The Gene for the Intermediate Chain Subunit of Cytoplasmic Dynein Is Essential in Drosophila

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ABSTRACT

The microtubule motor cytoplasmic dynein powers a variety of intracellular transport events that are essential for cellular and developmental processes. A current hypothesis is that the accessory subunits of the dynein complex are important for the specialization of cytoplasmic dynein function. In a genetic approach to understanding the range of dynein functions and the contribution of the different subunits to dynein motor function and regulation, we have identified mutations in the gene for the cytoplasmic dynein intermediate chain, *Dic19C*. We used a functional *Dic* transgene in a genetic screen to recover *X*-linked lethal mutations that require this transgene for viability. Three *Dic* mutations were identified and characterized. All three *Dic* alleles result in larval lethality, demonstrating that the intermediate chain serves an essential function in Drosophila. Like a deficiency that removes *Dic19C*, the *Dic* mutations dominantly enhance the rough eye phenotype of *Glued*[1], a dominant mutation in the gene for the p150 subunit of the dynactin complex, a dynein activator. Additionally, we used complementation analysis to identify an existing mutation, *shortwing* (*sw*), as an allele of the dynein intermediate chain gene. Unlike the *Dic* alleles isolated *de novo*, *shortwing* is homozygous viable and exhibits recessive and temperature-sensitive defects in eye and wing development. These phenotypes are rescued by the wild-type *Dic* transgene, indicating that *shortwing* is a viable allele of the dynein intermediate chain gene and revealing a novel role for dynein function during wing development.

CYTOPLASMIC dynein is a minus-end-directed microtubule motor involved in numerous intracellular motility events including retrograde axonal transport, the transport and positioning of vesicles and organelles, spindle assembly and morphogenesis, and nuclear migration. The dynein motor is a large complex composed of two heavy chain polypeptides and numerous intermediate and light chain subunits. The heavy chains compose the ATPase portion of the molecule, providing energy for movement along microtubules through the binding and hydrolysis of ATP (reviewed by Holzbaur and Vallee 1994). Electron microscopy analysis has shown that the heavy chains form two globular heads connected by thin stalks. The intermediate and light chain subunits are present as a complex at the base of the heavy chain stalk where they are in a position to interact with other cellular components and may participate in targeting the motor to specific cargoes (Vallee *et al.* 1988; King and Witman 1990; Steffen *et al.* 1996).

A role for the intermediate chain (IC) subunit in the attachment of dynein to cargo was first suggested by structural analysis of axonemal outer arm dynein. In the flagellar axoneme, dynein motor activity drives the sliding of adjacent outer doublet microtubules. As the heavy chain motor subunit moves along one outer doublet, the base of the motor complex remains attached to the adjacent outer doublet. Thus the transported cargo for axonemal dynein is another doublet microtubule attached through the base of the motor complex. Chemical crosslinking studies show that attachment through the base is mediated by direct binding of the intermediate chain subunit and α-tubulin within the A-tubule lattice of the outer doublet microtubule (King *et al.* 1991).

The homology between axonemal and cytoplasmic dynein intermediate chains has suggested a similar cargo-binding function for the IC subunit of cytoplasmic dynein (Paschal *et al.* 1992). Subsequently, *in vitro* binding of the cytoplasmic dynein intermediate chain to the p150-Glued subunit of dynactin was demonstrated in rat brain extracts (Karki and Holzbaur 1995; Vaughan and Vallee 1995). Dynactin, initially identified because of its ability to stimulate dynein-mediated vesicle motility (Schroer and Sheetz 1991), may act to couple dynein to cellular cargoes (reviewed by Karki and Holzbaur 1999). The interaction between the dynein intermediate chain and p150-Glued and the association of the Arp1 subunit of dynactin with the membrane skeleton component spectrin (Holleran *et al.* 1996) have suggested a model in which dynactin serves as a cargo adapter molecule for dynein attachment to vesicular cargo. Additional studies showing that dynactin function is re-

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quired during mitosis present the possibility that dynactin may also serve as a cargo adapter for dynein during cell division (Echeverri *et al.* 1996). Alternatively, the interaction of dynein IC and p150-Glued may affect motor processivity (King and Schroer 2000).

The diversity of cytoplasmic dynein heavy chains is limited, but the multiplicity of accessory subunits is proposed to modulate specific dynein functions. Evidence for the assembly of functionally different dynein complexes has been demonstrated for the dynein light intermediate chain (LIC) and light chain subunits (Tai *et al.* 1998, 2001; Tynan *et al.* 2000; Chuang *et al.* 2001). Two LIC genes have been identified in rat: one that binds to pericentrin and one that does not. In triple overexpression studies, Tynan *et al.* (2000) showed that the dynein heavy chain could bind to either LIC1 or LIC2, but not to both. Multiple alternatively spliced isoforms of the dynein intermediate chain have been identified (Vaughan and Vallee 1995; Pfister *et al.* 1996; Nurminsky *et al.* 1998) and it has been suggested that this isoform diversity contributes to functional specificity, perhaps by the formation of distinct dynein complexes with specific intermediate chain isoforms. Recent reports have also implicated dynein light chain subunits in binding directly to specific cargoes, suggesting that the dynein intermediate chain may act indirectly to modulate cargo attachment by association with specific light chain subunits. For example, the 14-kD light chain was found to bind rhodopsin in the rod cells of the vertebrate retina and may function in turnover of photoreceptor membrane (Tai *et al.* 1999). Despite these leads, the functional analysis of how accessory subunits might contribute to specifying dynein functions is limited.

As part of a systematic approach to understanding the functions of the intermediate chain subunit in the attachment of dynein to specific cargoes, we have previously cloned and characterized the gene *Dic19C* from Drosophila (Boylan *et al.* 2000). We showed that, similar to the dynein heavy chain, the dynein intermediate chain is present as a single gene that is expressed throughout Drosophila development. In addition, we found evidence for a dosage-sensitive interaction between the intermediate chain gene and a mutation in the p150/Glued subunit of dynactin. In this report, we use the *Dic* transgene in a screen to identify mutations in the dynein intermediate chain gene and investigate functions of the dynein intermediate chain during Drosophila development through analysis of the mutant phenotypes, both alone and in combination with the mutant *Glued*^{1}.

MATERIALS AND METHODS

Fly stocks: Transformation and mutagenesis experiments were performed using the stock *Df (1)w*^{67c23}, which carries the markers *yellow* (*y*) body and *white* (*w*) eyes (Lefevre and Green 1972). The construction and characterization of the *Dic* transgene *P(Dic*$^{+}$*)* is described in Boylan *et al.* (2000). Briefly, the complete dynein intermediate chain transcription unit with the endogenous promoter was assembled from genomic cosmids and subcloned into the transformation vector *pCaSpeR4* (Klemenz *et al.* 1987). Germline transformants were obtained, and the *Dic* transgene was determined to be functional by its ability to suppress the rough eye phenotype of the dominant mutation *Glued*^{1} (Boylan *et al.* 2000). The *sw*^{1} stock was obtained from the Bloomington Stock Center, as was a stock with the *X*-linked deficiency *Df(1)mal3* (breakpoints 19A1-2; 20E1-F). *Df(1)mal3* is maintained in males with the duplication *Dp (1;Y)mal106* (breakpoints 1A1; B2 and 18F; 20F4). To facilitate identification of the transgene in rescue experiments, a *y w sw* chromosome was generated by meiotic recombination; *y w sw* males were identified on the basis of the *shortwing* eye and wing phenotype and were tested for lethality over the deficiency *Df(1)mal3* or over the strong *Dic* allele *Dic*^{1}. The *Glued*^{1} stock was provided by Douglas Kankel (Yale University) and is described in Harte and Kankel (1982). Mutant alleles of the dynein heavy chain gene *Dhc64C* have been described previously (Gepner *et al.* 1996). Chromosomes and markers are described in Lindsley and Zimm (1992). All flies were raised on standard yeast-cornmeal-agar medium at 25°, unless stated otherwise.

Mutagenesis: *y w* males were mutagenized with EMS as previously described (Lewis and Bacher 1968). Males were starved for 1 hr and then fed 25 mm EMS in 1% sucrose overnight. Mutagenized males were mass mated to *C(1)DX* females, with attached-*X* chromosomes, homozygous for a second chromosome insertion of the *Dic* transgene *P(Dic*$^{+}$*)*. F_1 males, carrying a mutagenized *X* chromosome and heterozygous for the *Dic* transgene, were individually mated to attached-*X* virgin females without the *Dic* transgene. Pair matings were done in test tubes using yeast-sucrose food (Simmons *et al.* 1980) at 28°. Because initial screening showed that a high percentage of the F_1 males were infertile, some of the pair matings (1230 of 7748) were reared at 25° to determine if the fertility of the F_1 males could be improved by a lower rearing temperature. In the F_2 generation, segregation of the *Dic* transgene was followed using the *mini-w+* eye color marker. F_2 populations were screened for the absence of white-eyed males, which would indicate that the *Dic* transgene was required for viability. Mutant stocks were maintained as males in the presence of the *Dic* transgene or in females over the *X* chromosome balancer *FM7*.

Complementation tests and genetic analyses: To test for complementation between *shortwing* and the *Dic19C* alleles, virgin females of the genotype *y w Dic*$^{-}$*/FM7* were crossed to *sw/Y* males and examined for the presence of *y w Dic*$^{-}$*/sw* progeny. Crosses were done at 18°, 22°, 25°, and 28° for each *Dic19C* allele. To show that the phenotypes associated with the *Dic*$^{-}$*/sw* progeny could be rescued by the wild-type *Dic* transgene, *y w sw* males heterozygous for a second chromosome insertion of the *Dic* transgene (genotype *y w sw/Y; P(Dic*$^{+}$*)/+*) were crossed to *Dic*$^{-}$/FM7 virgin females. The *y w Dic*$^{-}$*/sw; P(Dic*$^{+}$*)/+* progeny were compared to *y w Dic*$^{-}$*/sw* progeny for number of adults as well as for the presence of eye and wing phenotypes. Again, crosses were done at 18°, 22°, 25°, and 28°. The rescue of the *sw* mutant phenotype by the *Dic* transgene was also tested by separately crossing *sw/Y* males to attached-*X* females without the *Dic* transgene and to attached-*X* females homozygous for a second chromosome insertion of the *Dic* transgene. For the comparison of *Dic*^{1}*/sw* to *Df/sw*, *Dic*^{1}*/FM7* females were crossed to *sw/Y* males at 22° and 18°, and *sw/FM7* females were crossed to *Df(1)mal3/Y Dp(1:Y)mal106* males at 22° and 18°.

Lethal phase analysis: To estimate the lethal stage of the

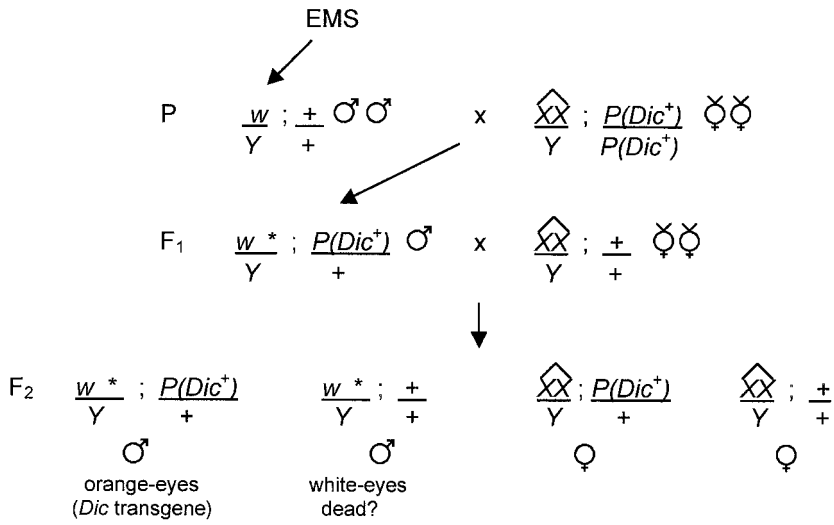

FIGURE 1.—Strategy for the identification of mutations in the cytoplasmic dynein intermediate chain gene. The screen strategy relies on an autosomal insertion of a dynein intermediate chain transgene *P(Dic$^+$)*, marked with the *mini-w$^+$* eye color gene, to rescue lethal mutations in the dynein intermediate chain gene. White-eyed males are mutagenized and mated to attached-*X* virgin females carrying a homozygous copy of the *P(Dic$^+$)* transgene inserted on chromosome *2*. The attached-*X* chromosome present in the parental females causes patroclinous inheritance of the mutagenized *X* chromosome (denoted by an asterisk) from fathers to sons. In the F_1 generation, males containing a mutagenized *X* chromosome and heterozygous for the *P(Dic$^+$)* transgene are individually mated to attached-*X* virgin females without the transgene. In the F_2 generation, segregation of the transgene allows populations to be scored for the absence of white-eyed males, indicating a mutation in the dynein intermediate chain gene.

Dic mutants, balanced virgin females for each *Dic* allele were crossed to wild-type (Oregon-R) males. From this cross, approximately one-quarter of the progeny would be expected to die due to the presence of the *Dic* mutation. After several days of mating, 2- to 4-hr egg collections were made on grape juice agar plates. Embryos were counted and transferred to fresh plates. After 36 hr, unhatched eggs were counted, and larvae were counted and placed on fresh food in culture vials. Subsequently the numbers of pupae and adult flies from each cross were counted. The total lethality was determined as a percentage of the collected embryos that died before reaching adulthood {$\%L = [(\text{no. of eggs} - \text{no. of adults})/\text{no. of eggs}] \times 100$}. The lethality for each stage of development was determined as a percentage of the total lethality {*e.g.*, % embryonic lethality = $[(\text{no. of eggs} - \text{no. of larvae})/(\text{no. of eggs} - \text{no. of adults})] \times 100$}.

Phenotypic analysis: Wild-type and mutant larvae were dissected in phosphate-buffered saline (PBS) as described in GINDHARDT *et al.* (1998). Filleted larvae were fixed in 4% formaldehyde in PBS + 0.1% Triton X-100 (PBT) for 30 min at room temperature and washed with several changes of PBT followed by blocking in antibody incubation buffer (PBT + 2% bovine serum albumin) for 1 hr at room temperature. All primary antibody incubations were performed overnight at 4°, and secondary antibody incubations were performed for 2 hr at room temperature. Primary antibodies used were mouse monoclonal anti-cysteine string protein, 1:100 (ZINSMAIER *et al.* 1994), and affinity-purified rabbit polyclonal anti-Drosophila kinesin heavy chain, 1:2000 (Cytoskeleton, Denver). Samples were washed for 1–2 hr in three changes of PBT after antibody incubations. The final wash was in 80% glycerol in PBT. Stained larvae were mounted in PermaFluor mounting medium (ThermoShandon, Pittsburgh).

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

Wings were dissected from adult flies, mounted in methylsalicylate and Canada balsam, and examined by brightfield microscopy with a Nikon Eclipse E800 microscope equipped with a ×4 objective. Digital images were collected using a CoolCam liquid-cooled three color CCD camera (Cool Camera Company, Decatur, GA) and Image Pro Plus software (Media Cybernetics, Silver Springs, MD).

RESULTS

Recovery of *Dic* mutations: We have previously shown that the cytoplasmic dynein heavy chain subunit is expressed throughout development (LI *et al.* 1994) and that mutations in the unique *Dhc64C* gene that encodes this subunit result in larval lethality (GEPNER *et al.* 1996). Similarly, the dynein intermediate chain is the product of a single gene, *Dic19C*, which is expressed throughout development (BOYLAN *et al.* 2000). We reasoned that mutations in this gene would also be lethal. Thus we used a modified F_2 screen to recover mutations in flies that were rescued by a wild-type *Dic* transgene. As shown in Figure 1, we screened for F_2 populations in which the *Dic* transgene was required for viability at 28°. The progeny of 3006 fertile F_1 males were examined, and three lethal mutations were identified. To retest these mutations, males carrying the mutant *X* chromosome and a single copy of the *Dic* transgene were crossed to attached-*X* females without a *Dic* transgene. Analysis of the resultant progeny showed that for each of the three mutations, the *Dic* transgene was required for viability at 28° (Table 1). The mutations were all tested for viability at lower temperatures (18°, 22°, and 25°) and were found to be inviable at all temperatures (data not shown). Consequently, none of the mutations identified are temperature sensitive.

We previously identified the cytological location of the dynein intermediate chain gene as polytene region 19C (BOYLAN *et al.* 2000). As expected, all mutants identified in the screen were found to be lethal over *Df(1)mal3*,

TABLE 1

Transgene rescue of *X*-linked lethal mutations

Dic allele	F_2 progeny classes: *Dic*^{m}/*Y*; +/+ ♂	*Dic*^{m}/*Y*; *P(Dic*$^+$*)*/+ ♂	*XX/Y*; +/+ and *XX/Y*; *P(Dic*$^+$*)*/+ ♀[a]
Dic^1	0	44	111
Dic^2	2[b]	111	117
Dic^3	0	41	104

X-linked lethal mutations (*Dic*^{m}) were tested for rescue by the *Dic* transgene *P(Dic*$^+$*)*.

[a] Female classes with and without the *Dic* transgene were counted together. The presence or absence of the transgene cannot be scored in the attached-*X* females due to their wild-type eye color.

[b] These were determined to be *P(Dic*$^+$*)* flies that had lost the *mini-w*$^+$ marker.

TABLE 2

***Dic* mutations fail to complement a deficiency that removes polytene region 19C**

Dic allele	Progeny classes (total no. of adults)[a]: *Dic*^{m}/*Df* ♀	*Dic*^{m}/*YDp* ♂	*Dic*^{m}/*FM7* ♀	*FM7/YDp* ♂
Dic^1	0	76	92	77
Dic^2	0	106	108	98
Dic^3	0	125	154	133
sw^b	0	119	NA	NA

[a] Balanced *Dic* alleles (*Dic*^{m}) were mated to *Df(1)mal3/Dp(1:Y)mal106* males.

[b] Homozygous *shortwing* (*sw*) virgin females were mated to *Df(1)mal3/Dp(1:Y)mal106* males. NA, not applicable.

a deficiency of polytene region 19 that removes the intermediate chain gene (Table 2). To determine whether any interallelic complementation occurs between the *Dic* mutations, *inter se* crosses were performed. All three heteroallelic combinations of *Dic* alleles failed to complement for adult viability.

***Dic* mutants exhibit larval lethality:** To establish the time of development at which zygotic expression of the dynein intermediate chain is required, we examined the stage at which flies hemizygous for mutant *Dic* died. For each *Dic* mutant allele, females with one mutant gene and one wild-type gene were crossed to wild-type males. From this cross, approximately one-quarter of the progeny would be expected to die due to the presence of the *Dic* mutation. The stage of lethality was determined by counting the numbers of larvae, pupae, and adults resulting from the embryos collected for each cross (Figure 2). For all three *Dic* alleles, the mutations resulted in lethality predominantly at the larval stage. The weakest *Dic* allele recovered in the screen (*Dic*^{2}) lives to the third instar larval stage. These larvae exhibit a crawling defect that results in complete paralysis with the heads of the larvae poking up out of the food (Figure 2). Previous work has shown that mutations affecting either anterograde or retrograde axonal transport display abnormal larval crawling behavior and paralysis and the accumulation of vesicles and organelles within the axon (Hurd and Saxton 1996; Gindhardt *et al.* 1998; Bowman *et al.* 1999; Martin *et al.* 1999). To determine if the larval paralysis phenotype of the *Dic*^{2} mutation resulted from a defect in axonal transport, we looked for the presence of axonal "cargo jams" in *Dic*^{2} mutant larvae using antibodies to the anterograde motor, kinesin, and a synaptic vesicle component, cysteine string protein (Zinsmaier *et al.* 1994). Similar to mutations in the kinesin heavy and light chains, as well as the dynein heavy chain, we observed accumulations of cargoes in the segmental nerves of *Dic*^{2} mutant larvae that were recognized by both antibodies (data not shown).

***shortwing* is allelic to *Dic19C*:** In addition to the *de novo* isolation of mutations in the dynein intermediate chain gene, we examined the database for existing mutations in the region of the *Dic* gene that might represent additional *Dic* alleles. One candidate, the recessive mutant *shortwing* (*sw*), was mapped by linkage analysis to the *X* chromosome between positions 63.5 and 64 (Eker 1935). Further analysis of deficiencies in the region positioned the *sw* locus at polytene segment 19B (Lefevre 1981; Paradi *et al.* 1983). We previously showed that the *Dic* gene is also located near this region (Boylan *et al.* 2000). The *sw* mutant was originally identified in 1932, arising spontaneously in a stock of the second chromosome mutant *short-bristle* (Eker 1935). The *sw* phenotype was described as pleiotropic, with defects in eye and wing development at 25°. *sw* mutants have eyes that are variably smaller than those of wild type, elliptically shaped, and rough. The wing phenotype is also variable. The wings may have incisions of the medial, lateral, and posterior margins and may be reduced in size. Additionally, portions of the longitudinal or cross veins may be missing, veins may be bifurcated, or additional vein material may be present. Wings may also be outheld from the body. Previous work has shown that mutations in the dynein heavy chain gene, *Dhc64C*, also result in a rough eye phenotype (McGrail *et al.* 1995; Gepner *et al.* 1996). Taken together, the similar location of the *Dic* and *sw* mutations and the eye phenotype of other known components of the dynein complex suggested that *sw* might be allelic to *Dic19C*. To verify the chromosomal location of the *shortwing* mutation, *sw* virgin females were crossed to males carrying a deficiency that removes the dynein intermediate chain gene. No *sw/Df* progeny were recovered at 25° (Table 2) or 28° (data not shown). Some *sw/Df* flies were viable at 22° (9/167), and at 18° *sw/Df* females represent 39% of the progeny ($n = 107$). These results indicate that, although the *sw* mutation is homozygous viable, *sw* is

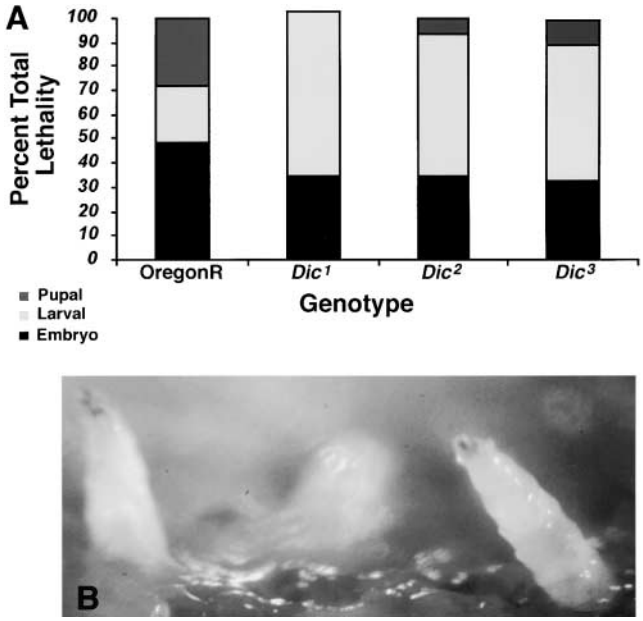

FIGURE 2.—The *Dic* mutants exhibit larval lethality. (A) Histogram summarizing results of the lethal phase analysis for *Dic* mutants. The percentage of the total lethality is plotted for each stage. Oregon-R ($n = 576$), Dic^1 ($n = 357$), Dic^2 ($n = 328$), and Dic^3 ($n = 390$). (B) Dic^2 mutant larvae live to the third instar stage, but display a larval crawling phenotype and become paralyzed with their heads poking out of the food.

TABLE 3

Complementation tests

Dic allele	Temp.	Progeny classes (total no. of adults)[a] Dic^m/sw ♀	Dic^m/Y ♂	*sw/FM7* ♀	*FM7/Y* ♂
Dic^1	28°	0	0	24	9
	25°	0	0	72	53
	22°	14	0	91	46
	18°	36	0	55	24
Dic^2	28°	55	0	52	42
	25°	74	0	91	61
	22°	69	0	59	66
	18°	61	0	61	31
Dic^3	28°	2	0	63	71
	25°	12	0	63	62
	22°	33	0	87	56
	18°	56	0	58	37

[a] Lethal *Dic* alleles (Dic^m) were tested for complementation with the viable allele *shortwing* (*sw*).

lethal over a deficiency that removes the dynein intermediate chain gene and the lethality is temperature sensitive.

To determine whether the nonlethal mutant *shortwing* is allelic to the lethal *Dic19C* mutations, we first conducted a complementation analysis. For each *Dic19C* allele, balanced females were crossed to *sw/Y* males at 18°, 22°, 25°, and 28°. As shown in Table 3, *sw* fails to complement the Dic^1 allele for viability at 25° and 28°. At 22° and 18° some *sw*/Dic^1 flies are viable; however, viability is reduced compared to the sibling class. *sw* in combination with Dic^3 also shows reduced viability at 22°, 25°, and 28°. In addition to reduced viability, *sw* flies in combination with these two *Dic* alleles display abnormal wing and eye development, similar to the phenotype of *sw* mutant males (Figures 3 and 4). The weakest of the *Dic19C* lethal alleles, Dic^2, complements *sw* for viability at all temperatures; however, at 28° the *sw*/Dic^2 flies have a visible wing and eye phenotype. These results show that *sw* fails to complement mutations in *Dic19C* and suggests that the *sw* mutation is an allele of the dynein intermediate chain gene. Moreover, the failure of these mutants to complement is temperature sensitive and varies with the strength of the lethal *Dic* allele.

To demonstrate rescue of the *sw* eye and wing phenotypes by the *Dic* transgene, *sw* mutants were tested using a modification of the original screen for *Dic* mutations. *sw/Y* males were separately crossed either to attached-*X* females homozygous for a second chromosome insertion of the *Dic* transgene or to attached-*X* females without the *Dic* transgene, at 25° and 28°. In the absence of the *Dic* transgene at 28°, many of the *sw* males had a visible *sw* phenotype; however, none of the flies displayed a wing or eye phenotype in the presence of the *Dic* transgene (Figure 4), showing that the *sw* phenotype is rescued by the *Dic* transgene. Furthermore, although *sw/Y* males are viable at both 25° and 28°, they appear to be present in reduced numbers in the absence of the *Dic* transgene [43% of the progeny ($n = 239$) compared to 55% in the presence of the *Dic* transgene ($n = 285$)], suggesting that in addition to the eye and wing phenotype, the *sw* mutation also affects viability.

Similarly, the lethal and visible phenotypes associated with the *Dic* alleles in *trans*-heterozygous combination with *sw* are also rescued by the *Dic* transgene. To demonstrate the transgene rescue of the lethal *Dic* alleles over *sw*, the complementation tests were repeated using *y w sw* males heterozygous for a second chromosome insertion of the *Dic* transgene (genotype *y w sw/Y; P(*Dic^+*)/+*). The results of these crosses are shown in Table 4. For all *Dic* alleles in *trans*-heterozygous combination with *sw*, the lethal and visible phenotypes are rescued by the *Dic* transgene. The identification of sw as an allele of the dynein intermediate chain is also supported by the observation that the levels of the intermediate chain polypeptide appear reduced in the mutant background (data not shown). However, neither the analysis of genomic DNA nor immunoblots have suggested that the *Dic* mutants produce aberrant truncated gene products.

***Dic* mutations interact with a mutation in dynactin:** As a simple test for interactions between the *Dic* mutations and other components of dynein or dynactin, the *Dic* mutations were crossed to recessive lethal alleles of

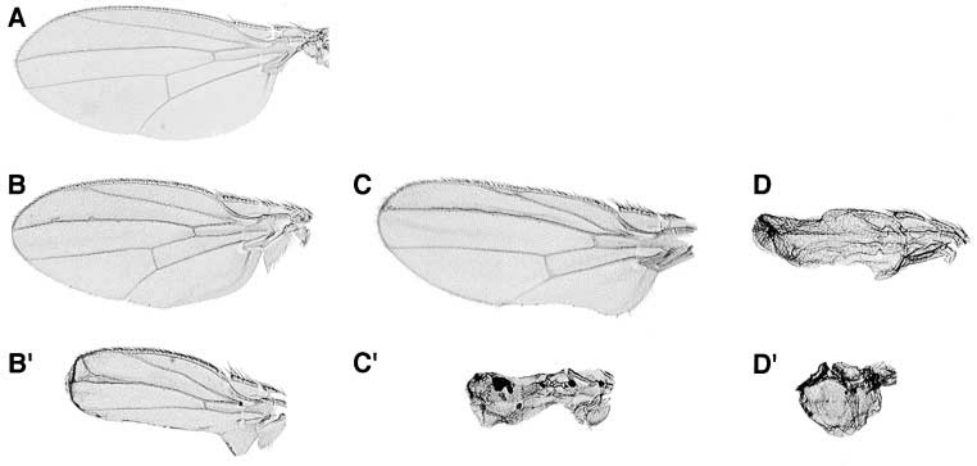

Figure 3.—The lethal *Dic* alleles fail to complement the *sw* wing phenotype. Examples of the variable wing phenotype of the lethal *Dic* alleles in combination with the viable allele, *shortwing* (*sw*). Shown are mild and severe wing defects for each allelic combination. (A) The wing phenotype is rescued by a copy of the wild-type *Dic* transgene. (B and B′) Dic^2/sw displays a mild wing phenotype at 28°. The Dic^3/sw (C and C′) and Dic^1/sw (D and D′) flies have a more severe wing phenotype and have reduced viability compared to Dic^2/sw.

the dynein heavy chain gene, *Dhc64C*, and mutations in the gene for the p150-Glued subunit of dynactin (*Glued* gene). No dominant interactions were observed between any of the lethal *Dic* alleles and recessive alleles of the dynein heavy chain gene or recessive alleles of the *Glued* locus. We have previously shown that the dominant mutation $Glued^1$ exhibits a dosage-sensitive interaction with the dynein intermediate chain gene. A deficiency that removes the *Dic19C* gene dominantly enhances the rough eye phenotype of $Glued^1$, and a duplication of the *Dic* region suppresses the rough eye phenotype (Boylan *et al.* 2000). Similar to the deficiency, all three lethal *Dic* alleles were found to be dominant enhancers of the rough eye phenotype of $Glued^1$ (Figure 5). To determine whether the nonlethal *Dic* allele *sw* also shows a genetic interaction with $Glued^1$, homozygous *sw* females were crossed to $Gl^1Sb/TM6B, D$ males. As shown in Table 5, *sw* females in *trans*-heterozygous combination with $Glued^1$ are viable at all temperatures tested. However, these flies have an enhanced rough eye compared with the $Glued^1$ mutation alone (Figure 5), and the enhanced phenotype is reversed in the presence of the *Dic* transgene. In males in which only mutant *Dic* is present, the combination of *sw* and $Glued^1$ is lethal.

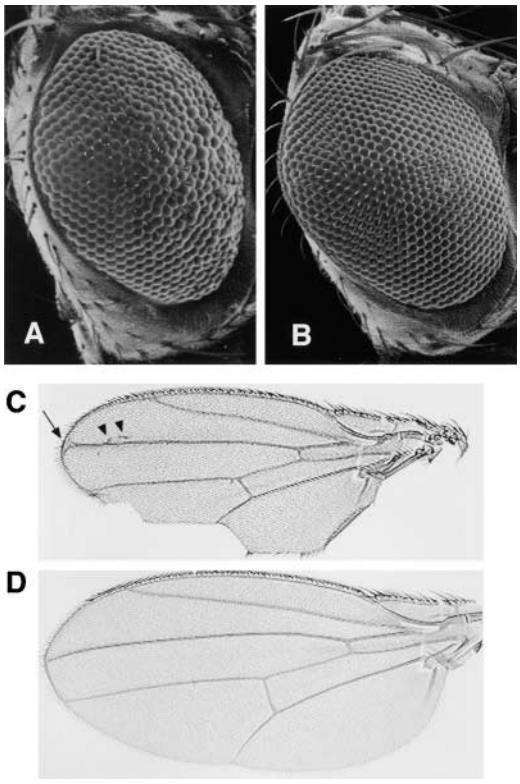

Figure 4.—The rough eye phenotype caused by *shortwing* is rescued by the *Dic* transgene. (A) Scanning electron micrograph (SEM) showing the rough eye phenotype of *sw/Y*. (B) SEM of an *sw* eye in the presence of the *Dic* transgene [genotype *sw/Y; P(Dic*$^+$*)/+*]. (C) The wing phenotype of *shortwing* is variable, including defects in wing size and venation and incised margins. Arrow, bifurcated wing vein; arrowheads, ectopic vein material. (D) The wing defects are rescued by the wild-type *Dic* transgene [genotype *sw/Y; P(Dic*$^+$*)/+*].

DISCUSSION

Our results provide the first direct evidence of an essential function for the intermediate chain subunit of cytoplasmic dynein. Previous analysis of dynein heavy chain mutations in mouse and Drosophila has demonstrated that dynein function is essential in these organisms (Gepner *et al.* 1996; Harada *et al.* 1998). In yeast and filamentous fungi, however, dynein heavy chain mutations are not lethal, but have defects in nuclear migration and spindle orientation (Eshel *et al.* 1993; Li *et al.* 1993; Plamann *et al.* 1994; Xiang *et al.* 1994). Similar to the yeast mutations in the heavy chain, mutations in the *Saccharomyces cerevisiae* dynein intermediate chain gene (*pac11*) are not lethal, but are synthetic lethal in combination with mutations in the kinesin gene, *cin8* (Geiser *et al.* 1997). Attempts to generate dynein

TABLE 4

Transgene rescue of *sw/Dic^m*

Temp.	*Dic* allele	Progeny classes (total no. of adults)[a] *Dic^m/y w sw; +/+* ♀	*Dic^m/y w sw; P(Dic$^+$)/+* ♀	*Dic^m/Y; +/+* ♂	*Dic^m/Y; P(Dic$^+$)/+* ♂	*FM7/y w sw; +/+* ♀	*FM7/y w sw; P(Dic$^+$)/+* ♀	*FM7/Y; +/+* ♂	*FM7/Y; P(Dic$^+$)/+* ♂
25°	*Dic^1*	0	50	0	54	63	62	47	28
25°	*Dic^3*	2	59	0	44	64	56	56	59
25°	*Dic^2*	35[b]	35	0	49	30	33	18	27
28°	*Dic^2*	1[c]	45	0	43	30	49	31	34
28°	*sw*	10[d]	24	1	32	23	31	25	22

[a] *Dic* mutant alleles (*Dic^m*).
[b] Some flies with mild wing phenotype.
[c] Severe wing phenotype.
[d] Rough eyes, reduced fertility, mild wing phenotype.

heavy chain and intermediate chain knockouts by homologous recombination in Dictyostelium failed, consistent with an essential function for both subunits (Koonce and Knecht 1998; Ma *et al.* 1999). In Dictyostelium, mutants overexpressing truncations of the *Dic* gene by a conditional promoter exhibited defects in Golgi dispersion, interphase microtubule organization, cell division, and centrosome replication and separation (Ma *et al.* 1999). Whether the intermediate chain subunit is required for all dynein functions is not known.

To identify mutations in the dynein intermediate chain gene, we used a modification of the screen reported by Fehon *et al.* (1997) in which they isolated mutations for a region of the *X* chromosome using a cosmid transgene as an autosomally linked duplication of the *X* chromosome. By substituting a transgene containing a single transcription unit for a larger genomic cosmid transgene, we were able to identify mutations within a single complementation group. Screening the progeny of ~3000 fertile F_1 males identified three mutations in the dynein intermediate chain gene. This recovery rate corresponds well with previous predictions of

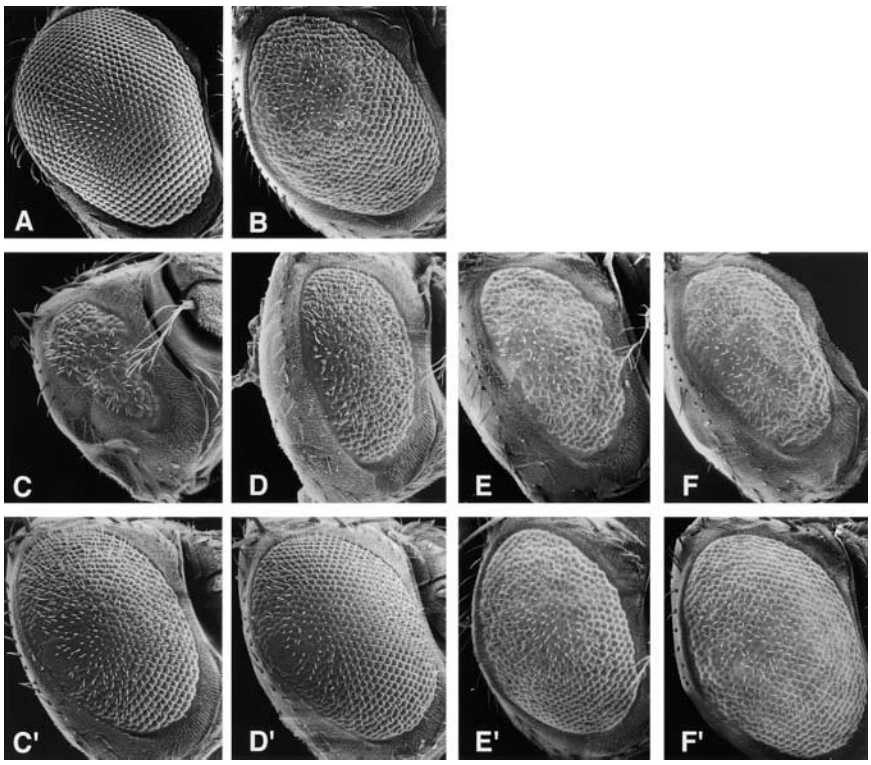

Figure 5.—Mutations in the dynein intermediate chain gene enhance the rough eye phenotype of *Glued^1*. Scanning electron micrographs of Drosophila heads showing (A) a wild-type eye and (B) the dominant rough eye phenotype of *Glued^1*. The *Glued^1* phenotype is dominantly enhanced by *Dic* mutant alleles (C) *Dic^1*, (D) *Dic^2*, (E) *Dic^3*, and (F) *sw*. The enhanced rough eye is reversed by the addition of the *Dic* transgene, *P(Dic$^+$)*, shown in C′, D′, E′, and F′. Genotypes: (A) wild type, (B) *Gl^l/+*, (C) *Dic^1/+; Gl^l/+*, (C′) *Dic^1/+; P(Dic$^+$)/+; Gl^l/+*, (D) *Dic^2/+; Gl^l/+*, (D′) *Dic^2/+; P(Dic$^+$)/+; Gl^l/+*, (E) *Dic^3/+; Gl^l/+*, (E′) *Dic^3/+; P(Dic$^+$)/+; Gl^l/+*, (F) *sw/+; Gl^l/+*, and (F′) *sw/+; P(Dic$^+$)/+; Gl^l/+*.

TABLE 5

shortwing* interacts with *Glued[1]

	Progeny classes (total no. of adults)			
Temp.	*sw/+; Gl[1] Sb/+* ♀	*sw/Y; Gl[1] Sb/+* ♂	*sw/Y; +/TM6B, D* ♂	*sw/+; +/TM6B, D* ♀
28°	63	0	19	68
25°	58	0	42	88
22°	54	0	27	78
18°	25	0	32	36

one lethal mutation for every 1000 F_1 progeny scored (Greenspan 1997); however, it is approximately one-third the number of mutations recovered in the screen by Fehon *et al.* (1997). One consequence of screening by this method is that only *X* chromosomes with single lethal hits in the *Dic* gene will be identified. Chromosomes containing multiple lethal hits will not be recovered. While this obviates the need to remove second-site lethals from the mutagenized chromosome, it could reduce the number of *Dic* mutations identified.

The lethal phase analysis shows that the *Dic* mutations result in larval lethality. A similar lethal phase has been observed for mutations in the dynein heavy chain. Strong alleles of the dynein heavy chain (*Dhc64C*) die as first instar larvae, and somatic clone analysis of *Dhc64C* mutations demonstrates that dynein function is required for cell viability (Gepner *et al.* 1996). These results suggest that the maternal contribution of dynein is sufficient to allow the completion of embryogenesis without a zygotic contribution of gene product. Although all three *Dic* alleles die as larvae, the weakest *Dic* allele (*Dic[2]*) lives to a late larval stage, while two *Dic* alleles (*Dic[1]* and *Dic[3]*) appear to die as first instar larvae. Although none of the *Dic* alleles identified appears to be a null allele, the relative efficiency of screening for *Dic* mutants should allow for identification of additional alleles.

In addition to lethality, one of the *Dic* mutations displays a larval crawling defect. This may result from progressive larval paralysis, as the mutant larvae become stiff with their heads poking out of the food like spikes. Similar crawling and paralysis phenotypes have been identified in mutations in the kinesin heavy chain and kinesin light chain genes (Hurd and Saxton 1996; Gindhardt *et al.* 1998). The kinesin mutant larvae show axonal organelle jams, suggesting that the larval paralysis is due to a defect in axonal transport. Mutations in the dynein heavy chain (*Dhc64C*) have also been shown to disrupt axonal transport, causing larval paralysis (Martin *et al.* 1999). Additionally, screens for mutants with sluggish larval crawling behavior identified a gene, *roadblock*, in which the larval crawling phenotype was shown to be due to a mutation in the dynein light chain LC7. The *roadblock* mutants also accumulate axonal cargo and, additionally, have severe axonal loss and nerve degeneration (Bowman *et al.* 1999). Not surprisingly, we also observed accumulations of cargo in the axons of *Dic* mutant larvae, suggesting that the larval paralysis is due to a defect in axonal transport.

The genetic analysis of the mutant *sw* strongly suggests that it represents a viable allele of the cytoplasmic dynein intermediate chain gene, *Dic19C*. *sw* fails to complement the lethal *Dic* alleles in a temperature-sensitive manner. A copy of the wild-type *Dic* transgene rescues the lethal and visible phenotypes resulting from non-complementation of sw and the *Dic* alleles. In addition, the complementation behavior of *sw* with the lethal *Dic* alleles provides a way to gauge the relative strength of the lethal alleles. For example, Figure 3 shows a range of wing phenotypes for the combinations of *sw* with the lethal *Dic* alleles from mild (*Dic[2]/sw*) to severe (*Dic[1]/sw*). The weakest lethal allele, *Dic[2]*, fully complements *sw* at 25°, but fails to complement the *sw* wing and eye phenotype at 28°. The stronger alleles, *Dic[3]* and *Dic[1]*, fail to complement *sw* for viability at 25°. At lower temperatures, *Dic[1]/sw* and *Dic[3]/sw* adults are viable, but exhibit the *sw* eye phenotype and display severe defects in wing development. By this test, the allele *Dic[1]* is the strongest of the lethal alleles, although comparison of *Dic[1]/sw* to Df/*sw* suggests that *Dic[1]* is not a null allele.

Using a deficiency that removes the intermediate chain locus and a *Dic* genomic transgene (Boylan *et al.* 2000), we have previously shown that the rough eye phenotype of the *Glued[1]* mutation is sensitive to the dosage of the dynein intermediate chain gene. The *Glued[1]* mutation was initially identified on the basis of the dominant rough eye phenotype (Plough and Ives 1935) and was subsequently shown to be due to a truncation of the p150 subunit of dynactin caused by a transposon insertion in the *Glued* gene (Swaroop *et al.* 1985). The truncated Glued polypeptide is unable to assemble into a functional dynactin complex (McGrail *et al.* 1995); however, it retains the region identified as important for interaction with the dynein intermediate chain (Vaughan and Vallee 1995). Consequently the *Glued[1]* truncation could act as a "poison" to dynein function, by uncoupling dynein from its cargo. The interaction between *Glued[1]* and *Dic* depends on the dosage of *Dic*, suggesting a model where *Glued[1]* acts by reducing the level of dynein intermediate chain available for cargo binding below a threshold required for normal eye development. Similar to a deficiency for the intermediate chain locus, the lethal dynein intermediate chain alleles identified in our screen and the viable allele *sw* all dominantly enhance the rough eye phenotype of *Glued[1]*. This result indicates that the intermediate chain alleles are all loss-of-function mutations that reduce the level of wild-type intermediate chain available to interact with the dynactin complex, causing an enhanced rough eye phenotype. Additional evidence for a dosage-sensitive

TABLE 6

Phenotypes associated with dynein intermediate chain and heavy chain mutations

Genotype	Phenotype	Reference
sw(Dic)/Y	Wings have defects in size and venation; eyes rough and elliptical	This study
Dic^{2}/Y	Larval paralysis	This study
Dhc^{3-2}/Dhc^{6-10}	Eyes small and rough; bristles short and thin	McGrail *et al.* (1995) Gepner *et al.* (1996)
Dhc^{4-19}/Dhc^{8-1}	Eyes darker in color and rounder in shape than those of wild type; bristles short and thin with bent tips	Gepner *et al.* (1996)
Dhc^{3-2}/Dhc^{6-12}	Female sterile; male fertile	Gepner *et al.* (1996) McGrail and Hays (1997)
Dhc^{6-6}/Dhc^{6-10}	Female sterile	McGrail and Hays (1997)
Dhc^{6-10}/Dhc^{6-12}	Female sterile; male sterile	Gepner *et al.* (1996)
$Dhc^{x18}/Df(3L)10H$	Short bristles, female sterile	K. L. M. Boylan and T. S. Hays (unpublished observations)

interaction between the dynein intermediate chain and $Glued^{1}$ comes from the observation that in males, the combination of the viable *Dic* allele, *sw*, with $Glued^{1}$ is lethal. Moreover, this result shows that the interaction between the dynein intermediate chain and Glued is essential for viability and is not restricted to eye development.

The wing defects present in the *sw Dic* mutant identify a novel dynein phenotype. Our previous analysis of mutations in the dynein heavy chain (*Dhc64C*) has revealed heteroallelic combinations of alleles that complement for viability but have phenotypes in the eye and bristles and during oogenesis (Gepner *et al.* 1996; McGrail and Hays 1997), but no wing phenotypes have been observed. A major question raised by this observation is whether the wing phenotype reflects a tissue-specific function for the dynein intermediate chain and dynein transport. This seems unlikely since *sw/sw* females fail to exhibit a wing phenotype. An alternative explanation is that different tissues require different levels of dynein function during development. However, if this were the case one might expect the mutant phenotypes to "accumulate" on the basis of the level of dynein function provided by a particular mutant allele. For example, if the level of dynein function required for oogenesis is higher than that required for proper eye development, then all dynein mutants with a rough eye phenotype might also be expected to exhibit female sterility. As shown in Table 6, this is not the case, suggesting that the different dynein mutant alleles affect different aspects of dynein function. Consistent with the explanation that levels of dynein function account for different phenotypes in different tissues would be the prediction that noncomplementation may arise between *Dhc* and *Dic* mutations. However, so far we have failed to observe any such genetic interactions between *Dic* mutations and mutations in other dynein subunits. Further experiments will be necessary to establish whether, in addition to its essential functions, the dynein intermediate chain subunit serves tissue-restricted functions. The identified *Dic* alleles will provide new tools to identify interacting components that, similar to Glued (dynactin), play a role in regulating dynein function and its interaction with specific cargoes.

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