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

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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, ayl. 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 ayl 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 ayl 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 ayl 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; Finnegar 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 retroelements, as for example the L1 element of the mouse (Evans and Palmitter 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 copia 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; Bronner 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 microtia family (Lankenau 1998) are transcribed in the lampbrush loop pairs Threading and Pseudonucleolus in primary spermatocytes (Huijser et al. 1988). More recently, it has been demonstrated that an antisense transcript of microtia is found in spermatocytes (Lankenau et al. 1994). This transcript might be involved in the regulation of transcription frequencies of microtia 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
elements are located in the lampbrush loop pair Noset 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 Noset DNA (Vogt et al. 1988; Vogt and Henning 1986a,b; Hochstenbach et al. 1993a,b, 1994a).

MATERIALS AND METHODS

Drosophila stocks: Both the D. hydei Tübingen wild-type strain and the D. hydei wild-type strain were from our laboratory collection. D. hydei males of the genotype X/ma(yn)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 ma(yn)Q1 Y chromosome was cytogenetically normal, carrying a sterile allele of gene Q on the short arm (Hackstein et al. 1982; Hackstein and Henning 1982). During subsequent maintenance of the chromosome in males of the genotype T(1;Y)59/ma(yn)Q1, the short arm became deleted (J. F. H. Hackstein, personal communication). T(1;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/ma(yn)Q1 males used for isolation of RNA were obtained by crossing T(1;Y)59/ma(yn)Q1 males to virgin wild-type females. Absence of the short arm was confirmed by inspection of neuroblast metaphases of X/ma(yn)Q1 third instar larvae and by the failure of an ay1 repeat probe to hybridize to Southern blots of genomic DNA of X/ma(yn)Q1 adults. Repeats of the Y-specific ay1 family are located exclusively on the short arm of the Y chromosome (Vogt and Henning 1983). Flies were grown at 18° or 24° as described (Hochstenbach et al. 1995a).

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 Henning (1989). Plasmid DNA was isolated according to a boiling procedure recommended by Stratagene.

Nucleic acid probes: Two probes were used for the detection of Noset transcripts. As a probe for detecting transcripts of the Y-specific ay1 family of repetitive DNA sequences, we used an EcoRI DNA fragment of 893 bp that represents the sequence complexity of this family (Vogt and Henning 1986a). This particular repeat is called ay1. As a probe for detecting transcripts of the Y-associated DNA sequences of the Noset loop pair we used the 5.8-kb BamHI-EcoRI DNA fragment of the genomic clone DnNo90 (Hochstenbach et al. 1993a). Both DNA fragments were subcloned in pBluescript II K5+ plasmid vectors (Stratagene). Integrity of RNA samples was verified using Dmk2-30, a 1.2-kb DNA 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 dyeoxy chain-termination method, all following procedures provided by Amerham. 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]-dCTP, following conventional methods (Sambrock 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 Henning (1989). Approximately 20 μg total RNA was loaded in each lane.

Transcript in situ hybridization: Transcripts 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 sequential incubations with rhodamin-conjugated sheep anti-digoxigenin Fab-fragments (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).

RESULTS

Co-transcription of ay1 and Y-associated DNA sequences in the Noset lampbrush loop pair: The gypsy elements were identified in genomic clones that were isolated as potential segments of the lampbrush loop pair Noset. 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 Henning 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.
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 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 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 associated DNA sequences hybridize to Nosese transcripts in primary spermatocytes (Hochstenbach et al. 1993a).

The hybridization pattern of the shared associated DNA sequence on Nosese 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 Nosese 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 Nosese loop pair, consistent with our analysis of the genomic DNA of the lambrush 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 transcribed in a lambrush 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 hybridized in a similar pattern to RNA fragments heterogeneous in size, with the largest fragments 10-
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 (Hochstenbach et al. 1983; Trapitz et al. 1988). The size heterogeneity is expected given the fact that the growing nascent loop transcripts 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 3, 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 Nos es transcription unit not only the ayl repeats (Lischitz 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 Nos es 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 (Marler et al. 1986) and D. viridis (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' 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 (Spana et al.
Figure 3.—Only one strand of ayl 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/yW(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 ayl (lanes 1–4) or for DhNo90BE5.8 (lanes 5–8). These blots were stringently washed in 0.02 M sodium phosphate buffer at 50°C 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 ayl probe hybridizes to testis transcripts of a heterogeneous size [but only if the short arm of the Y chromosome is present (lanes 1–5)] and so does the probe for the coding 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 ayl, 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 Nososes transcrip- tion unit have random orientations relative to adjacent ayl 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 Nososes transcripts. To confirm this finding, we determined the orientation of the ayl repeats immediately flanking the gypsy elements in clones DhNo90 and DhNo86 by partial sequence determination of ayl repeat clusters. DhNo19 was completely sequenced as its restriction map revealed the presence of at least three separate clusters of ayl repeats (Hochstenbach et al. 1993a). Comparisons of the orientations of adjacent gypsy and ayl sequences show that the gypsy fragments in DhNo90 and DhNo86 are indeed transcribed from the same strand of DNA as the ayl repeats in these clones (Figure 1), suggesting that DhNo90 and DhNo86 represent genuine segments of the Nososes.

In contrast, DhNo19 contains six different gypsy fragments, with only two in the same orientation as the ayl 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 Nososes 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 Nososes transcription unit. This finding emphasizes that ayl 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 ayl repeats than predicted by the 260-kb length estimate for the Nososes 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 ayl have been identified, it is the only clone with gypsy sequences in the opposite orientation relative to ayl (Hochstenbach 1994; Hochstenbach et al. 1994b).

Distribution of Nososes transcripts during male germ cell development: Because retrotransposon transcripts encode proteins, we investigated whether the Nososes transcripts are transported from the nucleus to the cyto-
plasm. We used the ay1 probe to follow the distribution of the loop transcripts during spermatogenesis in wild-type males of \textit{D. hydei}. Identical results were obtained using the \textit{Dh}No90BE5.8 \textit{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 \textit{Y} chromosome is not active (HENNING 1967, 1985), and, as expected, we did not detect transcripts containing ay1 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 (HENNING 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 \~24 hr, the loop pairs start to unfold. In the nuclei of such cells the \textit{Nooses} can be seen as a small round loop pair in close proximity to the nucleus (Figure 5A). Subsequently, the \textit{Nooses} unfold, together with the other loop pairs (Figure 5, B and C). During stage II, lasting \~50 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 ay1 probe is reduced in intensity and residual \textit{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 \textit{Nooses} transcripts are rapidly degraded at the end of meiotic prophase. While some, probably proteinaceous, remnants of the \textit{Pseudo-nucleolus} and the \textit{Clubs} may still be detectable during the first meiotic division (HENSS and MEYER 1968), almost no ay1-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 \textit{Nooses} loop pair contains defective \textit{gypsy} elements: We have identified defective truncated members of the \textit{gypsy} retrotransposon family as transcribed constituents in the \textit{Y} chromosomal lambrush loop pair \textit{Nooses} of \textit{D. hydei} and in a related loop pair of \textit{D. eohydi}. Within the loop-forming transcription unit the \textit{gypsy} elements occur interspersed between members of the ay1 family of \textit{Y}-specific repetitive DNA sequences. The
assess the copy number of the...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 DhNo9BE5.8, 10 of the related sequence in DhNo86H3.8 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 DhNo9BE5.8 or DhNo86H3.8, 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 micropin family, found in the loop pairs Threads and Pandoraneus, that are formed by fertility genes A and C, respectively, have also lost their protein-coding capacity (Hugser et al. 1988). It is remarkable that also in the case of micropin 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

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 (Grono 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 DhNo9BE5.8, 10 of the related sequence in DhNo86H3.8 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 DhNo9BE5.8 or DhNo86H3.8, 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.

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retrotransposons into genes usually result in mutations (Green 1988). In \textit{gypsy}-induced mutations, the binding of the \textit{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 \textit{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 \textit{gypsy} element in clone DhNo90 has lost the binding sites for the \textit{su(Hw)} protein (also see Hochstenbach et al. 1994b). The \textit{gypsy} element of this clone was also identified in three additional \textit{ayl}-containing clones (Hochstenbach et al. 1993a), and therefore, several copies related to this cloned fragment occur in the \textit{Nooses} loop. We do not know whether all the \textit{gypsy} elements in the loop have lost their capacity to bind the \textit{su(Hw)} protein. However, a probe containing the \textit{su(Hw)}-binding sites from the \textit{D. melanogaster} element failed to reveal male-specific DNA fragments in \textit{D. hydei} (Hochstenbach et al. 1994b), suggesting that there are no such DNA sequences on the Y chromosome. This would make plausible why the \textit{gypsy} elements do not interfere with gene function.

In Miller spreads the \textit{Nooses} loop can be seen as a single transcription unit (Grond et al. 1983). Hence, the \textit{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 \textit{gypsy} and the transcriptional termination signals in the 3' LTR (Arkhipova et al. 1988; Jarrell and Meselson 1991) are either deleted, mutated, or nonfunctional in the context of lampbrush loop transcription. Consistent with the first possibility, the \textit{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 \textit{gypsy} elements for fertility gene function:** Mutations or deletions in fertility gene \textit{Q}, forming the \textit{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 \textit{gypsy} elements transcribed in the \textit{Nooses} for the function of the associated fertility gene \textit{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 \textit{ayl} and \textit{gypsy}, seems to be required for gene function.

The detailed sequence analysis of \textit{ayl} repeats and \textit{Y}-associated \textit{gypsy} elements indicates that, in general, point mutations or deletions of these sequences are unlikely to interfere with the function of gene \textit{Q}. The \textit{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 \textit{ayl} repeats are heterogeneous in size and they do not share an extended conserved DNA region (Vogt and Henning 1986a,b; Wlaschek et al. 1988; Papenbrock 1991; Hochstenbach 1994).

In this context it is of interest that \textit{ayl} and \textit{gypsy} are absent in the lampbrush loops of most other \textit{Drosophila} species. The \textit{ayl} repeats are present only in \textit{D. hydei} and its closest relatives \textit{D. noahydei} and \textit{D. eohydei} (Hareven et al. 1986; Vogt et al. 1986). \textit{Gypsy} is also transcribed in a loop pair of \textit{D. eohydei} (Figure 2) and in a loop pair of \textit{D. viridis} (data not shown), but we failed to detect transcription of \textit{gypsy} in the lampbrush loops of other species with \textit{gypsy} elements in the genome, as for instance \textit{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 Henning 1990 for discussion).

Following earlier suggestions by Hardy et al. (1981) and Goldstein et al. (1987) that the loop-forming fertility genes \textit{k1-3} and \textit{k1-5} on the \textit{Y} chromosome of \textit{D. melanogaster} encode dynein-like proteins, Gepner and Hays (1993) have shown that one member of the dy­ ein \(\beta\)-heavy chain gene family is located in the region containing \textit{k1-3}. Alyes et al. (1973) have isolated EMS-induced temperature-sensitive alleles of several of the loop-forming fertility genes of \textit{D. melanogaster}, \textit{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 \textit{D. hydei}, including gene \textit{Q} (Hackstein et al. 1982). At the restrictive temperature the temperature-sensitive allele \textit{su(Hw)}\textit{Q} of gene \textit{Q} forms a morphologically normal \textit{Nooses} loop pair, at least at the level of the light microscope, in which both \textit{ayl} and \textit{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 \textit{Nooses} loop pair, we have no indication that this loop contains protein-coding sequences (Hochstenbach 1994). As discussed by Henning (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 \textit{Nooses}, as detected by \textit{ayl} or \textit{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 (Henning 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-amanin (HENNING 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 (LIFSCHTITZ 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 HENNING 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 progress of the transcriptional apparatus or induce heterochromatin formation (ZUCKERKANDL and HENNING 1995). With respect to the Neos, the orientations of the ay1 and gypx 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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