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Genetics

Structural genes on the Y chromosome of *Drosophila melanogaster*

(spermatogenesis/dynein/sperm axoneme/fertility genes)

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ABSTRACT **Testis proteins of *Drosophila melanogaster* deficient for six different Y-chromosome regions were fractionated by means of a sodium dodecyl sulfate/polyacrylamide gel system designed to separate high molecular weight polypeptides (M_r, >200,000). Analysis of the banding patterns indicates that the three regions containing fertility genes *kl-2*, *kl-3*, and *kl-5* are responsible for three different high molecular weight polypeptides. Several observations indicate that these polypeptides are structural components of the sperm axoneme. They are present in seminal vesicles, which are highly enriched for mature sperm. They are first detected during development at a time when the first spermatids are elongating. Finally, deletion of either *kl-5* or *kl-3* leads to the absence of the outer dynein arm of the peripheral doublets of the axoneme. Although absence of the *kl-2* region eliminates the third polypeptide, an associated structural defect in the axoneme has yet to be identified. The three polypeptides are in the M_r 300,000–350,000 range, and their mobilities are similar to those of dynein polypeptides from *Chlamydomonas* axonemes. Experiments using dosage variation and a temperature-sensitive sterile mutation in *kl-5* suggest that the Y-chromosome regions contain the coding sequences for the polypeptides.**

The Y chromosome of *Drosophila melanogaster* is required for male fertility; males lacking a Y (X0 males) are phenotypically normal but sterile (1). Marsh and Wieschaus (2) were able to recover progeny from the descendants of XY pole cells that had been transplanted to the polar cap of X0 embryos, demonstrating that X0 somatic tissues are capable of supporting the development of functional sperm from XY primordial germ cells and, therefore, that Y-chromosome function is necessary only in the male germ line. Genetic studies (refs. 3–6; M. Gatti and S. Pimpinelli, personal communication) indicate that the submetacentric Y contains six complementation groups required for male fertility, four on the long arm (Y^L) and two on the short arm (Y^S). From the terminus of Y^L to that of Y^S, these fertility factors are designated *kl-5*, *kl-3*, *kl-2*, *kl-1*, *ks-1*, and *ks-2*; *kl-4*, tentatively identified by Brosseau (3), has not been confirmed (4).

Attempts to delineate the role of the Y chromosome by ultrastructural examination of spermatogenesis in X0 males (7, 8) have been inconclusive. Many abnormalities were observed, but no structure was reported to be consistently missing or abnormal in all developing spermatids. These authors concluded from the general breakdown of spermatogenesis seen in X0 testes that the Y-chromosome fertility genes are not responsible for particular structural proteins, but rather that they exert a "regulatory" or "morphogenetic" influence over sperm development. Recent studies of Hardy *et al.* (9) indicate that the complexity of the phenotype reported in earlier publications was due in part to the simultaneous deletion of six fertility genes and in part to the authors' having examined late stages of spermiogenesis, when indirect consequences of the deficiency tend to obscure the primary lesions. Hardy *et al.* (9) sought to identify the earliest ultrastructural departure from normality in spermatogenesis of males singly deficient for each of the six fertility genes; the material studied comprised four contiguous, nonoverlapping deficiences in Y^L and two in Y^S produced by combining complementary elements of X–Y translocations (ref. 4; see Fig. 1). (From the terminus of Y^L, these deficiencies were designated A through D in Y^L and F and G in Y^S; they contain fertility genes *kl-5*, *kl-3*, *kl-2*, *kl-1*, *ks-1*, and *ks-2*, respectively. In this communication, we refer to these deficiencies according to the fertility gene contained.) The absence of either the *kl-5* or the *kl-3* region results (*i*) in the reduction of a specific structure from the primary spermatocyte nuclei (aggregates of tubuli or reticular material, respectively) and (*ii*) in the absence of the outer dynein arms associated with the A microtubules of the nine peripheral doublets of the axoneme. Deficiency for the *kl-2* region leads to the formation of crystals in the primary spermatocytes and to the abnormal distribution of chromosomes and mitochondria at meiosis. The first abnormalities detected in males deficient for the *kl-1* and *ks-1* regions occurred late in spermiogenesis and may be secondary consequences of unrecognized earlier abnormalities. Absence of the *ks-2* region leads to misalignment of the developing axoneme and the nebenkern, with drastic effects on subsequent spermatid development.

The observation that absence of the Y chromosome leads to defective spermiogenesis suggested to earlier investigators that it might be possible to identify specific Y-encoded gene products. Their attempts to identify specific testis polypeptides whose presence depends on the presence of the Y chromosome were unsuccessful. Two-dimensional gel analyses by Ingman-Baker and Candido (10) and Kemphues and Kaufman (11) failed to identify any polypeptide in XY males that is absent from X0 males, although some quantitative differences were noted. These observations seemed to agree with the hypothesis that the Y chromosome is regulatory in function and is not responsible for major structural proteins of the sperm. However, the observation that two of the Y-chromosome deficiencies result in specific loss of the outer dynein arms, coupled with the knowledge that these arms contain high molecular weight polypeptides in all organisms examined to date (12, 13), prompted us to examine this class of polypeptides in the testes of *Drosophila melanogaster* lacking all or part of the Y chromosome.

MATERIALS AND METHODS

Y-Chromosome Aneuploidy. The *T(X;Y)* chromosomes used in the generation of Y-chromosome deficiencies and duplications were induced by irradiation of *y w f*/$B^S Y y^+$ sperm from *y w f*/$B^S Y y^+$/*Y* males [ref. 4; see Lindsley and Grell (14) for descriptions and symbols of chromosomes and markers]. They are reciprocal translocations having the X-chromosome breakpoint in the proximal heterochromatin and the Y breakpoints

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distributed as shown in Fig. 1. Males bearing both elements of the translocation and no free Y, which may be symbolized X^DY^P/Y^DX^P, are fertile. In translocations with breaks in Y^L, the X^DY^P element is marked with y^+ and the Y^DX^P element is marked with B^S. Stocks have X^DY^P, $y\ w\ f.y^+/Y^DX^P$, B^S males and $C(1)A$, y/Y^DX^P, B^S females. Kennison (4) tested the elements of the fertile translocations and, where possible, determined whether the X-chromosome heterochromatin breakpoint was either proximal to, distal to, or within the *bb* locus (Fig. 1).

Two strategies were utilized to produce Y-chromosome aneuploids. The first produces males that contain either one or the other element of the translocation and, therefore, are deficient for one or more Y-chromosome regions. X^DY^P/Y^DX^P males produce X/Y^DX^P sons when crossed to free X-chromosome females and $X^DY^P/0$ sons when crossed to $C(1)/RM/0$ females. The second approach allows the production of duplications or deficiencies for a single Y-chromosome region; this is accomplished by combining complementary elements from different translocations. For example, a *kl-2* deficiency is made by combining the Y^DX^P, B^S element of *W27* stock females with the X^DY^P, $y\ w\ f.y^+$ element of *E15* stock males (Fig. 1). The sons of such a cross, which are $w\ f\ B^S$, have all the Y-chromosome fertility factors except *kl-2* and, incidentally, have one dose of bb^+, derived from Y^S. A duplication for *kl-2* results from the reciprocal cross; it carries three doses of bb^+. This method allows all regions of the Y chromosome to be tested because the translocation that provides the proximal limit of one deficiency or duplication provides the distal limit of the more proximal adjacent deficiency or duplication.

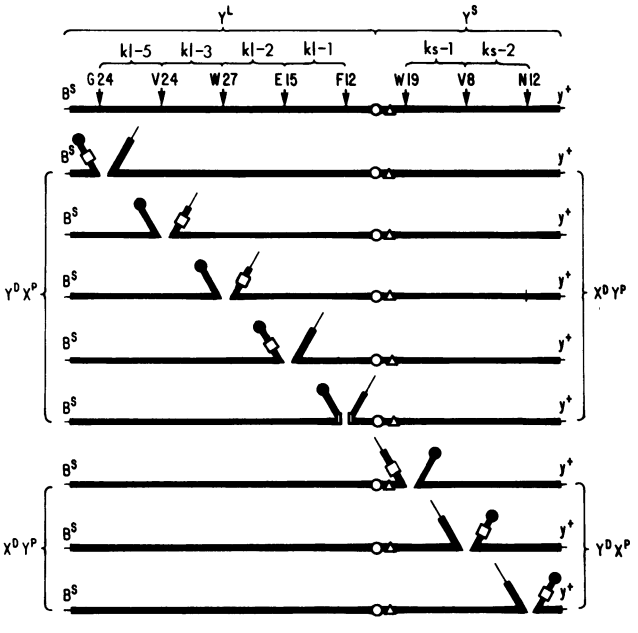

FIG. 1. Generation of Y-chromosome deficiencies. Designation and relative positions (arrows) of the Y-chromosome breakpoints of *T(X;Y)*s used in the generation of a series of contiguous Y-chromosome deficiencies. Horizontal lines, Y chromosome; diagonal lines, X chromosome; heavy lines, heterochromatin; thin lines, euchromatin; ○, Y-chromosome centromeres; ●, X-chromosome centromeres; △, *bb* locus on the Y chromosome; □, *bb* locus on the X chromosome. Interstitial deficiencies in the Y chromosome are generated by combining the left-hand element (marked B^S) of one *T(X;Y)* with the right-hand element (marked with y^+) of the one directly below it in the diagram. Terminal Y^L deficiencies are generated by recovering the right-hand element of the translocation in the absence of the left-hand element; the complementary deficiency is generated by combining the left-hand element with a normal X chromosome. The diagram is modified from Hardy *et al.* (9) with the permission of Springer-Verlag. [Note that the position of the X-chromosome breakpoint of *T(X;Y)W27* is corrected from distal to bb^+ (9) to proximal to bb^+ (4)].

Gel Electrophoresis. Testes were dissected from larval, pupal, or adult flies 1 day old or less. Testes together with the seminal vesicles were separated from the vas deferens and accessory glands in adults. Mature sperm were sampled by isolating seminal vesicles alone from males that had been allowed to accumulate sperm for 2–3 days in the absence of females. The dissecting medium was either Ephrussi–Beadle Ringer's or Hoyle's buffered saline. Generally 5–10 flies were dissected, and the testes immediately were placed in sample buffer (4.27% $NaDodSO_4$/3% Trizma base/0.008% disodium EDTA/10% sucrose/0.7% bromphenol blue/1% 2-mercaptoethanol, pH 8.8), submerged in boiling water for 1–2 min, and then frozen. The gel system used was that of Piperno and Luck (15), which consists of a 2.8% polyacrylamide stacking gel, a 3.2% polyacrylamide separation gel containing a 0–4 M urea gradient, and the discontinuous Tris borate/Tris sulfate/Tris chloride buffer system developed by Neville (16) and modified by Piperno and Luck (15). Gels were vertical slabs 0.75 mm thick and either 9 or 12 cm long and 15 or 18 cm wide. Gels were stained with silver by the method of Merril *et al.* (17). Mobilities were compared with those of dynein from *Chlamydomonas* axonemes provided by S. Dutcher (15, 18).

Radiolabeling. Testes (5–10) from 2- to 3-day-old males were dissected in Ephrussi–Beadle Ringer's solution and put into labeling medium consisting of serum-free modified Eagle's medium prepared without methionine to which [^{35}S]methionine was added. The specific activity of the medium was 5–10 mCi per ml (1 Ci = 3.7×10^{10} becquerels), and 1–2 μl were used per testis. Labeling of protein synthesis was carried out for 4–6 hr, after which time the tissue appeared relatively healthy and the sperm retained some motility. After labeling, testes were washed three times in 25-μl drops of Ephrussi–Beadle Ringer's solution, placed in sample buffer, boiled, and then frozen. After electrophoresis, the gels were fixed in 50% methanol/12% acetic acid, washed in 10% acetic acid for at least 30 min, dried, subjected to autoradiography on Kodak XAR-5 film, and developed in Kodak D19. Densitometry was carried out with a Gelman ACD-8 scanning densitometer.

RESULTS

Specific High Molecular Weight Polypeptides Are Correlated with Specific Regions of the Y Chromosome. The gel system that we utilized allows excellent resolution of polypeptides of M_rs > 200,000. Fig. 2 shows the results of electrophoresis and silver staining of testis extracts from normal XY males compared with those from X0 males. It is evident that five bands that were present in XY extracts were absent from X0 extracts; the same five bands were found in XY males of all strains of *D. melanogaster* examined. The presence of the bands labeled 2, 3, and 5 was consistently Y chromosome dependent; the relationship of the other two to the Y chromosome is problematical. In extracts of [^{35}S]methionine-labeled testes from XY males, bands 2, 2′, 3, and 5 were labeled; only these four will be considered henceforth. In similar extracts of X0 testes there was no incorporation in the positions of bands 2, 3, and 5. Band 2′ was reduced or absent in silver-stained gels of X0 extracts but was observed in extracts of [^{35}S]methionine-labeled X0 testes, indicating that it represents a polypeptide that is synthesized but not accumulated in the absence of the Y chromosome. Fig. 2 also shows the results from an extract of *Chlamydomonas reinhardtii* axonemes; the three prominent polypeptides are dynein components I, II, and V—all demonstrated to be subunits of the outer dynein arm by both biochemical and genetic criteria (15, 18). We note that these have mobilities comparable to those of three of the Y-dependent polypeptides, bands 2, 3, and 5.

In order to delineate further the regions of the Y chromosome responsible for the observed polypeptide differences, we sub-

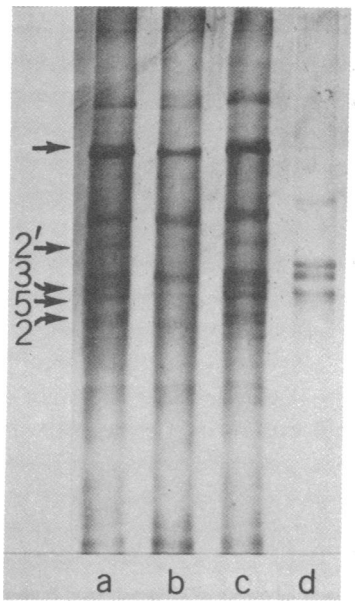

FIG. 2. $NaDodSO_4$/urea-gradient/polyacrylamide gel electrophoresis of silver-stained high molecular weight polypeptides from extracts of *Drosophila* testes (lanes a–c) and *Chlamydomonas* axonemes (lane d). Lanes: a, XY males; b, X0 males; c, XY males; d, *Chlamydomonas* axonemes. Arrows identify the bands discussed in text.

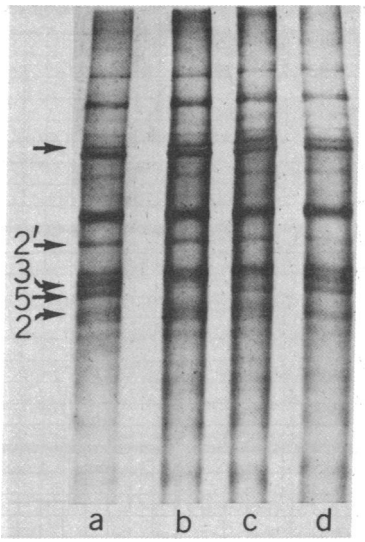

FIG. 3. Gel electrophoresis of silver-stained high molecular weight polypeptides from testes of males deficient for regions containing *kl-5*, *kl-3*, and *kl-2*. Lanes: a, XY males; b, *kl-5*-deficient males ($X^DY^PV24/0$) (These males also lack the region of the Y chromosome distal to the *G24* breakpoint; however, this region is not essential for male fertility, and flies lacking this region alone show no change in the polypeptide pattern); c, *kl-3* deficiency (X^DY^PW27/Y^DX^PV24); d, *kl-2* deficiency (X^DY^PE15/Y^DX^PW27).

divided the chromosome using the translocations indicated in Fig. 1. Preparations of testes from males carrying the separated elements of the translocations indicated that bands 2, 3, and 5 depend on the regions of Y^L containing *kl-2*, *kl-3*, and *kl-5*, respectively. These conclusions were confirmed and are shown here by determinations made on testes of males deficient for these regions one at a time. Polypeptides 5, 3, and 2 were removed one at a time, in both silver-stained (Fig. 3) and labeled preparations, by deficiencies for *kl-5*, *kl-3*, and *kl-2*. We refer to these as the kl-5, kl-3, and kl-2 polypeptides. In addition, band 2′ is reduced in silver-stained but not in [^{35}S]methionine-labeled preparations of testes from males deficient for *kl-2*. We refer to it as the 2′ polypeptide. Deficiencies for other Y-chromosome segments are without detectable effect on the pattern of high molecular weight testis polypeptides.

Y Chromosome-Dependent High Molecular Weight Polypeptides Are Components of Sperm. We argue that the kl-2, kl-3, and kl-5 polypeptides are structural components of sperm on two grounds. First, deficiencies for *kl-3* and *kl-5* are correlated with the absence of specific structures from the axoneme—i.e., the outer dynein arms of the peripheral doublets (9); the absence of *kl-2* has not yet been associated with a missing structure. Second, the presence of all three polypeptides is strictly correlated with the presence of axonemes in the sample. The polypeptides are absent from larval testes in which no elongating spermatids can be found and from the testes of males homozygous for *ms(3)sa* [male sterile (3) spermatocyte arrest (3–44) mapped by K. Kemphues, personal communication], a mutant that arrests spermatogenesis premeiotically and accumulates primary spermatocytes. They are present, however, in young pupal testes, where spermatid elongation is in progress; in isolated seminal vesicles, in which the major cell type is mature spermatozoa; and in preparations of isolated axoneme (unpublished data). By similar arguments polypeptide 2′ is likely to be a sperm component.

Structural Genes for High Molecular Weight Polypeptides Are Present on the Y Chromosome. In general, in *Drosophila* there is a strict correspondence between structural gene dosage and the amount of the corresponding polypeptide synthesized or accumulated (19). If the *kl-5*, *kl-3*, and *kl-2* deficiencies truly contain coding sequences for their correlated polypeptides, then alterations in dosage of these Y-chromsome regions should be mirrored by alterations in the amount of polypeptide produced. To test this, flies containing duplications for these regions were produced. Testes were dissected from these flies and from flies containing the parental translocations, proteins being synthesized were labeled *in situ* with [^{35}S]methionine, and extracts were subjected to electrophoresis and autoradiography. Sample autoradiographs and corresponding densitometer scans are shown in Fig. 4. Increases in the dosage of specific segments of the Y chromosome were accompanied by corresponding increases in the rates of synthesis of specific polypeptides. Duplications for the *kl-5* or *kl-3* regions stimulated the synthesis of the kl-5 or kl-3 polypeptides relative to the other polypeptides in the same region of the gel. The situation with respect to the *kl-2* region was more complex; duplication for this region was accompanied by increased labeling of the kl-2 polypeptide; in addition, incorporation into the polypeptide 2′ was often greatly stimulated. Synthesis of the kl-5 and kl-3 polypeptides may have been weakly stimulated. We conclude that *kl-5*, *kl-3*, and *kl-2* duplications lead to increased rates of synthesis of the kl-5, kl-3, and kl-2 polypeptides, respectively, in accordance with expectations from the hypothesis that the coding sequences for these polypeptides are on the Y chromosome. We cannot explain the response of the polypeptide 2′ to duplication for the *kl-2* region. We note that, although the results from labeling and autoradiography are variable, the data taken together support the above conclusions.

Ayles *et al.* (20) have isolated a number of temperature-sensitive male-sterile mutants on the Y chromosome. We examined one of these, *ms(Y)B119*, which K. Livak (personal communication) has mapped to the *kl-5* region. This mutant, which is presumed to be an allele of *kl-5* based on phenotypic and genetic considerations, provides information pertinent to the question of whether the coding sequence for the kl-5 polypeptide resides in the *kl-5* region or is controlled by a gene that resides in that region. If the *kl-5* product is required for the expression of an X-linked or autosomal gene that codes for the

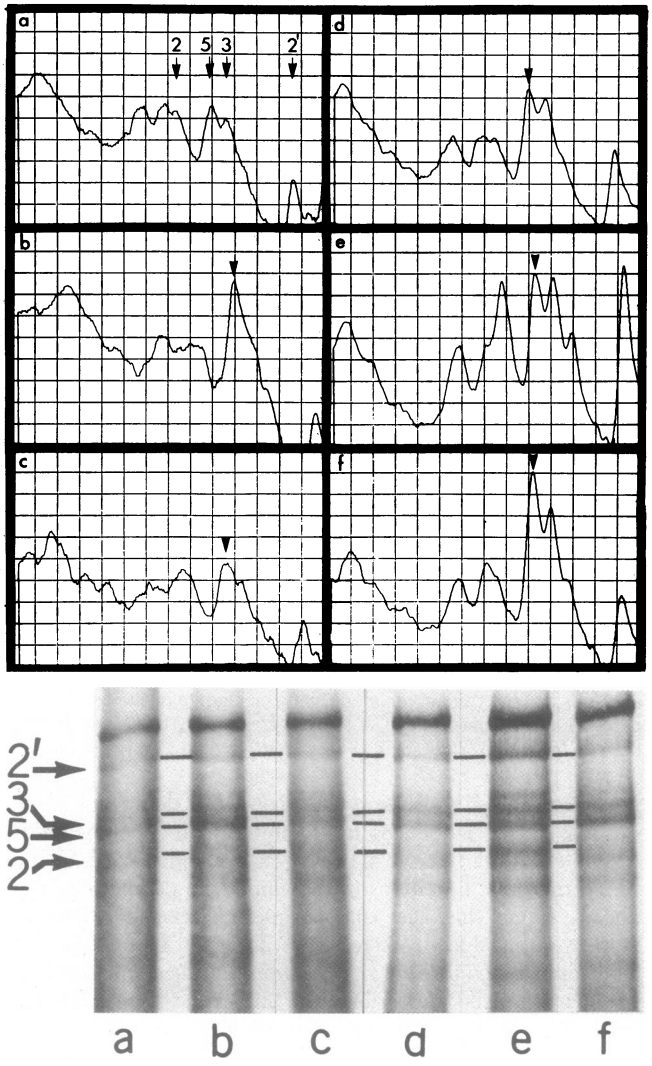

FIG. 4. Autoradiographs and densitometer scans of gels from testes labeled with [^{35}S]methionine. Lanes: a, *T(X;Y)G24*; b, duplication for *kl-5* (X^DY^PG24/Y^DX^PV24); c, *T(X;Y)V24*; d, *T(X;Y)W27*; e, duplication for *kl-2* (X^DY^PW27/Y^DX^PE15); f, duplication for *kl-5* and *kl-3* (X^DY^PG24/Y^DX^PW27). The scan readings from left to right correspond to the gel contents proceeding from the bottom to the top; the arrowheads point to the peak corresponding to the *kl-5* band in each case. Scans a–c are from one gel and d–f are from another.

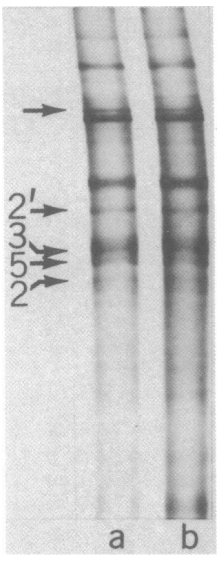

FIG. 5. Gel electrophoresis of silver-stained high molecular weight polypeptides from testes of wild-type and *ms(Y)B119* males raised at 28–29°C. Lanes: a, *X/ms(Y)B119*; b, X/Y.

polypeptide rather than itself containing the coding sequence, then the phenotype of *ms(Y)B119*-bearing males reared at restrictive temperatures should mimic that of *kl-5* deficiencies, resulting in the failure to produce the polypeptide. On the other hand, were *kl-5* the structural gene for the polypeptide, a conditional mutation could lead to the synthesis of a defective polypeptide of normal size. We found the *kl-5*-associated polypeptide present in apparently normal quantities in *ms(Y)B119*-bearing males raised at restrictive temperature (see Fig. 5); however, outer arm assembly was drastically reduced. In *ms(Y)B119*-bearing males raised at permissive temperature, outer arms were observed in all axoneme cross sections, whereas at restrictive temperature, only rarely did an axoneme cross section contain an outer arm. We conclude that *ms(Y)B119* produces a temperature-sensitive kl-5 polypeptide; however, the possibility remains that outer arm assembly and the kl-5 polypeptide are controlled by separate but closely linked genes.

DISCUSSION

The experiments described in this paper provide evidence that the Y chromosome of *D. melanogaster* codes for three polypeptides in M_r range of 300,000–350,000. The only previous evidence in favor of a Y chromosome-dependent polypeptide was presented by Hennig *et al.* (21) for *Drosophila hydei*. They describe a polypeptide in the M_r 300,000 range from the testes of *D. hydei* that is absent in males that lack the short arm and the proximal portion of the long arm of the Y chromosome. Subdivision of *D. hydei* testes into pre- and postmeiotic segments demonstrated that the polypeptide was more abundant in postmeiotic cells. Furthermore, their observations strongly support the proposition that the structural gene for the polypeptide is Y chromosome linked as well. They demonstrated that *Drosophila neohydei* produces an analogous protein with different mobility and that, in hybrids and their backcross progeny, the polypeptide phenotype is correlated with that of the species from which the Y chromosome was derived and is independent of the X chromosome and autosomal constitution. Several comparisons of the testicular proteins of XY and X0 males of *D. melanogaster* have failed to detect any differences (10, 11). However, the methods used were incapable of resolving polypeptides of high molecular weight.

Dynein polypeptides can be defined as the polypeptide components of the axonemal dynein arms. Isolated arms and polypeptide complexes making up the arms are associated with ATPase activity and, in general, contain high molecular weight polypeptides. The definition of the polypeptide components of the dynein arms in many organisms has been accomplished by biochemical analysis of salt or low-ionic-strength dialysis extraction of axonemes; these treatments result in the selective extraction of the dynein arms from the axoneme (reviewed in refs. 12 and 13). In a few organisms, genetic analysis has been useful in characterizing the dynein arms. In *C. reinhardtii*, two loci have been identified by paralyzed-flagella mutations that remove specific ATPase activities, the outer dynein arms, and a specific array of polypeptides from isolated axonemes. Another locus has been identified by a paralyzed-flagella mutation that results in loss of a different set of ATPase activities, reduces the inner dynein arms in the axoneme, and loses another set of polypeptides (18, 22). In humans, recessive conditions are known that lead to the immotile-cilia syndrome. This condition, recognized by upper respiratory defects, often includes sperm immotility among its symptoms. Ultrastructural analyses have associated a variety of axonemal defects, including the lack of some or all of the dynein arms, with different examples of this

syndrome (23, 24). Electrophoretic analysis of sperm from a patient lacking dynein arms demonstrated the reduction of a particular set of high molecular weight polypeptides (25).

In *Drosophila* we describe two adjacent Y-chromosome deficiencies, each of which sterilizes males, removes the outer arm from the axoneme of the sperm flagella, and removes a specific axonemal polypeptide of M_r 300,000–350,000. We propose that these polypeptides are dynein arm components on the basis of their molecular weights and of their requirement for outer arm assembly. We also argue that the structural genes for these polypeptides are carried on the Y chromosome.

We have presented arguments in *Results* that the structural genes for the Y chromosome-dependent polypeptides are Y linked. One of these arguments is based on the observation that the rate of incorporation of [^{35}S]methionine into each polypeptide is responsive to the dose of the particular region of the Y chromosome responsible for its appearance. When *kl-5* or *kl-3* is duplicated, specific stimulation of kl-5 or kl-3 polypeptide synthesis is observed; when deficient, these polypeptides are absent. Duplication of *kl-2* leads to enhanced synthesis of both the kl-2 and the 2′ polypeptides. Also, because the kl-2 polypeptide is removed by the *kl-2* deficiency, we infer that its structural gene is in the *kl-2* region. As yet a sperm structure dependent upon the presence of the kl-2 polypeptide has not been identified. We do not understand the control of the 2′ polypeptide, whose synthesis is stimulated by a duplication (but not eliminated by a deficiency) of the *kl-2* region. A second argument that the structural genes are Y linked comes from the analysis of *ms(Y)B119*, a temperature-sensitive male sterile mutant presumed to be an allele of *kl-5*. This mutant produces a polypeptide of the normal size and in apparently normal quantities in males raised under either restrictive or permissive conditions, whereas it assembles outer arms only under permissive conditions. Although it is possible to imagine that polypeptide synthesis and outer-arm assembly are under the control of different genes and that the temperature-sensitive mutant affects the one required for outer arm assembly, the more parsimonious hypothesis is that both phenotypes result from lack of the same gene function and that the polypeptide produced by the temperature-sensitive mutant is rendered unsuitable for incorporation into the outer-arm structure at restrictive temperatures. Once incorporated, the mutant polypeptide seems insensitive to restrictive conditions because males carrying the mutant Y chromosome raised under permissive conditions do not become sterile immediately upon exposure to restrictive temperatures (K. Livak, personal communication). We favor the above interpretation of the action of *ms(Y)B119* over the hypothesis that *kl-5*$^+$ is required for the activation of an X-linked or autosomal structural gene for the polypeptide. Were this the case, then *ms(Y)B119* would be expected to mimic a deficiency for the *kl-5* region at restrictive temperature; i.e., it would fail to activate the structural gene. The appearance of the polypeptide in males raised at restrictive temperature is inconsistent with this supposition. In total then, gene-dosage response and the conditional allele of *kl-5* indicate that the three polypeptides of high molecular weight are encoded by the Y chromosome, each one from a different region.

Our analysis has depended mostly upon deficiencies and duplications involving large chromosome segments one might argue that more than one gene necessary for sperm development is contained in each region. However, extensive genetic analysis has shown that male-sterile Y-chromosome rearrangements and point mutations within a region fail to complement, whereas those in different regions complement completely (4, 5). Therefore, the simplest interpretation is that each region contains a single gene necessary for sperm development and fertility. By this interpretation, both the *kl-5* and *kl-3* regions contain one gene coding for a high molecular weight polypeptide component of the axonemal outer dynein arm, and the *kl-2* region contains a gene coding for a high molecular weight spermatid polypeptide required for the completion of spermatogenesis.

It is puzzling that genes which encode components of a structure as basic as the axoneme may be located on the Y chromosome and, thus, be confined to males of the species. It may be that these genes specify sperm-specific components and that other axonemal structures are encoded by non-Y-linked genes. Alternatively, it is possible that in *Drosophila* dynein arms occur only in sperm. Axonemal structures have been reported in chordotonal organs and in the nerve cells that innervate bristles; however, published micrographs do not reveal dynein arms in these structures (26–28). Finally, our arguments to the contrary notwithstanding, we cannot completely exclude the possibility that the Y-linked genes merely regulate the activity of genes located elsewhere in the genome.

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