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Genetics

Drosophila telomere transposon HeT-A produces a transcript with tightly bound protein

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ABSTRACT **Telomeres from *Drosophila* appear to be very different from those of other organisms. A transposable element, HeT-A, plays a major role in forming telomeres and may be the sole structural element, since telomerase-generated repeats are not found. The structure of the HeT-A element, deduced from cloned fragments of DNA, suggests that transposition of the element is mediated by a polyadenylylated RNA intermediate. We now report analyses of HeT-A transcripts. The major RNA is of the appropriate size and strandedness to serve as a transposition intermediate. This RNA is found in cultured cells and in intact flies and is unusual in that it is associated with protein after treatments that apparently remove all protein from other RNAs.**

Chromosomes from organisms as divergent as humans and *Tetrahymena* end in variable numbers of distinctive short repeats. These repeats, produced by enzymes (telomerases), provide a mechanism for circumventing the problem of replicating ends of linear DNA (1, 2). Surprisingly, *Drosophila*, the organism in which telomeres were defined (3), appears to have a different mechanism to maintain chromosome ends. This involves a transposable element, HeT-A, that has moved onto the ends of all the "healed" broken chromosomes studied (4–7). The ends of unbroken chromosomes show linked chains of HeT-A elements, apparently from multiple transpositions (6–8). HeT-A transposition appears to be absolutely limited to chromosome ends, either natural ends or breaks (8). The structure of the HeT-A element suggests it is a retroposon. We now report an RNA with the sequence predicted for a transposition intermediate. The RNA, found in cultured cells and intact animals, is tightly, and perhaps covalently, associated with protein.

Because HeT-A elements exist in linked chains with insertions of other sequences and with some elements truncated on the distal end, the structure of the intact element was not apparent in early studies (4). Recently alignment of cloned HeT-A sequences has allowed the structure of the element to be deduced (7). These alignments show an element of ≈6 kb. The exact size is uncertain because, although the sequences of the elements are highly conserved, each element studied has had several insertions or deletions that can be easily detected in sequence comparisons (6). The deduced structure (7) has a consistent polarity with respect to the end of the chromosome. On the DNA strand running 5′ to 3′ toward the centromere, each element begins at the distal end with a region (≈700 bp) containing distinctive A-rich structures, followed by two overlapping open reading frames (≈2.7 kbp), then by a long region of imperfect repeats (≈2.5 kbp), and finally by an oligo(A) linking sequence to the next, more proximal element or to other chromosome sequences. (See the diagram in Fig. 1.) This structure is similar to that of LINES (long interspersed repetitive elements) retroposons (10). It suggests that HeT-A elements transpose by means of a polyadenylylated RNA intermediate and that each element joins to the chromosome end by means of the poly(A) tail of this RNA. To test this hypothesis we looked for an RNA with the characteristics deduced from the structures of the cloned HeT-A elements.

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MATERIALS AND METHODS

Extraction of HeT-A RNA from Cultured Cells. Cultured *Drosophila melanogaster* cells were grown in Schneider's *Drosophila* medium (GIBCO/BRL) supplemented with 10% (vol/vol) fetal calf serum (HyClone). RNA was extracted with 8 M guanidine hydrochloride followed by phenol/chloroform, 1:1 (vol/vol), and was fractionated on 1% agarose gels (11).

Extraction of RNA from Intact Flies. RNA was extracted from adult male or female flies by homogenizing flies in the presence of proteinase K (to remove the protein associated with the HeT-A RNA) and then extracted with phenol/chloroform (12). When phenol/chloroform was used in the initial stages of fractionation, HeT-A RNA was not recovered, presumably because its associated protein draws it into the interphase.

Attempts To Remove Protein from HeT-A RNA. Ribonucleoprotein (RNP) was pelleted from solutions of RNA prepared from cultured cells by the guanidine hydrochloride protocol. Pelleting was accomplished by centrifugation at 100,000 × *g* for 60 min. RNP was suspended in H_2O and treated for 30 min with (*i*) 10 mM dithiothreitol, (*ii*) 2.5 mM EDTA, (*iii*) 5 mM EDTA, (*iv*) 0.5% SDS at 37°C, and (*v*) 10 μg of proteinase K in 0.5% SDS at 37°C. Preparations were then glyoxylated and fractionated on 1% agarose gels (11).

Preparation of Probes for RNA (Northern) Hybridization Analysis. Single-strand RNA probes were transcribed *in vitro* from cloned fragments by using either T7 or T3 polymerase. Fragments were from HeT-A elements, U06947 and U06920, in the data base. Sequences of the cloned fragments on the map in Fig. 1 *Lower* were as follows: probe 1, U06947 nucleotides 6110–7421; probe 2, U06920 nucleotides 825–3504; and probe 3, U06920 nucleotides 2152–3504. Other experiments showed hybridization to the ≈6-kb RNA with a probe containing nucleotides 580–824 of U06920 (the most 5′ region of the element).

RESULTS AND DISCUSSION

Northern hybridization of total-cell RNA from cultured *Drosophila* cells reveals the predicted HeT-A RNA. The major RNA detected appears by gel migration to be a homogeneous band of ≈6 kb, but the size of the transcript was obscured in the initial experiments by an unusual association of this RNA with protein. Those experiments showed clearly that HeT-A sequences were transcribed; all probes for the strand expected to be the transposition intermediate gave essentially identical patterns of hybridization. Each probe detected a

Abbreviation: RNP, ribonucleoprotein.

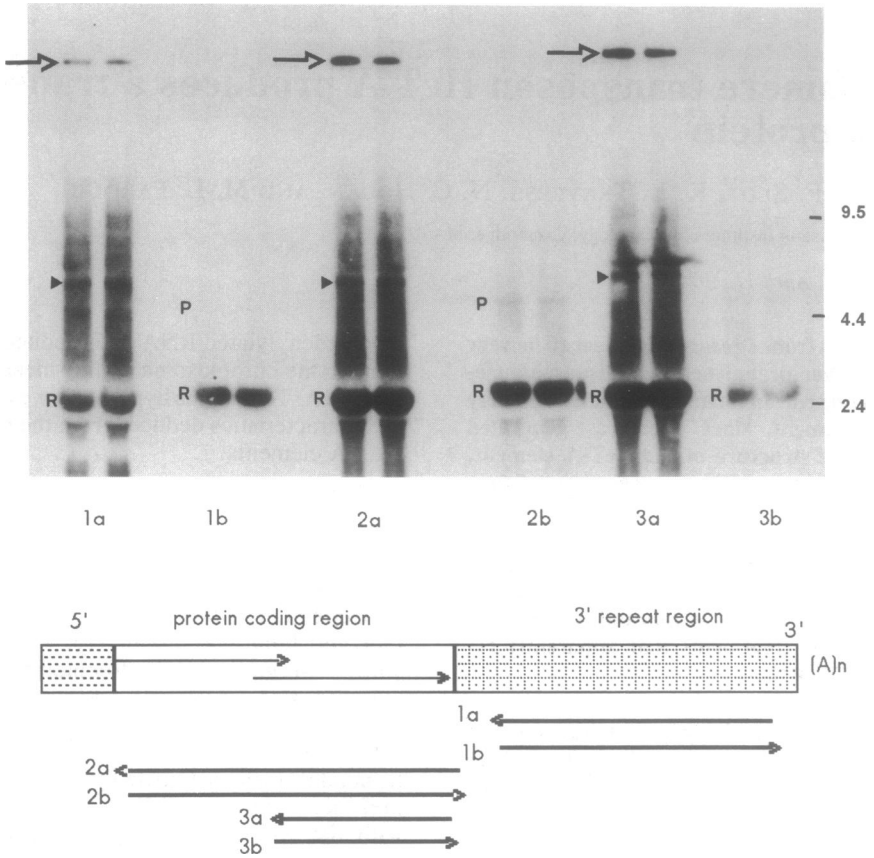

FIG. 1. Only sense-strand transcripts of HeT-A elements are detected in *Drosophila* cells. (*Upper*) Autoradiograph of gel-fractionated total RNA from cultured *Drosophila* cells (Schneider line 3) probed with ^{32}P-labeled RNA transcribed from different parts of the HeT-A sequence. Numbers under each pair of duplicate lanes indicate the part of the sequence transcribed, as mapped in *Lower* below the diagram of the structure of the complete HeT-A element. Lanes marked "a" were probed with RNA that would detect the sense strand of HeT-A. Lanes marked "b" were probed with ^{32}P-labeled RNA that would detect the antisense strand. Only sense strands are detected, although probes for the two strands were of equal specific activity and all hybridized well to DNA blots. The three different probes (1, 2, and 3) show similar patterns of hybridization, except for some smaller RNAs migrating ahead of the ribosomal RNA. Most of the hybridization is broadly spread around a region where an ≈6-kb RNA (arrowhead on left) would be expected to migrate. Note that all a lanes also show strong hybridization over the wells of the gel (arrows at the top). Probes that should detect antisense transcripts (the b lanes) show binding only over the ribosomal RNA (R) and its precursor (P). This binding appears to be nonspecific; it is seen with all the probes, in both the a and the b lanes. [The transcripts migrating below ribosomal RNA may represent degradation or partial transcripts of intact HeT-A elements or may be derived from other HeT-A-related sequences (9) in the genome.] RNA markers (kb) are indicated on right.

significant amount of material that did not enter the gel, as well as a smear of hybridization extending from the 9.5-kb marker to just above the ribosomal RNA. There was no specific hybridization with any probes for the opposite strand of HeT-A (Fig. 1). Opposite-strand probes bound only to ribosomal RNA. Such binding appears to be nonspecific because it is seen with many RNA probes, including both the sense and antisense probes used here. We had not expected to see such clear evidence that the opposite strand was never transcribed. Telomeres have many copies of HeT-A. At least a few of these have other types of elements transposed into or adjacent to them (13, 14). It would not have been surprising to see some irrelevant read-through from adjacent promoters into the nontranscribed strand. The lack of nonspecific transcription may suggest that the HeT-A promoter is exceptionally adapted to its heterochromatic environment in the telomere region.

The presence of transcripts that do not enter the gel suggests that HeT-A RNA might be in a RNP complex, although this RNA had been prepared by treatment with guanidine hydrochloride followed by phenol/chloroform and was free of protein contamination by all conventional tests. The protein association was confirmed by digesting the preparation with proteinase K. This treatment releases HeT-A RNA from the material that does not enter the gel and from complexes migrating above ≈6 kb (Fig. 2*A*). After the protease treatment, HeT-A RNA is found predominantly as a single band of ≈6 kb. This is consistent with the size predicted from the DNA sequence, and the RNA appears to contain the complete HeT-A sequence on the basis of hybridization with different parts of the sequence (Fig. 1 and data not shown). The oligo(A) sequence at the proximal end of each element suggests that the HeT-A transcript should be polyadenylylated. This prediction, also, is fulfilled; a significant portion of the HeT-A transcripts have poly(A) tails long enough to allow retention on an oligo(dT) column (data not shown).

The association between HeT-A RNA and protein is very tight; RNA is released only by protease treatment. SDS alone does not release the RNA but enhances the effect of proteinase K. EDTA and dithiothreitol do not release the RNA (Fig. 2*B*). These results suggest that the protein may be covalently attached to the RNA. Such associations are not unknown (e.g., refs. 15 and 16) but are relatively rare. The protein in the association is not yet identified, but it is obviously important to do so.

Studies of vertebrate cells have suggested that telomerase activity in the intact animal may be controlled differently than in immortal cultures (17, 18). Do cultured *Drosophila* cells reflect the situation in flies? We have been able to detect the

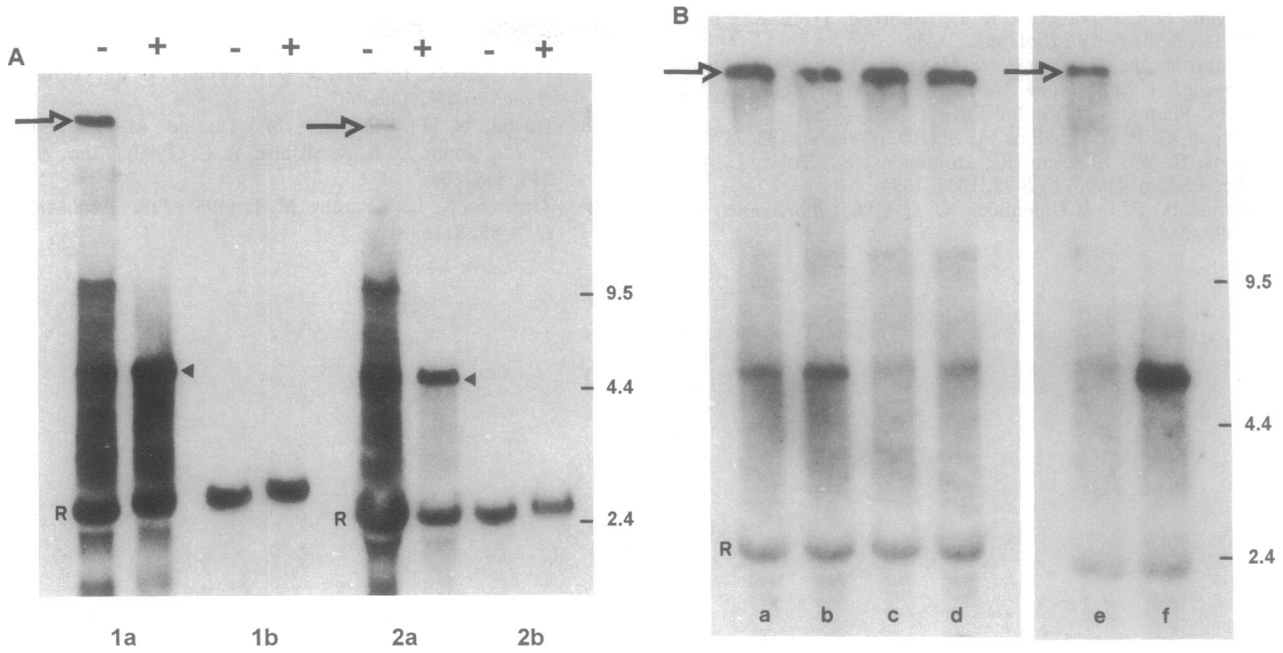

FIG. 2. HeT-A transcripts have a very tight, possibly covalent, association with protein. RNP was concentrated by centrifuging RNA preparations such as those used for Fig. 1 at 100,000 × *g* for 60 min. RNP pellets were dissolved in H_2O and subjected to various treatments. Only protease digestion of pellets releases HeT-A RNA from complexes remaining in the well (arrows) and from others migrating above the ≈6-kb band (arrowhead indicates 6 kb) (*A*) RNP pellets were divided; half of the preparation was incubated with proteinase K for 30 min (lanes marked +), and half was untreated (lanes marked −). After gel fractionation, each pair of lanes was probed with single-stranded RNA. Probes are identified (1, 2, or 3) under the lanes of the autoradiograph as in Fig. 1. Proteinase K releases RNA migrating above ≈6 kb and increases the intensity of the ≈6-kb band. In most experiments the ≈6-kb band is sharp, indicating a relatively homogeneous population of RNA (lane 2a), but when lanes are overloaded (lane 1a), a smear of presumed degradation products is detected. As in Fig. 1, probes for antisense RNA (lanes b) show only sticking to ribosomal RNA (R). (*B*) Evidence that many treatments do not release HeT-A RNA from RNP. An RNP pellet, prepared as in *A*, was incubated with no treatment (lane a), 10 mM dithiothreitol (lane b), 2.5 mM EDTA (lane c), 5.0 mM EDTA (lane d), 0.5% SDS (lane e), and proteinase K plus 0.5% SDS (lane f). Only proteinase K releases HeT-A RNA (arrow) from the complex in the well (arrowhead). This blot was probed with probe 2a. RNA markers (kb) are indicated on right.

≈6-kb HeT-A transcript in RNA preparations from both adult males and females (Fig. 3). Because the extracts were made from whole animals, transcripts could derive from germ cells or somatic cells or both.

These experiments have revealed a HeT-A transcript with the features predicted for a transposition intermediate, although they do not prove the function of this RNA. The RNA is the appropriate strand and contains all sequences known to be in the telomeric element. RNA is detected in both males and females, consistent with evidence that HeT-A transposition has occurred in both sexes. "Healing" of the terminal deletions in RT stocks occurred in males (4). The broken ring C(1)A chromosome acquired new telomeres in a female (19). Both events must have occurred in the germ line. The presence of HeT-A RNA in cultured cells raises the possibility that transposition can also occur in somatic cells.

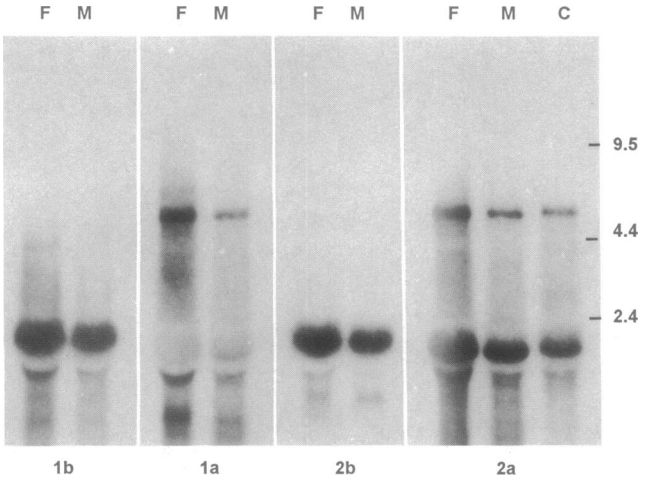

FIG. 3. HeT-A transcripts are found in male and female flies. Autoradiograph of total RNA prepared from adult males (lanes M) and females (lanes F). The RNA preparations were gel-fractionated and probed with ^{32}P-labeled single-stranded RNA probes as in Fig. 1. Only probes for the sense strand (lanes a) detect RNA of ≈6 kb. This RNA comigrates with the HeT-A RNA from cultured cells (lane C). RNA markers (kb) are indicated on right.

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