Cytoplasmic Dynein Function Is Essential in *Drosophila melanogaster*

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Manuscript received October 15, 1995
Accepted for publication December 8, 1995

ABSTRACT

The microtubule motor cytoplasmic dynein has been implicated in a variety of intracellular transport processes. We previously identified and characterized the Drosophila gene *Dhc64C*, which encodes a cytoplasmic dynein heavy chain. To investigate the function of the cytoplasmic dynein motor, we initiated a mutational analysis of the *Dhc64C* dynein gene. A small deletion that removes the chromosomal region containing the heavy chain gene was used to isolate EMS-induced lethal mutations that define at least eight essential genes in the region. Germline transformation with a *Dhc64C* transgene rescued 16 mutant alleles in the single complementation group that identifies the dynein heavy chain gene. All 16 alleles were hemizygous lethal, which demonstrates that the cytoplasmic dynein heavy chain gene *Dhc64C* is essential for Drosophila development. Furthermore, our failure to recover somatic clones of cells homozygous for a *Dhc64C* mutation indicates that cytoplasmic dynein function is required for cell viability in several Drosophila tissues. The intragenic complementation of dynein alleles reveals multiple mutant phenotypes including male and/or female sterility, bristle defects, and defects in eye development.

DYNEIN is a multisubunit ATPase that functions as a motor that powers microtubule-based motility. Axonemal dyneins drive the sliding of microtubules in cilia and flagella, and were the first dynein motors to be identified and characterized (reviewed in PORTER and JOHNSON 1989). More recently, a cytoplasmic dynein motor has been described in cells that do not contain cilia or flagella (reviewed in WALKER and SHEETZ 1993; HOLZBAUR and VALLEE 1994; SCHROER 1994). Cytoplasmic dynein has been implicated in a variety of cellular processes including chromosome movements (PFARR *et al.* 1990; STEUER *et al.* 1990; HYMAN and MITCHISON 1991), spindle morphogenesis (VAISBERG *et al.* 1993), nuclear positioning (ESHEL *et al.* 1993; LI *et al.* 1993; PLAMANN *et al.* 1994; XIANG *et al.* 1994), and minus-end directed cytoplasmic organelle transport (VALE *et al.* 1985; PASCHAL and VALLEE 1987; SCHROER *et al.* 1989; DILLMAN and PFISTER 1994). Cytoplasmic dyneins consist of two heavy chain polypeptides as well as several intermediate and light chains (PASCHAL *et al.* 1987; KING and PATEL-KING 1995; reviewed in WALKER and SHEETZ, 1993). The heavy chains are extraordinarily large polypeptides of >500 kD that use the energy derived from ATP hydrolysis to translocate along the microtubule lattice. Recent mutational analyses in *Saccharomyces cerevisiae* (ESHEL *et al.* 1993; LI *et al.* 1993), *Aspergillus nidulans* (XIANG *et al.* 1994), and *Neurospora crassa* (PLAMANN *et al.* 1994) have demonstrated the requirement for cytoplasmic dynein in nuclear migration and division. However, little is known about the functional repertoire of cytoplasmic dynein in metazoan cells and tissues.

To examine the range of cytoplasmic dynein function in multicellular organisms, we previously characterized the principal cytoplasmic dynein heavy chain gene in *Drosophila melanogaster* (HAYS *et al.* 1994; LI *et al.* 1994). The dynein heavy chain gene has been designated *Dhc64C* due to its location on the left arm of chromosome *3*. Northern analysis has revealed that *Dhc64C* is expressed throughout embryogenesis, in adult ovaries and testes, as well as at other developmental stages (LI *et al.* 1994). The spatial distribution of cytoplasmic dynein during embryogenesis (HAYS *et al.* 1994) and oogenesis (LI *et al.* 1994) has been examined by immunofluorescence. In early embryos, dynein is associated with mitotic spindle microtubules in both the syncytial blastoderm and the cellularized blastoderm, consistent with a proposed role for cytoplasmic dynein in force generation within the mitotic spindle apparatus. Later in embryogenesis, dynein is enriched in the developing central nervous system, where it could have a cellular and/or developmental function. A developmental role for the dynein motor in the transport of morphogens during oogenesis has also been implicated by the preferential localization of dynein to, and within, the developing oocyte (LI *et al.* 1994).

Despite the clues that such localizations provide to potential dynein functions, the mechanisms by which dynein-mediated transport contributes to the embryonic development of multicellular organisms remain to be determined. Moreover, whether and how dynein motor function is differentially regulated in specific cells and tissues is completely unknown. To begin to

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address these questions, we have isolated and characterized mutations in the Drosophila dynein heavy chain gene *Dhc64C*.

MATERIALS AND METHODS

Stocks: The chromosomes *ru h th ss bx* and *In(3LR)DcxF, ru h D e* were obtained from the Bowling Green Stock Center, *mwh jv ca* and *TM6B, h Hu D e ca* were provided by L. CROSBY (Harvard University), and *iso-1* (BRIZUELA *et al.* 1994) and *w-c* were provided by J. TAMKUN (University of California, Santa Cruz). The deficiencies *Df(3L)GN24* (63F4-7;64C13-15) and *Df(3L)GN50* (63E1-2;64B1-7) were obtained from the Indiana Stock Center; *Df(3L)10H, st* e^4 (64B10-12;64C5-9) and *Df(3L)ems*^{13}, *st ri in pp* (64B1-2;64E) (GARBE *et al.* 1993) were provided by J. GARBE (University of California, Berkeley). The FLP and FRT stocks used in this study were obtained from the Indiana Stock Center and are described in XU and RUBIN (1993). Markers and other chromosomes used are described in LINDSLEY and ZIMM (1992). Mutations were maintained over the balancer chromosomes *TM6B, h Hu D e ca*, or *TM6B, Tb Hu e ca*. Flies were raised on standard yeast-cornmeal-agar medium at 25° unless otherwise indicated.

Nomenclature and complementation tests for mutations identified in 64C: In accordance with LINDSLEY and ZIMM (1992), each of the eight lethal genes identified in the interval of 64C that is removed by the deficiency *Df(3L)10H* has been designated *l(3)64C* followed by a lowercase letter (*a–h*) for each gene. The positions of the genes with respect to one another has not been determined. *l(3)64Ca* corresponds to the cytoplasmic dynein heavy chain gene and has been named *Dhc64C* (*Dynein heavy chain 64C*) to reflect the identity of the polypeptide encoded by the gene. The alleles recovered in each lethal gene are indicated by superscripted designations that refer to the experiment and sequence in which they were originally isolated. For example, $Dhc64C^{6\text{-}10}$ (alternatively, $l(3)64Ca^{6\text{-}10}$) refers to a dynein allele that was the 10th lethal mutation to be isolated from the sixth mutagenesis experiment.

For complementation analysis, five females heterozygous for one mutation were crossed to five males heterozygous for the second mutation. Each mutation was crossed to at least one member of every group and to all members of its own group. The number of progeny in the test class and all sibling classes were recorded. As originally isolated, the two mutations that define group 7, $l(3)64Cg^{1\text{-}4}$ and $l(3)64Cg^{3\text{-}5}$, were lethal over *Df(3L)10H*. It appears that since the time of their initial identification and characterization, both mutations have partially reverted, have acquired modifying mutations, or have lost enhancer mutations originally in their background. Recently, $l(3)64Cg^{3\text{-}5}/Df(3L)10H$ flies have shown good viability and $l(3)64Cg^{1\text{-}4}$ is only semilethal over *Df(3L)10H*. In addition, heterozygous $l(3)64Cg^{1\text{-}4}/l(3)64Cg^{3\text{-}5}$ flies no longer fail to complement but show very low viability. Although we have not analyzed the basis of the reversion to viability, we have retained the *l(3)64Cg* complementation group since the mutations were originally recovered as lethal in combination with *Df(3L)10H*. A second anomaly was observed in the characterization of the dynein mutation $Dhc64C^{6\text{-}8}$ that resulted in the isolation of an additional dynein allele, $Dhc64C^{6\text{-}12}$. The $Dhc64C^{6\text{-}12}$ mutation appears to have arisen by a spontaneous event.

Mutagenesis: *ru h th ss bx* or *mwh jv ca* males were treated with EMS as described by LEWIS and BACHER (1968). Briefly, males were starved for 1.5 hr and then fed 25 mM EMS in 1% sucrose overnight. EMS-treated males were mass mated to *Df(3L)10H, st* e^4*/DcxF, ru h D e* virgin females, and balanced male progeny (*ru h th ss bx/DcxF*) were mated singly to two *Df(3L)10H, st* e^4*/DcxF* virgin females in vials or test tubes at 25° (Figure 3). If the mutagenized chromosome was lethal over the deficiency *Df(3L) 10H*, as evident by the absence of the D^+ progeny class, siblings that carried the balanced mutagenized chromosome (*ru h th ss bx/DcxF*) were stocked and subsequently retested.

An attached-X assay (CROSBY and MEYEROWITZ 1986) was used to estimate the number of lethal hits per chromosome arm produced by the EMS. Males were treated with EMS as described above and mass-mated with attached-X females. The percentage of *X* chromosomes with lethal hits was estimated from the number of surviving males as compared to the number of sibling females in the progeny from the mass mating. In three of the six separate mutagenesis experiments conducted, the attached-X assay indicated that 48, 66, and 42% of the *X* chromosomes contained lethal hits. These values are consistent with previously reported values (GRIGLIATTI 1986).

A second screen to isolate temperature-sensitive lethal mutations in *Dhc64C* involved a slight modification of the above strategy. EMS-treated mutagenized chromosomes (*ru h st* p^p e^s *ss*) were recovered over the third chromosome balancer *TM6B, D Hu e ca*. Individual males were mated at 29° to virgin females heterozygous for the recessive lethal dynein allele $Dhc64C^{4\text{-}19}$, which was recovered in the original screen described above. The absence of the critical class of progeny, *ru * h st* p^p e^s *ss/ mwh* $Dhc64C^{4\text{-}19}$ *jv ca* (where * indicates a new mutation in *Dhc64C*), was scored by the absence of D^+ flies. In pair matings that did not give rise to D^+ flies, the new dynein mutation was recovered from the sibling males. Potential temperature-sensitive dynein mutants were retested at 25° and 29°. Although no strict temperature-sensitive mutations were recovered from an initial screening of 1800 chromosomes, several additional recessive lethal *Dhc64C* alleles were recovered. One of these dynein alleles, $Dhc64C^{8\text{-}1}$, is reported here.

Construction of a *Dhc64C* transgene [*P*(*Dhc*$^+$)] for transformation rescue: A cosmid genomic clone, *pCS3*, which contains the entire cytoplasmic dynein heavy chain gene and portions of at least one flanking gene at each end, was isolated previously (LI *et al.* 1994). The region encompassing *Dhc64C* is ~19 kb in length. We constructed a transgene that consisted of *Dhc64C* genomic DNA derived from *pCS3* cloned into the *P*-element vector *pCaSpeR4*. This DNA construct contained the predicted dynein transcription unit with a small amount of flanking sequences but no additional transcripts. It was constructed by the modification and reassembly of four subcloned fragments of *pCS3*. All of the junction sites generated during construction of the transgene were subsequently verified by sequencing. The *Not*I fragment containing the complete transcription unit was cloned into the *P*-element vector *pCaSpeR4* (KLEMENZ *et al.* 1987) to generate the plasmid *pDN17* that was used in *P* element-mediated germline transformation. The wild-type *Dhc64C* transgene contained within *pDN17* will be referred to as *P*(*Dhc*$^+$).

***P* element-mediated transformation:** Transformation was performed by microinjection of *w-c* Drosophila embryos with both plasmid *pDN17* (600 μg/ml) and helper plasmid *pπ25.7wc* (75 μg/ml) (KARESS and RUBIN 1984). Four independent transformants were obtained, the chromosomal locations of the *P*-element inserts were determined by linkage analysis, and stocks were established over the appropriate balancer chromosomes (*FM6, CyO,* and *TM3* or *TM6B*). The number of *P*-element inserts in each transformed line was determined by genomic Southern blot analysis. The transformed line that contains *P*(*Dhc*$^+$) on the *X* chromosome will be referred to as *P*(*Dhc*$^+$)^X in this study.

Southern blot and *in situ* polytene hybridization analysis: Genomic DNA was prepared from female adult flies as detailed in RASMUSSON *et al.* (1994). DNA samples from the equivalent of four to five flies were digested with *Hind*III, separated in 1.0% agarose gels, and transferred to Magnagraph filters (Micron Separations) by standard methods (SAMBROOK *et al.* 1989). Filters were hybridized with the same digoxigenin-labeled *Dhc64C* probe used for *in situ* hybridization, and the signals were detected using the chemiluminescent substrate "Lumiphos" according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis IN). To identify deficiencies that remove the dynein heavy chain gene, larval polytene chromosome squashes were hybridized with a digoxigenin-labeled 1-kb DNA fragment from the 3′ end of *Dhc64C*. Preparation of chromosome squashes and *in situ* hybridization were carried out as described in RASMUSSON *et al.* (1994).

Lethal phase and phenotypic analyses of the Dhc64C mutants: To examine the lethal stage of the dynein mutants, each of the *Dhc64C* alleles were balanced over a *TM6B, Tb* chromosome and mated with *Df(3L)10H/TM6B, Tb* flies. Larvae and pupae hemizygous for the dynein mutation ($Dhc64C^{*}$/*Df(3L)10H*) were Tb^{+} and easily distinguished from their heterozygous Tb siblings. For each dynein allele we scored the presence or absence of Tb^{+} larvae and pupae.

For selected dynein alleles we further examined the developmental stage at which lethality was observed. Analysis of the *Df(3L)10H* deficiency indicated a semidominant lethality associated with the deficiency. To circumvent this lethality, we scored the lethal phase in flies that were doubly heterozygous for two separate dynein alleles or homozygous for a single allele. In the case of flies homozygous for the $Dhc^{6\text{-}6}$, $Dhc^{6\text{-}10}$, or $Dhc^{4\text{-}19}$ mutations, we confirmed that the observed lethality was due to the dynein mutation by demonstrating that the wild-type dynein transgene was able to rescue the homozygous lethality of each allele. We determined the percentage lethality during embryogenesis, larval stages, and the pupal stage for the following *Dhc64C* genotypes: $Dhc^{4\text{-}19}$/$Dhc^{5\text{-}8}$, $Dhc^{6\text{-}10}$/$Dhc^{6\text{-}6}$, $Dhc^{6\text{-}10}$/$Dhc^{6\text{-}10}$, $Dhc^{6\text{-}6}$/$Dhc^{6\text{-}6}$, and $Dhc^{4\text{-}19}$/$Dhc^{4\text{-}19}$. To assess the stage of lethality, each mutation was outcrossed to OregonR wild-type flies (+/+), and male progeny carrying a *Dhc* allele (*e.g.*, $Dhc^{4\text{-}19}$/+) were then mated to outcrossed virgin female progeny carrying the second *Dhc* allele (*e.g.*, $Dhc^{5\text{-}8}$/+). After two to three days of mating, the flies were moved to fresh egg lay plates and allowed to lay eggs for 12–24 hr. Two to four hundred embryos were selected and transferred to a fresh plate. After 36 hr the number of unhatched eggs was counted, and the larvae counted and transferred to a fresh vial. The larvae were left to pupate, the number of pupae counted, and then subsequently the number of adult flies that eclosed were recorded. The total percentage of lethality (%L) was determined from the formula: %L = [(# eggs − # adults)/# eggs] × 100. The lethality at each stage is expressed as a percentage of the total lethality (%L). The percentage of total lethality that occurred at a particular stage (*e.g.*, % embryonic lethality) was determined by the formula: % embryonic lethality = [(# eggs − # larvae)/(# eggs − # adults)] × 100.

Cytological analysis: Staged collections of mutant embryos were examined by immunocytochemical methods. Antibody probes that recognize the *engrailed* and *snail* gene products were provided by Drs. SEAN CARROLL (HHMI, University of Wisconsin, Madison, WI) and AUDREY ALBERGA (Institut de Chimie Biologique, Strasbourg, France), respectively. The monoclonal antibody BP102 recognizes a central and peripheral nervous system antigen and was obtained from Dr. COREY S. GOODMAN (University of California, Berkeley, Berkeley, CA). The dynein heavy chain antibodies were previously characterized (HAYS *et al.* 1994). The identification of the hemizygous mutant embryos was facilitated by the use of a third chromosome balancer stock carrying a *P*-element insert that expresses the reporter β-galactosidase under the *ftz* promoter. Embryos hemizygous for a dynein mutation lacked the balancer chromosome and therefore did not express β-galactosidase. Embryos were double-labeled with antibodies against the molecular marker of interest (snail, engrailed, BP102, or dynein) and antibodies against β-galactosidase. The control sibling classes were recognized by the presence of the *ftz*-striped pattern of β-gal expression. Embryos were prepared for immunolocalization as described in HAYS *et al.* (1994). Embryos were examined on a Zeiss Axioskop equipped with differential interference contrast optics.

Clonal analysis of cell lethality: To assess the requirement for dynein function in somatic cell viability, clones of cells homozygous for the dynein allele $Dhc64C^{4\text{-}19}$ were induced by mitotic recombination. The *mwh* $Dhc64C^{4\text{-}19}$ *jv ca* chromosome, which is lethal free with the exception of the *Dhc64C* mutation, was used for the analysis of somatic clones generated with the FRT/FLP recombinase system (GOLIC 1991; XU and RUBIN 1993). Using the FRT stock 80-NM (XU and RUBIN 1993), an FRT insert located at the base of 3L at position 80B was recombined onto the $Dhc64C^{4\text{-}19}$ chromosome. Recombination events between $Dhc64C^{4\text{-}19}$ and the FRT site were selected for on the basis of resistance to G418 and loss of the *mini-w*$^{+}$ marker carried on a *P* element located at 70C on the 80-NM chromosome. $Dhc64C^{4\text{-}19}$ FRT recombinants were identified by testing for lethality of the recombinant chromosome over the deficiency *Df(3L)10H,* and for rescue of homozygous *mwh* $Dhc64C^{4\text{-}19}$ *jv* FRT flies by a wild-type *Dhc64C* transgene. An *X* chromosome bearing the FLP recombinase behind the hsp70 promoter (*yw P*[*ry*$^{+}$, *Hsp70:FLP*]) was crossed into the appropriate FRT stock. For detection of clones in the wing and in thoracic and abdominal epidermis, the FRT stock 80-*y*$^{+}$ (XU and RUBIN 1993) was used. This chromosome carries a *y*$^{+}$ body color marker on a *P* element located at position 66E. For detection of clones in the eye, the FRT stock 80-NM was used. Males of the genotype *yw P*[*ry*$^{+}$ *Hsp70:FLP*]/*Y; P*[*y*$^{+}$] *P*[*ry*$^{+}$ *Hsp70:neo FRT*]/*TM3, Sb* or *yw P*[*ry*$^{+}$ *Hsp70:FLP*]/*Y; P*[*mini-w*$^{+}$] *P*[*ry*$^{+}$ *Hsp70:neo FRT*]/*TM3, Sb* were crossed with virgin *yw; mwh* $Dhc64C^{4\text{-}19}$ *jv P*[*ry*$^{+}$ *Hsp70:neoFRT*]/*TM3, Sb* and *yw P*(Dhc^{+})*; mwh* $Dhc64C^{4\text{-}19}$ *jv P*[*ry*$^{+}$ *Hsp70:neoFRT*]/*TM3, Sb* females. Eggs were collected for 24 hr and aged to first, second, or third instar larval stages. Larvae were heat shocked for 1 hr at 37° in a water bath to induce expression of FLP recombinase and mitotic recombination. Progeny of the genotype *yw P*[*ry*$^{+}$ *Hsp70: FLP*]/*yw; P*[*y*$^{+}$] *P*[*ry*$^{+}$ *Hsp70:neo FRT*]/*mwh* $Dhc64C^{4\text{-}19}$ *jv P*[*ry*$^{+}$ *Hsp70:neoFRT*] and *ywP*[*ry*$^{+}$ *Hsp70: FLP*]/*yw P*(Dhc^{+})*; P*[*y*$^{+}$] *P*[*ry*$^{+}$ *Hsp70:neo FRT*]/*mwh* $Dhc64C^{4\text{-}19}$ *jv P*[*ry*$^{+}$ *Hsp70:neoFRT*] were dissected, and body parts were mounted in methylsalicylate and Canada balsam and examined on a Zeiss Axioskop microscope equipped with differential contrast optics. Cells homozygous for the *Dhc64C* mutation were scored in the wing and in thoracic and abdominal epidermis in the presence and the absence of the wild-type *Dhc64C* transgene [*P*(Dhc^{+})] located on the *X* chromosome. Clones were identified using the *multiple wing hair* marker in the wing, and the markers *yellow* and *javelin* in the bristles of the epidermis. Body parts were scored either positive or negative for the presence of clones. Flies were counted positive if they contained one or more clones of at least one trichome in the wing or a single bristle in the thorax. In the abdomen, clones were counted only if two or more adjacent bristles were seen. Single bristle abdominal clones appear to reflect spontaneous recombination in the abdominal histoblasts, which occurs in the absence of FLP recombinase (RIPOLL 1977) (data not shown). The

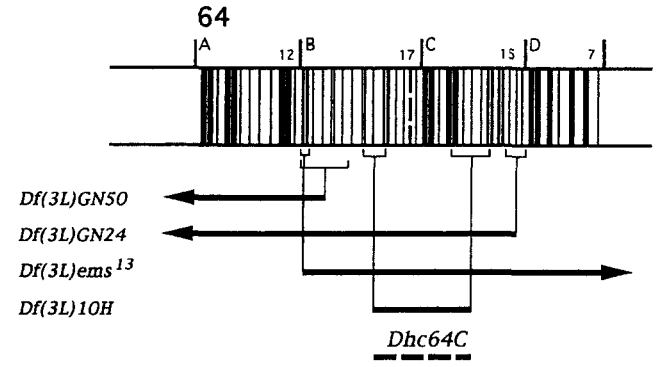

FIGURE 1.—The 64BC region. The arrows indicate the regions removed by the deletions listed to the left. The drawing is derived from the chromosome drawings in LINDSLEY and ZIMM (1992). ---, the location of the dynein heavy chain gene, *Dhc64C*, as determined by *in situ* hybridization to larval polytene chromosomes and RFLP analysis.

number of clones per fly was not recorded. Progeny of the genotype *yw P[ry⁺ Hsp70:FLP]/yw; P[mini-w⁺] P[ry⁺Hsp70:neo FRT]/mwh Dhc64C⁴·¹⁹ jv P[ry⁺ Hsp70:neoFRT]* and *yw P[ry⁺Hsp70:FLP]/yw P(Dhc⁺); P[mini-w⁺] P[ry⁺Hsp70:neo FRT]/mwh Dhc64C⁴·¹⁹ jv P[ry⁺Hsp70:neoFRT]* were examined for the presence of clones in the eye using a stereomicroscope. Clones were identified in the presence of the *P(Dhc⁺)* transgene by the occurrence of pale yellow patches in an orange background.

RESULTS

Essential genes in 64C: To screen for recessive lethal mutations in the *Dhc64C* dynein locus, we first identified deficiencies that removed *Dhc64C*. Figure 1 summarizes the approximate breakpoints of deficiencies that have previously been characterized in the 64C region. The identification of a restriction fragment length polymorphism (RFLP) in the middle of *Dhc64C* enabled us to use RFLP analysis to score for the loss of the *Dhc64C* gene. Flies of the strain *iso-1* lack a *Hind*III site present in the dynein gene of several other laboratory strains including the third chromosome balancers *TM8* and *TM6B*. Figure 2A shows a Southern blot of *Hind*III-digested genomic DNA from flies heterozygous for one of two 64BC deficiencies, *Df(3L)GN24* or *Df(3L)10H*. The blot was hybridized with a digoxigenin-labeled 1-kb *Dhc64C* DNA fragment. The *Dhc64C* probe hybridized to a unique 10-kb *Hind*III fragment in the *iso* stock and a 3.4-kb *Hind*III fragment in *Df(3L)GN24/TM8* and *Df(3L)10H/TM6B* flies. The probe hybridized to a 2.3-kb fragment in all stocks. Both the 10- and the 3.4-kb bands were present in the DNA from *iso/TM8* and *iso/TM6B* flies. Since *Df(3L)GN24/iso* and *Df(3L)10H/iso* flies contain only the 10-kb band characteristic of DNA from *iso* flies, and not the 3.4-kb band, then both *Df(3L)GN24* and *Df(3L)10H* remove the DNA fragment identified by the RFLP. Southern blots of *Df(3L)10H/TM6B, Df(3L)GN24/TM8* and *iso* DNA were hybridized with probes that cover the entire *Dhc64C* gene and no other differences were detected (data not shown).

In situ hybridization to larval polytene chromosomes was used to further characterize deficiencies in the 64C region of the cytological map. The deficiency *Df(3L)GN24* was large enough to interfere with synapsis and resulted in a "looping out" of the wild-type homologue in the region of the deficiency (Figure 2B). The *Dhc64C* probe hybridized only to the looped out region, demonstrating that *Df(3L)GN24* removed the *Dhc64C* gene. *In situ* hybridization experiments showed that *Df(3L)ems¹³* also removed the *Dhc64C* gene, while *Df(3L)GN50* did not. It was difficult to determine whether *Df(3L)10H* deleted *Dhc64C* by *in situ* hybridization to *Df(3L)10H/+* larvae, since the deficiency was too small to interfere with synapsis enough to cause a visible looping out. However, chromosomes from *Df(3L)10H/TM6B, D Hu* larvae were examined. Since *TM6B* is multiply inverted, it promotes asynapsis, thus making it possible to visualize both the deficiency and balancer chromosomes. In all such larval chromosomes examined, hybridization was detected only in the 64C region of the inverted homologue, confirming that *Df(3L)10H* deletes *Dhc64C*. Of the three deficiencies that did remove the dynein gene, *Df(3L)10H* represented the smallest deletion and was therefore subsequently used in the screen to recover dynein mutations.

A standard F_2 mutagenesis scheme was used to isolate EMS-induced recessive lethal mutations within the region removed by *Df(3L)10H* (Figure 3). A total of 4820 chromosomes were screened in six different experiments, producing a total of 39 mutations that were lethal in combination with the *Df(3L)10H* chromosome. Each of the mutations was also lethal in combination with *Df(3L)GN24*, a larger deficiency that also removes the dynein gene. The lethal mutations from this screen were assigned to eight complementation groups based on *inter se* crosses between the mutants (Table 1). *l(3)64Ca* (group 1) was the largest with 16 original members, and *l(3)64Ch* (group 8) was the smallest with only one member. Two additional alleles of *l(3)64Ca, Dhc⁶·¹²* and *Dhc⁸·¹*, were subsequently identified (see MATERIALS AND METHODS) and are included in Table 1. Since *l(3)64Ch* contains only one member, all of the essential genes in this region may not have been identified. The relative positions of the eight lethal complementation groups, *l(3)64Ca–h*, have not been determined.

Identification of the *Dhc64C* complementation group by transformation rescue: The cytoplasmic dynein heavy chain is >500 kD, which suggests that the *Dhc64C* gene could represent a large target for EMS mutagenesis. The assignment of a disproportionate number of the recovered lethal mutations to *l(3)64Ca* (group 1) suggested that this complementation group might represent the dynein gene. Transformation rescue experiments were conducted to unequivocally determine which complementation group identified *Dhc64C*.

To rescue lethal *Dhc64C* mutations, we first recovered germline transformants containing a *Dhc64C* transgene.

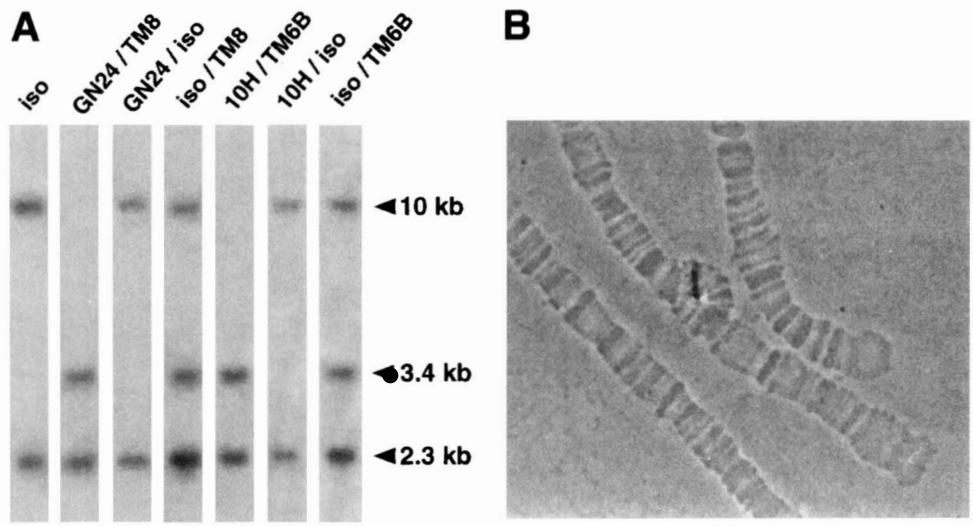

FIGURE 2.—Mapping of *Dhc64C* within the 64BC region. (A) Southern blot analysis of DNA from flies containing deletions of the 64BC region. DNA extracts were digested with *Hind*III. The blot was prepared and hybridized with a 1-kb *Dhc64C* DNA probe as described in MATERIALS AND METHODS. The relevant genotype of the fly strain from which DNA extracts were derived is indicated above the lane. The absence of hybridization of the probe to a 3.4-kb *Hind*III fragment in flies of the genotype *GN24/iso* and *10H/iso* reflects the deletion of the *Dhc64C* gene. (B) The deficiency *Df(3L)GN24* deletes *Dhc64C*. Polytene chromosomes from *Df(3L)GN24/+* larvae were hybridized with the digoxigenin-labeled *Dhc64C* probe. The central chromosome arm pictured is the distal end of 3L. The *Dhc64C* probe hybridized to the looped out region of the wild-type homologue.

Previous experiments had identified a cosmid clone that included the entire *Dhc64C* gene as well as two flanking genes (LI *et al.* 1994). In addition, a reporter gene construct had indicated the 5′ sequences required for dynein expression (LI *et al.* 1994). Subclones of the cosmid genomic DNA were modified to eliminate the flanking genes, and the single dynein transcription unit was reassembled in the *P*-element vector *pCaSpeR4* (Figure 4A; see also MATERIALS AND METHODS). The reconstructed dynein transgene, designated $P(Dhc^+)$, included the endogenous *Dhc64C* promoter, the entire coding sequence, and the 3′ polyadenylation signal. The 28-kb *P*-element construct containing the transgene was introduced into flies by *P* element-mediated germline transformation.

Four independent transformant lines were obtained, each of which carried a single insert of the dynein transgene. As shown in Figure 4B for the transformant $P(Dhc^+)^X$, Southern blot analysis of genomic DNA using a probe specific for the transgene detected a unique band in each transformed line. In genetic linkage tests, we determined that one transformant line $[P(Dhc^+)^X]$ contained the transgene on the *X* chromosome, while

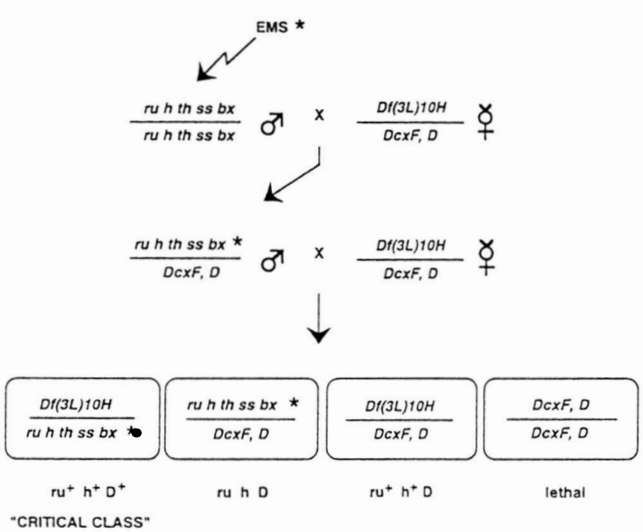

FIGURE 3.—Scheme for the isolation of lethal mutations in the *Df(3L)10H* region. EMS-treated *ru h th ss bx* (or *mwh jv ca*) males were mass-mated to *Df(3L)10H/DcxF* virgin females. Male *ru h th ss bx/DcxF* progeny were mated singly to *Df(3L)10H/DcxF* virgin females, and the progeny were scored for the absence of the D^+ progeny class. For details, see MATERIALS AND METHODS.

TABLE 1

Lethal complementation groups

Group	No. of alleles	Allele name[a]
1 *l(3)64Ca*	18	1-1,3-2,4-3,4-6,4-16,4-18, 4-19,4-22,5-6,5-7,5-8, 5-12,6-4,6-6,6-8,6-10, 6-12,8-1
2 *l(3)64Cb*	6	1-2,1-6,3-7,6-1,6-2,6-7
3 *l(3)64Cc*	5	1-3,3-10,4-4,5-2,5-5
4 *l(3)64Cd*	4	3-6,5-3,6-3,6-5
5 *l(3)64Ce*	3	3-8,4-12,4-15
6 *l(3)64Cf*	2	2-2,2-4
7 *l(3)64Cg*	2	1-4,3-5
8 *l(3)64Ch*	1	3-9

[a] Each allele was derived from a single mutagenized male. The first number in each mutant name corresponds to the experiment in which it was isolated.

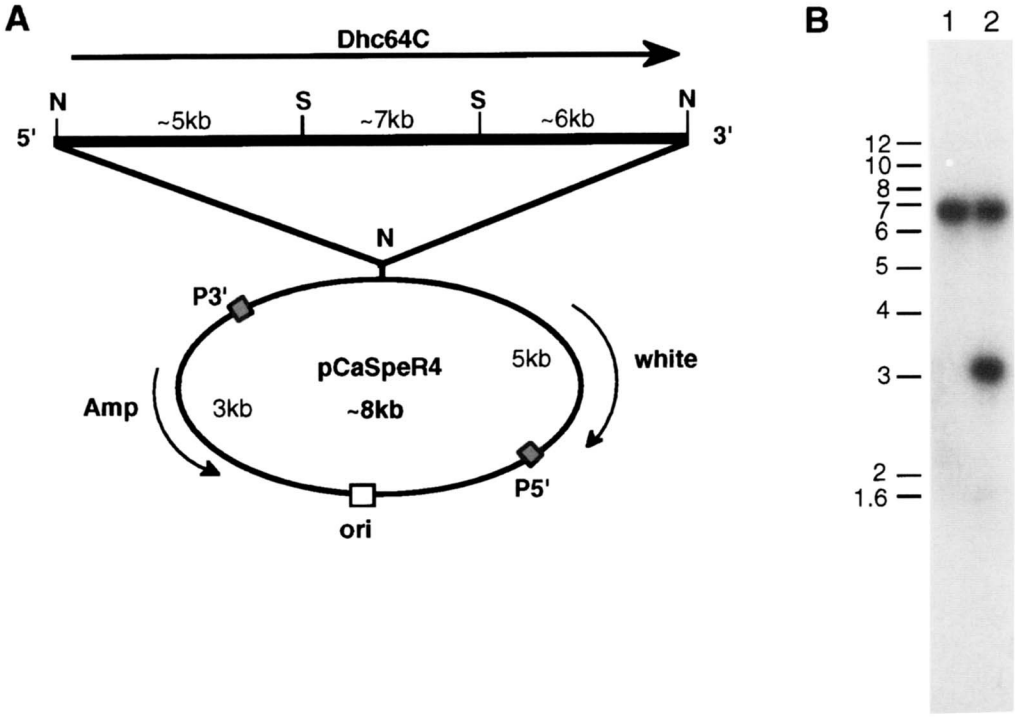

FIGURE 4.—Construction and transformation of the *Dhc64C* transgene used for rescue of the dynein mutants. (A) Map of the *Dhc64C* transgene construct *P(Dhc*$^+$*)*. The construct contains an ~19-kb insert derived from genomic DNA cloned into the vector *pCaSpeR4*. The insert, comprised of three *Not*I/*Sph*I (N, *Not*I; S, *Sph*I) fragments of ~5–7 kb each, includes the complete dynein transcription unit and no additional genes (see MATERIALS AND METHODS). The *P*-element inverted repeats (P3′ and P5′), the bacterial origin of replication (ori) and ampicillin resistence gene (Amp), and the eye color marker gene *white* are also present on the *pCaSpeR4* vector. (B) Genomic DNA analysis of the transformant *P(Dhc*$^+$*)*^{X}. Genomic DNA was digested with *Eco*RI and probed with a genomic fragment that recognizes both the endogenous and transgenic copy of the *Dhc64C* gene. The endogenous copy of the *Dhc64C* gene lacks an *Eco*RI restriction enzyme site that is present in the *P(Dhc*$^+$*)* transgene construct. The *w-c* stock (lane 1) used for transformation shows hybridization of the probe to only the endogenous copy of *Dhc64C*. For the transformed line *P(Dhc*$^+$*)*^{X} (lane 2), the probe hybridizes to both the endogenous *Dhc64C* gene, as well as the second copy of the *Dhc64C* gene present on a unique ~3-kb fragment derived from the single *P*-element insert.

the other three lines contained the insert on chromosome *3*. For the *X*-chromosome insert and one of the third chromosome transformants, flies homozygous for the insert chromosome were viable and fertile, indicating that the insertion event did not disrupt a vital gene. Moreover, these results suggest that four copies of the wild-type *Dhc64C* gene (two copies of the transgene and the two endogenous genomic genes) do not severely affect fly viability or fertility. We have also shown that flies with six copies of *Dhc64C* (four transgenes plus two genomic copies) are also viable and fertile (K. BOYLAN and T. S. HAYS, unpublished data).

To determine which of the eight complementation groups identified the *Dhc64C* gene, *P(Dhc*$^+$*)*^{X} flies were crossed to mutations in each of the complementation groups. Males heterozygous for the deficiency *Df(3L)10H* and carrying the dynein transgene on the *X* chromosome were crossed with virgin female flies that carried the lethal mutation to be tested. In the resultant progeny, all the female flies will inherit the *X* chromosome from their fathers and so will contain the dynein transgene. Female progeny that also inherited the *Df(3L)10H* chromosome from their fathers and the lethal dynein mutation (*m*) from their mothers will die unless rescued by the *X*-linked dynein transgene. As an internal control, all of the male progeny will inherit their *X* chromosome from their mothers and therefore will not contain the wild-type dynein transgene. In the absence of the transgene, male progeny of the genotype *m/Df(3L)10H* will not be rescued and this class of progeny should be absent (Figure 5).

The dynein transgene *P(Dhc*$^+$*)*^{X} only rescued the hemizygous lethality of alleles in the complementation group *l(3)64Ca* (group 1; Table 1). For all *l(3)64Ca* (group 1) mutations, *P(Dhc*$^+$*)*^{X}*; m/Df* female flies survived (where *m* refers to the lethal mutation and *Df* is *Df(3L)10H*), whereas sibling *w/Y; m/Df* males did not. For each of the alleles, the number of rescued female flies of the genotype *P(Dhc*$^+$*)*^{X}*; m/Df* were similar to the number of female flies in the sibling classes (Table 2). These results indicate that the viability of *l(3)64Ca* (group 1) alleles appears to be completely restored by the wild-type dynein transgene.

We repeated the rescue experiments by crossing *P(Dhc*$^+$*)*^{X}*/P(Dhc*$^+$*)*^{X}*; Df(3L)10H/TM6B, D* females to *w/Y; m/TM6B, D* males. These experiments showed that

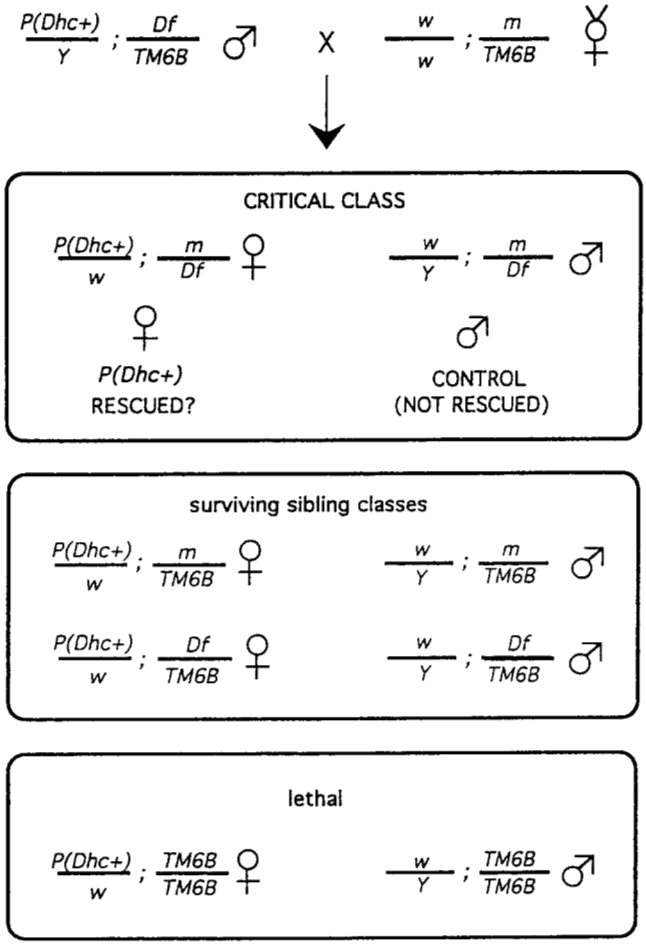

FIGURE 5.—Scheme for the rescue of cytoplasmic dynein heavy chain mutants. *P(Dhc$^+$)/Y; Df/TM6B, D Hu* males were crossed to *w; m/TM6B, D Hu* females. *Df* is *Df(3L)10H* and *m* refers to a lethal mutation. For mutations in *Dhc64C*, D^+ Hu^+ females should survive but D^+ Hu^+ males should not. For mutations outside *Dhc64C*, all D^+ Hu^+ flies should die. Results from this cross are shown in Table 2.

for *l(3)64Ca* (group 1), *P(Dhc$^+$)^X* could rescue both male and female critical class progeny (data not shown). Since the *Dhc64C* transgene, *P(Dhc$^+$)^X*, was able to rescue the lethality of *l(3)64Ca* mutations, we conclude that this complementation group consists of lethal mutations in the cytoplasmic dynein heavy chain gene, *Dhc64C*. These mutations will subsequently be referred to as *Dhc64C* (*Dhc*) mutations.

To attempt to locate the lesions associated with the *Dhc64C* mutations, genomic DNA blots and protein immunoblots were performed on flies heterozygous for each of the mutations. Digested DNA from adult flies heterozygous for each of the mutations were hybridized with a *Dhc64C* probe that included the entire gene. No differences were detected between the mutations indicating that none of the mutations were associated with large genetic rearrangements or deletions (data not shown). To detect aberrant heavy chain polypeptides, cytoplasmic extracts were prepared from the ovaries of heterozygous mutant females and subjected to immunoblot blot analysis using antibodies that specifically recognize the dynein heavy chain (HAYS *et al.* 1994; LI *et al.* 1994). Here again, no differences were observed between the mutants, suggesting that none of the mutations were nonsense mutations that resulted in premature termination of translation and a detectably truncated heavy chain polypeptide (data not shown). However, our immunoblot analyses do not eliminate the possibility of small truncations of the heavy chain that would not have been resolved or that are unstable.

Cytoplasmic dynein zygotic function is required for larval and pupal development: To determine the stage of development at which zygotic dynein function is required, we first examined whether embryos hemizygous for each dynein allele in combination with the deficiency *Df(3L)10H* could survive to larval and pupal stages. Each of the *Dhc64C* mutations were balanced over the *TM6B, Tb Hu* chromosome and mated to females of the genotype *Df(3L)10H/TM6B, Tb Hu*. The presence of non-*Tb*, *i.e.*, Tb^+, larvae or pupae were scored in the resultant progeny. The *Dhc64C* alleles Dhc^{1-1}, Dhc^{3-2}, Dhc^{4-3}, Dhc^{4-6}, Dhc^{4-16}, Dhc^{4-18}, Dhc^{4-19}, Dhc^{4-22}, Dhc^{5-6}, Dhc^{5-7}, Dhc^{5-8}, Dhc^{5-12}, and Dhc^{6-4} were characterized as strong alleles since only *Tb* larvae were observed. The *Dhc64C* alleles Dhc^{6-6}, Dhc^{6-8}, Dhc^{6-10}, Dhc^{6-12}, and Dhc^{8-1} were characterized as weak alleles since both Tb^+ larvae and pupae were observed. These weak alleles therefore support partial larval development with lethality occuring during the second or third instar, or complete larval development with a pupal or pharate adult lethal phase.

We subsequently retested strong and weak alleles of the dynein heavy chain gene to examine more closely whether dynein function was required during embryogenesis. The deficiency *Df(3L)10H* is associated with a semidominant maternal effect that results in 30% embryonic lethality (J. GEPNER and T. S. HAYS, unpublished data). While this did not affect our scoring of the presence or absence of Tb^+ larvae and pupae as described above, it prohibited interpreting the lethality of hemizygous embryos in terms of dynein dysfunction. To avoid the semidominant lethality associated with *Df(3L)10H*, we instead scored the lethal phase for embryos doubly heterozygous for strong and weak dynein alleles. The results of these experiments are given in Table 3. In the case of the strong allele Dhc^{4-19}, most (~75–80%) of the lethality occurred during larval stages in both homozygous and transheterozygous (Dhc^{4-19}/Dhc^{5-8}) mutant embryos. In the case of the weak dynein allele Dhc^{6-10}, the lethality was more evenly divided between pupal (55%) and larval (42%) stages. For the dynein alleles tested relatively low levels (3–12%) of embryonic lethality were observed, suggesting that the maternal complement of cytoplasmic dynein heavy chain is indeed sufficient for embryonic development. The total lethality observed for both strong and weak alleles was observed to exceed the expected 25%

TABLE 2

Transformation rescue of lethal mutations

Mutant	Group	Genotype					
		P(Dhc⁺)/w; m/Df[a]	*w/Y; m/Df*	*P(Dhc⁺)/w; m/TM6B*	*w/Y; m/TM6B*	*P(Dhc⁺)/w; Df/TM6B*	*w/Y; Df/TM6B*
1-1	1	33	1[b]	25	31	34	43
3-2	1	59	0	58	55	59	61
4-3	1	79	0	76	61	77	58
4-6	1	72	0	47	61	39	32
4-16	1	100	0	77	100	72	75
4-18	1	60	0	56	45	44	37
4-19	1	56	0	37	48	50	31
4-22	1	47	0	65	62	35	33
5-6	1	54	0	66	59	42	50
5-7	1	34	0	45	55	39	36
5-8	1	53	0	61	43	36	35
5-12	1	38	0	47	50	34	36
6-4	1	62	0	70	57	54	43
6-6	1	54	0	50	57	21	24
6-8	1	38	20[c]	48	21	45	20
6-10	1	40	0	50	43	29	27
6-12	1	48	44[c]	61	39	58	28
8-1	1	68	0	67	61	66	69
1-2	2	0	0	51	67	65	36
3-7	2	0	0	72	68	57	46
1-3	3	0	0	73	79	78	74
3-10	3	0	0	38	53	35	39
3-6	4	0	0	36	37	32	29
6-3	4	0	0	100	88	108	83
3-8	5	0	0	49	52	73	57
4-15	5	0	0	57	70	68	44
2-2	6	0	0	94	91	71	77
2-4	6	0	0	58	52	53	32
3-9	8	0	0	72	78	73	66

[a] *m* refers to the lethal mutation; *Df* is *Df(3L)10H; P(Dhc⁺)* refers to the dynein heavy chain transgene.
[b] There was one *Dichaete*⁺ male with orange eyes that probably resulted from nondisjunction.
[c] The rescue of these two *Dhc* alleles was performed using *P(Dhc⁺)/P(Dhc⁺); Df/TM6B* females. As described in the text this results in the rescue of both males and females.

of the embryos that should comprise the critical class (Dhc^{-}/Dhc^{-}). The elevated level of lethality observed in the Oregon R wild-type strain (Table 3, row 1) suggests that the Oregon R genetic background used in the outcross of the dynein mutations contributed to the higher levels of lethality in the analysis of the dynein alleles. It should be also be noted that even the strong dynein alleles are not complete loss-of-function mutations. This

TABLE 3

Determination of lethal phases of *Dhc64C* mutations

Genotypes of parents		No. of individuals at each stage				Total lethality (%)	Lethal phase (% of total lethality)		
Maternal	Paternal	Eggs	Larvae	Pupae	Adults		% embryonic	% larval	% pupal
+/+	+/+	230	221	203	199	13	29	58	13
$Dhc64C^{4\text{-}19}$/+	$Dhc64C^{4\text{-}19}$/+	378	374	283	258	32	3	76	21
$Dhc64C^{4\text{-}19}$/+	$Dhc64C^{5\text{-}8}$/+	288	283	212	200	31	6	81	13
$Dhc64C^{6\text{-}6}$/+	$Dhc64C^{6\text{-}6}$/+	300	286	231	180	40	12	46	42
$Dhc64C^{6\text{-}10}$/+	$Dhc64C^{6\text{-}10}$/+	376	373	329	271	28	3	42	55
$Dhc64C^{6\text{-}10}$/+	$Dhc64C^{6\text{-}6}$/+	300	299	291	272	9	4	28	68

Balanced stocks containing the *Dhc64C* mutations were outcrossed to Oregon R (+/+) to generate the parental genotypes. Parents were mated for several days and then egg collections were taken and followed through development to determine the lethal phase (see MATERIALS AND METHODS).

Dhc64C Complementation Analysis.

Mutant	1-1	3-2	4-3	4-6	4-16	4-18	4-19	4-22	5-6	5-7	5-8	5-12	6-4	6-6	6-8	6-10	6-12	8-1	10H
1-1	-	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	AL	L	L
3-2		-	L	L	L	L	L	L	L	L	L	L	L	L	L	AL	V	AL	L
4-3			-	L	L	L	L	L	L	L	L	L	L	L	L	L	AL	V	L
4-6				-	L	L	L	L	L	L	L	L	L	L	L	L	AL	AL	L
4-16					-	L	L	L	L	L	L	L	L	L	L	L	AL	AL	L
4-18						-	L	L	L	L	L	L	L	L	L	L	AL	AL	L
4-19							-	L	L	L	L	L	L	L	L	L	AL	V	L
4-22								-	L	L	L	L	L	L	L	L	AL	AL	L
5-6									-	L	L	L	L	V	V	V	V	V	L
5-7										-	L	L	L	L	L	L	AL	AL	L
5-8											-	L	L	L	L	L	AL	AL	L
5-12												-	L	L	L	L	AL	AL	L
6-4													-	L	L	L	AL	AL	L
6-6														-	V	V	V	AL	L
6-8															-	L	L	V	L
6-10																-	V	V	L
6-12																	-	V	AL
8-1																		-	L
10H																			-

FIGURE 6.—Complementation chart of adult flies heterozygous for *Dhc64C* alleles. *Inter se* crosses were performed between all *Dhc64C* (group 1) alleles. The viability of adult progeny heterozygous for two *Dhc64C* mutations is indicated as V (viable), AL (adult-lethal), or L (lethal). 10H refers to the third chromosome deficiency *Df(3L)10H*.

is most simply indicated by the complementation pattern of the allele Dhc^{8-1}. As shown in Figure 6, the Dhc^{8-1} allele is hemizygous lethal with *Df(3L)10H* but complements all *Dhc64C* mutations with the exception of Dhc^{1-1}. This observation indicates that even the strong *Dhc64C* alleles retain partial function. Therefore, we cannot rule out the possibility that a complete loss of function, or null mutation, in *Dhc64C* might reveal a requirement for zygotic dynein function late in embryogenesis.

Our lethal phase studies suggest that a maternal contribution of cytoplasmic dynein is sufficient to support most of embryogenesis. To support this conclusion, we characterized the distribution of three previously characterized molecular markers in the dynein mutants Dhc^{3-2} and Dhc^{4-19}. The *snail* (*sna*) gene product is required for gastrulation and is expressed shortly after cellularization of the blastoderm in a band along the ventral midline of wild-type embryos (LEPTIN and GRUNEWALD 1990). *engrailed* (*en*) encodes a homeodomain protein that is prominently detected in the central and peripheral nervous system around 8 hr of development (PATEL *et al.* 1989). The third molecular marker is also present in the developing nervous system and can be detected by the monoclonal antibody BP102 (KLAMBT *et al.* 1991). The distributions of all three molecular markers in the hemizygous dynein mutant backgrounds were indistinguishable from the patterns observed in heterozygous sibling progeny (data not shown). The wild-type distribution of the three molecular markers suggests that morphogenesis during at least the first 16 hr of embryonic development is not grossly defective in the dynein mutants. These results further indicate that the maternal endowment of cytoplasmic dynein to the embryo is sufficient to support the majority of embryogenesis in the absence of a zygotic contribution.

Intragenic complementation between *Dhc64C* alleles reveals additional zygotic and maternal functions for cytoplasmic dynein: Figure 6 shows the complementation behavior for the reported *Dhc64C* alleles. Most combinations of *Dhc64C* alleles are lethal, but several combinations of alleles result in viable adults. While several intragenic combinations produce pupae that eclose, these adults frequently die within the first day after eclosure. We refer to this phenotype as adult-lethal (AL). In other cases intragenic complementation results in adults with normal viability (V). As shown in Figure 6, complementation involves the alleles Dhc^{5-6}, Dhc^{6-6}, Dhc^{6-8}, Dhc^{6-10}, Dhc^{6-12}, *and* Dhc^{8-1}. To ask whether the complementation results simply from the combined activity of two hypomorphic alleles that together provide sufficient amounts of dynein function, we have compared the homozygous and double heterozygous phenotypes for the dynein alleles Dhc^{6-6} and Dhc^{6-10}. As indicated in Table 3, both dynein alleles are homozygous lethal. We determined that the lethality in both homozygotes can be rescued by a dynein transgene confirming that the lethality is due to the dynein mutations. In contrast, flies that are doubly heterozygous for the

TABLE 4

Adult phenotypes in heteroallelic *Dhc64C* mutants

Heteroallelic combination	Phenotypes
Dhc^{3-2}/Dhc^{6-10}	Eyes are small and rough; bristles are short and thin
Dhc^{4-19}/Dhc^{8-1}	Eyes are darker in color and rounder in shape than wild type; bristles are short and thin with bent tips
Dhc^{3-2}/Dhc^{6-12}	Female sterile; male fertile
Dhc^{6-10}/Dhc^{6-12}	Female sterile; male sterile

two alleles are viable. The two mutant dynein heavy chains are functional in the presence of one another, but individually are nonfunctional. This result suggests that the products of the two dynein alleles exhibit protein complementation and provide genetic evidence for the known dimeric nature of the dynein heavy chains within the native dynein motor complex. The nature of the lesions present in the mutant heavy chains may shed light on functional domains in the dynein polypeptide.

In addition to the implications for dynein structure, the intragenic complementation of dynein alleles has revealed additional processes that require dynein function. Defects in bristle formation, eye development, and fertility can be observed in adult flies that are transheterozygous for certain of the complementing dynein alleles. Table 4 summarizes the range of adult phenotypes observed in flies doubly heterozygous for *Dhc64C* mutants, listing as examples those combinations of dynein alleles that result in the most severe adult phenotypes. The underlying cellular defects that contribute to the observed phenotypes remain to be determined.

Mutations in cytoplasmic dynein act as cell lethals: We have tested the hypothesis that dynein function is required for cell viability by generating somatic clones of cells that are homozygous for the strong dynein allele Dhc^{4-19}. We previously determined that the lethality in flies homozygous for the *mwh* $Dhc64C^{4-19}$ *jv ca* chromosome can be rescued by a wild-type dynein transgene. This control experiment indicated that no additional lethals were present on the chromosome that would produce misleading results in the clonal analysis. Mitotic clones were generated in the wing, eye, thoracic, and abdominal epidermis using the FRT/FLP recombinase system (GOLIC 1991; XU and RUBIN 1993). These experiments were carried out in the presence or absence of the dynein transgene $P(Dhc^{+})^{X}$ introduced on the *X* chromosome. The mutation *multiple wing hair* (*mwh*) was used to score clones produced in the wing, the eye color marker *white* (*w*) indicated the presence of clones in the eye, and the mutations *yellow* (*y*) and *javelin* (*jv*) served as clonal markers in the thoracic and abdominal epidermis of adult flies. As indicated in Figure 7, we recovered numerous large clones of homozygous mutant cells in several tissues when the wild-type dynein transgene was present. In the absence of the transgene, we failed to observe clones in any of the tissues examined except the wing, where some small clones were infrequently observed. The small mutant clones detected in the wing tissue most likely reflect the perdurance of the wild-type dynein product for a few cell divisions following the recombination event, the production of the clone, and the loss of dynein gene activity. Figure 8 presents examples of the clones obtained in the imaginal wing disc. The greatly reduced viability of somatic homozygous mutant clones in all four tissues examined, except in the presence of the $P(Dhc^{+})$ transgene, indicates that dynein function is required in a cell autonomous manner for cell viability.

DISCUSSION

Lethal mutations in the Drosophila cytoplasmic dynein heavy chain gene were isolated and provide direct evidence that cytoplasmic dynein motor function is essential in a multicellular organism. Animals that are homozygous for mutations in cytoplasmic dynein result in larval or pupal lethality, suggesting a delayed requirement for zygotic dynein function. The postembryonic, zygotic-lethal phenotype is readily explained by a maternal endowment of dynein supporting the majority of embryogenesis. In this regard, we have previously shown that *Dhc64C* is abundantly transcribed during oogenesis and that the heavy chain polypeptide accumulates in the oocyte (LI *et al.* 1994). GATTI and BAKER (1989) have described the phenotype of late larval/pupal lethality as diagnostic of mutations in essential cell cycle functions. Our clonal analysis shows that dynein function is required for cell viability in the eye, thorax, abdomen, and wing, suggesting that cytoplasmic dynein is required autonomously in all cells, perhaps during cell division.

Mutations in the cytoplasmic dynein heavy chain have also been isolated in *S. cerevisiae* (ESHEL *et al.* 1993; LI *et al.* 1993), *A. nidulans* (XIANG *et al.* 1994), and *N. crassa* (PLAMANN *et al.* 1994). In contrast to the observation in Drosophila, dynein heavy chain mutations in these fungi are not lethal. For example, yeast cells in which the dynein gene has been disrupted grow more slowly than wild type and a portion of those (14–38% at 11°) (LI *et al.* 1993) appear bi- and multinucleate. In yeast, cytoplasmic dynein appears to function in the proper migration of nuclei during mitosis (ESHEL *et al.* 1993; LI *et al.* 1993). Dynein function in yeast may be nonessential due to other redundant mechanisms involved in nuclear migration. Alternatively, either dynein-mediated nuclear migration is more critical in metazoan cells, or the dynein motor has been utilized for other essential cellular and/or developmental functions.

The characterization of multiple cytoplasmic dynein heavy chain alleles and their pattern of intragenic com-

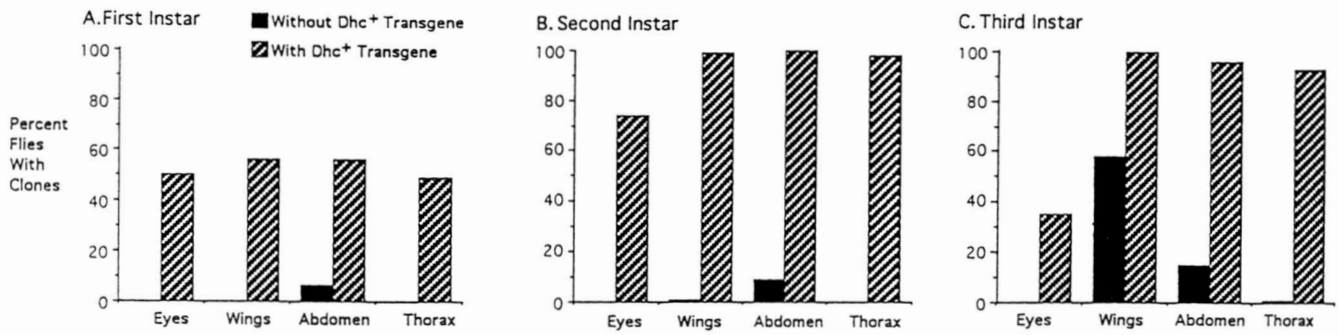

FIGURE 7.—Frequency of somatic clones of homozygous mutant $Dhc^{4\text{-}19}/Dhc^{4\text{-}19}$ cells in the presence or absence of the $P(Dhc^{+})$ transgene. Using the FRT/FLP recombinase system (see MATERIALS AND METHODS), recombination was induced in heterozygous $Dhc^{4\text{-}19}/+$ animals during first (A), second (B), and third (C) instar larval stages. Clones were scored in the eye, wing, thoracic and abdominal epidermis in the presence (▨) or absence (■) of the $P(Dhc^{+})X$ transgene. For clones in the eye, 250–300 flies of each genotype were scored. For clones in the wing, thorax, and abdomen, a total of at least 46 flies from three separate experiments were dissected and scored. Flies were counted positive if they contained one or more clones in at least one eye, or at least one bristle clone in the wings and thorax. In the abdomen, clones were counted only if two or more adjacent bristles were seen. Single bristle abdominal clones appear to reflect spontaneous recombination in the abdominal histoblasts.

plementation has revealed phenotypes in oogenesis, spermatogenesis, bristle formation, and eye development. Based on an asymmetric pattern of distribution during oogenesis, we have previously speculated that cytoplasmic dynein may be required for the directional transport of cytoplasmic constituents to the presumptive oocyte within the syncytial egg chamber. The female sterile and maternal effect lethal phenotypes observed for certain interallelic combinations of dynein alleles are consistent with this hypothesis. The male sterile phenotype may result from a similar defect in cytoplasmic transport among the cohort of 16 primary spermatocytes that also retain cytoplasmic bridges during spermatogenesis and spermatid differentiation. In addition to the female and male sterile phenotypes, adult flies doubly heterozygous for certain of the isolated dynein alleles exhibit abnormally short bristles with aberrant tapering at the distal tips. The morphology of the bristle is determined by the cuticle surrounding the cytoplasmic extension of the trichogen (bristle) cell. Electron microscopy reveals that the cytoplasmic extension contains a central core of microtu-

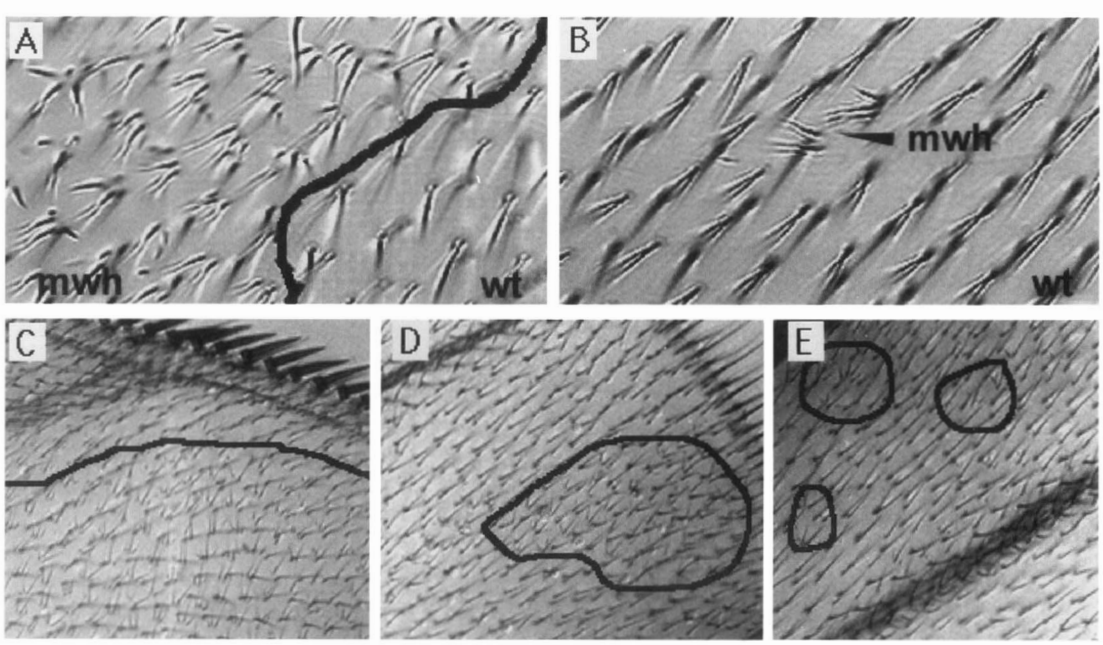

FIGURE 8.—Micrographs of somatic $Dhc^{4\text{-}19}$ clones in wing tissue. (A and B) Representative $Dhc^{4\text{-}19}/Dhc^{4\text{-}19}$ clones in the wing marked with *mwh* in the presence (A) or absence (B) of the $P(Dhc^{+})$ transgene. In the absence of the transgene, small clones were seen only when recombination was induced late in larval development (arrow, B) and may reflect perdurance of the wild-type dynein gene product. (C–E) Cells in the wing disc divide continuously throughout larval development. This is reflected in the size and number of clones detected when recombination is induced at different larval stages. Shown are mitotic clones induced in the presence of the $P(Dhc^{+})$ transgene during the first (C), second (D), and third (E) larval instars. Induction of recombination at later stages of development resulted in a higher frequency of clones that were smaller in size.

bules aligned with the long axis of the bristle and surrounded by multiple spherical islands of actin filament bundles lying in specific positions adjacent to the cell membrane (TILNEY *et al.* 1995). These microtubule and microfilament arrays are thought to support the cytoplasmic extension of the bristle cell and to participate in the secretion of the surrounding cuticle (OVERTON 1967). In this regard, the shortened bristles observed in the dynein mutant backgrounds may result from the failure to extend the bristle cell cytoplasm or from a compromised secretion of cuticle. Another phenoytpe observed in adult flies doubly heterozygous for dynein alleles is the disruption of the hexagonal array of ommatidia in the eye. This defect reflects the failure to complete and/or maintain the proper differentiation of the ommatidial cluster of cells (MEYEROWITZ and KANKEL 1978; RENFRANZ and BENZER 1989).

The rescue of each mutant phenotype by the introduction of a wild-type dynein transgene demonstrates that the observed phenotypes are due to dynein dysfunction. However, further analysis will be required to reveal the underlying basis for each phenotype. In particular, it will be important to discriminate between the possible tissue- and cell-specific functions of dynein that contribute to morphogenetic processes, and those functions ubiquitously required for cell viability and/or cell division. While multiple phenotypes may imply multiple functions, we cannot exclude the possibility that defects in a single dynein-mediated process could account for all the observed phenotypes. For example, different cell types might require different levels of dynein-mediated organelle transport. A partial loss-of-function allele might still provide the proper threshold of transport for one cell, but not another. Further analysis of the cellular defects associated with the observed dynein mutant phenotypes should shed light on the functional range of the dynein motor.

Intragenic complementation has classically been taken as evidence that the encoded products of the two alleles are capable of forming a homodimer (CRICK and ORGEL 1964; CHOVNICK 1989). The complementation of dynein alleles therefore provides *in vivo* evidence that confirms previous biochemical studies demonstrating the homodimeric nature of the heavy chain polypeptides in the native dynein motor complex. Our results further suggest that the complementing mutations reside in separable domains of the dynein polypeptide and that only a single copy of each functional domain is required for partial function of the assembled homodimer. According to this model, dynein complexes that consist of two heavy chain polypeptides, each of which is defective in a different domain, may still be functional. Dynein complexes that consist of two heavy chain polypeptides, each of which is defective in the same domain, would be nonfunctional. For example, $Dhc^{5\text{-}6}$ might eliminate the function of "domain 1", $Dhc^{6\text{-}6}$ might affect "domain 2", and $Dhc^{6\text{-}8}$ and $Dhc^{6\text{-}10}$ could both alter "domain 3." Thus, $Dhc^{5\text{-}6}$ could complement $Dhc^{6\text{-}6}$, $Dhc^{6\text{-}8}$ and $Dhc^{6\text{-}10}$ since they affect different domains, but $Dhc^{6\text{-}8}$ and $Dhc^{6\text{-}10}$ fail to complement each other since they destroy the same domain. Consistent with this model, all the complementing mutations are lethal as hemizygotes. One possibility is that during assembly of the dynein homodimer, the functional copy of each domain provides a template on which the mutant domain is allowed to properly fold and recover activity. As an alternative explanation for intragenic complementation, it is also possible that certain of the complementing mutations are simply hypomorphic, resulting in heavy chain polypeptides that have a low level of activity. According to this explanation, flies hemizygous for a hypomorphic allele fail to survive because they do not retain enough dynein activity, but two such hypomorphic alleles provide enough dynein activity for adult survival. We have discounted this explanation directly in the lethal phase analyses of the complementing alleles $Dhc^{6\text{-}6}$ and $Dhc^{6\text{-}10}$. Homozygotes bearing either of the two complementing alleles die as larvae or pupae. The lethality can be rescued by the introduction of the wild-type dynein transgene. In contrast, flies that are doubly heterozygous for $Dhc^{6\text{-}6}$ and $Dhc^{6\text{-}10}$ survive as healthy adults without the dynein transgene. A third interpretation of the interallelic complementation is that a particular allele "suppresses" a mutation in a second allele by an allele-specific interaction that restores function. It is more difficult to understand the observed complementation pattern in terms of specific suppression, because one would have to argue that $Dhc^{5\text{-}6}$ was able to specifically restore function to several alleles, some of which are able to restore function to each other.

The analysis of the molecular lesions present in the complementing alleles may help to relate structural and functional domains within the dynein heavy chain. For example, certain of the *Dhc64C* alleles that complement one another also interact genetically with a dominant mutation in the $p150^{Glued}$ component of dynactin (MCGRAIL *et al.* 1995). Dynactin is a multisubunit complex that has been proposed to regulate dynein motor activity (GILL *et al.* 1991; SCHROER and SHEETZ 1991). We have shown that certain dynein alleles act as dominant suppressors of the $Glued^{1}$ mutation, while other alleles act as dominant enhancers of the $Glued^{1}$ mutation. A molecular understanding of the lesions in the dynein alleles may provide clues to the mechanism by which the dynactin complex associates with and regulates cytoplasmic dynein-mediated transport.

Cytoplasmic dynein complexes from rat, chick and cow have been shown to consist of two heavy chains as well as several smaller polypeptides (PASCHAL *et al.* 1987; COLLINS and VALLEE 1989; STEUER *et al.* 1990). To date, only one cytoplasmic dynein heavy chain gene has been detected in *Dictyostelium discoideum* (KOONCE *et al.* 1992), *S. cerevisiae* (ESHEL *et al.* 1994; LI *et al.* 1994), *A. nidulans*

(XIANG *et al.* 1994), *N. crassa* (PLAMANN *et al.* 1994), *Caenorhabditis elegans* (LYE *et al.* 1995) and *Rattus norvegicus* (MIKAMI *et al.* 1993), suggesting that a single gene encodes both heavy chain polypeptides. In Drosophila, *Dhc64C* appears to be the major cytoplasmic dynein heavy chain gene. Northern analysis shows that *Dhc64C* is the only dynein heavy chain highly expressed outside the testis, although low levels of other dynein heavy chain transcripts can be detected outside the testis using PCR methods (RASMUSSON *et al.* 1994). Regardless of whether additional cytoplasmic dynein isoforms remain to be identified, the fact that mutations in *Dhc64C* are lethal indicates that other dynein heavy chain genes in Drosophila cannot strictly substitute for *Dhc64C* function.

In summary, we report the isolation of mutations in eight essential genes in the 64B/C region of the third chromosome of *D. melanogaster.* Since one of our lethal complementation groups had only one member and two groups had only two members each, we may not have saturated the region for lethal mutations. In fact, attempts to isolate lethal mutations in this region by *P*-element insertion have uncovered an additional lethal complementation group that appears to be distinct from the mutations reported here (M. MCGRAIL, J. GEPNER and T. HAYS, unpublished observations). The lethal mutations in one of the complementation groups identify the Drosophila cytoplasmic dynein heavy chain gene *Dhc64C* as an essential gene. Further characterization of the *Dhc64C* mutations will enable us to define cellular and developmental processes requiring cytoplasmic dynein function, to begin to dissect functional domains within this extraordinarily large polypeptide and to identify other components that interact specifically with the cytoplasmic dynein heavy chain.

We thank JIM GARBE for generously providing deficiencies in the 64C region, especially *Df(3L)10H.* We also thank Drs. LYNN CROSBY and JOHN TAMKUN for stocks and helpful suggestions. We also thank Dr. JIM KENNISON for the *iso-1* stock, and Drs. COREY GOODMAN, AUDREY ALBERGA, and SEAN CARROLL for antibodies. This work was supported by the March of Dimes, the Pew Charitable Trusts, and National Institutes of Health (NIH) grant GM-44757 to T.S.H. Support for M.M. and K.B. was provided in part by NIH Research Training grant 5T32-07323 and National Science Foundation Research Training grant DIR91-11-44. M.-G.L. was supported by NIH postdoctoral fellowship GM-17485.

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Communicating editor: R. S. HAWLEY