Regulation of Cytoplasmic Dynein Function In Vivo by the *Drosophila* Glued Complex

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Abstract. The *Drosophila Glued* gene product shares sequence homology with the p150 component of vertebrate dynactin. Dynactin is a multiprotein complex that stimulates cytoplasmic dynein–mediated vesicle motility in vitro. In this report, we present biochemical, cytological, and genetic evidence that demonstrates a functional similarity between the *Drosophila* Glued complex and vertebrate dynactin. We show that, similar to the vertebrate homologues in dynactin, the Glued polypeptides are components of a 20S complex. Our biochemical studies further reveal differential expression of the Glued polypeptides, all of which copurify as microtubule-associated proteins. In our analysis of the Glued polypeptides encoded by the dominant mutation, *Glued*^{1}, we identify a truncated polypeptide that fails to assemble into the wild-type 20S complex, but retains the ability to copurify with microtubules. The spatial and temporal distribution of the Glued complex during oogenesis is shown by immunocytochemistry methods to be identical to the pattern previously described for cytoplasmic dynein. Significantly, the pattern of Glued distribution in oogenesis is dependent on dynein function, as well as several other gene products known to be required for proper dynein localization. In genetic complementation studies, we find that certain mutations in the cytoplasmic dynein heavy chain gene *Dhc64C* act as dominant suppressors or enhancers of the rough eye phenotype of the dominant *Glued*^{1} mutation. Furthermore, we show that a mutation that was previously isolated as a suppressor of the *Glued*^{1} mutation is an allele of *Dhc64C*. Together with the observed dependency of Glued localization on dynein function, these genetic interactions demonstrate a functional association between the *Drosophila* dynein motor and Glued complexes.

THE minus-end directed microtubule motor cytoplasmic dynein has been implicated in numerous intracellular motile processes including mitotic chromosome movements (Pfarr et al., 1990; Steuer et al., 1990; Hyman and Mitchison, 1991), mitotic spindle morphogenesis (Vaisberg et al., 1993), nuclear positioning (Plamann et al., 1994; Xiang et al., 1994), nuclear and mitotic spindle migration during cell division (Eschel et al., 1993; Li et al., 1993), perinuclear localization of the Golgi complex (Corthésy-Theulaz et al., 1992), and retrograde axonal vesicle transport (Paschal and Vallee, 1987; for review see Holzbaur and Vallee, 1994). While it seems clear that cytoplasmic dynein is likely to participate in the transport of multiple cytoplasmic organelles and/or cargoes, there is much to be learned about the mechanisms that regulate specific functions of the dynein motor within cells. One essential aspect of dynein regulation that is a focus of current research is how the cytoplasmic dynein motor is targeted to particular cellular cargoes. In this regard, recent work has drawn attention to the role of the dynactin complex (Gill et al., 1991; Schroer and Sheetz, 1991) as a potential regulatory component involved in the coupling of the dynein motor complex to membrane-bound vesicles.

Dynactin is a 20S complex that has been biochemically characterized in chicken and bovine brain extracts (Gill et al., 1991; Schroer and Sheetz, 1991; Paschal et al., 1993). The complex includes a doublet of 150/160–kD polypeptides, a 45-kD actin-related protein Arp-1/centractin, conventional actin, actin-capping protein, and polypeptides of 62, 50, 27, and 24 kD (Gill et al., 1991; Schroer and Sheetz, 1991; Lees-Miller et al., 1992; Paschal et al., 1993; for review see Schroer, 1994). Although the dynactin complex is not required to support dynein-mediated microtubule gliding in vitro, dynactin has been shown to stimulate cytoplasmic dynein–mediated in vitro vesicle motility (Gill et al., 1991; Schroer and Sheetz, 1991). The predicted amino acid sequence of the p150 component also contains a conserved motif that is found in the endosome linker protein CLIP-170 (Pierre et al., 1992), which is postulated to participate in connecting the motor cargo complex to the microtubule lattice. These results have fostered a model in which the dynactin complex acts to regulate dynein function at the level of cargo attachment.

Recent studies in *Neurospora crassa* (Plamann et al.,

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1994) and *Saccharomyces cerevisiae* (Clark and Meyer, 1994; Muhua et al., 1994) have provided molecular and genetic evidence for the existence of dynactin-related subunits in these organisms, as well as their involvement in a common cellular process together with cytoplasmic dynein. In *N. crassa*, the isolation of suppressors of a mutation in the *cot-1* locus, which encodes a cAMP-dependent protein kinase (Yarden et al., 1992), identified a number of loci including the genes encoding the cytoplasmic dynein heavy chain (*ro-1*) and homologues of the p150 (*ro-3*) and Arp-1 (*ro-4*) subunits of dynactin (Plamann et al., 1994). In a wild-type background, the *ro-1*, *ro-3*, and *ro-4* mutations share a phenotype characterized by a defect in nuclear migration (Plamann et al., 1994). In the budding yeast *S. cerevisiae*, disruption of the cytoplasmic dynein heavy chain gene, and/or the Arp-1 homologue *ACT5*/*ACT3*, results in defects in mitotic spindle orientation and nuclear migration (Eschel et al., 1993; Li et al., 1993; Clark and Meyer, 1994; Muhua et al., 1994). Despite this evidence for similar phenotypes produced by mutations in the dynein heavy chain gene and genes encoding apparent dynactin-related subunits, the nature of the interaction between dynein and dynactin in vivo remains to be determined.

The *Drosophila Glued* gene shares significant homology with the gene encoding the p150 subunit of the dynactin complex in rat and chicken (Swaroop et al., 1987; Gill et al., 1991; Holzbaur et al., 1991). The *Glued* locus was initially identified by the spontaneous mutation, *Glued*^{1} (*Gl*^{1}),[1] which results in a dominant rough eye phenotype in adult flies (Plough and Ives, 1935). In heterozygotes the *Gl*^{1} mutation causes a severe disruption in the organization of the retina and in the retinal projections to the optic lobe (Meyerowitz and Kankel, 1978). In homozygous animals the *Gl*^{1} mutation is recessive lethal, demonstrating that the *Glued* gene is also essential for normal development in *Drosophila.* Screens for revertants of the dominant rough eye phenotype of *Gl*^{1} recovered additional recessive lethal mutations at the *Glued* locus (Harte and Kankel, 1982). In addition to late embryonic and larval lethal phenotypes, several of these new *Glued* alleles displayed a temperature-sensitive maternal effect, suggesting that *Glued* is required not only for proper nervous system and eye development, but during the early development of the egg or embryo (Harte and Kankel, 1982). In mosaic analyses using a null mutation of *Glued*, somatic clones of homozygous *Glued* mutant tissue were not recovered, suggesting that the *Glued* gene product has an essential function within the cell. Alternatively, *Glued* might be required for the successful differentiation and incorporation of particular cell types into developing tissues (Harte and Kankel, 1982).

The *Drosophila Glued* gene is expressed throughout development (Swaroop et al., 1986), consistent with the genetic data suggesting that *Glued* has an essential role in *Drosophila* development (Harte and Kankel, 1982). The molecular characterization of the dominant *Gl*^{1} mutation revealed the insertion of a transposon into the *Glued* coding sequence, resulting in the production of a truncated message that could potentially encode a truncated polypeptide (Swaroop et al., 1985). The dominant nature of the *Gl*^{1} mutation suggests that the truncated *Glued* transcript encodes a protein product that acts as a "poison" to disrupt the function of the wild-type polypeptide.

We previously cloned *Dhc64C*, a gene encoding a *Drosophila* cytoplasmic dynein heavy chain (Li et al., 1994), and subsequently isolated recessive lethal mutations in the gene (Gepner, J., M.-g. Li, S. Ludmann, C. Kortas, K. Boylan, M. McGrail, and T. S. Hays, manuscript in preparation). In addition to the recessive lethality, we have noticed additional phenotypes in adult flies that are doubly heterozygous for certain combinations of the dynein mutations. These phenotypes include female sterility, male sterility, bristle defects, and a rough eye phenotype similar to that observed in flies that contain the dominant *Gl*^{1} mutation (Gepner, J., manuscript in preparation). The *Dhc64C* mutations provide tools to analyze the regulation and function of the dynein motor. In this report, we use mutations in both the dynein heavy chain and *Glued* genes to investigate the interaction between the *Drosophila* cytoplasmic dynein motor and the dynactin-related Glued complex. We identify and characterize the Glued polypeptides as components of a 20S complex similar to vertebrate dynactin and present cell biological and genetic observations that indicate the physical and functional interaction of the Glued and dynein motor complexes.

Materials and Methods

Fly Stocks and Genetic Analyses

The *Dhc64C* mutations used in this study were isolated by a standard F2 screen for recessive lethal mutations under the deficiency *Df(3L)10H* (64B10-12; 64C5-9) which removes the chromosomal region containing the dynein gene. The third chromosome deficiency *Df(3L)10H* was provided by J. Garbe (University of California, Berkeley). For the purposes of the studies described here, the identity of the dynein mutations is established by the rescue of the recessive lethal phenotype of the dynein mutations by an X-linked P element insert containing a genomic copy of the *Dhc64C* transcription unit (designated *P*(*Dhc*$^{+}$); see Table III). Molecular clones containing the intact *Dhc64C* transcription unit were previously reported (Li et al., 1994). A detailed description of the isolation and phenotypic characterization of the dynein mutations will be reported elsewhere (see also McGrail, M., J. Gepner, M.-g. Li, S. Ludmann, K. Boylan, S. Iyadurai, and T. S. Hays. 1994. *Mol. Biol. Cell Abstracts.* 5:131*a*).

The stocks *Gl*^{1} *Sb*, *Df(3L)Gl*$^{+R2}$, and *Su(Gl)102* were provided by Dr. Douglas Kankel, Yale University, and are described in Harte and Kankel (1982). The alleles *egalitarian*^{WU50}, *Bicaudal-D*^{R26}, *cappuccino*^{RK12}, and *spire*^{RP48}, were provided by Dr. Trudi Schüpbach, Princeton University, and are described in Manseau and Schüpbach (1989), Suter et al. (1989), and Schüpbach and Weischaus (1991). The second chromosome deficiency *Df(2L)TW119*, which removes the *Bicaudal-D* gene, was obtained from Dr. Ruth Steward, Rutgers University, and is described in Steward et al. (1987).

For the analysis of cytoplasmic dynein and Glued localization in the female-sterile combination of dynein alleles *Dhc64C*$^{6\text{-}6}$/*Dhc64C*$^{6\text{-}12}$, virgin females of the genotype *Dhc64C*$^{6\text{-}6}$/*TM6B, D Hu* were crossed with males of the genotype *Dhc64C*$^{6\text{-}12}$/*TM6B, D Hu*, and female progeny that were wild type for the dominant marker mutations *Dichaete* (*D*, outheld wings) and *Humeral* (*Hu*, extra hairs on the shoulder) were selected. In the analysis of cytoplasmic dynein and Glued localization in the *Bicaudal-D*^{R26} mutant, *Bicaudal-D*^{R26}/*Df(2L)TW119* females were recovered from the cross *Bicaudal-D*^{R26}/*CyO* × *Df(2L)TW119*/*CyO*. Markers and other chromosomes used are described in Lindsley and Zimm (1992).

Analysis of the genetic interactions between the *Dhc64C* alleles and the *Gl*^{1} mutation was performed by crossing heterozygous virgin females from the stock *Gl*^{1} *Sb*/*TM6B, D Hu* with heterozygous males of the following genotypes: *Df(3L)10H*/*TM6B, D Hu*; *Dhc64C*$^{6\text{-}6}$/*TM6B, D Hu*; *Dhc64C*$^{6\text{-}8}$/*TM6B, D Hu*; *Dhc64C*$^{6\text{-}10}$/*TM6B, D Hu.* In the progeny, flies

1. *Abbreviation used in this paper*: *Gl*^{1}, *Glued*^{1}.

heterozygous for the *Gl*^{1} mutation and the dynein mutation were identified by the presence of adults which show the dominant bristle phenotype caused by the *Sb* (*Stubble*, short blunt bristles) mutation, but were wild type for the dominant wing and bristle phenotypes of the *D* (*Dichaete*) and *Hu* (*Humeral*) mutations. To examine the effect of an additional copy of the wild-type *Dhc64C* gene in flies heterozygous for the *Gl*^{1} mutation and the dynein alleles *Dhc64C*$^{6-8}$ or *Dhc64C*$^{6-10}$, an X chromosome bearing the wild-type *Dhc64C* transgene was separately crossed into the *Dhc64C*$^{6-8}$ and *Dhc64C*$^{6-10}$ backgrounds and stocked. Males of the genotype *P*(*Dhc*$^{+}$)/*Y*; *Dhc64C*$^{6-8}$/*TM6B*, *D Hu* and *P*(*Dhc*$^{+}$)/*Y*; *Dhc64C*$^{6-10}$/*TM6B*, *D Hu* were crossed to virgin *Gl*^{1} *Sb*/*TM6B*, *D Hu* females. All of the female progeny from this cross carry one X chromosome bearing the *Dhc64C* transgene *P*(*Dhc*$^{+}$).

Complementation analyses between the *Su*(*Gl*)*102* mutation and the *Dhc64C* mutations were performed by crossing heterozygous *Su*(*Gl*)*102*/*TM6B*, *D Hu* virgin females with males heterozygous for the deficiency *Df*(*3L*)*10H* or the appropriate *Dhc64C* allele, and *TM6B*, *D Hu*. The critical class was scored by the absence of the *TM6B*, *D Hu* third chromosome balancer dominant marker mutations *Dichaete* (*D*) and *Humeral* (*Hu*) in adult progeny.

To test the ability of the dynein transgene to rescue the lethality of *Su*(*Gl*)*102* in combination with *Df*(*3L*)*10H* and the *Dhc64C* alleles *Dhc64C*$^{4-6}$, *Dhc64C*$^{4-16}$, and *Dhc64C*$^{6-6}$, the following crosses were performed: heterozygous *w*, *P*(*Dhc*$^{+}$ *w*$^{+}$)/*Y*; *Su*(*Gl*)*102*/*TM6B* males were crossed with heterozygous, virgin *w*/*w*; *Df*(*3L*)*10H*/*TM6B*, *w*/*w*; *Dhc64C*$^{4-6}$/*TM6B*, *w*/*w*; *Dhc64C*$^{4-16}$/*TM6B* or *w*/*w*; *Dhc64C*$^{6-6}$/*TM6B* females. Rescue of the critical class (*Su*(*Gl*)*102*/mutant) was scored by the presence of *w*$^{+}$ *Dichaete*$^{+}$ *Humeral*$^{+}$ adults in the progeny from each cross. Because the wild-type dynein transgene is carried on the X chromosome and is contributed by the male, only female progeny inherit the wild-type dynein transgene and should be rescued. The absence of male progeny that carry the dynein mutations but lack the paternally derived X-linked wild-type dynein transgene demonstrates that the rescue of lethality in the female progeny is due to the presence of the dynein transgene.

RNA Blot Analyses

Total RNA was isolated from ovaries and testes, staged collections of embryos, larvae, pupae, and heads, as described previously (Li et al., 1994). 25 μg total RNA was run on a 7.5% agarose–formaldehyde denaturing gel, and blotting, hybridization, and washing conditions were as described previously (Li et al., 1994). A 1.8-kb BamHI fragment from the *Glued* cDNA (Swaroop et al., 1987), provided by Dr. Alan Garen, Yale University, was used to prepare a DNA probe labeled with [^{32}P]dATP using random hexamer primers (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). A probe derived from the RP49 gene (Vaslet et al., 1980) was used to monitor loading and to verify integrity of the RNA.

Antibody Production and Purification

An anti-Glued rat polyclonal serum was prepared using a bacterially expressed Glued fusion protein. A 1.8-kb BamHI fragment of the *Glued* cDNA, provided by Dr. Alan Garen, which encodes the ~600 COOH-terminal residues of the *Glued* open reading frame (Swaroop et al., 1987), was cloned in frame into the expression vector pGEX1 (Smith and Johnson, 1988) and expressed in the bacterial strain HB101. Inclusion bodies containing the glutathione *S*-transferase–Glued fusion protein were prepared as described (Li et al., 1994) and fractionated by SDS-PAGE. Gel slices corresponding to the glutathione-*S*-transferase–Glued fusion protein were excised and used to elicit a polyclonal serum in rat as described (Li et al., 1994). The anti-Glued antiserum was affinity purified over an affinity column prepared with a Glued fusion protein expressed in the vector pET5b (Novagen, Inc., Madison, WI) in the bacterial strain BL21 (Novagen, Inc.). The affinity column was generated by coupling the fusion protein to Actigel (Sterogene Bioseparations, Inc., Arcadia, CA) resin according to the supplier's instructions. The affinity-purified antibodies were stored in PBS containing 1 mg/ml BSA.

Microtubule-associated Protein Preparations

Ovaries from well-fed 3-d-old wild-type OregonR females and heterozygous *Gl*^{1}/*TM6B* females were dissected in EBR buffer (130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM Hepes, pH 6.9), placed on ice until 100 μl of ovaries were obtained, quick frozen in liquid nitrogen, and stored at −80°C until use. Heads and embryos were collected from the wild-type OregonR and the *Gl*^{1}/*TM6B* stocks, quick frozen in liquid nitrogen, and stored at −80°C until use. Microtubule-associated proteins from each tissue were prepared as described previously (Hays et al., 1994). Briefly, ~1 ml packed ovaries, 6 ml packed heads, or 15 ml packed embryos, were rinsed three times in PMEG buffer (100 mM Pipes, pH 6.9, 5 mM MgOAc, 5 mM EGTA, 0.1 mM EDTA, 0.5 mM DTT, 0.9 M glycerol) plus protease inhibitors (10 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 0.1 μg/ml each of soybean trypsin inhibitor, *n*-tosyl L-arginine methylester, and benzamidine). Tissues were homogenized on ice in a glass homogenizer in 1.5 vols PMEG plus protease inhibitors. The homogenate was spun at 125,000 *g* for 40 min at 4°C, and the supernatant was pulled from between the top lipid layer and the insoluble pellet at the bottom of the tube. All subsequent steps were carried out at 4°C. Microtubules were assembled in the high-speed supernatant by the addition of GTP to 1.0 mM and taxol to 20 μM, plus 20 μM cytochalasins B and D to inhibit assembly of filamentous actin. After 15 min incubation with gentle rocking, endogenous ATP was depleted from the extract by the addition of hexokinase and glucose to final concentrations of 10 U/ml and 100 mM, respectively, and the extract incubated further for 45 min. The extract was underlaid with a 1/4 vol 15% sucrose cushion and centrifuged at 37,000 *g* for 30 min in a rotor (SW50.1; Beckman Instruments, Inc., Fullerton, CA) to pellet microtubules and microtubule-associated proteins. The microtubule pellet was washed once by resuspension in PMEG plus taxol and recentrifuged. The microtubule pellet was extracted with PMEG containing 10 mM Mg-ATP and 20 μM taxol, and recentrifuged at 37,000 *g* for 15 min in an SW50.1 rotor plus adapters for 5 × 41 mm open-topped ultraclear tubes (Beckman Instruments, Inc.).

Sucrose Density Gradient Centrifugation

1 mg total protein of a high-speed extract (125,000 *g*) from ovary homogenates, and 2 mg total protein of a high-speed extract from embryo and head homogenates, were sedimented through 11.5 ml 5–20% sucrose gradients prepared in PMEG buffer plus protease inhibitors as described previously (Hays et al., 1994). The gradients were spun at 4°C in a rotor (SW40; Beckman Instruments, Inc.) for 16 h at 230,000 *g*, and then collected into 0.5-ml fractions. The sedimentation standards thyroglobulin (19S), catalase (11S), and cytochrome (2S) (Sigma Chemical Co., St. Louis, MO) were run in parallel on a separate gradient.

Immunoblotting

SDS-PAGE and immunoblotting were carried out as described by Laemmli (1970) and Towbin et al. (1979). Proteins were electrophoresed on 0.75 mm, 7.5% polyacrylamide slab gels prepared with a 1:100 ratio of bis-acrylamide/total monomer. To better resolve the migration of the truncated Glued polypeptide from the wild-type, samples shown in Fig. 3 were run on 6.5% polyacrylamide gels. Gels were stained with Coomassie brilliant blue or electroblotted to PVDF membrane (Millipore Corp., Burlington, MA). Blots were probed with the affinity-purified anti-Glued antibody diluted 1:100, or the affinity-purified anti-dynein heavy chain antibody PEP1 (Li et al., 1994) diluted 1:500, in PBS/0.05% Tween-20 containing 0.2% I-Block (Tropix, Bedford, MA). Alkaline phosphatase–conjugated secondary antibodies were diluted in the same. Blots were developed with NBT and BCIP (Sigma Chemical Co.) in alkaline phosphatase buffer (100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris, pH 9.5), or with a nonradioactive chemiluminescence detection system (Tropix) according to the supplier's instructions. Images of gels were captured on an IRIS Indigo Video board installed on a Silicon Graphics R3000 Indigo workstation (Silicon Graphics Computer Systems, Mountain View, CA). Images of blots were obtained from blots and x-ray films scanned into a MacIntosh Power PC 8100 (Apple Computer Corp., Cupertino, CA) with a flat bed scanner (ScanJet IIc; Hewlett Packard, Corvallis, OR) using Adobe Photoshop 3.0 software (Adobe System, Inc., Mountain View, CA). Hardcopy image prints were produced on a dye sublimation printer (Phaser IISDX; Tektronix, Inc., Beaverton, OR).

Immunolocalization

Ovaries were dissected, fixed, and prepared for immunocytochemistry as described previously (Li et al., 1994). Briefly, ovaries were dissected from 2–3-d-old females in EBR and fixed for 5 min in 100 μl devitellinizing buffer/600 μl heptane using the method of Cooley et al. (1992). Devitellinizing buffer = 1 vol buffer B/1 vol 36% formaldehyde/4 vol H_2O. Buffer B contains 100 mM KH_2PO_4/K_2HPO_4, pH 6.8, 450 mM KCl, 150 mM NaCl, and 20 mM $MgCl_2.6H_2O$. After fixation, ovaries were rinsed three times in PBS, rinsed three times in PBS/0.1% Triton X-100 (PBT), and washed for

1–2 h in PBT with gentle rocking. The ovarioles were then teased apart, washed for 1–2 h on a rotating wheel (Cole-Parmer Instrument Co., Chicago, IL), and blocked in PBT containing 1% BSA for 1–2 h at room temperature. The ovarioles were double labeled with the affinity-purified rabbit anti-dynein antibody PEP1 (Li et al., 1994) diluted 1:5 or 1:50, and the affinity-purified rat anti-Glued antibody diluted 1:5. The anti-dynein and anti-Glued antibodies were detected with anti–rabbit FITC-conjugated (Boehringer Mannheim Biochemicals, Indianapolis, IN) and anti–rat Texas red–conjugated (Jackson Immunoresearch Laboratories, West Grove, PA) secondary antibodies, respectively. Antibody incubations were performed overnight at 4°C. The secondary antibodies were used at a final dilution of 1:100 after preabsorbing against fixed embryos at a dilution of 1:10. All antibodies were diluted in PBS/0.1% Triton X-100 containing 1% BSA. Ovarioles were mounted in a solution of PBS/90% glycerol containing 1 mg/ml *p*-phenylenediamine (Sigma Chem. Co.), and examined on a diaphot (Nikon Inc. Instrument Group, Melville, NY) microscope with a confocal imaging system (MRC-600; Bio-Rad Laboratories, Hercules, CA), using a 60×/1.4 planapochromat lens.

SEM Analysis

Drosophila heads were dehydrated in an ethanol series as described (Carthew and Rubin, 1990) and prepared for scanning electron microscopy by critical point drying and coating with gold-palladium. Images were recorded on film (type 55; Polaroid Corp., Technical Imaging Products, Cambridge, MA).

Results

Drosophila Glued Gene Product Is a Component of a 20S Complex

To initiate our analysis of the *Drosophila Glued* gene, we characterized the expression profile of *Glued* during development. Our results, like those reported by Swaroop et al. (1986), show that the *Glued* transcript is expressed throughout embryonic development. Our analysis also shows that *Glued* is expressed in heads, testes, and ovaries of adult flies (Fig. 1 *a*). The developmental profile of the *Glued* gene is highly similar to that of the cytoplasmic dynein heavy chain gene, *Dhc64C* (Li et al., 1994). Both the *Glued* and *Dhc64C* transcripts are abundantly expressed in the ovaries of adults, and are present at high levels in 0–2-h embryos (Fig. 1 *a*, and Li et al., 1994). The high level of *Glued* transcript present in 0–2-h embryos indicates that, like *Dhc64C*, there is a substantial maternal contribution of *Glued* in the early embryo.

The polypeptides encoded by the *Glued* gene exhibit biochemical characteristics similar to those of the homologous vertebrate gene products. As shown in Fig. 1 *b*, we used an affinity-purified rat polyclonal serum specific for the *Drosophila* Glued polypeptides (see Materials and Methods) to determine whether the polypeptides were components of a multiprotein complex. Analysis of *Drosophila* ovary cytosol by sucrose density gradient centrifugation revealed that a doublet of polypeptides of ~145/160–kD are recognized by the Glued antibody, and sediment in the ~20S fraction of the sucrose gradient (Fig. 1 *b*, *bottom*). The apparent molecular weights of the Glued polypeptides and their sedimentation as a 20S particle are similar to the characteristics of the homologous p150 subunits in vertebrate dynactin (Gill et al., 1991; Schroer and Sheetz, 1991; Paschal et al., 1993). In comparison, the cytoplasmic dynein complex in ovary extracts sediments at a lower S-value as detected by antibodies that recognize the heavy chain polypeptide (Fig. 1 *b*, *top*). The *Drosophila* Glued polypeptides, like the homologous vertebrate polypeptides, also exhibit an ATP-sensitive microtubule association. In the absence of endogenous nucleotide, the Glued polypeptides are detected in microtubule protein prepared from ovary extracts (Fig. 1 *c*, lane *5′*). The Glued polypeptides are enriched in the fraction of proteins released from taxol-stabilized microtubules by extraction with 10 mM ATP (Fig. 1 *c*, lane *8′*). These results show that the *Drosophila Glued* gene and the related vertebrate genes encode polypeptides with similar biochemical properties.

Glued and Cytoplasmic Dynein Colocalize Throughout *Drosophila* Oogenesis

The *Drosophila* ovary consists of ~15 ovarioles, each of which contains a series of egg chambers of increasing developmental age (for reviews on *Drosophila* oogenesis, see King, 1970; Spradling, 1993). The egg chamber is formed at the tip of the ovariole in the germarium, where the stem cells reside. Oogenesis begins with a stem cell division, which produces a cystoblast that will then undergo four rounds of cell division with incomplete cytokinesis to produce a cyst of 16 cells that remain connected by intercellular bridges called ring canals. As the 16-cell cluster moves posteriorly through the germarium, one of the 16 cells is specified to become the oocyte, while the other 15 adopt the fate of nurse cells. Somatically derived follicle cells migrate around the 16-cell cluster, eventually enveloping the cluster as it buds off the germarium to form the stage 1 follicle, or egg chamber. As the egg chamber matures, the nurse cells supply the oocyte with the materials required for growth. Ultimately, during stages 10B-11 the nurse cells transfer their entire cytoplasmic content into the oocyte and subsequently degenerate as formation of the mature egg is completed.

We have previously reported that during *Drosophila* oogenesis cytoplasmic dynein accumulates in the presumptive oocyte in region 2b of the germarium, and is asymmetrically localized to the posterior pole of the oocyte during stage 9 of oogenesis (Li et al., 1994). The temporal and spatial pattern of dynein distribution during oogenesis provides an excellent opportunity to examine the association of the Glued complex and cytoplasmic dynein. We compared the distribution of the dynein and Glued polypeptides during oogenesis by immunocytochemical double labeling of ovaries with the affinity-purified anti-Glued antibody and the affinity-purified anti-dynein antibody PEP1 (Li et al., 1994). The pattern of Glued localization is indistinguishable from that of cytoplasmic dynein (Fig. 2, *a–d*). Glued is initially evenly distributed throughout all cells in the anterior of the germarium (Fig. 2 *c*, *arrowhead*), then accumulates in the single cell destined to become the oocyte in region 2b of the germarium (Fig. 2 *c*, *arrow*). Similar to the cytoplasmic dynein heavy chain, the Glued polypeptides remain enriched in the oocyte as the egg chamber matures and later during stage 9 become localized to the posterior pole of the oocyte (Fig. 2, *b* and *d*, *arrowheads*).

Glued Localization in Oogenesis Requires Cytoplasmic Dynein

To address whether the colocalization of dynein and Glued

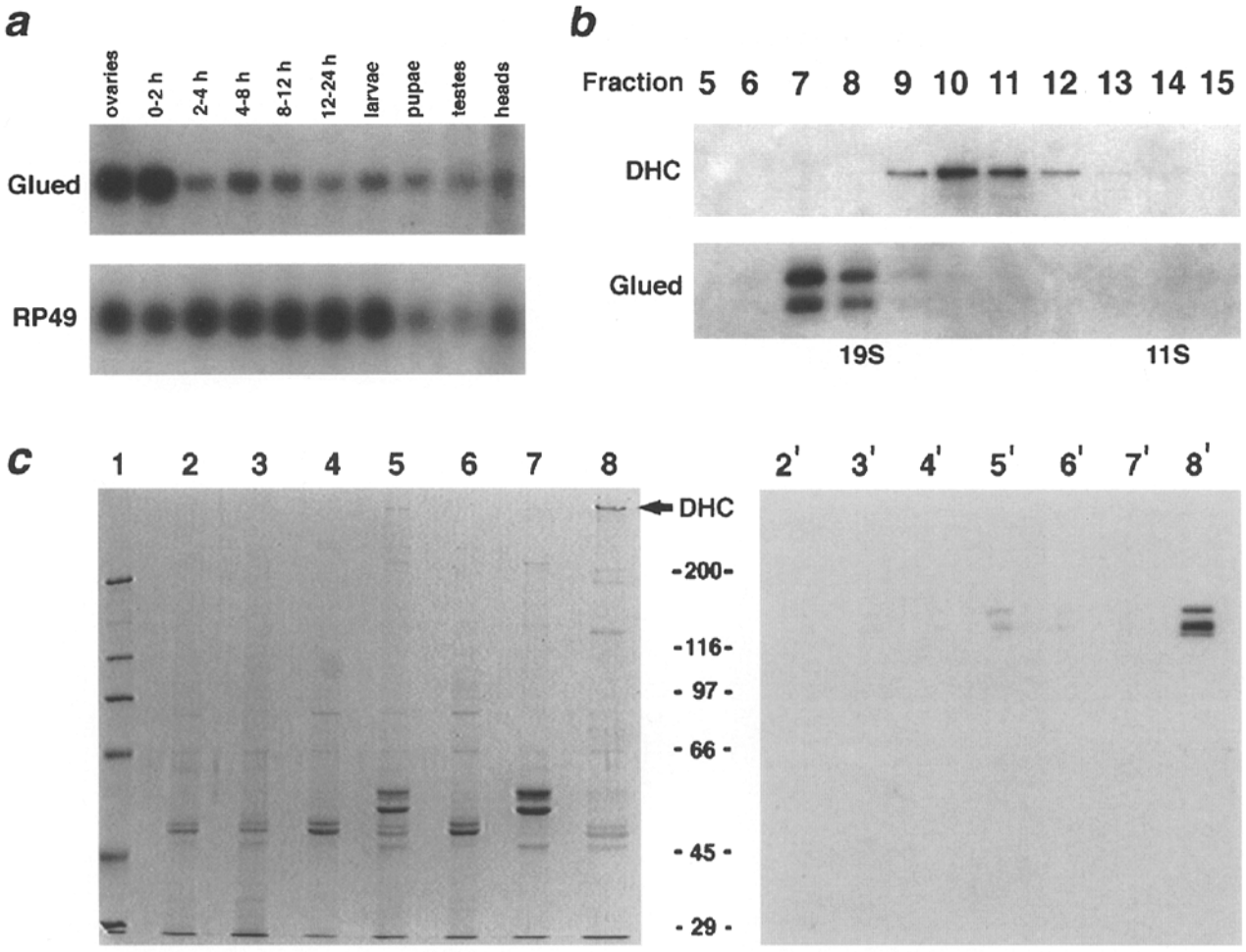

Figure 1. Developmental profile of the *Glued* mRNA and behavior of the *Glued* gene product. (*a*) *Top*: RNA blot containing total RNA isolated from adult ovaries; 0–2, 2–4, 4–8, 8–12, and 12–24-h embryos; larvae (mixed instars); pupae; and adult testes and heads. The blot was hybridized with a ^{32}P-labeled DNA probe produced from the *Glued* cDNA. *Bottom*: as a control to monitor equal loading of the RNA, the blot was subsequently rehybridized with a probe derived from the *Drosophila RP49* gene (Vaslet et al., 1980). (*b*) Immunoblot demonstrating the behavior of the dynein and Glued polypeptides on a sucrose density gradient. 1 mg total protein from a high-speed ovary extract was fractioned on a 5–20% sucrose density gradient, then collected into 0.5 ml fractions. 15-μl samples from each fraction of the gradient were analyzed in duplicate on separate gels by SDS-PAGE and immunoblotting with the affinity-purified anti-dynein heavy chain antibody PEP1 (Li et al., 1994) or the affinity-purified anti-Glued antibody. Sedimentation of the cytoplasmic dynein heavy chain peaks at ~18S (*top*, fractions 9–12). The Glued antibody detects a ~145/160-kD doublet present in a ~20S complex (*bottom*, fractions 7–8). Numbers above the panels indicate the gradient fractions; the positions of the 19S and 11S sedimentation standards are indicated below. *Left*, bottom of gradient. (*c*) The Glued polypeptides sediment with microtubules and are enriched with cytoplasmic dynein after ATP extraction of the microtubule pellet. Samples containing 1 μg total protein from each fraction of a microtubule-associated protein preparation from ovary extracts were run, in duplicate, on separate 7.5% polyacrylamide gels. Lanes *1–8*, Coomassie blue–stained gel. Lanes *2′–8′*, immunoblot of duplicate gel probed with the affinity-purified anti-Glued antibody. (Lane *1*) molecular weight markers; (lanes *2* and *2′*) homogenate; (lanes *3* and *3′*) high speed pellet; (lanes *4* and *4′*) high speed supernatant; (lanes *5* and *5′*) taxol-stabilized microtubule pellet; (lanes *6* and *6′*) supernatant of microtubule pellet; (lanes *7* and *7′*) microtubule pellet after ATP extraction; (lanes *8* and *8′*) ATP-extracted microtubule-associated proteins. The high molecular weight band in lane *8* is the cytoplasmic dynein heavy chain polypeptide (*arrow*, *DHC*; Li et al., 1994). The affinity-purified anti-Glued antibody detects a doublet of polypeptides that sediment with the microtubule pellet (lane *5′*) and are enriched in the ATP elution fraction (lane *8′*) with cytoplasmic dynein. The relative molecular mass of each marker is indicated between the panels.

in oogenesis reflects an association of the dynein and Glued complexes, we asked whether Glued localization is dependent on the function of cytoplasmic dynein. To do this, we made use of mutations in the cytoplasmic dynein heavy chain gene that disrupt the localization of dynein during oogenesis. Flies that are doubly heterozygous for the mutations *Dhc64C*$^{6\text{-}6}$ and *Dhc64C*$^{6\text{-}12}$ are female sterile (data not shown). In ovaries derived from these sterile females, the dynein heavy chain does not accumulate at the posterior pole of the oocyte in stages 9 and 10 egg chambers, but is mislocalized in a punctate pattern at the anterior margin of the oocyte (*arrows*, Fig. 2, *e* and *f*). In addition, dynein is frequently concentrated in patches within the cytoplasm of the nurse cells (*arrowheads*, Fig. 2, *e* and *f*). In this mutant combination of *Dhc64C* alleles the posterior localization of the Glued polypeptides is similarly dis-

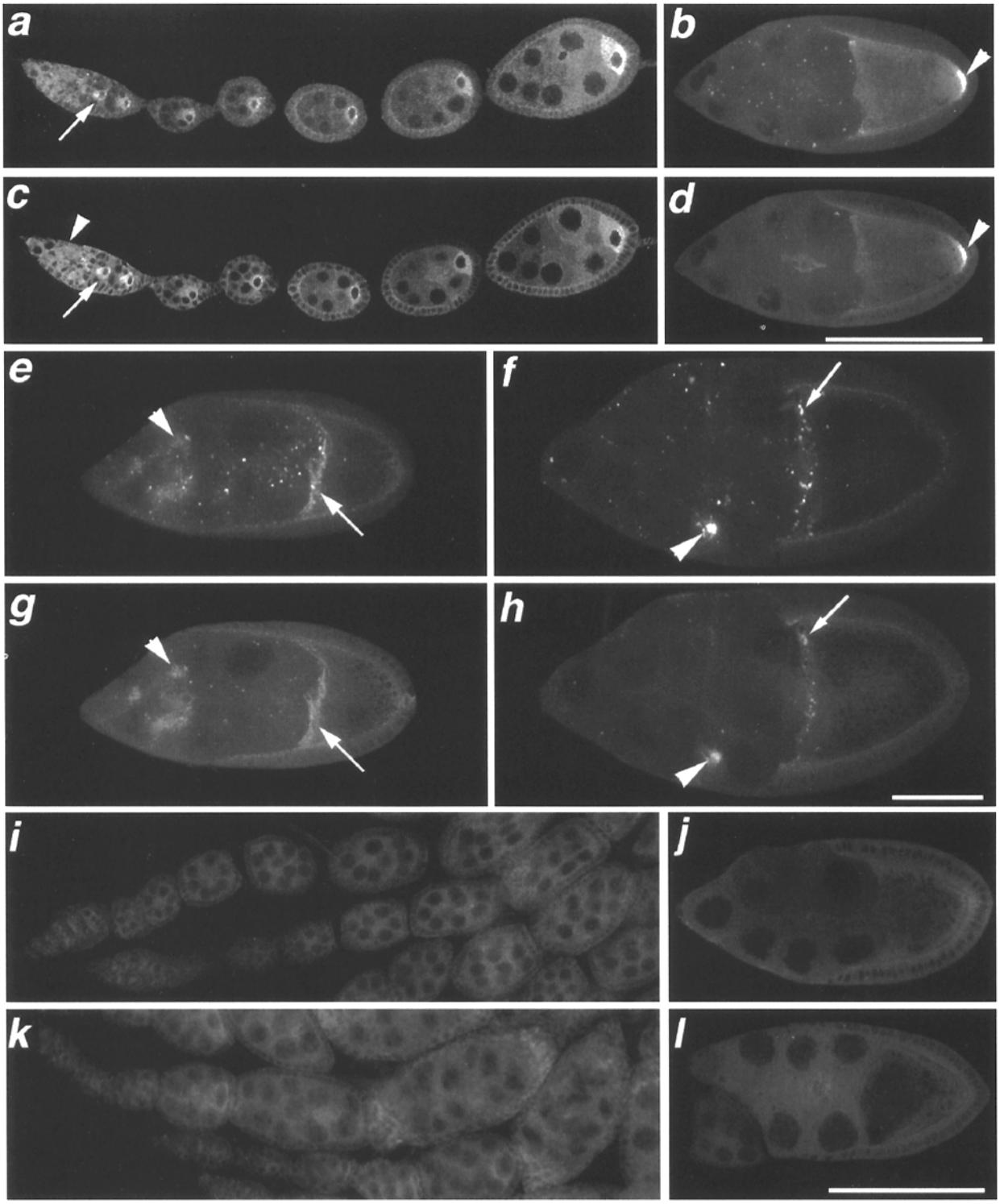

Figure 2. The Glued polypeptides colocalize with cytoplasmic dynein during oogenesis and have the same genetic requirements for localization. (*a–d*) Double-label immunolocalization of cytoplasmic dynein (*a* and *b*) and Glued (*c* and *d*) in ovaries from wild-type Oregon R females. The Glued polypeptides are evenly distributed in the mitotically active cells in the anterior of the germarium (*arrowhead*, *c*) and are differentially localized to the pro-oocyte (*arrow*, *c*) coincident with cytoplasmic dynein (*arrow*, *a*; Li et al., 1994). The Glued polypeptides remain enriched in the oocyte as the egg chamber matures during its passage down the ovariole. Later in oogenesis, like cytoplasmic dynein (*arrowhead*, *b*; Li et al., 1994), the Glued polypeptides are asymmetrically localized to the posterior pole of the oocyte in the stage 9 egg chamber (*arrowhead*, *d*). (*e–h*) Double-label immunolocalization of cytoplasmic dynein (*e* and *f*) and Glued (*g* and *h*) in stages 9 and 10 egg chambers from heteroallelic $Dhc64C^{6\text{-}6}/Dhc64C^{6\text{-}12}$ females. (*e* and *f*) In egg chambers from $Dhc64C^{6\text{-}6}/Dhc64C^{6\text{-}12}$ females, dynein is not localized to the posterior pole of the stage 9 and stage 10 oocyte. Instead, dynein is mislocalized in a punctate pattern at the anterior margin of the oocyte (*arrows*, *e* and *f*), and in bright patches within the nurse cell cytoplasm (*arrowheads*, *e* and *f*). (*g* and *h*) Like cytoplasmic dynein, localization of the Glued polypeptides to the posterior pole of the oocyte is disrupted in the $Dhc64C^{6\text{-}6}/Dhc64C^{6\text{-}12}$ mutant egg chambers. Glued is mislocalized with dynein to the anterior margin of the oocyte (*arrows*, *g* and

rupted, and Glued is colocalized with the mislocalized dynein at the anterior margin of the oocyte (*arrows*, Fig. 2, *g* and *h*) and to the patches within the nurse cells (*arrowheads*, Fig. 2, *g* and *h*). Earlier in oogenesis both dynein and Glued accumulate in the developing oocyte as in wild-type egg chambers (data not shown). The coincident mislocalization of the dynein and Glued polypeptides due to mutations in the cytoplasmic dynein heavy chain gene provides strong evidence that the localization of Glued is dependent on its association with the cytoplasmic dynein motor complex.

Glued and Cytoplasmic Dynein Localization Exhibit Similar Genetic Requirements in Oogenesis

To examine further the association of dynein and Glued in vivo, we asked whether mutations in other genes that disrupt the localization of dynein during oogenesis also affect the distribution of the Glued polypeptides. We have previously shown that mutations in the genes *Bicaudal-D* (*Bic-D*) and *egalitarian* (*egl*) disrupt the accumulation of dynein to the presumptive oocyte (Li et al., 1994). Loss-of-function mutations in either of these genes prevent oocyte differentiation and result in egg chambers containing 16 cells that develop as nurse cells (Suter et al., 1989; Schüpbach and Weischaus, 1991). Like the cytoplasmic dynein heavy chain polypeptide, the Glued polypeptides fail to accumulate in a single cell in egg chambers derived from females homozygous mutant for the egl^{WU50} mutation, or hemizygous for the $Bic\text{-}D^{R26}$ mutation (Fig. 2, *i* and *k*).

We also examined the effect of mutations in *cappuccino* (*capu*) and *spire* (Schüpbach and Weischaus, 1991) on the accumulation of Glued during oogenesis. The *capu* and *spire* genes are required for both dorsoventral and anteroposterior axis formation during oogenesis. Mutations in these two maternal effect loci cause a disruption in the accumulation of the posterior group gene products, *staufen* protein and *oskar* mRNA, to the posterior pole of the oocyte (Manseau and Schüpbach, 1989). We have previously shown that both the *capu* and *spire* gene products are also required for the enrichment of cytoplasmic dynein at the posterior pole of stage 9 oocytes (Li et al., 1994). As shown in Fig. 2, we observe a similar disruption of Glued accumulation at the posterior pole in oocytes derived from females that are homozygous mutant for either $capu^{RK12}$ or $spire^{RP48}$ (Fig. 2, *j* and *l*). The observations that the Glued and cytoplasmic dynein heavy chain polypeptides exhibit similar genetic requirements for their proper localization during oogenesis is consistent with the physical association of the two complexes.

A Dominant Mutation in the Glued Gene, Gl^1, Encodes a Truncated Polypeptide that Associates with Microtubules

The dominant mutation in the *Glued* locus, Gl^1, was previously shown to be caused by the insertion of a transposable element in the 3′ coding sequence of the *Glued* gene (Swaroop et al., 1985). Analysis of *Glued* expression in heterozygous $Gl^1/+$ flies revealed, in addition to the wild-type *Glued* transcript, a truncated message presumably caused by premature termination of transcription due to the inserted transposon (Swaroop et al., 1985). Like other *Glued* alleles, the Gl^1 mutation is a recessive lethal mutation and homozygous animals die in late embryogenesis or early larval stages of development (Plough and Ives, 1935; Harte and Kankel, 1982). In addition, the Gl^1 mutation produces a dominant rough eye phenotype in heterozygous adults (Plough and Ives, 1935). This dominant phenotype was shown to be dosage dependent. The addition of small chromosomal duplications carrying extra copies of the wild-type *Glued* gene into heterozygous $Gl^1/+$ flies rendered the severity of the eye phenotype less extreme in a dosage-sensitive manner (Harte and Kankel, 1982). The dosage dependence of the eye phenotype, together with the molecular nature of the lesion, suggests that the Gl^1 mutation encodes a truncated polypeptide that acts as a poison product. In support of this model, we have identified the truncated protein product encoded by the Gl^1 mutation and examined the association of the truncated polypeptide with microtubules and the Glued complex.

To identify the product of the Gl^1 mutation, we compared the Glued polypeptides in microtubule-associated proteins prepared from wild-type versus heterozygous $Gl^1/+$ tissues. These studies provided a number of new findings. First, the pattern of Glued polypeptide species encoded by the wild-type *Glued* gene is tissue dependent. The simplest pattern is observed in extracts from adult heads, in which the predominant species of Glued polypeptide is a single band which migrates at ~160 kD on SDS-PAGE (*upper arrow*, Fig. 3 *a*, lane marked *head*, +/+). This species is larger than the predicted molecular mass of 148 kD based on the cDNA sequence (Swaroop et al., 1987). An additional lower molecular mass species of ~145 kD is present in minor amounts (*lower arrow*, Fig. 3 *a*, lane marked *head*, +/+). In contrast, the 145-kD species in ovaries appears more abundant than the 160-kD form (Fig. 3 *a*, lane marked *ovary*, +/+). In embryos, the 160-kD and 145-kD species are present in relatively equal amounts (Fig. 3 *a*, lane marked *embryo*, +/+). In all three tissues, a third species that migrates at ~135 kD is present in minor amounts (*arrowhead*, Fig. 3 *a*). The presence of a single Glued transcript in each of these tissues suggests that the multiple Glued polypeptides may arise from the posttranslational modification of a single wild-type product, as opposed to different products resulting from alternative splicing. Second, each of the wild-type Glued species sediments with microtubules (Fig. 3 *b*, lanes marked +/+) and is enriched in microtubule-associated proteins eluted with ATP (Fig. 3 *c*, lanes marked, +/+; see also Fig. 1 *c*). Lastly, a substantial portion of the Glued polypeptides in each tissue re-

h) and to bright patches within the nurse cell cytoplasm (*arrowheads*, *g* and *h*). (*i–l*) Glued localization, like cytoplasmic dynein (Li et al., 1994), requires the genes *egalitarian*, *Bicaudal-D*, *cappuccino*, and *spire*. The Glued polypeptides are not enriched in a single cell in egg chambers from $egalitarian^{WU50}/egalitarian^{WU50}$(*i*) or $Bicaudal\text{-}D^{R26}/Df(2L)TW119$(*k*) mutant females. In $cappuccino^{RK12}/cappuccino^{RK12}$(*j*) or $spire^{RP48}/spire^{RP48}$(*l*) females, the Glued polypeptides are not asymmetrically localized to the posterior pole of the stage 9 oocyte. The localization of Glued to the oocyte through stage 8 of oogenesis is completely normal in $cappuccino^{RK12}$ and $spire^{RP48}$ mutants (data not shown). In all panels, anterior is at left. Magnification is identical in panels *a–d*, *e–h*, and *i–l*. Bars shown in d and l, 100 μm; bar in h, 50 μm.

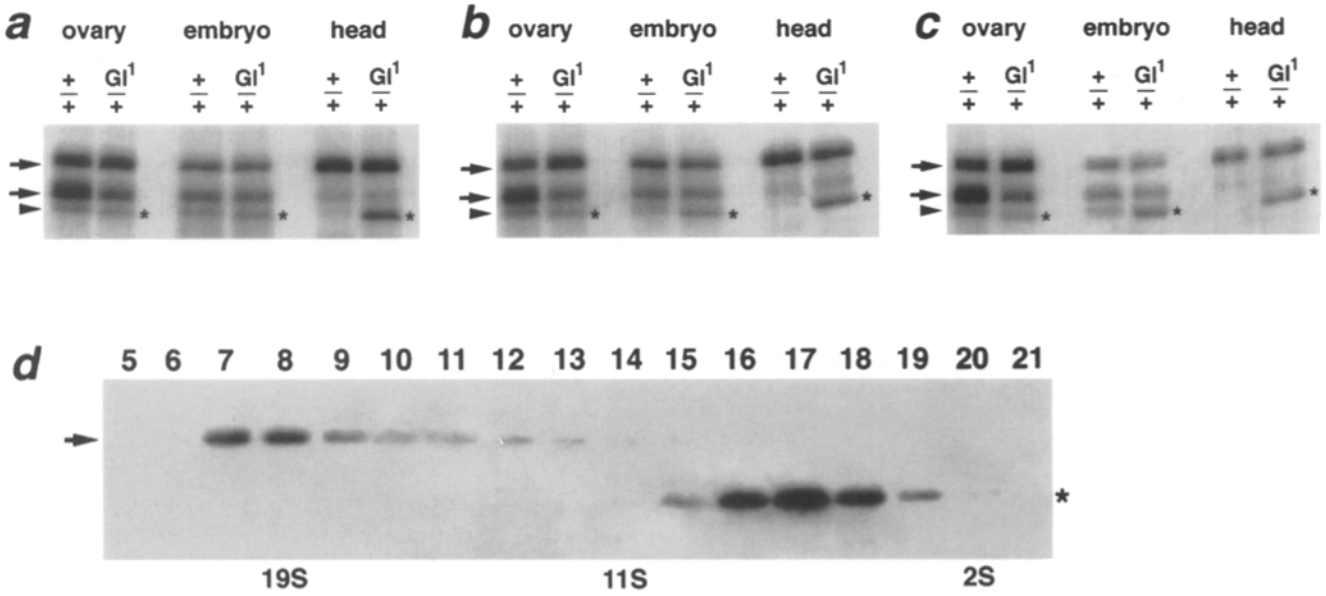

Figure 3. Behavior of the truncated polypeptide encoded by the dominant mutation Gl^1. (*a–c*) Immunoblots of supernatants (*a*), taxol-stabilized microtubule pellets (*b*), and ATP-eluted, microtubule-associated proteins (*c*) prepared from ovary, embryo, and head extracts, from wild-type (+/+) and heterozygous Gl^1/+ (Gl^1/+) animals. To better resolve the difference in migration between the wild-type and mutant Glued polypeptides samples were run on 6.5% polyacrylamide gels. After SDS-PAGE, gels were blotted and the membranes probed with the affinity-purified anti-Glued antibody. 45 μg total protein of the supernatant and taxol-stabilized microtubule pellet samples (*a* and *b*) were loaded per lane, in comparison to 8 μg total protein of the ATP-eluted, microtubule-associated protein samples (*c*). The anti-Glued antibody detects an additional polypeptide which migrates at ~130 kD in samples derived from Gl^1/+ tissues (*asterisks*, *a–c*). Like the wild-type Glued species of ~160 kD (*upper arrow*), ~145 kD (*lower arrow*), and ~135 kD (*arrowhead*), the truncated polypeptide sediments with microtubules (*b*) and is present in the ATP-eluted, microtubule-associated proteins (*c*). A substantial portion of both the wild-type and truncated Glued polypeptides do not sediment with the taxol-stabilized microtubules (*a*). (*d*) Immunoblot demonstrating the behavior of the truncated Glued polypeptide on a sucrose density gradient. 2 mg total protein from a high-speed (125,000-*g*) extract from Gl^1/+ heads was fractioned on a 5–20% sucrose density gradient, then collected into 0.5-ml fractions. 68-μl samples from each fraction of the gradient were run on a 6.5% polyacrylamide gel which was blotted and probed with the affinity-purified anti-Glued antibody. In extracts from Gl^1/+ heads, the ~160-kD wild-type Glued polypeptide sediments in the 19–20S fractions of the gradient (*arrow*). In contrast, the truncated Glued polypeptide sediments as a 6–7S particle distinct from the wild-type product (*asterisk*). Bottom of the gradient is at left. Numbers above the panel indicate gradient fractions. The positions of the 19S, 11S, and 2S sedimentation standards are indicated below the panel.

main unbound in the supernatant after microtubule sedimentation (Fig. 3 *a*, lanes marked +/+), similar to the behavior described for the dynactin complex from chicken (Gill et al., 1991).

The analysis of microtubule-associated protein preparations from heterozygous Gl^1/+ tissues reveals a truncated Glued polypeptide of ~130 kD in addition to the wild-type polypeptides. The truncated polypeptide is most easily distinguished in the samples derived from head extracts, in which the truncated polypeptide is most abundant (*asterisks*, Fig. 3 *a–c*, lanes marked Gl^1/+). In comparison to wild type, the additional polypeptide present in Gl^1/+ extracts migrates slightly faster than the smallest wild-type species of ~135 kD. The presence of this additional polypeptide in extracts from heterozygous Gl^1/+ tissues indicates that the ~130-kD polypeptide represents the truncated protein encoded by the Gl^1 mutation. The variation in the amount of the mutant Glued polypeptide between ovaries, embryos, and heads may reflect variability in the expression of the *Glued* gene, or in the stability of the truncated Glued product, in these different tissues. Like the wild-type Glued polypeptides, the ~130-kD Glued polypeptide sediments with microtubules (*asterisks*, Fig 3 *b*, lanes marked Gl^1/+) and is enriched in the ATP-eluted microtubule-associated proteins (*asterisks*, Fig. 3 *c*, lanes marked Gl^1/+). This result suggests that the COOH-terminal ~20 kD of the wild-type Glued polypeptide is not strictly required for the ability of Glued to sediment with microtubules.

To examine further the behavior of the mutant Glued polypeptide, we analyzed the sedimentation of the truncated polypeptide on sucrose density gradients. Extracts from wild-type and heterozygous Gl^1/+ tissues were fractionated and analyzed by SDS-PAGE and immunoblotting with the affinity-purified Glued antibody. In wild-type head extracts the predominant 160-kD Glued polypeptide sediments in the 20S fraction of the gradient, similar to the sedimentation profile of the major 145/160–kD doublet of Glued polypeptides present in wild-type ovaries (see Fig. 1 *b*) and embryos (data not shown). In head extracts from Gl^1/+ flies, the truncated Glued polypeptide of ~130 kD sediments at ~6–7S (*asterisk*, Fig. 3 *d*), while the 160-kD wild-type Glued polypeptide remains in the 20S fraction of the gradient (*arrow*, Fig. 3 *d*). A similar result was obtained from the fractionation of extracts from Gl^1/+ embryos (data not shown). The distinct sedimentation values of the wild-type and mutant Glued polypeptides indicate that the truncated Glued product does not associate with

the wild-type polypeptide in an ~20S complex. Based on the predicted molecular mass of ~130 kD for the truncated polypeptide, its sedimentation behavior suggests that the mutant Glued polypeptide may be present in the cytoplasm as a monomer. However, our experiments do not exclude the possible association of the truncated Glued protein with other component polypeptides of the Glued complex.

Mutations in Dhc64C Display a Rough Eye Phenotype Similar to the Gl^1 Mutation

The *Drosophila* adult eye is composed of ~800 ommatidia arranged in a precise hexagonal pattern (Fig. 4, *a* and *a'*; for a review on *Drosophila* eye development, see Dickson and Hafen, 1993). Each of the ommatidia, or unit eyes, is made up of ~20 cells, including eight photoreceptor cells, four cone cells which secrete the lens material, several pigment and associated cells, and a single hair cell. The ordered array of ommatidia in the adult eye arises from a precise spatial and temporal sequence of cell divisions, cell shape changes, and cell signaling events during the differentiation of the eye imaginal disc epithelium in the third larval instar.

We have identified several heteroallelic combinations of *Dhc64C* mutations that produce viable adults displaying a rough eye phenotype. For example, as shown in Fig. 4 (*b* and *b'*), the heteroallelic combination of *Dhc64C* alleles $Dhc64C^{3-2}/Dhc64C^{6-10}$ disrupts the shape, size, and organization of the ommatidia within the eye. This eye phenotype is similar to that observed for the dominant Gl^1 mutation (Plough and Ives, 1935; Meyerowitz and Kankel, 1978; Harte and Kankel, 1982) (Fig. 4, *c* and *c'*). The Gl^1 rough eye phenotype is apparently due to the failure to complete and/or maintain the differentiation of clusters of neuronal precursor cells within the developing retinal epithelium during the third larval instar (Renfranz and Benzer, 1989). The similarity between the rough eye phenotypes of heteroallelic combinations of *Dhc64C* mutations and the dominant Gl^1 mutant suggests that the dynein and Glued complexes are components of a common cellular pathway involved in the development of the adult eye.

Mutations in Dhc64C Act To Suppress or Enhance the Rough Eye Phenotype of Gl^1

To obtain more direct evidence that dynein and Glued interact in vivo, we asked whether mutations in the *Dhc64C* gene could act to modify the rough eye phenotype of the dominant mutation Gl^1. In the analysis of flies doubly heterozygous for a *Dhc64C* allele and the Gl^1 mutation, we have found that certain *Dhc64C* alleles act as dominant suppressors of the Gl^1 rough eye phenotype, while other *Dhc64C* alleles act as dominant enhancers of the Gl^1 phenotype (Table I and Fig. 4). For example, in flies doubly heterozygous for the $Dhc64C^{6-6}$ allele and the Gl^1 mutation, the rough eye phenotype is largely suppressed except in the most posterior portion of the eye (Fig. 4 *e*), and the shape and packing of ommatidia is very similar to that observed in wild-type flies (Fig. 4 *e'*). In contrast, the $Dhc64C^{6-8}$ and $Dhc64C^{6-10}$ alleles enhance the rough eye phenotype of Gl^1; the eyes are significantly smaller (Fig. 4 *g*) and the remaining ommatidia are more severely disrupted than those observed in flies carrying only the Gl^1 mutation (Fig. 4 *g'*). The specificity of these genetic interactions is demonstrated by the observation that a deficiency that removes the *Dhc64C* gene, *Df(3L)10H*, in heterozygous combination with Gl^1 does not alter the Gl^1 rough eye phenotype (Fig. 4, *f* and *f'*; Table I). Moreover, the *Dhc64C* alleles do not display a phenotype in combination with a deletion that entirely removes the *Glued* gene, Gl^{+R2} (Table I; Harte and Kankel, 1982), or with other recessive lethal alleles of *Glued* (data not shown). The observed allele specificity indicates that the dominant genetic interactions depend on the presence of the mutant dynein and *Glued* gene products.

To gain insight into the nature of the interaction between the enhancer alleles of *Dhc64C* and Gl^1, we examined the effect of an additional copy of the wild-type *Dhc64C* gene on the enhancement of the Gl^1 rough eye phenotype by the $Dhc64C^{6-8}$ and $Dhc64C^{6-10}$ alleles. The appropriate crosses were conducted to introduce a wild-type *Dhc64C* transgene carried on the X chromosome into flies that were also doubly heterozygous for the Gl^1 mutation and the $Dhc64C^{6-8}$, or $Dhc64C^{6-10}$ allele. The increased dosage of wild-type dynein heavy chain resulted in the reversal of the enhanced rough eye phenotype (Table I and Fig. 4 *h*). The shape, size, and alignment of the ommatidia were less disrupted (Fig. 4 *h'*) and more nearly resembled the original Gl^1 rough-eyed phenotype. This result demonstrates that the activity of the wild-type *Dhc64C* gene product can functionally compete with the product of the *Dhc64C* enhancer allele. We conclude that the genetic interaction observed between the *Dhc64C* and *Glued* mutations reflects the normal interaction of the dynein and Glued complexes in vivo, rather than an aberrant (neomorphic) activity associated with the mutant polypeptides.

A Previously Isolated Suppressor of Gl^1, Su(Gl)102, Is a Dhc64C Allele

Our observations that certain known *Dhc64C* mutations interact with Gl^1 suggested that mutations originally isolated as suppressors of Gl^1 might identify additional *Dhc64C* alleles. One candidate was the *Su(Gl)102* mutation (Harte and Kankel, 1982), which was previously recovered in a screen for EMS-induced revertants of the Gl^1 rough-eyed phenotype (Fig. 4, *d* and *d'*). The *Su(Gl)102* mutation is recessive lethal and maps within the interval between *roughoid* and *hairy* on the left arm of chromosome three (Harte and Kankel, 1982). Significantly, this interval includes the deficiency *Df(3L)10H* which removes the *Dhc64C* gene. To examine whether the *Su(Gl)102* mutation is an allele of *Dhc64C*, we performed a number of complementation tests. We found that *Su(Gl)102* is lethal in combination with the deficiency *Df(3L)10H*, as shown by the absence of progeny of the genotype *Su(Gl)102/Df(3L)10H* in the cross between heterozygous *Su(Gl)102/TM3* and *Df(3L)10H/TM6* flies (Table II, column labeled Critical Class). This result shows that the *Su(Gl)102* mutation lies within the region uncovered by the deficiency. Similar crosses were performed between the *Su(Gl)102* mutation and each of the *Dhc64C* alleles, and show that *Su(Gl)102* is lethal in combination with all but two of the *Dhc64C* alleles that we had isolated (Table II, column la-

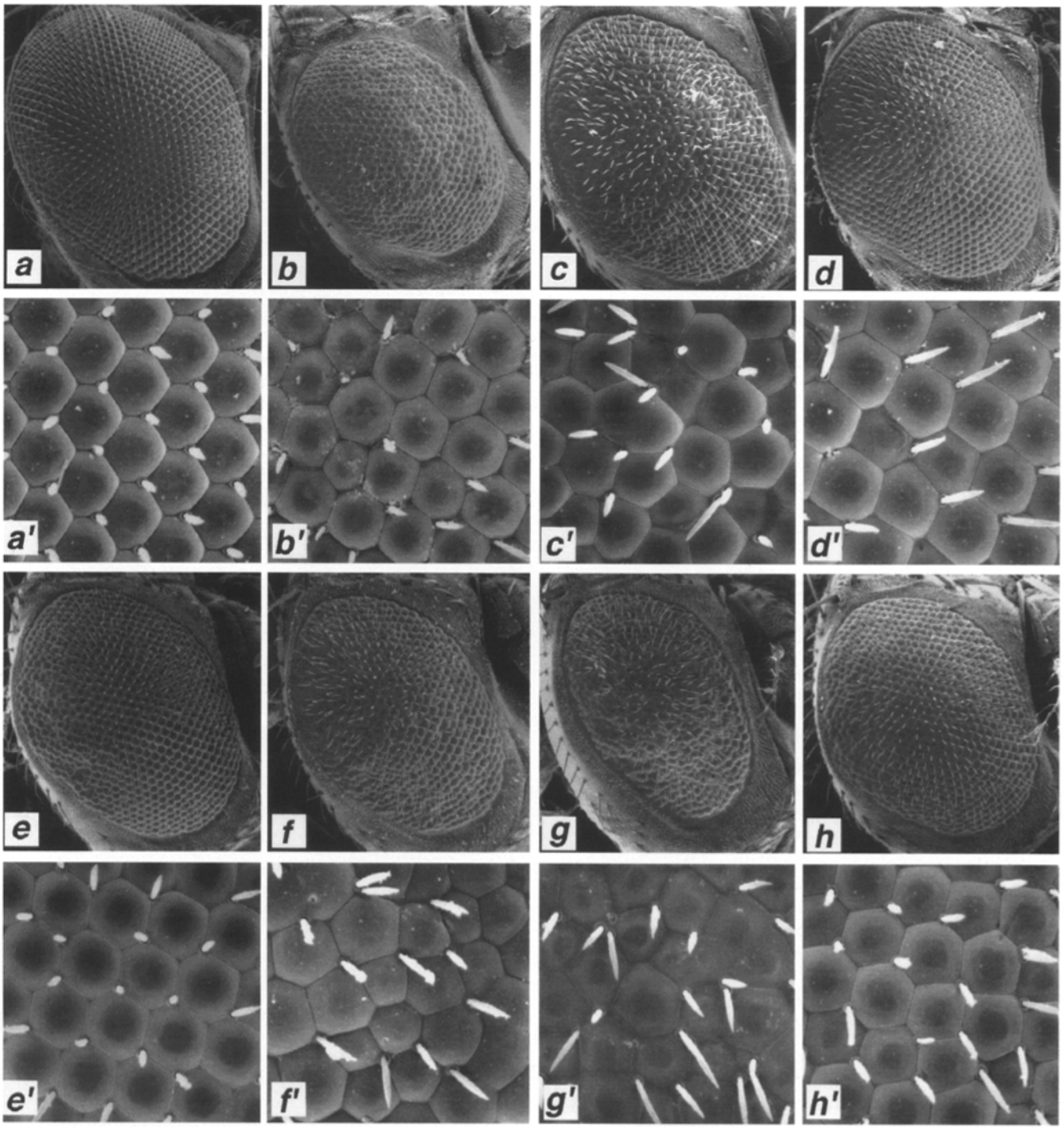

Figure 4. Low (*a–h*) and high (*a'–h'*) magnification SEM micrographs documenting the genetic interaction between *Dhc64C* alleles and the Gl^1 mutation. (*a* and *a'*) An eye from a wild-type OregonR adult fly. (*b* and *b'*) The heteroallelic combination of *Dhc64C* alleles $Dhc64C^{3-2}/Dhc64C^{6-10}$ produces a rough eye phenotype. (*c* and *c'*) The phenotype of the dominant mutation Gl^1, in which the ommatidia appear disorganized, misshapen, and the alignment of bristles is disrupted. (*d* and *d'*) The *Su(Gl)102* mutation suppresses the Gl^1 rough eye phenotype; the bristles and ommatidia in the eye are normally aligned. (*e* and *e'*) The $Dhc64C^{6-6}$ allele suppresses the Gl^1 rough eye phenotype, except in the most posterior region of the eye, similar to the *Su(Gl)102* mutation (*d* and *d'*). (*f* and *f'*) The third chromosome deficiency, *Df(3L)10H*, in combination with Gl^1 does not alter the rough eye phenotype. (*g* and *g'*) The $Dhc64C^{6-10}$ allele enhances the Gl^1 rough eye phenotype in that the eye is smaller, has fewer ommatidia of abnormal shape and size, and duplicated or missing bristles. A similar result is obtained with the $Dhc64C^{6-8}$ allele (data not shown). (*h* and *h'*) Addition of a wild-type copy of the *Dhc64C* transgene can rescue the enhancement of Gl^1 by the $Dhc64C^{6-10}$ allele. The alignment of ommatidia and bristles is less disrupted than in the $Dhc64C^{6-10}/Gl^1$ combination (*g* and *g'*). The same result is obtained when the *Dhc64C* transgene is present in $Dhc64C^{6-8}/Gl^1$ flies (data not shown). The genotypes of the flies represented in each panel are (*a* and *a'*) wild-type OregonR; (*b* and *b'*) $Dhc64C^{3-2}/Dhc64C^{6-10}$; (*c* and *c'*) $+/Gl^1$; (*d* and *d'*) $Su(Gl)102\ +/+\ Gl^1$; (*e* and *e'*) $Dhc64C^{6-6}\ +/+\ Gl^1$; (*f* and *f'*) $Df(10H)3L\ +/+\ Gl^1$; (*g* and *g'*) $Dhc64C^{6-10}\ +/+\ Gl^1$; (*h* and *h'*) $P(Dhc^+)/w;\ Dhc64C^{6-10}\ +/+\ Gl^1$. Crosses to generate flies of the above genotypes are described in Materials and Methods.

Table I. Summary of Eye Phenotypes in Flies Doubly Heterozygous for Glued^{1} and Dhc64C Mutations

Genotype*	Eye phenotype‡
$+/Gl^{1}$§	Rough eyes, misshapen ommatidia, abnormal bristle distribution
$Dhc64C^{6-6}/Gl^{1}$	Gl^{1} rough eye is suppressed, normal hexagonally shaped ommatidia except in the most posterior of the eye
$Dhc64C^{6-8}/Gl^{1}$	Gl^{1} rough eye is enhanced, eyes are reduced in size, frequently fused ommatidia
$Dhc64C^{6-10}/Gl^{1}$	Gl^{1} rough eye is enhanced, eyes are reduced in size, frequently fused ommatidia
$P(Dhc^{+})$; $Dhc64C^{6-10}/Gl^{1}$‖	Rough eyes, similar to the original Gl^{1} rough eye phenotype
$Df(3L)10H/Gl^{1}$	Gl^{1} rough eye phenotype is neither suppressed nor enhanced
$Dhc64C^{6-6}/Gl^{+R2}$¶	Wild-type eyes

*Flies doubly heterozygous for the *Glued^{1}* (Gl^{1}) mutation and a *Dhc64C* mutation ($Dhc64C^{6-6}$, $Dhc64C^{6-8}$, or $Dhc64C^{6-10}$) were generated by crossing heterozygous virgin females of the genotype $Gl^{1}/+$ with heterozygous $Dhc64C^{6-6}/+$, $Dhc64C^{6-8}/+$, $Dhc64C^{6-10}/+$, or $Df(3L)10H/+$ males. The small third chromosome deficiency, *Df(3L)10H*, removes the *Dhc64C* gene (Gepner, J., M.-g. Li, S. Ludmann, C. Kortas, K. Boylan, M. McGrail, and T. S. Hays, manuscript in preparation).

‡See also Fig. 4 micrographs.

§The dominant mutation *Glued^{1}* (Plough and Ives, 1935; Meyerowitz and Kankel, 1978) results in a rough eye phenotype in adult flies heterozygous for the *Glued^{1}* allele.

‖The Dhc^{+} transgene also reverses the enhancement of the Gl^{1} rough eye phenotype in doubly heterozygous $Dhc64C^{6-8}/Gl^{1}$ flies.

¶The recessive lethal Gl^{+R2} allele is a small deficiency that removes the *Glued* gene (Harte and Kankel, 1982). Doubly heterozygous $Dhc64C^{6-8}/Gl^{+R2}$ and $Dhc64C^{6-10}/Gl^{+R2}$ flies also have wild-type eyes.

beled Critical Class). The lethal phenotype of the *Su(Gl)102* mutation in combination with the *Dhc64C* mutations strongly suggests that *Su(Gl)102* is a *Dhc64C* allele. Interestingly, *Su(Gl)102* is viable in combination with the two *Dhc64C* alleles, $Dhc64C^{6-8}$ and $Dhc64C^{6-10}$, which act as enhancers of the Gl^{1} mutation (Tables I and II). To confirm that *Su(Gl)102* is an allele of *Dhc64C*, we have shown that the lethality of the *Su(Gl)102* mutation in combination with other *Dhc64C* alleles is rescued by a wild-type *Dhc64C* transgene. As shown in Table III, flies doubly heterozygous for *Su(Gl)102* and a *Dhc64C* allele, and which also carry one copy of a wild-type *Dhc64C* transgene on the X chromosome (designated $P(Dhc^{+})$), are viable (column labeled $P(Dhc^{+})/+$; *m/Su(Gl)102*, where *m* indicates the appropriate *Dhc64C* allele). The wild-type dynein transgene also rescues the lethality of *Su(Gl)102* in combination with the deficiency, *Df(3L)10H* (Table III). These results demonstrate that an independently isolated suppressor of the Gl^{1} mutation, *Su(Gl)102*, is a *Dhc64C* allele. The characterization of *Dhc64C* alleles that act as allele-specific, dominant second-site modifiers of the Gl^{1} mutation suggests that the functional interaction of the dynein motor complex and Glued complex is mediated by their physical interaction in vivo.

Discussion

Previous work has described the structural similarity between the *Drosophila Glued* gene and the gene encoding the p150 subunit of the dynactin complex in rat and chick (Swaroop et al., 1987; Gill et al., 1991; Holzbaur et al., 1991). In this report, we have demonstrated that the products of the *Drosophila Glued* gene are subunits of a 20S complex similar to the homologous components in vertebrates. In addition, we have provided cell biological evidence for the physical association of the Glued and cytoplasmic dynein motor complexes. Specific mutations in the dynein heavy chain gene, *Dhc64C*, result in the coincident mislocalization of both the dynein heavy chain and Glued polypeptides in late stage oocytes. Finally, our analysis of genetic interactions between a dominant *Glued* mutation, Gl^{1}, and *Dhc64C* mutations demonstrates the functional interaction in vivo between the *Drosophila* Glued and dynein complexes.

Similar to the Glued-related polypeptides in chicken (Gill et al., 1991), the three major species of *Drosophila* Glued polypeptides with apparent molecular masses of 160, 145, and 135 kD, show tissue-specific variations in abundance (Fig. 3). Although multiple electrophoretic species of Glued polypeptides are present, we and others detect a single copy of the *Glued* gene (data not shown; see Swaroop et al., 1986), as well as a single predominant transcript throughout development (Fig. 1). While these results suggest that the multiple Glued polypeptides in *Drosophila* may result from posttranslational modifications, we cannot exclude the possibility that alternate transcripts of low abundance contribute to the heterogeneity

Table II. Suppressor of Glued^{1} Mutation, Su(Gl)102, Is Lethal in Combination with Dhc64C Mutations

	Genotypes of progeny classes (total number of surviving adults)			
		Sibling classes		
Genotypes of mated flies*	Critical class‡	*Su(Gl)102/TM6*	*m/TM3*	*TM3/TM6*
	m/Su(Gl)102			
Df(3L)10H/TM6 ♂ × *Su(Gl)102/TM3* ♀	0	107	99	62
$Dhc64C^{4-6}$/*TM6* ♂ × *Su(Gl)102/TM3* ♀	0	104	149	24
$Dhc64C^{4-16}$/*TM6* ♂ × *Su(Gl)102/TM3* ♀	0	28	37	7
$Dhc64C^{6-6}$/*TM6* ♂ × *Su(Gl)102/TM3* ♀	0	183	228	71
$Dhc64C^{6-8}$/*TM6* ♂ × *Su(Gl)102/TM3* ♀	37§	75	79	21
$Dhc64C^{6-10}$/*TM6* ♂ × *Su(Gl)102/TM3* ♀	148§	133	154	56

*Males (♂) heterozygous for the deficiency *Df(3L)10H* or a *Dhc64C* mutation were mated with virgin females (♀) heterozygous for the *Su(GL)102* mutation. *TM6* and *TM3* refer to multiply marked balancer chromosomes which are wild type at the *Dhc64C* locus.

‡Critical class [*m/Su(Gl)102*] refers to the class of progeny that carries both the *Su(Gl)102* mutation and the *Dhc64C* allele (*m*). The abscence of progeny of this genotype demonstrates that *Su(Gl)102* fails to complement, or is lethal in combination with, the deficiency *Df(3L)10H* or the *Dhc64C* alleles $Dhc64C^{4-6}$, $Dhc64C^{4-16}$, and $Dhc64C^{6-6}$.

§The presence of the critical class in these two crosses demonstrates that the *Su(Gl)102* mutation is viable in combination with the Dhc^{6-8} and Dhc^{6-10} alleles.

Table III. Su(Gl)102 Mutation is a Dhc64C Allele

Genotypes of mated flies*	Genotypes of Progeny Classes (total number of surviving adults)‡					
	P(Dhc⁺)/+; m/Su(Gl)102	*+/Y; m/Su(Gl)102*	*P(Dhc⁺)/+; Su(Gl)102/TM6*	*+/Y; Su(Gl)102/TM6*	*P(Dhc⁺)/+; m/TM6*	*+/Y; m/TM6*
	♀§	♂‖	♀	♂	♀	♂
+/+; *Dhc64C$^{4-6}$/TM6* ♀ × *P(Dhc⁺)/Y; Su(Gl)102/TM6* ♂	115	0	101	99	76	80
+/+; *Dhc64C$^{4-16}$/TM6* ♀ × *P(Dhc⁺)/Y; Su(Gl)102/TM6* ♂	89	0	78	92	67	88
+/+; *Dhc64C$^{6-6}$/TM6* ♀ × *P(Dhc⁺)/Y; Su(Gl)102/TM6* ♂	97	0	64	99	76	98
+/+; *Df(3L)10H/TM6* ♀ × *P(Dhc⁺)/Y; Su(Gl)102/TM6* ♂	76	0	98	85	61	44

*Virgin females (♀) heterozygous for the *Dhc64C* mutation or the deficiency *Df(3L)10H* were mated with males (♂) heterozygous for the *Su(Gl)102* mutation. The wild-type *Dhc64C* transgene, *P(Dhc⁺)*, is on the paternal X chromosome. *Y*, Y chromosome; +/+, maternal X chromosome pair.

‡At the top of each column the genotype of the progeny class is indicated. Note that female and male progeny are shown in separate columns. Because male progeny inherit their X chromosome from the mother, only female progeny inherit the *Dhc⁺* transgene present on the paternal X chromosome. The presence of female progeny of the class *P(Dhc⁺)/+; mSu(Gl)102*§ demonstrates that the *Dhc⁺* transgene rescues the lethality of the *Su(Gl)102* mutation in heterozygous combination with the dynein mutations (*m*). In the absence of the *Dhc⁺* transgene, males‖ doubly heterozygous for *Su(Gl)102* and the dynein mutation (*m*) do not survive.

of polypeptides. In this regard, multiple transcripts have been described for the *Glued* homologue in chicken (Gill et al., 1994) and human (Tokita, M. K, V. M. Y. Lee, and E. L. F. Holzbaur. 1993. *Mol. Biol. Cell. Abstracts.* 4:162). In our characterization of the *Drosophila* Glued polypeptides we show that each of the polypeptides partially copurifies with microtubules and is present in the 20S Glued complex. In addition, we show that the truncated polypeptide encoded by the Gl^1 mutation also copurifies with microtubules. In this case, the failure of the truncated polypeptide to incorporate into the 20S complex suggests that the microtubule association observed for the Glued polypeptides is independent of their presence in the 20S complex. Although the multiple Glued polypeptides are not distinguished by their biochemical properties, the functional significance of the variation in expression of each species is suggested by the apparent restriction of the Gl^1 dominant phenotype to the eye. In extracts derived from head tissue of wild-type flies, a single 160-kD species is the predominant Glued polypeptide. In Gl^1/+ heterozygous flies, the mutant truncated polypeptide is more abundant in head extracts than in the other tissues examined. Whether a dominant effect of the truncated polypeptide is limited to the eye because of the level of its expression, or alternatively reflects an eye-specific function of the 160-kD species, remains to be determined.

The dominant Gl^1 mutation was previously shown to result from the insertion of a transposable element that causes the premature termination of the *Glued* transcript (Swaroop et al., 1985). In Gl^1/+ heterozygous flies, both the wild-type transcript of 6.0 kb and a shorter transcript of 5.1 kb are detected. The shorter transcript has been proposed to encode a truncated polypeptide that lacks a COOH-terminal domain. Since the *Glued* gene is required for normal development (Harte and Kankel, 1982), the expression of a dominant phenotype in Gl^1/+ adult flies suggests the defective gene product functionally competes with the wild-type product, rather than completely blocking the function of the wild-type Glued polypeptide. Consistent with this hypothesis, gene dosage analysis has shown that the mutation behaves as an antimorphic mutation, most likely as the result of the truncated Glued polypeptide acting to poison the function of the wild-type *Glued* gene product. This "poison product" or dominant-negative mechanism is distinct from dominant phenotypes that can arise from alterations in the amount of wild-type gene product (Lindsley et al., 1972) or that arise from the misexpression of a mutant gene (Struhl, 1981). As pointed out by Herskowitz (1987), for proteins whose activity depends on multiple functional sites, a mutant variant that retains only a subset of these sites could act to compete with the wild-type counterpart for its substrate or ligands. The relative stoichiometry of the major components in the bovine brain and chicken dynactin complex is 1.5–2:5:10 (p150/160:p50:Arp-1/actin) (Paschal et al., 1993; Schafer et al., 1994). The extended regions of heptad repeat sequences present in the Glued polypeptide may form coiled-coil structural domains that mediate the dimerization of the polypeptide and/or the association of the Glued polypeptides with the 20S complex. In this regard, the dominant effect observed for the truncated Glued polypeptide could result from its dimerization with an intact Glued polypeptide and its incorporation into a defective Glued complex.

In support of a dominant negative mechanism underlying the Gl^1 phenotype, we have identified a truncated ~130-kD Glued polypeptide in cytoplasmic extracts of tissues from heterozygous Gl^1/+ flies. However, the sedimentation of the truncated polypeptide in the 6–7S fractions on sucrose density gradients demonstrates that the truncated polypeptide cannot assemble into the 20S Glued complex. We conclude that the dominant phenotype is not due to the truncated polypeptide acting as a structural poison to render the Glued complex defective. The separation of the wild-type and truncated Glued polypeptides in two distinct peak fractions of 20S and 7S, respectively, suggests that partial complexes containing less than a full complement of Glued subunits do not form. If this were the case, one would expect both the wild-type and/or truncated polypeptides to cosediment or to be spread across the gradient fractions.

How can the dominant and dosage-sensitive effect of the truncated Glued polypeptide be explained? One possibility is that although the truncated polypeptide fails to incorporate into the Glued complex, it does compete with the wild-type Glued polypeptide for some other limiting component that is required for the assembly and/or function of the complete Glued complex. As a consequence of the titration of such a limiting component, the level of wild-type Glued complex might fall below a required threshold. An alternative possibility is that the dominant effect could be mediated by the ability of the truncated

polypeptide to compete with the wild-type 20S Glued complex for binding sites on the dynein complex and/or microtubules. In the context of previously proposed models in which the dynactin complex acts as a cargo adaptor (Gill et al., 1991; Paschal et al., 1993; Holzbaur et al., 1994), the truncated Glued polypeptide may interfere with the native Glued complex in the docking of cellular cargoes to cytoplasmic dynein and/or microtubules.

The sedimentation behavior of the mutant Glued product demonstrates that the COOH-terminal ~20-kD domain missing from the truncated protein is required for the association of the wild-type Glued polypeptide with itself and/or the native 20S Glued complex. The transposon insertion in the Gl^{1} mutation is located in a region close to or within the conserved COOH-terminal stretch of ~130 residues which exhibit α-helical coiled-coil characteristics (Swaroop et al., 1987). The interruption, or absence, of this potential coiled-coil domain may prevent the mutant polypeptide from dimerizing with the wild-type Glued product, and could account for its distinct sedimentation on the gradient. Alternatively, the inability of the truncated polypeptide to incorporate into a particle with a higher sedimentation value may reflect the absence of the conserved cluster of charged residues LKEK, which lies COOH-terminal to the site of transposon insertion and would be missing in the mutant polypeptide. In vitro studies of the p150 component of dynactin from rat suggest that the homologous sequence, KKEK, mediates the binding of p150 to the centractin/ARP-1 subunit of the dynactin complex (Waterman-Storer et al., 1995).

In contrast to the potential role in dimerization of the Glued polypeptide and/or its incorporation into the Glued complex, the COOH-terminal ~20-kD domain is not strictly required for association of the Glued polypeptide with microtubules. We have shown that the truncated Glued product pellets with microtubules and elutes from microtubules with ATP-dependent, microtubule-associated proteins. These observations are consistent with studies by Waterman-Storer et al. (1995) reporting the existence of a microtubule-binding motif within the NH_2-terminal 39–150 residues of the p150 subunit of dynactin from rat. However, our study does not distinguish between the direct binding of the truncated polypeptide to microtubules and an indirect association mediated by an unknown component present in the cytoplasmic extract.

Although we have not yet determined the specific molecular lesions in the dynein alleles that enhance or suppress the dominant Gl^{1} eye phenotype, the genetic interactions exhibit several characteristics that provide insight into the nature of the mutations. We have shown that certain *Dhc64C* alleles act to suppress the rough eye phenotype, while other alleles act to enhance the rough eye phenotype of the Gl^{1} mutation. The observed dominant interactions are likely not due to mutations that up-regulate *Dhc64C* expression, as additional copies of a wild-type *Dhc64C* transgene in heterozygous Gl^{1} flies have no effect on the severity of the Gl^{1} rough eye phenotype (data not shown). Moreover, since a deficiency that entirely removes the *Dhc64C* gene does not modify the severity of the Gl^{1} rough eye phenotype, the suppressor and enhancer alleles do not represent complete loss-of-function alleles. In other words, the specificity of the genetic interactions between certain dynein alleles and the Gl^{1} mutation suggests that the mutant dynein heavy chains retain some wild-type activity. In addition, the suppressor and enhancer *Dhc64C* alleles fail to produce eye phenotypes when present in combination with a deletion of the *Glued* gene (Gl^{+R2}), or in combination with other recessive lethal *Glued* alleles. This allele specificity indicates that the dominant interactions require the presence of both the mutant *Dhc64C* gene product and the truncated Glued polypeptide. The analysis of the lesions in the interacting *Dhc64C* alleles may help to identify domains in the dynein heavy chain involved in the association of the Glued and dynein motor complexes.

What molecular mechanism can account for the ability of certain dynein alleles to enhance the Gl^{1} rough eye phenotype, while other alleles suppress the phenotype? Given the allele-specific characteristics of the genetic interactions and the truncated product encoded by the Gl^{1} mutation, we favor a model in which the truncated Glued product retains the ability to associate with the dynein complex, thereby competing with the wild-type Glued complex and its attached cargo for association with dynein. The dynein alleles that suppress the dominant effect of the Gl^{1} mutation would do so by acting to eliminate or reduce the defective Glued product from interfering in dynein motor function. One possibility would be that the suppressor alleles encode dynein heavy chains that incorporate into a dynein complex that can associate with the defective Glued polypeptide as well as the wild-type Glued complex, but fails to associate with cellular cargoes. The remaining wild-type dynein heavy chain and Glued polypeptides in the transheterozygote would provide sufficient levels of functional complexes to ensure proper cargo transport within the cell. In contrast, the enhancer alleles may encode dynein heavy chains that incorporate into the motor complex and retain the ability to attach with cellular cargoes, but cannot support cargo transport. The enhanced rough eye phenotype would result from the combined effects of the truncated Glued product and the defective dynein motor competing with wild-type dynein and Glued complexes for the proper association and transport of cargoes.

Regardless of the detailed mechanism of interaction, the allele specificity of the observed suppression and enhancement implies that the truncated Glued product is capable of associating with the dynein complex. Additional evidence of the physical association between the Glued complex and cytoplasmic dynein is provided by our observation that the pattern of localization of the Glued polypeptides is dependent on dynein function. We show that mutations in the *Dhc64C* gene that disrupt the localization of dynein result in the coincident mislocalization of the Glued polypeptides. Furthermore, other genes required for dynein localization during oogenesis, including the *Bicaudal-D*, *egalitarian*, *cappucino*, and *spire* genes, are also required for proper Glued localization. Whether the association of the dynein and Glued complexes in vivo involves the direct binding of the Glued polypeptide to the dynein heavy chain, or to another subunit of the dynein complex, is not known. However, recent in vitro evidence indicates that the 74-kD intermediate chain subunit of cytoplasmic dynein can mediate the association of dynein

with the dynactin complex (Vaughan et al., 1995; Karki, S., K. T. Vaughan, R. B. Vallee, and E. L. F. Holzbaur. 1994. *Mol. Biol. Cell Abstracts.* H113.).

Examples of genetic interactions between genes whose products interact in the formation of complex structures or in common cellular processes have been reported in a number of organisms. In *Chlamydomonas*, extragenic suppressors of paralyzed flagellar mutations have identified loci that alter the outer and inner dynein arm structures (Huang et al., 1982; Porter et al., 1992; 1994), as well as regulatory components (Huang et al., 1982; Piperno et al., 1992, 1994; Gardner et al., 1994) of the flagellar axoneme. Similarly, in *Aspergillus nidulans*, extragenic suppressors of mutations in the β tubulin gene, *benA*, have identified α tubulin (Morris et al., 1979) and γ tubulin genes (Oakley and Oakley, 1989). In other examples, dominant enhancer, or second-site noncomplementing, mutations have revealed structural interactions between gene products. For example, mutations in the *Caenorhabditis elegans* collagen gene *sqt-1* (Kramer et al., 1988) show unusual dominant interactions with mutations in other loci, causing defects in cuticle structure and body shape (Kusch and Edgar, 1986). Like *sqt-1*, these loci might encode other collagens or components of the worm cuticle. In addition, mutations in the α tubulin genes of *S. cerevisiae* (Stearns and Botstein, 1988) and *Drosophila* (Hays et al., 1989) have been identified by their failure to complement mutant alleles of β tubulin genes. As in the examples cited above, the dominant allele-specific interactions between the *Drosophila Dhc64C* and *Glued* mutants reflect a functional interaction between the Glued and dynein complexes.

The eye phenotypes observed for the dominant Gl^1 mutation and certain intragenic combinations of *Dhc64C* alleles, as well as the enhancement and suppression of the Gl^1 rough-eyed phenotype by specific *Dhc64C* alleles, make clear the necessity for dynein and Glued function during *Drosophila* eye development. Previous studies have described the effect of Gl^1 on the projection of axons from the retina to the optic tectum in adult flies (Meyerowitz and Kankel, 1978). This defect could reflect the disruption of dynein function in retrograde axonal transport. Perhaps dynein transport is required for the delivery of a signal from the optic tectum back to the retinal cell body and nucleus that indicates formation of the proper synaptic connection. Alternatively, the defective projection of retinal axons could result from an earlier defect in dynein or Glued function during the development of the retinal epithelium. Differentiation of the eye disc epithelium is accompanied by a highly orchestrated series of events including the synchronization of cell cycles and divisions, cell shape changes, cell-specific patterns of nuclear migration, and the formation of the founder cell clusters that give rise to the ommatidia. Renfranz and Benzer (1989) have described an apparent failure to complete and/or maintain the ommatidial clusters of cells in Gl^1 eye imaginal discs in the third larval instar. Additional analysis will be required to reveal the underlying basis of the rough eye phenotype caused by the dynein and *Glued* mutants.

In addition to the eye phenotypes described above, certain alleles of both the *Glued* (Harte and Kankel, 1982) and *Dhc64C* (Gepner, J., M.-g. Li, S. Ludmann, C. Kortas, K. Boylan, M. McGrail, and T. S. Hays, manuscript in preparation) genes exhibit female-sterile phenotypes. Together with our observations that dynein and Glued colocalize during oogenesis and share similar genetic requirements for localization, the female-sterile phenotypes indicate a role for dynein and Glued in the development of the egg. There is growing evidence implicating microtubule motors and cytoskeletal components in the specification and differentiation of the oocyte (Cooley and Theurkauf, 1994). We are currently conducting genetic screens for additional second-site modifiers of *Dhc64C* and *Glued* mutations to identify other gene products that may provide a link between dynein motor function and the mechanisms that regulate specification and differentiation of cell fate.

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