

Drosophila melanogaster Male Germ Line-Specific Transcripts With Autosomal and Y-Linked Genes

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ABSTRACT

We have identified a set of related transcripts expressed in the germ line of male *Drosophila melanogaster*. Surprisingly, while one of the corresponding genes is autosomal the remainder are located on the Y chromosome. The autosomal locus, at 77F on chromosome arm 3L, corresponds to the previously described transcription unit 18c, located in the first intron of the gene for an RI subunit of cAMP-dependent protein kinase. The Y chromosome copies have been mapped to region h18-h19 on the cytogenetic map of the Y outside of any of the regions required for male fertility. In contrast to *D. melanogaster*, where Y-linked copies were found in nine different wild-type strains, no Y-linked copies were found in sibling species. Several apparently Y-derived cDNA clones and one Y-linked genomic clone have been sequenced. The Y-derived genomic DNA shares the same intron/exon structure as the autosomal copy as well as related flanking sequences suggesting that it transposed to the Y from the autosomal locus. However, this particular Y-linked copy cannot encode a functional polypeptide due to a stop codon at amino acid position 72. Divergence among five different cDNA clones ranges from 1.5 to 6% and includes a large number of third position substitutions. We have not yet obtained a full-length cDNA from a Y-linked gene and therefore cannot conclude that the *D. melanogaster* Y chromosome contains functional protein-coding genes. The autosomal gene encodes a predicted polypeptide with 45% similarity to histones of the H5 class and more limited similarity to cysteine-rich protamines. This protein may be a distant relative of the histone H1 family perhaps involved in sperm chromatin condensation.

SPERMATOGENESIS, the production of highly differentiated sperm cells from stem cell progenitors, is a complex process that is apparently similar in organisms as diverse as insects and mammals. In the case of *Drosophila melanogaster*, single stem cell derivatives (gonial cells) each give rise to 16 primary spermatocytes by mitotic division [see LINDSLEY and TOKUYASU (1980) for a comprehensive review]. The latter grow rapidly in size, during which time they accumulate RNA and protein for the postmeiotic phase of development. Although translation can be detected postmeiotically, it is generally accepted that the majority of gene transcription is restricted to premeiotic stages (OLIVIERI and OLIVIERI 1966; DAS, KAUFMAN and GAY 1964a). Postmeiotic development can thus be viewed as being, for the most part, genetically preprogrammed. After meiosis, the 64 spermatids embark upon a complex program of cellular rearrangement and differentiation, eventually becoming motile sperm. Elucidation of the molecular processes underlying this extreme example of cellular dif-

ferentiation represents a considerable challenge for developmental biologists.

As might be expected of such a complex process as spermatogenesis, many mutations are known that disrupt it. It has been estimated that 10% of *D. melanogaster* genes can be mutated by EMS to cause male-sterility (LINDSLEY and LIFSCHYTZ 1972). LIFSCHYTZ (1987) has divided these "fertility genes" into two broad classes: genes that express products specific to the male germ line, and genes whose function is required in both the germ line and soma. He suggests that the germ line is especially sensitive to perturbation of gene activity required for basic cellular functions. In support of this, some male-sterile mutations have been found to be alleles of lethal mutations [reviewed by LIFSCHYTZ (1987)]. Despite the availability of mutants, classical genetic analysis of sperm development can be problematic due to the preprogrammed nature of spermatogenesis. Pleiotropic effects of mutations in genes expressed premeiotically can cause a multiplicity of general defects later in sperm development, thereby thwarting a traditional epistatic analysis (HARDY, TOKUYASU and LINDSLEY 1981).

An alternative line of enquiry has been the characterization of genes identified solely on the basis of

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their specific expression in the male germ line. Analysis of the *mst87F* gene family, for example, and of the gene encoding testis-specific β -tubulin, has identified sequences that confer germ line-specific transcriptional and translational regulation on reporter genes (SCHAFER *et al.* 1990; MICHIELS *et al.* 1989). There are also enhancer-trap lines in which germ line-specific genes have been tagged by β -galactosidase staining (GONCZY, VISWANATHAN and DINARDO 1992), and that in addition provide valuable markers for the various stages of sperm development. Coupled with immunological studies of germ line-specific proteins, studies such as these are likely to make an increasingly important contribution.

Only a very small proportion of the *D. melanogaster* "fertility genes" are male-specific in the sense of being on the *Y* chromosome. Despite accounting for approximately 12% of the male genome, the *Y* appears to be composed of mainly satellite DNA sequences (BONACCORSI and LOHE 1991). It is entirely heterochromatic (HEITZ 1933; KAUFMANN 1934; PIMPINELLI, SANTINI and GATTI 1978), and is dispensable for normal somatic development. It is, however, essential for male fertility (BRIDGES 1916), six relevant complementation groups being known. Four of these fertility factors (*kl-1*, *kl-2*, *kl-3* and *kl-5*) are on the long arm, and two (*ks-1* and *ks-2*) are on the short arm (KENNISON 1981; HAZELRIGG, FORNILI and KAUFMAN 1982; GATTI and PIMPINELLI 1983). At least three (*kl-3*, *kl-5* and *ks-1*) are associated with giant "lampbrush" loops reminiscent of those found in the spermatocyte nuclei of *Drosophila hydei* (BONACCORSI *et al.* 1988), a species that has been the focus of relatively intense study because of its large spermatocytes. The *Y* chromosome of *D. hydei* has between 7 and 16 complementation groups necessary for male fertility [see HACKSTEIN (1987) for a discussion], 5 of which are associated with lampbrush loops (HENNIG 1985).

In terms of its molecular structure the *Drosophila Y* chromosome remains poorly described. Most studies have focused on the *D. hydei* lampbrush loop containing fertility factors where it has been shown that the loops represent large single transcription units composed, in part, of loop-specific repetitive sequences (GROND, RUTTEN and HENNIG 1984; DE LOOS *et al.* 1985; HARAVEN, ZUCKERMAN and LIFSCHYTZ 1986; WLASCHEK, AWGULEWITSCH and BUNEMANN 1988). In *D. melanogaster* more limited studies suggest that approximately 80% of the *Y* chromosome is composed of simple satellite sequences. Here, however, the repetitive sequences are not loop-specific (BONACCORSI and LOHE 1991). LIVAK (1990) has studied the *Ste* and *Su(Ste)* loci where he has shown that the transcription and splicing of mRNA from the X-linked *Ste* locus is regulated by related *Su(Ste)* sequences located in region h11 of the *Y* chromosome. *Su(Ste)* contains re-

gions of sequence identity with *Ste* interspersed with nonhomologous sequences (LIVAK 1990). DANILEVSKAYA and colleagues (1991) have sequenced DNA from the *Su(Ste)* region which shows homology to *Ste* and is also related to the He-T family of repetitive sequences (TAVERSE and PARDUE 1989). Thus it may be that X-linked *Ste* sequences have been transposed to the *Y* chromosome where they have diverged and been recruited to regulate *Ste*. Finally GOLDSTEIN, HARDY and LINDSLEY (1982) presented evidence to suggest that the fertility factors *kl-3* and *kl-5* encode polypeptide components of the sperm tail axoneme, the structure that provides the mechanical force for sperm movement. However, they could not entirely rule out the possibility that the relevant genes are elsewhere in the genome and are merely regulated in some way by *kl-3* and *kl-5*. In this respect it is of interest to note that male-sterile lesions in *D. melanogaster* loop-forming fertility factors have been identified which appear to have no effect on the morphology of the loops (BONACCORSI *et al.* 1988). Thus it is still an open question as to whether or not the *Drosophila Y* chromosome contains conventional protein coding genes. In summary, for neither *D. melanogaster* or *D. hydei* are we certain of the molecular structure or function of the majority of the *Y* chromosome and the role that it plays in spermatogenesis remains obscure.

This report describes the characterization of a family of *D. melanogaster* transcripts specific to the male germ line. Surprisingly, while one of the corresponding genes is on the third chromosome, the remainder are located on the *Y* chromosome. We have determined the sequence of genomic DNA including one *Y* chromosomal locus, and find that its structure is very similar to that of the autosomal locus. It appears unable to encode a functional polypeptide, however. The sequence of a near full-length cDNA clone derived from the autosomal locus (KALDERON and RUBIN 1988) translates as a polypeptide with similarity both to the vertebrate histone H1/H5 class of proteins and to mammalian cysteine-rich protamines.

MATERIALS AND METHODS

***Drosophila* methods:** *Drosophila* stocks were maintained at 25° on standard cornmeal/yeast/agar medium. Mutant nomenclature is as described by LINDSLEY and ZIMM (1992). Agametic males were generated by crossing *Oregon R* males with virgin *bw tud^{wc8}/bw tud^{wc8}* females (BOSWELL and MAHAWOLD 1985). Genotypic females somatically transformed to maleness were generated by crossing *cn tra2^B bw/CyO*; *B^Y* males with virgin *Df(2L)trix/CyO* females (BELOTE and BAKER 1983; GORALSKI, EDSTROM and BAKER 1989). Single-sex populations for Northern blot analysis were generated by use of the *cin* strategy (DIBENEDETTO *et al.* 1987). *XO* and *XXY* individuals were generated by crossing *Y^s.Y^L*, *In(1)EN, y w f* males with virgin *Oregon R* females. Elements of *T(X;Y)s* were separated by crossing *T(X;Y)* males with virgin *Oregon R* females (KENNISON 1981). For breakpoints in either *Y^L* or *Y^s*, the *Y^DX^P* element segregates to male

progeny and the X^{DY^P} element to female progeny. Males carrying Y chromosome deficiencies were generated by crossing attached $XY/Df(Y)$ males to virgin $y w f$ females, male progeny carry the Y chromosome deficiency. Wild-type flies were obtained from the Cambridge stock collection.

Nucleic acid isolation: Large and small scale preparation of *Drosophila* genomic DNA was carried out essentially as described by ASHBURNER (1989). Total *Drosophila* RNA from various developmental stages was isolated, with minor modifications, by the method of CHOMCZYNSKI and SACCHI (1987). Purification of poly(A⁺) mRNA by oligo-dT cellulose chromatography, subcloning of cDNA and genomic DNA fragments, plasmid DNA and bacteriophage λ DNA isolation, were all carried out as described by SAMBROOK, FRITSCH and MANIATIS (1989).

Gel electrophoresis, blotting and hybridization: DNA and RNA were separated by electrophoresis on agarose and formaldehyde/agarose gels respectively, and were blotted onto Hybond-N membranes, as described by SAMBROOK, FRITSCH and MANIATIS (1989). Hybridization was carried out at 42° in 50% formamide, 1% sodium dodecyl sulfate (SDS), 5 × SSPE, 10 × Denhardt's reagent 100 μ g/ml denatured salmon sperm DNA. ³²P-labeled DNA probes were prepared from gel-purified restriction fragments by random-priming (FEINBERG and VOGELSTEIN 1983). Blots were washed in 0.1 × SSC, 1% SDS at 65° (high stringency), or in 2 × SSC, 1% SDS at 55° (low stringency).

DNA sequencing: Double-stranded plasmid DNA sequencing was carried out by the Sequenase 2 method, according to the manufacturers recommendations (U.S. Biochemical Corp.). Nested deletions of plasmids were generated as described by HENIKOFF (1984). DNA sequence analysis was performed with the assistance of the GCG suite of computer programs (DEVEREUX, HAEBEREL and SMITHIES 1984).

Male-specific genes: λ gS8 and λ gS9, two EMBL3 derivatives containing fragments of *D. melanogaster* genomic DNA, were isolated in a screen for phage that hybridize with cDNA representing male, but not female, third instar larvae (RUSSELL 1989). Although the inserts are non-overlapping, restriction fragments that hybridize with male cDNA cross-hybridize with one another under conditions of high stringency. Two cDNA clones (cS1, 530 bp; cS8, 479 bp) were isolated from a male third instar larval cDNA library (RUSSELL 1989) via homology with a 3.2-kb *Hind*III fragment of λ gS9 that encompasses the male-specific region (Figure 1).

RESULTS

In the course of screening a *D. melanogaster* genomic DNA library for genes expressed in male but not female third instar larvae, we isolated two clones (λ gS8 and λ gS9; Figure 1) with substantial homology between their male-specific regions, despite having non-overlapping inserts (RUSSELL 1989). As we show below these two clones contain related sequences which show male restricted expression. Furthermore we demonstrate that in the genome these sequences are found at a single autosomal site and at several copies on the Y chromosome. The sequence of a 2.0-kb *Bam*HI-*Hind*III fragment including the entire male-specific region of λ gS8 was found to be that of a previously characterized locus, 18c (KALDERON and RUBIN 1988;

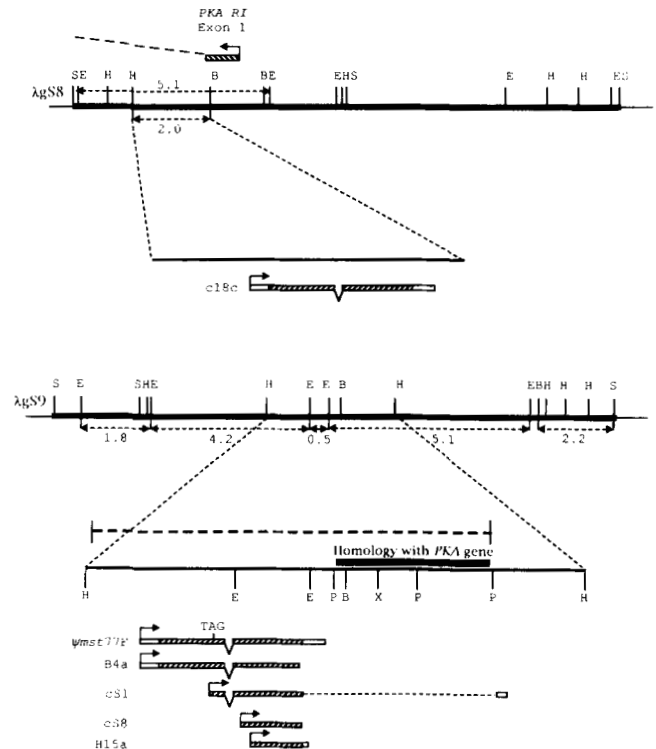


FIGURE 1.—The *Drosophila* genomic DNA clones λ gS8 (autosomal; 77F) and λ gS9 (Y -linked), their relationships to the cDNA clones c18c and cS1, and to the gene encoding the RI subunit of PKA (see also KALDERON and RUBIN 1988). Exons are shown as boxes (with the proviso discussed in the text concerning what appears to be an extra exon of cS1). Relative directions of transcription are indicated by arrows. c18c appears to represent an authentic transcript of the autosomal locus defined by λ gS8 (see text). λ gS8 also contains a component of the functional RI gene from 77F as shown. Other relationships are not intended to imply the origin of a particular cDNA from the genomic locus in question, merely a correspondence in terms of overall DNA sequence. Thin lines in the maps of genomic DNAs represent vector arms. Shaded boxes in the maps of cDNA clones represent putative coding sequences, open boxes represent 5' and 3' untranslated sequences. ψ mst77F represents the conceptual mRNA from the λ gS9 clone with the position of the stop codon indicated. The hatched box above λ gS8 represents the first exon of the RI gene (note that a small proportion of RI transcripts have sequences internal to the box spliced out). In the case of the 77F locus, this exon is separated from subsequent exons by an intron \approx 12 kb in length (beyond the limits of the *Drosophila* DNA component of λ gS8). The 3.2-kb *Hind*III fragment shown in expanded form below the map of λ gS9 includes all of the homology with male cDNA. It has been sequenced in its entirety. The region of *known* homology between λ gS9 and the 77F locus begins at nucleotide position 47 and extends for 2411 bp and is shown as a dotted line above the 3.2-kb *Hind*III fragment (see also Figure 6). The extent of homology with the PKA RI subunit is indicated with a black bar. The location of the 3' end of cS1 is shown and is also included in Figure 6. B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sal*I. Sizes of restriction fragments are given in kilobases.

EMBL accession no. X16962), mapping to cytological region 77F on the left arm of chromosome 3 (see Figure 6 for the sequence). 18c is contained within the first intron of a gene encoding a regulatory (RI) subunit of cAMP-dependent protein kinase (PKA). As

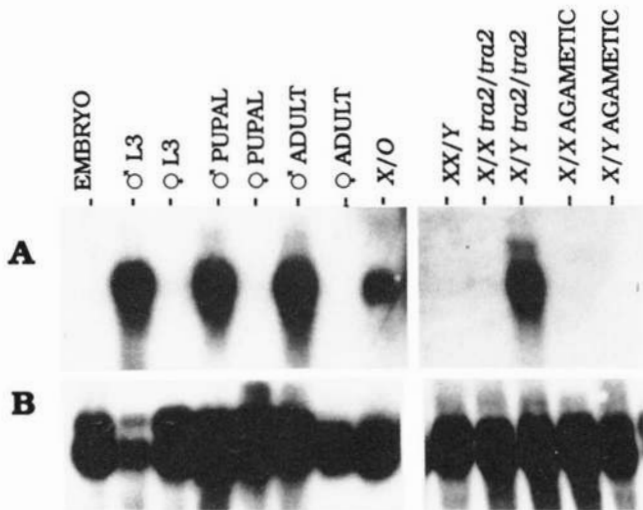


FIGURE 2.—Northern blots representing approximately 2 μ g of poly(A⁺) mRNA per lane from the indicated stages and genotypes. (A) Putative Y-linked cS1 probe and (B) same blot reprobed with cloned DNA from the actin 5C gene (FYRBERG *et al.* 1981). L3, third instar larvae.

the transcription of 18c is restricted to males we propose to rename it *mst77F* (male-specific transcript) in accordance with accepted practice.

Transcriptional analysis: The cDNA clone cS1 was used to probe Northern blots of poly(A⁺) mRNA prepared from various developmental stages, as well as from agametic and sexually transformed individuals (Figure 2). A broad band, suggestive of a heterogeneous collection of mRNAs of average size 1400 nucleotides, is seen in lanes representing wild-type male, but not female, third instar larvae, pupae and adults. The band is not seen in lanes representing adult male offspring of females homozygous for the mutation *tudor* (*tud^{wc8}*). Such males have no germ line (BOSWELL and MAHAWOLD 1985). Nor is it seen in adult genotypic females homozygous for the *tra2* mutation (pseudomales) which, though transformed somatically to maleness, have a female-like or undifferentiated germ line in which few spermatocytes can be detected (STEINMANN-ZWICKY, SCHMIDT and NOTHIGER 1989). In both agametic males and pseudomales the somatic component of the gonad is phenotypically male, albeit underdeveloped (BOSWELL and MAHAWOLD 1985; BROWN and KING 1960). Taken together these data suggest that the transcript(s) detected by cS1 are either expressed in the male germ line, or are dependent upon the male germ line for their expression. Support for the former hypothesis is provided by preliminary results from *in situ* hybridization to larval testis, in which cS1 detects transcripts present in spermatocytes (S. R. H. RUSSELL, unpublished data).

Figure 2 also shows the relative levels of transcript(s) in XXY females (none detectable), and in XO males (detectable but at reduced intensity). Three possibili-

ties could account for the latter observation: (a) a general reduction in cell metabolism, associated with disruption of spermatogenesis in XO individuals, results in reduced transcription or stability of germ-line transcript(s); (b) Y-linked copies are in some way required for maximal transcription of the autosomal locus; (c) Y-linked copies are themselves transcribed, giving rise to poly(A⁺) RNAs similar in size to the autosomal transcript. Isolation of cDNA clones appearing to represent Y-linked copies suggests that the latter are indeed transcribed (see below). Finally, KALDERON and RUBIN (1988) presented evidence that the *mst77F* family is transcribed in adult heads, although at relatively low levels compared with those in adult bodies. We have not formally ruled out such a possibility, but given the above data it is probable that their result was due to a contamination of heads with bodies (D. KALDERON, personal communication).

Y-linked sequences with homology to *mst77F*: KALDERON and RUBIN (1988) noted that *mst(77)F* sequences were repeated in the genome and to investigate their distribution carried out *in situ* hybridization to squashes of salivary gland polytene chromosomes. Hybridization was seen only at 77F, suggesting to them that the sequences related to *mst77F* are all clustered within this region of chromosome 3. Hybridization to the Y chromosome would not have been observed in these experiments. We have obtained evidence for Y-linked copies as follows. The cDNA clone cS1 was used to probe Southern blots of DNA isolated from *Oregon R* males and females. In addition to a common band, due to a 5.1-kb *EcoRI* fragment that spans the *mst77F* locus, seven extra bands are detected in lanes representing male DNA (10 kb, a 5.1-kb band which comigrates with the autosomal band, 4.5, 4.2, 3.8, 3.1 and 0.5 kb; Figures 3 and 4). Thus, sequences related to cS1 are present on the Y chromosome, a conclusion that is supported by similar analysis of DNA isolated from XO males and XXY females (Figure 4A). In the case of three of the male-specific bands (0.5, 4.2 and 5.1 kb) fragments of the same sizes and hybridization characteristics occur within the genomic clone λ gS9. Identical results are obtained when the KALDERON and RUBIN c18c clone is used as a probe (not shown). The lack of overlap between the *Drosophila* DNA components of λ gS8 and λ gS9 genomic clones is therefore explained by the latter being derived from the Y chromosome.

To investigate the generality of our observations, we examined DNA prepared from *D. melanogaster* isolated from a range of geographical locations, as well as from various sibling species. In the case of nine additional *D. melanogaster* strains, both male and female DNAs contain a 5.1-kb *EcoRI* fragment that hybridizes with cS1, while additional fragments are evident in male DNA. The hybridization patterns of

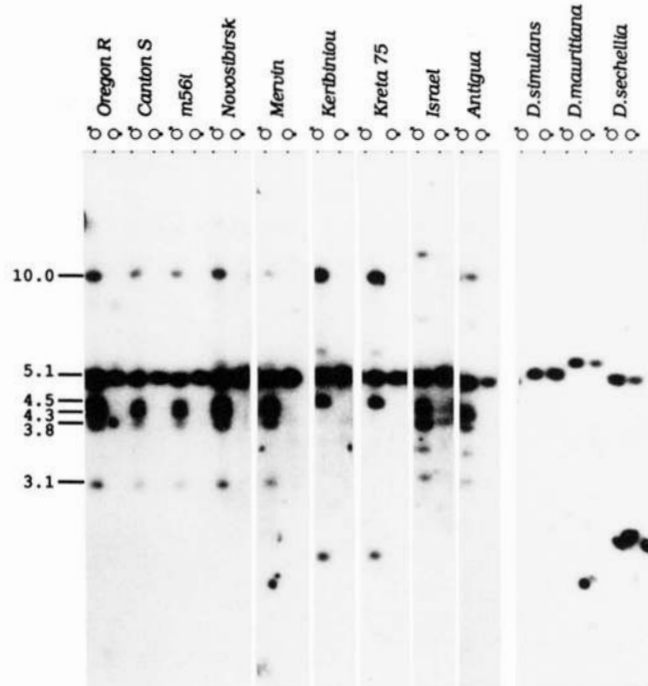


FIGURE 3.—Southern blot of *Eco*RI-cleaved male and female genomic *Drosophila* DNA probed with the putative Y-linked cS1 cDNA clone. The first 18 lanes are DNAs from *D. melanogaster* strains; the blot was washed at high stringency. The remaining lanes, containing DNA from sibling species, were washed at low stringency. All lanes derive from the same blot, which has been rearranged for clarity. Sizes in kilobases refer to the male-specific bands of *Oregon R* DNA. (Note that the 0.5-kb fragment referred to in the text is not visible on this exposure. The relevant band can be seen in Figure 4A.)

Canton S, *m56i*, *Novosibirsk* and *Mervin* DNAs (Figure 3) are all similar to the *Oregon R* pattern. *Keribiniou* and *Kreta 75* DNAs exhibit a simpler pattern with four male-specific bands, two of which appear to have *Oregon R* counterparts (Figure 3). *Israel* and *Antigua*, as well as the attached-XY strain used for generating XO and XXV individuals, the *T(X;Y)* strains and the *Df(Y)* strains described below, exhibit a more complex pattern of at least 10 male-specific bands, some of which appear to have *Oregon R* counterparts (Figures 3 and 4). In contrast, none of the three sibling species we have tested (*Drosophila simulans*, *Drosophila sechellia* and *Drosophila mauritiana*) shows evidence of Y-linked copies (Figure 3), their DNAs exhibit only a single band in both males and females. *In situ* hybridization to *D. simulans* polytene chromosomes indicates a single site of hybridization at 77F (not shown). In summary, the Y-linked sequences appear to be unique to *D. melanogaster*, and thus may have "transposed" to the Y chromosome after present day *D. melanogaster* and its sibling species diverged from a common ancestor.

We have also prepared DNA from males lacking different regions of the Y chromosome. The entirely heterochromatic Y of *D. melanogaster* consists of a long

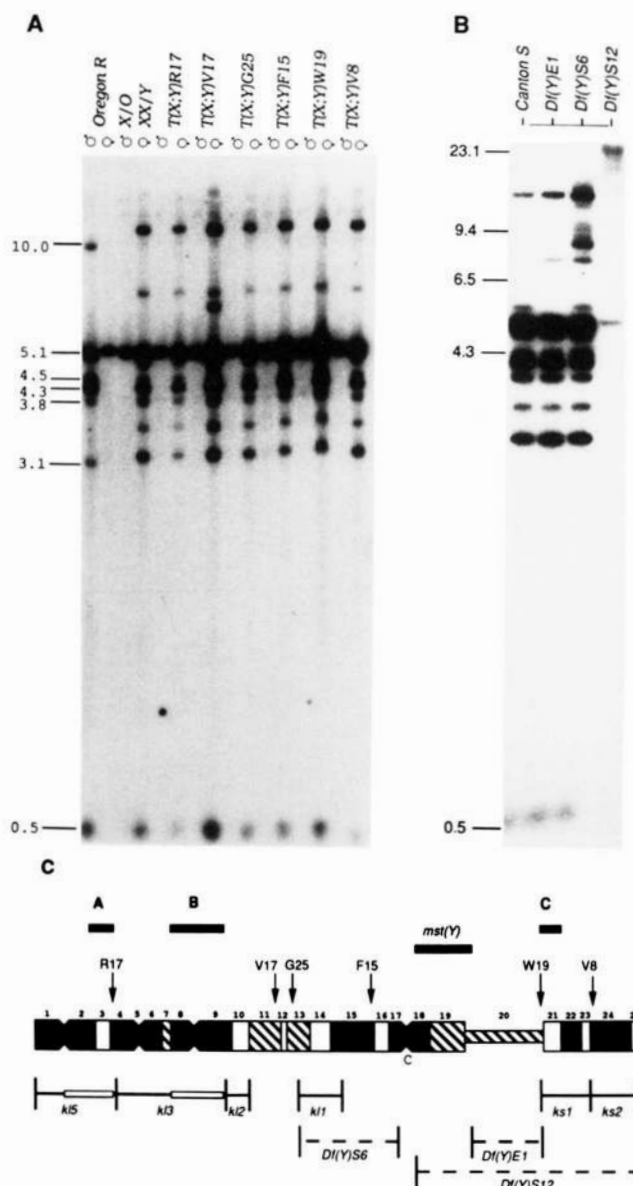


FIGURE 4.—Restriction site and cytogenetic analyses. (A) Southern blot of *Eco*RI-cleaved male and female DNA isolated from *D. melanogaster* strains as indicated and probed with the putative Y-linked cS1 cDNA clone. The blot was washed at high stringency. Sizes in kilobases refer to the male-specific bands of *Oregon R* DNA. (B) Southern blot of *Eco*RI-cleaved DNA from males carrying the indicated Y chromosome deficiencies. The blot was probed with the autosomally derived cDNA clone c18c (KALDERON and RUBIN 1988) and washed at high stringency. The intensity of the 5.1-kb fragment in *Df(Y)S12* is of lower intensity than expected whether the c18c or cS1 probes are used; however, we note a substantial portion of the DNA remains undigested, and we have obtained the same result on two separate occasions and believe that it is an experimental artifact. (C) A cytogenetic map of the Y chromosome (redrawn after BONACCORSI *et al.* 1988), showing the *T(X;Y)* and *Df(Y)* breakpoints. Lamprush loop-forming regions (A, B and C) are indicated above the map. The locations of fertility factors are shown with the lines representing their maximal extent and the open boxes their minimal extent. The centromere and the region containing the *mst77F* homology are also indicated.

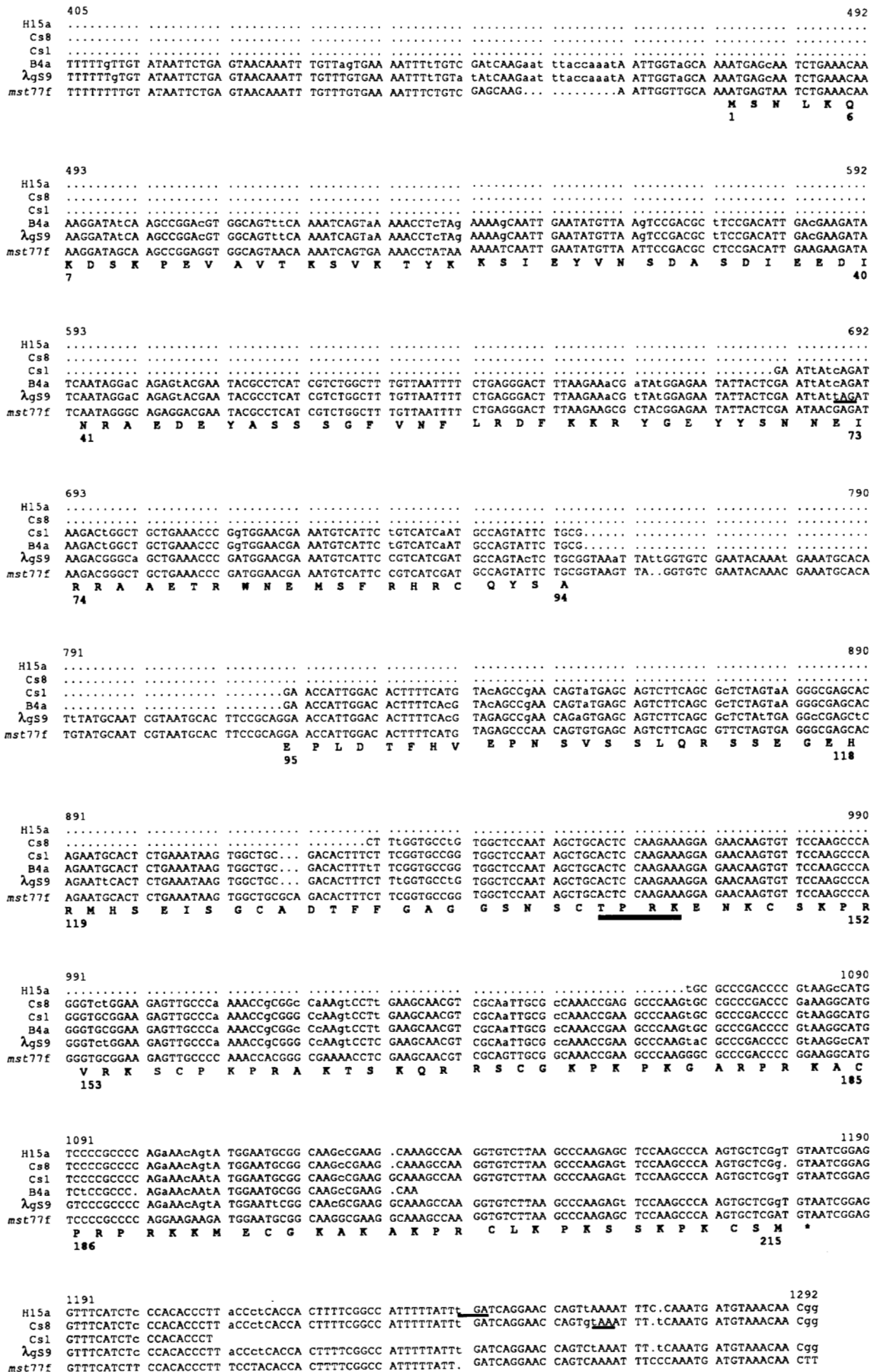


FIGURE 5.—Alignment of indicated genomic and cDNA sequences with respect to the sequence of *mst77f* using GCG PILEUP with default parameters. Differences from *mst77f* are indicated in lower case. Numbering is taken from KALDERON and RUBIN (1988). The 5'

arm, Y^L , and a short arm, Y^S (Figure 4C). Radiation-induced translocation between the Y and the X generates hybrid chromosomes [$T(X;Y)$'s] of two types. $Y^D X^P$ chromosomes (D , distal; P , proximal) have a centromere-less portion of the Y appended to a centromere-containing portion of the X . Conversely, $X^D Y^P$ chromosomes have a centromere-containing portion of the Y appended to a centromere-less portion of the X . We analysed a series of strains carrying different reciprocal translocations between either the long or the short arm of the Y chromosome and centromeric heterochromatin of the X (KENNISON 1981). Males having both hybrid chromosomes lack no genetic information and are fertile if the Y breakpoint does not interrupt a fertility factor (see Figure 1 of GOLDSTEIN, HARDY and LINDSLEY 1982 for a diagrammatic representation). Crossing such males with wild-type females, however, causes $Y^D X^P$ chromosomes to segregate in male progeny and $X^D Y^P$ chromosomes to segregate in female progeny. Six $T(X;Y)$'s were studied in all, representing breakpoints throughout the Y chromosome (Figure 4C). We followed the sequences of interest by hybridization as above to Southern blots of the respective DNAs (Figure 4A). In the case of Y^L breaks (R17, V17, G25 and F15), homology segregated with female progeny. Thus, all of the Y -linked members must lie proximal to (*i.e.*, on the centromere side of) the F15 breakpoint. In the case of Y^S breaks (W19 and V8) homology again segregated with the female progeny. Thus all of the Y -linked members must lie proximal to the W19 breakpoint.

To localize the Y linked copies more precisely we investigated males bearing three different Y chromosome deficiencies (Figure 4C). Compound $XY/Df(Y)$ males were crossed with virgin $y w f$ females and the DNA from male progeny analyzed by Southern blotting (Figure 4B). While the normal complement of male-specific fragments is present on the $Df(Y)E1$ and $Df(Y)S6$ chromosomes none is present on the $Df(Y)S12$ chromosome. In summary, all of the Y -linked members of the $mst77F$ family reside on the short arm of the Y chromosome, between the proximal boundaries of $Df(Y)S12$ and $Df(Y)E1$. This region, h18-h19 on the cytogenetic map (GATTI and PIMPINELLI 1983), is indicated in Figure 4C. It does not include any known fertility determinants.

A gene "family": That KALDERON and RUBIN's (1988) clone c18c represents an authentic transcript

of the autosomal $mst77F$ locus is suggested by the fact that the two sequences differ at only a single, noncoding, position. They also sequenced two other cDNAs (B4a and H15a) that, although clearly related to $mst77F$, are sufficiently different both from it and from each other that they seemed likely to represent independent loci. We have also isolated and sequenced two cDNA clones (cS1 and cS8), and have sequenced a segment of λ gS9 DNA (see legend to Figure 1) that includes the entire male-specific region. Taken together these sequences appear to represent five additional loci which our Southern blot data indicate derive from the Y chromosome. An alignment of the various sequences with respect to that of $mst77F$, together with the sequence of the 215-amino acid product deduced for $mst77F$ (via the sequence of c18c), is shown in Figure 5.

The member of the family represented by genomic clone λ gS9 retains the intron/exon structure of the autosomal locus (the single intron comprises residues 757–818), but is unable to encode a full-length polypeptide due to a stop codon at amino acid residue 72 (equivalent to residues 688–690 of the $mst77F$ sequence and underlined in Figure 5). There is also an insertion of 12 bp just 5' to the putative initiation codon, an in-frame deletion of nucleotide residues 918–920 (that would cause deletion of an alanine residue if a full-length translation product was generated), and a number of single-base substitutions.

cDNA B4a (698 bp) extends 5' to include the $mst77F$ translation initiation codon, and the 12-bp insertion observed in λ gS9. In the other direction the homology with $mst77F$ extends to nucleotide position 1134 where the B4a cDNA terminates (KALDERON and RUBIN 1988). It is not known whether the cDNA showed evidence of a poly(A) tail since the 3' end of the clone was not sequenced in full (D. KALDERON, personal communication). As in the case of λ gS9, B4a has an in-frame deletion of residues 918–920, together with a number of single-base substitutions. There are also deletions of residues 1100 and 1131, the effect of which is a change of reading frame beyond amino acid residue 188. According to KALDERON and RUBIN, the putative B4a product would terminate after a further 10 amino acids. Taking into account the in-frame deletion, the B4a product would thus be 18 amino acids shorter than the $mst77F$ product.

boundary represents residue 48, the 3' boundary residue 863 of the polyadenylated cDNA clone c18c (total length 1031 bp). The sequence of c18c can be derived merely by removing the intron (residues 757–818) from the $mst77F$ sequence. Homology between $mst77F$ and λ gS9 extends considerably further both 5' and 3' (see Figure 6). B4a extends 5' to position 399, and 3' to position 1124. cS1 diverges from $mst77F$ beyond position 1209 and terminates after a further 60 bp with evidence of a poly(A) tail. cS8 diverges beyond position 1340 and terminates after a further 69 bp with no evidence of a poly(A) tail. H15a diverges both upstream of position 1068, and beyond position 1280 (terminating after a further 27 bp). The sequence of the putative $mst77F$ translation product (deduced from c18c) is also shown. The translation stop codon is indicated by an asterisk (*), and the putative phosphorylation site is underlined. Also underlined is the premature stop codon in the λ gS9 sequence (residues 688–690). See text for other details.

47 TTGAAAAAAGGAAGTACGCTATTCTATTCTTGAAGATCAGATACGCGTTTCTCAGCTAGTCGGATTGCAAAACAGAACTACTAAAACCTTTTCAAGACG 146
-21 TTGAAAAAAGGAAGTACGCTATTCTATTCTTGAAGATCAGATACGCGTTTCTCAGCTAGTCGGATTGCAAAACAGAACTACTAAAACCTTTTCAAGACA 79
147 AACGGACGCGACTATGGCCAGCTCGTTAGATATTGATAAAGAAATGTATATCCTTAAACATTTTCTCCTACAATACCTTGAACGAATCTAGAATACAAA 246
80 CACGGACGCGCAATATGGCCAGCTCGACTGATCTAGTCAAGA AACATTAACATTTTCTCCTACAATATCTTGAACGAATATAGTATACAAA 173
247 AAT . ACCATGGAATATGGCCGCGATCGGGAATGTACTTCGAAATCAGCCACATTGTTGAAAAAGGAACCTTTTCATGATCATAGTAGCATTGAG 345
174 AATAACCATGGAATATGGCCGCGATCGGGAATGTACTTCGAAACAGCCAACTTGGTTGAAAAAGGAACCTTTTCTGATCATAGTAGAATTGAG 273
346 CCTCCATTCAAATCCAATCTTCATATCCCACTCTGAAGACAGCATA . ATCAGTTA CCTTGAAGTACGAAACAACTAAAATAATTCGATAA 436
274 CCTTCATTCAAATCCAATTTCCAGGCTCCAGCTGAAGCCAGCATAACAGTTAGTACACTACCTTTGAAACGAGGAACAACTAAAATAATTCGATAA 373
437 AATTGAGTACAGTTTTTTATACATAT . . . TTTTTTTGTGTATAATTCTGAGTAAACAAATTTGTTTGTGAAAAATTTTGTATATCAAGAATTTACCAAT 533
374 AATTGAGTACAGTTTTTTATACATATATAATTTTTTTTTGTATAATTCTGAGTAAACAAATTTGTTTGTGAAAAATTTCTGTCGAGCAAG 461
534 AATTGGTAGCAAAATGAGCAATCTGAAACAAAAGGATATCAAGCCGCGAGTGGCAGTTTCAAATCAGTAAAAACCTCTAGAAAAGCAATGAATATGTT 633
462 AATTGGTTGCAAAATGAGTAACTGAAACAAAAGGATAGCAAGCCGAGGTTGGCAGTAAACAAATCAGTAAAAACCTATAAAAAATCAATGAATATGTT 561
^{msc77F start}
634 AAGTCCGACGCTCCGACATGACGAAGATATCAATAGGACAGAGTACGAATACGCCCTCATCGTCTGGCTTTGTTAATTTCTGAGGGACTTTAAGAAAC 733
562 AATTCCGACGCTCCGACATGAAAGAAATATCAATAGGGCAGAGGCAATACGCCCTCATCGTCTGGCTTTGTTAATTTCTGAGGGACTTTAAGAAAC 661
734 GTTATGGAGAAATATTACTCGAATATTAGATAAGACGGGCGACTGAAACCCGATGGAACGAAATGTCATTCCGTCATCGATGCCAGTACTCTGCGgtaaa 833
662 GCTACGGAGAAATATTACTCGAATAACGAGATAAGACGGGCTGCTGAAACCCGATGGAACGAAATGTCATTCCGTCATCGATGCCAGTATTCTGCGgtaa 761
834 ttattggtgtcgaatacaaatgaaatgcacatttctgcaatcgtaatgcacttccgcagAACCATTTGGACACTTTTACAGTAGAGCCGAACAGAGTGTAG 933
762 tta . . ggtgtcgaatacaaacgaaatgcacatgtagtaatgcacttccgcagAACCATTTGGACACTTTTACAGTAGAGCCGAACAGTGTAG 859
934 CAGTCTTACGCGCTTATTGAGGCGGAGCTCAGAAATCACTCTGAAATAAGTGGCTGC . . . GACACTTTCTTTGGTGCCTGTGGCTCCAATAGCTGCCT 1030
860 CAGTCTTACGCGTCTTAGTGAAGGCGAGCACAGAAATGCACTCTGAAATAAGTGGCTGCGCAGACACTTTCTTGGTGCCTGTGGCTCCAATAGCTGCCT 959
1031 CCAAGAAAGGAGAACAGTGTCCAAGCCAGGGTCTGGAAGAGTGTCCCAAACCCGCGGCAAGTCCCTCGAAGCAACGTCGCAATTTGCCCAAAACCGA 1130
960 CCAAGAAAGGAGAACAGTGTCCAAGCCAGGGTCTGGAAGAGTGTCCCAAACCCGCGGCAAGTCCCTCGAAGCAACGTCGCAATTTGCCCAAAACCGA 1059
1131 AGCCCAAGTACGCCCGACCCGTAAGGCATGTCCCGCCCGAAGCAGTATGGAATTGCGCAACGCGAAGCCAAAGCCAAAGGTGCTTAAAGCCCAAGAG 1230
1060 AGCCCAAGGCGCCCGACCCCGAAGGCATGTCCCGCCCGAAGCAGTATGGAATTGCGCAACGCGAAGCCAAAGCCAAAGGTGCTTAAAGCCCAAGAG 1159
1231 TTCCAAGCCCAAGTGTCTGGTGTAAATCGGAGGTTTCATCTCCACACCCCTTACCCTCACCCTTTTCGGCCATTTTTATTGATCAGGAACAGCTCTAAA 1330
1160 CTCCAAGCCCAAGTGTCTGGTGTAAATCGGAGGTTTCATCTCCACACCCCTTACCCTCACCCTTTTCGGCCATTTTTA . TTGATCAGGAACAGTCAAAA 1258
^{msc77F stop}
1331 TTT . TCAAATGATGTAACACACGGGCGTTGCTAGAATCTATGAACCTTGAACAACTCAGTAAACAGGAAATGAGAAAATCGGAAAGAAATCTAATCTG 1429
1259 TTTCCAAATGATGTAACAACTTGGTGTGCTAGAATCTATGAACCTTGAAGAACTCCAGTGAACAGGAAATTAAGAAATC 1340
1430 AATCGGCGCAAAAGGGAGCTCAAACAGATCTGATAAACCAAGGACCTAAGACAGGAAGGCCAAAGCATTGATCCTCAAGGCAACGATAAGCGATTGACT 1529
1341 GATCATACGGATCTGATAAACCAAGGACATAACACAGGAAGGCCAAAGCATTGATCCTCAAGGCAACGATAAGCGATTGACT 1423
1530 ATTTAGAAATAATGGTTTTATGTCTGATCAA TTATAATTTGGGTTTCGGCCCTTACCCTC 1588
1424 GTTTAGAAATAAGGTTTTATGTCTGATCAAATTTCTACCCATATTTTATCTACCAATATATGGTATTATTATAATTTGGTTTTGGACTCACCTC 1523
^{poly A signal} ^{RI Exon}
1589 TCGAGTTTCTGAAAGTACTGTCTGAGGACTGCACG ACGTGTCTGCAGACGGACAACCTGGACGATGAAGTCTTGGAGACTCGCTGG 1675
1524 TC . AGTTTCTGAAAGTACTGTCTGAGGACTGCACGGGATTCTCGGCTCTGCAGACGCACAGCTGGACGATGCAGTCTTGGAGACTCGCTGG 1616
1676 ATCCCATTCGATGGATGTAGTGTTC GCTCTGCTTTCACGCGATTTGGCCATCATGTAGGACATGTGGTACGGTGGAGTGGAAATGG 1763
1617 ATCCCATTCGCTGGATGTAGTGTTCGCACTCCCGCAGGCTCTGCTCCTCCAGCGTTTGGCCATCATGTAGGACATGTGGTACGGTGGAGTGGATGG 1716
1764 ATTCGGAATGGGTTTGAATGAATGGCGGTGATGATGAGATTGTGATAGGG AAAATATACGTATGTACGTAT 1836
1717 ATTCAGAGTGGGTTTGAATGAATGGCAGTGTGATGAGATGGTGTGTTGGAACAGCTTTCAAGCCCGCCTACGCAAAATATACGTATGTAGGTAT 1816
1837 GGATGATATATATTATGTACATAACGTTTGTGTGGGTAGTTATCTA . . GAGGGTTAATGTTTCGCTGGCAAACTCGGTTAAAAGCACTTGATAAATTTGCC 1934
1817 GGATGATATATATTATGTACATAACGTTTGTGTGGGTAGTTATGAAATGTTGTTTTCGCTGGCAAACTCGGTTAAAAGCAC . TGATAAATTTGGC 1915
1935 TATCT . TCGGACTGCCGCTTTGTCTAAGCCTGCTGC . TTATCAACCCG . TTGCTGTGACCCG . TTCGAGCTTT . . . CTGG . . TGTGGCAGCTGTG 2025
1916 TATATCTTGAAGTCTGCCGCTTTGTCTAAGCCTGCTGCTTTATCAACCCGTTGCTGTGACCCGTTTCGAGCTTTTCTTGGCTTTTGGCACCCTTT 2015
2026 GTT CACACACAGCACAACCTCCGCTTTCACCTTGGCAAAAT CAGGTTTCTGGACTTTTTGCAATCACTGGA 2098
2016 GTTCCGTTTTCGACACACAGCACAACCCGTTTTCACCTTGGCAAAAT TACGTTTCCGGACCTCTTGAATGCTCTAATGATTAATGCCCTTAGCCC 2115
2099 GGCAGCATATGGATTGCTCGATGGCTGTCAGAAAGTGTAACTAAGTT TTTTTCACGCAGTACAAAG 2168
2116 CGCACTCCCGCAGCATATGGATTGATCGATGGCTGTGGAAGTCACTTAAGTATTTTTCATAAACTTAACCAATTTTCAGCAGCTCAAGC 2215
2169 TT . TTTTTTTTTTTGGGCTCAGGATAGTGGTGGCGCTGATTTAGTACCCGTTTATCAATAGGTATCACTTT . GCCTCAGACTTATCGATGACAA 2265
2216 TTGTTGTTTTTTTTGGGCTGCATGCAGTGGTGGCGCTGATTTGGTCA . CGTTCATATCGATAGGTTTCACTTGGCAACACTGCTTATCGATACAT 2315

FIGURE 6

How many of the sequences represent functional loci? Assuming that *mst77F* is indeed functional, and that c18c represents its transcript, the locus represented by λ gS9 is unlikely to encode a functional polypeptide due to the premature stop codon at amino acid residue 72. Both H15a and cS8 show no homology with *mst(77)F* at their 3' ends and if translated, both would result in polypeptides having carboxy termini that are longer and substantially different in character than that of the *mst77F* polypeptide. Until we have full-length cDNA clones we cannot comment further on the genes encoding these cDNAs. cS1 which may be incomplete at its 5' end due to premature termination of cDNA synthesis shares the same termination codon and basic structure as *mst77F*, and thus may represent a functional locus. Finally, B4a, despite having a frameshift at amino acid residue 189 and diverging from *mst77F* within the coding domain, is able to encode a polypeptide that is neither radically different in length from the *mst77F* product, nor altogether different in terms of the nature of its terminal residues. Thus it too may encode a polypeptide with functional similarity to *mst77F*.

Structure of the locus represented by λ gS9: To learn more about the nature of the event(s) that gave rise to *mst77F*-like genes on the Y chromosome, we have sequenced the entire 3.2-kb *Hind*III fragment of λ gS9, shown in expanded form in Figure 1. This fragment, containing all of the homology with male cDNA and only a single member of the *mst77F* family, has more extensive similarity with DNA in and around the *mst77F* locus than might have been expected. The extended 77F and λ gS9 sequences, aligned in Figure 6, have approximately 92% sequence identity. 5' to the *mst(77)F* gene the homology extends at least 500 nucleotides upstream of the initiation codon. In addition the intron-exon structure of the male-specific gene is conserved, there are only 5 base changes within the 62-bp intron (Figure 5). This indicates, at least in the case of this particular Y-linked copy, that presence on the Y chromosome is not due to integration of a reverse-transcript of a *mst77F* mRNA. At the 3' end, homology between the 77F locus and λ gS9 extends for several hundred base pairs beyond the poly(A) addition site of c18c, and into transcribed sequences of the gene for the RI subunit of PKA. Polymorphism (notably insertion/deletion) is most evident 3' to the *mst77F* gene. It may be that sequence drift in the 5' region has been subject to more constraint due to functional selection of some kind, for example important regulatory sequences may need to be conserved for appropriate expression. Assuming that c18c represents a prematurely terminated cDNA of a 1.4-kb polyadenylated mRNA (see below), some of the 5' region is likely to correspond to 5'-untranslated sequences. A search for promoter motifs up-

stream of the c18c 5' end provides little compelling evidence for standard promoter elements. A 5/8 match to the TATA-box consensus is found centered around nucleotide 320, 30 bp upstream of the c18c 5' end and a 7/9 match to the CAAT-box consensus is present around position 197, 153 bp upstream of the c18c 5' end. In the absence of primer extension data, however, we cannot firmly identify the transcription start-site of *mst77F*. We have also searched for promoter elements present in other male germ line-specific genes such as β_2 -tubulin and *mst87F* but failed to detect any similarities.

We have also investigated the nature of λ gS9 sequences which flank the region with autosomal homology. The 2.2-kb *Bam*HI-*Sal*I fragment of Figure 1 detects a family of repetitive DNA sequences containing a few Y-linked and many autosomal copies (Figure 7). The two relatively intense bands visible in lane 1 (male DNA) have not been investigated further. The 1.8-kb *Eco*RI fragment of Figure 1 detects a fragment of its own size in both male and female DNAs (Figure 7). The intensity of the corresponding band suggests that the 1.8-kb fragment lies internal to a repetitive DNA sequence. Data from limited restriction mapping are consistent with the repetitive sequence being a transposable element of the *calypso* class (COTE *et al.* 1986). In summary λ gS9 DNA, outside of the region of extended homology with the 77F locus, has characteristics that might be expected of the heterochromatic Y chromosome.

The *mst(77)F* gene product: The c18c cDNA clone sequenced by KALDERON and RUBIN (1988) contains a long open reading frame (ORF) which translates as a 215-amino acid polypeptide. They assigned no function to this putative translation product but noted that it contained a basic C-terminal domain. We have used the computer programs Prosrch and BLAST to search the protein databases with the sequence of the *mst77F* polypeptide. Using Prosrch (COLLINS, COULSON and LYALL 1988) to search the Pir28 protein database, highest scores (100–120) were obtained against various examples of the vertebrate histone H1/H5 family. Chicken erythrocyte H5, in particular, can be aligned with the *mst77F* polypeptide to display 26% identity and 45% similarity when conservative changes are allowed (Figure 8A). It should not be overlooked, however, that correspondence over the C-terminal 75 amino acids is in large part due to the predominance of basic residues in this region of both proteins. The contribution of amino acid constitution to the match is further emphasized by relatively high GCG-BESTFIT quality scores between a randomized *mst77F* polypeptide and chicken H5. Finally, the *mst77F* polypeptide is only slightly more like chicken H5 than like chicken H1 or *Drosophila* H1 (\approx 20% identity, 35% similarity). In contrast, likeness between

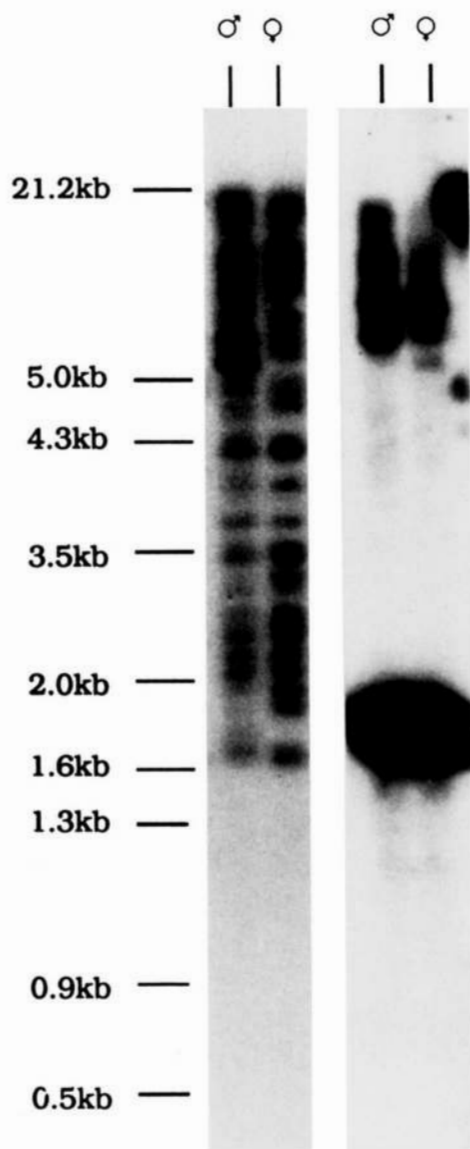


FIGURE 7.—Southern blots of *EcoRI*-cleaved male and female *Oregon R* DNA washed at high stringency. The left hand two lanes were probed with the 2.2-kb *BamHI-SalI* fragment of λ gS9, and the right hand lanes with the 1.8-kb *EcoRI* fragment (Figure 1). The two probes were of comparable specific activity and the two blots, each representing the same amount of the same pair of cleaved DNAs, were exposed for autoradiography for the same time.

chicken H5 and either chicken H1 or *Drosophila* H1 is significantly greater ($\geq 35\%$ identity, 50% similarity).

If it is a relative of H1 and H5, the *mst77F* polypeptide is a rather distant one. Secondary structure predictions, however, made using the computer programme ALB (PTITSYN and FINKELSTEIN 1989), tend to support the identification of the *mst77F* polypeptide as a member of the H1/H5 family. The first 10 amino acids are very polar. Subsequent sequence suggests a globular head region (residues 11–135) in which the dominant secondary structure is α -helix. This is fol-

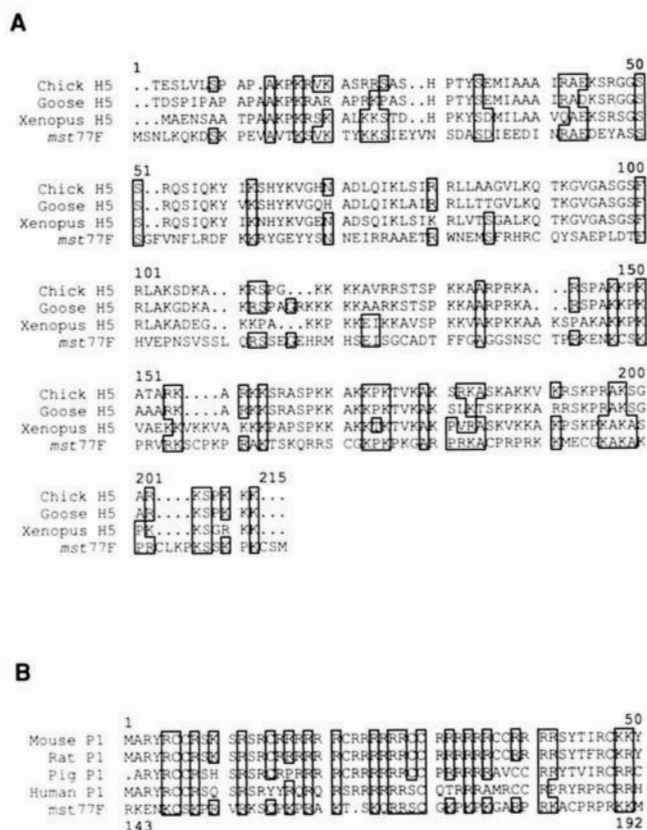


FIGURE 8.—Alignments of polypeptide sequences. (A) Alignment of the indicated polypeptide sequences using the GCG PILEUP program with default parameters. The *mst77F* sequence, to which the numbering refers, is the putative translation product of cDNA clone c18c [KALDERON and RUBIN (1988); Swissprot accession number: P16909]. Identities between the c18c translation product and the other polypeptides are boxed. PIR accession numbers are as follows: chicken H5, A29179; goose H5, A02588; Xenopus H5, JT0403. (B) Alignment of the putative *mst77F* polypeptide with mature mammalian protamine 1 sequences using GCG PILEUP with default parameters. Cysteine and basic (arginine and lysine) residues shared with the *mst77F* polypeptide are boxed. Numbering above the figure refers to rat protamine P1. Numbering below the figure refers to the *mst77F* polypeptide. Swissprot accession numbers are as follows: rat, P10118; mouse, P02319; human, P04553; pig, P19757.

lowed by a basic tail (residues 136–215) in which lysine, arginine and proline are frequently clustered, and that would be unstructured in free solution. Overall the structure is reminiscent of the those of H1 and H5, both of which have disordered N- and C-terminal domains flanking a central globular domain [see ISENBERG (1979) and BRADBURY (1992) for reviews]. Phosphorylation of members of the H1 family, occurring at serine or threonine residues in coordination with the cell division cycle is thought to activate their chromatin-condensing functions (HOHMANN 1983; BRADBURY 1992). In addition a consensus kinase motif of the form S(T)PK(R)K(R) has been identified in the N and C termini of histone H1, it is expected that phosphorylation of this site would abolish the interaction between H1 and DNA (CHURCHILL and SUZUKI

1989). The *mst77F* polypeptide contains one such sequence (residues 141–144, underlined in Figure 5), close to the predicted boundary between the globular domain and the basic tail.

Using the computer program BLAST (ALTSCHUL *et al.* 1990) to search the Swissprot protein database with the sequence of the *mst77F* polypeptide gave slightly different results. As well as identifying members of the H1/5 class high scores were also obtained between a segment of the basic tail of the *mst77F* polypeptide and cysteine-rich protamines from rat, pig, mouse and man. Similarities were approximately 50% (Figure 8B), in large part reflecting the predominance of basic residues but also involving cysteines. Protamines are short and predominantly basic polypeptides that complex with DNA. They are the major component of the sperm chromatin of many organisms (BLOCH 1969), their role being to assist compaction of the genome into the restricted volume of the sperm head. The cysteine-rich variety is typical of protamines found in mammalian sperm (SUBIRANA 1983; YELICK *et al.* 1987). The cysteines are thought to stabilize compacted chromatin via disulfide bridges (BEDFORD and CALVIN 1974). Although it is rare for cysteine residues to occur within histones H1 and H5, the *mst77F* polypeptide has 10, 8 of which are fairly regularly spaced throughout its basic tail (see Figure 8A).

Phosphorylation of serine residues by protamine kinase is thought to stimulate the binding of protamines to DNA where they replace histones or transition proteins, become dephosphorylated and condense DNA (BELLVE, ANDERSON and HALEY-BOWDOIN 1975; OLIVA and DIXON 1991). Although serine residues make up 10% of the basic tail and 12% of the full-length *mst77F* polypeptide, a relatively high serine content is also a feature of the H1/H5 family as a whole.

In summary, the *mst77F* polypeptide has characteristics suggestive of a distant member of the histone H1/H5 family. The C-terminal basic tails of H1 and H5 ("linker" histones) play a role in chromatin condensation via interaction with DNA. In H1 the basic residue is almost exclusively lysine. In H5, associated in chicken erythrocytes with chromatin inactivation, there are significant amounts of the more basic residue, arginine. In this respect the *mst77F* polypeptide is more like H5 than H1. The relationship between the *mst77F* polypeptide and cysteine-rich protamines seems relatively tenuous. It does, however, serve to point out the interspersion of cysteine residues within the basic tail, a feature that distinguishes the *mst77F* polypeptide from all other members of the H1/H5 family.

DISCUSSION

Male-specific transcripts with autosomal and Y-linked genes: We have identified a family of genes

transcribed in male but not female *D. melanogaster*. Transcription of any of the copies requires the presence of a male germ line, and preliminary *in situ* hybridization data suggest that this is restricted to spermatocytes (S. R. H. RUSSELL, unpublished). The member of the family that has been characterized in greatest detail is *mst77F*, present at cytological position 77F on the left arm of chromosome 3 (KALDERON and RUBIN 1988). It is contained within the large intron of the gene for an RI subunit of cAMP-dependent PKA (Figure 1). Southern blot analysis of male and female DNAs (Figures 3 and 4) points to *mst77F*-like sequences being present on the Y chromosome of *D. melanogaster*. Evidence that these represent members of an *mst77F* gene family is provided by genomic DNA clone λ gS9, and by cDNAs B4a, cS1, cS8 and H15a (the Y group). The insert of λ gS9 (Figure 1), clearly derived from the Y chromosome, contains a single *mst77F*-like gene ($\approx 8\%$ divergence) having the intron-exon structure of the autosomal locus embedded within related flanking sequences (Figures 5 and 6). Each of the four cDNA sequences differs sufficiently from the sequence of the autosomal transcript ($\approx 5\%$ divergence) that they too must represent Y-linked members (Figure 5).

As to the number of Y-linked loci we can consider the number of bands detected by cS1 in Southern blots of male *Oregon R* DNA (Figures 3 and 4). There are eight bands in total, one of which represents the autosomal *mst77F* locus (5.1 kb). Three of the remaining bands (0.5, 4.2 and 5.1 kb) represent the family member present within λ gS9 (Figure 1), leaving four (3.1, 3.8, 4.5 and 10 kb) to accommodate further members of the family. Thus we estimate that there are five Y-linked loci assuming that each non- λ gS9 band represents a single locus. It appears to be the case that some *Canton S* derivatives have additional Y-linked members, since the Y chromosomes of the XXY, T(X;Y) and Df(Y) individuals represented in Figure 4 were derived from a *Canton S* background (M. GATTI, personal communication).

Although members of the Y group are all more closely related to one another than they are to the autosomal *mst77F* locus (apparent at the DNA sequence level in terms of shared nucleotide substitutions, insertions and deletions), divergence still varies between 1.5 and 6% over the region of available overlap between any two members of the group (Figure 5). Can this be taken to indicate that each DNA clone from the Y group represents an independent locus, or might it be argued that some of these clones represent strain differences at a single locus? Our own sequences (λ gS9, cS1 and cS8) all represent an *Oregon R* population maintained in Glasgow while the cDNA clones c18c B4a and H15a and the genomic clone 18c are derived from a *Canton S* background (KALDERON

and RUBIN 1988). Although the pattern generated by hybridization of cS1 to *Canton S* DNA resembles that of *Oregon R* DNA (Figure 4A), sequence polymorphisms of this level may still be present which would not be revealed by the sensitivity of the Southern blotting experiments. Thus we cannot determine how many *Y* loci are represented by the cloned DNA. In conclusion, although there is more than one family member on the *Oregon R Y* chromosome, strain differences at a single locus may account for the differences between some of our cloned DNA.

Are the *Y*-linked members functional? In all of the preceding discussion, the assumption has been made that the autosomal *mst77F* locus is functional, and we have thus used its sequence as a standard against which to compare sequences representing *Y*-linked members. This assumption cannot be proven on the basis of the available data. It is relevant, however, that of several sibling *Drosophila* species we have investigated, all except *D. melanogaster* have a single member of the *mst77F* family (Figure 3), and in the case of *D. simulans* it resides at the cytological position equivalent to 77F of *D. melanogaster*. For reasons of karyotype similarity the same is likely to be true of *D. mauritiana* and *D. sechellia*. Presumably it is a functional locus in these species.

Accepting *mst77F* as functional, we must conclude that the locus represented by λ gS9 would be unable to encode a functional polypeptide due to its premature stop codon (Figure 5). Moreover, for reasons of either truncation or divergence, none of the cDNAs representing *Y*-linked members contains a full-length coding domain (Figure 5). We may reasonably question, therefore, whether any of the *Y*-linked members are capable of producing a functional polypeptide. Only in the case of the loci represented by cS1, and possibly that represented by B4a, does this seem likely.

Expression of *Y*-linked members: KALDERON and RUBIN (1988) discussed a second gene within the large RI intron, though they did not report its sequence. This is 13a, transcribed in the same direction as *mst77F*. Its 3' end lies approximately 350 bp upstream of the most 5' nucleotide present in 18c, a cDNA representing the autosomal *mst77F* locus (KALDERON and RUBIN 1988). This places the 3' end of 13a very close to the 5' boundary of the sequence shown in Figure 5, and delimits the initial 350 bp as a region that one might reasonably investigate for *mst77F* promoter activity. Motifs suggestive of known promoter elements are not convincing; however, a complete analysis awaits primer extension and transformation studies.

Isolation of cDNA clones representing *Y*-linked members demonstrates that at least some of the latter are transcribed. The prevalence of the clones within cDNA libraries implies transcription at levels compa-

able to the autosomal locus, in agreement with the reduced levels of transcripts in *XO* males (Fig. 2). Moreover, a Northern blot of poly(A⁺) RNA isolated from *XY* individuals has no more bands than does the blot of *XO* RNA, suggesting that *Y*-linked transcripts are all polyadenylated mRNAs of approximately the same size as transcripts of the autosomal gene (Figure 2). There is evidence for polyadenylation from at least some cDNA sequences. Although the *Y*-linked member represented by λ gS9 has a domain corresponding to the putative *mst77F* promoter region, there are a number of insertions and deletions (Figure 6). Single base substitution, by comparison, is not significantly more apparent than within the coding domain. These data tend to suggest that *Y*-linked members are expressed conventionally from *mst77F*-like promoters, and with the same temporal and developmental specificity as the autosomal locus.

Genetic screens for male-sterile mutations (either deletions or EMS induced) have provided no evidence for essential fertility functions in the region of the chromosome where the *Y*-linked members map (Figure 4C; KENNISON 1981, 1983; GATTI and PIMPINELLI 1983). Even if one or more *Y*-linked members were able to give rise to a functional polypeptide, however, this could be accounted for by full or partial complementation of fertility by the autosomal locus. We are initiating a mutational analysis of the autosomal locus in order to address this question.

Finally, the apparently normal expression of the autosomal copy in *XO* males observed on Northern blots (Figure 2) would tend to rule out any role for *Y*-linked copies in splicing transcripts of the autosomal locus. This is in contrast with the *Y*-linked *Su(Ste)* locus of *D. melanogaster*, which is required for appropriate splicing of transcripts of the *X*-linked *Stellate* locus (LIVAK 1990).

Origin of *Y*-linked members: Members of the *Y* group are all more closely related to one another than to *mst77F* suggesting that they result from gene duplication following the transposition of a single copy of the *mst77F* locus to the *Y*. This is particularly apparent in the case of the in-frame 918–920 deletion which is present in all of the putative *Y* copies (Figure 5). In this respect it is worth noting that the autosomal locus is itself in an unusual genomic location within the intron of another gene. As the autosomal location appears to be the same in the case of *D. melanogaster* and *D. simulans* it is probable that the autosomal copy transposed to the 77F locus in a common ancestor and then to the *D. melanogaster Y* after divergence. The significant proportion of third base changes over the *mst77F* coding domain, reflecting a high proportion of synonymous/conservative changes, implies that there may have been at one time selection for function of the *Y* loci.

Transposition to the *Y* involved not merely the *mst77F* locus. The family member represented by λ gS9, for example (Figures 1 and 6), is embedded within a 77F-like domain that extends downstream to include the first exon of the gene for the RI subunit of PKA. In the case of other family members, correspondence with 77F may extend at least several hundred base pairs further in this direction (S. F. GOODWIN, unpublished). Upstream correspondence between λ gS9 and the autosomal locus extends for at least several hundred bp beyond the most 5' nucleotide found in cDNAs of the *mst77F* family (Figure 6).

Although transposition must have played a role in generating the *Y*-linked members of the *mst77F* family, there is a fairly high degree of conservation of their genomic organization across a range of *D. melanogaster* isolates from different geographic locations. Such conservation would not be expected for actively transposing sequences, which tend to show considerable variation between strains (RUBIN 1983; ISING and BLOCK 1984), and might be taken to suggest some form of functional constraint. On the other hand, since we are ignorant of the mechanism by which transposition has taken place, it may merely be an accident of history. This situation is in sharp contrast to the *Y*-associated sequences isolated from *D. hydei* which are not conserved between different *hydei* strains (BRAND and HENNIG 1989; HARAVEN, ZUCKERMAN and LIFSCHYTZ 1986).

Y-linked copies are not present in the sibling species *D. simulans*, *D. mauritiana* and *D. sechellia*. *D. simulans* and *D. mauritiana* also have a substantially lower copy number of *Y*-linked *Su(Ste)* sequences than does *D. melanogaster* (LIVAK 1984), and the *D. simulans Y* is devoid of functional *rRNA* genes (LOHE and ROBERTS 1990). Thus the *Y* chromosomes of even very closely related species can be substantially different. Such differences may in some way drive or consolidate reproductive isolation.

The *mst77F* gene product: The putative *mst77F* product has a sequence and predicted secondary structure suggestive of a distant relative of the histone H1/H5 ("linker" histone) family, complemented by an array of cysteine residues in its basic tail that invites comparison with mammalian cysteine-rich protamines (Figure 8). We are not aware of such a structure having been described previously. Indeed, cysteines are notable by their absence from all but the occasional member of the H1/H5 family, and have never been previously observed in the basic tail (WELLS and MCBRIDE 1989). By analogy with the cysteine-rich protamines, their role in the *mst77F* polypeptide may be tight compaction of chromatin via disulfide bridges. In this context it is also worth noting the relatively high arginine:lysine ratio of the basic tail. Such a feature is characteristic of H5 rather than H1,

and again may reflect a role in relatively tight compaction of chromatin since chicken erythrocyte H5, the archetypal member of the H5 group, is implicated in chromosome inactivation.

A role in sperm chromatin condensation? Prior to this report, only two *D. melanogaster* histone genes lying outside the somatic histone gene cluster on chromosome 2 had been described. These are the H2A variant, H2vd (VAN DAAL *et al.* 1988), and the H3 variant, H3.3 (FRETZIN *et al.* 1991). For many other organisms, in contrast, several variants of both core and linker histones are known, often developmentally regulated (STEIN, STEIN and MARZLUFF 1984; WU *et al.* 1986). The range of variation is generally greater for linker histones than for core histones (ISENBERG 1979). Though the physiological basis of this variation is, on the whole, poorly understood, a substitution of somatic histones for sperm-specific DNA-binding proteins during the latter stages of spermatogenesis has been described for many species (SUBIRANA 1983). Sperm-specific substitutes range from relatively slight variants of the somatic histones as seen in sea urchins and starfish, through to the short basic protamines found in mammals and some species of fish. They appear to mediate the compaction of chromatin into the small volume of the sperm head. In the case of *D. melanogaster*, several studies have suggested a histone transition during spermatogenesis (DAS, KAUFMANN and GAY 1964a,b; HAUSCHTECK-JUNGEN and HARTL 1982). In *D. hydei*, moreover, somatic H1 immunoreactivity is undetectable during postmeiotic stages of spermatogenesis (KREMER, HENNIG and DIJKHOF 1986), suggesting replacement by some other protein(s). The *mst77F* polypeptide is the first candidate for a specialized DNA binding protein involved in compaction of *Drosophila* sperm chromatin.

Clearly further experimentation if required to determine the precise role of the *mst77F* polypeptide. One possibility is that, like the cysteine-rich protamines, it is involved in the terminal stages of sperm chromatin condensation. Alternatively, it may function as a transition protein to assist partial chromatin condensation prior to the appearance of protamines. Such proteins have been described for rat (GRIMES *et al.* 1977). Yet another possibility is that the *mst77F* polypeptide is a precursor that is subsequently processed to yield an active polypeptide, as in the case of mouse and human protamine 2 (YELICK *et al.* 1987; AMMER, HENSCHEN and LEE 1986). A combination of biochemical and immunohistochemical studies will help to resolve these questions.

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