Evidence for horizontal transmission of the mobile element jockey between distant Drosophila species

(retroposon/internal promoter/Drosophila funebris)

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Communicated by Maxine Singer, August 31, 1990 (received for review July 13, 1990)

ABSTRACT We addressed the possibility of the horizontal transfer of long interspersed element (LINE)-like mobile elements by studying the distribution of the Drosophila melanogaster LINE-like element jockey in different Drosophila species. Outside the D. melanogaster group jockey was detected only in the distantly related species Drosophila funebris. Cloning and sequencing of this element from D. funebris revealed the existence of the two open reading frames highly similar to those of jockey from D. melanogaster. Elements from both species are transcriptionally active and contain evidence of horizontal transmission of jockey into D. funebris.

Transposition of long interspersed element (LINE)-like mobile elements (1–3) is an important source of mutations, at least in Drosophila (4) and humans (5, 6). Previously we have cloned the original copy of jockey, a member of this family of mobile elements, from Drosophila melanogaster ctMRE strain, where it is involved in the generation of the mutant phenotype (7). The sequence of jockey contains two open reading frames (ORFs) similar, respectively, to the gag and pol genes of retroviruses (8). There are two types of jockey in the genome of D. melanogaster: the full-size elements of about 5 kilobases (kb) and the copies with an internal deletion, which are approximately 2.5 kb long (8). Two polyadenylated transcripts of jockey were detected at different stages of Drosophila ontogenesis and in cell culture. These transcripts have the same length as the genomic copies of jockey and correspond to the coding strand. Experiments with α-amanitin showed that jockey is transcribed by RNA polymerase II. We demonstrated also that the transcription of jockey is due to an internal promoter located downstream of the site of initiation of the transcription (9). Such an internal location of the promoter allows it to be preserved in the course of replication by means of reverse transcription and accounts for the distribution of jockey and probably other LINEs throughout the genome. However, nothing is known about the origin of LINEs or the modes of mechanisms of their spreading through different organisms. The fact that the putative reverse transcriptase of jockey is much more similar to the transcriptases from mammalian and Trypanosoma LINEs than to those from Drosophila long terminal repeat (LTR)-containing retroelements (8) suggests a close evolutionary relationship between LINEs from different species. A possible explanation for such a close relationship might be that this type of mobile element is capable of horizontal transmission between different organisms. We have chosen jockey as an active and well-studied LINE-like mobile ele-

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RESULTS AND DISCUSSION

Screening of genomic DNA from a number of different species of the genus Drosophila with probes derived from different regions of jockey from D. melanogaster revealed decreasing hybridization within the D. melanogaster group which correlates with the phylogenetic relationships of the species (Fig. 1). A similar pattern of distribution has been described for other mobile elements, including the LINE-like I element (15, 16). No reproducible hybridization was detected with DNA from almost all tested species outside the D. melanogaster species group. The only exception was the signal seen with D. funebris DNA (Fig. 1, lane FU) which remained even after higher-stringency washes. This observation suggests that jockey may have been transferred horizontally between Drosophila species. To investigate this possibility we studied the structure of this element and its distribution in species related to D. funebris.

We prepared a phage library from D. funebris DNA and screened it with DNA of jockey from D. melanogaster. Two clones containing presumably full-length copies were selected for subcloning and sequencing. The complete sequence of one of the copies (Fig. 2) revealed two ORFs with 61% and 69% overall protein identity to the corresponding ORFs of jockey from D. melanogaster. The 5' ORF-1 (gag) encodes a conserved region in the C-terminal half of its predicted protein that includes the proposed zinc-finger-like structure (8). The N-terminal part of this ORF-1 has little similarity to that of the element from D. melanogaster; this suggests the absence of conserved functional domains in this part of the protein. The protein homology of the ORF-2 (pol) is rather uniform, comparing the D. funebris and D. melanogaster elements, and does not reveal any conserved structures in addition to those identified previously (8). Sequencing of 5' and 3' nontranslated regions of a second cloned copy has shown that both copies are identical in these regions and that their sizes coincide with those of the full-length copy of jockey from D. melanogaster.

It was of particular interest to compare the DNA sequences from the 5' nontranslated region of jockey because the D. melanogaster element contains an internal RNA polymerase II promoter in that region (9). In addition to the first few nucleotides, which are absolutely necessary for the transcriptional activity of the promoter (9) and show homology in both elements (Fig. 3B), there is a fully conserved 6-bp sequence, GGACGT, containing a short palindromic. To determine whether this sequence is important for promoter function, we constructed two plasmids based on the sequence of the promoter of the D. melanogaster jockey. One construct contained the 6-bp core (CAT1) and one did not (CAT2). In both clones the jockey segment was fused to the reporter CAT gene of Escherichia coli (Fig. 3B). The results of the CAT assay after transfection of D. melanogaster tissue
culture cells (Fig. 3C) showed that deletion of the 6-bp core abolished the transcriptional activity of the internal promoter. jockey is also actively transcribed in D. funebris (Fig. 3A), and we believe that this promoter element is involved in the regulation of jockey transcription in D. funebris as well, and this would explain the conservation of the core. C. McLean and D. Finnegan noticed (personal communication) that an almost identical 6-bp sequence, at the same position with respect to the site of initiation, is present in the downstream regulatory elements of the P2 promoter of Antennapedia, and in several LINE-like mobile elements of Drosophila. For these mobile elements the existence of an internal promoter has been suggested (4, 9). It seems likely that the same protein(s) are required for transcription of these mobile elements and genes in D. melanogaster, and of jockey and other genes in D. funebris. We also found two long regions of conservation in the right part of the 5' nontranslated region (Fig. 2), but their functional activity remains to be determined.

Southern hybridization experiments (Fig. 4) with a probe derived from the newly cloned element showed that jockey is present in all four strains of D. funebris tested in approxi-

FIG. 2. Sequence of the jockey element from D. funebris. The amino acid sequences of two ORFs are shown above the nucleotide sequence. The conservative sequences in the 5' nontranslated region, the zinc-finger domains, and the region of homology between predicted reverse transcriptases of different LINEs are underlined.

attachment in the internal transcribed region.
Fig. 3. jockey is transcribed in D. funebris and contains conserved promoter elements. (A) Northern blot containing 5 μg of poly(A)⁺ RNA from D. melanogaster (lane Me) and D. funebris (lane Fu) was hybridized with the randomly labeled 1.4-kb Pst I fragment of D. funebris jockey (Fig. 4, fragment A) and washed in 0.1x SSC/0.5% SDS at 50°C. The region homologous to this probe is absent from copies of D. melanogaster jockey with internal deletion (8), and therefore only full-length 5-kb transcript is detected in lane Me. Increasing the stringency of washing (0.1x SSC, 68°C) leads to disappearance of the signal in lane Me (not shown). (B) Comparison of the promoter regions of jockey from D. melanogaster and D. funebris. The first nucleotide of the D. funebris jockey is indicated above its sequence. The design of the constructs used in the transfection experiments is shown on the bottom. (C) A 6-bp conserved sequence is essential for the activity of jockey promoter. CAT assays were hybridized in extracts of D. melanogaster cultured cells (Schneider 2) transfected with the above constructions. Lanes 1 and 2 correspond to constructs CAT1 and CAT2, respectively. CM, chloramphenicol; AcCM, acetyl derivatives of CM.

Fig. 4. Map of jockey from D. funebris (Lower) and autoradiogram of the Southern blot with DNA from different strains of the D. funebris group probed with a jockey fragment from D. funebris (Upper). The 908-bp HindIII-Xba I fragment (indicated as B on the map) was used as a probe. Because this fragment is not an internal HindIII fragment, it gives different bands for different copies of the element when hybridized to HindIII digests of genomic DNAs. The following strains from the Bowling Green collection were used (stock number and origin are in parentheses): 1–4, D. funebris (1911.1, Mexico; Uméa Drosophila Stock Center, Sweden; 1911.0, Kentucky; 1911.6, Alaska); 5, D. subfunebris (1951.51, California); 6, D. microspina microspina (1911.2, Mexico); 7, D. microspina limpiensis (1921.0, Arizona); 8, D. multisima (1941.0, Japan); 9, D. melanogaster (Oregon R). In addition, no signal was detected in lanes 5–8 when other fragments of jockey were used as probes (data not shown).

The absence of jockey in sibling species from the same D. funebris group makes the explanation of this distribution by means of a vertical transmission from common ancestor unacceptable. Polyspermy should also be excluded because of the significant morphological differences between D. melanogaster and D. funebris. This leaves the horizontal transmission model as the only one that is consistent with the observed distribution of jockey. Southern hybridization of DNA of different species from within the D. melanogaster group with a probe from jockey from D. funebris indicates that the homology with DNA from D. melanogaster is the highest (data not shown)—i.e., D. melanogaster is the most likely species from which jockey was transferred to D. funebris.

The possibility of the horizontal transmission of the LINE-like I element in Drosophila has been discussed earlier (15). The distribution of I elements correlates with the phylogenetic relationship between species and is confined to the D. melanogaster group (15, 16). On the basis of population studies, Kidwell suggested (17) that the complete and active I factor appeared in natural populations of D. melanogaster in the 1930s–1940s. It was suggested (18) that it may have occurred through the reactivation of some inactive element(s).
which is present in all strains of *D. melanogaster* or, alternatively, by acquisition from a sibling species in which active elements also exist (19). However, morphological differences between sibling species in this group do not prevent mating (20), so the I element could, as suggested in ref. 15, be acquired by means of polyspermy or, even more directly, in rare cases when interspecies matings give rise to fertile offspring. Thus, information available now does not provide evidence for the authentic horizontal transmission of I element. So far, experimental evidence for the horizontal transfer of mobile elements has been obtained only for the P element from *Drosophila*, which codes for a transposase rather than a reverse transcriptase and whose transposition does not, presumably, involve an RNA intermediate (for review see ref. 21). It was shown (see ref. 22 and references therein) that P elements invaded *D. melanogaster* (only one species from the group with the same name) from *D. willistoni*, which belongs to another group (see phylogenetic tree, Fig. 1), approximately 50 years ago or earlier (17). The idea that LTR-containing retrotransposons transfer horizontally was proposed recently, on the basis of the similarities between ORFs encoding putative reverse transcriptases in mobile elements from different species (23, 24). We have found active LTR-containing retroelement gypsy in *D. virilis* (unpublished results). The features of the gypsy element and its distribution between different *Drosophila* species lead us to the conclusion that it is also capable of horizontal transmission. Thus, horizontal transfer appears to be a general feature of mobile elements and could play a significant role in eukaryotic evolution.

We thank M. F. Singer for advice and help throughout; M. M. Green for discussions; and M. F. Singer, S. Haynes, and I. B. Dawid for critical reading of the manuscript. L.J.M. thanks V. Corces for support while working in his laboratory.