*Proc. Natl. Acad. Sci. USA*
Vol. 85, pp. 2051–2055, April 1988
Biochemistry

# Microcloning reveals a high frequency of repetitive sequences characteristic of chromosome 4 and the β-heterochromatin of *Drosophila melanogaster*

(molecular cloning/polytene chromosomes/euchromatin–heterochromatin transition zones)

GEORGE L. GABOR MIKLOS*†, MASA-TOSHI YAMAMOTO*‡, JANE DAVIES*§, AND VINCENZO PIRROTTA¶

*Developmental Neurobiology Group, Research School of Biological Sciences, The Australian National University, G.P.O. Box 475, Canberra, Australian Capital Territory 2601, Australia; and ¶Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

*Communicated by M. M. Green, October 14, 1987*

ABSTRACT **Microdissection and microcloning of the euchromatin–heterochromatin transition region of the *Drosophila melanogaster* polytene X chromosome and part of the euchromatin of chromosome 4 reveals that they share certain features characteristic of β-heterochromatin, which is morphologically defined as the loosely textured material at the bases of some polytene chromosome arms. Both are mosaics of many different middle-repetitive DNA sequences interspersed with single-copy DNA sequences. Sixty percent of cloned inserts derived from division 20 and about 40 percent from subdivisions 19EF of the X chromosome harbor at least one repetitive DNA sequence in an average insert of 4.5 kilobases. No repeats have significant cross-hybridization to any of the eleven satellite DNAs, or to the clustered-scrambled sequences present in pDm1. The repetitive elements are, in general, confined to the β-heterochromatic regions of polytene chromosomes, but some are adjacent to nomadic elements. Chromosome 4, however, has some repeats spread throughout its entire euchromatin. These data have implications for the structure of transition zones between euchromatin and heterochromatin of mitotic chromosomes and also provide a molecular basis for reexamining some of the unusual classical properties of chromosome 4.**

Eukaryotic chromosomes are generally characterized by a concentration of highly repetitive DNA sequences in the regions flanking centromeres (1, 2). In *Drosophila melanogaster*, the proximal half of the metaphase X chromosome is totally heterochromatic and consists predominantly of repetitive DNAs (3, 4). Within this expanse of tandem arrays there are no lethal genetic complementation groups, nor any loci required for either male or female viability. Except for the ribosomal genes it is a genetically impoverished region. The distal euchromatic half of the X chromosome, by contrast, consists of ≈1000 vital genetic complementation groups (5). How these very different genic and nongenic regions are resolved at their zone of contact in molecular terms in either mitotic or polytene chromosomes is not known for any eukaryote.

The genetic and cytological attributes of a transition zone have been described in detail for only one eukaryotic chromosome—namely, the X chromosome of *D. melanogaster*, where the mitotic equivalent of polytene chromosome divisions 19EF and 20 has been extensively mutagenized (6–8). By microcloning (9) this region of the X chromosome, as well as part of chromosome 4, we have found that the proximal part of the X chromosome contains a high proportion of middle repetitive DNA sequences, which are, in general, restricted to the β-heterochromatin. Details of these repetitive DNA sequences in the β-heterochromatin of the X chromosome and the euchromatin of chromosome 4 are conveyed in this report.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

## MATERIALS AND METHODS

**Microdissections and Microcloning.** A chromosomal fragment was microdissected (9) from the 19EF–20A region (Fig. 1) of a single salivary gland polytene chromosome of a $gt^{x11}/gt^{1}$ larva. The DNA was extracted, cleaved with *Eco*RI, and cloned into bacteriophage λNM1149 (12). The phage were plated on strain POP13b *Escherichia coli* (9) to select against nonrecombinant plaques. Four serial microdissections from the 19EF–20 region of another single salivary gland polytene chromosome (Fig. 1) were also made.

DNA extractions from different *Drosophila* genotypes, from bacteriophage clones, or from bacteria harboring plasmid subclones were done as described (13). Restriction endonuclease cleavages, Southern blots, plaque lifts, *in situ* hybridizations, and biotinylated probe syntheses have been described (13, 14). Plasmids pDm1 (15, 16), pC2 (17), pI407 (18), pπ25.1 (19), and other plasmids harboring all, or part of, the mobile elements *copia* (20), *297* (20), *mdg3* (20), and *hobo* (pSDH4.2) (21) are described in the literature.

## RESULTS

In an earlier study we initiated "chromosomal walks" in the 19EF region to clone selected loci of neurological interest. However, most of these walks terminated after a short distance by running into thick nests of repetitive sequences (ref. 13 and G.L.G.M., unpublished work). We therefore used the microdissection technique to obtain clones representing this entire region; thus we could not only isolate these genes but also further examine the unusual molecular structure of this euchromatin–heterochromatin transition zone. Two different microdissections were done. In the first approach, we excised the 19EF–20A region from a single polytene chromosome and constructed a mini-library. However, *in situ* hybridization experiments revealed that the excision contained DNA sequences from chromosome 4, part of which probably underlay the X chromosome during microdissection. In a second experiment, we sliced serial fragments from the base of another single polytene X chromosome and constructed a mini-library from each of these four contiguous chromosomal fragments.

**Mini-Libraries.** Microcloning of the 19E–20A chromosome region (with approximate cut positions diagrammed in Fig. 1) yielded the mini-library (termed 19F+102) containing 227 putative recombinant clones. Sequential microdissection and microcloning of another single polytene X chromosome gave four mini-libraries (termed 1, 2, 3, and 4) that contained 98,

†To whom reprint requests should be addressed.
‡Present address: Department of Cell Genetics, National Institute of Genetics, Mishima, Shizuoka 411, Japan.
§Present address: Department of Genetics, University of Glasgow, Glasgow G11 5JS, Scotland.

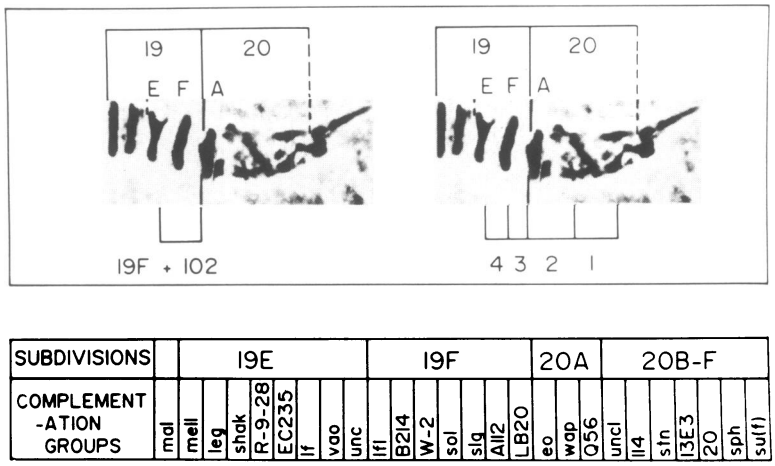


FIG. 1. Photographic maps by Lefevre (10) of polytene chromosome divisions 19 and 20 showing the approximate microdissection cuts for the 19F+102 and 1, 2, 3, and 4 mini-libraries together with the cytogenetic positions of lethal and visible complementation groups in this area (5, 6, 8, 11).

395, 138, and 364 putative recombinant clones respectively (Fig. 1).

**Mini-Library 19F+102.** We chose 115 of the 227 clones in this mini-library for analysis. When hybridized to nick-translated *Drosophila* embryonic or adult head DNA, 60% of these clones gave positive signals that indicated the presence of repetitive sequences. The nonrepetitive clones had a median-insert size of 3 kilobases (kb), whereas the repetitive clones had a median-insert size of 6 kb. Seventeen of the 115 clones had no inserts or inserts of <300 base pairs. The remaining 98 clones had inserts averaging 4.5 kb, and these were subcloned into the plasmid vector pACYC184.

Twenty-one subclones (a–u) were labeled with $^{32}P$ and hybridized to Southern blots of DNA from a wild-type strain (NN) cut with *Eco*RI (Fig. 2). Seven clones (a–g), hybridized as a single band, ranging in size from 1.4 to 3.6 kb, and these therefore contain single-copy DNA sequences and, at most, a very short tract of repetitive DNA. Clones h–u, however, hybridized as multiple bands, background smears, or both, with molecular sizes ranging from <1 kb to fragments of 20 kb or more.

When the 21 subclones were hybridized *inter se*, only two pairs cross-hybridized under standard conditions [3× SSC, (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7), 69°C]. Clone d hybridized to clone e, and clone o hybridized to clone u. The remaining 17 subclones either did not cross-hybridize or cross-hybridized only slightly. Thus, there were ten predominantly different DNA sequence families of the subclones harboring repetitive DNA sequences. The internal redundancy of the mini-library was also examined by hybridizing each of 43 clones to the array of 115 recombinants; the minimum size of the cloned genomic fragment was estimated in this way to be 300 kb, approximately half of which derives from the 19EF–20A fragment and the other half from the fourth chromosome.

We also challenged the 115 clones with the mobile elements *copia, 297, hobo, mdg 3*, the *I* element, and the *P* element. Four of the 115 clones were positive; one clone cross-hybridized with *297*, one with *hobo*, and the other two clones to the *I* element.

We analyzed most of the clones giving single bands, depicted in Fig. 2, as well as others in the 19F+102 mini-library by *in situ* hybridization to polytene chromosomes and by deficiency mapping in Southern hybridizations (13). Approximately half of these single-copy genomic DNA-containing clones originate from 19EF–20A, whereas the remaining clones derive from single bands in subdivisions 102A, 102B, and 102C on chromosome 4.

Clones harboring repetitive sequences could be divided into two distinct categories of *in situ* patterns. The first is illustrated in Fig. 3*a* by a member of the clone u family (termed Dr.D). Clone Dr.D hybridizes to the β-heterochromatin at the bases of all polytene chromosomes (Fig. 3*a*), along the *entire* euchromatic length of chromosome 4, and in some strains to 82C of chromosome 3 (Fig. 3*b*, arrow).

Examination of the normal cytological appearance of the hybridizing regions is informative. Fig. 4 illustrates the photographic representation by Lefevre (10) of the bases of the various polytene chromosomal arms. The β-heterochromatic areas of divisions 20, 40, 41, 80, 81, and 101 are diffuse and poorly banded. Note that clone Dr.D hybridizes exclusively to these regions and all along the euchromatin of chromosome 4 (Fig. 4, yellow bars).

A variant of this pattern is represented by clone r, which has no significant cross-hybridization to clone Dr.D. Clone r

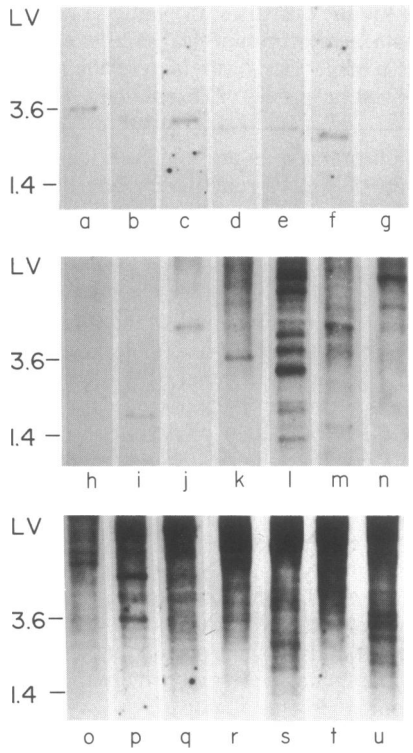


FIG. 2. Autoradiographs of 21 individual Southern blot hybridizations (a–u) of wild-type total genomic DNA from embryos cleaved with *Eco*RI and challenged singly with $P^{32}$-labeled DNA from plasmid subclones (a–u) from the 19F+102 mini-library. The molecular mass markers of 1.4 and 3.6 kb are the smallest- and largest-cloned unique DNA inserts in this sample. LV, position of the largest DNA fragments (>23 kb) that run at limiting velocity (LV) under these electrophoretic conditions.

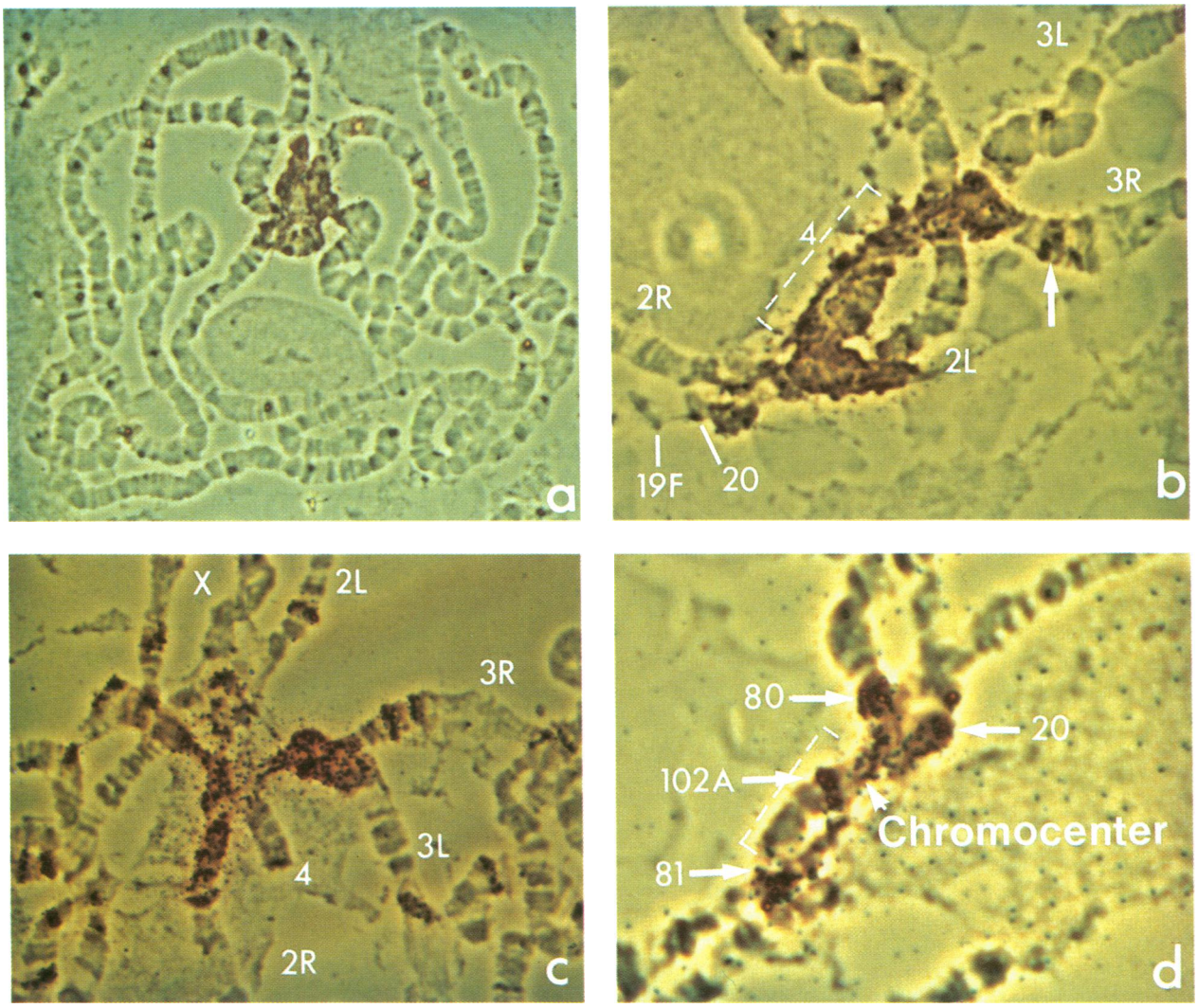


FIG. 3. *In situ* hybridization to polytene chromosomes of biotinylated probes assayed using the alkaline phosphatase detection system. The β-heterochromatin is darkly stained, and the chromosomal arms are as designated. Hybridization patterns are shown for clone Dr.D (*a* and *b*), clone s (*c*), and clone ℓ (*d*).

hybridizes to the β-heterochromatin of all chromosomes, throughout the euchromatin of chromosome 4, and, in addition, to a number of euchromatic sites that vary between three different strains (data not shown). Because clone r also hybridizes to the *I* element (18), at least the euchromatic hybridization is probably due to its nomadic element content.

Clone s hybridizes to the β-heterochromatin of all chromosomes except chromosome 4 (Fig. 3*c*). The extent of its hybridization is shown by the black bars of Fig. 4. Part of clones s may also contain a putative mobile element because it gives rise to ≈50 sites of hybridization in the euchromatic arms, which vary between three different strains. All three strains, however, share the pattern of hybridization to β-heterochromatin.

Sequences hybridizing to clone ℓ are concentrated at the *junctions* between β-heterochromatin and euchromatin of all chromosome arms (Fig. 3*d*), although it does hybridize weakly to the more proximal β-heterochromatin of all chromosomes (Fig. 4, red bars).

Clone t, which does not hybridize to any of the foregoing clones, hybridizes to the β-heterochromatin of all chromosomes, uniformly along the euchromatin of chromosome 4, carries a putative nomadic element, but gives stronger hybridization signals in some areas such as 102C of polytene chromosomes than in other regions (data not shown).

The second category of hybridization patterns is found with clones k, m, n, p, and q. Although these clones are repetitive, they hybridize to single sites on chromosome 4—namely, subdivisions 102C, 102B, 102B, 102B, and 102C, respectively, and to nowhere else in the genome (data not shown). The 102B and C regions of chromosome 4 thus contain at least five different localized repetitive DNA sequence families.

In addition, we have tested the 19F + 102 mini-library for hybridization to two other clones. The first is pDm1, which represents some of the clustered-scrambled repeats of the genome (15, 16). We find that pDm1 does not hybridize to any of our 115 clones; yet, its published pattern of *in situ* hybridization (15) has some similarities to that of clone s in that both clones hybridize to the β-heterochromatin of all chromosomes except for chromosome 4. The previously reported euchromatic sites of hybridization for pDm1 were nearly all at sites of "intercalary heterochromatin" as defined by Ananiev *et al.* (22).

We have also probed the mini-library with the type I insertion sequence (clones pC2) found originally in the ribosomal genes (17). This sequence hybridizes to two of our 115 clones, both of which originate in 19E8 (13).

Of the ten repetitive non-cross-hybridizing clones, five derive from localized areas on chromosome 4, whereas five others hybridize to DNA sequences in the β-heterochro-

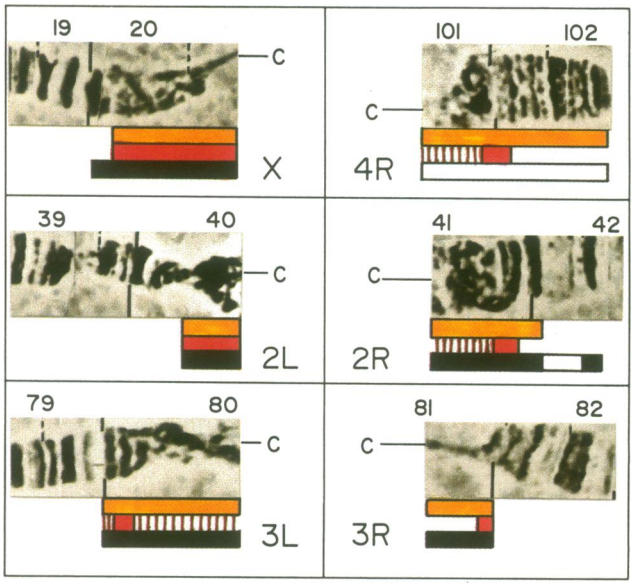


FIG. 4. Diagrammatic comparisons of the extents of *in situ* hybridizations of the clones of Fig. 3 to the photographic maps of Lefevre (10). Hybridizations to the β-heterochromatic regions are as follows: clone Dr.D (yellow bars), clone ℓ (red bars; striping represents weak hybridization), and clone s (black bars).

matin. Some hybridize throughout the euchromatin of chromosome 4. Three of the last five clones contain DNA from putative mobile elements.

We have furthermore screened genomic libraries of Oregon-R, Canton-S, and *Drosophila simulans* (Guatemala) with probes of clones Dr.D and ℓ (which are exclusively β-heterochromatic). For both probes about 7% of the clones per genome equivalent in *D. melanogaster* and about 5% in *D. simulans* give positive signals, although the intensities vary from strong to weak.

**Mini-Libraries 1, 2, 3, and 4.** These mini-libraries represent sequential cuts from the X chromosome beginning from the β-heterochromatin deep in division 20 (mini-library 1) through to subdivision 19E (mini-library 4). Measurements of the internal redundancy of the four mini-libraries yielded a total cloned genomic region of ≈1500 kb. Judged by hybridization with total labeled Canton-S genomic DNA, respectively, 57, 59, 38, and 38% of the clones in mini-libraries 1, 2, 3, and 4 were repetitive.

We also challenged the 995 clones with the eleven cloned and sequenced satellite DNAs known to constitute most of the heterochromatic DNA of the genome (4). Each radiolabeled cloned satellite was hybridized under stringent conditions (at $\approx t_m - 14$; $t_m$ = melting temperature) and we found only five weak signals. One satellite DNA probe (1.672-1) hybridized strongly to seven clones in mini-libraries 2 and 3, but this satellite clone contains part of a *297* mobile element (A. Lohe, personal communication), to which we can attribute the hybridization.

We also assayed the four mini-libraries with radioactive probes made from repetitive clones ℓ, q, and u from the 19F + 102 mini-library. Clone ℓ yields five strong positives in libraries 1 and 2; clone q, which derives from subdivision 102C, gives three strong positives in libraries 2 and 3; and clone u produces eleven strong positives distributed among all four libraries (data not shown). There are more signals for each probe, but these are of much reduced intensities.

The type I insertion sequence contained in clone pC2 yielded 12 strong positives in mini-library 4, but the *I* element was not represented in the four mini-libraries (data not shown).

We have also challenged mini-libraries 1, 2, 3, and 4 with a clone containing the simple repeat $(GATA)_n$ which we know from our *in situ* hybridizations to be distributed throughout the β-heterochromatin of the X chromosome in region 20B–F (data not shown). None of the 995 clones hybridize to this sequence. Thus, there are simple sequences in the microdissected region that have not been cloned in λNM1149, probably because they occur in areas in which *Eco*RI sites are spaced at distances of >11 kb.

Finally, we have used a large number of chromosomal rearrangements in divisions 19 and 20 (5–8, 11, 13, 14) to accurately pinpoint more than 50 of the individual clones in mini-libraries 1, 2, 3, and 4 by deficiency mapping in Southern hybridizations and by *in situ* hybridization. All clones derive solely from the base of the X chromosome and provide cloned entry points for all complementation groups between legless (subdivision 19E2) and stoned (subdivision 20B) (Fig. 1). Mini-libraries 1 and 2, in particular, are bona fide samples of the β-heterochromatin of subdivisions 20B–F, a region with a minimum of 10 lethal complementation groups (Fig. 1) interspersed among a large number of repetitive elements.

## DISCUSSION

Electron microscopic studies reveal that polytene chromosomes radiate from a compact block of α-heterochromatin that is transcriptionally inactive, through a number of transcriptionally active diffuse blocks of β-heterochromatin into the conventionally banded euchromatic arms (23). In organisms where it is present, α-heterochromatin is generally composed of highly repetitive sequences typical of satellite DNAs (1, 24). The composition of β-heterochromatin, however, has been less well defined. Previous genetic data (6) indicate that the β-heterochromatin of the X chromosome contains at least 10 genetic loci distributed throughout subdivisions 20B–F. β-heterochromatin has also been proposed to be disproportionately endowed with nomadic elements (25), and the data of Young (26) agree with this postulate. According to these data, one-third to one-half of the β-heterochromatin may consist of repetitive sequences, most of which are mobile (or once were mobile) elements.

Our microcloning results strongly support the repetitive nature of β-heterochromatic regions because 60% of our clones contain repetitive DNA sequences. By contrast, large euchromatic regions such as those represented by the ace rosy walk (27) or the bithorax complex walk (28) are essentially single-copy sequences. However, not all euchromatic regions are so free of repeats. In the regions adjacent to the white locus, 16% of the clones are repetitive (29). Although the chromosome-walking method used in sampling these regions differs from our microcloning techniques, the large disparity in frequency of repetitive sequences is, nevertheless, remarkable. It should be noted that the clones obtained by microdissection and microcloning in our particular vector are biased against sequences that contain no *Eco*RI sites, and hence repetitive regions devoid of such sites [such as the $(GATA)_n$ arrays] can remain underrepresented.

What then is the nature of the repetitive sequences found in the β-heterochromatin? First, none of our clones hybridize to, or are contiguous with, the eleven satellite DNA families, and none hybridize to the clustered-scrambled sequences found in pDm1 (15, 16). Second, the repetitive sequences found among our clones fall into four classes: (*i*) Some clones correspond to known mobile element families. (*ii*) Some clones hybridize *in situ* preferentially or exclusively to the β-heterochromatin of all chromosomes. A striking feature of many of these β-heterochromatic-specific repetitive clones is that they also hybridize *in situ* along the *entire euchromatic length of chromosome 4*, suggesting that much of this chromosome has properties in common with β-heterochromatin. Some clones,

while preferentially hybridizing *in situ* to the β-heterochromatin, also hybridize to a number of euchromatic sites, suggesting that they contain β-heterochromatin-specific sequences into which nomadic elements have been inserted. (*iii*) Some clones, though repetitive, hybridize exclusively to discrete bands in the euchromatin of chromosome 4. (*iv*) At least one family of nonsatellite simple sequences $(GATA)_n$ occurs in the β-heterochromatin of the X chromosome (data not shown), but these sequences were not recoverable using our microcloning technique.

Furthermore, nomadic element sequences are found interspersed among β-heterochromatic sequences at a relatively high frequency. The tendency of nomadic elements to insert in, or near, other repetitive sequences has been noted (30–33). For example, Di Nocera and Dawid (31) isolated a clone containing multiply nested elements: a G sequence had inserted into an *F* element, which, in turn, had inserted into a type I sequence. Part of this array had then been amplified. In another example, clone λ T-A contains a cluster of different repetitive DNA sequences as well as part of a mobile element, and this clone hybridizes *in situ* predominantly to both telomeric and β-heterochromatic regions (33).

We deduce, therefore, that the bulk of the β-heterochromatin is largely the end product of the insertion, deletion, and amplification of mobile elements into each other, as well as into regions containing unique sequences. This agrees with Rubin's general suggestion (20) that clustered-scrambled repeats represent the graveyards of nomadic elements. Precisely why these graveyards should be so extensive at heterochromatin–euchromatin transition zones remains unknown, but the low frequencies of meiotic recombination in such regions may be one contributing factor. Clearly β-heterochromatin harbors some repetitive arrays, such as the $(GATA)_n$ sequences, the genesis and turnover characteristics of which probably differ from those of conventional nomadic elements. Charlesworth *et al.* (34) have hypothesized that repetitive sequences are likely to persist longest in chromosomal regions where recombination is infrequent.

Not all sequences in β-heterochromatin, however, are without function because genic sequences are distributed throughout the molecular debris of region 20 (Fig. 1). Furthermore, sequences such as those in clone ℓ and others that occur at the very junctions of β-heterochromatin and euchromatin (35) may well have a structural function.

Finally, the DNA-sequence organization of the euchromatin of chromosome 4 has been a surprise and may, in some part, be due to the absence of meiotic crossing-over in this chromosome. Some sequences concentrated in the β-heterochromatic regions of the other chromosomes are distributed uniformly throughout the euchromatin of the fourth chromosome. Furthermore, other parts of chromosome 4, such as regions 102A, 102B, and 102C contain clusters of localized repeats. Even type I insertion sequences are found at subdivision 102C (36).

The fourth chromosome may well be primarily hetrochromatic in genetic action and the repetitive sequences distributed throughout its euchromatin may play a significant role in position-effect phenomena. Similarity in the distribution of repetitive sequences in β-hetrochromatin and the euchromatin of chromosome 4 might help elucidate the unusual genetic properties of both types of chromatin.

We gratefully acknowledge the help of D. Finnegan, S. Beckendorf, R. Appels, A. Lohe, and G. Rubin in providing us clones; M. Meselson for the *D. simulans* library; and J. Gibson for *Drosophila* stocks. We also thank M. Whittaker, C. Brockl, and B. Gray for expert technical assistance and Marilyn Miklos for preparation of the manuscript.

1. Miklos, G. L. G. (1985) in *Evolutionary Biology*, ed. MacIntyre, R. J. (Plenum, New York), pp. 241–321.
2. Singer, M. F. (1982) *Int. Rev. Cytol.* **76,** 67–112.
3. Hilliker, A. J. & Appels, R. (1982) *Chromosoma* **86,** 469–490.
4. Lohe, A. R. & Brutlag, D. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 696–700.
5. Lefevre, G. & Watkins, W. (1986) *Genetics* **113,** 869–895.
6. Schalet, A. & Lefevre, G. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, A. & Novitski, E. (Academic, London), pp. 847–902.
7. Lifschytz, E. (1978) *Genetics* **88,** 457–467.
8. Lefevre, G. (1981) *Genetics* **99,** 461–480.
9. Pirrotta, V., Jackle, H. & Edstrom, J. E. (1983) in *Genetic Engineering, Principles and Methods*, eds. Hollander, A. & Setlow, J. K. (Plenum, New York), Vol. 5, pp. 1–17.
10. Lefevre, G. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, London), pp. 31–66.
11. Miklos, G. L. G., Kelly, L. E., Coombe, P. E., Leeds, C. & Lefevre, G. (1987) *J. Neurogenet.* **4,** 1–19.
12. Murray, N. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weinberg, R. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 395–432.
13. Miklos, G. L. G., Healy, M. J., Pain, P., Howells, A. J. & Russell, R. J. (1984) *Chromosoma* **89,** 218–227.
14. Green, M. M., Yamamoto, M. & Miklos, G. L. G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4533–4537.
15. Wensink, P. C., Finnegan, D. J., Donelson, J. E. & Hogness, D. S. (1974) *Cell* **3,** 315–325.
16. Wensink, P. C., Tabata, S. & Pachl, C. (1979) *Cell* **18,** 1231–1246.
17. Kidd, S. J. & Glover, D. M. (1980) *Cell* **19,** 103–119.
18. Bucheton, A., Paro, R., Sang, H. M., Pelisson, A. & Finnegan, D. J. (1984) *Cell* **38,** 153–163.
19. O'Hare, K. & Rubin, G. M. (1983) *Cell* **34,** 25–35.
20. Rubin, G. M. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, London), pp. 329–361.
21. McGinnis, W., Shermoen, A. W. & Beckendorf, S. K. (1983) *Cell* **34,** 75–84.
22. Ananiev, E. V., Gvozdev, V. A., Ilyin, Y. V., Tchurikov, N. A. & Georgiev, G. P. (1978) *Chromosoma* **70,** 1–17.
23. Lakhotia, S. C. & Jacob, J. (1974) *Exp. Cell Res.* **86,** 253–263.
24. Gall, J. G., Cohen, E. H. & Polan, M. L. (1971) *Chromosoma* **33,** 319–344.
25. Ananiev, E. V., Barsky, V. E., Ilyin, Y. V. & Ryzic, M. V. (1984) *Chromosoma* **90,** 366–377.
26. Young, M. W. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6274–6278.
27. Hall, L. M. C., Mason, P. J. & Spierer, P. (1983) *J. Mol. Biol.* **169,** 83–96.
28. Bender, W., Akam, M., Karch, F., Beachy, P. A., Peifer, M., Spierer, P., Lewis, E. B. & Hogness, D. S. (1983) *Science* **221,** 23–29.
29. Pirrotta, V., Hadfield, C. & Pretorius, G. H. J. (1983) *EMBO J.* **2,** 927–934.
30. Dawid, I. B., Long, E. L., Di Nocera, P. P. & Pardue, M. L. (1981) *Cell* **25,** 399–408.
31. Di Nocera, P. P. & Dawid, I. B. (1983) *Nucleic Acids Res.* **11,** 5475–5482.
32. Scherer, G., Tshudi, C., Perera, J., Delius, H. & Pirrotta, V. (1982) *J. Mol. Biol.* **157,** 435–451.
33. Young, B. S., Pession, A., Traverse, K. L., French, C. & Pardue, M. L. (1983) *Cell* **34,** 85–94.
34. Charlesworth, B., Langley, C. H. & Stephan, W. (1986) *Genetics* **112,** 947–962.
35. Donnelly, R. J. & Keifer, B. I. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7172–7176.
36. Peacock, W. J., Appels, R., Endow, S. & Glover, D. (1981) *Genet. Res. Camb.* **37,** 209–214.