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Transcription of a satellite DNA on two *Y* chromosome loops of *Drosophila melanogaster*

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Abstract. Primary spermatocyte nuclei of *Drosophila melanogaster* exhibit three giant lampbrush-like loops formed by the *kl-5*, *kl-3* and *ks-1* *Y* chromosome fertility factors. Detailed mapping of satellite DNA sequences along the *Y* chromosome has recently shown that AAGAC satellite repeats are a significant component of the *kl-5* and *ks-1* loop-forming regions. To determine whether these simple repeated sequences are transcribed on the loop structures we performed a series of DNA-RNA in situ hybridization experiments to fixed loop preparations using as a probe cloned AAGAC repeats. These experiments showed that the probe hybridizes with homologous transcripts specifically associated with the *kl-5* and *ks-1* loops. These transcripts are detected at all stages of development of these two loops, do not appear to migrate to the cytoplasm and are degraded when loops disintegrate during the first meiotic prophase. Moreover, an examination of the testes revealed that the transcription of the AAGAC sequences is restricted to the loops of primary spermatocytes; the other cell types of *D. melanogaster* spermatogenesis do not exhibit nuclear or cytoplasmic labeling. These experiments were confirmed by RNA blotting analysis which showed that transcription of the AAGAC sequences occurs in wild-type testes but not in *X/O* testes. The patterns of hybridization to the RNA blots indicated that the transcripts are highly heterogeneous in size, from large (migration at limiting mobility) to less than 1 kb. We discuss the possible function of the AAGAC satellite transcripts, in the light of the available information on the *Y* chromosome loops of *D. melanogaster*.

Introduction

The formation of sperm cells from the relatively undifferentiated gonial cells is one of the most elaborate and fascinating processes in animal development. In *Drosophila melanogaster* spermatogenesis is controlled by several hundred euchromatic genes that are capable of mutating to male sterility (for reviews see Lindsley and Tokuyasu 1980; Lifschytz 1987). However, it is not currently clear whether most of these genes identify functions that are specific for spermatogenesis or functions shared by spermatogenesis and other developmental pathways (Lindsley and Tokuyasu 1980; Lifschytz 1987).

A set of genes that is specifically required for spermatogenesis comprises the six *Y* chromosome male fertility factors (Lindsley and Tokuyasu 1980; Lifschytz 1987). Four of these genes map to the long arm of the entirely heterochromatic *Y* chromosome (*YL*) and two to the short arm (*YS*). Starting from the extremity of *YL* they are designated *kl-5*, *kl-3*, *kl-2*, *kl-1*, *ks-1* and *ks-2*; the existence of *kl-4*, postulated in earlier studies (Brosseau 1960), has not been confirmed (Kennison 1981; Hazelrigg et al. 1982; Gatti and Pimpinelli 1983). These fertility factors are essential for normal sperm differentiation. In males deficient for one or more of these genes spermatids undergo an extensive elongation process but spermatogenesis is not completed and sperm degenerate before maturation (Lindsley and Tokuyasu 1980; Lifschytz 1987; Hardy et al. 1981).

Cytogenetic studies have shown that three of the male fertility factors, *kl-5*, *kl-3* and *ks-1*, have extremely large physical dimensions. Each of these genes is defined by noncomplementing breakpoints and deficiencies scattered over chromosomal regions containing about 3.000 kb of DNA (Gatti and Pimpinelli 1983; Bonaccorsi et al. 1988). More recently we have shown that the *kl-5*, *kl-3* and *ks-1* fertility factors develop giant lampbrush-like loop in primary spermatocytes (Bonaccorsi et al. 1988). These loops are filamentous structures which begin to form in young spermatocytes, grow throughout spermatocyte development, reach their maximum size in mature spermatocytes and disintegrate prior to the first meiotic metaphase (Bonaccorsi et al. 1988). Deficiency and breakpoint mapping experiments revealed that the loop-forming sites map within the *kl-5*, *kl-3* and *ks-1* fertility factors, suggesting that each loop

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is an integral part of a different fertility gene, probably representing the cytological manifestation of its activity (Bonaccorsi et al. 1988).

About 20% of *D. melanogaster* genome is composed of highly repetitive satellite DNA and this type of DNA is almost exclusively restricted to the heterochromatic regions of the chromosomes (for review see Peacock et al. 1977; Brutlag 1980; Lohe and Roberts 1988). No specific functions have been thus far associated with satellite DNA; however, it has been suggested that long arrays of tandemly repeated satellite sequences are the principal component of the *Y* chromosome fertility factors (Gatti and Pimpinelli 1983). To define the actual relationships between satellite DNA and the fertility factors eight cloned satellite sequences (Lohe and Brutlag 1986) have recently been mapped along the *Y* chromosome by in situ hybridization (Bonaccorsi and Lohe, in preparation). One of these sequences, the AAGAC repeat, was mapped to regions h3 and h21 of the *Y* chromosome. Region h3 corresponds to the proximal third of the *kl-5* locus and is responsible for the formation of the *kl-5* loop, while region h21 represents about one-third of the *ks-1* locus and forms the *ks-1* loop (Bonaccorsi et al. 1988).

In the present study we investigated whether the AAGAC repeats which map to the *kl-5* and *ks-1* loop-forming regions (Bonaccorsi and Lohe, in preparation) are transcribed during the development of these structures. DNA/RNA in situ hybridization experiments to fixed loop preparations and RNA blotting analysis clearly show that the AAGAC satellite DNA is specifically and abundantly transcribed on the *kl-5* and *ks-1* loops. These satellite transcrips are tightly associated with the loops throughout primary spermatocyte development, do not appear to migrate to the cytoplasm and are degraded along with the loops during the first meiotic prophase. These findings, coupled with the available information on the *Y* loops, lead us to envisage a concrete biological function for a highly repetitive, simple sequence satellite DNA. We believe that the AAGAC transcripts of the *kl-5* and *ks-1* loops participate in providing a structural framework for the compartmentalized accumulation of non *Y*-encoded proteins involved in sperm differentiation.

Materials and methods

In situ hybridization. Preparation of primary spermatocytes for DNA-RNA hybridization followed the procedure of Bonaccorsi et al. (1988), with the exception that they were fixed by methanol/acetone at $-20°$ C and then treated with 1% Triton, 0.5% acetic acid in PBS before being extensively washed in PBS (a detailed account of this technique is given in Ashburner 1989). After being air dried the slides were treated with proteinase K according to Brahic and Hasse (1978) except that HCl treatment was omitted. Approximately 5×10^4 cpm of tritium-labeled nick-translated (Maniatis et al. 1982) plasmid 1.686-198 DNA (Lohe and Brutlag 1986) in 5 μl of hybridization solution (50% formamide in $3 \times$ SSC) were applied per slide. ($1 \times$ SSC is 0.15 M NaCl, 0.015 M sodium citrate). Slides were incubated overnight at 40° C, washed twice in 50% formamide in $3 \times$ SSC at the same temperature and extensively rinsed in $2 \times$ SSC at room temperature. Slides were then dehydrated in an ethanol series and dipped in Kodak NTB2 emulsion. After the desired exposure they were developed in Kodak D19. To establish stringent hybridization conditions and avoid cross-hybridization between closely related satellite sequences (Lohe and Brutlag 1986), a hybridization temperature about 13° C below the mean melting temperature of the AAGAC sequence was chosen (see Bonaccorsi and Lohe, in preparation). RNase and DNase digestions on slides were performed as follows. For digestion with RNase slides were incubated for 30 min at room temperature in a 2 μg/ml solution of pancreatic RNase A diluted in $2 \times$ SSC and washed twice in $2 \times$ SSC. For digestion with DNase slides were incubated for 30 min at 4° C in a 20 ng/ml solution of pancreatic DNase I (Sigma) dissolved in 50 mM Tris-HCl, pH 7.9, 5 mM $MgCl_2$, 10 mM 2-β-mercaptoethanol; the reaction was stopped in 10 mM Tris-HCl, pH 7.4.

RNA blotting. Testes of newly emerged males were dissected in Ringer's solution and immediately homogenized in 0.1 M Tris-HCl, 2% SDS, 0.2 M EDTA, 5% ethanol, pH 9.5 on ice (M. Jacobs-Lorena, personal communication). The solution was extracted with phenol (saturated with 1 M Tris-HCl, pH 9.5), extracted twice with chloroform and ethanol precipitated. RNA obtained in this way was loaded directly onto the gel, or digested with DNase, or digested with both RNase and DNase. Digestions were performed as follows: RNA was dissolved in 20 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$ and treated with DNase I (Promega, RQ1, 1 unit) predigested with proteinase K (Tullis and Rubin 1980) or treated with 2 μg of RNase followed by addition of DNase I. After incubation at 37° C for 1 h, the solution was made to 15 mM EDTA, pH 7.5 and 1% SDS, 50 μg proteinase K was added and incubation continued for 1 h further. The RNA was then reextracted with phenol and chloroform and precipitated with ethanol. In all cases RNA was electrophoresed on a 0.7% agarose-formaldehyde gel (Maniatis et al. 1982) and transferred to nitrocellulose (Thomas 1980). RNA from 25 testes was loaded per lane (approximately 5 μg RNA) and intact 18S and 28S bands were visible in gels stained with ethidium bromide in the absence of RNase treatment. Plasmid 1.686-198 (Lohe and Brutlag 1986) was labeled by nick-translation with [^{32}P]dCTP (Maniatis et al. 1982), and hybridization was carried out overnight in $3 \times$ SSC, 50% formamide at 40° C. The filter was washed in the hybridization solution at 40° C, then in $2 \times$ SSC at room temperature, and exposed overnight to X-ray film (Kodak XAR-5) with an intensifying screen.

Results

Localization and transcription of AAGAC repeats

The AAGAC repeats, cloned from the 1.686 g/cm^3 *D. melanogaster* satellite DNA, only comprise 18% of this satellite but account for 2.4% of the genome; thus they represent a major satellite DNA in *D. melanogaster*. In situ hybridization experiments on mitotic chromosomes from brain neuroblasts have shown that the AAGAC sequences map to the *kl-5* and *ks-1* loop-forming regions (see Fig. 1). These repeated sequences also hybridize with the non-loop-forming regions h4–h6, h10 and h24–h25 of the *Y* chromosome, with the centromeric heterochromatin of chromosome *2* and, very weakly, with chromosomes *3*, *4* and *X* (Lohe and Roberts 1988; Bonaccorsi and Lohe, in preparation).

To ask whether the AAGAC repeats are transcribed on the *kl-5* and *ks-1* loops we performed DNA-RNA hybridization experiments, according to the technique originally described by Pukkila (1975). DNA from the 1.686-198 clone, consisting of about 85 tandelmly ar-

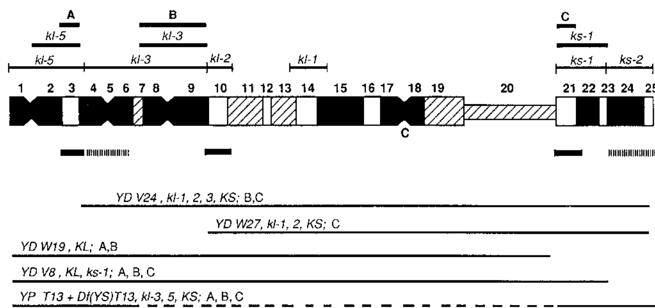

Fig. 1. Localization of the fertility factors, the loop-forming regions and the AAGAC repeats along the *Y* chromosome of *Drosophila melanogaster*. The diagram is a schematic representation of the *Y* chromosome of *D. melanogaster* stained with Hoechst 33258, showing its division into 25 regions (Gatti and Pimpinelli 1983). *Filled segments* indicate bright fluorescence, *hatched segments* indicate dull fluorescence and *open segments* indicate no fluorescence. *C* centromere. The *thin horizontal lines above the diagram* indicate the maximum physical sizes of the fertility factors and the *thick lines above them* their minimum physical size (Gatti and Pimpinelli 1983; Bonaccorsi et al. 1988). The *thick lines in the upper row* correspond to the loop-forming regions. The *bars* just below the diagram indicate the localization of the AAGAC repeats as defined by in situ hybridization experiments on mitotic chromosomes (S. Bonaccorsi and A. Lohe, manuscript in preparation). For a fine localization of these repeats these experiments were performed on a series of different *Y*-autosome translocations (Gatti and Pimpinelli 1983) so that the AAGAC DNA was mapped with respect to the translocation breakpoints. The extension of each bar is defined by the breakpoints used for mapping. A *filled bar* indicates that the AAGAC repeats are located throughout most of the region. A *hatched bar* indicates a location somewhere within the region. The *horizontal lines below the diagram* indicate *Y* chromosome deficiencies. The fertility factor(s) and the loop(s) missing in each deficiency are indicated beside its designation (the fertility genes on *YL* are collectively designated as *KL* and those on *YS* as *KS*). *YDV24*, *YDW27*, *YDW19* and *YDV8* designate the *Y*-distal *X*-proximal elements of *T(X;Y)V24*, *T(X;Y)W27*, *T(X;Y)W19* and *T(X;Y)V8*, while *YPT13* designates the $X^D Y^P$ element of *T(X;Y)T13*. This translocation carries a deletion of *YS* which removes the *ks-1* loop. For a detailed description of these translocations see Kennison (1981) and Bonaccorsi et al. (1988)

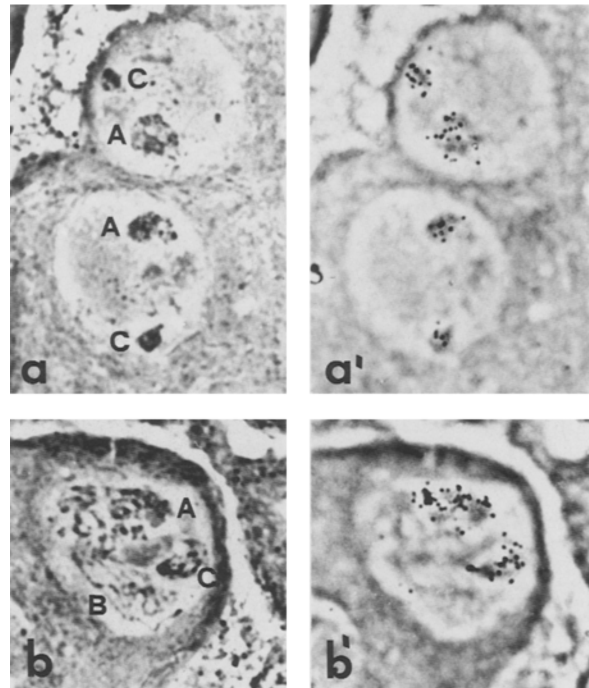

Fig. 2a, b. In situ hybridization of ^{3}H-labeled DNA of the 1.686-198 clone to the transcripts associated with the *kl-5* and *ks-1* loops of *Drosophila melanogaster*. After hybridization and autoradiography the slides were exposed for 5 days before development. **a, a'** Two young wild-type spermatocyte nuclei photographed before (**a**) and after (**a'**) hybridization showing only the *kl-5* (*A*) and *ks-1* (*C*) loops; the *kl-3* loop appears later during spermatocyte development (Bonaccorsi et al. 1988). Note that the *kl-5* and *ks-1* loops are clearly and specifically labeled. **b, b'** Mature wild-type spermatocyte nucleus photographed before (**b**) and after (**b'**) hybridization showing labeling restricted to the *kl-5* and the *ks-1* loops; the *kl-3* loop (*B*) is clearly devoid of grains. The *kl-5* and *ks-1* loops are easily distinguishable from the *kl-3* loop which is less compact and contains a thinner filament (Bonaccorsi et al. 1988)

ranged copies of the AAGAC sequence (Lohe and Brutlag 1986) was nick-translated with ^{3}H-labeled nucleoside triphosphates and used as a probe to detect homologous transcipts on fixed loops. After 5 days of autoradiographic exposure the *ks-1* and *kl-5* loops are specifically labeled (Fig. 2). The AAGAC transcipts are detected at all stages of the development of these two loops and are degraded when loops disintegrate prior to first meiotic metaphase. Moreover, an examination of the testes revealed that the transcription of AAGAC repeats is restricted to primary spermatocytes; the other cell types of *D. melanogaster* spermatogenesis do not exhibit nuclear or cytoplasmic labeling.

To check whether our hybridization technique effectively detects AAGAC RNA transcripts we treated our testes preparations with RNase and DNase before incubation with the labeled 1.686-198 DNA. Pretreatment with DNase does not affect the hybridization pattern, whereas RNase digestion results in the absence of silver grains (data not shown), indicating that the DNA probe is in fact hybridizing to RNA transcripts.

Specificity of satellite transcription

To investigate whether only the AAGAC sequences of the *kl-5* and *ks-1* loop-forming regions (regions h3 and h21 respectively) are specifically transcribed in primary spermatocytes, we performed additional DNA/RNA hybridization experiments making use of males carrying different *Y* chromosome deficiencies (the deficiencies used are diagrammed in Fig. 1). As shown in Fig. 1 males carrying a normal *X* and the *Y*-distal element of *T(X;Y)V24* (henceforth abbreviated in $X/Y^D X^P V24$ males) are deficient for regions h4–h25 and exhibit the *kl-5* loop only. $X/Y^D X^P W27$ males are deficient for regions h10–h25 and show the *kl-5* and *kl-3* loops, whereas the $X/Y^D X^P W19$ males, deficient for regions h1–h20, only exhibit the *ks-1* loop. The $X/Y^D X^P V8$ males and those carrying the *Y*-proximal element of *T(X;Y)T13* without an extra free *Y* chromosome (abbreviated in $X^D Y^P T13/O$) carry different deficiencies of *Y* chromosome material but exhibit identical cytological phenotypes in primary spermatocytes. These males have primary spermatocytes indistinguishable from those of *X/O* males (*X/O* males carry a normal *X* and no *Y* chromosome), containing no loops but only some compact pro-

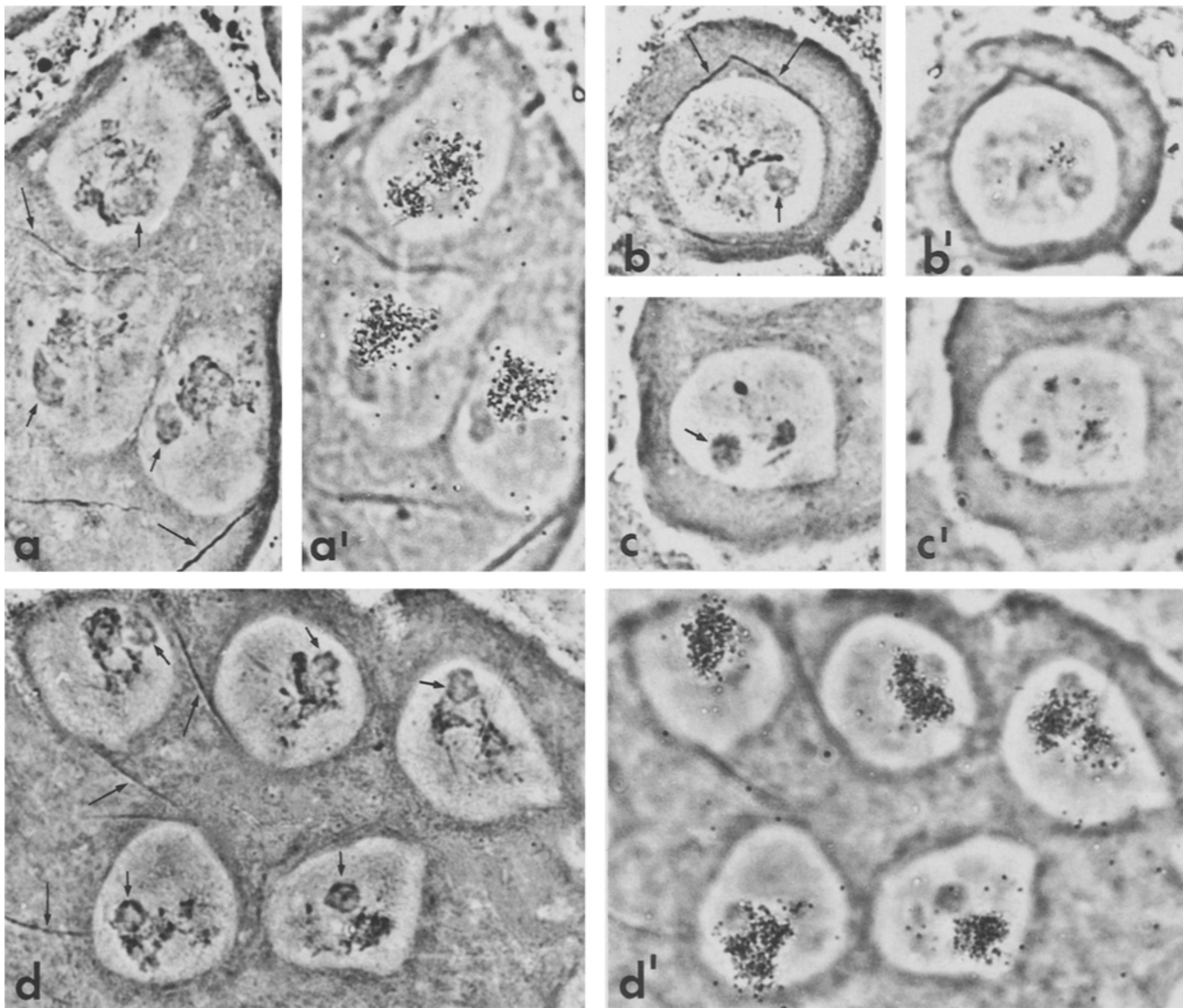

Fig. 3a–d. In situ hybridization of ^{3}H-labeled DNA of the 1.686-198 clone to primary spermatocyte nuclei of different genetic constitutions. Exposure times ranged from 15 to 20 days. **a, a'** Spermatocyte nuclei of $X/Y^{D}X^{P}V24$ males showing only a heavily labeled *kl-5* loop. **b, b'** and **c, c'** *X/O* (**b, b'**) and $X^{D}Y^{P}T13/O$ (**c, c'**) primary spermatocytes showing the "*X/O* granules" and no loops. Note that in both cases there are a few silver grains associated with the *X/O* granules. **d, d'** Partial cyst from $X/Y^{D}X^{P}W19$ males carrying only the *ks-1* loop. Note that in each spermatocyte nucleus the loop is covered by silver grains and that no grains are present on the cytoplasm. **a, b, c, d** and **a', b', c', d'** Photographs taken before and after autoradiography, respectively. The *short arrows* point to the nucleolus; the *long arrows* indicate the proteinaceous crystals caused by the deficiency of region h11 of the *Y* chromosome (Hardy et al. 1984)

teinaceous granules. These structures, which have been named "*X/O* granules", are not observed in wild-type mature spermatocytes and appear when the *kl-5*, the *ks-1* or both these loops are missing (Bonaccorsi et al. 1988). It has been suggested that these granules are accumulations of proteins which in the wild type are bound to the *kl-5* and *ks-1* loops (Bonaccorsi et al. 1988).

In the experiments with these deleted *Y* chromosomes the slides were exposed for 15–21 days to detect low levels of hybridization. In $X/Y^{D}X^{P}V24$ and $X/Y^{D}X^{P}W27$ males the only consistently labeled structure within the nucleus is the *kl-5* loop (Fig. 3a). Although these males differ in the *Y* material they contain and in the loops present in their spermatocytes, they do exhibit identical labeling patterns, suggesting that the AAGAC sequences located in the h4–h6 region are not transcribed.

In the $X/Y^{D}X^{P}W19$ spermatocytes the *ks-1* loop is heavily and specifically labeled and no other nuclear structures are consistently associated with silver grains (Fig. 3d). The $X^{D}Y^{P}T13/0$, the $X/Y^{D}X^{P}V8$ and the *X/O* males exhibit identical labeling patterns. The spermatocyte nuclei of these males are usually completely unlabeled. However, in about 20% of the testes examined, some spermatocyte nuclei exhibit a few silver grains consistently associated with the *X/0* granules (Fig. 3b, c). Together, these observations indicate that the AAGAC sequences located in regions h10 and h24–h25 are not transcribed. The erratic labeling on the *X/O* granules could be due to a very limited transcription of the autosomal AAGAC sequences or to the binding of the probe to the proteins of the granules.

The examination of the testes of $X/Y^{D}X^{P}V24$, $X/Y^{D}X^{P}W27$ and $X/Y^{D}X^{P}W19$ males confirmed that hy-

bridization with the AAGAC repeats is restricted to primary spermatocytes; spermatogonia, secondary spermatocytes, spermatids and sperm do not exhibit labeling. Moreover, even in the more heavily labeled spermatocytes silver grains are exclusively located on the loops, while the rest of the nucleus and the cytoplasm are completely unlabeled (Fig. 3a', d'). This observation indicates that the bulk of the AAGAC RNA transcripts is tightly associated with the loop structures and does not migrate to the cytoplasm.

Size of the AAGAC transcripts

To obtain information on the size distribution of the AAGAC transcripts and to check whether some transcription occurs in *X/O* males, transcription was examined by RNA blotting and hybridization of total RNA isolated from the testes of adult *X/O* and *X/Y* males (Fig. 4). RNA from *X/O* males showed a smear of weak hybridization but this disappeared upon DNase digestion (Fig. 4). Hence the hybridization results from minor DNA contamination of the RNA preparations since AAGAC repeats are abundant on chromosome *2* (Lohe and Roberts 1988). RNA preparations from *X/Y* males that had been digested with DNase before electrophoresis showed strong hybridization to the probe and this hybridization signal was sensitive to RNase digestion (Fig. 4). These data show that the *Y* chromosome AAGAC repeats, but not those located in the autosomal heterochromatin, are abundantly transcribed in testes. Moreover, the satellite transcripts show a wide variation in size, from large (migration at limiting mobility) to less than 1 kb.

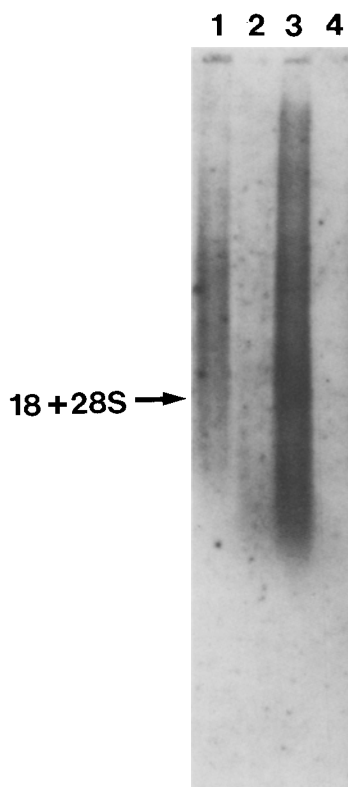

Fig. 4. RNA blotting analysis of size distribution of the AAGAC RNA transcripts in *X/Y* and *X/O* testes of *Drosophila melanogaster*. *Lane 1* total RNA from *X/O* testes; *lane 2* total RNA from *X/O* testes digested with DNase; *lane 3* total RNA from *X/Y* testes digested with DNase; *lane 4* total RNA from *X/Y* testes digested with DNase and RNase. The *arrow* indicates the position of the 18S and 28S ribosomal bands which are clearly visible in gels stained with ethidium bromide

Discussion

Taken together our results indicate that the simple sequence AAGAC satellite DNA is specifically transcribed during spermatogenesis of *D. melanogaster*. Transcription of the AAGAC sequences only occurs on the loops; the AAGAC sequences of non-loop-forming regions of the *Y* chromosome and those located on the autosomes are not transcribed. Long AAGAC transcripts are accumulated on the *kl-5* and *ks-1* loops and do not appear to migrate to the cytoplasm. These transcripts disappear along with the loop during the first meiotic prophase.

Transcription of satellite DNA has thus far been reported only in amphibia (Varley et al. 1988a, b; Diaz et al. 1981; Diaz and Gall 1985; Macgregor and Sessions 1986; Wu et al. 1986). Satellite DNAs composed of relatively long tandemly repeated sequences (220–360 bp) have been shown to be transcribed on the lampbrush chromosomes during the oogenesis of several species of newts (Varley et al. 1980a, b; Diaz et al. 1981; Diaz and Gall 1985; Macgregor and Sessions 1986) and frogs (Wu et al. 1986). In *Notophthalmus viridescens* the copies of satellite DNA are interspersed among the histone gene clusters and are transcribed coordinately with these genes (Diaz et al. 1981; Diaz and Gall 1985). It has been proposed that transcription of satellite DNA may result from failure of normal transcription termination from upstream histone genes (Diaz et al. 1981; Diaz and Gall 1985) and that such failure of termination may be widespread at the lampbrush chromosome stage (Diaz et al. 1981; Diaz and Gall 1985; Macgregor and Sessions 1986; Wu et al. 1986). However, recent studies in *N. viridescens* have shown that transcription often initiates upstream of the first histone gene promoter, questioning the readthrough model for satellite DNA transcription (Bromley and Gall 1987). In any case, it is presently unclear whether satellite DNA transcription on amphibian lampbrush loops is merely an accidental phenomenon due to failure of transcription termination or whether it has some biological function during oogenesis (Diaz et al. 1981; Wu et al. 1986).

The molecular organization of the *Y* chromosome loops of *D. melanogaster* is still largely unknown and a model for satellite DNA transcription in this organism is currently difficult to envisage. However, it is less difficult to envisage a biological function for satellite DNA transcripts. Based on the observation that loops are extremely large [each of the *kl-5* and *ks-1* loop-forming regions contains more than 1000 kb of DNA (Bonaccorsi et al. 1988)] and accumulate large amounts of proteins

we have suggested that they fulfill a protein-binding function rather than a coding function (Bonaccorsi et al. 1988). This suggestion has been recently substantiated by the finding that the *kl-3* loop accumulates a sperm-specific protein which probably belongs to the tektin family (C. Pisano, S. Bonaccorsi and M. Gatti, unpublished results). It is possible that the *kl-5* and *ks-1* loops play similar roles by sequestering and storing other proteins involved in the differentiation of the enormously long *Drosophila* sperm tail. The AAGAC transcripts could participate in providing a structural framework for the compartmentalized accumulation of these proteins.

Similar hypotheses have been advanced about the biological role of the *Y* chromosome loops of *D. hydei* (Glätzer 1984; Hennig 1985). The *Y* chromosome of this species develops five giant loops which have been extensively studied at the cytological, genetical and molecular levels (for reviews see Hennig 1985; Lifschytz 1987). Genomic DNA clones from all the *Y* loops have been isolated in *D. hydei* and shown to be actively transcribed on the loops (Lifschytz 1979; Vogt et al. 1982; Lifschytz et al. 1983; Hareven et al. 1986; Vogt and Hennig 1986a, b; Huijser and Hennig 1987; Trapitz et al. 1988). The analysis of these clones revealed that they identify several families of moderately repetitive DNA, each composed of related but heterogeneous elements. However, no real simple sequence highly repetitive satellite DNA appears to be transcribed on *D. hydei* loops.

Evolutionary conservation studies on the loop DNA of *D. hydei* have shown that they do not share homology with *D. melanogaster* DNA (Hareven et al. 1986; Vogt et al. 1986). However, the *Y* loops of *D. melanogaster* and *D. hydei* accumulate proteins which react with the same antibodies (Glätzer 1984; Hulsebos et al. 1984; Bonaccorsi et al. 1988; C. Pisano, S. Bonaccorsi and M. Gatti unpublished results). These findings suggest that, despite the fact that they contain different DNAs, the *Y* loops of the two species fulfill comparable protein-binding functions.

The molecular characterization of the *Y* loops is at its very beginning and the hypothesis that they serve a function for the storing of sperm proteins needs further support. We are currently pursuing the identification of the molecular components of the *Y* loops of *D. melanogaster* and trying to determine how these components interact. We believe that these studies can elucidate the peculiar functioning of the loop-forming *Y* chromosome fertility factors and possibly assign a concrete biological role to the loop satellite DNA.

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