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A Molecular Genetic Analysis of the Interaction between the Cytoplasmic Dynein Intermediate Chain and the Glued (Dynactin) Complex

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The microtubule motor cytoplasmic dynein performs multiple cellular functions; however, the regulation and targeting of the motor to different cargoes is not well understood. A biochemical interaction between the dynein intermediate chain subunit and the p150-Glued component of the dynein regulatory complex, dynactin, has supported the hypothesis that the intermediate chain is a key modulator of dynein attachment to cellular cargoes. In this report, we identify multiple intermediate chain polypeptides that cosediment with the 19S dynein complex and two differentially expressed transcripts derived from the single cytoplasmic dynein intermediate chain (*Cdic*) gene that differ in the 3′ untranslated region sequence. These results support previous observations of multiple *Cdic* gene products that may contribute to the specialization of dynein function. Most significantly, we provide genetic evidence that the interaction between the dynein intermediate chain and p150-Glued is functionally relevant. We use a genomic *Cdic* transgene to show that extra copies of the dynein intermediate chain gene act to suppress the rough eye phenotype of the mutant *Glued*[1], a mutation in the p150-Glued subunit of dynactin. Furthermore, we show that the interaction between the dynein intermediate chain and p150-Glued is dependent on the dosage of the *Cdic* gene. This result suggests that the dynein intermediate chain may be a limiting component in the assembly of the dynein complex and that the regulation of the interaction between the dynein intermediate chain and dynactin is critical for dynein function.

INTRODUCTION

The intracellular trafficking of organelles and macromolecular complexes plays a major role in many cell and developmental processes. The directed transport of cellular components along polarized microtubule arrays uses two major classes of motor enzymes, the kinesins and the dyneins, which each move unidirectionally along the microtubule tracks. These motors are required for multiple cellular tasks, such as the transport and positioning of organelles and vesicles during interphase, the assembly of the mitotic spindle, and chromosome movement (reviewed by Karki and Holzbaur, 1999). Given these diverse functions, a major question that remains to be answered is how the association of motors with specific cargoes is regulated.

Mechanisms that account for the targeting of dyneins and kinesins may differ. For the kinesins, the multiple cellular functions are provided at least in part by multiple kinesin-related heavy chain polypeptides and associated light chains (reviewed by Goldstein, 1993; Moore and Endow, 1996). Sequence differences outside of the motor domain of the kinesin heavy chains contribute to the targeting of distinct kinesins to specific functions, either directly or by association with other proteins. For example, in *Drosophila*, the kinesin-like protein Nod contains a DNA-binding motif in the nonmotor domain that localizes it to chromosomes during female meiosis (Ashfar *et al.*, 1995). The mechanisms that target the dyneins are less clear. Only three subfamilies of dynein heavy chains have been identified: the axonemal inner and outer arm dynein heavy chains and the cytoplasmic dynein heavy chains. The cytoplasmic dynein heavy chains include two isoforms, a ubiquitously expressed isoform (DHC 1a) and isoform DHC 1b, which appears to be more functionally restricted (Criswell *et al.*, 1996; Vaisberg *et al.*, 1996; Pazour *et al.*, 1999; Porter *et al.*, 1999). The heavy chains are the force-producing subunits that interact with microtubules and hydrolyze ATP. The intermediate, light-intermediate, and light chain subunits are located in a position to interact with other cellular components; their assembly

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Abbreviations used: IC, intermediate chain; MAPs, microtubule-associated proteins.

and regulation may mediate the targeting of cytoplasmic dyneins to specific cargoes (see review by King, 2000).

The dynein intermediate chain may participate in the attachment of dynein to cellular cargoes by several different mechanisms. In flagellar axonemes, a direct attachment to cargo has been demonstrated for an axonemal dynein intermediate chain (IC) by chemical cross-linking of the *Chlamydomonas* IC78 to α-tubulin (King *et al.*, 1991). Alternatively, the dynein IC may act indirectly through dynein light chain subunits to interact with other cellular proteins that modulate dynein function. For example, the light chain LC8 has been shown to bind the mammalian Bim protein, suggesting a role for dynein in the regulation of the apoptosis pathway (Puthalakath *et al.*, 1999). Similarly, the 14-kDa light chain was found to bind rhodopsin in the mammalian eye and may function in turnover of photoreceptor membrane (Tai *et al.*, 1999). Furthermore, in *Drosophila*, a family of at least five light chains related to the LC7 gene *roadblock* has been identified; these light chains are proposed to modulate specific dynein functions (Bowman *et al.*, 1999). Because the light chains are thought to interact with the dynein ICs directly (Mitchell and Rosenbaum, 1986; King *et al.*, 1991, 1996, 1998; King and Patel-King, 1995), a role for the IC may be the regulation and assembly of dynein complexes targeted for specific functions.

The dynein IC may also function through interaction with cargo adapters, such as the Glued (dynactin) complex. Dynactin was originally identified by its ability to stimulate dynein-mediated vesicle motility in vitro (Gill *et al.*, 1991) and was subsequently shown to bind to dynein through the IC subunit (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995). Dynactin is a large complex that is composed of eight or more subunits, including p150-Glued, Arp1 (centractin), and p50-dynamitin (reviewed by Holleran *et al.*, 1998). Holleran *et al.* (1996) demonstrated an association between centractin and a Golgi-specific isoform of spectrin in cells overexpressing the centractin subunit as well as in rat brain cytosol. Together, these data support a model in which dynactin serves as an adapter molecule that binds the IC and links the dynein motor to vesicular cargo.

Previous work has provided evidence for multiple alternatively spliced dynein IC transcripts that may mediate the differential interaction of dynein and cytoplasmic cargoes. In *Drosophila*, the cytoplasmic dynein IC is encoded by a single X-linked gene that is expressed throughout development (Nurminsky *et al.*, 1998a; this work). Nurminsky *et al.* (1998a) have provided evidence that the single *Cdic* (cytoplasmic dynein intermediate chain) gene gives rise to multiple alternatively spliced *Cdic* transcripts, some of which show a tissue-specific distribution. The reported splice variants differ only within small 5′ exons, and overexpression of *Cdic–GFP* constructs in transfected cells suggests that some of the variant IC isoforms may be targeted to different locations within the cell (Nurminsky *et al.*, 1998a). Analyses of cytoplasmic dynein ICs in rat, human, and mouse have revealed two homologous genes in each species that display transcript variants resulting from the alternative splicing of 5′ exons (Paschal *et al.*, 1992; Vaughan and Vallee, 1995; Pfister *et al.*, 1996; Crackower *et al.*, 1999). These data suggest that the dynein IC gene, and its variant products, may be important in the subcellular targeting of the dynein motor (Vaughan and Vallee, 1995; Pfister *et al.*, 1996; Nurminsky *et al.*, 1998a). In this report, we identify a triplet of IC polypeptides present in the dynein motor complex and a novel transcript variant. Furthermore, we provide new evidence for an in vivo interaction between the cytoplasmic dynein IC and the p150-Glued component of dynactin. We show that the interaction between p150-Glued and dynein is dependent on the dosage of the IC gene. This result raises the possibility that the IC subunit is a limiting component in the assembly and function of the dynein motor complex that could be subject to regulation by other interacting proteins.

MATERIALS AND METHODS

PCR and 3′ Rapid Amplification of cDNA Ends Analysis

Degenerate oligonucleotide primers were designed based on dynein IC sequences available from rat IC1 (X66845), *Chlamydomonas* IC70 (X55382), and two human expressed sequence tags (T06737 and T09431): primer 1 (sense): 5′-C-(G/A/T/C)-G-A-(A/G)-T-A-(T/C)-G-T-(G/A/T/C)-T-T-(T/C)-C-A-(T/C)-T-G-3′; primer 2 (antisense): 5′-A-C-(G/A/T/C)-A-C-(A/G)-A-A-(A/G)-T-T-(A/G)-T-T-(G/A/T/C)-A-C-(A/G)-T-C-3′. Primers were synthesized on an Applied Biosystems (Foster City, CA) 392 DNA synthesizer. Amplification reactions (50 μl) contained 2 μl of genomic DNA (~50 ng), 0.2 mM dNTPs, 0.5 μM of each degenerate primer, buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl, 0.1% Triton X-100), and 1.5 U of *Taq* DNA polymerase (Promega, Madison, WI). Reactions underwent 35 cycles of amplification at 50°C for 2 min, 72°C for 3 min, and 95°C for 1 min. Amplification products were analyzed by running 8 μl on an agarose gel and staining with ethidium bromide. The resulting PCR product was gel purified and ligated into pBluescript II KS (Stratagene, La Jolla, CA) after the addition of *Eco*RI linkers. Plasmids containing inserts of the appropriate size were sequenced with the use of the vector primers.

Analysis of the 3′ end of the *Cdic* transcript was performed with the use of the 3′ rapid amplification of cDNA ends (RACE) kit (Life Technologies–BRL, Gaithersburg, MD) according to the supplier's instructions. Nested gene-specific primers for amplification of *Cdic* cDNAs were synthesized (Life Technologies–BRL custom primers): an outer primer: 5′-AAC-TCC-GAC-TAC-GTG-ATG-GAC-G-3′; and an inner primer: 5′-AAG-CTG-TAC-GTG-TAC-GAC-GTG-G-3′. One microgram of total RNA from *Drosophila* embryos, ovaries, or testes was used for first-strand cDNA synthesis with an oligo(dT) adapter primer. Two microliters of the target cDNA was amplified with the use of the outer gene–specific primer and the universal amplification primer supplied in the 3′ RACE kit. Nested PCR was performed on 1 μl of the primary amplification product with the use of the inner gene–specific primer and the universal amplification primer. Reactions underwent 30 cycles of amplification as described above. Amplification products were analyzed by Southern hybridization with a radiolabeled probe to the 3′ end of the *Cdic* cDNA. Products of the predicted size were gel purified and cloned into the pGEMT-easy vector (Promega) according to the supplier's instructions.

Isolation of cDNA Clones and DNA Sequencing and Analysis

A λ ZAP cDNA library constructed from *Drosophila* ovaries by random and oligo(dT) priming (Hazelrigg and Tu, 1994) was screened by hybridization with an IC PCR probe excised from a single subclone by digestion of the plasmid with restriction enzymes. The resulting DNA fragment was gel purified and radiolabeled with [^{32}P]dATP with the use of random hexamers (Pharmacia LKB Biotechnology, Piscataway, NJ). Positive clones were identified after high-stringency hybridization and washing (described below),

and plasmids were obtained by in vivo excision according to the supplier's instructions.

Both strands of the ovary cDNA were sequenced by a combination of the dideoxy chain termination method (Sanger *et al.*, 1977) with the use of Sequenase 2.0 polymerase (United States Biochemical, Cleveland, OH) and automated cycle sequencing (Applied Biosystems 377 automated DNA sequencer, Perkin Elmer-Cetus, Norwalk, CT). A series of nested deletions was generated with the use of exonuclease III and S1 nuclease (Henikoff, 1987) and sequenced with the use of plasmid vector primers. Gaps in the sequence were filled in by synthesizing specific primers for sequencing from the full-length cDNA. Sequence was assembled with AssemblyLIGN (Oxford Molecular Group, Madison, WI) and analyzed with the use of GCG Wisconsin Package version 10.0 (Genetics Computer Group, Madison, WI) and MacVector version 6.0 (Oxford Molecular Group). Sequence data are available from EMBL/GenBank/DDBJ under accession number AF26337.

DNA and RNA Blot Analyses

Genomic DNA for Southern blot analysis was prepared as described previously (Rasmusson *et al.*, 1994) from an isogenic *Drosophila* stock, *iso-1* (Tamkun *et al.*, 1991). Seven micrograms of DNA was digested with restriction enzymes, electrophoresed on a 0.8% agarose gel, and transferred to a nylon membrane (Zeta-Probe, Bio-Rad, Richmond, CA). Hybridizations to radiolabeled cDNA probes were carried out by standard methods (Sambrook *et al.*, 1989). Final washes were done at high stringency (0.1× SSC, 0.1% SDS at 65°C). Isolation of total RNA from *Oregon-R* flies and subsequent blot analysis was done as described previously (Li *et al.*, 1994). Twenty micrograms of total RNA was run on a 1.5% agarose, 9.25% formaldehyde denaturing gel and transferred, probed, and washed as for Southern blotting except that the final wash was done in 0.2× SSC, 0.1% SDS. A probe derived from the *RP49* gene (Vaslet *et al.*, 1980) was used to monitor loading and to verify the integrity of the RNA.

Protein Preparation

Microtubule-associated proteins (MAPs) were prepared from 0- to 20-h *Drosophila* embryos as described previously (Hays *et al.*, 1994). Briefly, 12.5 ml of packed embryos were homogenized on ice in a Dounce homogenizer in 1.5 volumes of PMEG buffer (100 mM piperazine-*N*,*N*′-bis[2-ethanesulfonic acid], 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). A 125,000 × *g* extract was prepared, from which dynein was enriched by affinity with taxol-stabilized microtubules and released with 10 mM Mg-ATP.

For sucrose density gradient separation of proteins, frozen ATP-release fraction from embryo MAPs was thawed and clarified by centrifugation at 100,000 × *g* for 30 min at 4°C. MAPs (0.3 mg) in 550 μl volume were sedimented through an 11.2-ml 5–20% sucrose gradient prepared in PMEG buffer with protease inhibitors as described previously (Hays *et al.*, 1994). The gradients were centrifuged at 230,000 × *g* for 15 h and collected into 0.5-ml fractions. Sedimentation standards were run in parallel on a separate gradient.

Immumoprecipitation experiments were carried out on extracts from hand-dissected ovaries. Homogenization, incubation, and wash steps were in 50 mM HEPES, pH 7.4, 150 mM KCl, 0.9 M glycerol, 0.5 mM DTT, and 0.1% Triton X-100 supplemented with protease inhibitors as described above plus 2 mM PMSF. mAbs to the rat cytoplasmic dynein IC (MAB 1618, Chemicon, Temecula, CA) or the *Drosophila* dynein heavy chain (P1H4; McGrail and Hays, 1997) were allowed to bind to protein A–Sepharose (Sigma-Aldrich, St. Louis, MO) and then incubated with equal amounts of ovary extract (0.6 mg of total protein in 400 μl) for 3 h at 4°C. Beads were washed three times, the last two in buffer lacking Triton X-100. Each pellet was eluted into 20 μl of SDS-PAGE sample buffer, and the entire volume was loaded onto a gel for blot analysis. Equal volumes of supernatants were analyzed by blot analysis (total protein, ~25 μg).

Immunoblots and Immunolocalization

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% slab gels prepared with a 1:100 ratio of bisacrylamide to total monomer. Gels were electroblotted to polyvinylidene difluoride membranes (Millipore, Burlington, MA). Blots were probed with mAbs to the rat cytoplasmic dynein IC (MAB 1618, Chemicon) or the *Drosophila* dynein heavy chain (P1H4). Proteins were detected with the use of alkaline phosphatase–linked secondary antibodies with a chemiluminescence detection system (Applied Biosystems), followed in some experiments by color development with the use of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrate.

For immunofluorescence microscopy, ovaries were dissected from 2- to 4-d-old *Oregon-R* females and fixed and stained as described by McGrail and Hays (1997). The anti-IC mAb 1618 (Chemicon) was diluted 1:50. Egg chambers were examined on a Nikon (Garden City, NY) diaphot microscope with an MRC-600 confocal imaging system (Bio-Rad) with the use of a 60 × 1.4 planapochromat lens.

Construction of a Cdic Genomic Transgene

Cosmids containing genomic DNA from the cytological region 19B-C were provided by the European *Drosophila* Genome Project (Maduñeo *et al.*, 1995). Two overlapping cosmids, 15E10 and 58G1, were used to assemble the 11-kilobase (kb) *Cdic* genomic transgene [called *P(Cdic$^+$)*]. Briefly, a *Xba*I–*Sal*I subclone of cosmid 58G1 was directionally cloned into the *Xba*I–*Xho*I-digested transformation vector *pCaSpeR4* (Klemenz *et al.*, 1987). This plasmid was opened by digestion with *Xba*I, and a 9-kb *Spe*I–*Xba*I fragment from cosmid 15E10 was inserted. The orientation of the insert and the integrity of the *Xba*I junction were verified by sequencing.

Germline transformants were made by microinjection of *Drosophila Df (1)w-c* embryos with the *Cdic19C* genomic transgene (60 μg/ml) and helper plasmid *p-π* 25.7*wc* (7.5 μg/ml) (Karess and Rubin, 1984) in injection buffer (10 mM Tris, pH 8.0, 1 mM EDTA). Multiple independent transformant lines were recovered and analyzed as described previously (Gepner *et al.*, 1996).

β-Galactosidase Reporter Construction and Expression

An ~2-kb *Bam*HI–*Bgl*II fragment was isolated from a *Spe*I–*Xba*I subclone of genomic cosmid 15E10 in pBluescript II KS (Stratagene) and excised with the use of the *Bam*HI site in the multiple cloning region of the vector. This fragment was ligated into the *Bam*HI site of the P-element vector *pCaSpeR-βgalAUG* (Thummel *et al.*, 1988). The orientation of the insert was determined by restriction mapping and verified by sequencing. Germline transformants of the reporter construct were made and analyzed as for the *Cdic* genomic transgene described above. β-Galactosidase activity during oogenesis was assayed by the protocol of Cheung *et al.* (1992) for transformant and control [*Df (1)w-c*] females as described previously (Li *et al.*, 1994).

Fly Stocks and Genetic Analyses

The *Glued* mutant stock *Glued*^{1} *Sb* was provided by Dr. Douglas Kankel (Yale University, New Haven, CT) and has been described by Harte and Kankel (1982). The X chromosome–deficiency stock *Df (1)mal 3* (breakpoints 19A1-2; 20E1-F) was obtained from the Bloom-

ington *Drosophila* stock center (Bloomington, IN) and maintained in males with the duplication *Dp (1;Y)mal 106* (breakpoints 1A1; B2 and 18F; 20F4). Progeny containing the *Glued*[1] mutation were identified with the use of the dominant genetic marker *Sb* (Stubble, short blunt bristles), and the presence of the dynein IC transgene was determined with the use of the *mini-w+* eye color marker. Progeny of the deficiency crosses were evaluated in females, which are heterozygous for the deficiency and do not carry the Y-linked duplication.

Scanning Electron Microscopy

Drosophila heads were dehydrated in an ethanol series as described by Carthew and Rubin (1990) and prepared for scanning electron microscopy by critical point drying and sputter coating with gold with the use of a Fullam sputter coat device (Ernest F. Fullam, Inc., Schenectady, NY). Images were recorded on type 55 Polaroid film (Technical Imaging Products, Cambridge, MA).

RESULTS

The Drosophila Cytoplasmic Dynein IC Is Present in the Dynein Motor Complex

To establish the presence of a dynein IC subunit in the *Drosophila* dynein complex, a mAb against the rat cytoplasmic dynein IC (Dillman and Pfister, 1994) was used to probe an immunoblot of fractions from a preparation of MAPs from *Drosophila* embryos (Figure 1A, upper panel). The antibody recognizes a triplet of polypeptides of ~74 kDa that are enriched in taxol-stabilized microtubule pellets prepared from embryo extracts (Figure 1A, lane 5). Similar to the dynein heavy chain, the IC polypeptides are released from microtubules in the presence of ATP (Figure 1A, lane 7) and migrate on sucrose density gradients as part of a 19S complex (Figure 1, B and C). These data further suggest that the differences between these IC polypeptides do not affect their assembly into the 19S complex. We also performed immunoprecipitation experiments with *Drosophila* ovary extracts and found that antibodies directed against the dynein heavy chain are able to precipitate dynein IC in a complex with the heavy chain subunit (Figure 1D). In reciprocal experiments, the IC antibody precipitates a complex that contains both the dynein heavy chain and IC polypeptides (Figure 1D). Comparison of the relative amounts of IC and heavy chain subunits in the resultant supernatants and pellets suggests that a free pool of IC and heavy chain subunits may exist in ovary extracts. Alternatively, this may reflect the partial disruption of the complex under the conditions used for our analyses (Figure 1D).

Our previous characterization of the distribution of the dynein heavy chain during *Drosophila* oogenesis has shown that the heavy chain polypeptide is enriched in the oocyte relative to the nurse cells in early-stage egg chambers and becomes localized to the posterior of the oocyte during later stages of development (Li *et al.*, 1994). As shown in Figure 2, when the IC antibody is used to probe whole-mount preparations of *Drosophila* ovaries, the dynein IC polypeptide exhibits a distribution similar to that of the heavy chain polypeptide. The IC subunit accumulates in the presumptive oocyte in early egg chambers and at stage 9 becomes concentrated at the posterior of the oocyte. These results are consistent with the biochemical analyses and indicate that the IC is associated with the dynein heavy chain in situ.

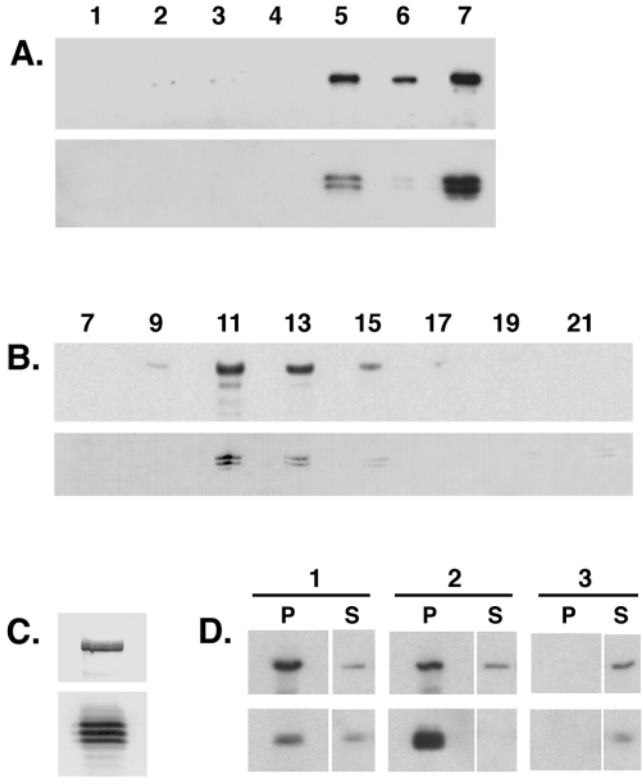

Figure 1. The *Drosophila* cytoplasmic dynein IC associates with the dynein heavy chain. (A) The dynein IC is enriched in a standard preparation of MAPs from *Drosophila* embryos. Equal total protein from each step in the purification was analyzed by Western blotting with the use of an antibody that recognizes the cytoplasmic dynein heavy chain (upper panel) and an antibody that recognizes the dynein IC (lower panel). Lane 1, homogenate; lane 2, low-speed supernatant (57,000 × *g*); lane 3, high-speed supernatant (125,000 × *g*); lane 4, microtubule-depleted supernatant; lane 5, microtubule pellet; lane 6, Mg-ATP–extracted microtubule pellet; lane 7, Mg-ATP supernatant. (B) Embryo ATP MAPs were fractionated over a 5–20% sucrose density gradient and collected into 23 aliquots. Equal volumes of alternate fractions were analyzed by Western blotting. Upper panel, anti-dynein heavy chain; lower panel, anti-dynein IC. Fraction numbers are indicated above the appropriate lanes. Sedimentation standards were as follows: 19S, fraction 11; 11S, fraction 17; 2S, fraction 22. (C) The 19S dynein peak fraction from the sucrose gradient shown in B. The IC can be resolved as a triplet of polypeptides by one-dimensional PAGE. Upper panel, anti-dynein heavy chain; lower panel, anti-dynein IC. (D) Antibodies against the dynein heavy chain immunoprecipitate the dynein IC (column 1, P). Similarly, antibodies against the dynein IC immunoprecipitate the dynein heavy chain (column 2, P). No heavy chain or IC polypeptide is precipitated when beads alone are used (column 3). P, pellet; S, supernatant.

The Drosophila Cytoplasmic Dynein IC Is Encoded by a Single-Copy Gene That Encodes Multiple Transcripts

As a first step in the genetic analysis of IC function, we cloned the cytoplasmic dynein IC gene in *Drosophila*. Degenerate oligonucleotide primers were designed based on dynein IC sequences from rat, *Chlamydomonas*, and a human

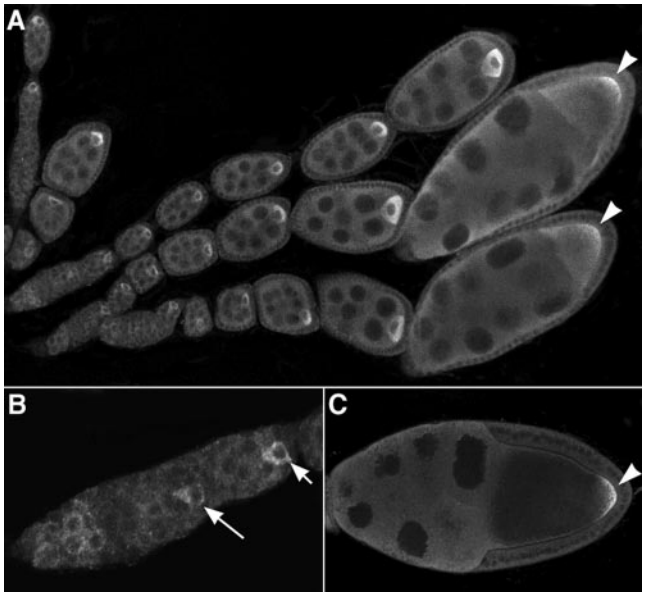

Figure 2. Localization of the dynein IC polypeptide during *Drosophila* oogenesis. Ovaries dissected from 2-d-old wild-type females were fixed and stained with a mAb to the rat cytoplasmic dynein IC. (A) The dynein IC is concentrated in the oocyte relative to the nurse cells in the developing egg chambers. In stage 9 and 10 egg chambers, the dynein IC is localized to the posterior of the oocyte (A and C, arrowheads). (B) The dynein IC is seen uniformly distributed in regions 1–2a of the germarium. The IC becomes enriched in the presumptive oocyte as early as region 2b in the germarium (large arrow).

Table 1. Dynein intermediate chain sequence similarities with *Drosophila Cdic19C*

	Percent identity (% similarity)	Amino acid residues (range, 1–642)
Cytoplasmic		
Mouse IC2	56 (65)	2–640
Rat IC2a	55 (65)	4–640
Rat IC1	51 (60)	2–640
Human IC1	51 (59)	2–640
Caenorhabditis elegans	40 (50)	3–641
Dictyostelium	43 (50)	74–639
Yeast	30 (43)	361–530
Axonemal		
Chlamydomonas IC78	28 (38)	246–623
Chlamydomonas IC70	28 (38)	233–628
Sea urchin IC2	25 (35)	209–642
Sea urchin IC3	25 (36)	216–623

Amino acid sequence comparisons of the *Drosophila* dynein intermediate chain with both cytoplasmic and axonemal intermediate chains from other species. Sequences were aligned using the GCG program BESTFIT with the use of default parameters. Accession numbers are: *Drosophila*, AF263371, and Cdic2a, AF070692 (Nurminsky *et al.*, 1998a); rat IC1, X66845 (Paschal et al., 1992); rat IC2a, U39044 (Vaughan and Vallee, 1995); mouse IC2, AF063231; human IC1, AF063228 (Crackower *et al.*, 1999); *C. elegans*, cosmid CELC17H12, AF045642 (*C. elegans* Genome Sequencing Consortium, 1998); *Dictyostelium*, U25116 (Ma *et al.*, 1999); yeast, U16820 (Geiser *et al.*, 1997); *Chlamydomonas* IC78, U19120 (Wilkerson *et al.*, 1995); *Chlamydomonas* IC70, X55382 (Mitchell and Kang, 1991); sea urchin IC2, D38538; sea urchin, IC3, D28863 (Ogawa *et al.*, 1995).

expressed sequence tag. Amplification reactions were run with the use of fly genomic DNA as a template, and the predicted 250-base pair (bp) PCR product was recovered from an agarose gel, purified, and subcloned. Sequence analysis of six independent clones revealed the presence of identical 258-bp genomic inserts. The genomic IC PCR fragment was used subsequently to recover clones from a *Drosophila* ovarian cDNA library. Five overlapping clones were identified and characterized by restriction mapping and partial sequence analysis. One clone of 2.8 kb appeared to be full length and was chosen for further characterization. The coding sequence of the full-length ovary IC cDNA clone predicts a protein of 643 amino acids with a molecular mass of 71 kDa. The 169-bp 5′ untranslated region that lies upstream of the predicted translational start codon contains stop codons in all three reading frames. The predicted amino acid sequence of the *Drosophila* IC gene product is highly similar to cytoplasmic dynein IC sequences from other organisms and is considerably less similar to axonemal isoforms (Table 1). The sequences of numerous IC cDNA variants in *Drosophila* have been reported by Nurminsky *et al.* (1998a). Comparison of the sequence of the ovary cDNA with the sequences of these cDNAs shows it is identical to *Cdic* isoform 2a, which is constitutively expressed (Nurminsky *et al.*, 1998a; accession number AF070692) and is not specific to the ovary.

Using probes derived from the *Drosophila Cdic* cDNA, we identified *Cdic* transcripts of 2.8 and 2.4 kb that are differentially expressed (Figure 3A). The 2.8-kb transcript is expressed at relatively constant levels in all *Drosophila* tissues and stages examined and is recognized by overlapping cDNA probes that span the entire length of the 2.8-kb cDNA clone (our unpublished data). Two different 2.4-kb transcripts can be distinguished with the use of cDNA probes from coding and noncoding regions of the cDNA. Detection of one 2.4-kb transcript is limited to ovaries and early embryos, suggesting a maternal pattern of expression. The maternal 2.4-kb transcript is recognized by cDNA probes derived from the 5′ untranslated region (UTR) or the *Cdic* coding sequence (Figure 3A, panel 1) but is not recognized by a probe that contains only the 3′ UTR sequence of the 2.8-kb cDNA (Figure 3A, panel 2). This result suggests that the shorter 2.4-kb transcript in the ovary and early embryos differs from the 2.8-kb transcript in the length of the 3′ UTR. Consistent with this interpretation, both short and long polyadenylated 3′ cDNA fragments are amplified by 3′ RACE with the use of an oligo(dT) and a gene-specific primer at the 3′ end of the *Cdic* coding sequence (our unpublished data). The sequence of the shorter 3′ cDNA tail is fully contained within the longer sequence and ends ~400 bp from the 3′ end of the long sequence. A second 2.4-kb transcript is detected in RNA from testis. The testis-specific transcript can be distinguished from the maternally expressed transcript because it is not recognized by the 5′ cDNA probe. A similar testis-specific dynein IC transcript that lacks the 5′ coding region has been described (Nurmin-

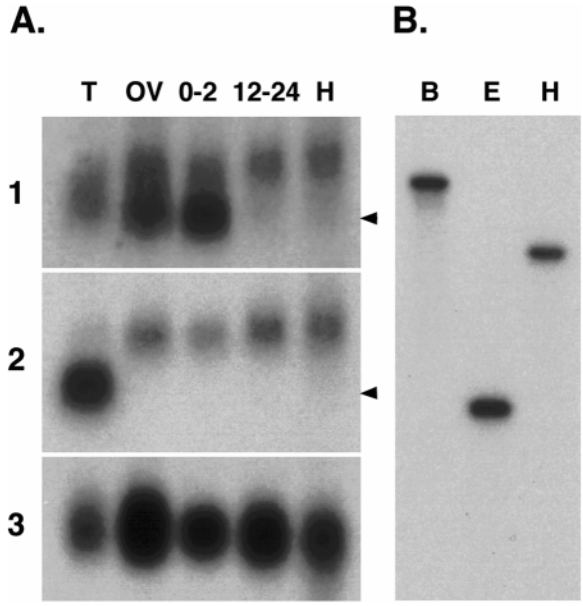

Figure 3. The *Drosophila* dynein IC is represented by a single gene that is expressed throughout development. (A) Northern analysis of *Drosophila* total RNA shows the presence of two transcripts. Blots of total RNA from various tissues were hybridized to probes derived from the 5′ end (panel 1) and the 3′ UTR (panel 2) of the *Cdic* cDNA. Transcripts of ~2.4 and 2.8 kb are shown. T, testes; OV, ovaries; 0–2, 0- to 2-h embryos; 12–24, 12- to 24-h embryos; H, heads. Arrowheads indicate the mobility of the 2.4-kb RNA molecular size standard. Blots were rehybridized with RP49 as a loading control (panel 3). (B) Southern blots of *Drosophila* genomic DNA digested with different restriction enzymes and hybridized to the 5′ *Cdic* probe used in the Northern blot analysis. A single band is identified in each lane, showing that the dynein IC is represented by a single gene in the *Drosophila* genome. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII.

sky *et al.*, 1998a,b). Our results identify additional variation in *Cdic* transcripts that arises at the 3′ end of the molecule and complement previous demonstrations of alternative splicing of small 5′ exons (Vaughan and Vallee, 1995; Nurminsky *et al.*, 1998a). Although our RNA blot analysis detects only two separate hybridization bands, the broadness of each band is consistent with previous reports of alternative splicing of small exon sequences within the N terminus (Vaughan and Vallee, 1995; Pfister *et al.*, 1996; Nurminsky *et al.*, 1998a).

To detect the presence of additional IC-related genes in the *Drosophila* genome, we used both Southern analysis and BLAST sequence analysis. Blots of *Drosophila* genomic DNA were hybridized with a *Drosophila* IC probe derived from the 5′ region of the cDNA that is divergent between cytoplasmic and axonemal IC sequences (Figure 4). Under both high- and low-stringency hybridization conditions, the 5′ probe identifies a single restriction enzyme fragment on genomic DNA blots (Figure 3B), suggesting the presence of a single cytoplasmic dynein IC gene in *Drosophila*. In situ hybridization to larval polytene chromosomes was conducted to identify the cytological location of the dynein IC gene. The IC PCR fragment hybridizes to a unique site at the proximal end of the X chromosome in region 19C (our unpublished data). During the course of our analysis, the presence of an annexin–dynein IC tandem repeat present in region 19F was reported (Benevolenskaya *et al.*, 1995), and subsequently Nurminsky *et al.* (1998a) reported the presence of the adjacent single-copy *Cdic* gene. Sequence comparisons and a search of the recently completed *Drosophila* genomic sequence confirm that a single complete IC gene resides at the polytene chromosome region 19C. Our database searches identified three other sequences in the *Drosophila* genome (Adams *et al.*, 2000) with high similarity to axonemal dynein ICs (Table 2). Two of these sequences, positioned at polytene positions 57B and 34BC, predict proteins that are most closely related to the *Chlamydomonas* IC78 and IC70, respectively (Mitchell and Kang, 1991; Wilkerson *et al.*, 1995). Furthermore, these genomic sequences contain expressed sequence tags that are similar to the *Chlamydomonas* IC orthologues, suggesting that these regions are expressed. The third genomic region (62AB) shows the greatest similarity to *Chlamydomonas* IC78; however, no expressed sequence tag sequences were identified from this region. The completion of the fly genome sequence has revealed additional putative axonemal dynein IC homologues at polytene regions 7D, 61A, 66A, and 68C (Adams *et al.*, 2000; Goldstein and Gunawardena, 2000). This information substantiates the finding that although multiple axonemal dynein IC genes are represented in the genome, the cytoplasmic dynein IC is encoded by a single gene in *Drosophila*.

Construction of a Cdic Transgene

To pursue a functional analysis of the dynein IC, a genomic transgene was constructed. Cosmids in the cytological region of the *Cdic* gene were analyzed by DNA blot hybridization (Figure 4). Using probes derived from the 5′ and 3′ ends of the 2.8-kb *Cdic* cDNA, we identified two overlapping cosmids, 15E10 and 58G1, that span the complete dynein IC cDNA. These cosmids were further analyzed by a combination of restriction mapping and sequencing of selected subclones. Cosmid clone 15E10 ends less than 1 kb from the 3′ end of the *Cdic* cDNA. Cosmid 58G1 was determined to contain the full-length dynein IC gene and extends farther 3′ to include one or more copies of a flanking genomic rearrangement between the dynein IC gene and annexin X (Benevolenskaya *et al.*, 1995).

The boundaries of the *Cdic19C* transcription unit were further defined by hybridizing blots of RNA from various *Drosophila* tissues with genomic DNA fragments extending 5′ and 3′ from the *Cdic19C* gene. We identified two transcription units that flank the *Cdic19C* gene. A 1.4-kb transcript expressed in embryos was identified ~5 kb upstream of the *Cdic19C* cDNA (Figure 4A). Southern analysis and sequencing of genomic subclones 3′ to the *Cdic19C* transcription unit confirmed the presence of a previously identified genomic rearrangement containing tandem repeats of a 3′ fragment of the dynein IC gene fused to a truncation of the *Drosophila* annexin X gene (Benevolenskaya *et al.*, 1995). The *annexin–Cdic* gene fusion borders the intact dynein IC gene (Nurminsky *et al.*, 1998a). Genomic subclones from within the dynein IC transcription unit recognized only the 2.8- and 2.4-kb *Cdic* transcripts (our unpublished data).

An 11-kb genomic transgene containing only the *Cdic19C* transcription unit was constructed from subclones of cosmids 15E10 and 58G1. The transgene includes 2.0 kb of

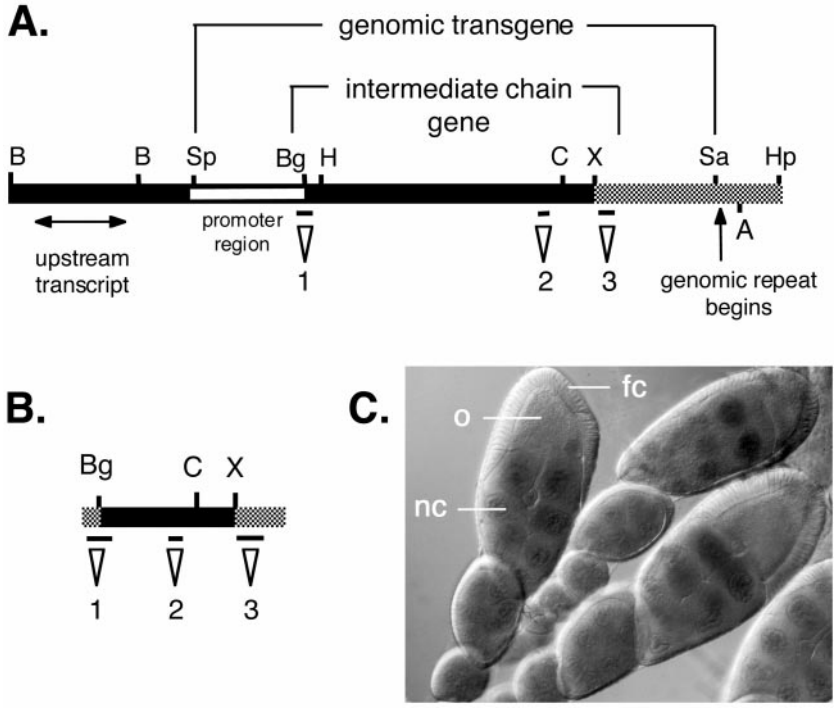

Figure 4. Molecular map of the *Cdic* genomic region 19C and cDNA. (A) Restriction map of the genomic cosmids used in construction of the *Cdic* genomic transgene and a diagram of the *Cdic* transcription unit showing the location of neighboring transcripts. The black bar indicates DNA from cosmid 15E10, and the gray bar indicates DNA from cosmid 58G1. The open bar indicates the region used for promoter fusion to β-galactosidase. The positions of the probes used for blot hybridizations are indicated: probe 1, 5′ cDNA; probe 2, PCR product; probe 3, 3′ UTR. (B) Restriction map of the IC cDNA showing the relative positions of the probes. The gray bar represents the 5′ and 3′ UTRs, and the black bar indicates the coding sequence. Regions 1, 2, and 3 are described in A. (C) Micrograph of ovaries from transformed females expressing the *Cdic* promoter fusion stained for β-galactosidase. LacZ staining is restricted to the nurse cell nuclei and is not seen in the oocyte or the somatic follicle cells surrounding the egg chamber. nc, nurse cell; o, oocyte; fc, follicle cell. B, *Bam*HI; Sp, *Spe*I; Bg, *Bgl*II; H, *Hin*dIII; C, *Cla*I; X, *Xba*I; Sa, *Sal*I; A, *Aat*I; Hp, *Hpa*II.

sequence 5′ to the predicted start of the coding sequence and extends 3′ to the beginning of the annexin–dynein repeat. The transgene was transformed into flies by P-element–mediated germline transformation. Twelve independent transformant lines were recovered that contain single transgene insertions, as determined by genomic DNA blot analysis with the use of probes for the *Cdic19C* gene. In parallel, 2.0 kb of genomic DNA upstream from the dynein IC gene was cloned separately into the transformation vector pCasPeRβgalAUG and shown to be sufficient for expression of the β-galactosidase reporter gene in transgenic flies (Figure 4C); we conclude that this 2-kb region contains a functional endogenous *Cdic* promoter.

Table 2. Dynein intermediate chain–related sequences from the *Drosophila* genome[a]

Genomic region	Related IC	Blast probability
57B6-13 (DS02397)	*Chlamydomonas* IC78	1.4×10^{-83}
	Cdic19C	4.1×10^{-8}
	Chlamydomonas IC70	3.3×10^{-7}
34B6-C2 (DS08787)	*Chlamydomonas* IC70	2.8×10^{-51}
	Cdic19C	1.3×10^{-6}
62A10-B5 (DS04848)	*Chlamydomonas* IC78	6.3×10^{-16}
	Cdic19C	0.0021

BLAST analysis of the *Drosophila* genome sequence from the Berkley *Drosophila* Genome Sequencing Project identifies three regions of the *Drosophila* genome with similarity to axonemal dynein intermediate chains (*Chlamydomonas* IC70 and IC78) as well as to the *Drosophila* cytoplasmic dynein intermediate chain gene at region 19C (*Cdic19C*). Amino acid sequences from the known intermediate chain genes were compared with the *Drosophila* genomic nucleotide sequence with the use of the program TBLASTN. BLAST probability represents the probability that the sequence similarity identified is due to chance.

[a] The completion of the *Drosophila* genome sequence has identified additional related sequences that may be present at genomic regions 7D, 61A, 66A, and 68C (Adams *et al.*, 2000; Goldstein and Gunawardena, 2000).

The Cytoplasmic Dynein IC Interacts with p150-Glued In Vivo

Biochemical experiments have shown a direct interaction between the cytoplasmic dynein IC and the p150-Glued subunit of dynactin (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995). To determine whether this biochemical interaction is functionally relevant in vivo, we asked whether additional copies of the dynein IC could affect the dominant rough eye phenotype of *Glued*[1], a mutation in the p150-Glued subunit of dynactin (Figure 5). The wild-type *Drosophila* eye is composed of ~800 ommatidia organized in a highly ordered array (Figure 5A) (for a review of *Drosophila* eye development, see Dickson and Hafen, 1993). In flies containing the dominant mutation *Glued*[1], the organization of the ommatidia is disrupted, causing a rough-eye phenotype (Figure 5B) (Harte and Kankel, 1982). To cross the *Cdic* transgene into the *Glued*[1] background, males containing a single copy of the dynein IC transgene, *P(Cdic*$^+$*)*, were mated to *Glued*[1] mutant virgin females (genotype *w/w; Gl*[1] *Sb/TM6B, D, Hu*). When this additional copy of the dynein IC gene is introduced, the rough-eye phenotype of *Glued*[1] is suppressed (Figure 5C). The degree of suppression was assessed visually by the extent of disruption of the omma-

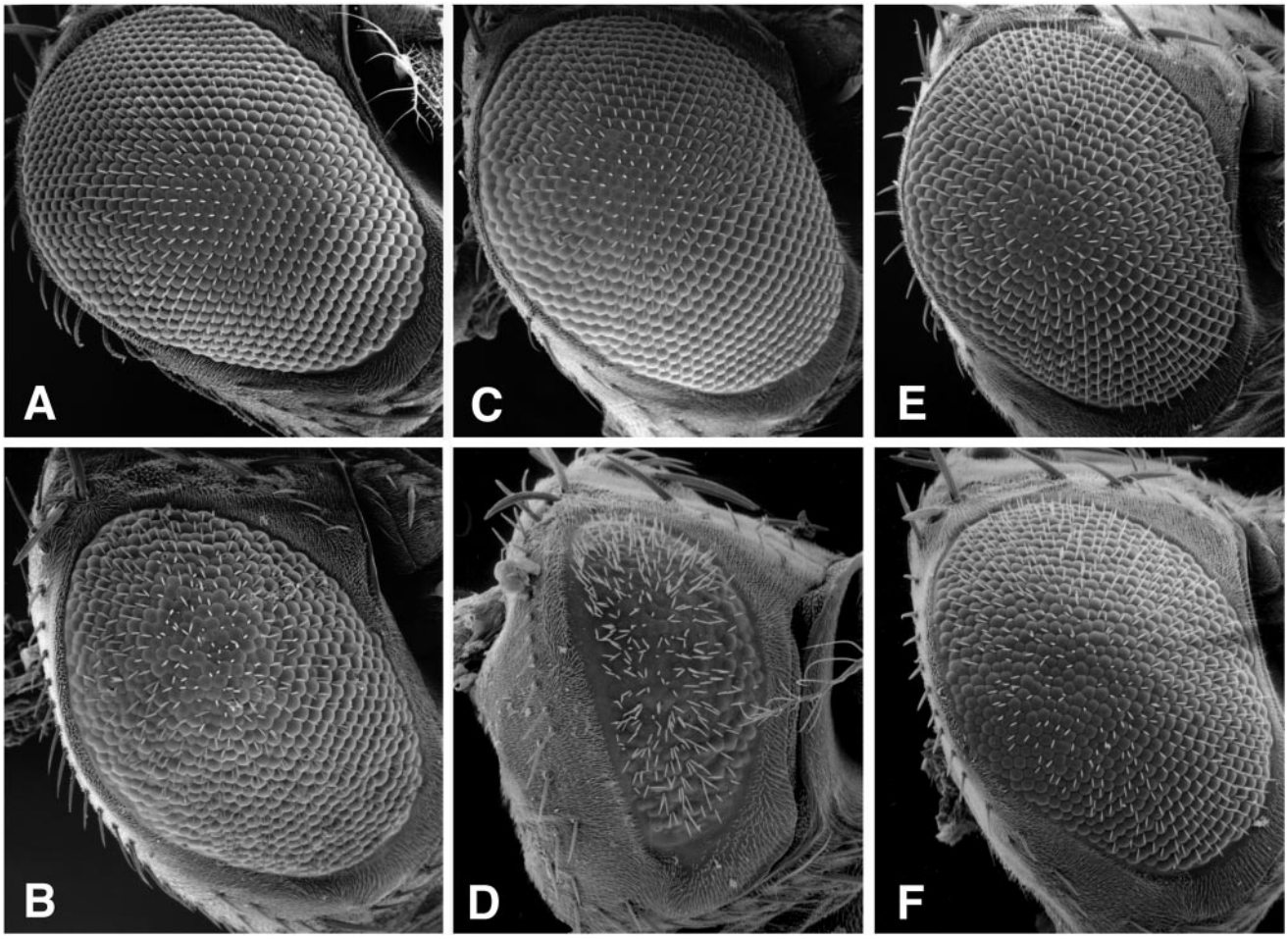

Figure 5. The dynein IC shows a dosage-sensitive interaction with the rough-eye phenotype of *Glued*^{1}. Scanning electron micrographs of *Drosophila* eyes. (A) Wild-type eye: +/+. (B) The dominant mutation *Glued*^{1}: *Gl*^{1}/+. (C) A dynein IC transgene suppresses the rough-eye phenotype of *Glued*^{1}: *P(Cdic*$^+$*)/+; Gl*^{1}/+. (D) A deficiency of the dynein IC locus dominantly enhances the *Glued*^{1} phenotype: *Df (1)mal3/+; Gl*^{1}/+. (E) A duplication of the *Cdic* locus suppresses the *Glued*^{1} rough eye: *Dp (1;Y)mal106/+; Gl*^{1}/+. (F) The dynein IC transgene reverses the genetic interaction between the deficiency and *Glued*^{1}: *Df (1)mal3/+; P(Cdic*$^+$*)/+; Gl*^{1}/+ (compare with D).

tidial packing and by alteration in eye size. Nine independent *P(Cdic*$^+$*)* transformant lines were tested against *Glued*^{1}, and all suppressed the rough-eye phenotype, although because of position effects the extent of suppression varies. Generally, for an individual line, suppression was stronger in males than in females. Given that the *Cdic19C* locus is X-linked, this finding suggests that some element of dosage compensation is retained in the transgene. Moreover, we observed the level of suppression to be dependent on the dosage of the *P(Cdic*$^+$*)* transgene. The *Glued*^{1} rough-eye phenotype was less severe in flies homozygous for the transgene than in flies heterozygous for the transgene or in flies hemizygous (i.e., containing only a single copy) for the endogenous *Cdic* gene (Figure 5).

To further investigate the functional significance of the dosage of the dynein IC gene on the behavior of the *Glued*^{1} mutation, we analyzed additional lesions that affect the *Cdic* gene. An X chromosome deficiency of region 19C [*Df (1)mal3*] was found to dominantly enhance the rough-eye phenotype of *Glued*^{1} mutant flies (Figure 5D). Conversely, a duplication that spans the 19C region [*Dp (1;Y)mal 106*] acts to suppress the *Glued*^{1} rough eye (Figure 5E). To show that the dynein IC gene was removed by the deficiency that interacts genetically with *Glued*^{1}, we used restriction fragment-length polymorphisms and DNA blot analysis to distinguish the X chromosomes (our unpublished data). Results of the restriction fragment-length polymorphism analysis show that the deficiency that enhances the *Glued*^{1} rough eye removes the dynein IC gene. Accordingly, the duplication for region 19C that suppresses *Glued*^{1} includes the dynein IC gene.

To demonstrate the specificity of the interaction between *Glued*^{1} and the *Cdic* gene, we show that the *Cdic19C* transgene, *P(Cdic*$^+$*)*, acts to reverse the severe rough-eye phenotype seen in flies carrying the *Glued*^{1} mutation and the *Cdic19C* deficiency. Females of the genotype *Df/+, P(Cdic*$^+$*)/+, Glued*^{1}/+ (Figure 5F) have a rough-eye phenotype approximately equivalent to that of the *Glued*^{1} mutation

alone (Figure 5B). This result shows that it is the reduction in dosage of the dynein IC gene and not some other gene under the deficiency that enhances the *Glued*^{1} rough eye. Together with the observations that an increased dosage of the *Cdic19C* gene acts to suppress the *Glued*^{1} eye phenotype, our results provide in vivo evidence that the dosage of the dynein IC gene can modulate the level of dynein function in the presence of the *Glued*^{1} gene product.

DISCUSSION

Our biochemical analyses of *Drosophila* cytoplasmic dynein show that the IC is a bona fide subunit in the dynein complex. The IC polypeptide cosediments in the 19S cytoplasmic dynein complex, associates with microtubules in an ATP-sensitive manner, and coimmunoprecipitates with the dynein heavy chain in cytoplasmic extracts. We show that at least three IC polypeptides can be distinguished by one-dimensional SDS-PAGE and that each is present in the 19S dynein complex. These multiple polypeptides are encoded by a single gene that is expressed throughout development. New functional evidence indicates that the interaction between dynein and dynactin is sensitive to the dosage of the dynein IC gene, *Cdic19C*. Our results extend previous in vitro biochemical studies and raise the possibility that dynein function may be regulated by the level of IC available for interaction with the p150-Glued subunit of dynactin or other adapter complexes.

As reported previously (Vaughan and Vallee, 1995; Nurminsky *et al.*, 1998a), the pairwise comparisons of cytoplasmic IC sequences reveal several conserved domains of potential functional significance. At the N terminus, the first 50 amino acids of the IC polypeptides are predicted to form a coiled-coil structure. A second region of homology (amino acids 70–95) contains a serine-rich region that forms a putative region for phosphorylation (Vaughan and Vallee, 1995; Nurminsky *et al.*, 1998a), and a third N-terminal region (amino acids 121–135; *Drosophila* sequence) contains a conserved motif of unknown function present only in cytoplasmic ICs (Nurminsky *et al.*, 1998a). Across the C-terminal half of the polypeptide, cytoplasmic IC sequences also display high sequence similarity to axonemal IC sequences. This similarity is proposed to reflect the presence of multiple WD repeats that are characteristic of all known dynein ICs (Ogawa *et al.*, 1995; Wilkerson *et al.*, 1995; Nurminsky *et al.*, 1998a; Yang and Sale, 1998). These observations are consistent with the model that the C-terminal domain in both axonemal and cytoplasmic ICs is important for interactions with the heavy chain subunit, whereas the divergent N-terminal region is important for isoform-specific interactions such as cargo binding (Paschal *et al.*, 1992).

One mechanism that contributes to the divergence of N termini in cytoplasmic dynein IC polypeptides is the alternative splicing of IC transcripts. As suggested previously, such splice variants could provide a diversity in IC isoforms that contributes to the targeting of the dynein motor to specific cargoes and functions (Vaughan and Vallee, 1995; Pfister *et al.*, 1996; Nurminsky *et al.*, 1998a). Indeed, Nurminsky *et al.* (1998a) report that alternative splicing of small 5′ exons in the single *Drosophila* IC gene accounts for at least 10 distinct transcripts. Our data do not directly address this source of heterogeneity in IC transcripts, but the relatively broad banding of the IC transcripts on RNA blots is consistent with the presence of additional splice variants. Our analysis of *Cdic* cDNAs does provide evidence for at least two differentially expressed transcripts. The short, maternally expressed 2.4-kb transcript differs from the 2.8-kb ubiquitously expressed transcript only in the length of the 3′ UTR. Elements of 3′ UTRs are commonly involved in regulatory mechanisms used in early development (Seydoux, 1996; Wickens *et al.*, 1997). How such 3′ UTRs contribute to the regulation of mRNA translation and/or the stability of dynein subunits and how these mechanisms might affect dynein function during development have not been investigated for any dynein subunit. Although the diversity in *Cdic* transcripts is intriguing as a regulatory mechanism for the functional specialization of cytoplasmic dynein, the physiological significance of these variations remains to be demonstrated.

Our genetic analysis of the dynein IC gene provides previously lacking evidence that the association between the dynein IC and p150-Glued polypeptides is functionally significant in *Drosophila*. Purified IC and p150-Glued have been shown to interact physically in rat and *Xenopus* extracts (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995; Steffen *et al.*, 1997) and when overexpressed in *Dictyostelium* (Ma *et al.*, 1999). However, similar associations between the yeast homologues of IC and p150-Glued were not observed (Kahana *et al.*, 1998). This and previous data showing that the dynein and dynactin complexes are separable by ion exchange chromatography (Schroer and Sheetz, 1991) suggest a low-affinity or regulated interaction between the dynein and dynactin complexes.

The transgenic expression of the *Cdic19C* transcription unit has shown that the interaction between IC and p150-Glued is sensitive to the dosage of the IC gene. Additional copies of the IC gene can suppress the dominant rough eye of *Glued*^{1}, and removal of the *Cdic19C* locus by a deletion can enhance the *Glued*^{1} rough-eye phenotype. Previous molecular analysis of *Glued*^{1} has shown that the mutation results from the insertion of a transposon in the 3′ end of the *Glued* gene that creates a premature translation stop codon and the production of a truncated Glued polypeptide (Swaroop *et al.*, 1985, 1987; McGrail *et al.*, 1995). The truncated Glued polypeptide is unable to incorporate into a 20S complex like the wild-type dynactin complex (McGrail *et al.*, 1995), but it retains the region that interacts with the dynein IC (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995; Ma *et al.*, 1999). The dosage-sensitive interaction between the dynein IC and *Glued*^{1} suggests that the dominant negative effects of *Glued*^{1} are mediated by titrating the level of dynein IC below a threshold required for normal eye development. The association of the dynein IC with a truncated p150-Glued polypeptide would have the effect of "uncoupling" dynein from its cargo. It remains possible that p150-Glued or dynactin may act in one or more pathways unrelated to dynein function. In this case, the truncated Glued polypeptide could "poison" another process, and the associated rough-eye phenotype might not reflect dynein dysfunction. This explanation seems unlikely for two reasons. First, recent analysis of IC truncations in *Dictyostelium* have shown that similar defects occur when the truncated IC is unable to associate with either the dynein heavy chain or the dynactin complex, suggesting that the association of dynein with dynactin is

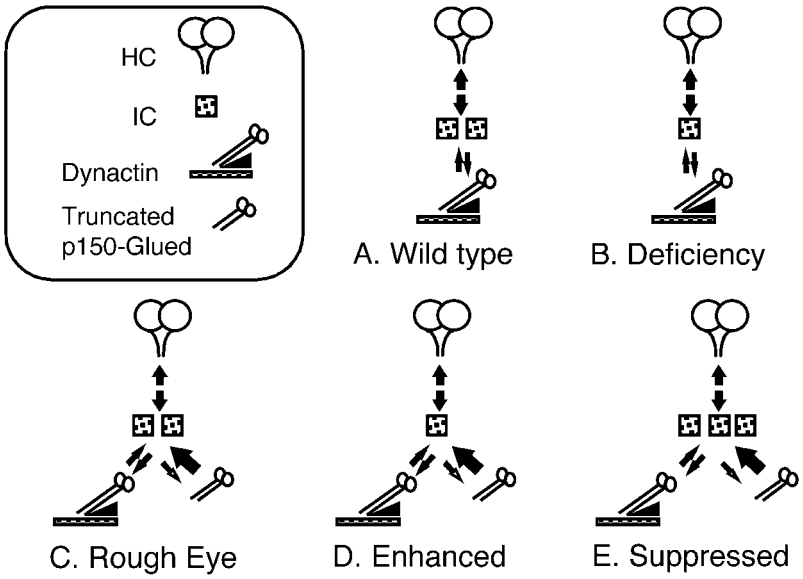

Figure 6. Model for the in vivo interaction between the dynein IC and *Glued*[1]. A cartoon showing a model for the dosage-sensitive effect of the dynein IC on the rough-eye phenotype of *Glued*[1]. (A) In the wild type, the dynein IC associates with the heavy chain subunit and mediates the interaction with the p150-Glued subunit of dynactin. (B) In the presence of a deficiency that removes one copy of the dynein IC gene, the level of dynein IC is reduced, but it is still present at a level sufficient to support the interaction with dynactin, and no phenotype is detected. (C) In the case of *Glued*[1], association of the truncated p150-Glued subunit with the dynein IC is favored. The truncated Glued polypeptide binds to the dynein IC but is unable to assemble into the dynactin complex or associate with cargo. Because of a limited pool of the IC subunit, the level of dynein capable of transporting cargo is reduced below a threshold, resulting in a rough-eye phenotype. (D) In the presence of the *Glued* mutation, when the level of dynein IC is reduced by a deficiency, the level of dynein-mediated transport is further reduced, and the rough-eye phenotype is enhanced. (E) With the addition of a dynein IC transgene, the level of dynein able to associate with cargo is increased, and the rough-eye phenotype is suppressed.

required for multiple cellular functions (Ma *et al.*, 1999). Additionally, in previous work we have shown that specific alleles of the dynein heavy chain can act to enhance or suppress the *Glued*[1] rough-eye phenotype (McGrail *et al.*, 1995). Although the molecular nature of the lesions in the heavy chain mutants is not established, it is interesting to speculate that the *Dhc* alleles that act as dominant modifiers of the *Glued*[1] phenotype may do so by altering the association between the dynein heavy chain and IC polypeptides. This prediction is also consistent with the observation that, in contrast to the activity of the *Cdic19C* transgene, additional copies of a wild-type dynein heavy chain transgene do not suppress the *Glued*[1] phenotype.

The demonstration of a dosage-sensitive interaction between *Cdic* and *Glued* emphasizes the possibility that this interaction is important for regulating dynein function. The *Cdic19C* locus is not haplo-insufficient, because flies with only a single wild-type copy of the gene exhibit no phenotype. Yet in the presence of two copies of the wild-type *Cdic* gene, the *Glued*[1] mutant yields a rough-eye phenotype. To account for these observations, we infer that the interaction between the IC and the truncated p150-Glued polypeptide in some way prevents or diminishes the interaction between IC and wild-type p150-Glued. This could occur through the enhanced binding and sequestration of IC by the mutant p150-Glued polypeptide (Figure 6). Consequently, the level of IC subunit available for association with wild-type dynactin complex, or for assembly into the dynein motor complex, would be reduced and dynein function diminished. The increased affinity of a mutant protein for its substrate has been described for the actin-binding protein Sac6p (Sandrock *et al.*, 1997).

In the case of the truncated Glued polypeptide, an enhanced binding to IC could reflect the loss of a regulatory site that modulates the association of the IC and Glued polypeptides. For example, in vertebrates, both the IC subunit and p150-Glued are phosphorylated (Dillman and Pfister, 1994; Pfister *et al.*, 1996; Huang *et al.*, 1999). In our analysis of *Drosophila* cytoplasmic IC, we observe a triplet of IC polypeptides of ~74 kDa in ovary and embryo extracts that is consistent with the presence of phosphorylated variants of the cytoplasmic IC. Phosphorylation of the N-terminal serine residues in the p150-Glued polypeptide has been proposed to regulate the interaction of p150-Glued with microtubules or the dynein IC (Waterman-Storer *et al.*, 1995; Farshori and Holzbaur, 1997). In *Drosophila*, we previously observed the presence of multiple p150-Glued polypeptides produced from a single transcript, suggesting posttranslational modification of the wild-type p150-Glued gene product. In contrast, the mutant *Glued*[1] gene appears to produce a single truncated polypeptide (McGrail *et al.*, 1995). Although the serine-rich cluster is retained within the truncated Glued polypeptide, an altered conformation of the mutant protein could modify the phosphorylation of these sites. Alternatively, C-terminal phosphorylation sites deleted from the truncated Glued polypeptide may be important for the regulation of interactions between the IC and Glued polypeptides. A better understanding of the nature of this interaction may provide insight into whether dynactin represents an adapter complex that couples dynein to specific functions or serves a more universal function in the regulation of dynein-based motility (King and Schroer, 2000).

The presence of multiple IC isoforms in human, mouse, rat, and *Drosophila* (Vaughan and Vallee, 1995; Pfister *et al.*, 1996; Nurminsky *et al.*, 1998a; Crackower *et al.* 1999) suggests that the IC may play a role in functional specification of the dynein complex; however, the association of different IC isoforms with distinct binding partners has not yet been demonstrated. With regard to the observed interaction between IC and p150-Glued, Vaughan and Vallee (1995) have provided preliminary evidence that the IC variants, IC-1A and IC-2B, can both bind the p150-Glued subunit of dynactin in vitro. At least in this one case, the domain involved in splicing appears to be separable from the binding of IC to dynactin. The dosage-

sensitive interaction between the dynein IC and the p150-Glued subunit of dynactin suggests that the IC subunit may be a limiting component in the coupling of dynein to adapters and cellular cargoes. The observation that the dominant *Glued*[1] phenotype is restricted to the eye indicates that IC levels are spatially and temporally regulated during development. For example, the IC polypeptide may be limiting during eye development such that the truncated Glued polypeptide significantly disrupts dynein function. In contrast, an excess pool of "free" IC may be maternally loaded into embryos and competitively block the effect of the Glued "poison" product in early development. Further genetic and biochemical analyses of the dynein IC may identify additional gene products that regulate the availability of the dynein IC for interaction with such adapters and so regulate dynein function. The genetic and molecular reagents developed in this study of the *Drosophila* dynein IC subunit will facilitate the investigation of such regulatory mechanisms in a developmental context.

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