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Genetics

HeT DNA: A family of mosaic repeated sequences specific for heterochromatin in *Drosophila melanogaster*

(transposable elements/repeated DNA/telomeres)

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ABSTRACT **HeT DNA is a complex family of repeated DNA found only in pericentric and telomeric heterochromatin. In contrast to other DNA families that have been specifically associated with heterochromatin, HeT DNA is not principally a family of tandemly repeated elements. Much of the HeT DNA family appears to be a mosaic of several different classes of large sequence elements arranged in a scrambled array; however, some elements of the family can be found in tandem repeats. In spite of the variable order of the different elements in HeT DNA, the sequence homology between different members of each class of element is extremely high, suggesting that the members are evolving in a concerted fashion. Sequence analysis suggests that some elements in the HeT family may make up a novel family of heterochromatin-specific transposable elements and that the mosaic organization of the elements may be produced by retroposition and other mechanisms involved in the transposition of mobile elements. We suggest that such mechanisms may be a general feature for the maintenance of chromosome structure.**

Heterochromatin is defined by its differential chromatin staining and greater compaction in both metaphase and interphase chromosomes (1–3). A wide variety of studies have associated heterochromatin with additional characteristics. Heterochromatin contains few, if any, genes that can be identified in conventional mutation analyses. It has the ability to affect the expression of genes moved into its environment (i.e., to produce position-effect variegation). Heterochromatin is generally late-replicating and is also underreplicated in some tissues. It is susceptible to x-ray-induced breakage and has a tendency toward ectopic pairing with other heterochromatin. Although it is often assumed that these are general characteristics of heterochromatin, no DNA sequence from heterochromatin has yet been shown to display them all (4, 5).

The tandemly repeated sequences of mouse satellite DNA were the first DNA sequences of heterochromatin to be identified (6). Since that time, so many tandem repeated sequences have been mapped to heterochromatic regions in chromosomes of animals and plants (7, 8) that it is sometimes assumed that heterochromatin and tandemly repeated DNA are synonymous. However, the sequences that have been studied do not make up the whole of the heterochromatin in any organism. For instance, mouse satellite DNA makes up only 7% of the mouse genome, yet the pericentric heterochromatin occupies at least 20% of the chromosomes (9).

The chromosomes of *Drosophila melanogaster* offer a particularly favorable system for the analysis of heterochromatin. In the polytene chromosomes two classes of pericentric heterochromatin, α and β, can be distinguished on the basis of differential staining (3). The α-heterochromatin contains the tandemly repeated satellite sequences. The sequences of α-heterochromatin from all of the chromosomes aggregate into one or two small clusters in the chromocenter. They undergo little, if any, polytene replication, suggesting that any function that these sequences might have is dispensable in polytene nuclei (10). The sequences of β-heterochromatin are also fused in the chromocenter but they are both less condensed and less underreplicated than α-heterochromatin. Although α- and β-heterochromatin are easily distinguished in polytene chromosomes, the heterochromatin of *Drosophila* metaphase chromosomes does not show a similar subdivision. *Drosophila* metaphase chromosomes resemble metaphase chromosomes of other organisms in their response to C-band staining for heterochromatin. Thus it seems likely that the polytene chromosomes provide us an opportunity to study aspects of chromosome structure that will also hold true for organisms without polytene nuclei.

Most, if not all, of the transposable elements (TEs) that have been identified in *Drosophila* have some homology with sequences in the β-heterochromatin; however, that TEs are tolerated at many sites in the euchromatic arms suggests that they are not responsible for the features of β-heterochromatin (11–13). We have therefore sought sequences that are found only in heterochromatic regions as better candidates for structural elements in these parts of the chromosomes. The family of sequences that we have found, HeT DNA, is found exclusively in the telomeric and pericentric heterochromatin (14). Strikingly, HeT DNA is a family of clustered middle-repetitive DNA that differs from the known families of clustered repeats in that much of the HeT family is not found in tandem repeats; instead it appears to be an irregular mosaic of elements that are highly conserved within the genome.*

MATERIALS AND METHODS

DNA preparation, restriction digests, agarose gel electrophoresis, and hybridization were done by standard procedures (15). Isolation of the clone λT-A was described (14).

DNA probes were oligolabeled (16) using [^{32}P]dATP for Southern blot hybridization, and [^{3}H]dATP plus [^{3}H]dTTP for *in situ* hybridization.

DNA sequences were determined by the dideoxy chain-termination technique (17) and compared using programs from the National Biomedical Research Foundation and the University of Wisconsin Genetics Computer Group.

In situ hybridization was performed as described (14).

RESULTS

The Sequences of HeT DNA Are Mosaics of Fragments with Well Conserved Sequences. The initial clone of HeT DNA contained 9.4 kilobases (kb) of DNA that, by *in situ* hybrid-

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Abbreviation: TE, transposable element.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M37704 and M37705).

ization, showed homology only with the heterochromatin of the chromocenter and with the last band on each telomere. Restriction mapping of the initial clone (λT-A) gave no evidence of tandem repetition within the cloned segment; however, there were two regions of internal cross-hybridization. When total *Drosophila* genomic DNA was digested and Southern blots were hybridized with fragments of λT-A, the results showed that the cloned sequences were part of a complex family of middle-repetitive DNA (14).

Although other families of middle-repetitive DNA from heterochromatin have been composed of tandem repeats, the HeT family does not appear to have this structure. We have used HeT sequences to screen both the Maniatis library (18) and an unamplified library constructed in a host–vector system chosen to prevent preferential loss of tandem repeated DNA sequences (19). From the Maniatis library we obtained only a few clones, one of which contained 3-kb tandem repeats with homology to part of the λT-A sequence. This clone maps specifically to the pericentric region of the Y chromosome (20). The unamplified library yielded a number of clones. Our restriction analyses of these clones indicates that tandem repeats are very rare and the irregular repeat structure of the λT-A clone is typical of the family. We have therefore chosen λT-A for our initial sequence analysis of HeT DNA.

The HeT sequences within λT-A have been divided into three *Hin*dIII fragments, A, B, and C (Fig. 1). Fragments A and C show significant cross-hybridization, indicating a region of shared sequence within these two fragments. When fragments A and C were used to probe genomic blots of *Drosophila* DNA, they showed similar multiple band patterns. The similarity of the hybridization patterns suggests that the sequences shared by these two fragments are a major component of the repeated DNA segments recognized by these two fragments. Fragment B, on the other hand, shares no detectable homology with A and C but is also present in multiple copies in the genome (14). Fragments A and B were therefore chosen as representative members of different elements of the HeT family for analysis and sequencing to begin the investigation of the structure of the repetitive elements of this family.

Subfragments of HeT DNA Differ in Their Relative Levels in the *Drosophila* Genome. Fragments A and B are both fairly large, 3.6 and 1.2 kb, respectively. To determine whether the repeated element within each of these fragments was confined to a small portion of the DNA embedded in unique sequences, fragments A and B were subdivided further (Fig. 2) and these subfragments were used to probe blots of *Hin*dIII-restricted genomic DNA (Fig. 3). The results indicate that the sequences contain at least four classes of repeated elements. Subfragments from A show a striking difference in the number of bands of hybridization on the genomic blots. Subfragments A2–A4 belong to a highly repetitive class. A5 and A6 belong to a second, less repetitive class that is most often associated with the first class. A1, on the other hand, exhibits a pattern distinctly different from the other pieces of fragment A and thus appears to belong to a third class. The three subfragments of B apparently make up

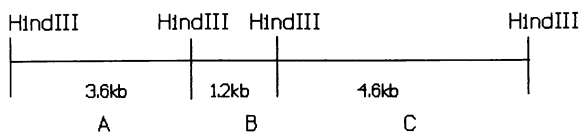

FIG. 1. Map of the HeT DNA sequences cloned in λT-A. The DNA segment consists of three contiguous *Hin*dIII fragments totaling 9.4 kb. The *Hin*dIII fragments have been renamed A (3.6 kb), B (1.2 kb), and C (4.6 kb) for ease in reference. The fragments were originally designated 3 (= A), 5 (= B), and 2 (= C) (14).

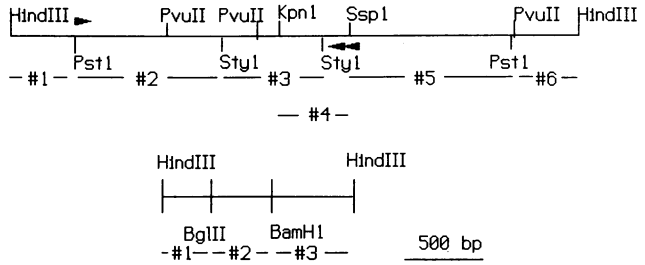

FIG. 2. Maps of fragment A (*Upper*) and fragment B (*Lower*). Subfragments used for these studies are indicated by number and the restriction sites used to generate each subfragment are noted. Arrowheads in the map of fragment A indicate runs of adenines at the 3′ end of each duplicated sequence. Each arrowhead indicates the 3′ → 5′ direction of the run. bp, Base pairs.

a fourth class. Further subdivision of these sequences will be needed to completely define the classes.

Our analyses with the subfragments have identified a 1.8-kb region within fragment A (subfragments A2–A4) as the most abundant segment of fragment A in the genome. This region has been shown by cross-hybridization to be the region that fragment A shares with fragment C (data not shown), supporting our analysis that the most repeated elements within A and C are the same. As will be discussed below, the A2–A4 region contains a sequence termed the HeT-A box. The pattern of subfragment hybridization, furthermore, shows that the most repetitive segment within fragment A (A2–A4) exists in many different sequence environments. The results also indicate that, although subfragments A5 and A6 are less abundant than A2–A4, A5 and A6 are associated with the A2–A4 repetitive element in most of the places where A5 and A6 are found within the genome. Subfragment A1, on the other hand, seems to be found most often in DNA environments that do not include the highly repetitive element from fragment A2–A4.

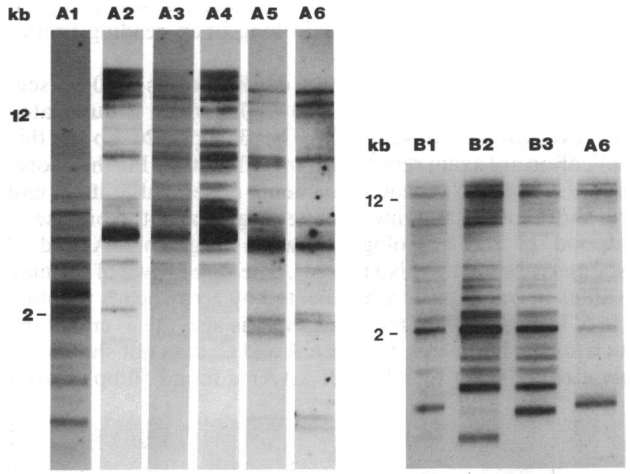

FIG. 3. Autoradiographs comparing the patterns of restriction fragments hybridizing with subfragments of HeT DNA. DNA from embryos (Oregon-R stock) was cleaved with *Hin*dIII and fractionated in agarose gels. Each lane was probed with the fragment indicated. Because of differences in the efficiency of probe labeling, patterns should be compared qualitatively rather than quantitatively. Different exposures have been chosen for the different probes to allow for comparison of the band pattern. Lanes in *Left* were all cut from the same blot. Lanes in *Right* are from a second blot. Comparisons should be made only within a blot. Blots differ in that DNA was made from different population cages of Oregon-R flies and that the running conditions of the two gels were different. Both variables can affect the pattern of hybridizing fragments.

The three subfragments from B show nearly indistinguishable patterns of hybridization on genomic blots. The most obvious differences are in the very small fragments (smaller than fragment B). The similarity of the patterns indicates that these subfragments are most often found linked to each other in the genome. Subfragment B1 lies adjacent to A6 and the similarity of their hybridization patterns on genomic blots indicates that they are frequently in this relationship in the genome (Fig. 3).

Subfragments of HeT DNA Differ in Their Relative Abundances in Different Heterochromatic Regions. The subfragments from fragments A and B that were used as probes for the genomic blots in Fig. 3 were also used as probes for *in situ* hybridizations to polytene chromosomes. Prior results had shown that fragment A hybridizes strongly to both the telomeres and the pericentric heterochromatin, whereas fragment B hybridizes primarily to the telomeres (14). The *in situ* hybridizations with the subfragments are consistent with these results and the results from the genomic blots (Fig. 4). Subfragment A1, which shows the pattern most different from the other subfragments of A on the genomic blots, hybridizes mostly to the pericentric region and also shows hybridization to the telomere of the X chromosome, but no hybridization is observed on the other telomeres. Conversely, subfragments A2–A4, which contain the highly repetitive element in fragment A, hybridize to all of the telomeres but not to the pericentric region. A5 and A6 show hybridization to the telomeres, as well as some hybridization to the pericentric heterochromatin.

All three subfragments from B show hybridization to all of the telomeres, a pattern similar to that of the complete fragment B, although we have not detected any chromocentral hybridization with the subfragments.

The Sequences of HeT DNA Are Mosaics of Fragments That Do Not Contain Tandem Repeats. Fragments A and B were completely sequenced on both strands (Fig. 5). The sequences of these fragments show no indication of tandem repeats or any simple repetitive pattern. Fragment A has regions that have a very high A+T content, but B shows no such distinctive characteristics. All sequences were analyzed for open reading frames, but no long open reading frames were found on either strand.

The only significant region of duplication is a 60-bp segment within fragment A, bp 1943–2002, which is duplicated and reversed at bp 428–487. The 3′-most 20 bp of this duplication are again repeated at bp 2014–2033. Furthermore, each of these two repeated segments is defined at its 3′ end by a short row of adenine residues that is also the precise 3′ endpoint of the homology between fragments A and C discussed above (see also ref. 21). The three rows of adenine residues in fragment A are indicated by arrowheads pointing 3′ to 5′ in Fig. 2. (This 60-bp segment is also the 3′ end of the much longer homology between A and C; data not shown). A polyadenylylation signal (AATAAA) is found 50 bp in front of this row of adenine residues (although not in the case of the 20-bp duplication at bp 2014–2033), suggesting that this element may transpose via an RNA intermediate.

Recently, broken chromosomes ends in *Drosophila* have been found to add HeT sequences to the broken ends, resulting in "healing" of the broken chromosome (21, 22). The sequences added on to the broken ends are a specific subset of the HeT family and have been named the HeT-A box. The most repetitive element within fragment A (subfragments A2–A4) is a member of the HeT-A-box set. Like the other members of this set, the box is defined at its 3′ end by a short row of adenines, the same residues that define the end of the homology between fragments A and C. The 5′ end of members of the HeT-A-box set is less well defined; the region of homology extends for different distances from the 3′ end when different members of the HeT-A set are compared. It is the 3′ end of the HeT-A sequence that is duplicated in the 60- and 20-bp repeats in fragment A.

DISCUSSION

HeT DNA Is a Novel Heterochromatin Sequence Family. The mosaic sequence structure seen in HeT DNA is distinctly different from the tandem repeat structure of most of the sequences that have been associated with heterochromatin in other studies. However, we think it likely that families with mosaic sequence structure will also be found in heterochromatin in other organisms. Detection of the HeT family in *Drosophila* was facilitated by the giant polytene chromosomes, which allow cytological resolution of heterochromatin that is not possible with metaphase chromosomes. *In situ* hybridization to these chromosomes shows clearly that HeT DNA is specifically localized to heterochromatic regions. The hybridization experiments were carried out under conditions in which we can easily detect 40 bp of homologous sequence in the euchromatic portions of the polytene chromosomes. (For example, a subcloned 40-bp segment of the yellow locus gives a strong signal.) Such experiments show no hybridization from any part of the HeT DNA in the euchromatic regions of any of the >20 *D. melanogaster* stocks that have been analyzed, so we can say with some certainty that HeT DNA sequences are restricted to heterochromatin. In wild-type chromosomes the heterochromatic regions are located in telomeric and pericentric regions. Chromosomal rearrangements that move heterochromatic regions also move sites of HeT DNA (ref. 22; unpublished results).

Elements of HeT DNA Differ in Their Distribution Within the Heterochromatin. Although *in situ* hybridization can be used to map DNA sequences quite precisely in the euchromatic regions of the polytene chromosomes, there is some uncertainty in studies of the heterochromatic regions. The uncertainty is due to the variable polytenization of heterochromatic regions. As first suggested by Heitz (2, 3) and later

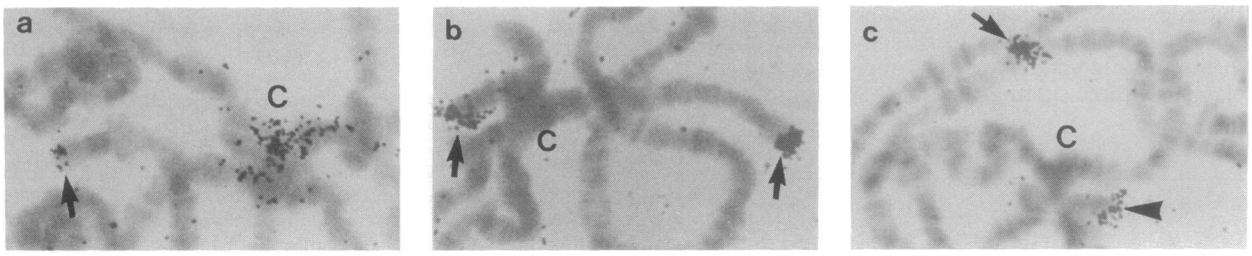

FIG. 4. Autoradiographs illustrating the relative distribution of sequences hybridizing to HeT DNA fragments in telomeres and pericentric heterochromatin. (*a*) Fragment A1 shows hybridization to the pericentric heterochromatin of the chromocenter (C) and to the telomere of the X chromosome (arrow). Other telomeres (not shown) do not show hybridization. (*b*) Fragment A3 shows hybridization only to telomeres (arrows). The chromocenter (C) does not label. (*c*) Fragment B3 shows a hybridization pattern identical to that of A3. Four telomeres are ectopically associated to produce a single cluster of hybridization (arrow). Chromosome 4 shows label over the telomere (arrowhead).

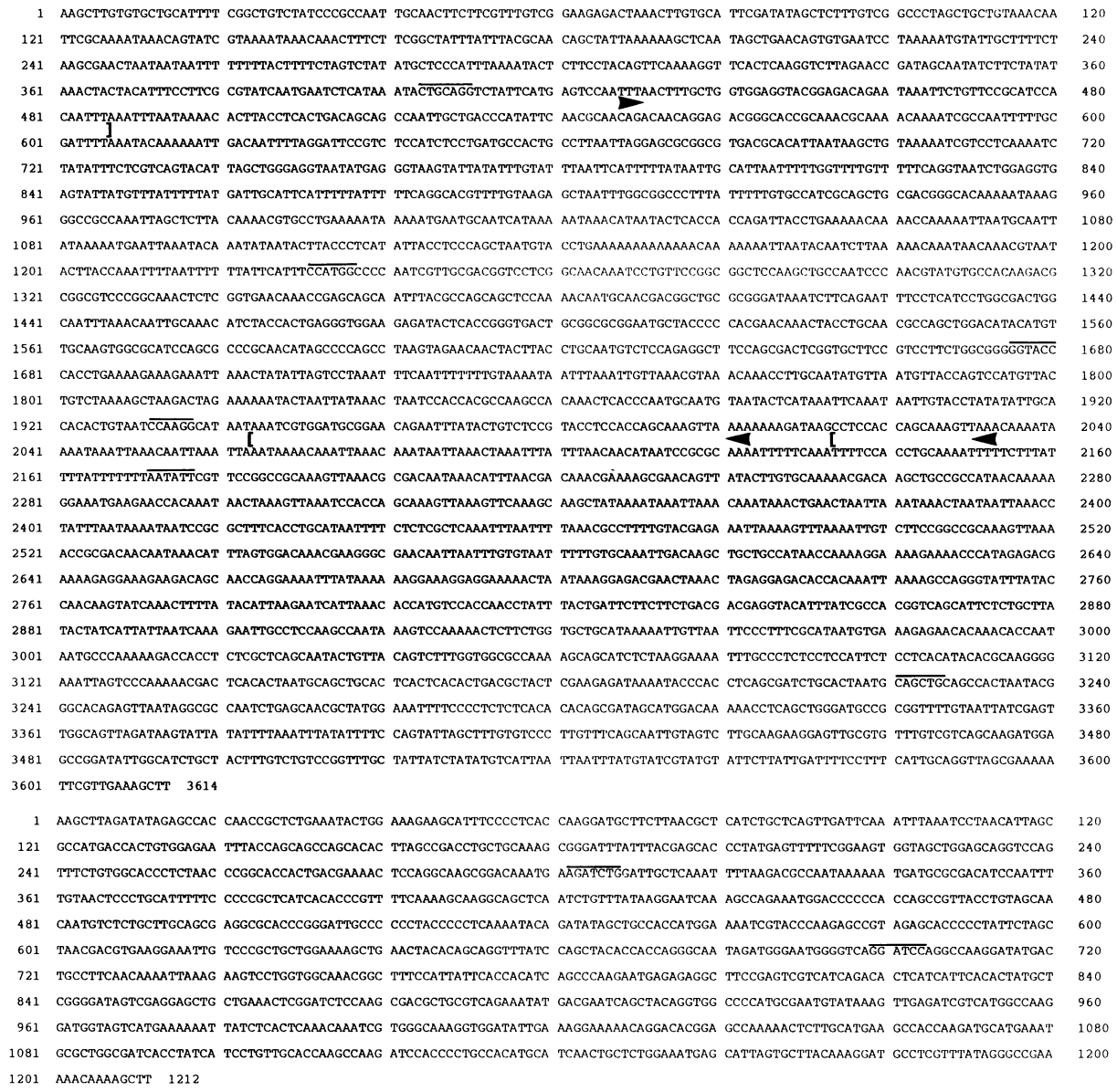

FIG. 5. Sequence of HeT DNA fragments A and B. (*Upper*) The sequence of fragment A begins with subfragment A1 and continues through A6. (*Lower*) The sequence of fragment B begins with B1 and continues through B3. Restriction sites used to generate the subfragments used for hybridization studies are overlined. Arrowheads mark the adenine runs at the 3′ end of duplications; brackets mark the 5′ ends.

confirmed by quantitative studies (23), there is very significant underreplication of sequences in the pericentric heterochromatin. Underreplication of satellite DNA sequences in α-heterochromatin has been analyzed (10), but little is known about the level of replication of other heterochromatic sequences. Although the telomeric heterochromatin has not been studied, it too is likely to be underreplicated. Our preliminary early studies of the elements in fragment B show that some of the restriction fragments with homology to B are very underreplicated in polytene nuclei while others appear to be fully polytenized (14). Other studies (unpublished results) suggest that the pattern of underreplication is affected by the genetic background of the stock. This problem will require more study. In the meantime, studies on the chromosomal distribution of HeT DNA elements must be qualified; regions that do not show hybridization to a particular element may either have lower levels of the sequence or have undergone less polytenization.

Our studies allow several conclusions, in spite of the qualifications that must be placed on hybridization analysis of heterochromatic regions. To control for effects of the genetic background we used chromosomes from both Oregon-R and gt-1 stocks. Our Oregon-R stock shows very low HeT DNA hybridization to the telomere of *3R*. Our gt-1 stock carries a *3L* telomere that shows very low HeT DNA hybridization. In both cases, as with other stocks that show low labeling telomeres, all subfragments of HeT DNA show the same low hybridization to the stock-specific underlabeled chromosome, suggesting that all elements being studied are lost or underreplicated as a unit on the telomeres.

Although all of the subfragments studied appear to be treated as a unit on telomeres, the subfragments do show differences in their relative representation when telomeres are compared with pericentric heterochromatin. These differences are seen in both the Oregon-R and the gt-1 stocks and thus do not appear to be stock-specific features. All three subfragments of B, and the subfragments of A that form part of the HeT-A box (A2–A4), show strong hybridization to telomeres and no hybridization to the pericentric regions. In contrast, subfragment A1 shows strong hybridization to peri-

centric regions and to the telomere of the X chromosome but not to the other telomeres. The strong association of HeT-A-box sequences with telomeres is consistent with the evidence that these sequences are involved in the healing of broken chromosome ends. On the other hand, the sequences in A1 lie just outside the 3′ end of the box and may be a more general heterochromatic element. That all of the subfragments of λT-A hybridize to the telomere of the X chromosome suggest that the original clone may have come from this region.

Is There a Relationship Between HeT Elements and TEs? The most notable feature of the three repeated motifs seen in the sequence of fragment A is that they resemble TEs. The Het-A box has a string of adenine residues of variable lengths at the putative 3′ end and a variable 5′ end. These are features associated with a set of TEs, the best known of which are the LINE elements from mammalian genomes, but which also include the *Drosophila* TEs *I*, *G*, *D*, *jockey*, and *Doc*. Retroposons of the LINE class have open reading frames that are thought to encode proteins involved in their transposition; however, all of these families have a significant number of defective members that no longer have the long open reading frames (24–26). We have been unable to detect any significant open reading frames in the HeT-A-box region sequenced. While it is certainly possible that fragment A contains an imperfect copy of the HeT-A box, it is also possible that all HeT-A boxes are retroposons that do not encode the mechanisms for mobilization but instead respond to a master transposition mechanism.

The duplication of the 3′-most 60 bp of the HeT-A box that begins at bp 428 might be considered a HeT-A box with an extreme truncation of the 5′ end. However, this duplication appears to lack one of the distinguishing features of retroposons; it does not show a clear example of a short duplication of the target site at both ends (24–26). Although this element has an 8-bp direct duplication at both ends, most of this duplication appears to lie within the element instead of directly flanking it. The second, shorter duplication of this same region (bp 2014–2033) could be an even more truncated version; this 20-bp duplication lacks any clear evidence of target-site duplication.

Subfragments of the HeT DNA sequence hybridize to heterochromatic sites on multiple chromosomes under very stringent conditions. These results indicate that the sequences are evolving together, suggesting that elements of this family are capable of movement or of exchanging information by gene conversion. The four new telomeres that have been added at sites of chromosome breakage in *Drosophila* (21, 22) give evidence that HeT DNA can indeed move onto broken chromosome ends through a mechanism that does not require sequence homology on the broken end. The sequence structure of the healed RT chromosomes (21) suggests that the HeT DNA moved on to those ends by retroposition. These unusual situations are the only ones where there is any evidence that movement of HeT DNA has occurred to regions outside the heterochromatin.

The HeT DNA family contains elements with structural similarity to TEs; however, we consider these elements to be a novel class of TEs because the HeT DNA elements have a chromosomal restriction not seen with other TEs. The HeT DNA elements are completely limited to heterochromatin in all of the *D. melanogaster* stocks studied, yet they can move to euchromatic regions in the special situation of a broken chromosome end.

The β-heterochromatin appears to contain many members of families of known TEs. Many, if not all, of the TEs that have been studied show *in situ* hybridization to this region of the polytene chromosomes, but all have also been found in euchromatic regions, at least in some stocks of *D. melanogaster* (11–13, 25). Attempts to clone sequences specifically from β-heterochromatin have yielded clones with homology to TEs (12, 27). The most obvious explanation for the prevalence of regions of TE homology in β-heterochromatin is that TEs that insert in this region become trapped. However, our studies of HeT DNA raise the interesting possibility that at least part of the TE sequence homology in the β-heterochromatin is due to sequences that are, in fact, a functional part of the heterochromatin, rather than accidental occupants. The sequences in the heterochromatin would be components of transposition mechanisms that the cell uses to maintain the structure of heterochromatic regions. If the cell does utilize such mechanisms for chromosome structure, it may well be that the known TEs have simply appropriated parts of the mechanism to move themselves around. In the original studies of TEs, McClintock (28) noted that conditions that disrupted chromosome structure led to mobilization of TEs. It is possible that disruption of chromosomes activates these cellular mechanisms.

We emphasize that our studies show HeT DNA to be a complex and heterogeneous family. Sequence analysis has allowed us to define one class of these elements, the HeT-A-box class. This class accounts for only part of the repeated elements. Other parts of the HeT DNA clearly have different sequences and may well have different structures.

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