtubules (16) and a highly charged C-terminal motif that is involved in the binding of p150Glued to centrinactin (16) (Fig. 1). The conservation of these functional domains supports the contention that the two polypeptides may be involved in analogous cellular functions in vertebrates and Drosophila. However, related proteins have been identified that also share extensive sequence identity with the microtubule binding domain of p150Glued, including the human endosome-microtubule linker protein, CLIP-170 (28) and the yeast microtubule binding protein, BIK-1 (29). Glued, p150Glued, and CLIP-170 also share remarkable similarity in predicted secondary structure outside of this domain, including extensive predicted α-helices with heptad repeats of hydrophobic amino acids (Fig. 1). Therefore, based on sequence comparisons alone it is unclear whether Glued and p150Glued polypeptides are related members of a large family of microtubule binding proteins, or if they share a homologous function in the dynactin complexes of Drosophila and rat, respectively.

To date, there has been no biochemical or cell biological characterization of the Glued gene product in Drosophila. Therefore, we sought to determine whether Glued and p150Glued are members of functionally homologous dynactin complexes. To approach this question, antibodies were raised to recombinant Glued protein and used to examine the biochemical properties and cellular distribution of Glued in the embryonically derived Drosophila Schneider 2 (S-2) cell line (30). We find that the Glued polypeptide is a member of an oligomeric, microtubule-associated, dynactin complex which localizes to the centrosome of cultured cells. In addition, like p150Glued, we demonstrate that Glued is capable of a direct interaction with Drosophila cytoplasmic dynein in S-2 cell cytosol. The similarity in biochemical behavior and subcellular localization of these two proteins indicates that they are involved in analogous cellular functions, and in turn, that dynactin function is essential for cell viability in Drosophila.

**EXPERIMENTAL PROCEDURES**

Preparation of Cytosolic Supernatants—S-2 cells, cultured as described (30), were harvested in mid-log phase, pelleted, rinsed once in PHEM buffer (50 mM PIPES, 150 mM HEPES, 1 mM EGTA, pH 7.0) and homogenized in 1.5 volumes of homogenization buffer (PHEM with 0.26 M dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 10 μM n-tosyl-L-arginine methyl ester and 1 μM pepstatin A) in a Dounce homogenizer. The homogenate was centrifuged for 30 min at 45,000 × g in a Ti60 rotor at 4°C, followed by centrifugation for 60 min at 4°C in a Ti-60 rotor at 145,000 × g. The resulting cytosolic extract was used fresh for all experiments. For immunoprecipitation experiments, cytosol was prepared in RIPA buffer without SDS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate), with the protease inhibitors described above. To generate rat brain cytosol, frozen rat brains were homogenized in 1.5 volumes of PHEM buffer with protease inhibitors, and the homogenate was processed similarly.

Sucrose Gradient Fractionation of S-2 Cytosol—A half-milliliter of fresh S-2 cytosol was loaded onto a 12-ml 5–25% linear sucrose density gradient in PHEM buffer or RIPA buffer without SDS and centrifuged at 110,000 × g for 17 h at 4°C in a Ti SW-41 rotor (Beckman). Gradients were calibrated with catalase (11.3 S), thyroglobulin (19 S), and bovine liver glutamate dehydrogenase (26.6 S) as standards. One milliliter fractions were collected and analyzed by SDS-PAGE and immunoblotting.

Isolation of Microtubule-associated Proteins—ATP was depleted from freshly prepared S-2 or rat brain cytosol by incubation for 15 min at 37°C with 3 units/ml hexokinase and 50 mM glucose. Microtubules were then assembled in the cytosol by addition of taxol to 40 μM and incubation at 37°C for 15 min. Exogenous bovine brain microtubules, assembled from DEAE-purified tubulin dimers and stabilized with taxol (31), were added to the cytosol at a final concentration of 0.5 mg/ml and the incubation continued for 15 min. Microtubules were then pelleted through a 6% sucrose cushion at 65,000 × g in a Ti-60 rotor, washed in PHEM with 40 μM taxol, and extracted in a minimal volume of either 10 mM MgATP or 0.4 M NaCl in PHEM buffer. ATP and NaCl extracts were loaded onto 5–25% linear sucrose density gradients and run as above. Gradient fractions were concentrated by methanol precipitation and analyzed by SDS-PAGE and immunoblotting.

Construction of Glued Affinity Matrix and Affinity Chromatography—A 2.0-kilobase BamHI fragment of the Glued cDNA (kind gift of Dr. A. Garen, Yale University) that codes for amino acids 599–1339 from the C terminus of Glued (Fig. 1) was subcloned into the pGEM3Z bacterial expression vector (Novagen). The protein was expressed in Escherichia coli strain BL-21 (Novagen), and purified by the His-Tag (Novagen) system on a Ni2+ affinity column under denaturing conditions as described by the manufacturer (Qiagen). The purified protein was dia-
lyzed into coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl, pH 8), and bound overnight at 4°C to CH-activated Sepharose 4B (Pharmacia Biotech Inc.). Following blocking and washing according to manufacturer's specifications (Pharmacia), 0.5 ml of the affinity matrix was packed into a column, equilibrated in PHEM buffer, and the column was loaded with 2 ml of S-2 cytosol. The column was washed with 200 bed volumes of PHEM plus 50 mM NaCl, and eluted with a step gradient of PHEM plus 0.5 M NaCl and then PHEM plus 1.0 M NaCl. The eluates were concentrated by methanol precipitation and analyzed by SDS-PAGE and immunoblotting.

Antibody Production and Immunocytochemistry—The bacterially expressed and purified fragment of the Glued cDNA was used to immunize rabbits. Polyclonal antibodies were affinity purified against the Glued affinity matrix described above. The purified antibodies were eluted from the affinity matrix in 100 mM triethylamine, and transferred into phosphate-buffered saline (PBS, 50 mM NaPO₄, 150 mM NaCl, pH 7.4) with or without 1% bovine serum albumin by gel filtration over a NAP-10 column (Pharmacia). Affinity purified antibodies were used for all experiments described.

For immunoprecipitation, protein A-agarose beads (Life Technologies, Inc.) were loaded with anti-Glued or anti-p150Glued antibodies (16) in PBS at 4°C overnight. Cytosol was pre-cleared by incubation with un-loaded protein A beads for 30 min at 4°C, prior to incubation with antibody-bound beads for 3 h at 4°C, with agitation. The beads were then loaded extensively with RIPA buffer without SDS and eluted with sample buffer without β-mercaptoethanol. Protein eluates were analyzed by SDS-PAGE and electroblotted onto Immobilon-P (Millipore).

For some immunoprecipitation experiments, Western blots were probed with biotin-LC-hydrazide (Pierce) conjugated primary antibodies followed by goat anti-rabbit IgG and then rabbit peroxidase anti-peroxidase (Jackson Immunologicals) and developed similarly. The anti-contractin antibody has been described previously (18), and the anti-dynein heavy chain antibody DD1 (17) was the kind gift of Dr. E. Vainsberg.

For immunofluorescent localization of the Glued polypeptide, coverslips of S-2 cells were fixed at ~20°C in methanol with 1 mM EGTA, blocked for at least 1 h in PBS with 10% normal goat serum and 1% bovine serum albumin, and rinsed in PBS with 1% bovine serum albumin. Coverslips were then incubated for 1 h at 37°C in primary antibody, rinsed in PBS, incubated for 1 h in fluorophore-conjugated secondary antibody (Jackson Immunologicals) that had been preadsorbed against fixed S-2 cells, rinsed, and mounted in Tris-buffered Mowiol 4–88 (Calbiochem) containing 0.5% 3-5-propyl gallate (Kodak) to retard photobleaching. Microtubules were localized with the monoclonal anti-yeast tubulin antibody YL1/2 (Serotec) and centrosomes were localized with rabbit anti-Drosophila γ-tubulin antibody (Ref. 32, the kind gift of Dr. Y. Zheng). Immunofluorescence was performed on the Leica TCS laser scanning confocal microscope equipped with a 100×/1.4 oil objective, a krypton-argon laser and fluorescein and Texas red filters. Contrast enhancement and “sharpening” convolutions were applied to images using the Leica software prior to recording on T-MAX 100 film using a Focus Imagecorder Plus (Focus Graphics).

RESULTS

Glued Is a Member of a Microtubule-associated Polypeptide Complex—In order to characterize the biochemical behavior of the Glued protein in Drosophila S-2 cells, we raised polyclonal antibodies to the C-terminal half of the Glued protein, expressed in E. coli. Affinity purified antibodies reacted specifically with a doublet of 150/135 kDa in whole S-2 cell cytosol (Fig. 2). The presence of an immunoreactive doublet of polypeptides of 150 and 135 kDa is also characteristic for p150Glued isolated from various vertebrate species (2, 3). As a test for immunologic relatedness between Glued and rat p150Glued, the anti-Glued antibodies were used to probe a 0.4 M NaCl extract of rat brain microtubules, which is highly enriched in p150Glued (21). The anti-Glued antibody reacted solely with a 150-kDa polypeptide in the rat sample (Fig. 2). The immunoreactive rat polypeptide was identical in mobility to a 150-kDa polypeptide recognized when immunoblots were re-probed when polyclonal antibodies to rat p150Glued (16) (not shown). However, the anti-p150Glued antibodies did not cross-react with Glued protein in S-2 cytosol.

Mammalian p150Glued may be isolated by extraction of rat brain microtubules with either ATP or 0.4 M NaCl (21). In order to examine the microtubule binding properties of the Glued protein, microtubules were assembled from endogenous tubulin in S-2 cytosol by addition of taxol. Because of the low level of tubulin in S-2 cells as compared to neuronal tissue, excess exogenous taxol-stabilized bovine brain microtubules were also added to the assay. After incubation, the microtubules and associated proteins were isolated by centrifugation, and following a buffer wash, the microtubules were extracted with either 10 mM ATP or 0.4 M NaCl, and the resulting fractions were examined by immunoblot with the anti-Glued antibody (Fig. 3, A and B). The Glued polypeptide was observed to remain soluble in the cytosol until microtubules were assembled, and then to co-sediment with microtubules (Fig. 3, A and B). Extraction of the pellet with either 10 mM MgATP or 0.4 M NaCl released the 150-kDa Glued protein from microtubules into the supernatant. In contrast, most of the 135-kDa Glued polypeptide did not co-sediment with microtubules (Fig. 3B), and therefore very little of the 135-kDa protein was extracted from microtubules by either ATP or salt (Fig. 3B). The ATP- and salt-sensitive microtubule binding of the Glued polypeptide was compared to the behavior of rat brain p150Glued under similar conditions (Fig. 3A and B). The p150Glued polypeptide co-sedimented with microtubules, and was nearly fully released with either ATP or elevated ionic strength, as had been previously noted for the rat protein (7, 21).

The ATP eluate of microtubules from S-2 cell extracts was further fractionated on 5–25% sucrose density gradients. Immunoblots of the gradient fractions indicated that Glued sedimented at 20 S (Fig. 3C). This result suggests that microtubule-associated Glued polypeptide is part of a large protein complex. To determine whether the Glued polypeptide exists in S-2 cytosol exclusively as a component of a macromolecular complex, whole cytosol was fractionated on a 5–25% sucrose density gradient. Analysis of gradient fractions by immunoblotting revealed that both the 150- and 135-kDa Glued polypeptides sedimented solely as a peak at ~20 S (Fig. 4). The absence of detectable immunoreactivity at a lower sedimentation coefficient suggests that there is not a significant fraction of free Glued subunits in S-2 cytosol.

To further examine the native protein interactions of the Glued polypeptide, immunoprecipitations from S-2 cytosol were performed under non-denaturing conditions. Anti-Glued antibodies were observed to precipitate proteins of 150, 143,
Drosophila Glued Is in a Dynactin Complex

![Diagram](image)

**Fig. 3.** Partitioning of the Drosophila Glued and rat p150Glued proteins during a microtubule-associated protein preparation from S-2 cells (S-2) or rat brain (Rat). Samples from the preparation were analyzed by 8% SDS-PAGE, electroblotted to Immobilon P, the blot stained with Coomassie Blue (A), destained, and probed with anti-Glued (S-2, B) or anti-p150Glued antibodies (Rat, B). S-2 cell cytosol was prepared by two successive rounds of centrifugation (45k, 45,000 × g; 145k, 145,000 × g; S, supernatant; P, pellet). ATP was depleted in the cytosol, microtubules were assembled, and exogenous, taxol-stabilized, bovine brain microtubules were added. Microtubules were then sedimented and washed (MT; S, post-microtubule supernatant; P, buffer washed microtubule pellet). The microtubule pellet was then extracted of associated proteins with either 10 mM ATP (ATP; S, ATP extract of microtubules; P, post-ATP extracted pellet) or 0.4 M NaCl (NaCl; S, NaCl extract of microtubules; P, post-NaCl extracted pellet). Rat brain (Rat) was processed similarly, but only the 10 mM ATP (ATP, S and P) and 0.4 M NaCl (NaCl, S and P) extracts of microtubules are shown for comparison. The ATP extract of S-2 microtubules was then sedimented through a 5-25% sucrose gradient and the fractions analyzed by 8% SDS-PAGE and immunoblotting with anti-Glued antibodies (C). Arrowhead corresponds to the position of a 150-kDa molecular mass marker.

135, 72, and 45 kDa (Fig. 5). This was compared to immuno-precipitations from rat brain cytosol performed in parallel with anti-rat p150Glued antibodies, which precipitated detectable polypeptides of 150, 135, and 45 kDa as assayed by Coomassie staining. Western analysis of the S-2 cell immunoprecipitate demonstrated that the 150/143/135-kDa triplet was reactive with anti-Glued antibodies, while in the immunoprecipitate from rat brain, the 150/135-kDa polypeptides reacted with anti-p150Glued antibodies. Antibodies to Dictyostelium cytoplasmic dynein (17) and human cincentractin (18) reacted weakly with bands of >300-kDa and 45-kDa polypeptides in the Drosophila immunoprecipitate, respectively (not shown), while the 45-kDa rat protein also reacted with anti-centrin antibodies (not shown).

Glued Interacts Directly with Cytoplasmic Dynein—We have recently demonstrated using proteins isolated from rat, that a biochemical interaction between the dynactin complex and cytoplasmic dynein is mediated by direct binding of p150Glued to the dynein intermediate chain (18). In that study, an affinity column with a fragment of rat p150Glued polypeptide covalently bound to an inert matrix was capable of specifically retaining intact cytoplasmic dynein from whole brain cytosol. Also, an affinity column with bound rat dynein intermediate chain was found to retain the dynactin complex. To determine if the Glued protein from Drosophila was capable of a similar interaction, a Glued affinity column was tested for its ability to retain cytoplasmic dynein from S-2 cytosol. To form the affinity matrix, a 2.1-kilobase pair fragment of the Glued cDNA that encoded amino acids 599-1319 of the Glued polypeptide was expressed in E. coli, purified, and covalently bound to Sepharose beads. When S-2 cytosol was passed over the matrix, several polypeptides were specifically retained through extensive washing, and eluted with 0.5 M NaCl and 1.0 M NaCl (Fig. 6). Prominent bands noted in the eluate were of >300 and 72 kDa, similar in molecular mass to the >300 kDa heavy chain and 74 kDa intermediate chain of rat cytoplasmic dynein (7). The identity of the >300 kDa protein as dynein heavy chain was confirmed by immunoblot with anti-cytoplasmic dynein heavy chain antibodies (17). Available antibodies to the dynein intermediate chain did not cross-react with Drosophila proteins. A prominent band at 55 kDa was seen in the eluate of the Glued affinity column, as well as in control columns with covalently bound bovine serum albumin (not shown). The 55-kDa protein was identified by Western blot as tubulin. Because the affinity chromatography was performed at −21°C, it is likely that under these conditions tubulin polymerizes in the cytosol, and we speculate that due to their length, the polymerized microtubules become nonspecifically trapped in the affinity matrix.

Glued Localizes to the Centrosome of S-2 Cells—In various types of cultured vertebrate cells, p150Glued displays centrosomal and punctate cytoplasmic staining in interphase cells (2, 3, 16), while it is localized to the spindle poles, kinetochore fibers and kinetochores during mitosis (2, 14). To determine if the Glued protein is localized to similar structures in S-2 cells, its distribution was examined by indirect immunofluorescence. Methanol-fixed S-2 cells labeled with anti-Glued antibodies revealed a punctate cytoplasmic distribution of the Glued protein with one or more bright spots adjacent to the nucleus (Fig. 7A). In addition, Glued was localized to the spindles of mitotic S-2 cells (Fig. 7, C and D) that had been double labeled with anti-Glued and anti-tubulin antibodies. When the distribution of Glued was compared to that of Drosophila γ-tubulin (32), an integral component of the centrosome, both proteins localized primarily to perinuclear spots (Fig. 7B). The observation that the anti-γ-tubulin antibody labeled more than one perinuclear spot per cell suggests that multiple centrosomes may be common in S-2 cells. Apparent co-localization of γ-tubulin and Glued was observed, but could not be confirmed directly as both primary antibodies had been raised in rabbit. Staining with secondary antibodies alone produced a continuous, low level of background fluorescence throughout the nucleus and cytoplasm of S-2 cells (not shown).

**DISCUSSION**

We have examined the immunocytochemical and biochemi-cal behaviors of the Glued protein in the embryonically derived S-2 cell line from Drosophila. The results of these studies indicate that Drosophila Glued and mammalian p150Glued are functionally homologous members of a dynactin complex and that each interacts directly with the microtubule-based motor protein, cytoplasmic dynein. Immunocytochemical evidence for the similarity between the two polypeptides was demonstrated by inter-species antibody cross-reactivity, as well as similar subcellular localization patterns. Polyclonal antibodies raised to the C-terminal region of the Glued protein react with rat p150Glued. This indicates that the two share epitopes in regions that are only 24% identical at the primary sequence level (see Fig. 1) and that higher order structure may be conserved between Glued and p150Glued.
In biochemical assays, we have observed that like rat p150 Glued, Drosophila Glued is a microtubule binding protein that can be extracted from microtubules with either ATP or elevated ionic strength, and that both proteins exist exclusively as members of multimeric 20 S complexes in their respective species. This distinguishes Glued from the related microtubule binding protein, CLIP-170, which exists in cytosol as a dimer, with a sedimentation coefficient of 5.7 S (28). The data presented here also demonstrates that there are functionally distinct isoforms of this polypeptide in Drosophila. We noted that it was primarily the 150-kDa form of the Glued protein that associated with microtubules, while the 135-kDa form did not. Based on observations made on the p150 and p135 isoforms expressed in human neurons, we speculate that p135 in Drosophila is an alternatively spliced isoform lacking the microtubule-binding motif that we previously characterized in the rat p150 Glued polypeptide (16).

In addition to its ability to associate with microtubules, the Glued polypeptide was also found to be capable of binding to intact cytoplasmic dynein complex from S-2 cytosol. This was demonstrated by the specific retention of cytoplasmic dynein heavy chain, as well as several polypeptides similar in molecular mass to vertebrate cytoplasmic dynein subunits, by an affinity matrix constructed of a bacterially expressed fragment of the Glued polypeptide. This result provides further insight into the recent observation of a genetic interaction between Dhc64C, the gene encoding cytoplasmic dynein heavy chain, and Glued (44) in Drosophila, in that it demonstrates a biochemical interaction between the two gene products.

We have recently used a similar method to demonstrate in

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that the interaction between the dynactin complex and cytoplasmic dynein is mediated by direct binding of p150\textsuperscript{Glued} to the 74-kDa dynein intermediate chain (18). In that study, we showed that the 766 amino acids spanning the predicted extended \(\alpha\)-helix (amino acids 133–899) of p150\textsuperscript{Glued} (see Fig. 1) were sufficient for mediating the interaction with cytoplasmic dynein (18). In the present study, the Glued affinity matrix was made with a 720-amino acid C-terminal fragment (amino acids 599–1319, see Fig. 1) of the Glued protein. This suggests that the minimal dynein intermediate chain binding domain may be represented by the overlap, amino acids 599–899, in the constructs used in the respective binding experiments in the rat and Drosophila systems.

Immunoprecipitations performed under nondenaturing conditions with the anti-Glued antibody were used to further characterize the polypeptides that interact with Glued in the Drosophila 20 S complex. In this experiment, we observed the co-immunoprecipitation of polypeptides of 150, 143, and 135 kDa, which reacted with our anti-Glued antibody. The 143-kDa form of Glued may represent a differentially phosphorylated form of the protein or a proteolytic fragment of the 150-kDa protein. That this form was found only in the immunoprecipitation experiments may be due to the use of a different buffer system than was utilized for the rest of the experiments in this study. The 45-kDa protein that was observed in the anti-Glued immunoprecipitate is likely to be the Drosophila centractin homolog, 87C, as described by Fyrberg et al. (33). The identity of the 45-kDa protein is substantiated by its weak cross-reactivity with an anti-human centractin antibody. We also noted that dynein heavy chain was present in the anti-Glued immunoprecipitate, and this was most likely mediated by the Glued-centractin dynein interaction demonstrated more directly by affinity chromatography. While co-immunoprecipitation of dynein and the dynactin complex was not observed in the parallel immunoprecipitation experiment in rat brain, the anti-rat p150\textsuperscript{Glued} antibody used in this experiment was raised to the middle third (amino acids 133–899) of the p150\textsuperscript{Glued} protein (16). We have preliminary results indicating that this antibody specifically blocks the dynein-dynactin complex interaction,\(^3\) thus it is probable that the antibody would also block the co-immunoprecipitation of the two complexes.

The demonstration that the product of the Drosophila gene Glued is functionally homologous to the vertebrate p150\textsuperscript{Glued} polypeptide is significant, because the well characterized mutations at the Glued locus may now be considered as resulting from a disruption in dynactin complex function. As the null mutation is lethal (25), this indicates that expression of the dynactin complex is required, and that the complex plays an essential role in higher eukaryotes. This is in marked contrast to the recent observations in Saccharomyces cerevisiae and Neurospora crassa, in which disruption of dynactin complex function resulted in nonlethal phenotypes (10–12). In yeast, mutations in either dynein heavy chain or Arp1 (centractin) resulted in the mis-segregation of duplicated nuclei between mother and bud (8–11), while in filamentous fungi, disruption of either dynein or dynactin complex results in the block of nuclear migration along the hyphae (12, 13).

In higher eukaryotes, however, disruption of dynactin complex function has more severe consequences. The principal phenotype of adult heterozygote mutants of the dominant GI\(^1\) allele is disruptions in neuronal systems such as the optic lobe and eye (24). There is a body of evidence from biochemical and cell biological studies that both p150\textsuperscript{Glued} and cytoplasmic dynein play a role in basic neuronal function. Both of these polypeptide complexes are highly enriched in brain tissue from a variety of sources. In rat, in situ hybridization studies have shown that the p150\textsuperscript{Glued} component of the dynactin complex is expressed at high levels in developing brain and eye tissues (34), while in Drosophila embryos, cytoplasmic dynein is concentrated in the developing nervous system (35).

In the neuron, cytoplasmic dynein is a microtubule-based motor molecule that is believed to participate in the retrograde transport of organelles in the axon (36–38), and the dynactin complex may mediate this process. The involvement of the dynactin complex in axonal transport is supported by our recent observations that p150\textsuperscript{Glued} is localized along the length of axonal processes in cultured human neuronal cells.\(^1\) Thus, it may be that the defects in optic neuronal architecture and the aberrant cell patterning observed in Glued mutants (24) are the result of a defect in retrograde transport along the axon. Growth factors required for the survival of neurons (39) are transported from the growth cone to the cell body by fast retrograde transport (40). Disruption of growth factor transport may be the direct cause of aberrant neuronal outgrowth observed in the GI\(^1\) mutant.

In addition to the strong evidence for the involvement of Glued in neuronal function, the cell lethal phenotype of GI\(^1\) homozygotes observed in mosaic analyses (27), as well as the lethality of the null mutation at this locus (24, 25) suggest that the Glued gene product is also required for an essential function in non-neuronal cells. There is mounting evidence from a variety of species that cytoplasmic dynein participates in some aspect of mitosis, such as spindle assembly, nuclear migration, or chromosome-to-pole motility (8–13, 14, 17, 41). It is therefore likely that the dynactin complex is also involved in cell division. This is supported by the observations in Drosophila that cytoplasmic dynein in somatic cells (35) and oocytes (42), as well as Glued in S-2 cells (this study), all localize to spindle poles and kinetochore microtubules during mitosis.

The demonstration of a Drosophila dynactin complex also sheds light on the molecular mechanisms of the Glued mutations. Genetic analyses of Glued strongly suggests that certain mutations affect the ability of the gene product to participate in protein-protein interactions as a member of an oligomeric complex (27). The original dominant negative GI\(^1\) mutation arises from the insertion of a B104 transposon into the coding region of the gene, resulting in a truncated protein in which native sequence is substituted by transposon-coded sequence at the C

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\(^3\) C. M. Waterman-Storer, S. Kuznetsov, S. Karki, D. G. Weiss, G. M. Langford, and E. L. F. Holzbaur, manuscript submitted.
terminus of Glued (43). This results in the truncation of a highly conserved motif in Glued which has been implicated in contractin binding (16). Thus, the protein product of Gloted may be unable to form dynactin complexes due to an inability to bind to contractin. However, cellular transfections with similar truncations of p150Gloted did not produce a dominant negative phenotype (16), and so the substitution of native sequence by transposon-coded sequence in Gloted may be responsible for disrupting dynactin complexes, or may produce poisoned complexes that are unable to interact with either cytoplasmic dynein or microtubules. Our demonstration of a dynactin complex in Drosophila will now allow the targeted analysis of the function of the dynactin complex at the level of the organism as well as during development.

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