Bari-1, a New Transposon-Like Family in *Drosophila melanogaster* With a Unique Heterochromatic Organization

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ABSTRACT

We have identified a new middle repetitive DNA family in *Drosophila melanogaster*. This family is composed of a 1.7-kb element, called *Bari-1*, that shows common characteristics with many transposable elements. *Bari-1* is present in a few euchromatic sites that vary in different stocks. However, it is peculiar in that most copies are homogeneously clustered with a unique location in a specific heterochromatic region close to the centromere of the second chromosome. The molecular analysis of different copies coming from the euchromatin and the heterochromatin has revealed that, independent of their location, all possess the same open reading frame. The putative protein encoded by *Bari-1* shares similarity with the transposase of the *Tc1* transposon of *Caenorhabditis elegans*. We compare the *Bari-1* organization with other mobile DNA families and discuss the possibility of some functional role for the heterochromatic cluster.

THE constitutive heterochromatin comprises a substantial portion of the genomes of higher organisms, but its biological role is still an enigma [see PARDUE and HENNIG (1990) for a discussion]. The general view is that such material, very rich in both satellite and middle repetitive DNA, is genetically inert but may harbor normal genes at very low density. Genetic, cytological and molecular studies in *Drosophila melanogaster*, however, have suggested that some heterochromatin could be biologically active, but that heterochromatic and euchromatic information may be organized differently (PIMPINELLI *et al.* 1986). For example, in this species some of the heterochromatic *Y*-linked fertility factors have enormous physical dimensions ranging from 2,000 to 4,000 kb (GATTI and PIMPINELLI 1983). Moreover, the fertility factors are mainly, if not exclusively, composed of transcribed satellite DNA (BONACCORSI *et al.* 1990). Another large, heterochromatic, middle repetitive element called *ABO* (PIMPINELLI *et al.* 1985), that is undetectable by ordinary genetic analysis, has also been identified because of its interaction with mutations of the *abnormal oocytes* (*abo*) euchromatic locus (SANDLER 1970).

A heterochromatic locus on the second chromosome that has been characterized is *Responder* (*Rsp*) (GANETZKY 1977). *Rsp* is one of the main elements of the segregation distortion system [for review, see HARTL and HIRAIZUMI (1976) CROW (1979), SANDLER and GOLIC (1985) and TEMIN *et al.* (1991)], a well known case of naturally occurring meiotic drive (SANDLER and NOVITSKI 1957). *Rsp* causes degeneration of spermatids carrying it (NICOLETTI 1968; TOKUYASU, PEACOCK and HARDY 1977) when the other homolog carries the euchromatic *Segregation distorter* (*Sd*) element with which it interacts in primary spermatocytes. Genetically, the *Rsp* locus has different allelic states (MARTIN and HIRAIZUMI 1979; HIRAIZUMI and THOMAS 1984; TEMIN and MARTHAS 1984) with different degrees of sensitivity to *Sd*. Complete deletion of *Rsp* results in complete insensitivity (GANETZKY 1977). *Rsp* can also be split into two sensitive pieces by chromosome rearrangements (LYTTLE 1989). Cytogenetic analysis of chromosome rearrangements using banding techniques maps *Rsp* to a single brightly fluorescent heterochromatic region (h39) close to the centromere of the second chromosome. *Rsp* sensitivity is correlated with the size of this region, suggesting that it is repetitive (PIMPINELLI and DIMITRI 1989). Cloning of a 120-bp AT-rich satellite DNA whose abundance correlates with *Rsp* sensitivity has confirmed this prediction (WU *et al.* 1988).

Another observation related to *Rsp* induced us to perform the studies reported here. WU, TRUE and JOHNSON (1989) reported that an insensitive chromosome with only the h39 region deleted is homozygous semilethal. They suggested that the *Rsp* satellite DNA has functions important for viability. This conclusion was, however, based on the assumption that the h39 region is entirely composed of *Rsp* DNA repeats. Because of the importance of this issue we decided to test that assumption by molecular analysis of the h39 region.

The results demonstrate that the h39 region does not contain only the *Rsp* satellite DNA, but also contains a closely linked DNA family that is markedly different from all others previously described in *Drosophila melanogaster*. It consists of a repeated array of

a 1.7-kb element that we have named *Bari-1*. Its structure is analogous to that of the class II mobile elements but its mobility has not yet been demonstrated. *Bari-1* is unusual in that the majority of the copies are in a unique heterochromatic location as a single tandem array, with a few copies located at a variety of euchromatic sites. Moreover the heterochromatic copies retain an intact open reading frame (ORF) and the heterochromatic and euchromatic copies are nearly identical. *Bari-1* shares homology and some structural features with the *Tc1* transposon of *Caenorhabditis elegans* (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983; ROSENZWEIG, LIAO and HIRSH 1983).

MATERIALS AND METHODS

DNAs and hybridization conditions: DNAs from adults flies of different genotypes were extracted by the standard phenol and chloroform method, digested with restriction enzymes and Southern blotted as in MANIATIS, FRITSCH and SAMBROOK 1982. The Responder *Xba*I probe used in this study is from the 2.5-kb *Eco*RI fragment of clone H0 of WU *et al.* (1988). The plasmid containing this 2.5-kb fragment was *Xba*I digested and the 240-bp fragment purified by electroelution. Differential hybridizations were performed as follows: 0.5 μg of genomic DNA from *cn bw* or *R-16* stocks was labeled by oligo-priming and hybridized separately on two identical panels containing restricted DNA of both strains. When *cn bw* DNA was used as a probe, it was diluted 200-fold with cold *R-16* DNA to enhance the hybridization bands specific to the *cn bw* DNA.

Cloning and subcloning: With the procedure outlined above, we found a few bands which hybridized differentially using different restriction enzymes. The 1.7-kb *Sma*I band was chosen because it was relatively far from other repeated sequences which did not show differential hybridization. An electroeluted 1.7-kb *Sma*I band was ligated to a *Sma*I-cut pUC8 vector. Transformed clones were then hybridized in replica (MANIATIS, FRITSCH and SAMBROOK 1982) with total DNA following the same procedure used in Southern blots. One clone containing a single 1.7-kb *Sma*I fragment (named *Bari-1*) and positive in this analysis was further characterized.

An *Oregon-R* library in EMBL4 vector (kindly provided by V. PIRROTTA) was screened (MANIATIS, FRITSCH and SAMBROOK 1982) with labeled *Bari-1* probe. Among 50,000 plaques, 50 positive plaques were selected. A rapid analysis of such clones to determine their heterochromatic or euchromatic origin was done as follows. Miniprep λ clone DNA was partially digested with *Hin*dIII or *Kpn*I that cut only once in the cloned *Bari-1* fragment (see Figure 10a). Those phage that showed a ladder of 1.7 kb as major bands when hybridized with the *Bari-1* probe were classified as containing multiple copies of the *Bari-1* element and are most probably heterochromatic. Phage not containing the 1.7-kb ladder were grouped in another class. Totally digested DNA from all positive phage was also examined to verify the presence of the 1.7-kb band in the first group and its absence in the second group. The same filters were also hybridized with total *Oregon-R* DNA to look at all repeated sequences contained in the inserts. Combining the results of partial and total digests, four heterochromatic (λ B/1; λ B/19; λ B/23; λ B/25) and four euchromatic (λ B/31; λ B/33; λ B/34 λ B/36) clones were selected for this study. *In situ* hybridization (see below) was then done to verify the genomic location of each phage.

Single monomers of 1.7 kb from heterochromatic clones were subcloned from the λ clones as *Hin*dIII fragments. Four independent subclones (pB1-1, pB1-2, pB19-1, pB23-3) were used for sequencing. Euchromatic fragments harboring a single 1.7-kb monomer were subcloned and named as follow: 6.0-kb *Bam*HI from λ B/34 (pB/91F); 4.8-kb *Eco*RI from λ B/33 (pB/55F); 2.5-kb *Eco*RI from λ B/36 (pB47D); 4.5-kb *Eco*RI/*Xba*I from λ B/31 (pB82A).

DNA sequencing and computer analysis: The original 1.7-kb *Sma*I fragment and the four heterochromatic 1.7-kb *Hin*dIII fragments (pB1-1, pB1-2, pB19-1 and pB23-3) were further fragmented by using the enzymes *Bgl*II, *Kpn*I, *Sma*I and *Hin*dIII (see Figure 10a). The small fragments were subcloned in pUC19 and the double strand DNA was sequenced using M13 forward and reverse primers.

The dideoxy chain-termination method (SANGER, NICKLEN and COULSON 1977) was used with Sequenase Version 2.0 (U.S. Biochemical Corp.) enzyme. Due to the high A + T content of *Bari-1* (64%) very few ambiguities were found and these were resolved by reading the opposite strand. The termini of euchromatic clones (pB/91F, pB/55F, pB/47D and pB/82A) were sequenced by cloning the fragments obtained by digestion with *Bgl*II (for the left end) and *Hin*dIII (for the right end) with the closer restriction site. In addition, the complete element present in pB/47D was sequenced by subcloning the internal *Bgl*II-*Sma*I, *Sma*I-*Kpn*I and *Kpn*I-*Hin*dIII fragments. Sequencing reactions were run on denaturing 6% polyacrylamide wedge gels. With few exceptions, every base was read at least three times.

The DNA sequence analyses have been performed using GLORIA package developed by the Bioinformatics Group from CNR Research Area of Bari (Italy) and resident at the Italian EMBnet node in Tecnopolis (Valenzano, Bari, Italy).

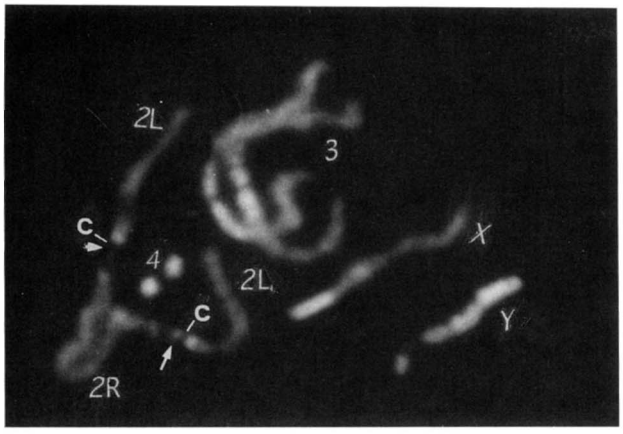
Cytology and *in situ* hybridization: Mitotic chromosome preparations and Hoechst 33258 staining were as described in PIMPINELLI and DIMITRI (1989).

In situ hybridization on mitotic and polytene chromosomes from third instar larvae were performed according to PARDUE (1986). DNA fragments flanking the *Bari-1* element in the euchromatic phage were used to determine their cytological origin; in particular, from λ B/33 a 4.8-kb *Eco*RI fragment; from λ B/34 a 7.5-kb *Bam*HI fragment; from λ B/31 a 1-kb fragment; and from λ B/36 a 7-kb *Eco*RI fragment. None of these DNA fragments hybridizes with the *Bari-1* probe nor do they contain other repeated sequences.

RESULTS

The heterochromatic h39 region of *D. melanogaster* contains the *Rsp*-associated *Xba*I repeats and another tandemly repeated sequence: To analyze the heterochromatic h39 region, we used an X-ray-generated derivative of the standard *cn bw Rsp*-sensitive chromosome. This chromosome, identified here as *R-16*, is insensitive to *Sd* because it carries a deficiency of just the h39 region located close to the centromere of the second chromosome (Figure 1). *R-16* seem to be homozygous semilethal (WU, TRUE and JOHNSON 1989).

Our strategy was to look for additional tandemly repeated sequences by comparing the restriction patterns of DNA from the *cn bw*, h39-positive chromosome and from the *R-16*, h39-negative chromosome. If there are repeated elements, in addition to the



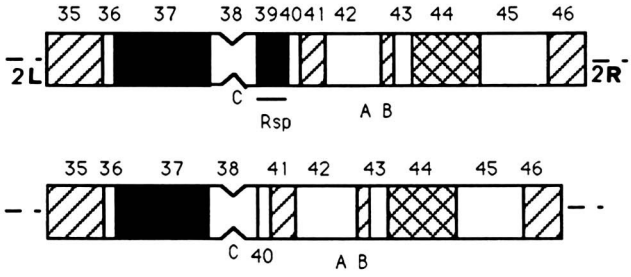


FIGURE 1.—Fluorescence pattern of the *D. melanogaster* mitotic heterochromatin. Larval neuroblast metaphase heterozygous for the *cn bw* chromosome and its X-ray derivative *R-16* stained with Hoechst 33258 fluorochrome. The *R-16* chromosome clearly lacks (arrowhead) the fluorescent h39 region close to the centromere in the *cn bw chromosome* (arrow), also shown in the diagrammatic representation of the two chromosome's heterochromatin. The diagram is according to PIMPINELLI and DIMITRI (1989). The dark areas correspond to bright regions; the crosshatched areas to moderately bright regions; the hatched areas to dull regions; and the open areas to nonfluorescent regions.

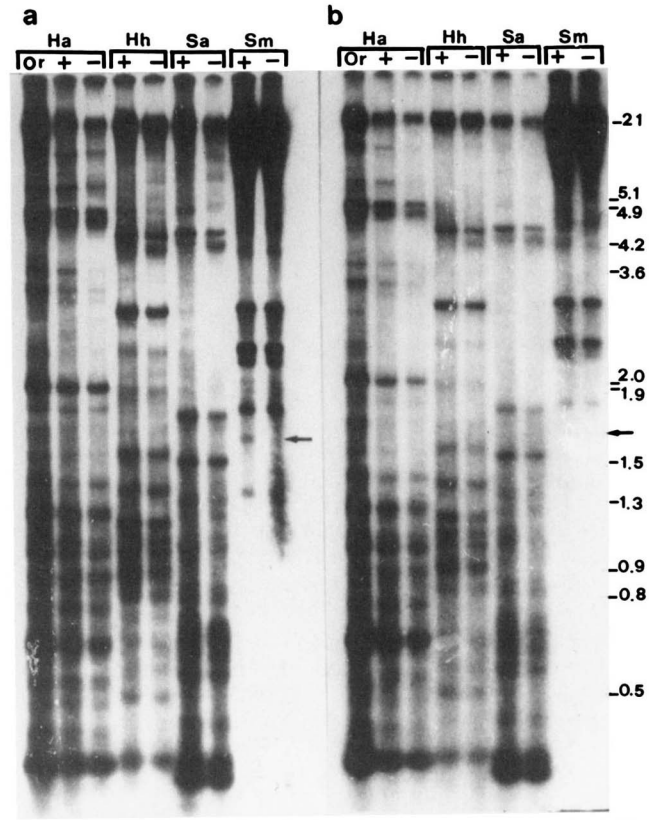


FIGURE 2.—Strategy for cloning DNA sequences from the h39 region. Four μg of DNA from the wild-type *Oregon-R* (Or), *cn bw* (+) and *R-16* (−) stocks, were digested by the enzymes *Hae*III (Ha), *Hha*I (Hh), *Sau*3a (Sa) or *Sma*I (Sm). Two identical panels were hybridized with total *cn bw* DNA (a) or total *R-16* DNA (b). A 1.7-kb *Sma*I band is present only in the *cn bw* lane probed with *cn bw* DNA. The arrows point to the 1.7-kb position in all the lanes. Numbers refer to the kb of λ *Eco*RI-*Hin*dIII marker DNA.

*Responder Xba*I satellite, that are cut by a restriction enzyme, h39-positive DNA should yield a band that is not seen with *R-16* DNA. Whether a distinctive fragment would be visible in an ethidium bromide-stained gel depends on the number of repeats and the resolution of the agarose gel, but it can, in any case, be identified by hybridization with labeled total h39-positive DNA. All ethidium bromide patterns (not shown) of restricted DNA from h39-positive (+) and h39-negative (−) chromosomes were very similar. Careful inspection, however, revealed the presence of a 1.7-kb fragment in only the (+) lane of a *Sma*I digest. The results of hybridization experiments are shown in Figure 2. When probed with labeled DNA, a 1.7-kb *Sma*I fragment was able to hybridize with total (+) DNA (Figure 2a) but not with total (−) DNA (Figure 2b), suggesting that *R-16* is missing a repeated sequence.

To demonstrate that the 1.7-kb *Sma*I repeat is from the h39 region we cloned it. From a preparative *Sma*I digestion of *cn bw* DNA, the 1.7-kb band was eluted from the gel and ligated to pUC8. Clones containing inserts were then hybridized in replica with total (+) and (−) DNAs. A clone, that we called *Bari-1*, was selected on the basis of its hybridization with (+) but not (−) DNA.

When *Bari-1* was used as a probe for *in situ* hybridization to *cn bw* and *R-16* mitotic chromosomes, only a region close to the centromere of the *cn bw* second chromosome was labeled while the *R-16* chromosome remained unlabeled (Figure 3, a and c). The same pattern of hybridization was also observed after *in situ* hybridization using the *Xba*I repeat (Figure 3, b and d). Thus, this suggests that there are not any other *Bari-1* tandem arrays outside the 2R heterochromatin.

The organization and mapping of the *Bari-1* repeat: The tandem organization of *Bari-1* in the h39 region is demonstrated by the results shown in Figure 4. Partial digests of genomic DNA obtained using *Hin*dIII, a restriction enzyme that cuts only once in the cloned fragment (see the map in Figure 11), produce a ladder of bands when hybridized with the *Bari-1* probe.

All the major bands are multiples of 1.7 kb, and no difference was found between *cn bw* and wild-type, *Oregon-R*, stocks. Within the resolution of the gel, the

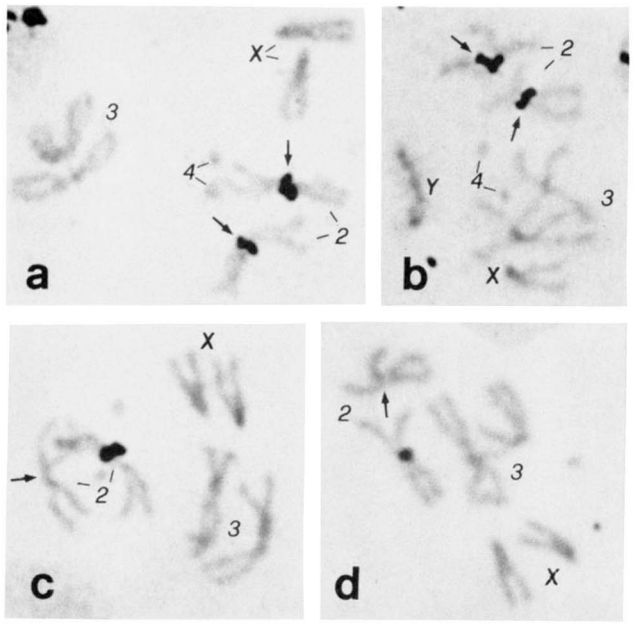


FIGURE 3.—*In situ* hybridization on mitotic chromosomes with (a, c) *Bari-1* and (b, d) *Xba*I probes. (a and b) Homozygous *cn bw* metaphases. Note the signal with both probes on a region close to the *cn bw* centromere (arrows). (c and d) Heterozygous *cn bw/R-16* metaphases. Note a complete absence of any signal on the *R-16* chromosomes with both probes (arrows).

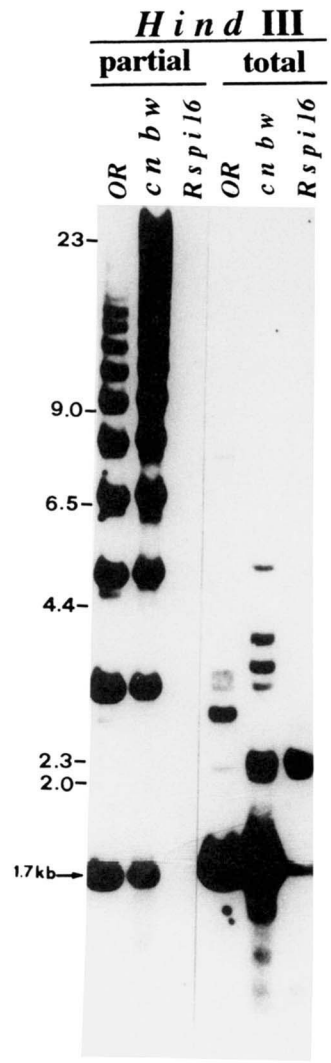


FIGURE 4.—The organization of the heterochromatic *Bari-1* cluster. Genomic DNA, partially or totally digested with *Hin*dIII, was separated in an agarose gel, Southern blotted and probed with *Bari-1*. The bands in the partial digests are multiples of 1.7 kb. Up to 16 bands were counted in a not overexposed autoradiogram in the *cn bw* lane. The difference between the *cn bw* and *Oregon-R* lanes, in the high molecular weight range, is due to the different amount of the partial digested DNA loaded. A few faint bands outside of the ladder are visible in the total digest. The position of λ *Hin*dIII marker DNA is also shown.

presence of 16 bands suggests a very homogeneous tandem array of the *Bari-1* element. In addition, an overexposure of a complete digest revealed a few bands outside the ladder. It is interesting to note, for example, that the band at 2.5 kb present in the total digest of DNA from *R-16* and *cn bw* lanes is absent in the *Oregon-R* lane, suggesting that some of the *Bari-1* repeats have variable locations outside the tandem array, either at h39 and/or elsewhere in the genome.

The structural relationship of *Bari-1* and the *Rsp* sequences was analyzed at cytological level by *in situ* hybridization on transpositions between the second chromosome heterochromatin and the *Y* chromosome and other second chromosome heterochromatic rearrangements. The main conclusions of such an analysis were that *Bari-1* repeats are clustered, are closely linked to but not interspersed with *Xba*I repeats, map distal to the *Xba*I array, and are unrelated to *Rsp*.

The transpositions were obtained by LYTTLE (1989) from irradiated males carrying the *T(2;Y)CB25* translocation. This translocation has one breakpoint in the right arm of the original *cn bw* chromosome proximal to h39, and the other breakpoint in the tip of the long arm of the *Y* chromosome distal to all of the fertility factors (see Figure 5a). Following irradiation, LYTTLE isolated a series of resealed second and *Y* chromosomes carrying reciprocal pieces of heterochromatin. The resealed *Y* chromosomes that we have used carry different portions of the h39 region; they were: *CB25-42*, *CB25-24*, *CB25-1* and *CB25-22*. Cytologically, as shown in Figure 5a, the *CB25-42 Y* chromosome is structurally normal, *CB25-24* carries part of the h39 region, *CB25-1* carries almost the entire h39 region and *CB25-22* carries, besides h39, another distal heterochromatic segment from *cn bw*.

We tested males carrying these *Y* chromosomes for *Bari-1* elements by both Southern blot analysis and *in situ* hybridization to mitotic metaphases. The mapping of these elements was then refined by restriction and cytological analyses of additional rearranged and natural population-derived chromosomes. The Southern blot analysis was done in an *R-16* homozygous background so that the only h39 segments present were those translocated to the *Y* chromosomes. The same type of restriction analysis, using the same chromosome rearrangements, had already been done for the *Xba*I repeats (WU *et al.* 1988). To allow direct com-

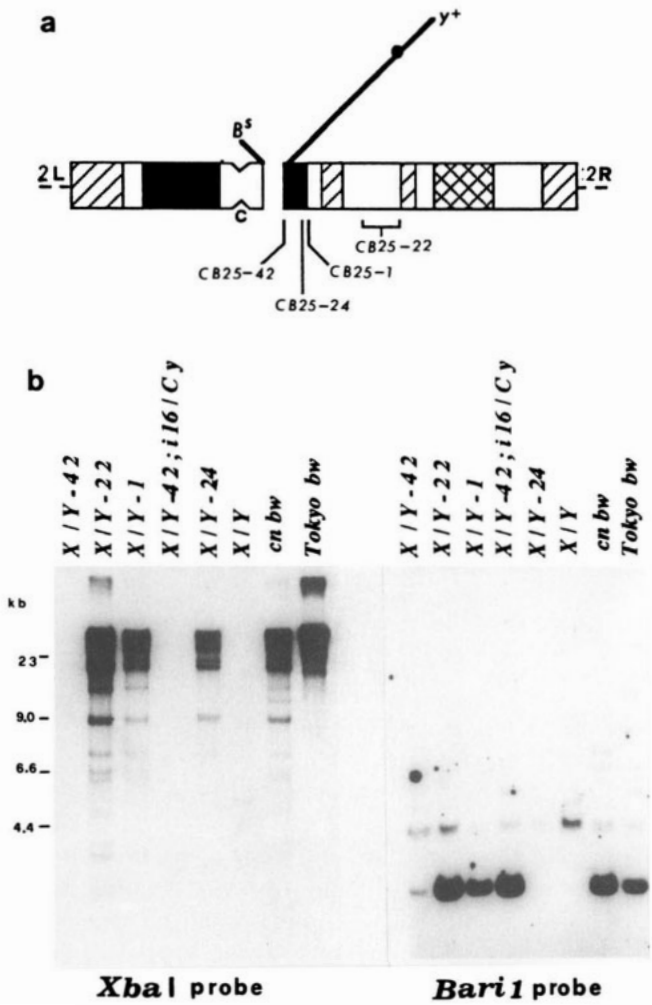


FIGURE 5.—Molecular mapping of *Bari-1*. (a) Diagrammatic representation of the *T(Y;2)CB25* translocation. The diagram also indicates the breakpoints of the four transpositions used for mapping *Bari-1*. The resealed free *Y* chromosomes carry different portions of 2R heterochromatin corresponding to the regions between the indicated breakpoint lines and the original *CB25* right half break. (b) DNA from males carrying the different *Y* transpositions, or homozygous for the *Rsp* sensitive *cn bw* or the *Rsp* supersensitive *Tokyo-bw* second chromosomes, were *Hin*dIII digested and probed with the *Xba*I (left panel) and *Bari-1* (right panel) repeats. All genotypes were in a homozygous *R-16* background except for lanes 4, 7 and 8.

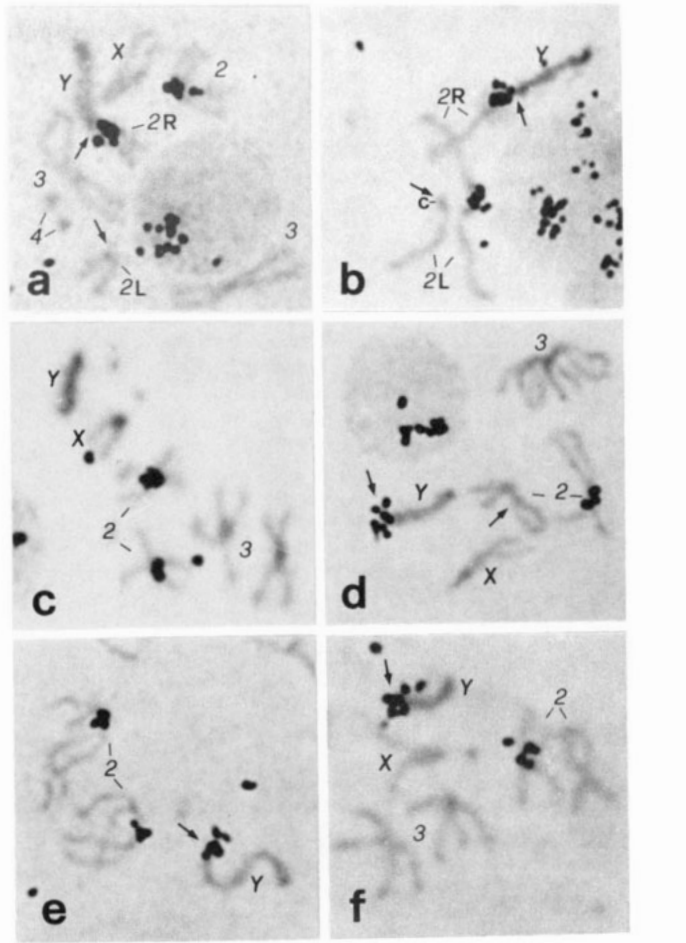


FIGURE 6.—*In situ* hybridization with *Bari-1* and *Xba*I probes on the *T(Y;2)CB25* translocation and two *Y* derivatives. In the *CB25* translocation, both the *Bari-1* sequences (a) and the *Xba*I repeats (b, where only the heterozygous translocation is shown) seem to be entirely translocated to the *Y* chromosome (arrows). There is no signal on the centromeric area of the translocated second chromosome (arrows). When the *CB25-24* transposition containing both the resealed *Y* and second chromosomes, is hybridized with the Bari-1 probe (c) there is an absence of any signal on the *Y* chromosome, but labeling are both the resealed and the original *cn bw* chromosomes. It is clear that the *Bari-1* cluster has been relocated to the resealed second chromosome. Conversely, with the *Xba*I probe (d) labeling is present on the *Y* chromosomes but is totally absent on the resealed second chromosome (compare with the labeling of the original *cn bw* chromosome) indicating that the distal breakpoint of the resealed *Y* proximal translocation was distal to almost the entire *Xba*I array. (e) partial metaphase showing the *CB25-1 Y* chromosome and the *Cy* and *cn bw* second chromosomes hybridized with *Bari-1* probe. All chromosomes are labeled with similar intensity. (f) Metaphase of the same genotype as in (e) hybridized with the *Xba*I probe. In this case as well, the *CB25-1 Y* chromosome is heavily labeled; however, the *Xba*I probe does not hybridized to the *Rsp* insensitive *Cy* chromosome even though the *Bari-1* probe did.

parison with *Bari-1*, we repeated those experiments and, for the first time, did *Xba*I-probe *in situ* hybridizations as well. Figure 5b shows the Southern blot results. Two identical panels were hybridized with the *Xba*I (left panel) and *Bari-1* (right panel) probes. The structurally normal *CB25-42 Y* chromosome (lane 1) does not have any copies of the *Xba*I sequence and also lacks *Bari-1*. The presence of a faint 1.7-kb band is probably due to the *R-16* background (see also Figure 4). Comparison of *CB25-22* (lane 2) and *CB25-1* (lane 3) with the original *cn bw* chromosome (lane 7) indicates that both transpositions carry approximately the whole array of both the *Xba*I and *Bari-1* repeats. *CB25-24* (lane 5), however, carries the majority of the *Xba*I sequences but completely lacks *Bari-1* elements. It is clear that the *Bari-1* repeats are clustered between the *CB25-24* and *CB25-1* distal breakpoints and are closely linked to the *Xba*I repeats.

The cytological analysis, by *in situ* hybridization, illustrated in Figure 6, gave the same results. In the *T(2;Y)CB25* translocation the signal is present only in the *Y* proximal half translocation with both the *Bari-*

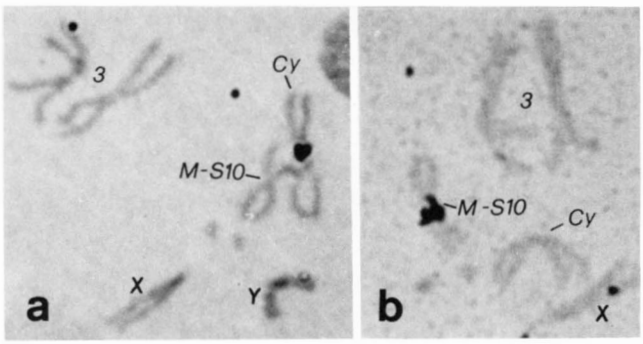


FIGURE 7.—*In situ* hybridization with *Bari-1* (a) and *Xba*I (b) probes on metaphases heterozygous for the *Cy* chromosome and *Df*(2Rh)*M-S2*^{10}, a partially *Rsp*-sensitive second chromosome deleted for almost the entire heterochromatin of the right arm. It is clear that, while the *Rsp*-insensitive *Cy* chromosome carries the *Bari-1* cluster (a) and lacks the *Xba*I array (b), *Df*(2Rh)*M-S2*^{10} lacks of the *Bari-1* cluster (a) but carries the *Xba*I array (b).

1 (Figure 6a) and *Xba*I (Figure 6b) probes. This indicates that the original autosomal breakpoint is proximal to both arrays. The resealed *CB25-24 Y* chromosome is void of any signal with the *Bari-1* probe (Figure 6c) but hybridizes with the *Xba*I probe (Figure 6d). The *CB25-1 Y* chromosome hybridizes with both the *Bari-1* (Figure 6e) and *Xba*I (Figure 6f) probes. This mapping was extended by analysis of the *Cy Rsp-insensitive* chromosome isolated from a natural population (HARTL 1975); the *Df*(2Rh)*MS2*^{10}, a partially *Rsp-sensitive* second chromosome (GANETZKY 1977) that is deficient for the entire heterochromatin of the right arm (MORGAN, SHULTZ and CURRY 1940); and the *Tokyo-bw Rsp-supersensitive* chromosome (HIRAIZUMI, MARTIN and ECKSTRAND 1980). The *Cy* chromosome lacks the *Xba*I repeats but carries the *Bari-1* cluster (Figure 5b, lane 4, and Figure 7) while *Df*(2Rh)*M-S2*^{10}, by both Southern analysis (data not shown) and *in situ* hybridization (Figure 7), carries the *Xba*I repeats but lacks the *Bari-1* cluster. These observations, along with the *Y* chromosome transposition results, clearly demonstrate that the *Xba*I and the *Bari-1* clusters are closely associated but not interspersed and that the *Bari-1* cluster is distal to the *Xba*I array. Moreover, this analysis, while it fully confirms the correlation between *Rsp* and the *Xba*I repeats (WU *et al.* 1988), also shows that *Rsp* is totally unrelated to *Bari-1*. The absence of any correlation between sensitivity to *Sd* and *Bari-1* is also indicated by the *Tokyo-bw Rsp-supersensitive* chromosome. This chromosome has a higher *Xba*I copy number than the *cn bw* chromosome, but the copy number of *Bari-1* seems to be lower than in *cn bw* (Figure 5b, lane 8).

To further examine the organization of the *Xba*I and *Bari-1* elements, we performed a Southern blot analysis on DNA from single flies collected from natural populations in three different geographic areas of Italy. One hundred flies were sampled for each population and gave similar results. Examples of this analysis are reported in Figure 8. The hybridization

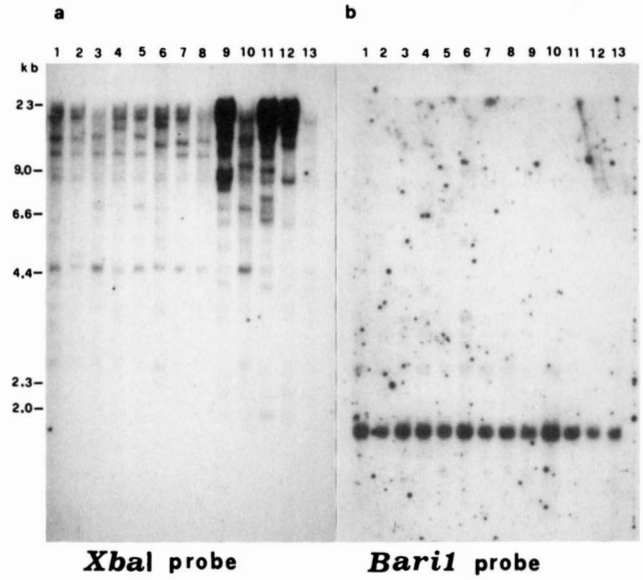


FIGURE 8.—Southern blot analysis of *Bari-1* and *Xba*I variation in a sample of 13 flies from a natural population collected near Rome. Single fly DNA was *Hin*dIII digested and probed with *Xba*I (a). The same filter was then stripped and reprobed with *Bari-1* (b).

with the *Rsp* satellite DNA showed different patterns in both the positions and intensities of the signals (Figure 8a). On the contrary, hybridization with *Bari-1*, on the same single fly DNAs, showed remarkably homogeneous patterns (Figure 8b). In addition, we performed an *in situ* hybridization analysis on mitotic chromosomes of samples coming from the same natural populations. The results showed that with both the *Xba*I and *Bari-1* probes the hybridization signals were always confined in the h39 region.

To see whether any *Bari-1* copies are located outside of the heterochromatin, we did *in situ* hybridizations to polytene salivary gland chromosomes of several Drosophila stocks. As shown for the *Canton-S* wild-type in Figure 9, *Bari-1* hybridized, as expected, to the chromocenter but it also hybridized at some euchomatic sites. We have also found variation in the euchromatic locations in different stocks (Table 1) suggesting that *Bari-1* may be mobile.

***Bari-1* is a new transposon-like element:** To characterize a complete element, we decided to isolate euchromatic sites harboring *Bari-1* by screening an *Oregon-R* λ library using *Bari-1* as a probe. Several positive plaques were isolated and classified as heterochromatic or euchromatic; inserts with more than one copy of *Bari-1* and whose flanking fragments, if any, were repetitive were classed as presuntive heterochromatic. Conversely, inserts were classed as euchromatic if they contained a single element and, more importantly, had flanking segments that *in situ* hybridized to a single polytene band. Several λ phages were selected, and four fragments harboring *Bari-1* were subcloned. Figure 10a shows the restriction maps of the subclones. Plasmids p91F, p55F and p47D all contain a single element. Plasmid p82A has only one

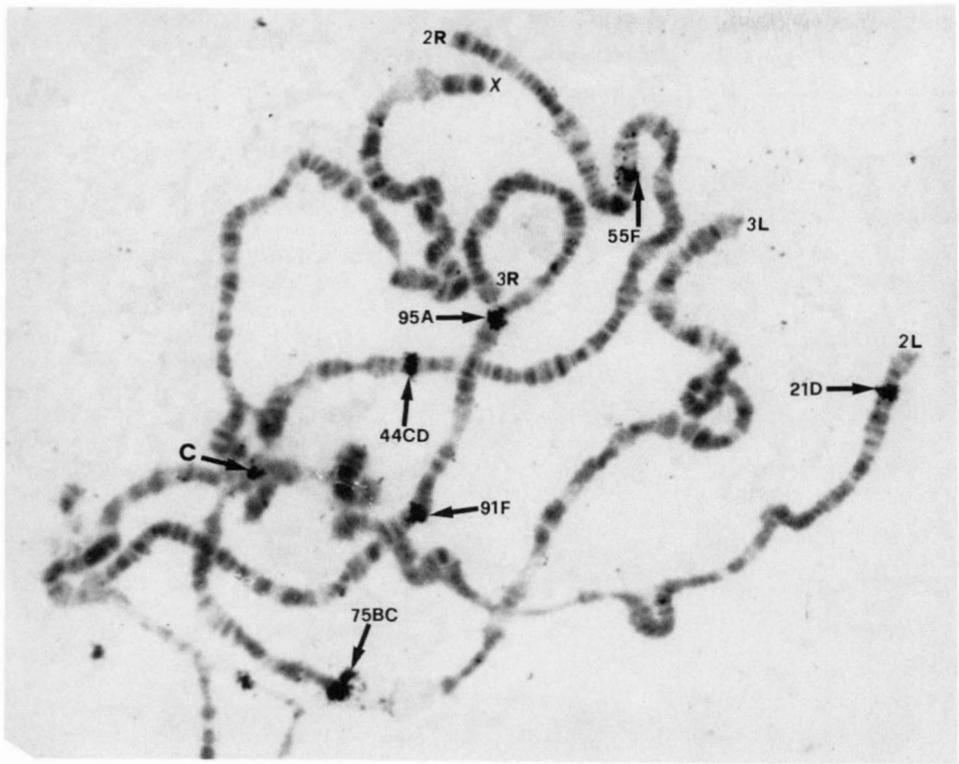


FIGURE 9.—*In situ* hybridization with *Bari-1* probe on polytene chromosomes of a wild-type *Canton-S* female. C indicates the chromocenter and the small numbered arrows identify the *Bari-1* euchromatic locations.

TABLE 1

Location of *Bari-1* elements in chromosomes of wild strains

Strain	Chromosome locations		
	X chromosome	Chromosome *2*	Chromosome *3*
Alma-Ata	4D, 8DE	34D, 44EF	82CD, 91F
Anapa-78		55F	67EF, 91F
Ashtarak	9CD	42B, 55F	91F
Aspra	3D	55F	91F
Boa Esperance		55F	65B, 91F
Canton-S		21D, 44CD, 55F	75C, 91F, 95A
Coff Harbour		34C, 34D, 42AB, 55F	69A, 82CD, 91F

half of the element since the original λ clone had one cloning site in the middle of *Bari-1*. The restriction maps of these subclones enable us to determine the orientation and the endpoints of the element in both the eu- and heterochromatic sites. The nucleotide sequence of one euchromatic element located in the 47D polytenic band of the second chromosome, is shown in Figure 11. The element is 1728 bp long and has almost perfect inverted repeats at the ends (24 matches out of 28 nucleotides). Moreover, all the euchromatic clones have a terminal TA dinucleotide at both ends because of their probable insertion in a TA target site (Figure 10b). There is one ORF in one strand of *Bari-1*. It has an ATG at position 379 and ends at position 1395 and could potentially encode a 339-amino acid polypeptide. In the other strand, two ORFs with coding capacities of 85 and 117 amino acids were found.

In the original heterochromatic *Sma*I clone the inverted repeats are located in the 0.4-kb *Bgl*II-*Hind*III fragment (see Figure 10a) in the expected tail to head fashion. The only difference from the euchromatic termini is a deletion of the last T nucleotide of the right end and the three initial ACA nucleotides of the left end (see Figure 10c). The sequences of four independent heterochromatic clones were identical to the *Sma*I clone and they are also identical to the euchromatic sequence except for the small differences in the terminal repeats.

The relationship of *Bari-1* and other repetitive sequences: A computer search in the EMBL data bank for similarity of the nucleotide sequence of *Bari-1* failed to find any significant homology. However, in the Swiss-Prot data bank we found a 26.6% identity (53% of homology, when conserved amino acid substitutions are considered) of the putative *Bari-1* 339-amino acid polypeptide and the putative transposase of the *Tc1* transposon of *Caenorhabditis elegans* (ROSENZWEIG, LIAO and HIRSH 1983). It has been reported that *Tc1* has some similarity with the *HB1* element of *D. melanogaster* (HENIKOFF and PLASTERK 1988) and with the *Uhu* element discovered in Hawaiian Drosophila (BREZINSKY *et al.* 1990). Neither *Uhu* nor *HB1* have functional open reading frames. Figure 12 shows the putative proteins of *Bari-1* and *Tc1* and reconstructed sequences of hypothetical proteins of *Uhu* and *HB1*. The most striking difference between *Bari-1* and *Tc1* is that the *Tc1* protein is shorter than that of *Bari-1* (273 amino acids *vs.* 339 amino acids). SCHUKKINK and PLASTERK (1990), however, have recently suggested that *Tc1* could encode

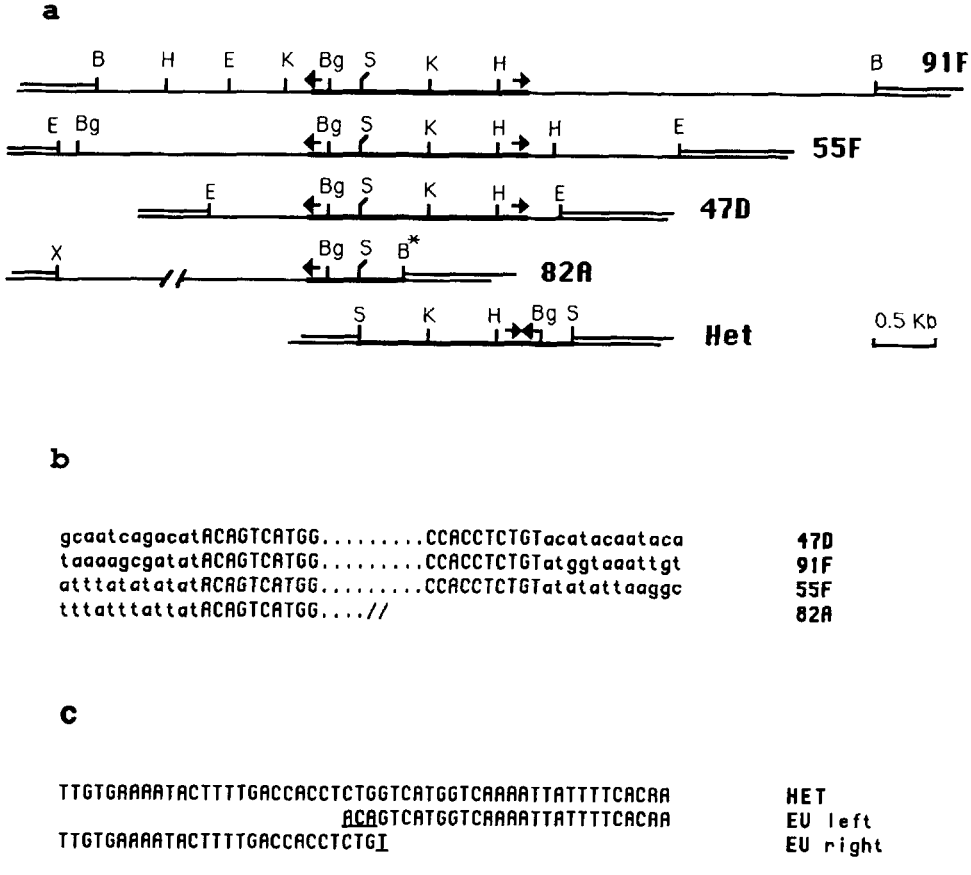


FIGURE 10.—Genomic fragments harboring *Bari-1* elements. (a) The restriction maps of the fragments subcloned from different euchromatic regions are aligned and compared with the original *Sma*I heterochromatic (Het) fragment. Note that the fragments from 91F, 55F and 47D harbor a complete *Bari-1* element (bold line). In the fragment from 82A, B* represents the original λ cloning site. Note, also, that the original heterochromatic clone (Het) is composed by the right portion of one element and the left part of the next one. B, *Bam*HI; H, *Hin*dIII; E, *Eco*RI; K, *Kpn*I; Bg, *Bgl*II; S, *Sma*I. Thin lines represent the flanking DNA, the open boxes represent the pUC vector and the arrows represent the inverted terminal repeats. (b) Sequences of ten nucleotides at the termini of the *Bari-1* elements (upper case) with the flanking DNAs from the different euchromatic sites (lower case). (c) A comparison of the right and left termini of euchromatic (EU) elements with those of the heterochromatic (HET) elements. In the heterochromatic clone the termini are juxtaposed in head-tail fashion and the underlined nucleotides are deleted.

a 335-amino acid protein. In that case, the *Tc1* protein would only be four amino acids shorter.

DISCUSSION

In the present work we demonstrate the existence, in *D. melanogaster*, of a new repeated element that we have called *Bari-1*. It belongs to the class II mobile elements (FINNEGAN 1989) and has an ORF with the coding capacity for a 339-amino acid polypeptide. The amino acid sequence of the putative *Bari-1* protein is similar to the transposase of the *Tc1* element of *C. elegans* (ROSENZWEIG, LIAO and HIRSH 1983). *Bari-1* and *Tc1* are similar in other ways as well. All analyzed copies of *Tc1* are inserted at a TA target site and have a TA duplication at the ends (RUAN and EMMONS 1987). Euchromatic copies of *Bari-1* also bear the TA duplication, suggesting that *Bari-1* could transpose by the same mechanism (for review see MOERMAN and WATERSON 1989). Although the four heterochromatic copies of *Bari-1* that we have sequenced are identical to the euchromatic copies in all other respects, the ACA terminal nucleotides of the left end and the T terminal nucleotide of the right end are deleted. Thus, the heterochromatic *Bari-1* elements retain coding capacity, but may have lost their mobility. Another *D. melanogaster* element called *HB1* shares some protein level similarity with *Tc1* (HENIKOFF and PLASTERK 1988) as does *Uhu* (BREZINSKY *et al.* 1990). Comparisons of *HB1* and *Tc1*, or *Uhu* and *Tc1*, however, required reconstruction of hypothetical proteins since *HB1* and *Uhu* are immobile and noncoding (BRIERLEY and POTTER 1985). BREZINSKI *et al.* (1990) have suggested the possibility of horizontal transmission, but if these elements have a shared evolutionary history, the distances are nevertheless so great that there has been complete divergence at the DNA sequence level. Whether the similarities of the putative polypeptides indicate a common origin, convergent evolution or selective constraints may not be easy to determine.

The organization of the heterochromatic copies of *Bari-1* is quite different from that of other heterochromatically located, transposon-like DNA families. The heterochromatic copies of these other families are generally rearranged and noncoding. They are also not present as homogeneous repeated arrays but are intermixed within the β-heterochromatin (reviewed in SPRADLING and RUBIN (1981), MIKLOS *et al.* (1988), VAURY, BUCHETON and PELISSON (1989) and DEVLIN, BINGHAM and WAKIMOTO (1990)]. The β-heterochromatin is a region at the hetero-euchromatic junction that remains visible in polytene chromosomes (HEITZ 1934). In contrast, the heterochromatic *Bari-1* copies are in a single repeated array in the α-heterochromatin. The α-heterochromatin forms the central part of the chromocenter in polytene chromosomes (HEITZ 1934) and is almost exclusively composed of satellite DNA (APPELS and PEACOCK 1978).

```
ACAGTCATGGTCAAAATTATTTTCACAAAGTGCATTTTTGTGCATGGGTCACAAACAGTTGC
63
TTGTGCAGCAAGTGGGGGGAGGTGAAATGCAAAAAAACTTTTGCTTTTGCAAATTCAAACCTATGCAGAGTCAGATGAA
142                                                    BglII
AGAAGAATTGAAAAAATAACTGTTCCTATGCGCAAGGAAGAGGCAAATGAAGAGATCTTTATCAGTTGTCAGAAGTATT
221
TGCACACGGTTTCGTCGCATCACAATTATTTTCACAACGCAATTTCTTCTTCAGTGATTGGTTTAGAGTGACAAGTGCC
299
GGTTTGTTTGCTTAAATACATTTAAATTATTGAATAAAAATTAGATTTAATCATTTTCCTATTACAGTTATTAAATAAA
379/1                                  SmaI
ATG CCC AAA ACA AAA GAG TTA ACA GTT GAG GCC CGG GCT GGT ATT GTT GCT AGG TTT AAA
Met pro lys thr lys glu leu thr val glu ala arg ala gly ile val ala arg phe lys
439/21
GCC GGT ACA CCT GCG GCC AAA ATA GCT GAA ATA TAT CAA ATT TCG CGT AGA ACT GTC TAC
ala gly thr pro ala ala lys ile ala glu ile tyr gln ile ser arg arg thr val tyr
499/41
TAC TTA ATA AAA AAG TTT GAT ACA GTT GGC ACA TTA AAA AAT AAA AAA AGA TCA GGC CGA
tyr leu ile lys lys phe asp thr val gly thr leu lys asn lys lys arg ser gly arg
559/61
AAA CCT GTG CTG GAC CAA AGG CAA TGC AGG CAA ATA CTT GGA GTT GTG GCG AAG AAT CCT
lys pro val leu asp gln arg gln cys arg gln ile leu gly val val ala lys asn pro
619/81
AGT GCC AGT CCG GTA AAA ATT GCC TTA GAA TCA AAA AAT ACA ATT GGC AAA CAA GTT AGT
ser ala ser pro val lys ile ala leu glu ser lys asn thr ile gly lys gln val ser
679/101
AGT TCT ACA ATT CGT CGC AGG CTA AAA GAA GCT GAT TTT AAG ACA TAC GTT GTT CGC AAA
ser ser thr ile arg arg arg leu lys glu ala asp phe lys thr tyr val val arg lys
739/121
ACG ATT GAG ATC ACA CCA ACC AAC AAA ACA AAA CGT CTT CGA TTT GCG TTG GAA TAT GTT
thr ile glu ile thr pro thr asn lys thr lys arg leu arg phe ala leu glu tyr val
799/141
AAG AAG CCT CTT GAC TTT TGG TTT AAT ATT TTA TGG ACT GAT GAG TCT GCA TTT CAG TAC
lys lys pro leu asp phe trp phe asn ile leu trp thr asp glu ser ala phe gln tyr
859/161
CAG GGG TCA TAC AGC AAG CAT TTT ATG CAT TTG AAA AAT AAT CAA AAG CAT TTG GCA GCC
gln gly ser tyr ser lys his phe met his leu lys asn asn gln lys his leu ala ala
919/181
CAG CCA ACC AAT AGA TTT GGT GGG GGC ACA GTC ATG TTT TGG GGA TGT CTT TCC TAT TAT
gln pro thr asn arg phe gly gly gly thr val met phe trp gly cys leu ser tyr tyr
979/201          KpnI
GGA TTC GGA GAC TTG GTA CCG ATA GAA GGA ACT TTA AAT CAG AAC GGA TAC CTT CTT ATC
gly phe gly asp leu val pro ile glu gly thr leu asn gln asn gly tyr leu leu ile
1039/221
TTA AAC AAC CAT GCT TTT ACG TCT GGA AAT AGA CTT TTT CCA ACT ACT GAA TGG ATT CTT
leu asn asn his ala phe thr ser gly asn arg leu phe pro thr thr glu trp ile leu
1099/241
CAG CAG GAC AAT GCT CCA TGC CAT AAG GGT AGG ATA CCA ACA AAA TTT TTA AAC GAC CTT
gln gln asp asn ala pro cys his lys gly arg ile pro thr lys phe leu asn asp leu
1159/261
AAT CTG GCG GTT CTT CCG TGG CCC CCC CAA AGC CCA GAC CTT AAT ATC ATT GAA AAC GTT
asn leu ala val leu pro trp pro pro gln ser pro asp leu asn ile ile glu asn val
1219/281
TGG GCT TTT ATT AAA AAC CAA CGA ACT ATT GAT AAA AAT AGA AAA CGA GAG GGA GCC ATC
trp ala phe ile lys asn gln arg thr ile asp lys asn arg lys arg glu gly ala ile
1279/301
ATT GAA ATA GCG GAG ATT TGG TCC AAA TTG ACA TTA GAA TTT GCA CAA ACT TTG GTA AGG
ile glu ile ala glu ile trp ser lys leu thr leu glu phe ala gln thr leu val arg
1339/321
TCA ATA CCA AAA AGA CTT CAA GCA GTT ATT GAT GCC AAA GGT GGT GTT ACA AAA TAT TAG
ser ile pro lys arg leu gln ala val ile asp ala lys gly gly val thr lys tyr
1399
TATTGTATTTATATAAAATAAAGAAATTCTTATGTTGAAATTAGATGTTAAGCTGAAATTTACTAAATTAAGTTGAGTG
1478                                                              HindIII
AAAATACTTTTGAAGCGCAATAAACATGTGAAAATACTATTGACAACTTGCATGCATATTTTCTTTTGCTTTAAGCTTT
1557
GTACTATGAACCGTTATCTTTCGTATTTCTTTTCGACTACCTTCTGCATAGATCAAGCTAAGCGATAAGAACTATTTCA
1636
GGCAAATCGGACAACAACAAGAAGAAATATAACAAAAAGAAGTTGAAGTTTGCAAATATTGTGCGTTGTGAAAATACTT
1715
TTGACCACCTCTGT
```

FIGURE 11.—Sequence of the *Bari-1* element. This sequence is that of the 1728-bp element from the polytene 47D euchromatic band. The 28-bp imperfect inverted terminal repeats are underlined. The putative protein is also shown. The numbers on the left, separated by a slashed line are, respectively, the numbers of nucleotides and amino acids. The position of restriction sites used for sequencing is also shown. (EMBL accession number X67681.)

Although the presence of non-satellite sequences in α-heterochromatin it has been already indicated (LOHE and BRUTLAG 1987) the our study of *Bari-1* provides the first glimpse of a specific type of non-satellite sequence within the α-heterochromatin. Most intriguingly, the tightly clustered heterochromatic *Bari-1* copies have intact ORFs and therefore retain coding potential. The h39 region, where the *Bari-1* cluster maps, is one of the heterochromatic regions that is active by genetic criteria. The *Responder* element (PIMPINELLI and DIMITRI 1989) that molecularly seems to correspond to an array of 120-bp satellite DNA (WU *et al.* 1988) maps there, and a deletion of this region is semilethal (WU, TRUE and JOHNSON 1989). The *Bari-1* cluster is adjacent to the *Rsp* sequences, but does not seem to be functionally involved

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          .         .         .         .         .         .         .         .         .         .
Bari1     MPKTKELTVEARAGIVARFKAGTPAAKIAEIYQISRRTVYYLIKKFDTVGTLKNKKRSGR
TC1       M-----------------------------------------------------------
HB1       M-----LILKLR-------KEGKTYKDIQKTLKCSAKMVSNAIK-Y----KWKPENRGTK
UHU       MVN-----------------------------LSTTTVFNIIRRFVDENRIEDKGRKAP
          *

                  .  .         .. ..  ..      .   ..  .  ......    ..    . ..
Bari1     KPVLDQRQCRQILGVVAKNPSASPVKIALESKNTIGKQVSSSTIRRRLKEADFKTYVVRK
TC1       --------DRNILRSAREDPHRTATDIQMIISSPNEPVPSKRTVRRRLQQAGLHGRKPVK
HB1       HKT-TDIEDRRIVSYSKVYRFASFRDI----KSELNLGISDVTIRRRLLNQNFSARSPRK
UHU       NKIFTEQEERRIIRKIRENPKLSAPKLTQQVQDEMGKKCSVQTVRRVLHNHDFNARVPRK
                *+*   + ++   ++++ ++  +++ +   *+++*** *++ ++++    *
          .  . ..  .. ..  .  ... .. ........ .   ..                .
Bari1     TIEITPTNKTKRLRFALEYVKKPLDFWFNILWTDESAFQYQGSYSKHFMHLKNNQKHLAA
TC1       KPFISKKNRMARVAWAKAHLRWGRQEWAKHIWSDESKFNLFGSDGNSWVR-RPVGSRYSP
HB1       VPLPSPRHIKARLSLAKTYLNWPVSKWRNILWTDGSKIMLFGGTGS-LQYIRPPNTEYHP
UHU       KPFISTKNKGTRMTFAKTHLDKDLEFWNTIIFEDESKFIIFGSDGRNYVR-RQSNTELNP
          +   + + +  *+ +* ++++   + * + + +* *  +  *  ++ +++ +   ++ ++

                .  ... .. . . .. . . .. .... .. .. ..      .   .
Bari1     Q---PTNRFGGGTVMFWGCLSYYGFGDLVPIEGTLNQNGYLLILNNHAFTSGNRLFPTTE
TC1       KYQCPTVKHGGGSVMVWGCFTSTSMGPLRRIQSIMDRFQYENIFETTMRPWALQN-VGRG
HB1       KHPVKTFNHGGPKIMVWACFFYNGMSLWIMIYGIIDQNAYVNILSDVLLSYSENI--PLK
UHU       KNLKATVKHGGGSVMVWACISAASVGNLVCIETTTDRNVDLSILKENLLQSAEKL-GIRR
          +    * + ** + * * *++  ++    +*++++++     *+++

          .  ....   ..      .       ... ..... ... .  ...      .
Bari1     WILQQDNAPCHKGRIPTKFLNDLNLAVLPWPPQSPDLNIIENVWAFIKNQRTIDKNRKRE
TC1       FVFQQDNDPKHTSLHVRSWFQRRHVHLLDWPSQSPDLNPIEHLWEELERRLGGIRASNAD
HB1       WTFQQDNDQKRRCKSAKNRFTQNRIDAMPWQAPPSHLNPIENLYGDIKQFVSKKSPTSKT
UHU       FRFYQDNDQKHKSGLVPSWLIWNCPHMII-PAQSPDVNVI-NLWDLLENN---IRNHRSN
           ++ ***+   ++    ++++  ++ +    +     * *    + ++++ +  ++++ +

                 ...   .    .. . . .. ... ...  ...
Bari1     GAIIEIAEIWSKLTLEFAQTLVRSIPKRLQAVIDAKGGVTKY
TC1       AKFNQLENAWKAIPMSVIHKLIDSMPRRCQAVIDANGYATKY
HB1       QIWQVVQDTWAKIPPKPCD-LVDFMPRGCKAVLANKGYPAKY
UHU       LK-NALLDEWSKISPETTRKLVSSMNNRLMEDIKAKGYHTKY
          + + ++++ *+ ++++  ++*+  + +        +* + **
```

FIGURE 12.—Comparison of the putative *Bari-1* polypeptide with the *Tc1* transposase and the reconstructed proteins from *HB1* and *Uhu* elements. Protein sequences in a one-letter code were aligned by the Clustal program (HIGGINS and SHARP 1988). Identical amino acids of the *Bari-1* and *Tc1* polypeptides are boldfaced. Asterisks indicate positions where the identical amino acids are present in all of the sequences. Dots indicated the positions where a *Bari-1* amino acid is also present in either *HB1* or *Uhu*. Plus signs indicate conservative substitutions between the *Bari-1* and *Tc1* polypeptides.

with segregation distortion. Instead *Bari-1* may account for the deleterious effect of the h39 deficiency. This suggestion is supported by our analysis of the molecular organization of the *Xba*I and *Bari-1* elements in natural populations. We found an extreme quantitative and structural variability of the *Rsp* satellite DNA, but a remarkable homogeneity of the heterochromatic *Bari-1* sequences. This absence of polymorphism could be due to functional constraints. It will clearly be necessary to map the semilethal effect to one or the other of the *Responder* and *Bari-1* heterochromatic arrays or even outside of them. Those experiments are now in progress.

To conclude, we want to stress that it is the detailed analysis of a specific, functionally identifiable heterochromatic region that has revealed this new repeated DNA family that is both tightly clustered and retains coding potential. The combination of classical genetic methodologies and cytological banding techniques has identified several active heterochromatic regions [for review, see PIMPINELLI *et al.* (1986)]. Approaches like those used here now permit their molecular analysis. That the molecular analysis of just one cytogenetically defined heterochromatic region has already identified two distinctive repeated arrays, *Rsp* and *Bari-1*, suggests that many similar studies will be needed if we are to have a satisfactory view of the organization of heterochromatin and an understanding of its biological role.

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