Stability of Tandem Repeats in the *Drosophila melanogaster Hsr-omega* Nuclear RNA

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Manuscript received August 27, 1994
Accepted for publication December 19, 1994

ABSTRACT

The *Drosophila melanogaster Hsr-omega* locus produces a nuclear RNA containing >5 kb of tandem repeat sequences. These repeats are unique to *Hsr-omega* and show concerted evolution similar to that seen with classical satellite DNAs. In *D. melanogaster* the monomer is ~280 bp. Sequences of $19^1/_2$ monomers differ by 8 ± 5% (mean ± SD), when all pairwise comparisons are considered. Differences are single nucleotide substitutions and 1–3 nucleotide deletions/insertions. Changes appear to be randomly distributed over the repeat unit. Outer repeats do not show the decrease in monomer homogeneity that might be expected if homogeneity is maintained by recombination. However, just outside the last complete repeat at each end, there are a few fragments of sequence similar to the monomer. The sequences in these flanking regions are not those predicted for sequences decaying in the absence of recombination. Instead, the fragmentation of the sequence homology suggests that flanking regions have undergone more severe disruptions, possibly during an insertion or amplification event. *Hsr-omega* alleles differing in the number of repeats are detected and appear to be stable over a few thousand generations; however, both increases and decreases in repeat numbers have been observed. The new alleles appear to be as stable as their predecessors. No alleles of less than ~5 kb nor more than ~16 kb of repeats were seen in any stocks examined. The evidence that there is a limit on the minimum number of repeats is consistent with the suggestion that these repeats are important in the function of the unusual *Hsr-omega* nuclear RNA.

THE *Hsr-omega* locus of *Drosophila melanogaster* produces one of the largest heat shock puffs. It is also constitutively active in almost every cell. It produces two distinct end products by alternative termination of transcription (Figure 1). Termination at the first polyadenylation signal yields a 1.9-kb RNA, omega-pre-c, which is spliced to give the 1.2-kb cytoplasmic transcript, omega-c. Termination at the second polyadenylation signal yields a >9-kb RNA, omega-n, that is not a precursor to a cytoplasmic RNA but instead remains within the nucleus. This second end product differs from any previously described nucleus-limited RNA; however, it seems likely that omega-n is the first of what will prove to be a class of RNAs with novel roles in the nucleus (PARDUE *et al.* 1990; HOGAN *et al.* 1994).

The amount of omega-n within the nucleus is regulated at both transcription and turnover. The combined effects of the two types of regulation allow the amount of omega-n to rise or fall rapidly in response to a variety of experimental manipulations (and presumably to changes in cellular conditions that occur in more natural situations). We have proposed that omega-n RNA acts by binding a protein, thereby affecting the activity of that protein. Because the levels of the RNA are modulated by cellular conditions, the activity of the protein would, in turn, be modulated by the same conditions. This proposal should soon be open to test. The omega-n transcript binds specifically to a 54-kD protein and this protein has recently been isolated (N. C. HOGAN and M. L. PARDUE, unpublished data).

Omega-n differs from the other transcripts of the *Hsr-omega* locus by possessing a large segment of short tandem direct repeats. These tandem repeats are responsible for the binding of the 54-kD protein (N. C. HOGAN and M. L. PARDUE, unpublished data). The region of tandem repeats makes up >50% of the length of the omega-n transcript and is composed of monomers of ~280 bp (GARBE *et al.* 1986; RYSECK *et al.* 1987). Thus the omega-n transcript is one of the many tandemly repeated sequences seen in eukaryotic genomes; however it has some unique features. Other tandem repeats that have been studied generally fall into two broad classes. One class consists of repeated genes, such as those encoding ribosomal RNAs or histones, where repetition amplifies the ability to produce a product that is needed in abundance. The second class consists of the so-called "satellite DNAs," tandemly repeated DNAs with no obvious coding function. All of these repeated DNAs share the intriguing feature of con-

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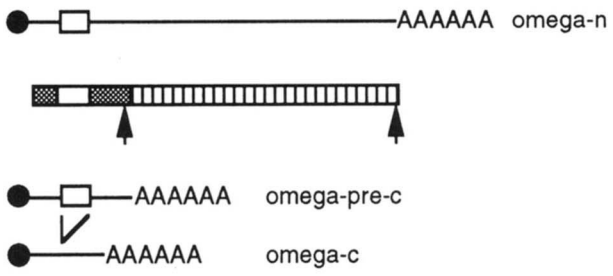

FIGURE 1.—Schematic diagram showing the structure and transcripts of *Hsr-omega*. The cross-hatched boxes represent exons 1 and 2 while the open box defines the sequences that are spliced out to make omega-c. Together these boxes, plus a short region 3′ of the first polyadenylation signal, compose the unique region of the transcription unit. The series of small open boxes represent the >5-kb segment of tandem direct repeats found only in omega-n. Arrows mark polyadenylation sites. All three transcripts have the same transcriptional start site (●). The largest transcript, omega-n, is colinear with the entire transcription unit, containing ~3.5 kb of unique region and >5 kb of tandem repeats. Omega-n is limited to the nucleus. The second transcript, omega-pre-c, made by alternative termination near the first polyadenylation signal, is also nuclear. However, omega-pre-c does not accumulate in the nucleus but is rapidly spliced and transported to the cytoplasm, yielding the third transcript, omega-c.

certed evolution. Within a species, members of a family of tandem repeats will show high levels of sequence identity whereas, between species, the sequences can diverge markedly.

In some ways the omega-n repeats resemble classical satellite DNAs. The classical satellite DNAs typically have repeat units of 100–400 bp and do not code for protein. Omega-n repeats are in the same size range and are also noncoding. Therefore in neither case is the concerted evolution constrained by the demands of an encoded protein sequence.

The omega-n repeats are entirely included within omega-n RNA, while classical satellite DNAs are generally thought not to be transcribed. However transcripts of satellite DNAs have been found in some cell-types (VARLEY *et al.* 1980; DIAZ and GALL 1985; EPSTEIN *et al.*, 1986) so omega-n repeats share this feature with at least some satellite DNAs in some cell types. The omega-n repeat regions differ from satellite regions in that there is only one cluster of omega-n repeats in the haploid genome and that cluster is small enough to be analyzed as a single entity. Satellite DNAs tend to be on multiple chromosomes and to be in large clusters, frequently adjacent to other satellites (*e.g.*, LOHE and ROBERTS 1988), complicating analyses. Thus omega-n repeats offer an experimental system that may give some useful insights into evolution and maintenance not only of this gene but also of a general class of tandem repeats.

MATERIALS AND METHODS

Drosophila stocks: *D. melanogaster* stocks were grown in either 40-ml vials or in half-pint bottles. Stocks were generally kept at 18°. Stocks used were Oregon R, ry^{506} (obtained from A. SPRADLING), gt-1 and gt-X11 (PARDUE and DAWID 1981), 215 (a wild-type stock of undetermined origin), and several stocks carrying the $Df(3R)e^{P1}$ chromosome, a chromosome isolated in 1983 (GARBE 1988). Stocks w5, w7, w9, and w15 were each derived from the gt-1 stock by making chromosome *3* homozygous. Similar stocks derived from the gt-X11 stock were 3L2, 3L8, 3L12, and 3L29. All the homozygous chromosome *3* lines were set up in March 1983. The ry^{506}, gt-X11, and $Df(3R)e^{P1}$ stocks are all derived from the Canton S strain but have been separated >5–20 years, depending on the stock. Canton S, Oregon R, and gt-1 are all derived from flies collected in very different locations long ago. They have been kept separate since that time (see LINDSLEY and ZIMM 1992). It is important to note that the allele frequency in our small populations can change rapidly so that DNA samples taken at different times may not show identical allele patterns.

DNA sequences and analyses: The *Hsr-omega* sequence was obtained from a cosmid clone, cDRM6, made from the Canton S stock and grown in a RecA$^-$ bacterial host strain (GARBE 1988). Thus, all the DNA that we have sequenced is from a single allele, cloned in cosmid cDRM6. Repeats were isolated from this clone by *Asu*II (now BstB1) digestion. This enzyme cuts once per repeat. Eight monomer repeats were cloned and sequenced. We assume these were a random sample of monomers in the array. Only two were identical in sequence. Two and a half additional repeats were cloned on a fragment that included part of the 3′ flanking sequences. Accession numbers are: U02277 (repeats plus 3′ flanking sequences), M14578-M14583 and M14556 (repeat monomers).

Multiple sequence alignments were made with the Multalign program (CORPET 1988). Sequence divergence was calculated from the alignments using only those positions where neither sequence had a gap. Other analyses were made with the programs COMPARE and DOTPLOT from the University of Wisconsin Genetics Computer Group (DEVEREUX *et al.* 1984).

Southern blot hybridization: DNA was fractionated and analyzed as described by GARBE and PARDUE (1986).

Northern hybridization: RNA from cultured cells was extracted by a guanidine-HCl protocol (HOGAN *et al.* 1994). RNA from adult flies was extracted by a protocol involving immediate protease digestion (DANILEVSKAYA *et al.* 1994). RNA was fractionated on 1% agarose gels, blotted onto nylon membrane, and probed with ^{32}P-labeled gel purified DNA fragments as previously described (HOGAN *et al.* 1994).

***In situ* hybridization:** *In situ* hybridization was performed as described (PARDUE and DAWID 1981). The probe used to localize the *Hsr-omega* repeats was a single monomer from cDRM6 cloned in pGEM3. Hybridization was overnight at 68° in 2× TNS (TNS: 0.15 M NaCl, 0.01 M Tris-HCl, pH 6.8).

RESULTS

The omega-n repeats are found only at the *Hsr-omega* locus: The omega-n repeats resemble classical satellite DNAs in the size of the repeat unit and in the lack of a significant open reading frame. However, the chromosome location of omega-n repeats is very different from that of a typical satellite DNA. Satellite DNAs are located in heterochromatic regions, usually on multiple chromosomes. The omega-n repeats are found at a single locus in the *D. melanogaster* genome. This is the *Hsr-omega* locus at polytene position 93D (in the center of the euchromatin on chromosome *3R*). Although tandem repeats are frequently associated with heterochro-

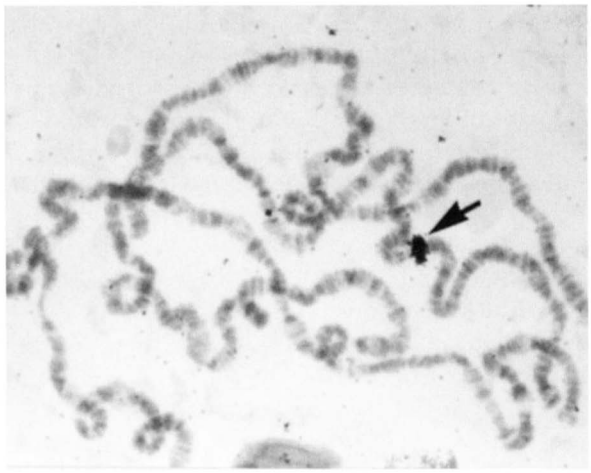

FIGURE 2.—The *Hsr-omega* repeats are found only in polytene region 93D. Autoradiograph showing hybridization of *Hsr-omega* repeat sequences to polytene chromosomes of *D. melanogaster*. Probe is detected only over region 93D (arrow). The chromosomes were probed with ^{3}H-labeled DNA from a cloned fragment containing one 280-bp monomer cloned in the plasmid pDRM30. Preparations from this experiment showed heavy label over the 93D locus after a 7-day exposure. The chromosome set shown here was allowed to expose for 96 days. The extended exposure did not reveal any new sites of hybridization by the repeat sequence.

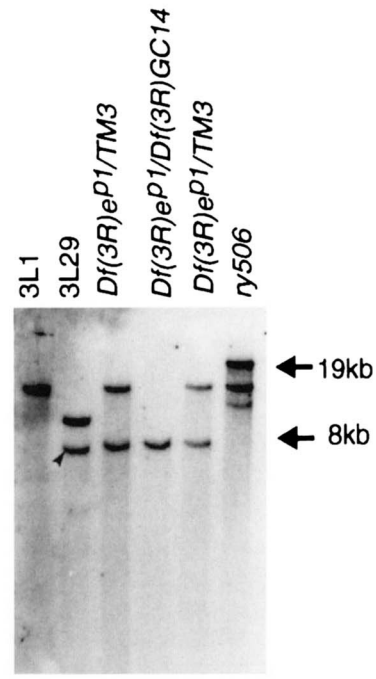

FIGURE 3.—The *Hsr-omega* repeats are clustered in the genome. Autoradiograph of a Southern blot probed with the *Hsr-omega* repeats probe. DNA was cleaved with *Hin*dIII and *Pst*I, which cut on either side of the repeat region. *Pst*I cuts 2154 bp 5′ of the first repeat. *Hin*dIII cuts 775 bp after the last complete repeat. Each lane contained an aliquot of DNA isolated from 25 flies from a single vial. The six populations analyzed are: 3L1, 3L29, $Df(3R)e^{P1}/TM3$, $Df(3R)e^{P1}/Df(3R)GC14$, $Df(3R)e^{P1}/TM3$, and ry^{506}. All six populations had been separated for >10 yrs. The ry^{506} population in this figure is distantly related to that sampled in Figure 5. The blot has been probed with the sequence of a single omega-n repeat cloned in the plasmid pDRM30. Each population shows 1–3 restriction fragments hybridizing with the probe. As discussed in the text, each fragment represents a different allele. Arrowhead indicates a new allele not seen in earlier samples of 3L29, as discussed in Figure 4. Note that the *Df(3R)GC14* chromosome has a deletion completely removing the Hsr-omega locus (MOHLER and PARDUE 1984; GARBE 1988). These fragments range in size between ~8 kb, the fragment on the $Df(3R)e^{P1}$ chromosome, to ~19 kb, the largest fragment in the ry^{506} population. Note that these fragments have ~3 kb of sequence flanking the repeat region. These measurements are only rough estimates because the gels are relatively insensitive in this size range.

matic regions, 93D shows none of the cytological characteristics attributed to intercalary heterochromatin (ZHIMULEV *et al.* 1982) and meiotic crossing over is not reduced in this region (LINDSLEY and SANDLER 1977).

The entire segment of omega-n repeats is within the *Hsr-omega* transcription unit. The repeats begin 870 bp after the site of termination of the precursor of the smaller *Hsr-omega* end product, omega-c. The last complete repeat ends 50-bp 5′ of the first potential polyadenylation signal of the omega-n transcript (HOGAN *et al.* 1994).

The chromosomal distribution of the omega-n repeats has been analyzed both by *in situ* hybridization to polytene chromosomes and by hybridization to total genomic DNA by Southern blot analysis. All of the hybridization detected on polytene chromosomes is in region 93D, the site of the *Hsr-omega* locus (Figure 2). Even after long autoradiographic exposures, which should reveal smaller regions of homology, no hybrid is detected at other chromosomal sites. In Southern blot experiments, probes for the omega-n repeat hybridize only to the restriction fragments predicted from the sequence of the *Hsr-omega* clone (Figure 3). When DNA is cleaved with enzymes that cut outside of the *Hsr-omega* repeats region, the repeats probe hybridizes only to a single fragment in some stocks. In other stocks, we detect two or more hybridizing fragments, raising the possibility that the populations sampled in these cases contain more than one allele. As discussed below, all of our evidence argues that these extra bands are additional alleles, differing in the number of repeats that they contain.

***Hsr-omega* alleles differ in numbers of repeats:** We have tested the possibility that different restriction fragments represented different alleles by analysis of stocks made homozygous for chromosome *3R*, which contains the *Hsr-omega* locus (Figure 4). The fragments found in the parental stocks segregated in the homozygous progeny as expected if each fragment were an allele, providing strong evidence that the restriction fragments identify alleles of slightly different size. Two points should be noted. First, the multiple alleles tend to fall into discrete size classes and appear as separate bands

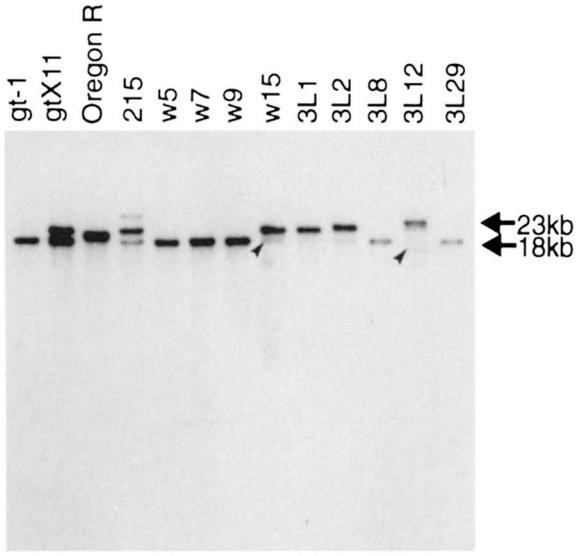

FIGURE 4.—Analysis of isolated alleles of *Hsr-omega*. Autoradiograph of a Southern blot prepared and probed as in Figure 3. DNA was cleaved with *Pst*I alone. Therefore hybridizing fragments are somewhat larger than the *Hin*dIII / *Pst*I fragments shown in Figure 3. For this blot, aliquots of DNA made from 50 flies were applied to each lane. Stocks analyzed were: gt-1, gtX11, Oregon R, 215, w5, w7, w9, w15, 3L1, 3L2, 3L8, 3L12 and 3L29. Each population in the w5-w15 series had been started by isolating a single chromosome from the gt-1 population in March, 1983. Each population in the 3L1–3L29 series had been started by isolating a single chromosome from the gtX11 population in March, 1983. DNA was prepared and analyzed in March, 1986. Note that the 3L1 and 3L29 populations were analyzed again in March, 1994 in the blot shown in Figure 3. The Oregon R and 215 stocks are unrelated to the others. Each of the derived lines has inherited one of the parental alleles. In the w15 population the parental allele (arrowhead) is now a minor allele and a new larger allele is now the major one in the population. In the 3L12 population, there is now a minor allele of smaller size (arrowhead). When analyzed in 1994, the 3L29 population had a second smaller allele (arrowhead in Figure 3).

on Southern blots rather than a smear of indistinguishable sizes. There may well be alleles that are not separated in this gel system. The remarkable thing is that there are many alleles that are clearly separated, in spite of the very limited resolution of fragments of this size on the gel. This separation suggests that changes in size can involve addition or loss of a significant number of repeats. Second, although the alleles vary in size, that variation remains within limits. The smallest repeat region that we have seen appears to be ~5 kb. The largest that we have seen appears to be about three times this size. The resolution of the gel in this size range is limited and absolute sizes are not very accurate.

The differences in size of the restriction fragments in the alleles could arise from restriction site polymorphisms, from changes in repeat number, or from insertions within the sequence. We can eliminate restriction site polymorphisms because these would not change the size of the omega-n RNA; in every case analyzed, we find that the sizes of the omega-n RNAs are those predicted from the sizes of the restriction fragments in the stock. Examples of Northern blot hybridization of stocks with different size alleles can be seen in Figure 5, B and C. On heavily loaded RNA blots, we have frequently detected minor populations of RNA differing in size from the predominant allele (see Figure 5B). These minor RNAs may come from minor fractions of the population carrying different alleles. It is also possible that the RNAs come from individuals mosaic for somatic changes or that RNAs smaller than the major RNA may represent degradation products. Because the changes in transcript size are seen in omega-n RNA but not omega-c or omega-pre-c, we conclude that the allele size changes seen by Southern blots reflect changes within the region of transcribed repeats.

In theory, the number of monomers in an array of tandem repeats can be altered by several mechanisms. Unequal exchanges between sister chromatids or between homologues would yield arrays with both larger and smaller numbers of repeats. Replication slippage can increase the length of an array. It is probable that the multiple *Hsr-omega* alleles result from some or all of these processes because the size differences between alleles appear to be produced by changes in the numbers of monomers. We cannot completely eliminate the possibity that rare increases may be due to insertion of unrelated sequences (such as transposable elements) into the repeat region, but we believe that the alleles studied have nothing but repeats in this region. We have approached the question of the homogeneity of the repeat region by restriction mapping. Enzymes that do not cut within the repeats do not cut any of the alleles. This observation suggests that the increases in length do not involve addition of unrelated sequences which should have sites for common enzymes. To increase the resolution of the analysis, we have used enzymes that cut once within each repeat. For the allele in the cosmid clone cDRM6, restriction fragments were detected by ethidium bromide staining. Because fragments were detected by DNA staining rather than by hybridization in this experiment, insertions should have been detected whether or not their sequences were known. The entire cDRM6 repeat region was digested to fragments of ~280 bp by enzymes cleaving once per repeat (data not shown). There were no fragments that would not be predicted under the hypothesis that the entire repeat region was composed of ~280 bp monomers (plus the deleted monomer discussed below). Therefore we conclude that the repeat region in the allele cloned in cDRM6 is homogeneous.

Uncloned alleles must be analyzed in the presence of total DNA and therefore we have had to use labeled probes to identify restriction fragments containing omega-n sequences in these analyses. Because unrelated sequences in the repeat region would not be detected by labeled probes, we must detect such sequences indi-

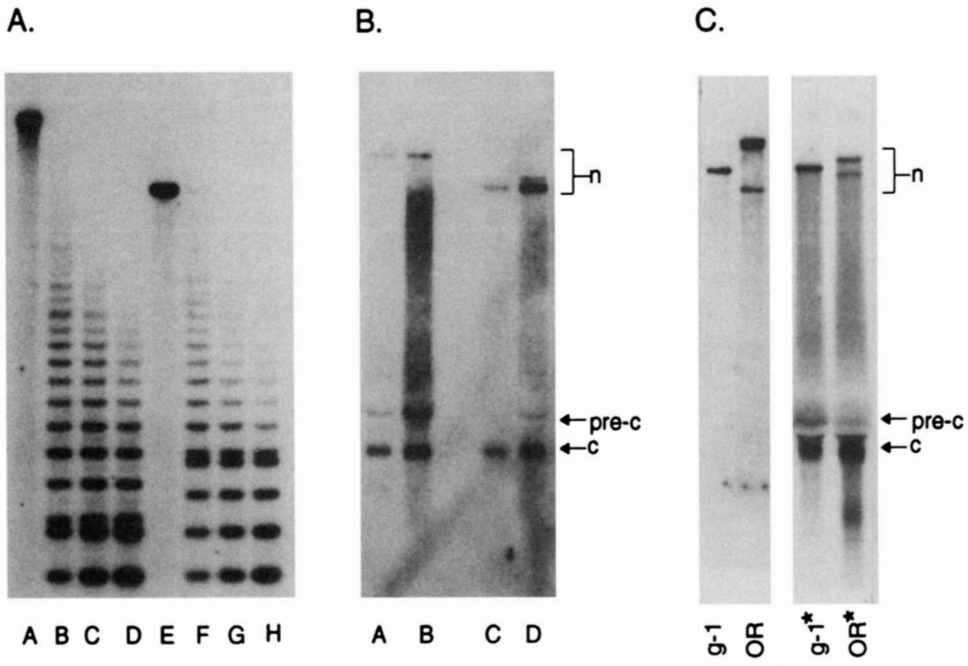

FIGURE 5.—Allelic variants differ in the number of repeats in the omega-n transcription unit. (A) Autoradiogram of partial restriction digests of DNA from the ry^{506} (lanes A–D) and $Df(3R)e^{P1}/Df(3R)GC14$ (lanes E–H) stocks. These stocks are only distantly related to those shown in Figure 3. DNA was completely digested with *Pvu*II to release the repeat region intact (lanes A and E), followed by increasing digestion with *Asu*II (BstB1), which cleaves once per repeat (lanes B–D and F–H). After electrophoresis in 0.7% agarose, fragments were transferred to a filter and probed with a 640-bp fragment from the 5′ flanking region of the repeats. This probe should show an array of fragments revealing the distances from the 5′ end of the repeat region to each of the sites of enzyme cleavage. Each locus shows a very regular ladder of bands spaced at ~280-bp intervals, indicating that the repeats are, for the most part, very regular. Each stock shows one shorter fragment which may be equivalent to the deleted repeat (nucleotides 110–200 of the consensus) found in the cDRM6 clone (see text). We estimate on the basis of densitometry of autoradiographs of filters probed with the omega-n repeat that the allele on the ry^{506} chromosome contains more than twice the number of repeats found on the $Df(3R)e^{P1}$ chromosome. The part of the repeat region that can be visualized from the 5′ end does not seem to have inserts of unrelated sequence. Similar experiments using a 3′ probe show no evidence of insertion in the region detected by that probe. (B) Omega-n transcripts reflect the size differences of the alleles from which they are transcribed. Autoradiogram of Northern blot hybridization to analyze *Hsr-omega* transcripts from the populations analyzed in A. Total RNA was isolated from third instar larvae and pupae of ry^{506} (lanes A and B, respectively) and from second and third instar larvae of $Df(3R)e^{P1}/Df(3R)GC14$ (lanes C and D, respectively). The probe was clone padm129, from the unique part of *Hsr-omega*, and therefore detects all *Hsr-omega* transcripts. The omega-pre-c and omega-c RNAs appear to be identical in size in the two stocks but the omega-n transcripts differ as expected from the Southern blot shown in A. There is a small fraction of RNA migrating above the major band in D. This may indicate that a small fraction of the population has a larger allele. (C) Comparison of DNA and RNA from a gt-1 population and an Oregon R population. Southern hybridization analysis with the repeats probe (lanes g-1 and OR) detects one allele in the gt-1 population and two in the Oregon R population. This Oregon R population is very distantly related to the one in Figure 3. Northern hybridization with the *Hsr-omega* repeats probe (lanes g-1* and OR*) detects the omega-n transcripts predicted from the allele sizes. The two Oregon R omega-n transcripts flank the single gt-1 transcript, as do the DNA fragments.

rectly. The indirect detection is accomplished by partial restriction digests (Figure 5A). Partial digests probed with sequences flanking one end of the repeat region should show an array of fragments revealing the distances from that end to each of the sites of enzyme cleavage. The pattern of this array of fragments would be affected by the presence of an unrelated insertion sequence even if the labeled probe did not bind to the insert. Partial digests of DNA from stocks carrying different *Hsr-omega* alleles showed nearly perfect arrays of fragments, each ~280 bp longer than the preceding one (Figure 5A). Such ladders are strong evidence of a regular array for at least as many repeats as can be detected on the blot. At least 21 repeats can be seen on the original of Figure 5A. The blots in Figure 5A were probed with sequence flanking the 5′ end of the repeat region and therefore survey repeats starting from the 5′ side. We have probed similar ladders of ry^{506} DNA with sequence flanking the 3′ region and can see at least 20 regular repeats (data not shown). Together the 5′ and 3′ analyses account for >10 kb of the 16 kb of repeats in this stock.

The only irregularities that we have seen in the repeat patterns are a very few shorter repeats found at different positions in different alleles (note those in Figure 5A). The sequence of one truncated repeat from cDRM6 was determined and found to be lacking sequences from position 10 to 200 of the consensus repeat sequence

(defined by BstB1 sites). The size of this truncated repeat is similar to that of the truncated repeat found in the two genomes analyzed in Figure 5. Because both of those genomes contain *Hsr-omega* loci derived from Canton S, as was cDRM6, it is possible that the truncated repeats seen in the partial digests are the same as the truncated repeat sequenced from the cosmid. The results of partial restriction analyses (Figure 5A) are consistent with the hypothesis that the differences in allele length are due to differences in numbers of repeats rather than insertion of unrelated sequences. These partial digests also show that alleles can differ in the placement of irregular repeats.

Alleles of *Hsr-omega* can be stable over many fly generations: Many of the *D. melanogaster* stocks analyzed have shown two or more allelic size variants of the *Hsr-omega* repeat region. Although variation occurs, it is clearly held within limits. No allele with less than ~5 kb of repeats has been detected. The largest repeat region found was in the ry^{506} stock, which appears to have a repeat region of ~16 kb. Most *Hsr-omega* alleles have repeat regions that seem to fall within a narrower range of 5–11 kb. It is notable that, although each DNA preparation is made from 50 flies (25 in some experiments), hybridization usually detects only two or three allelic fragments per stock. Therefore there appear to be very small numbers of alleles segregating within each population. This suggests that the generation of new alleles occurs relatively rarely and that new alleles are as stable as the older ones.

It is possible to make a very rough calculation of the frequency of production of new alleles by analysis of eight lines started from homozygous third chromosomes in March 1983 (Figure 4). The stocks were originally made for study of a different gene on chromosome *3*, but because *Hsr-omega* is also on this chromosome, they are also useful for the study of this locus. The *Hsr-omega* alleles in these stocks were first analyzed in March 1986. Four of the stocks were established from a population of the gt-1 stock that appeared to have a single allele of *Hsr-omega*; three of these homozygous lines showed alleles of the parental size. Five of the lines were from a parental population of the gt-X11 stock that appeared to have two alleles. Each of these homozygous stocks had a single major allele. In three of the new stocks, the alleles were the same size as the larger parental allele; in two stocks they were the size of the smaller parental allele. This is strong evidence that the numbers of repeats in the *Hsr-omega* loci of the parental lines and eight of the nine derived lines had not changed significantly over the generations since the chromosomes were made homozygous. The ninth new line, w15, was the only one in which the major allele showed evidence of changed repeat numbers. Three years after it was started this line had a repeat region that was slightly larger than that of the parental gt-1 stock. Longer exposures showed a second, minor, allele of the parental size. Another of the derived lines, 3L12, shows, in addition to its major allele of parental size, a second, less abundant, allele smaller than the other parental allele. Thus the w15 line appears to have undergone an increase in the number of repeats in the *Hsr-omega* transcription unit, while 3L12 appears to have acquired a new smaller allele. Two of the lines analyzed in 1986 were available for analysis in 1994. At that time 3L29 appeared to have acquired a new smaller allele (Figure 3).

All of the homozygous third chromosome lines have been kept at 18°, a temperature at which the generation time is ~6 wk (BIESSMANN and MASON 1988). Thus the stocks analyzed in 1986 had been through ~24 generations while stocks analyzed in 1994 had been through an additional 64 generations. All stocks were carried in vials and we estimate that at least 10–20 flies contribute to the gene pool in each generation of a stock. Because we made no attempt to select the parents in each generation, we assume that these were a random sample of the population in each generation. It is important to note, however, that the lines are going through a bottleneck in each generation and this can result in sharp swings in the frequency of alleles in a selectively neutral situation. If we assume that changes can occur in either sex, we can use these numbers to estimate that the March 1986 sample showed two size change events in ~8000 generations (15 flies × 2 chromosomes × 24 generations × 11 lines). The sample screened in 1994 had at least two events in ~4000 generations. The only other allele for which we have even partial records is the one on the $Df(3R)e^{P1}$ chromosome. That allele was isolated in 1983 and has been through 264 generations. The three stocks shown in Figure 3 were probably set up at about that time although we have no records. The three $Df(3R)e^{P1}$ stocks analyzed in Figure 3 appear to have identical *Hsr-omega* repeat regions, suggesting that the size has not changed since the chromosome was first isolated. This would suggest that this allele has undergone <1 change in ~8000 generations.

The sequence of the *Hsr-omega* repeat is conserved within the species, in contrast to its divergence between species: Eight monomer repeat units from the cosmid cDRM6 have been subcloned and sequenced (GARBE *et al.* 1986). The clones were made from isolated monomer repeats and we assume that they represent a random sample of the repeat array. The monomer sequences could be easily aligned to give a consensus of 281 bp. Pairwise comparisons between the eight sequences ranged from complete identity to 13% sequence divergence, with an average of 6 ± 3% divergence. Recently a subclone from the extreme 3′ end of the repeat region in cDRM6 has yielded the sequence of an additional two-and-a-half repeats (Figure 6). These additional repeats also fit the 281-bp consensus. They differ from each other by 5–12% and from the

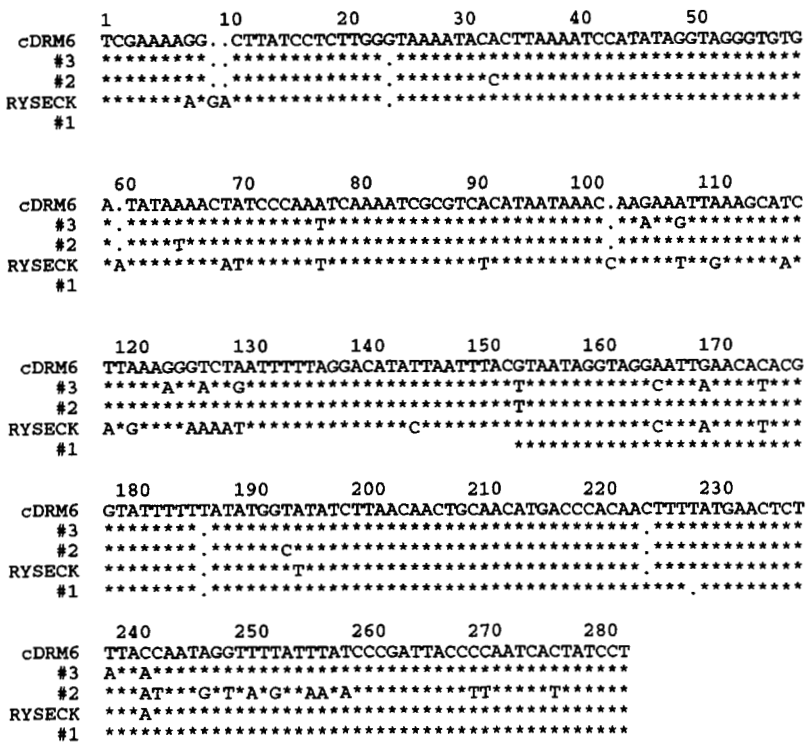

FIGURE 6.—The most 3′ monomers show no more divergence than do more interior sequences. The consensus sequences from previously published monomers are compared with the sequences of the last $2^1/_2$ complete monomers at the 3′ end of the repeat region from the cosmid cDRM6. cDRM6: consensus sequence derived from the seven randomly cloned monomers from cDRM6 published by GARBE *et al.* (1986) plus one additional monomer in this set. #1, #2, and #3: the last three complete monomers in cDRM6. They are ordered with #3 being the most 3′; #1 was truncated by the cloning of this terminal fragment. RYSECK: consensus derived from the nine random monomers published by RYSECK *et al.* (1987). Asterisks indicate positions identical to the top sequence. Dots indicate gaps introduced in the alignment.

consensus derived from the first eight elements by 2–7%. For these repeats the average sequence divergence is 6 ± 3%. In addition to the base changes, most repeats also differ by deletions/insertions of 1–3 nucleotides. Both base changes and deletion/insertion events seem to be randomly distributed over the monomer.

A set of nine *Hsr-omega* repeats has been sequenced by RYSECK *et al.* (1987), using DNA from populations in their laboratory in Heidelberg. This set of sequences can be aligned to give a consensus of 284 bp. The 284-bp consensus is not fundamentally different from the 281-bp consensus derived from cDRM6 repeats. Because of the way the consensus is derived, any new nucleotide insertion in a repeat will expand the consensus by 1 bp. Pairwise comparisons of the nine repeats published by RYSECK and collaborators show 1–22% nucleotide changes with an average of 10 ± 5% divergence. As with the monomers from cDRM6, the differences in the nine monomers are randomly distributed single nucleotide changes and small deletions/insertions. The consensus derived from this second set of sequences differs by 11% from the consensus of the cDRM6 monomers.

Both ends of the repeat region are flanked by short imperfect repeat sequences: The restriction enzyme BstB1 digests the repeat region of cDRM6 into monomers of ~280 bp. The sequences of the regions immediately flanking these monomers on either end of the repeat region show less than one monomer length of irregular sequence with similarity to the repeats (Figure 7). Beyond that region of imperfect similarity there is an abrupt change to an unrelated sequence. The region of imperfect similarity flanking the 5′ end of the repeat region contains three short segments of good sequence similarity to the repeat but the order of the segments is scrambled. There is a segment from the 3′ end of the repeat followed by a 5′ segment and a second 3′ segment. The middle segment of the monomer is not present in this flanking region. At the 3′ end of the repeat region, the flanking sequences show approximately half a monomer of sequence similarity to the repeats but the alignment within this segment would require more gaps than seen with other alignments.

There is some ambiguity in determining the sequences that flank the repeats because we do not know where the monomer actually begins. For convenience, the BstB1 site in each monomer has been taken as the start. By chance, the restriction site is almost exactly at the site at which alignment to the consensus sequence begins to break down rapidly at both ends of the repeat region. In theory the sequence of the extreme ends of the repeat region might identify the start of the repeats; however this does not seem to be the case. Both ends are imperfect. The fragment of homologous sequence immediately 5′ of the first BstB1 site is 91% identical to nucleotides 202–272 in the consensus repeat sequence. The fragment immediately 3′ of the last complete repeat is 82% identical to nucleotides 26–151 of the consensus. Thus no single start point will eliminate the ragged character of either end.

DISCUSSION

These studies show multiple alleles of *Hsr-omega*, differing in the length of the region of tandem repeats in

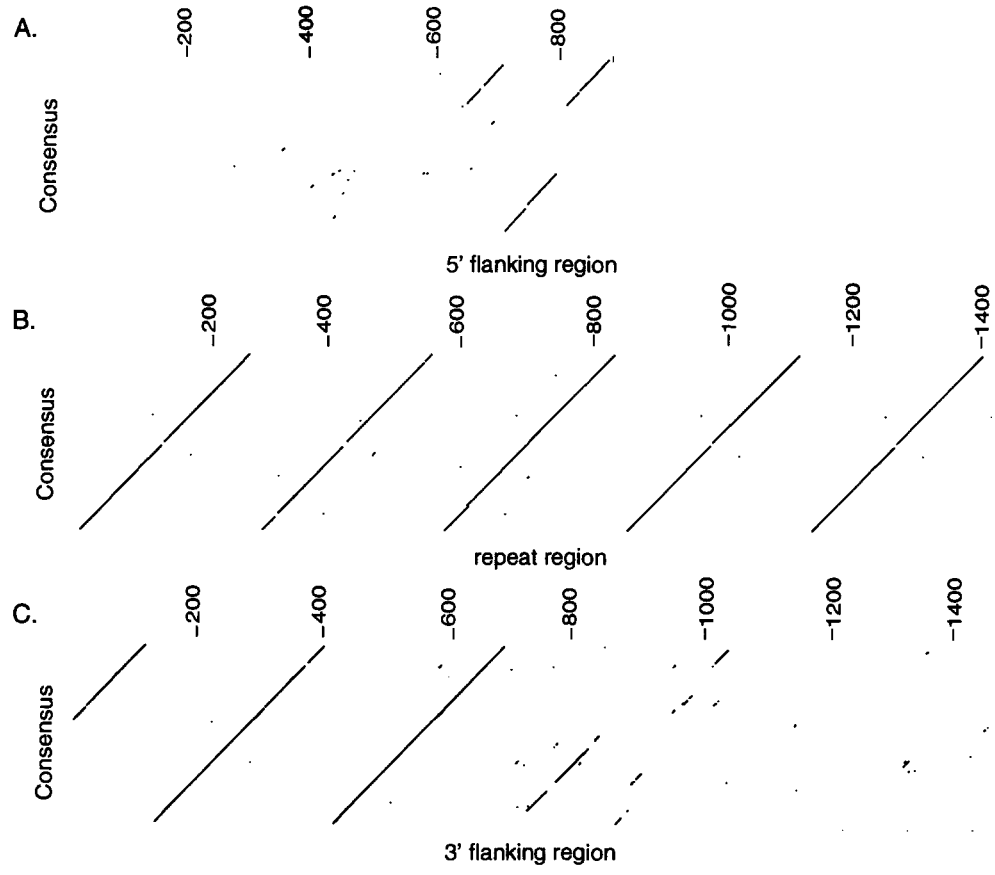

FIGURE 7.—The sequences flanking the repeat region show a short region of disrupted homology at each end. Dotplot comparison of the sequence of the consensus monomer (CONSENSUS) to A, the 880-bp immediately 5′ of the repeat region (5′ FLANKING REGION), to B, a model repeat region made by arbitrarily linking the sequences of five independently cloned monomers (REPEAT REGION), and to C, the 1460-bp containing $2^1/_2$ complete repeats plus the region immediately 3′ of them (3′ FLANKING REGION). The comparison was made using the GCG Compare program with a window of 21 bp and a stringency of 14. The 5′ repeat region shows three partial fragments of a monomer immediately before the BstB1 site that signals the beginning of the repeat region. At the 5′ edge of these fragments there is an abrupt loss of sequence similarity. The artificially constructed repeat region shows a very regular pattern. This construction was made by linking the sequences of five independently cloned monomers. We assume that these are a random sample of the sequences in the interior of the repeat region. The last complete repeats appear to be as regular as the interior ones shown above. As at the 5′ end, there is an abrupt loss of sequence similarity very soon after the last complete repeat.

the nuclear transcript, omega-n. The *Hsr-omega* alleles show changes in size, both increases and decreases, of a magnitude that allows the allelic differences to be easily detected, even by a relatively insensitive analysis. There may well be smaller changes that cannot be considered here. New alleles appear to be as stable as older ones. Within the size range of ~5–16 kb, we have seen no evidence of selection for the size of the repeat region. However, in extensive sampling of *D. melanogaster* stocks, we have not detected repeat regions that are either larger or smaller than this. Taken together, the evidence for change in the length of the repeat region and the failure to find alleles outside the 5- to 16-kb range argue that there is selection for both minimum and maximum numbers of repeats, although there is some latitude between these limits.

Sequence comparisons of $19^1/_2$ *Hsr-omega* repeat units show a strong conservation of the monomer sequence within *D. melanogaster*. This conservation contrasts sharply with the divergence seen between species (GARBE *et al.* 1986; RYSECK *et al.* 1987; K. L. TRAVERSE, F. SLOT, and M.-L. PARDUE, unpublished results). Pairwise comparisons between $10^1/_2$ monomers from the single allele in cDRM6 and similar comparisons between the 9 monomers cloned by RYSECK *et al.* (1987) show average divergences of <15%. The differences between omega-n repeats are mostly single base substitutions and 1–3 nucleotide insertion/deletion events. Base substitutions appear to be randomly distributed throughout the repeat and a significant fraction of those changes are found in more than one repeat. The types and pattern of changes in the omega-n repeats are similar to those found for other classical satellite sequences, such as human alpha satellite (DURFY and WILLARD 1989; ALEXANDROV *et al.* 1991) or the Drosophila 360 and 500 families (STRACHEN *et al.* 1985).

Although the omega-n repeats are highly conserved, there are satellite sequences that show still higher levels of conservation. In studies of the 360 and the 500 satellite families in five sibling species of the melanogaster species subgroup, STRACHEN *et al.* (1985) found intraspecific base changes ranging from 0.33 to 3.85% for the 360 family and from 0.7 to 3.85% for the 500 family. Human alpha satellite repeats can be divided into several families on the basis of sequence (ALEXANDROV *et al.* 1991). Comparisons of alpha repeats vary with the source of the monomers. Because they come from a single chromosomal locus, the omega-n repeats should be most analogous to alpha monomers from a single chromosomal array. DURFY and WILLARD (1989) have made comparisons for alphoid clusters cloned from at least six different *X* chromosomes and find that within clusters the monomers differ by 0.91%. This intracluster difference is significantly less than the differences between monomers from different *X* chromosomes or from different clusters. These authors suggest that homogenization forces may act within regions of about 15 kb. The *X* chromosome clusters were approximately the size of the omega-n repeat region but the monomers within the alphoid clusters were more homogeneous than the omega-n monomers.

This conservation of monomer sequence within the *D. melanogaster* locus contrasts sharply with the extreme divergence seen in the comparison between *D. melanogaster* repeats and those of *D. hydei* (GARBE *et al.* 1986; RYSECK *et al.* 1987). The *Hsr-omega* repeats of *D. hydei* differ in both size and sequence from those of *D. melanogaster*. The only obvious similarity between the two repeat regions is the repetition of a 9-nucleotide sequence, ATAGGTAGG, at slightly more than 100-bp intervals over >5 kb of the omega-n transcript in each species. The *D. hydei* omega-n monomer is ~115 bp and ATAGGTAGG is found once in each repeat. The *D. melanogaster* monomer is slightly more than twice the length of the *D. hydei* monomer but contains two copies of ATAGGTAGG with a spacing that distributes the conserved ATAGGTAGG along the *D. melanogaster* repeat region at slightly greater than 100-bp intervals. Thus, the two repeat regions have equivalent distributions of ATAGGTAGG in spite of the differences in size and sequence of their monomers. This interspecies comparison suggests that the ATAGGTAGG sequence has been held constant while the sequences surrounding it have changed. Although ATAGGTAGG is strongly conserved between Drosophila species, its conservation is not obvious within the *D. melanogaster* repeats. Changes in one or the other ATAGGTAGG regions are evident in eight of the $19^1/_2$ *D. melanogaster* monomers that have been sequenced to date.

If recombination is a major mechanism for maintaining the homogeneity of an array of tandem repeats, that array would be expected to contain the most different repeats at its extreme ends because these end repeats would be less frequently involved in recombination and therefore less homogenized with the rest of the array (SMITH 1976). There are several reports of repeated DNAs in which this expectation has been met. MARESCA and SINGER (1983) found that African Green Monkey alpha satellite repeats adjacent to junctions with deca satellite were more like the baboon and bonnet monkey repeats (and therefore presumably more like the ancestral repeat) than were repeats from interior positions. Tandem repeats within the coding regions of several proteins, Plasmodium S-antigen (COWMAN et al. 1985), involucrin (ECKERT and GREEN 1986) and Chironomus Balbiani ring protein (HOOG *et al.* 1988) show more difference from the consensus at the ends of the repetitive segment than in the interior regions. In contrast, the *D. melanogaster* simple sequence satellites show abrupt changes at junctions (LOHE and BRUTLAG 1987).

Omega-n repeats show no evidence that terminal repeats are more diverged than interior repeats. The sequences of the last $2^1/_2$ repeats in cDRM6 show an average amount of divergence in comparisons with other repeats and most changes seen in the terminal monomers are also seen in other repeats. The fragments of sequence flanking the last complete repeat at the ends of the repeat region are diverged; however, the structure of these fragments is not consistent with the hypothesis that their sequences have not evolved with the others because ends are less frequently included in recombinations or gene conversions with the rest of the array. The flanking fragments are incomplete and scrambled in a way that suggests that they may be products of a less controlled DNA patching and joining resulting from processes other than unequal crossover or gene conversion between tandem repeats. Perhaps the fragmentation of the flanking sequences occurred during an amplification and/or insertion of the repeats.

Our studies provide evidence that the alleles of *Hsr-omega* differ in the number of repeats. These differences could be caused by unequal recombination, either between sister chromatids or between homologous chromosomes or by replication slippage. Nothing in our results helps to decide between these three possibilities. There is strong evidence that changes in the number of repeats in genes for ribosomal RNA are accomplished by unequal sister chromatid exchange (TARTOF 1974; PETES 1980; ENDOW and KOMMA 1986). Recombination between homologous chromosomes does not appear to be the cause of most changes in the size of minisatellite loci (WOLFF *et al.* 1989). Mutations in mismatch repair increase the frequency of changes in the number of microsatellite repeats (STRAND *et al.* 1993), suggesting that these changes are due to replication slippage which is not followed by mismatch repair. Although the ribosomal RNA genes, the minisatellites, and the microsatellites are all tandemly repeated se-

quences, they differ in several ways. Monomer size decreases from thousands of bases (for the ribosomal genes), to tens of bases (for minisatellites), to dinucleotides (for microsatellites). There is reason to expect that the forces acting on monomers of different sizes may be different (STEPHAN 1989). It is not clear which, if any, of these repeats might be models for omega-n repeats. Our calculations of the frequency of changes in repeat numbers, 1/2000–4000 per fly generation, are similar to calculations for changes in the number of 250-bp repeats in the nontranscribed spacer of Drosophila ribosomal RNA genes, 1/3000 per generation (COEN *et al.* 1982). These rates are slightly lower than the rate measured for changes at a minisatellite locus, 14/1000 (JEFFREYS *et al.* 1990). However, any comparisons must take into account the increased efficiency with which changes can be detected in the minisatellite studies.

The *Hsr-omega* repeats differ from the typical tandem repeats in being located in the middle of the chromosome arm in a region that shows neither the cytological nor the recombinational characteristics of heterochromatin. Therefore it is interesting to compare this study with a recent study of the repeats at the *D. melanogaster Responder* locus, located in the heterochromatin of the right arm of chromosome *2* (CABOT *et al.* 1993), a region where exchanges between homologous chromosomes are expected to be rare. The ranges of pairwise estimates of genetic divergence for repeats are very similar to those that we measure for *Hsr-omega*. If the two arrays of tandem repeats are of similar ages, this similarity could mean that the mechanisms homogenizing these tandem repeats are similar, even though they are in chromosome regions that have very different tendencies toward recombination.

Concluding remarks: Tandem repeats with no obvious coding potential are frequently looked upon as junk. The *Hsr-omega* locus is very active in heat shock and also constitutively transcribed in all but a few cell types. The transcript carrying the repeats is closely regulated both at transcription and turnover and therefore likely to have a role in the cell (HOGAN *et al.* 1994). These studies of the maintenance of the *Hsr-omega* repeat region add additional evidence to this argument. We have found that the size of the repeat region can increase and also decrease, apparently by changes in the number of repeats. These changes are stable over one thousand or more chromosome generations. The cell appears to pay a price for maintaining these repeats because, in studies of *D. melanogaster* stocks collected in diverse parts of the world, we have never seen the length of the repeat region increase to more than 16 kb. On the other hand, no stock has <5 kb of the repeats, arguing that the cell needs a minimum amount. The conservation of the monomer sequence, even though it has no coding potential, is at least consistent with the possibility that the sequence has a function. We have proposed that this function is the binding of a protein and that the activity of that protein is controlled by binding omega-n. We hypothesize that the activity of the binding protein is indirectly controlled by the mechanisms that regulate transcription and turnover of the omega-n transcript. Because *Hsr-omega* transcription and turnover are very sensitive to changes in cell conditions (BENDENA *et al.* 1989; HOGAN *et al.* 1994), the activity of the protein would be responsive to those same changes. If this proposal can be verified it will raise the prospect that other noncoding repeats may have unsuspected functions. Two candidates for such functions are the tandem repeats in the nontranscribed spacer of Drosophila ribosomal RNA genes and the repeats in the *Responder* locus. For both the ribosomal genes (CLUSTER *et al.* 1987) and the *Responder* locus (WU *et al.* 1989), there is a correlation between the number of repeats in the genome and fitness, suggesting that these repeats, or something tightly linked to them, benefit the organism.

We are grateful to members of the Pardue laboratory and to KY LOWENHAUPT for comments on the manuscript. We thank JIM FIGUEIREDO for assistance with the manuscript.This work has been supported by a grant from the National Institutes of Health (to M.L.P.). Sequence analysis was done through the Whitaker College Computing Facility of the Massachusetts Institute of Technology.

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Communicating editor: V. G. FINNERTY