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Genetics

A fertility region on the Y chromosome of *Drosophila melanogaster* encodes a dynein microtubule motor

JANICE GEPNER AND TOM S. HAYS

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108

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ABSTRACT **A clone encoding a portion of the highly conserved ATP-binding domain of a dynein heavy-chain polypeptide was mapped to a region of the *Drosophila melanogaster* Y chromosome. Dyneins are large multisubunit enzymes that utilize the hydrolysis of ATP to move along microtubules. They were first identified as the motors that provide the force for flagellar and ciliary bending. Seven different dynein heavy-chain genes have been identified in *D. melanogaster* by PCR. In the present study, we demonstrate that one of the dynein genes, *Dhc-Yh3*, is located in Y chromosome region h3, which is contained within *kl-5*, a locus required for male fertility. The PCR clone derived from *Dhc-Yh3* is 85% identical to the corresponding region of the β heavy chain of sea urchin flagellar dynein but only 53% identical to a cytoplasmic dynein heavy chain from *Drosophila*. *In situ* hybridization to *Drosophila* testes shows *Dhc-Yh3* is expressed in wild-type males but not in males missing the *kl-5* region. These results are consistent with the hypothesis that the Y chromosome is needed for male fertility because it contains conventional genes that function during spermiogenesis.**

The *Drosophila melanogaster* Y chromosome, which has both a long arm (YL) and a short arm (YS), is 12–18% longer than the X chromosome at metaphase (1, 2). In contrast to the many genes localized to the smaller X chromosome, very few genes have been identified on the Y chromosome. The Y chromosome is predominantly heterochromatic and to a large extent is composed of simple repetitive sequences (3). In *Drosophila*, normal females contain two X chromosomes (XX) and males contain one X and one Y (XY). XO flies are viable and completely sterile males (1), demonstrating that the Y chromosome is essential only for male fertility. In fact, the presence of the Y chromosome is only necessary in germ-line cells, since the transplantation of XY pole cells into XO males results in normal fertility (4). Several laboratories have shown that there are six regions of the Y chromosome required for male fertility (5–7). The four fertility regions on YL have been designated *kl-1, kl-2, kl-3*, and *kl-5* and the two regions on YS are *ks-1* and *ks-2*. Chromosomal breaks within any one of these regions result in male sterility. The six loci appear to be separate functional units since breaks in any two of the regions complement each other. Two additional loci that have been identified on the Y chromosome are bobbed (*bb*) and Suppressor of Stellate [*Su(Ste)*]. The *bb* locus encodes clusters of ribosomal RNA genes (ref. 8; reviewed in ref. 9), and the *Su(Ste)* locus is required for correct splicing of the gene product of the *Ste* locus on the X chromosome (10).

Two main hypotheses have been proposed regarding the functions of the Y chromosome fertility regions. One hypothesis states that these loci are required for male fertility because of a regulatory or protein binding function (3, 6, 11, 12). Three of the regions—*kl-5, kl-3*, and *ks-1*—form lampbrush-type loops in primary spermatocytes (13). It has been suggested that these loops may sequester proteins needed for spermiogenesis. Alternatively, the fertility regions on the Y chromosome may contain conventional structural genes that encode products required for spermatogenesis (14–20). In particular, it has been suggested that at least two of the fertility loci may code for dynein heavy chains (21).

Flagellar dyneins are large multisubunit motors that power the microtubule sliding responsible for flagellar motility (reviewed in ref. 22). These dynein complexes include at least two heavy chains of ≈500 kDa, which contain hydrolytic ATP-binding sites. In 1981, Hardy *et al.* (18) showed that deletion of either the *kl-3* or *kl-5* region of the Y chromosome resulted in the loss of the outer arm dynein from the sperm flagellar axoneme. Moreover, Goldstein *et al.* (21) reported that testis extracts from males deleted for either *kl-3* or *kl-5* were missing polypeptides that were similar in electrophoretic mobility to dynein heavy chains from *Chlamydomonas*. In addition, duplications of either *kl-5* or *kl-3* were observed to increase the synthesis of the corresponding high molecular weight polypeptide and a temperature-sensitive sterile mutation in *kl-5* severely reduced dynein outer arm assembly at the restrictive temperature. Based on these results, they suggested that the *kl-5* and *kl-3* fertility regions of the Y chromosome encode dynein heavy chains. It was argued, however, that these loci may only regulate dynein rather than contain dynein structural genes (23).

Recent work in our laboratory has identified a dynein heavy-chain gene family in *D. melanogaster* (T.S.H., unpublished data). Primers located near the highly conserved putative hydrolytic ATP-binding site of dynein heavy chains were used in the PCR to amplify a family of seven dynein-like sequences of ≈400 bp. Each of these sequences corresponds to a separate gene as shown by genomic Southern blot analysis and *in situ* hybridization to larval polytene chromosomes. *Dhc-Yh3* was found to be present in males but not in females and was the only one of the seven genes that could not be localized by *in situ* hybridization. Since the Y chromosome is underreplicated in larval salivary glands and therefore not easily visualized, these results suggested that *Dhc-Yh3* is located on the Y chromosome.

In the present study, we have made use of a series of fertile translocations between the X and Y chromosomes (5) to determine the specific location of *Dhc-Yh3* on the Y chromosome. We demonstrate that dynein heavy-chain sequences are localized to the *kl-5* region of the Y chromosome, one of the two fertility loci previously hypothesized to encode a dynein heavy chain. Since dyneins are known to power flagellar motility, our results provide evidence that at least one of the Y chromosome fertility regions encodes a polypeptide required for spermatogenesis.*

MATERIALS AND METHODS

Fly Stocks. The X–Y translocation stocks, *T(X;Y)V24*, $y\,y^+$ *w f*, *T(X;Y;3)W27*, $y\,y^+\,w\,f\,B^S$, *T(X;Y)E15*, $y\,y^+\,w\,f\,B^S$, and

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*The sequence reported in this paper has been deposited in the GenBank data base (accession no. L23199).

T(X;Y)F12, y y^+ w f B^S (constructed and described in ref. 5), were obtained from the Bowling Green Stock Center. *T(X;Y;3)W27* is a reciprocal translocation between the X, Y, and third chromosomes (25) but behaves like a simple X–Y translocation for the purposes of these experiments. Segmental aneuploids (26) were constructed by crossing *T(Y;2)H116* and *T(Y;3)R71* males with females from Y-autosome translocation stocks with Y chromosome breaks in YS and autosomal breaks adjacent to *T(Y;2)H116* or *T(Y;3)R71*, respectively. All Y-autosome translocation stocks were from the Indiana Stock Center (Bloomington). The three Y chromosome deficiencies, *Df(Y)S7*, *Df(Y)S9*, and *Df(Y)S10*, were generously supplied by M. Gatti and are described in ref. 6. The *y sn^3 v car* and *C(1)M4/0* stocks were gifts from M. Simmons (University of Minnesota). The control stock for Southern analysis, *iso-1* marked with *y; cn bw* and made isogenic 2 years ago by J. Kennison (National Institutes of Health), was provided by J. Tamkun (University of California, Santa Cruz). All cultures were maintained at 21°C on standard medium.

Southern Blot Analysis. DNA extracts were prepared from 25 females or 40 males by standard methods. Southern blots were performed as described by Sambrook *et al.* (27). Each lane represents the DNA from four to eight flies. Filters were probed with the 392-bp *Dhc-Yh3* clone labeled with digoxigenin. The isolation and cloning of this segment of *Dhc-Yh3* DNA will be described (T.S.H., unpublished data). Digoxigenin labeling by the random primer method and hybridization of labeled probe to filters were performed essentially as described by the manufacturer (Boehringer Mannheim). Labeled DNA was detected by using Lumiphos (Boehringer Mannheim).

DNA Sequencing. *Dhc-Yh3* DNA was sequenced with Sequenase version 2.0 (United States Biochemical) as recommended by the manufacturer.

Whole Mount *in Situ* Hybridizations. Whole mount *in situ* hybridizations to testes were performed essentially as described (28) with the following modifications: Testes were fixed in 4% paraformaldehyde in PBT (130 mM NaCl/10 mM sodium phosphate, pH 7.2/0.1% Tween 20) containing 1% dimethyl sulfoxide for 20 min at room temperature. Prehybridization and hybridization were at 55°C. Fixed testes were probed with digoxigenin-labeled *Dhc-Yh3* DNA prepared as described for Southern blot analysis. In these experiments, wild-type males were Oregon-R. Males lacking *kl-5* were created using the cross in Fig. 2*B*.

RESULTS

To map *Dhc-Yh3* to a region of the Y chromosome, males with four different X–Y translocations (Fig. 1) were crossed to females with normal X chromosomes marked with *y* (Fig. 2*A*). These four translocations subdivide the Y chromosome into five sections and enabled us to determine whether

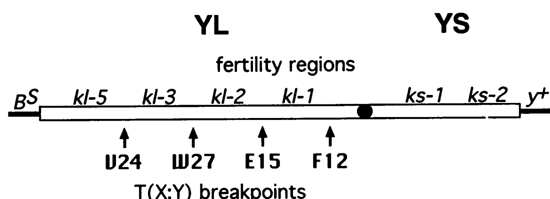

FIG. 1. *Drosophila* Y chromosome that was used to make the X–Y translocations (5). It is marked at the ends with B^S and y^+. The six regions required for male fertility are *kl-5*, *kl-3*, *kl-2*, and *kl-1* on YL and *ks-1* and *ks-2* on YS. The four fertile X–Y translocations used in this study—*T(X;Y)V24*, *T(X;Y;3)W27*, *T(X;Y)E15*, and *T(X;Y)F12*—have Y chromosome breakpoints between fertility regions. *T(X;Y)V24* has lost B^S.

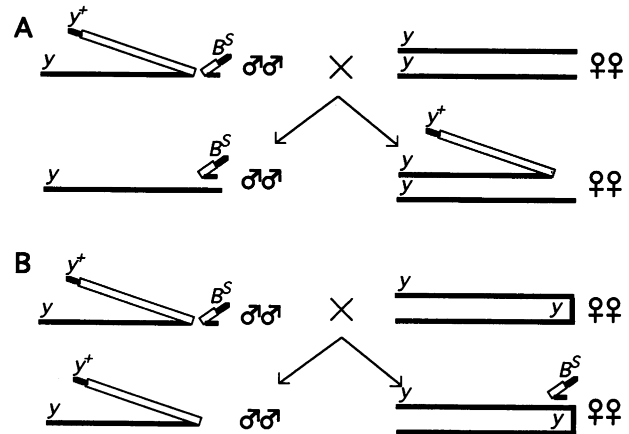

FIG. 2. Crossing schemes that separate the distal piece of YL from the remainder of the Y chromosome. (*A*) Males from each of the four X–Y translocation stocks with breakpoints in YL were crossed to *y sn^3 v car* females. Male progeny from this cross contain only the distal piece of YL; female progeny contain the rest of the Y chromosome. (*B*) *T(X;Y)V24* males were crossed to *C(1)M4/0* females. (These females have an attached X chromosome and no Y chromosome.) Female progeny from this cross contain the distal piece of YL; male progeny contain the remainder of the Y chromosome. (*A* and *B*) X chromosome is pictured as a solid line; Y chromosome is an open line.

Dhc-Yh3 is associated with one of the fertility regions on YL or with YS. In these translocations, the entire euchromatic portion of the X chromosome is attached to the proximal portion of the Y chromosome marked with y^+. The distal piece of the Y chromosome, marked with B^S, is attached to the X centromere and some centromeric X heterochromatin. The dominant markers enabled us to confirm the presence of YL or YS in the expected progeny classes.

Male progeny from the cross in Fig. 2*A* contain only the distal piece of the long arm of the Y chromosome and female progeny contain the rest of YL plus all of YS. In the case of *T(X;Y)V24*, male progeny contain only the most distal piece of YL, which includes a single male fertility locus, *kl-5*. Female progeny from this cross contain almost the entire Y chromosome with the exception of this distal piece of YL. Male progeny of *T(X;Y;3)W27* fathers contain about half of YL including both *kl-3* and *kl-5*. For *T(X;Y)E15*, male progeny contain three fertility regions—*kl-2*, *kl-3*, and *kl-5*. Finally, male progeny from *T(X;Y)F12* fathers contain all four YL fertility regions—*kl-1*, *kl-2*, *kl-3*, and *kl-5*. Female progeny from this cross contain YS plus a small amount of YL.

The male and female progeny derived from the crosses in Fig. 2*A* were used to localize *Dhc-Yh3* to a specific fertility region of the Y chromosome. The digoxigenin-labeled clone of *Dhc-Yh3* was hybridized to Southern blots of DNA prepared from the male and female progeny described above. For each cross, *Dhc-Yh3* was found in males but not in females (Fig. 3*A*). These results show that *Dhc-Yh3* is located in the region composed of *kl-5* and the tip of YL distal to *kl-5*, since this is the only Y region common to all male progeny and missing from all female progeny.

To confirm that *Dhc-Yh3* was located within the distal piece of YL containing *kl-5*, we showed that *Dhc-Yh3* is present in females that contain *kl-5* and that males missing *kl-5* lack *Dhc-Yh3* as well. This was accomplished by crossing *T(X;Y)V24* males to XX/O females (Fig. 2*B*). This cross produced females containing *kl-5* and the tip of YL and males that contained the rest of the Y chromosome, including all of the fertility regions except for *kl-5*. The Southern blot of the DNA isolated from these males and females is shown in Fig. 3*B*. In this case, females contained both *kl-5* and *Dhc-Yh3*. Similarly, males were missing both *kl-5* and *Dhc-Yh3*. Taken

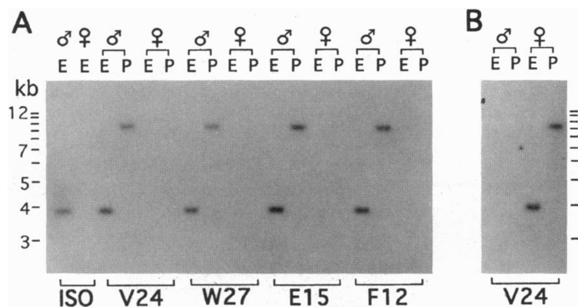

FIG. 3. Southern blots of DNA from progeny of the crosses in Fig. 2 hybridized with the digoxigenin-labeled *Dhc-Yh3* DNA fragment. DNA from flies of each genotype was digested with *Eco*RI (E) or *Pst* I (P). DNA from isogenic control flies was digested only with *Eco*RI and is shown on the left. Translocation of the male parent is indicated at the bottom. (*A*) DNA prepared from the progeny of the cross in Fig. 2*A*. For example, lanes 3–6 show DNA from the progeny of the cross between *T(X;Y)V24* males and *y sn³ v car* females. Note that, in every case, the males contain the distal piece of YL and also *Dhc-Yh3* DNA. (*B*) DNA from the progeny of the cross in Fig. 2*B*. Here the females contain the distal piece of YL and also *Dhc-Yh3*.

together, these results localize *Dhc-Yh3* to the distal piece of YL that includes the *kl-5* fertility region.

Dhc-Yh3 was further localized within the *kl-5* region by analyzing a series of Y chromosome deficiencies that have end points in *kl-5*. Gatti and Pimpinelli (6) have subdivided the Y chromosome into 25 cytological regions, designated h1–h25, which can be distinguished by fluorescent staining techniques. According to their analysis, *kl-5* extends from h1–h3 (Fig. 4). Their landmarks also define the regions of the Y chromosome deleted in the deficiencies tested here. *Df(Y)S10* removes h1–h10 and the tip of YL, *Df(Y)S7* removes h3–h8, and *Df(Y)S9* removes the proximal part of h3–h10 (6). Southern blot analysis of DNA from females carrying these deficiencies reveals that *Df(Y)S7* and *Df(Y)S10* remove *Dhc-Yh3* but *Df(Y)S9* does not (Fig. 5). This places the *Dhc-Yh3* clone within the region of *kl-5* that is between the end points of *Df(Y)S7* and *Df(Y)S9* or the distal two-thirds of region h3. Genomic DNA blot analysis of segmental aneuploids (data not shown; see ref. 26) also shows that *Dhc-Yh3* is located between the breakpoints of *T(Y;2)H116* in h3 and *T(Y;3)R71* in h1–h2 (Fig. 4). Since h3 is described as a nonfluorescent band or N-band, this would localize at least part of a structural gene in a nonfluorescent region of the Y

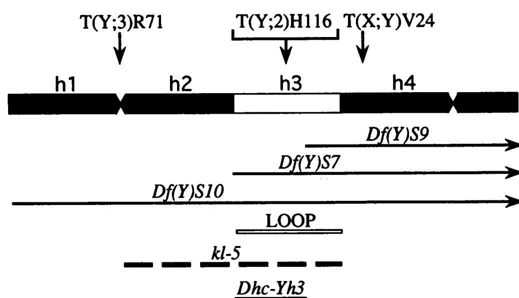

FIG. 4. Drawing of the distal end of YL fluorescently stained with Hoechst (see ref. 6). Solid areas are brightly fluorescent and open area in h3 is nonfluorescent. Thin lines below represent segments removed by Y chromosome deficiencies. Loop-forming region within *kl-5* is indicated as an open line (13). The limits of *kl-5* defined via noncomplementing rearrangements by Gatti and Pimpinelli (6) are shown with a broken line. Region containing the *Dhc-Yh3* DNA fragment is shown as a thick solid line. *T(Y;3)R71* and *T(Y;2)H116* (6) are rearrangements used to localize *Dhc-Yh3*, and *T(X;Y)V24* is the fertile translocation that marks the proximal limit of the *kl-5* region.

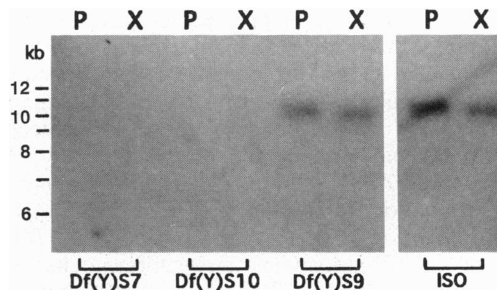

FIG. 5. Southern blot of DNA from female flies with Y chromosome deficiencies probed with the labeled *Dhc-Yh3* clone. DNA was digested with *Pst* I (P) or *Xho* I (X). Control DNA from isogenic males is shown in the two rightmost lanes. *Dhc-Yh3* is present in isogenic males and *Df(Y)S9* females; it is missing in *Df(Y)S7* and *Df(Y)S10* females.

chromosome. Thus N-band regions contain nonrepetitive unique sequence DNA in addition to G+C-rich satellite DNA (6). It has been noted that most male-sterile Y chromosome rearrangements involve breaks in N-banded regions (5, 6).

The partial clone of the *Dhc-Yh3* gene (T.S.H., unpublished data) shows greater homology to an axonemal dynein than to cytoplasmic dyneins. The deduced amino acid sequence of the cloned portion of *Dhc-Yh3* is compared in Fig. 6 to the corresponding regions of the β heavy chain of sea urchin flagellar dynein (29) and of *Dhc64C*, a cytoplasmic dynein heavy chain from *Drosophila* (M.-G. Li, M. Serr, and T.S.H., unpublished data). Within this stretch of the proteins, the predicted amino acid sequence of *Dhc-Yh3* is 85% identical to the sea urchin dynein β heavy chain. Comparison of the *Dhc-Yh3* partial sequence to a *Drosophila* cytoplasmic dynein heavy-chain isoform characterized as nonaxonemal shows significantly less homology (53% amino acid identity). Thus, within this region, *Dhc-Yh3* is highly homologous to a flagellar dynein isoform.

The expression of *Dhc-Yh3* has been demonstrated by Northern blot analysis and reverse transcriptase PCR (T.S.H., unpublished data). As expected, *Dhc-Yh3* expression was detected in adult males but not in embryos or adult females. To examine the distribution of *Dhc-Yh3* expression in testes, whole mount *in situ* hybridization was carried out on testes from wild-type males and males lacking *kl-5*. *Dhc-Yh3* expression was detected in normal males but not in males deleted for *kl-5* (Fig. 7). At low magnification, the pattern of *Dhc-Yh3* expression resembled a partial helix along the inner side of the middle of the anterior half of the testis (Fig. 7*A*). The pattern of staining shown in Fig. 7*A* was characteristic of all wild-type

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Dhc-Yh3  ITPLTDRCYI TLTQSLHLVM GGAPAGPAGT GKTETTKDLG    40
 urchin  .......... .......... S......... ..........  1866
 Dhc64C  Q.......L .M..A.ESRL ..S.F..... ....SV.A..  1909

Dhc-Yh3  RALGMMVYVF NCSEQMDYKS IGDIHKGLAQ TGAWGCFDEF    80
 urchin  ....I..... .......... C.N.Y..... ..........  1906
 Dhc64C  NQ..RF.L.. ..D.TF.FQA M.R.FV..C. V.........  1949

Dhc-Yh3  NRISVEVLSV VAVQVKCIQD AIKSKKQ--- ---TFSFLGE   114
 urchin  .......... .......V.. ..RD..E--- ---R.N.M..  1940
 Dhc64C  ..LEERM..A CSQ.IQT..E .L.YEMDSNK ESI.VELV.K  1989

Dhc-Yh3  HIALRTTVGV FITMNP                             130
 urchin  E.S.IPS..I ......                            1956
 Dhc64C  QVRVSPDMAI ......                            2005
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FIG. 6. Comparison of deduced amino acid sequence of the cloned segment of *Dhc-Yh3* (T.S.H., unpublished data) with the β heavy chain of sea urchin flagellar dynein (urchin; ref. 29) and a cytoplasmic dynein heavy chain from *Drosophila* (*Dhc64C*; M.-G. Li, M. Serr, and T.S.H., unpublished data). Dots are shown where the amino acid is identical to the *Dhc-Yh3* residue at that position. The putative hydrolytic ATP-binding site is underlined.

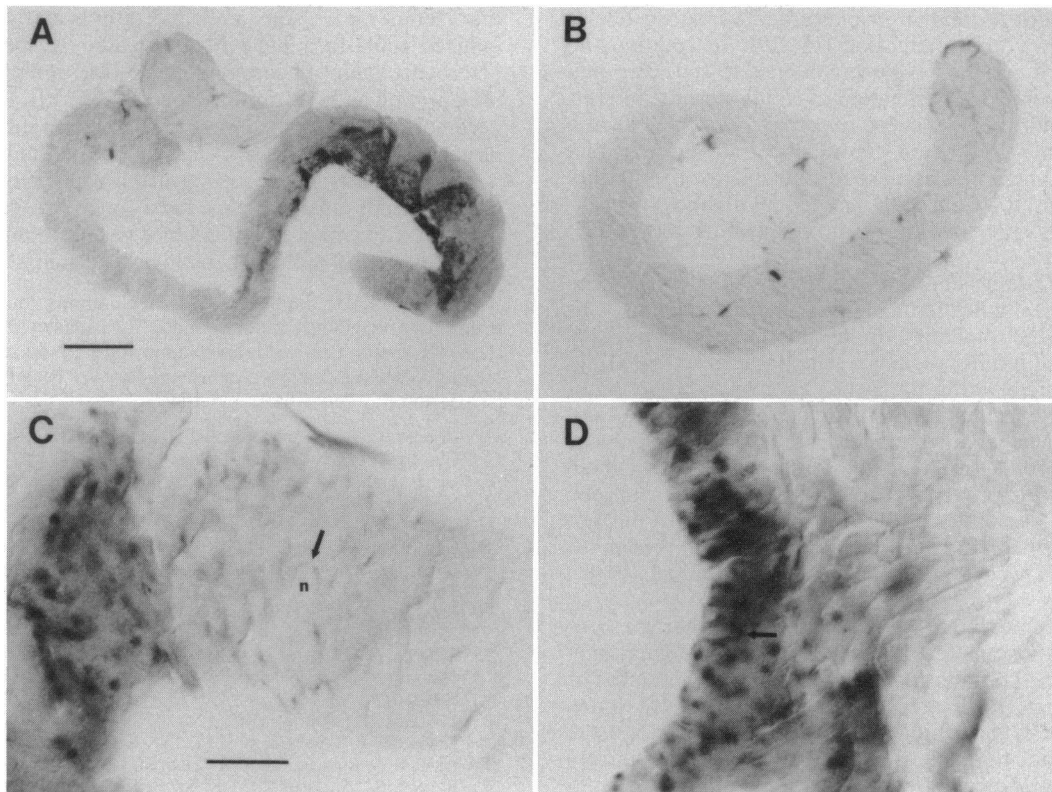

FIG. 7. *In situ* hybridization of digoxigenin-labeled *Dhc-Yh3* DNA to the testes of normal males and males lacking *kl-5*. (*A*) Testis of an Oregon-R male at low magnification. Staining can be seen along the inner side of the middle of the anterior half of the testis. (Bar = 100 μm.) (*B*) Testis of a male missing the *kl-5* region. No hybridization of *Dhc-Yh3* DNA was detected. (*C* and *D*) Close-ups of Oregon-R testes showing stain confined to the cytoplasm. (Bar = 20 μm.) (*C*) Near the apical end of the testis, early primary spermatocytes showed low levels of staining that circled the nucleus (n). (*D*) Late primary spermatocytes located more posteriorly show more intense staining around the nucleus in addition to darkly staining foci adjacent to nuclei (arrow).

testes examined and was coincident with the location of cysts of developing primary spermatocytes (17, 30, 31). The level and pattern of *Dhc-Yh3* expression varied with the age of the primary spermatocytes along the length of the testis. Early primary spermatocytes in the apical region of the testis showed a low level of hybridization that rimmed the nucleus (Fig. 7*C*). In late primary spermatocytes, *Dhc-Yh3* expression appeared to increase as indicated by the elevated levels of hybridization observed in more posterior regions of the testis. In addition, at these later stages, *Dhc-Yh3* transcripts appear to accumulate at cytoplasmic foci adjacent to nuclei (Fig. 7*D*). The significance of this accumulation is not clear. The increased expression of *Dhc-Yh3* that accompanies the progression of spermatogenesis is consistent with the expectation that the gene encodes a dynein used in the assembly and function of the sperm flagellar axoneme.

DISCUSSION

The results presented here provide evidence for the existence of a dynein heavy-chain gene, *Dhc-Yh3*, on the Y chromosome. This is one of only a few genes identified on the Y chromosome in *D. melanogaster*. Since dynein has been shown to function as a microtubule motor that powers flagellar movement in other systems (reviewed in ref. 22), we propose that *Dhc-Yh3* encodes a dynein polypeptide that is an essential functional component of the sperm flagellar axoneme. This conclusion is based on the fact that *Dhc-Yh3* is highly homologous to the corresponding region of a flagellar dynein heavy-chain isoform and that *Dhc-Yh3* is located within *kl-5*, a region on the *Drosophila* Y chromosome required for male fertility. We demonstrate that *Dhc-Yh3* is expressed in the testis in primary spermatocytes. It is generally accepted that the Y fertility loci function during the primary spermatocyte stage (see refs. 17 and 32 for reviews). Thus, the pattern and levels of expression of *Dhc-Yh3* are consistent with its proposed function in the sperm flagella.

The hypothesis that the *kl-5* locus encodes a flagellar dynein heavy chain is consistent with observations from previous studies. Mutation of the *kl-5* region has been reported to result in a lack of motile sperm (33), loss of sperm flagellar organization (11), loss of the outer dynein arms from the flagellar axoneme (18), and the absence of a high molecular weight protein that comigrates with *Chlamydomonas* dynein heavy chains on SDS/polyacrylamide gels (21). Our localization of a dynein heavy-chain sequence within *kl-5* provides direct evidence for the suggestion by Goldstein *et al.* (21) that *kl-5* contains a dynein heavy-chain structural gene. Our results help rule out the possibility that *kl-5* contains a gene that regulates dynein synthesis or assembly (23).

The partial clone of the *Dhc-Yh3* gene (T.S.H., unpublished data) has been shown to contain a putative hydrolytic ATP-binding domain that is 85% identical to the corresponding domain of a sea urchin flagellar dynein (29, 34). In contrast, this region of *Dhc-Yh3* is only 53%, 55%, and 54% identical to the same site in cytoplasmic dyneins from *Drosophila* (M.-G. Li, M. Serr, and T.S.H., unpublished data), *Dictyostelium* (35), and rat (36), respectively. Thus, within this highly conserved domain, *Dhc-Yh3* is more homologous to a flagellar dynein than to cytoplasmic dyneins.

The localization of the *Dhc-Yh3* clone to the *kl-5* fertility locus suggests that mutations within this region are sterile because they affect dynein function. The *kl-5* region appears to act as a single gene in complementation studies with rearrangement alleles (5, 6, 33) but there have been conflicting

reports on whether ethyl methanesulfonate-induced alleles show intragenic complementation (15, 19). In addition, no other potential functions have been mapped to *kl-5*. The only phenotype associated with deletion of *kl-5* is sterility and this is consistent with the phenotype expected from the loss of a flagellar dynein heavy chain. To demonstrate directly that alterations in dynein function are responsible for the sterility of *kl-5* mutations, it will be necessary to identify the molecular nature of the sterile mutations and show how they affect dynein function.

Brosseau (33) suggested that the fertility genes may have arisen from gene duplication and divergence of function. This was based on the similarity of the phenotypes of mutants missing different fertility regions. He hypothesized that all six genes may be involved in the same "pathway." Deletion of *kl-2, kl-3*, or *kl-5* results in both male sterility and the loss of high molecular weight polypeptides from testis (21). Similar to *kl-5*, deletion of *kl-3* results in the loss of outer arm dynein structures (18). Our previous characterization of a dynein gene family (T.S.H., unpublished data) almost certainly did not identify all of the *Drosophila* dynein genes. It remains a possibility that *kl-2* and *kl-3* encode additional dynein heavy chains.

There has been extensive discussion on whether the *Drosophila* Y chromosome fertility regions encode conventional structural genes. This controversy has arisen for a number of reasons. Among these are the unusual lampbrush loops formed by three of the fertility regions, the large size of these loci, and the fact that the Y chromosome appears entirely heterochromatic. Recent work by Hackstein *et al.* (20) suggests that Y chromosome loops are not essential for male fertility. They have shown in *Drosophila hydei* that Y chromosome mutations that alter the morphology of a fertility region loop nevertheless result in fertile males and, conversely, that sterile Y mutations may not affect loop morphology. This demonstrates that loop morphology is not always correlated with fertility.

If *kl-5* is required for male fertility due to the presence of a dynein gene, then the extraordinary size reported for *kl-5* must be explained. The *kl-5* region has been estimated to be 3000–4000 kb (6, 23) based on the distance between noncomplementing mutations at the locus. This is at least 2 orders of magnitude more DNA than would be needed to code for a typical dynein heavy-chain transcript of ≈14 kb. The extra DNA could be accounted for, however, by postulating the presence of distant enhancer elements or unusually large introns. The *kl-5* region has been shown to contain at least four satellite DNA sequences (3). These repetitive sequences may be in introns since they are transcribed but remain in the nucleus (37). Alternatively, it is possible that the size of the *kl-5* region was overestimated. It has recently been reported that when the rolled gene, which is normally located within heterochromatin on the *Drosophila* second chromosome, is moved to euchromatin, it is shut off (38). The sterile rearrangements used to estimate the dimensions of *kl-5* translocate the *kl-5* region to euchromatin (6). It is possible that in the new location, adjacent euchromatin acts at a distance to inactivate the *kl-5* locus.

The heterochromatic nature of the *Drosophila* Y chromosome is one reason why it has been considered genetically inert (23). In fact, functional loci have been identified in the centromeric heterochromatin of other chromosomes and their genomic organization appears to be very similar to that of the Y chromosome (reviewed in refs. 39 and 40). As in the case of the Y chromosome, relatively few functions have been identified in large regions of heterochromatic DNA on the X chromosome and autosomes. Hilliker (41) estimated that chromosome 2 centromeric heterochromatin contains genes at 1% the frequency found in euchromatin. Heterochromatic loci appear to act like single copy euchromatic genes in the sense that temperature-sensitive and ethyl methanesulfonate-induced mutations have been isolated in the centromeric heterochromatin of chromosome 2 (24) and chromosome 3 (41), as well as for Y fertility loci (14–16, 19). Because the Y chromosome is heterochromatic, it has been difficult to study its gene organization by classical genetic methods (14, 23). The discovery of a dynein sequence on the Y chromosome not only provides additional evidence for a functional gene in heterochromatin but may help facilitate genetic analysis of the Y chromosome by molecular biological techniques.

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