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Transcription of *gypsy* Elements in a Y-Chromosome Male Fertility Gene of *Drosophila hydei*

Ron Hochstenbach,^{*,1} Harry Harhangi,^{*} Karin Schouren,^{*} Petra Bindels,^{*} Ron Suijkerbuijk[†] and Wolfgang Hennig^{*}

^{*}Department of Molecular and Developmental Genetics, Faculty of Sciences, Catholic University of Nijmegen, NL-6525 ED Nijmegen, The Netherlands and [†]Department of Human Genetics, University Hospital Nijmegen, NL-6525 GA Nijmegen, The Netherlands

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ABSTRACT

We have found that defective *gypsy* retrotransposons are a major constituent of the lampbrush loop pair *Nooses* in the short arm of the Y chromosome of *Drosophila hydei*. The loop pair is formed by male fertility gene *Q* during the primary spermatocyte stage of spermatogenesis, each loop being a single transcription unit with an estimated length of 260 kb. Using fluorescent *in situ* hybridization, we show that throughout the loop transcripts *gypsy* elements are interspersed with blocks of a tandemly repetitive Y-specific DNA sequence, *ay1*. *Nooses* transcripts containing both sequence types show a wide size range on Northern blots, do not migrate to the cytoplasm, and are degraded just before the first meiotic division. Only one strand of *ay1* and only the coding strand of *gypsy* can be detected in the loop transcripts. However, as cloned genomic DNA fragments also display opposite orientations of *ay1* and *gypsy*, such DNA sections cannot be part of the *Nooses*. Hence, they are most likely derived from the flanking heterochromatin. The direction of transcription of *ay1* and *gypsy* thus appears to be of a functional significance.

ABOUT 40 families of transposable elements reside in the genome of *Drosophila melanogaster* (BERG and HOWE 1989; FINNEGAN 1990). The most abundant type of transposable elements are called retrotransposons, as they have structural similarity with retroviruses. At least 19 different families of retrotransposons have been identified in this species. They are implicated in the majority of spontaneous mutations (GREEN 1988), and a wealth of data exists on their structure and their genomic and phylogenetic distribution. Also studies on the mechanisms by which they affect normal patterns of gene expression have been carried out.

To cause heritable changes, retrotransposons must transpose within cells of the germ line. This requires an RNA intermediate, as has been shown for the IAP sequence of the mouse (HEIDMANN and HEIDMANN 1991) and also for several retrotransposons, as for example the L1 element of the mouse (EVANS and PALMITER 1991) and the *I* factor of *D. melanogaster* (JENSEN and HEIDMANN 1991; PÉLISSON *et al.* 1991). Therefore, such elements must be transcribed during oogenesis or spermatogenesis. The *I* factor is transcribed in the female germ line cells (LACHAUME *et al.* 1992; MCLEAN *et al.* 1993), and the *gypsy* retrotransposon of this species is transcribed in the somatic follicle cells that surround

the oocytes (PÉLISSON *et al.* 1994). However, little is known about the expression of retrotransposons in male germ line cells of *D. melanogaster*, even though the promoters of several retrotransposons have been identified (see for *mdg3* ARKHIPOVA *et al.* 1986, for *copla* SNEDDON and FLAVELL 1990, for *mdg1* ARKHIPOVA and ILYIN 1991, for *gypsy* JARRELL and MESELSON 1991). Some retrotransposons display localized expression during embryogenesis (BROOKMAN *et al.* 1992; FROMMER *et al.* 1994; BRÖNNER *et al.* 1995). For some families of retrotransposons, the developmental pattern of expression has been determined (PARKHURST and CORCES 1987), but since these studies were based on RNA extracted from entire animals, with males and females mixed, they reveal nothing about retrotransposon transcription in either the male or the female germ line.

Previous investigations of our laboratory on the molecular structure of the lampbrush loop-forming male fertility genes on the Y chromosome of *D. hydei* (reviewed by HENNIG *et al.* 1989; HENNIG 1990) have revealed that retrotransposons of the *micropia* family (LANKENAU 1993) are transcribed in the lampbrush loop pairs *Threads* and *Pseudonucleolus* in primary spermatocytes (HUIJSER *et al.* 1988). More recently, it has been demonstrated that an antisense transcript of *micropia* is found in spermatocytes (LANKENAU *et al.* 1994). This transcript might be involved in the regulation of transposition frequencies of *micropia* in the male germ line.

In this paper we show that defective members of the *gypsy* retrotransposon family are abundantly transcribed in the germ line of wild-type *D. hydei* males. These *gypsy*

Corresponding author: Wolfgang Hennig, Department of Molecular and Developmental Genetics, Faculty of Sciences, Catholic University of Nijmegen, Toernooiveld, NL-6525 ED Nijmegen, The Netherlands.

¹ Present address: Department of Microbiology and Evolutionary Biology, Faculty of Sciences, Catholic University of Nijmegen, Toernooiveld, NL-6525 ED Nijmegen, The Netherlands.

elements are located in the lampbrush loop pair *Nooses* that is associated with male fertility gene *Q* on the short arm of the *Y* chromosome. The *gypsy* elements are co-transcribed with repeats of the *Y*-specific *ay1* family of repetitive DNA sequences that was earlier identified as the major constituent of the *Nooses* DNA (VOGT *et al.* 1982; VOGT and HENNIG 1986a,b; HOCHSTENBACH *et al.* 1993a,b, 1994a).

MATERIALS AND METHODS

Drosophila stocks: Both the *D. hydei* Tübingen wild-type strain and the *D. eohydei* wild-type strain were from our laboratory collection. *D. hydei* males of the genotype *X/ms(Y)Q1* were used as a control, since they lack the short arm of the *Y* chromosome, and therefore, they lack fertility gene *Q*. Following its induction by EMS in 1979, the *ms(Y)Q1* *Y* chromosome was cytologically normal, carrying a sterile allele of gene *Q* on the short arm (HACKSTEIN *et al.* 1982; HACKSTEIN and HENNIG 1982). During subsequent maintenance of the chromosome in males of the genotype *T(X;Y)59/ms(Y)Q1*, the short arm became deleted (J. H. P. HACKSTEIN, personal communication). *T(X;Y)59* is a translocation of the short arm of the *Y* chromosome to the euchromatic arm of the *X* chromosome, complementing the absence of gene *Q*. It carries the markers *yellow*, *miniature*, and *cherry* (HACKSTEIN *et al.* 1982). The *X/ms(Y)Q1* males used for isolation of RNA were obtained by crossing *T(X;Y)59/ms(Y)Q1* males to virgin wild-type females. Absence of the short arm was confirmed by inspection of neuroblast metaphases of *X/ms(Y)Q1* third instar larvae and by the failure of an *ay1* repeat probe to hybridize to Southern blots of genomic DNA of *X/ms(Y)Q1* adults. Repeats of the *Y*-specific *ay1* family are located exclusively on the short arm of the *Y* chromosome (VOGT and HENNIG 1983). Flies were grown at 18° or 24° as described (HOCHSTENBACH *et al.* 1993a).

Isolation of nucleic acids: RNA was isolated from testes of 3- to 5-day old adult males by the method of CHIRGWIN *et al.* (1979) as described by BRAND and HENNIG (1989). Plasmid DNA was isolated according to a boiling procedure recommended by Stratagene.

Nucleic acid probes: Two probes were used for the detection of *Nooses* transcripts. As a probe for detecting transcripts of the *Y*-specific *ay1* family of repetitive DNA sequences we used an *EcoRI* DNA fragment of 393 bp that represents the sequence complexity of this family (VOGT and HENNIG 1986a). This particular repeat is called *ay1*. As a probe for detecting transcripts of the *Y*-associated DNA sequences of the *Nooses* loop pair we used the 5.8-kb *BamHI-EcoRI* DNA fragment of the genomic clone Dh.No90 (HOCHSTENBACH *et al.* 1993a). Both DNA fragments were subcloned in pBluescript II KS+ plasmid vectors (Stratagene). Integrity of RNA samples was verified using DmK2-30, a 1.2-kb cDNA clone containing parts of exons 16 and 17 of the *D. melanogaster* muscle myosin heavy-chain gene (GEORGE *et al.* 1989). This probe (kindly provided by Dr. K. MIEDEMA) hybridizes to major transcripts of 6.6 and 4.5 kb, and to less abundant transcripts of 6.1 and 4.2 kb in testis RNA of *D. hydei* (MIEDEMA 1994).

DNA sequence analysis: Restriction fragments for DNA sequencing were subcloned in M13mp18 or M13mp19 vectors, and sequences were determined using the dideoxy chain-termination method, all following procedures provided by Amersham. DNA sequences were analyzed using the software package of the University of Wisconsin Genetics Computer Group (DEVEREUX *et al.* 1984). For sequence database searches and DNA sequence alignments we used the programs FASTA and LFASTA, respectively (PEARSON and LIPMAN 1988).

Labeling of probes: Strand-specific RNA probes for *in situ* hybridization were prepared by *in vitro* transcription using either T3 or T7 polymerase (Stratagene) from linearized plasmid DNA, following protocols from Boehringer Mannheim. Such probes were labeled either by incorporation of digoxigenin-11-UTP or biotin-16-UTP (both from Boehringer Mannheim). Control hybridizations of these probes to plasmid DNA indicated comparable labeling of both strands (data not shown). RNA probes for hybridization to Northern blots were labeled by incorporation of [α -³²P]-UTP. Equal amounts of probe of each strand, labeled to comparable specific activities, were used. In some experiments single-stranded DNA probes were used for this purpose. Such probes were prepared from plasmid DNA using the Klenow fragment of *Escherichia coli* DNA polymerase, and they were labeled by incorporation of [α -³²P]-dCTP, following conventional methods (SAMBROOK *et al.* 1989).

Hybridization to Northern blots: Samples of testis RNA were denatured by glyoxal/dimethylsulfoxide, separated on 1–2% denaturing agarose gels, transferred to Hybond membranes (New England Nuclear), hybridized, and washed as described by BRAND and HENNIG (1989). Approximately 20 μ g total RNA was loaded in each lane.

Transcript *in situ* hybridization: Transcript *in situ* hybridization on squashed testis was performed by a modification of the method of TAUTZ and PFEIFLE (1988), as described in detail by HOCHSTENBACH *et al.* (1993a). If only a single probe was hybridized, we used digoxigenin for probe labeling. In this case probe detection was by an anti-digoxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim), and the probe was visualized by conventional phase contrast microscopy. If two probes were hybridized simultaneously, one probe was labeled with digoxigenin and the other with biotin. In this case probe detection was by indirect immunofluorescence, following essentially the procedure described by HOCHSTENBACH *et al.* (1993b), except that digoxigenin was detected by successive incubations with rhodamin-conjugated sheep anti-digoxigenin Fab-fragments (Boehringer Mannheim, 1:20 dilution), Texas Red-conjugated rabbit anti-sheep antibodies (Jackson Immunoresearch Laboratories, West Grove, PA, 1:100 dilution), and Texas Red-conjugated donkey anti-rabbit antibodies (Jackson Immunoresearch, 1:100 dilution). Probe visualization by fluorescence microscopy, digital image recording, and computer-assisted image processing were as described (HOCHSTENBACH *et al.* 1993b).

RESULTS

Cotranscription of *ay1* and *Y*-associated DNA sequences in the *Nooses* lampbrush loop pair: The *gypsy* elements were identified in genomic clones that were isolated as potential segments of the lampbrush loop pair *Nooses*. Our earlier molecular studies revealed that the *Y*-specific *ay1* family of repetitive DNA sequences accounts for about two-thirds of the 260 kb of DNA transcribed in this loop pair, but that, in addition, other DNA sequences are transcribed in the loops that are also present on other chromosomes. These sequences were therefore designated as *Y*-associated (VOGT and HENNIG 1983, 1986a,b; HOCHSTENBACH *et al.* 1993a,b). Using *ay1* repeats as a probe to screen genomic libraries, we recovered 300 kb of genomic DNA in plasmid, lambda and cosmid clones containing both *ay1* and *Y*-associated DNA sequences (HOCHSTENBACH *et al.* 1993a).

Three of the lambda clones are shown in Figure 1.

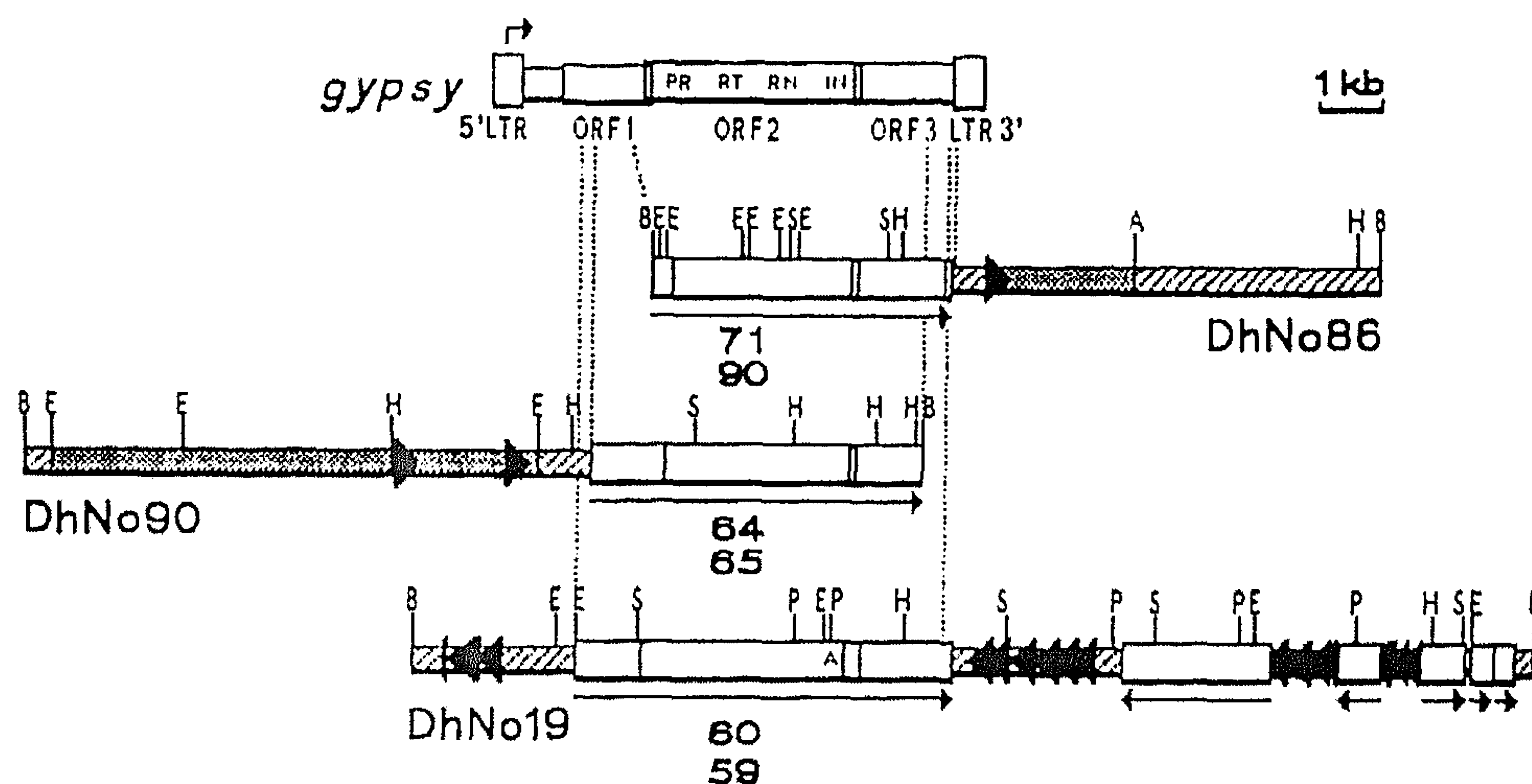


FIGURE 1.—Alignment of *D. hydei* Y-associated *gypsy* elements with the *gypsy* element of *D. melanogaster*. Restriction maps of three *ayI*-containing lambda clones are shown. In each clone *gypsy* sequences are indicated as open rectangles, and restriction fragments hybridizing to *ayI* probes are indicated by dark shading. Individual *ayI* repeats, as identified by sequence comparison with the basic 393-bp *ayI* repeat defined by VOGT and HENNIG (1986a), are depicted as black arrowheads, which also indicate the direction of transcription of *ayI* in the *Nooses* lampbrush loop pair. Restriction fragments that hybridize neither to *ayI* nor *gypsy* probes are hatched. The sequenced parts of these fragments have no obvious similarities to any sequence in the EMBL database (Release 40, September 1994). For each Y-associated *gypsy* sequence, the direction of transcription of the coding strand is indicated by an arrow. The numbers below the *gypsy* fragments indicate the percentage of sequence similarity to the corresponding sequences from the *gypsy* elements of *D. melanogaster* (upper numbers) and *D. virilis* (lower numbers). In the *D. melanogaster* *gypsy* element at the top, the LTR, open reading frames (ORF), as well as the positions of the protease (PR), reverse transcriptase (RT), ribonuclease (RN), and integrase (IN) activities encoded by ORF2 are indicated. The start site of *gypsy* transcription is marked by the small arrow above the 5' LTR. The vertical lines in the *gypsy* elements demarcate the limits of the different ORFs. The A in the largest *gypsy* sequence in DhNo19 indicates a poly(A)-tail that is located between ORF2 and ORF3. A more detailed analysis of these and other Y-associated *gypsy* sequences has been presented elsewhere (HOCHSTENBACH *et al.* 1994b). Restriction enzyme abbreviations are as follows: A, *AvaI*; B, *BamHI*; E, *EcoRI*; H, *HindIII*; P, *PstI* and S, *SalI*. The complete nucleotide sequence of DhNo19 has been submitted to the EMBL database under accession number X74538, the partial sequence of DhNo86 has been submitted under accession numbers X74539, X74540, X74541 and X74542, and the partial sequence of DhNo90 under accession numbers X74536, X74537 and X74543.

These clones have different restriction maps and hence, they do not overlap. Each of them contains *ayI* repeats that are organized in one to several clusters of tandem repeats. In addition, they share Y-associated DNA sequences. In clone DhNo86 the shared sequences are located in a 3.8-kb *BamHI-HindIII* fragment, in clone DhNo90 in a 5.8-kb *BamHI-EcoRI* fragment, and in clone DhNo19 in a 3.7-kb *EcoRI-EcoRI* fragment. These DNA fragments were designated DhNo86BH3.8, DhNo90BE5.8 and DhNo19EE3.7, respectively. On Southern blots of these clones, the Y-associated fragments cross-hybridize with one another after washing under nonstringent, but not under stringent conditions (HOCHSTENBACH *et al.* 1993a). Most of the copies on the other chromosomes are in the centromere-associated heterochromatin of the X chromosome and the autosomes. Using highly stringent conditions for *in situ* hybridization, the Y-associated DNA sequences hybridize to *Nooses* transcripts in primary spermatocytes (HOCHSTENBACH *et al.* 1993a).

The hybridization pattern of the shared Y-associated DNA sequence on *Nooses* transcripts is highly similar to that of *ayI*. This was shown by fluorescent transcript *in situ* hybridization, using a biotin-labeled, strand-specific RNA probe for *ayI* and a digoxigenin-labeled strand-

specific RNA probe for DhNo90BE5.8. This fragment was chosen because it is present in at least four different *ayI*-containing genomic clones (HOCHSTENBACH *et al.* 1993a), and it may therefore occur in multiple copies within the transcribed DNA of the loop. As shown in Figure 2, the two signals almost completely overlap and cover the entire *Nooses* loop pair. The slight differences in the patterns are due to the different sensitivities of detection at the different wave lengths. The overlap in signals indicates that both types of DNA sequences are interspersed throughout the *Nooses* loop pair, consistent with our analysis of the genomic DNA of the lampbrush loop. Moreover, no major parts of the transcription unit are devoid of either sequence. In this case partially differing patterns would be expected. Also in *D. eohydei*, a species closely related to *D. hydei* (WASSERMAN 1982), both sequences are cotranscribed in a lampbrush loop pair. This loop pair does not correspond to any of the four loop pairs previously described for this species (HENNIG 1978).

The results of the transcript *in situ* hybridization experiments were confirmed using Northern blots prepared from total testis RNA of *D. hydei* (Figure 3). Both probes hybridize in a similar pattern to RNA fragments heterogeneous in size, with the largest fragments 10-

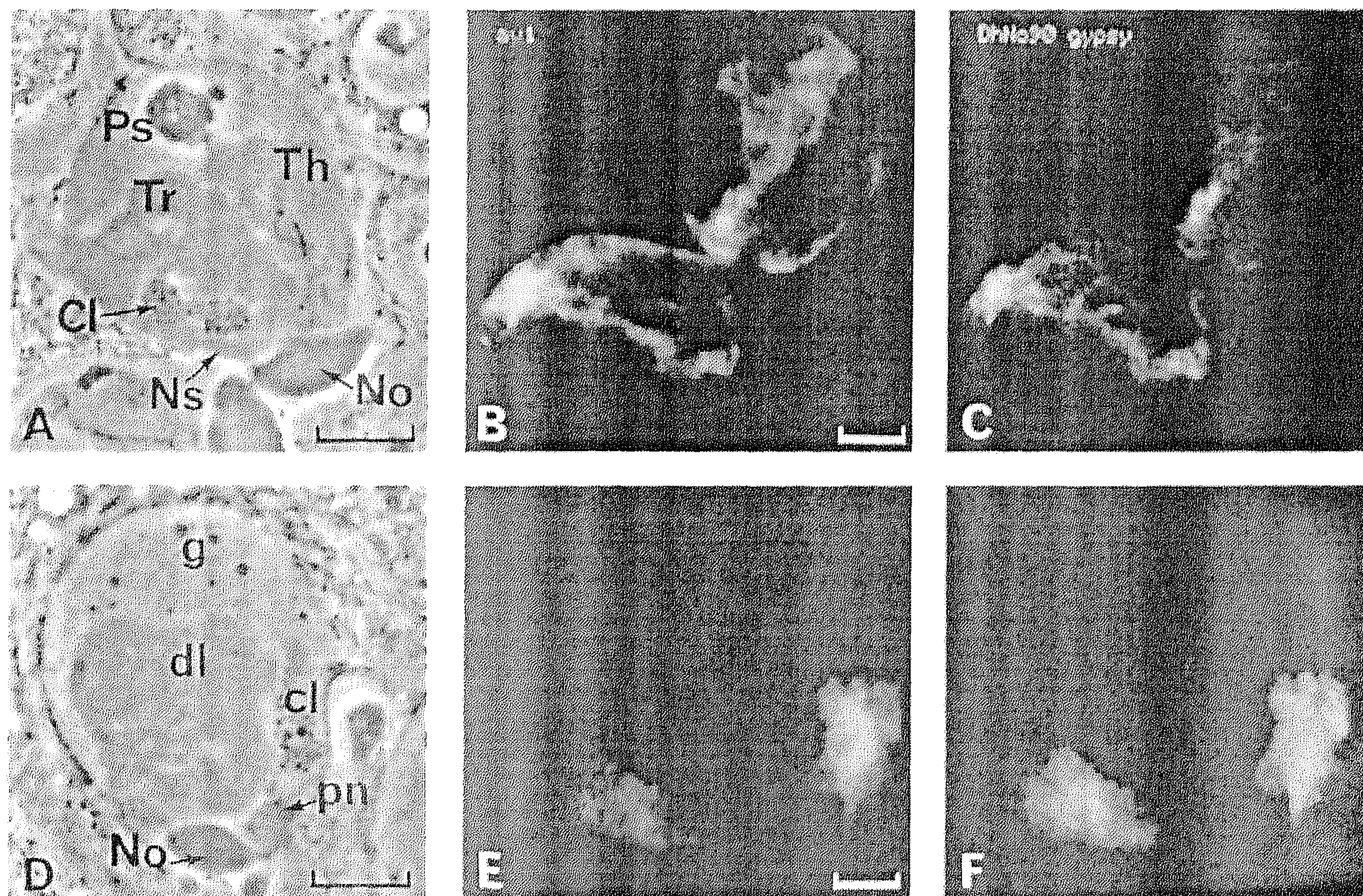


FIGURE 2.—Cotranscription of *ayl* and *gypsy* in the *Nooses* loop pair of *D. hydei* and in a loop pair of *D. eohydei*. In A and D a primary spermatocyte nucleus is shown for each species (phase contrast). Unfixed unstained nuclei are shown because cytology is severely distorted by *in situ* hybridization. The loop pairs of *D. hydei* (A) are as follows: Th, *Threads*; Ps, *Pseudonucleolus*; Cl, *Clubs*; Tr, *Tubular ribbons*; Ns, *Nooses* (HESS and MEYER 1968). Those of *D. eohydei* (D) are as follows: g, *granular loop*; cl, *club-like loop*; dl, *diffuse loop* and pn, *proximal loop* (HENNIC 1978). Nucleolus organizers are marked. Fixed testis tissue of *D. hydei* (B and C) and *D. eohydei* (E and F) was hybridized simultaneously with the biotin-labeled *ayl* probe and a digoxigenin-labeled probe for the DhNo90BE5.8 fragment, which contains a *gypsy* sequence. The *ayl* probe was detected by fluorescein isothiocyanate fluorescence (B and E), and the *gypsy* probe was detected by Texas Red fluorescence (C and F) (see MATERIALS AND METHODS). Two nuclei, each containing one labeled loop pair, are shown for each species. The opposite strand of both probes both fail to give detectable signals on testis transcripts *in situ* (see for *ayl* LIFSCHYTZ and HAREVEN 1985; TRAPITZ *et al.* 1988 and for *gypsy* HOCHSTENBACH *et al.* 1993a) or on Northern blots of testis RNA (Figure 3). In all figures bar, 10 μ m.

20 kb and the smallest only a few hundred bp in length. Using probes of the *ayl* family, such patterns were also observed in our earlier studies (VOGT *et al.* 1982) and by other investigators as well (LIFSCHYTZ *et al.* 1983; TRAPITZ *et al.* 1988). The size heterogeneity is expected given the fact that the growing nascent loop transcripts of the 260-kb *Nooses* transcription unit display a large size gradient (GROND *et al.* 1983). In addition, current biochemical methods of RNA isolation are not suited to isolate transcripts of several hundred kb in length without substantial degradation. However, hybridization with a *D. hydei* myosin-cDNA probe still allows the recognition of testis transcripts with sizes >6 kb (Figure 3). The patterns, therefore, indicate that both *ayl* and the *Y*-associated DNA segments are components of much larger primary transcripts. In testis RNA from males lacking an active gene *Q*, no hybridization is seen with either probe (Figure 3). Moreover, only one strand of DhNo90BE5.8 could be detected on the Northern blots, consistent with our earlier *in situ* hybridization experiments (HOCHSTENBACH *et al.* 1993a). Thus, within the *Nooses* transcription unit not only the *ayl* repeats (LIFSCHYTZ and HAREVEN 1985; TRAPITZ *et al.* 1988; PAPENBROCK 1991), but also all copies of the *Y*-associated DhNo90BE5.8

sequence, seem to have the same orientation. In addition, we have also found that the heterogeneous *ayl*-containing testis transcripts are not polyadenylated (HOCHSTENBACH 1994).

***Y*-associated DNA sequences of the *Nooses* loop pair are defective *gypsy* elements:** We sequenced DhNo90BE5.8, and the related sequences from DhNo19 and DhNo86. As shown in Figure 1, each of the three lambda clones contains a 4- to 5-kb-long DNA sequence with a high degree of similarity to the *gypsy* retrotransposon, known from *D. melanogaster* (MARLOR *et al.* 1986) and *D. virilis* (MIZROKHI and MAZO 1991). These *gypsy* elements, as well as all other *Y*-associated *gypsy* elements of *D. hydei* that have been sequenced so far, are defective. In particular, they have lost their protein coding capacity, since all open reading frames are destroyed by deletions or frame shifts, as shown by detailed sequence analysis (HOCHSTENBACH *et al.* 1994b). In addition, those DNA sequences that in complete *gypsy* elements control transcription are absent due to truncations at either the 5' end, the 3' end, or at both ends. For example, the 5' long terminal repeat (LTR), which contains the *gypsy* promoter (JARRELL and MESELSON 1991) as well as the binding sites for the protein encoded by the *suppressor of Hairy-wing* (*su(Hw)*) gene (SPANNA *et al.*

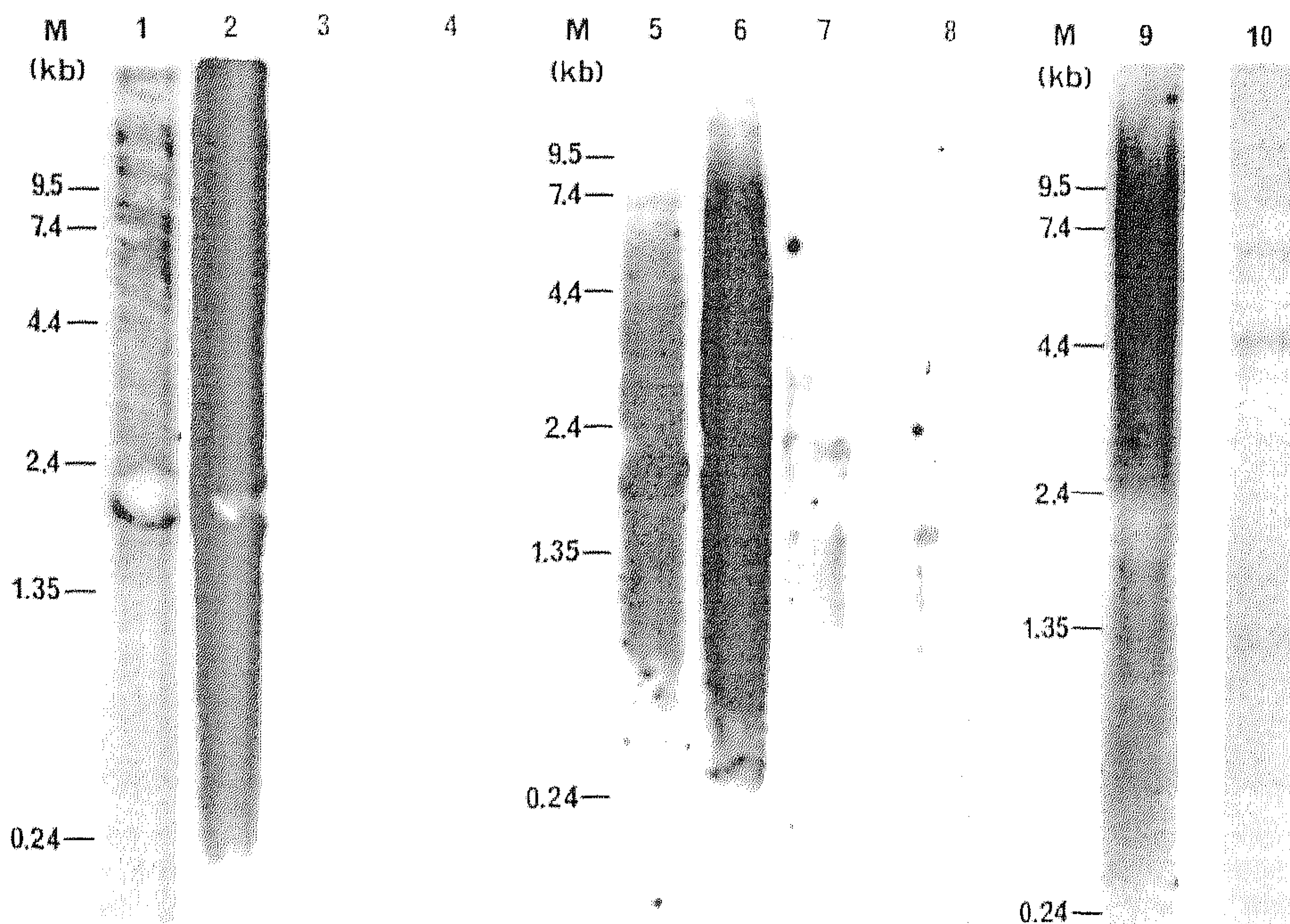


FIGURE 3.—Only one strand of *ay1* and only one strand of *gypsy* can be detected in testis transcripts. Twenty micrograms total testis RNA of wild-type *D. hydei* males (lanes 1, 2, 4, 5, 6, 8, 9 and 10) or of males of the genotype *X/ms(Y)Q1* (lanes 3 and 7) were loaded in each lane. The blots shown in lanes 1–8 were hybridized with [³²P]-labeled strand-specific RNA probes for *ay1* (lanes 1–4) or for DhNo90BE5.8 (lanes 5–8). These blots were stringently washed in 0.02 M sodium phosphate buffer at 50° and exposed for 48 hr using two intensifying screens. The blots shown in lanes 1 and 5 are shorter exposures of those in lanes 2 and 6, respectively. The *ay1* probe hybridizes to testis transcripts of a heterogeneous size [but only if the short arm of the *Y* chromosome is present (lanes 1–3)] and so does the probe for the coding strand of *gypsy* (lanes 5–7). At a level of ~2 kb comigrating ribosomal RNA causes a distortion of the signals. The blots shown in lanes 2 and 6 were stripped and subsequently hybridized under identical conditions with equal amounts of probes for the opposite strand of *ay1* and the noncoding strand of *gypsy*, and also exposed for 48 hr using two intensifying screens (lanes 4 and 8, respectively). As a control for the integrity of the RNA we used a probe for the *D. melanogaster* muscle myosin heavy chain gene. The blot shown in lane 9 was hybridized with a [³²P]-labeled strand-specific DNA probe for *ay1*, stripped, and then hybridized again with the myosin probe (lane 10).

1988), are absent in the *gypsy* element of DhNo90 and in the large *gypsy* element of DhNo19.

Y-associated *gypsy* elements outside of the *Nooses* transcription unit have random orientations relative to adjacent *ay1* repeats: The orientations of the *gypsy* elements in DhNo90BE5.8 and DhNo86BH3.8 with respect to the T3 and T7 promoters of the pBluescript vectors used for subcloning implied that the coding strand of *gypsy* is represented in the *Nooses* transcripts. To confirm this finding, we determined the orientation of the *ay1* repeats immediately flanking the *gypsy* elements in clones DhNo90 and DhNo86 by partial sequence determination of *ay1* repeat clusters. DhNo19 was completely sequenced as its restriction map revealed the presence of at least three separate clusters of *ay1* repeats (HOCHSTENBACH *et al.* 1993a). Comparisons of the orientations of adjacent *gypsy* and *ay1* sequences show that the *gypsy* fragments in DhNo90 and DhNo86 are indeed transcribed from the same strand of DNA as the *ay1* repeats in these clones (Figure 1), suggesting that DhNo90 and DhNo86 represent genuine segments of the *Nooses*.

In contrast, DhNo19 contains six different *gypsy* fragments, with only two in the same orientation as the *ay1*

repeats, which, on the other hand, all have the same orientation within the clone (Figure 1). Since only one strand of *gypsy* is detectable in *Nooses* transcripts, both by *in situ* hybridization (HOCHSTENBACH *et al.* 1993a) and by hybridization to Northern blots (Figure 3), it is unlikely that the genomic clone DhNo19 represents a part of the *Nooses* transcription unit. This finding emphasizes that *ay1* repeats that are interspersed by Y-associated DNA sequences are not necessarily located within the loop. Consistent with this conclusion, we have shown that the *Y* chromosome contains more DNA with interspersed *ay1* repeats than predicted by the 260-kb length estimate for the *Nooses* transcription unit (HOCHSTENBACH *et al.* 1993a,b). However, clones such as DhNo19 are exceptional, since from nine lambda and three cosmid clones in which both *gypsy* and *ay1* have been identified, it is the only clone with *gypsy* sequences in the opposite orientation relative to *ay1* (HOCHSTENBACH 1994; HOCHSTENBACH *et al.* 1994b).

Distribution of *Nooses* transcripts during male germ cell development: Because retrotransposon transcripts encode proteins, we investigated whether the *Nooses* transcripts are transported from the nucleus to the cyto-

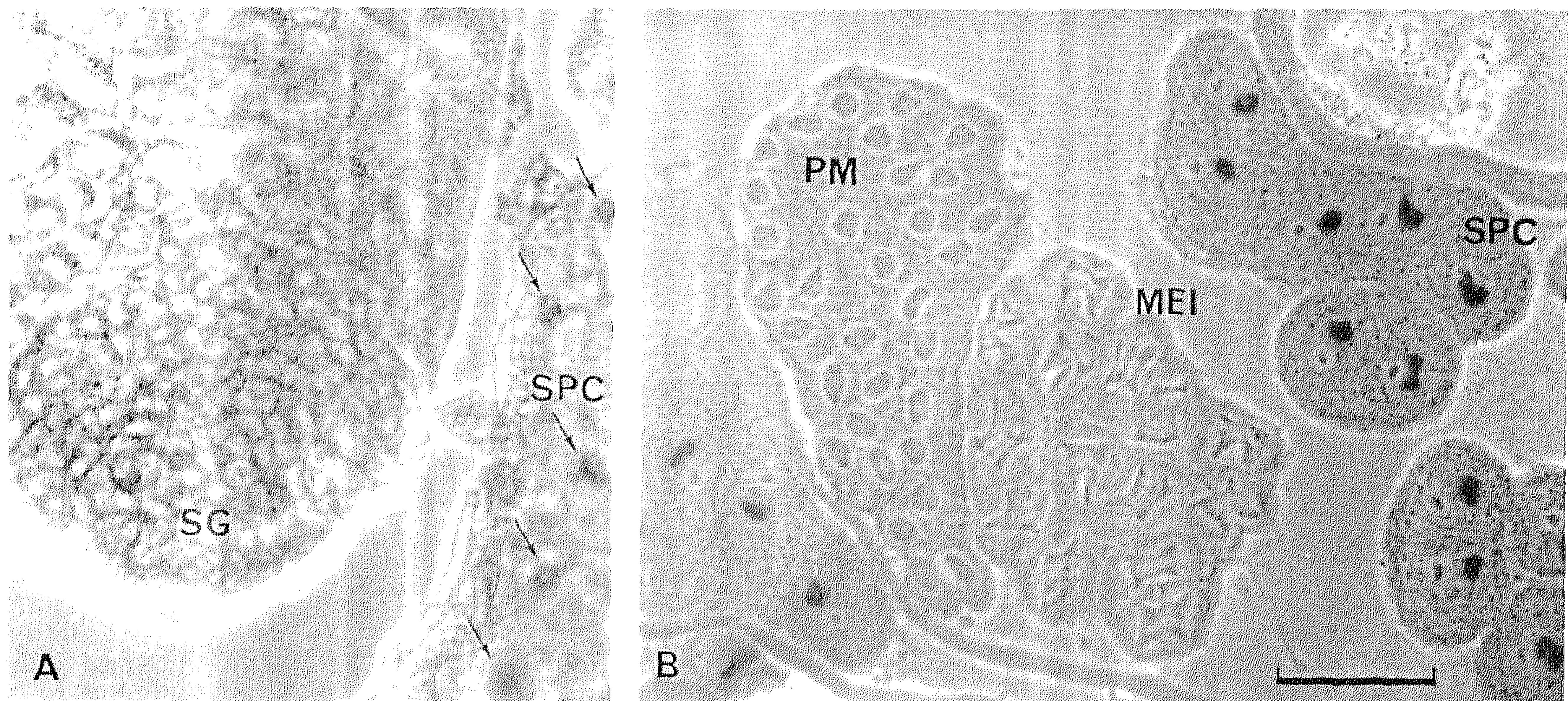


FIGURE 4.—Distribution of *Nooses* transcripts during spermatogenesis, as followed by *in situ* hybridization using the digoxigenin-labeled *ayl* probe. The probe was detected using an anti-digoxigenin antibody conjugated with alkaline phosphatase (see MATERIALS AND METHODS). (A) In the tip of the testis tube, spermatogonia (SG) fail to become labeled, whereas the nuclei of adjacent primary spermatocytes (SPC) contain a labeled *Nooses* loop pair (indicated by arrows). This is more clearly seen in B. Label is only detected in the nuclei of primary spermatocytes, but not in the cytoplasm of these cells. In the center of the figure, a cyst of secondary spermatocytes (containing almost the complete number of 16 cells) during anaphase II of meiosis is seen (MEI), and at the left, there is a complete cyst of 32 spermatids of an early postmeiotic stage (PM), with round or slightly oval Nebenkern derivatives. All cells of both cysts are completely free of label. Detailed descriptions of the different stages of spermatogenesis in *D. hydei* have been given by HESS and MEYER (1968), HENNIG (1985) and HENNIG and KREMER (1990). Phase contrast. Bar, 100 μ m.

plasm. We used the *ayl* probe to follow the distribution of the loop transcripts during spermatogenesis in wild-type males of *D. hydei*. Identical results were obtained using the DhNo90BE5.8 *gypsy* probe (data not shown).

Spermatogenesis starts in the tip of the testis tube where primordial germ cells differentiate into spermatogonia, which subsequently proliferate by mitotic divisions. In such cells the *Y* chromosome is not active (HENNIG 1967, 1985), and, as expected, we did not detect transcripts containing *ayl* in such cells (Figure 4A).

Spermatogonia develop into primary spermatocytes and they enter the meiotic prophase. Because the primary spermatocyte development includes more than half of the total time needed for spermatogenesis (HENNIG and KREMER 1990) we studied this phase in more detail (Figure 5). The different stages of primary spermatocyte development have been defined by HENNIG (1967) on the basis of their typical cytology. During stage I, which lasts \sim 24 hr, the loop pairs start to unfold. In the nuclei of such cells the *Nooses* can be seen as a small round loop pair in close proximity to the nucleolus (Figure 5A). Subsequently, the *Nooses* unfold, together with the other loop pairs (Figure 5, B and C). During stage II, lasting \sim 90 hr, all loops have reached their maximum size, and the primary spermatocytes enter a period of intense transcriptional activity (Figure 5D). Transcriptional activity decreases somewhat during stage III (27 hr), even though the loops remain

fully expanded. During the short stage IV (4 hr) the nuclei become round and RNA synthesis ceases. In cells of this stage the signal of the *ayl* probe is reduced in intensity and residual *Nooses* transcripts can be seen between the fragments of the other loop pairs (Figure 5E, left nucleus). Thus, together with the transcripts of the four other loop pairs, the *Nooses* transcripts are rapidly degraded at the end of meiotic prophase. While some, probably proteinaceous, remnants of the *Pseudonucleolus* and the *Clubs* may still be detectable during the first meiotic division (HESS and MEYER 1968), almost no *ayl*-containing transcripts have remained at the end of primary spermatocyte stage IV (Figure 5E, right nucleus). As expected, such transcripts are also not found in cells undergoing the second meiotic division and in spermatids undergoing postmeiotic development (Figure 4B).

DISCUSSION

The *Nooses* loop pair contains defective *gypsy* elements: We have identified defective truncated members of the *gypsy* retrotransposon family as transcribed constituents in the *Y* chromosomal lampbrush loop pair *Nooses* of *D. hydei* and in a related loop pair of *D. eohydei*. Within the loop-forming transcription unit the *gypsy* elements occur interspersed between members of the *ayl* family of *Y*-specific repetitive DNA sequences. The

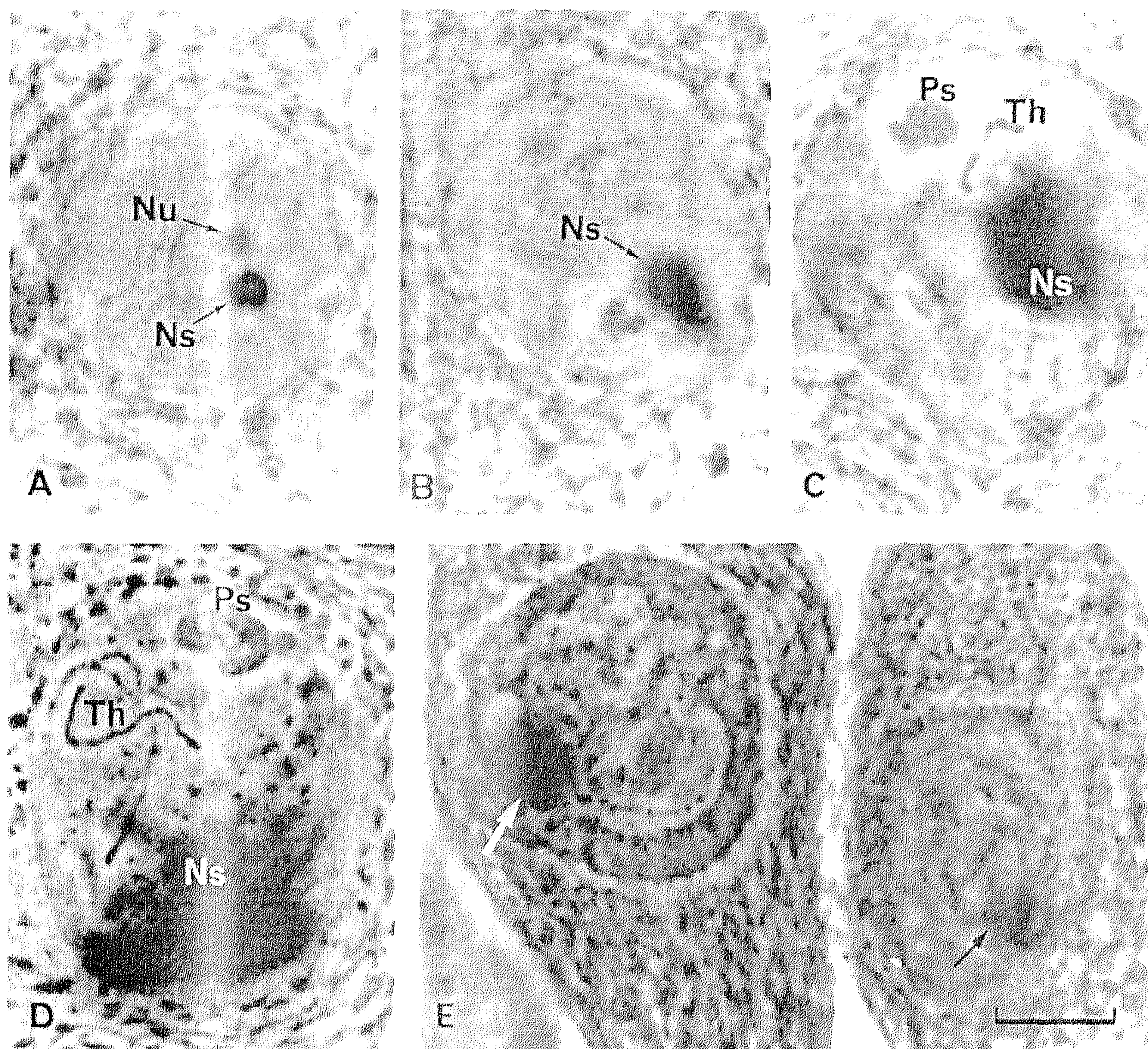


FIGURE 5.—Gradual unfolding of the *Nooses* loop pair during the successive stages of primary spermatocyte development. The *Nooses* loop pair was visualized by transcript *in situ* hybridization using the digoxigenin-labeled *ayl* probe. (A) In early Stage I primary spermatocytes, the *Nooses* loop pair (*Ns*) starts to unfold from a position close to the round nucleolus (*Nu*). The other loop pairs cannot be seen in this nucleus. (B and C) Subsequent, gradual unfolding of the *Nooses*, together with the other loop pairs, during stage I. Other loop pairs seen are the *Threads* (*Th*) and the *Pseudonucleolus* (*Ps*). (D) During stages II and III, the *Nooses*, as well as the other loop pairs, are seen at their maximum expansion. (E) During stage IV, the nuclei become round, and the loops are degraded. In the nucleus on the left, some residual *ayl*-containing transcripts are present (indicated by the white arrow). In the nucleus on the right, which is about to enter metaphase I, such transcripts (indicated by the black arrow) are barely detectable. Phase contrast. Bar, 10 μ m.

hybridization patterns of *ayl* and *gypsy* to the loop transcripts *in situ* are highly similar in both species, suggesting that both sequence types are intermingled throughout the entire transcription unit.

We can detect only the coding strand of *gypsy* and only one strand of *ayl* in the loop transcripts. If segments of the other strand are present as well, they are either too short or too highly diverged. Alternatively, they may escape detection because they are located at the very end of the transcription unit. It may also be argued that the inverted *ayl* or *gypsy* sequences are undetectable because they are spliced out from a giant primary loop transcript. However, from Miller spreads of nascent loop chromatin there is no indication that *Nooses* transcripts undergo splicing (GROND *et al.* 1983). In addition, such an explanation would also require that the inverted repeats are preferentially spliced out. Therefore, we conclude that most, if not all, copies of *ayl* and *gypsy* are present in only one orientation within the *Nooses*.

Probes containing *gypsy* sequences result in strong signals on *Nooses* transcripts, both *in situ* (Figure 2, also see HOCHSTENBACH *et al.* 1993a) and on Northern blots (Figure 3), suggesting that *gypsy* sequences represent a major part of the loop. We have no means to accurately assess the copy number of the *gypsy* elements in the *Nooses* loop. However, all *Y* chromosomal *gypsy* elements are clustered together in a distal region on the short

arm (HOCHSTENBACH *et al.* 1993b). From genomic Southern blots we estimated that this region contains ~ 10 copies of DhNo90BE5.8, 10 of the related sequence in DhNo86BH3.8 and at least two of the related sequence in DhNo19EE3.7 (HOCHSTENBACH *et al.* 1993a). If all these *Y* chromosomal copies of *gypsy*, as recognized by their hybridization to either DhNo90BE5.8 or DhNo86BH3.8, are located within the transcription unit, *gypsy* would represent more than half of the estimated 80–90 kb of *Y*-associated DNA of the 260-kb-long loop.

***gypsy* does not interfere with fertility gene function:** Male fertility gene *Q*, forming the loop pair *Nooses*, is not the only loop-forming fertility gene of *D. hydei* containing defective retrotransposons. Members of the *micropia* family, found in the loop pairs *Threads* and *Pseudonucleolus*, that are formed by fertility genes *A* and *C*, respectively, have also lost their protein-coding capacity (HUIJSER *et al.* 1988). It is remarkable that also in the case of *micropia* only the coding strand of the retrotransposon can be detected in the loop transcripts (LANKENAU *et al.* 1994). Thus, each loop-forming fertility gene appears to contain a few, or even only one family of retrotransposons, with all members in the same orientation within the loop-forming transcription unit.

An immediate question raised by these observations is why these retrotransposons do not interfere with the function of the respective fertility gene. Insertions of

retrotransposons into genes usually result in mutations (GREEN 1988). In *gypsy*-induced mutations, the binding of the *su(Hw)* protein, an ubiquitous nuclear zinc-finger protein (PARKHURST *et al.* 1988; SPANA *et al.* 1988; HARRISON *et al.* 1993), to its binding sites in *gypsy* is sufficient for mediating the mutagenic effects of the element on the expression of adjacent genes (GEYER *et al.* 1988; PEIFER and BENDER 1988; MAZO *et al.* 1989; GEYER and CORCES 1992; SMITH and CORCES 1992; ROSEMAN *et al.* 1993). We have shown that at least the *gypsy* element in clone DhNo90 has lost the binding sites for the *su(Hw)* protein (also see HOCHSTENBACH *et al.* 1994b). The *gypsy* element of this clone was also identified in three additional *ay1*-containing clones (HOCHSTENBACH *et al.* 1993a), and therefore, several copies related to this cloned fragment occur in the *Nooses* loop. We do not know whether all the *gypsy* elements in the loop have lost their capacity to bind the *su(Hw)* protein. However, a probe containing the *su(Hw)*-binding sites from the *D. melanogaster* element failed to reveal male-specific DNA fragments in *D. hydei* (HOCHSTENBACH *et al.* 1994b), suggesting that there are no such DNA sequences on the Y chromosome. This would make plausible why the *gypsy* elements do not interfere with gene function.

In Miller spreads the *Nooses* loop can be seen as a single transcription unit (GROND *et al.* 1983). Hence, the *gypsy* elements within the loop do not serve as secondary initiation sites for loop transcription, nor do they impede the normal progression of the RNA polymerase along the loop DNA. This suggests that the promoter sequences in the 5' LTR of *gypsy* and the transcriptional termination signals in the 3' LTR (ARKHIPOVA *et al.* 1986; JARRELL and MESELSON 1991) are either deleted, mutated, or nonfunctional in the context of lampbrush loop transcription. Consistent with the first possibility, the *gypsy* element in DhNo90 has a deletion of the 5' LTR, and the element in DhNo86 has almost completely lost its 3' LTR.

Significance of the *gypsy* elements for fertility gene function: Mutations or deletions in fertility gene *Q*, forming the *Nooses* loop pair, cause a developmental arrest of spermatogenesis at the end of the elongation stage, before spermatid individualization (HESS and MEYER 1968). Since the molecular basis of this effect is unknown, it is difficult to assess the role of the *gypsy* elements transcribed in the *Nooses* for the function of the associated fertility gene *Q*. However, mutant alleles of fertility genes that do not form a loop are sterile (LEONCINI 1977; HACKSTEIN *et al.* 1982, 1991). Therefore, the transcription of the repetitive loop constituents, such as *ay1* and *gypsy*, seems to be required for gene function.

The detailed sequence analysis of *ay1* repeats and Y-associated *gypsy* elements indicates that, in general, point mutations or deletions of these sequences are unlikely to interfere with the function of gene *Q*. The

gypsy elements, such as those shown in Figure 1, are randomly accumulating point mutations and deletions, and they have lost their protein coding potential (HOCHSTENBACH *et al.* 1994b). The *ay1* repeats are heterogeneous in size and they do not share an extended conserved DNA region (VOGT and HENNIG 1986a,b; WLASCHEK *et al.* 1988; PAPENBROCK 1991; HOCHSTENBACH 1994).

In this context it is of interest that *ay1* and *gypsy* are absent in the lampbrush loops of most other *Drosophila* species. The *ay1* repeats are present only in *D. hydei* and its closest relatives *D. neohydei* and *D. eohydei* (HAREVEN *et al.* 1986; VOGT *et al.* 1986). *Gypsy* is also transcribed in a loop pair of *D. eohydei* (Figure 2) and in a loop pair of *D. virilis* (data not shown), but we failed to detect transcription of *gypsy* in the lampbrush loops of other species with *gypsy* elements in the genome, as for instance *D. repleta* (HOCHSTENBACH *et al.* 1994b). Thus, it seems that the function of the loop-forming male fertility genes does not depend on the particular type of repetitive DNA sequences that are transcribed in the loops (also see HENNIG 1990 for discussion).

Following earlier suggestions by HARDY *et al.* (1981) and GOLDSTEIN *et al.* (1982) that the loop-forming fertility genes *k1-5* and *k1-3* on the Y chromosome of *D. melanogaster* encode dynein-like proteins, GEPNER and HAYS (1993) have shown that one member of the dynein β -heavy chain gene family is located in the region containing *k1-5*. AYLES *et al.* (1973) have isolated EMS-induced temperature-sensitive alleles of several of the loop-forming fertility genes of *D. melanogaster*, *k1-5* being one of these genes (GOLDSTEIN *et al.* 1982), and such alleles have been isolated by LEONCINI (1977) for several of the loop-forming genes of *D. hydei*, including gene *Q* (HACKSTEIN *et al.* 1982). At the restrictive temperature the temperature-sensitive allele *ms(Y)Q^{4s}* of gene *Q* forms a morphologically normal *Nooses* loop pair, at least at the level of the light microscope, in which both *ay1* and *gypsy* are transcribed (HOCHSTENBACH *et al.* 1994c). This would be expected if the mutant lesion is a point mutation or a small deletion in an exon of a protein-coding gene.

From our limited sample of DNA sequences from putative segments of the *Nooses* loop pair, we have no indication that this loop contains protein-coding sequences (HOCHSTENBACH 1994). As discussed by HENNIG (1993), such exons may be clustered at the very beginning or at the very end of the loop. It is even possible that the exons are distributed throughout the entire loop, separated by much larger introns that contain the rapidly evolving repetitive loop constituents, as proposed by HACKSTEIN *et al.* (1991). Our finding, however, that the transcripts of the *Nooses*, as detected by *ay1* or *gypsy* probes, lack a specific size, are not polyadenylated, remain within the nucleus, and are absent postmeiotically, when most proteins of the sperm are being made (HENNIG 1967), does not seem to be com-

patible with protein coding. Also the observation that loop transcription is sensitive to actinomycin-D but not to α -amanitin (HENNIG 1967) argues against protein coding by loop transcripts.

As shown by hybridization to loop transcripts *in situ* and on Northern blots, the repetitive loop constituents of *D. hydei* occur in one orientation within the loop-forming transcription units (LIFSCHYTZ and HAREVEN 1985; TRAPITZ *et al.* 1988). We do not know whether this merely reflects the evolutionary history of the loops, which were most likely generated by successive rounds of sequence amplification (see for discussion VOGT and HENNIG 1986b; HOCHSTENBACH *et al.* 1993a, 1994b). It is also possible that the distinct orientations of the repetitive DNA sequences is of a functional significance, as opposite orientations may lead to the formation of hairpin structures that could impede the progression of the transcriptional apparatus or induce heterochromatin formation (ZUCKERKANDL and HENNIG 1995). With respect to the *Nooses*, the orientations of the *ayl* and *gypsy* sequences within the transcription unit will greatly assist the reconstruction of the entire loop in an ordered set of overlapping genomic clones.

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