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Genetics

A spontaneously opened ring chromosome of *Drosophila melanogaster* has acquired He-T DNA sequences at both new telomeres

(heterochromatin/polytene chromosomes/repeated DNA)

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ABSTRACT **Ring chromosomes that have been opened to give linear chromosomes offer an opportunity to study the DNA sequences associated with new chromosome ends. The *Drosophila melanogaster* chromosome *C(1)A* was originally a ring chromosome, consisting of two linked X chromosomes, and thus had no telomeres. This chromosome has spontaneously opened in polytene region 13, a region near the middle of the euchromatic arm of the X chromosome. The opening of the ring has produced two new telomeres on the *C(1)A* chromosome. Each of the new telomeres has acquired He-T DNA sequences. He-T DNA is a complex family of repeated sequences found in the telomeric and pericentric heterochromatin of *D. melanogaster* chromosomes. He-T DNA sequences are detected, at various levels, in the most distal band on the end of each polytene chromosome in all *D. melanogaster* stocks. To our knowledge, these sequences have never been detected within the euchromatic chromosomal regions in any stock. The strong correlation between He-T DNA sequences and telomeric regions suggests that He-T sequences may have a role in organizing or maintaining the ends of chromosomes. The association of He-T DNA with newly acquired telomeres in a formerly euchromatic region, polytene region 13, strengthens this correlation.**

The genomes of eukaryotes contain several classes of repeated sequences. For example, some families of repeated DNA have well-defined chromosome positions, and these positions are at equivalent sites on multiple chromosomes. The best-known members of this class are the highly repeated "satellite" DNAs that are found in the centromeric heterochromatin of chromosomes from most organisms (1). Other families have also been found to be limited to particular chromosomal regions. These include families of repeats in yeast (2), rye (3), *Allium cepa* (4), and *Tetrahymena* mitochondrial DNA (5) that are found only adjacent to telomeres. In *Drosophila melanogaster* a family of repeated sequences, called He-T DNA, is limited to the regions around the telomeres and the centromeres (6). In *Xenopus laevis*, the large family of genes encoding the 5S ribosomal RNA genes is found at the telomeres of the long arms of all but two of the chromosomes (7, 8). [This discussion omits the very simple $C_{1\text{-}8}(T/A)_{1\text{-}4}$ repeats that have been found at the ends of chromosomes from several lower eukaryotes (9) and, more recently, from *Arabidopsis thaliana* (10) and humans (11). Repeats of the $C_{1\text{-}8}(T/A)_{1\text{-}4}$ type are thought to be added to the ends of DNA by a template-independent terminal transferase activity (12) and thus may have a very different origin from the sequences being considered here.]

A second class of repeated sequences in eukaryotes is also found at multiple sites, but the exact positions of these sites vary even among individuals of the same species. The sequences in this second class are now considered to be mobile elements (13). Some families of these mobile elements have terminal repeat structures resembling those of retroviruses; others do not. The common feature of mobile sequences is that each site occupied by one of these sequences (a "full" site) can also be found "empty" in other individuals.

The first class of repeated sequences, those with well-defined chromosome sites, must also have some degree of mobility to account for the dispersion and the continued co-evolution of the sequences. (This "mobility" could be by actual transposition or by gene conversion.) It seems likely that the chromosomal distribution of this class is limited by some mechanism. Perhaps the sequences have a function that is disruptive within the chromosome arms, leading to rapid loss of chromosomes (and cells) if the sequence is transferred to a position in the euchromatic arm. Alternatively, the movement of the sequences might be limited by the transposition mechanism itself. Perhaps transposition can occur only between two chromosome regions that are physically associated at the appropriate time in the cell cycle. It seems probable that each of these mechanisms limits some families of repeated sequences and not others.

Although it is now possible to construct minichromosomes in yeast to test questions of chromosome structure (14), this can not yet be done for the much larger, and probably more complex, chromosomes of higher eukaryotes. Tests of hypotheses about chromosome structure in higher eukaryotes can, however, be made by using some fortunate chromosomal rearrangements. One such chromosome is the *C(1)A* chromosome of *D. melanogaster*. The *C(1)A* chromosome was synthesized by Armentrout in 1964 as a ring with no telomeres (15). Since that time, *C(1)A* has opened into a linear chromosome and thus provides an opportunity to analyze two recently acquired telomeres.

MATERIALS AND METHODS

***Drosophila* Stocks.** *C(1)A* stock S10 and *C(1)RM* stock S12 were the kind gift of the late L. Sandler (University of Washington). Other stocks are described by Young *et al.* (6).

Recombinant Phage and Plasmids, Probe Preparation, and *in Situ* Hybridization. All methods are as described by Young *et al.* (6).

Mitotic Chromosome Preparation. Mitotic chromosomes from brains of third-instar larvae were prepared and stained with Hoechst 33258 as described by Gatti and Pimpinelli (16).

RESULTS

The *C(1)A* Chromosome Has Opened in Polytene Region 13E. The *C(1)A* chromosome was a spontaneous derivative of *C(1)TR94*, a ring composed of two X chromosomes joined at

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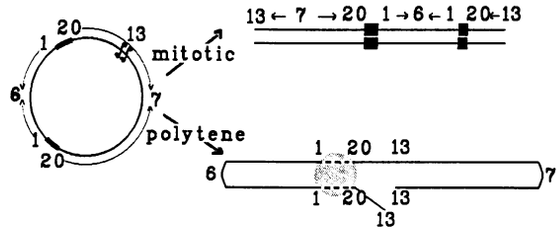

FIG. 1. Diagram showing the *C(1)A* ring, as well as the configuration of the sequence of the opened ring in mitotic and polytene chromosomes. None of the chromosomes is drawn to scale. To compare sizes of mitotic and polytene chromosomes, see Figs. 2 and 3. Thin lines indicate euchromatic regions. Heavier lines indicate heterochromatin. Relative sizes within each diagram are only approximate. The numbers indicate polytene regions or the equivalent positions on the mitotic chromosome. The mitotic chromosome contains two sister chromatids after DNA replication. The polytene chromosome is shown with a single chromatid. In the polytene chromosome, homologous regions of the chromatid are intimately paired; in the diagram these regions are slightly separated for clarity. The circle near the center of the polytene chromosome represents the fused heterochromatin of all of the chromosomes that makes up the chromocenter. The broken lines in the circle indicate that the *C(1)A* chromatid continues through the chromocenter. In a fraction of the polytene nuclei the homologues are not paired in region 20–13E (as in Fig. 3). Such asynapsis is indicated in the diagram to emphasize the new telomeres.

both ends. The derivative was apparently produced by a reverse crossing-over in polytene region 6F2–7A1. The new order of polytene bands was /1A–6F2/6F2–1A/20–7A1/7A1–20/. At both of the junctions between 1A and 20, heterochromatic DNA sequences joined the two chromosomes (15). The new chromosome order yields an unusually stable compound-ring X chromosome because exchange within the heterochromatin cannot produce a single X with a full complement of genetic material (Fig. 1). The rearrangement that produced *C(1)A* ensured that either single X produced by detachment at the heterochromatin would be duplicated for some genes and deficient for others and thus, if detachment occurred, the resulting single X chromosome would not be able to produce a viable individual.

At some time since its synthesis in 1964, *C(1)A* spontaneously opened to give a rod chromosome with two internal heterochromatic regions. This chromosome structure is diagrammed in Fig. 1. The linearized chromosome can be clearly

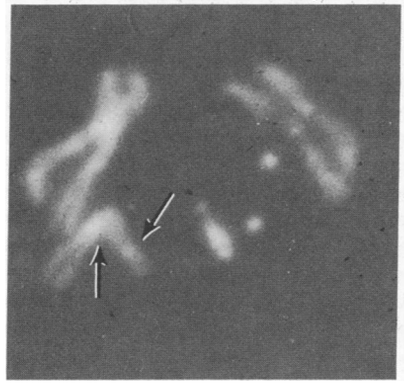

FIG. 2. Mitotic chromosomes from a female carrying the compound-X chromosome *C(1)A*. These larval brain chromosomes have been stained with Hoechst 33258. The compound-X chromosome is the large unpaired chromosome. Arrows indicate the two regions of brightly stained heterochromatin on the X; the region nearest the center of the X is much brighter than the other region. The compound-X chromosome is clearly linear. (The smaller unpaired chromosome is a Y fragment that this female is carrying.) (×2700.)

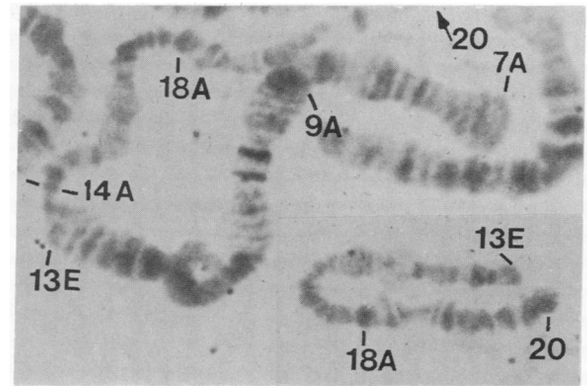

FIG. 3. Polytene *C(1)A* chromosome asynapsed in region 20–13E. The chromosome runs from the chromocenter (region 20) out to 7A, then folds back on itself and continues to the break in 13E. The second segment 20–13E has broken away from the chromocenter and was lying nearby. It is shown in the *Inset*. (Giemsa stain; ×950.)

seen in preparations of mitotic chromosomes from larval brain cells (Fig. 2). The *C(1)A* chromosome is approximately twice the size of the normal X and, in addition to the pericentric heterochromatin, it has a region of heterochromatin near the middle of one of the arms, an unusual feature for a *Drosophila* chromosome. In polytene nuclei, the pairing of homologous regions caused the original *C(1)A* ring to collapse into a rod with the two regions 1A–6F2 paired to form a single chromosome arm, separated by the chromocenter from the two regions 7A–20, which also paired to yield a single chromosome arm (Fig. 1). The heterochromatin of the two 1A/20 junctions is fused into the chromocenter. The opened ring *C(1)A* gives essentially the same polytene configuration as the closed ring; however, regions 20–13E do not pair in a fraction of the nuclei (Figs. 3, 6 *a* and *b*).

The fraction of nuclei showing asynapsis of regions 20–13E varies somewhat from experiment to experiment and may reflect larval growth conditions. Nuclei in which regions 20–13E do not synapse show that the linearization of *C(1)A*, which is seen in the mitotic chromosome, has occurred by a break in one of the two regions 13E. In nuclei where synapsis has been complete, it is possible to detect the break in 13E only on very-well-spread chromosomes because the association with the unbroken segment brings the two ends of the chromosome together very closely. This opening of *C(1)A* has formed two new chromosome ends in a euchromatic region that formerly had none.

Both of the Newly Acquired Telomeres Have Also Acquired He-T DNA Sequences. He-T DNA is a complex family of moderately repeated sequences. This family was originally identified from a 12-kilobase (kb) fragment of *D. melanogaster* DNA (Fig. 4) that had been cloned in a λ phage named λT-A (6). The fragment showed an unusual pattern of

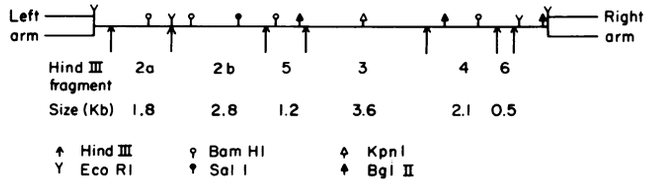

FIG. 4. Restriction map of *Drosophila* DNA cloned in λT-A. Cleavage sites for several restriction enzymes are given. The number designating each *Hin*dIII subfragment is given directly below the fragment and the size (in kb) of the fragment is also indicated. *Hin*dIII fragments 2, 5, and 3, plus all sequences that cross-hybridize with them, make up the He-T sequence family. Right and left arms of the λ phage are shown as boxes.

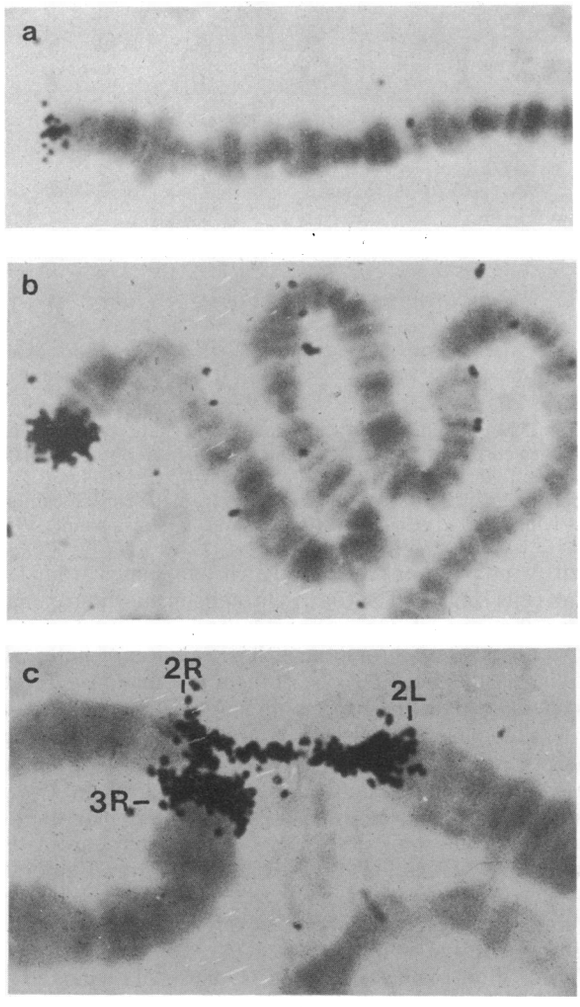

FIG. 5. Autoradiographs showing *in situ* hybridization of He-T DNA sequences to telomere regions of polytene chromosomes. (*a*) X chromosome tip from the gt-1 stock. This 7-day autoradiographic exposure shows label only over the most distal band. (*b*) X chromosome after a 228-day autoradiographic exposure. This slide is from the same experiment shown in *a*. There is a great increase in the label over the chromosome tip but no hybrid is detected anywhere in the euchromatic regions. (*c*) Tips of chromosomes *2L*, *2R*, and *3R* from a gt-1/gtXII hybrid larva (20), showing ectopic pairing between telomeres. The ectopically paired strands hybridize with He-T probes. (Exposure, 8 days.) All slides were hybridized with ^{3}H-labeled *Hin*dIII fragment 2 of λT-A, but similar results are obtained with fragments 3 and 5. The specific activity and concentration of the probe for the experiment shown in *c* differed from that used in *a* and *b*. Slides were stained with Giemsa stain.

hybridization to polytene chromosomes, hybridizing strongly to the most distal band on each telomere (Fig. 5) and to the pericentric heterochromatin and weakly to several euchromatic sites that differed from stock to stock. Analysis of subfragments of λT-A showed that the hybridization to the euchromatic sites was entirely due to sequences in fragment 4 of a *Hin*dIII digest (see Fig. 4). Fragment 4 appears to contain a conventional mobile element and has not been studied further. Instead we have concentrated on fragments 2, 3, and 5, which are contiguous and make up 9.4 kb of the original clone. None of these fragments shows hybridization to any euchromatic site. Restriction enzyme mapping and cross-hybridization of these fragments does not give evidence of the sort of simple repeats that have previously been associated with heterochromatin (1). Southern blot hybridization shows that each of these fragments can hybridize with many restriction fragments in the *D. melanogaster* genome; the sizes of some of the cross-hybridizing fragments differ from stock to stock, indicating some sorts of genetic change. In spite of the evidence that these sequences are changing, the chromosomal sites at which fragments 2, 3, and 5 are detected do not vary from stock to stock. Each of these fragments hybridizes only to the pericentric heterochromatin and to the last band of each telomere. The relative levels of hybridization to particular telomeres vary somewhat from stock to stock; however, we have preliminary evidence suggesting that the variation may reflect stock-specific differences of polytenization.

Because fragments 2, 3, and 5 have a distinctive chromosomal distribution that is not shared by any unrelated sequences that we are aware of, we consider the fragments and all sequences that cross-hybridize with them to be members of the He-T DNA family. Both of the previously reported sequences that hybridize to the telomeric bands of *D. melanogaster* have homology to He-T DNA (17, 18), suggesting that this is the major family in these regions.

The new telomeres on *C(1)A* have acquired He-T DNA sequences in the terminal bands. *In situ* hybridization to polytene chromosomes with any of the three He-T fragments, 2, 3, or 5, demonstrates the presence of the He-T sequences on both of the ends (Fig. 6). This is easy to see when the 20–13 region is asynapsed (Fig. 6 *a* and *b*). When the entire chromosome is synapsed, it is usually not possible to resolve two bands of hybridization; however, on some very stretched chromosomes the two telomeres may be pulled slightly apart (Fig. 6*c*).

Experiments with probes for known genes indicate that we could detect less than 50 base pairs of homology to He-T DNA in the euchromatic regions of these chromosomes. No internal sequences with homology to He-T sequences have been detected in any of the *D. melanogaster* stocks analyzed, including stocks carrying the chromosomes ancestral to those that were involved in the formation of the *C(1)A* ring. The He-T sequences that appear in region 13E of *C(1)A* are clearly correlated with the appearance of the new telomeres.

DISCUSSION

This study shows that the ring chromosome *C(1)A* has been opened to produce a linear chromosome with two telomeres. The new chromosome appears to have had an advantage over the original tandem ring because the linear chromosome has completely displaced the ring in the stock. Nevertheless, animals carrying the linear chromosome show some decrease in viability when compared to stocks carrying other X chromosomes (unpublished data). We find that the *C(1)A* stock produces significantly more males than females. Females carry the *C(1)A* chromosome; males do not. *C(1)A* has two complete copies of X chromosome material, and thus animals carrying this chromosome develop as females. In these stocks all males carry a single X chromosome, inherited from their father. (Zygotes that receive both the mother's compound-X and the father's single X yield triplo-X females that do not survive to produce progeny.) When we cross the *C(1)A* chromosome into other genetic backgrounds, the decreased viability follows the compound-X chromosome. The linear compound-X, *C(1)RM* (15), does not produce an equivalent loss in viability, suggesting that *C(1)A* does carry a defect. It is possible that the defect is due to semilethal mutations that have been induced on the *C(1)A* chromosome, perhaps by the event that opened the ring. However, it is also possible that the atypical configuration of heterochromatin, with a second region in the middle of one of the arms, in some way reduces the probability of perfect cell division and thus decreases the viability of females carrying *C(1)A*.

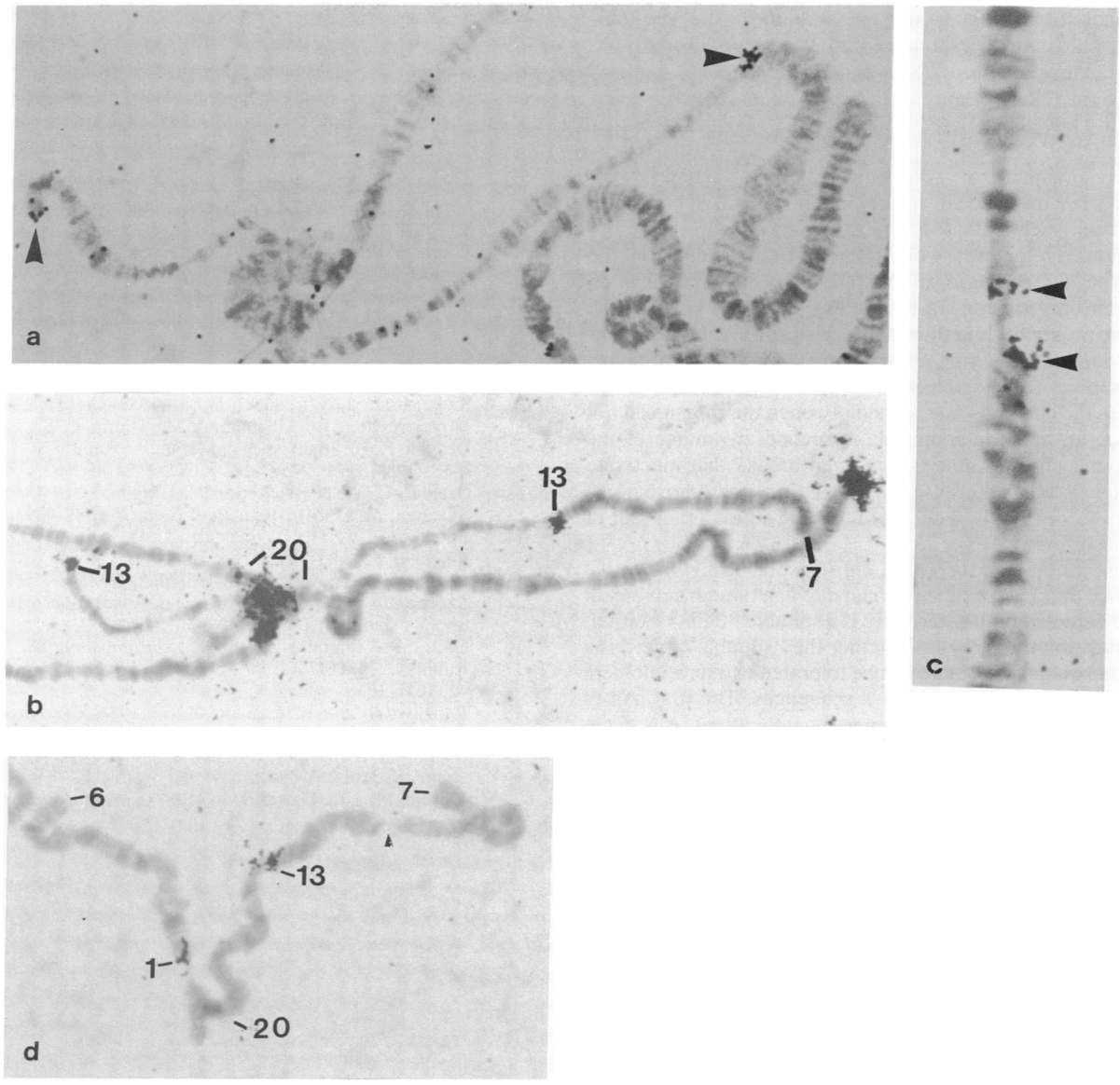

FIG. 6. Autoradiographs of polytene *C(1)A* chromosomes hybridized with He-T [^{3}H]DNA. (*a*) Autoradiograph of a portion of a polytene nucleus. Part of the *C(1)A* chromosome (regions 20–7–20) is shown. Region 20–13 is not synapsed, and the two half-width portions are well separated. Arrows indicate the two regions of He-T hybridization at each end of the opened ring chromosome. The probe was λT-A [^{3}H]DNA. (Exposure, 14 days; ×900.) (*b*) A second nucleus like that seen in *a*. In *b* the asynapsed segment has fallen on the opposite side of the chromocenter (flanked by regions 20) from its partner. Numbers indicate polytene regions. The fold-back synapsis of regions 7–13 produces a linear chromosome equal in width to the paired homologues of the nearby autosome. Hybridization of the He-T probe is seen on both new telomeres in region 13. The absence of hybridization on the fold-back in region 7 contrasts with the mass of label seen over the adjacent autosomal telomere. All other telomeres in this nucleus showed similar label. The probe was ^{3}H-labeled fragment 3. (Exposure, 14 days; ×750.) (*c*) A stretched *C(1)A* chromosome with all regions synapsed. The chromosome is extended enough to separate the two new telomeres and show two distinct regions of He-T hybridization (arrowheads). (Probe and exposure as in *a*; ×1500.) (*d*) Complete *C(1)A* chromosome that has been pulled away from the chromocenter. The homologous regions of the two parts have synapsed entirely so that the chromosome appears as a single linear structure. Numbers indicate polytene segments. The arrowhead marks the constriction seen in region 11A in many *D. melanogaster* stocks. The probe was ^{3}H-labeled fragment 3. Although He-T DNA is found on all of the telomeres on this preparation, no hybridized DNA is detected at either end of this X chromosome (regions 6 and 7) because the ends are not telomeres; instead they are fold-back regions of the chromosome (see polytene diagram in Fig. 1). The telomeres of this chromosome are in region 13 but, since the 7–20 segment that has been opened in region 13 is synapsed with the 7–20 segment that is unbroken, the telomeres are detected only by the hybridization of He-T DNA. The labeled DNA in region 1 is chromocentral DNA that was attached by the original construction of the double circular chromosome. Underreplication of this chromocentral DNA during polytenization causes the chromosome to narrow in this region. (Exposure, 2 days; ×800.)

It is not obvious how the break in region 13 occurred. It does not seem reasonable to invoke inversion or meiotic crossing-over as a means of moving telomeres to region 13. As explained above, *C(1)A* is passed through the female line. The ring contained both of the female's X chromosomes. These two homologues should pair with each other at meiosis, and neither homologue has a telomere. In some stocks, females carrying a compound-X also carry a free Y. The opening of the *C(1)A* ring might have occurred in such a female. However, there is no evidence that the Y chromosome has homology with the X chromosome sequences in region 13, so there is no particular reason to suspect that the telomeres were removed from the free Y. In addition, any hypothesis postulating exchange with preexisting telomeres must explain the acquisition of two telomeres in region 13, one on each side of the opening in the ring.

Perhaps the most attractive explanation for the new telomeres would involve a spontaneous break in the chromosome that somehow acquired DNA sequences capable of organizing a new telomere on each end. Sequences with such capability have been detected in yeast (19). It is possible that some fraction of the He-T sequences have the capacity for telomere organization. Alternatively, He-T sequences may be imported in response to the organizing activity of other sequences, such as $C_{1-8}(T/A)_{1-4}$. Sequences with the ability to organize telomeres would be difficult to detect in the wild-type *D. melanogaster* genotype because breaks on linear chromosomes lead to the loss of genetic material on acentric fragments and thus to inviable progeny. On the other hand, compound-X ring chromosomes are especially favorable material for the detection of new telomeres; any single break in the chromosome will simply open the ring; it will not produce an acentric fragment. A second advantage of the compound chromosome is that even breaks that inactivate vital genes may not cause loss of the new chromosome so long as there is a good copy of the gene on the other half of the chromosome.

These studies on the *C(1)A* chromosome offer suggestive evidence that He-T sequences can move, although movement can be detected only when there is a suitable "empty" target. One question that arises is whether the "suitable target" is a site where a new telomere can be tolerated or a new telomere that has not yet acquired He-T sequences. The first half of this question is now open to experimental attack, since it is possible to use *P*-element-mediated transposition to introduce He-T sequences into genotypes that have been constructed to allow synthesis of new telomeres.

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