Possible Horizontal Transfer of a Transposable Element from Host to Parasitoid

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Full-length mariner-like elements (MLEs) were identified from both a parasitoid wasp, Ascogaster reticulatus, and its moth host, Adoxophyes honmai. MLEs were detected in two related Tortricid moths, but not in another Ascogaster species. The MLEs of A. reticulatus and A. honmai were 97.6% identical in DNA sequence. This high similarity suggests a recent horizontal transfer, probably from the moth host to the wasp parasitoid, facilitated by the intimacy of the host-parasitoid relationship.

Introduction

The transposable element mariner was first identified in Drosophila mauritiana (Jacobson, Medhora, and Hartl 1986). Using degenerate primers and the polymerase chain reaction (PCR), Robertson and his colleagues found the presence of sequences homologous to mariner elements in a wide range of insect species (Robertson 1993; Robertson and MacLeod 1993). Since Robertson’s pioneering work, sequences homologous to mariner (mariner-like elements [MLEs]) have been found in a wide variety of animals, including invertebrates (Robertson and Lampe 1995a, 1995b) and vertebrates (Robertson 1995). Their broad but patchy distribution suggests the occurrence of horizontal transfers of MLEs (Maruyama and Hartl 1991; Robertson 1993; Robertson and MacLeod 1993).

The occurrence of horizontal transfer of MLEs is generally accepted (Hartl et al. 1997), however, there are many unanswered questions in MLE evolution. For example, the mechanisms of horizontal transfer remain unknown. Because the intimacy of parasitism may facilitate horizontal transfer (Kidwell 1992a), we have started to survey MLEs in parasitoid insects and their hosts. Here, we show that a parasitoid wasp and its moth host carry MLEs with 97.6% identity in nucleotide sequence. This high similarity and the lack of MLEs in a congeneric wasp suggest that MLEs were recently transferred horizontally from one of the species to the other one, most likely from the host to the parasitoid.

Materials and Methods

Insect Species

Colonies of the parasitoid wasp, Ascogaster reticulatus, and its moth host, the smaller tea tortrix Adoxophyes honmai, were obtained from stock cultures at the University of Tsukuba. Both species were maintained at 25°C ± 1°C and 60% ± 20% RH with a 16L : 8D photoperiod. The rearing conditions are described elsewhere (Honda, Kainoh, and Honda 1999). Other moth strains used in this study were maintained at the National Institute of Agro-Environmental Sciences. The rearing conditions for these moths are described elsewhere (Noguchi 1993).

PCR Amplification

Genomic DNA was extracted from adults of the parasitic wasp and pupae of the moth following methods modified from those used for Drosophila (Daniels and Strausbaugh 1986). The oligonucleotide primers used in this study are shown in table 1. The degenerate MAR 124F and MAR 276R primers were designed to amplify MLEs from insects, based on the conserved region of mariner transposases (Robertson 1993). The other primers were used to extend analyses of the PCR products amplified by the MAR 124F-MAR 276R primers (see below). PCR was performed under the following conditions. Template DNA (200 ng) was added to PCR buffer (Boehringer Mannheim) containing 200 μM of each dNTP, 2 mM oligonucleotide primers, and 0.5 U Taq DNA polymerase (Boehringer Mannheim) in 100 μl (total volume). Temperature cycling was carried out in a thermal cycler (Gene Amp 9600, Perkin Elmer) for 30 cycles (1 min at 95°C, 1 min at 55°C, and 1 min at 72°C).

Cloning and Nucleotide Sequencing of PCR Products

PCR products were ligated into the pCRII vector using a TA cloning kit (Invitrogen) and Escherichia coli INV α cells (Invitrogen) were transformed with the ligated plasmids. The nucleotide sequences of cloned PCR products were determined by an automated ABI PRISM 377 DNA Sequencer (Perkin Elmer) using a PRIZM Dye Deoxy Primer Cycle Sequencing Ready Reaction Kit (Perkin Elmer).
Isolation of Full-Length MLEs

Genomic libraries of both species were constructed using pUC 19 plasmids digested with PstI and EcoRI. Methods for probe labeling, hybridization, and signal detection are described below in the Southern Blot Analysis section. The nucleotide sequences of positive genomic clones were determined by primer walking using synthetic oligonucleotides.

Computer Analysis of the Sequences

The sequence alignment was performed using GENETYX-MAC, version 10.1 (Software Development). A similarity search for nucleotide and amino acid sequences was carried out with BLAST (Altschul et al. 1990) using the GenBank and EMBL databases. Phylogenetic reconstruction was performed by PAUP, version 4.0b6 (Sinauer), using maximum parsimony; bootstrap values for the nodes were determined using 100 replicates.

Southern Blot Analysis

The ProbeF and ProbeR primers, internal to the degenerate MAR 124F and MAR 276R primers, were designed from the sequences of the MLEs of the wasp and the moth (fig. 1). PCR fragments, amplified from moth genomic DNA using the ProbeF and ProbeR primers (table 1), were cloned into the pCR vector. One clone, designated pFR1, was labeled with digoxigenin-11-dUTP using the PCR DIG Labeling Kit (Boehringer Mannheim). Genomic DNA (10 μg) was digested with EcoRI, and DNA fragments were separated by electrophoresis in a 1% agarose gel and transferred to nylon membranes (Boehringer Mannheim) by capillary blotting. The membranes were prehybridized at 65°C for 4 h in 5× SSC containing 1% blocking reagent (Boehringer Mannheim), 0.02% SDS, and 0.1% N-lauroylsarcosine. The prehybridization buffer was replaced with fresh buffer containing heat-denatured probe. Hybridization was performed at 65°C for approximately 16 h. Membranes were washed twice with 2× SSC containing 0.1% SDS for 5 min at room temperature and twice with 0.1× SSC containing 0.1% SDS for 15 min at 65°C. Positive signals were detected using a DIG DNA Detection Kit following the methods recommended by the manufacturer (Boehringer Mannheim).

Results and Discussion

We surveyed MLEs in a parasitoid wasp, A. reticulatus, and its moth host, the smaller tea tortrix A. honmai, using degenerate mariner primers (Robertson 1993) (fig. 1a). Amplifications were obtained from genomic DNA of both the wasp and the moth (fig. 1b). Fragments of approximately the expected 500-bp size were observed, and these PCR products were cloned and sequenced. The fragments from the two species showed 98.7% identity with each other in DNA sequence. Furthermore, these sequences showed 84% identity at the nucleotide level and 77% identity at the amino acid level to the putative mariner transposase gene from the green lacewing Chrysoperla plumaturana (Robertson, Lampe, and MacLeod 1992; GenBank accession number L06041), identifying the cloned DNAs as representing MLEs from the moth and the wasp.

We extracted full-length genomic copies of these MLEs to examine them further. EcoRI/PstI-digested pUC19 genomic DNA libraries of the two species were screened with DIG-labeled PCR products and cloned into the pCR plasmid vector (Invitrogen) (fig. 2).
From the library for *A. reticulatus*, three complete genomic copies (named W1, W5, and W6) were isolated (fig. 2a). One of these elements, W1, carried a 57-bp duplication in the middle of the element, but these three copies were otherwise almost identical. Two elements (named M1 and M4) were isolated from the *A. honmai* library (fig. 2a). M1 and M4 were identical, except that M1 had an imperfect duplication of 7 bp and was truncated at the 3′ end of the element. (The 3′ deletion of M1 was after position 1105. We sequenced 890 bp further without finding more *mariner*-related sequence.) All of the clones had 25-bp inverted terminal repeats (ITRs) and a TA duplication at the target insertion sites (except for the 5′ end of W6, which had TT, and possibly the missing 3′ end of M1). These features are common to all *Tellmariner* family transposable elements. These MLEs all possessed stop codons within the putative open reading frame (ORF); therefore, they seem to be inactive. A phylogenetic tree demonstrates the relatedness of these MLE sequences to each other and to MLEs from other species obtained from GenBank by BLAST searching for the most similar sequences in the databases (fig. 3). The tree shown was constructed using maximum parsimony, but trees constructed using maximum likelihood and the unweighted pair grouping method with arithmetic means produced the same topology.

The putative ORF of the moth MLE M4 is apparently frameshifted by the insertion of a single A at position 629 (not a C at position 635, as previously reported [Yoshiyama et al. 2000]). If that A is removed, the conceptual translation of M4 is 85.1% identical to that of the lacewing (fig. 4). If we remove the 57-bp duplication in W1 and then construct a consensus sequence of the three wasp-derived MLEs, the conceptual translation thereof is 99.1% identical to that of M4, differing in only three amino acids (fig. 4). For each of these three amino acids, the wasp sequences agree with the lacewing sequence at that position. The wasp consensus translation is 85.9% identical to the lacewing sequence. We conclude that the ancestral MLE of the three wasp MLEs had a single ORF, the translation of which corresponds to that of the consensus we have derived.

Because the similarity between MLEs cloned from the moth and from the wasp was very high, we performed a PCR assay in order to test for the possibility that this similarity was a result of contamination by genomic DNAs. The PCR assays were carried out using primers designed from genomic flanking regions (fig. 1a) of these MLEs (fig. 5). PCR products of the expected sizes were observed when both primers and genomic DNA templates were from the same species, but no positive bands were detected in amplifications using a genomic DNA template from the other species. The identities of the PCR fragments were checked by cloning and partially sequencing them (data not shown). The results confirmed that no DNA contamination had occurred.

It is also possible that these MLEs existed non-chromosomally; that is, they might have been in the genomes of symbiotic organisms and/or viruses. In order to rule out such a possibility, Southern blot hybridization was performed (fig. 6a) using the labeled PCR products as probes (fig. 1a). Positive bands were observed in the genomes of both species. These bands were intense. Since the amount of genomic DNA of the moth or wasp should be much greater than any symbiont or virus DNA, this result indicates that the MLEs probably exist in the genomes of both the wasp and the moth.

The similarity between the MLEs of the moth and its parasitoid wasp can be explained by a horizontal transfer between the two species. Such high similarity between MLE sequences from two different species has been reported for MLEs belonging to the irritans family (Robertson and MacLeod 1993). Especially, the transposase region of MLE sequence of the hornfly differs by only 2 bp out of 1,044 from that of the lacewing. However, in this case, there is no obvious ecological relationship between these two species. On the other hand, the wasp *A. reticulatus* is an egg-larval parasitoid of moth species of the genus *Adoxophyes* (Watanabe 1967), and thus the two species have a very intimate physical relationship. The most plausible explanation is that a horizontal transfer occurred from one of these species to the other.

Several issues need to be addressed. The first question is that of what kind of intermediates are involved in horizontal transfer. Intermediate vectors are probably required for the horizontal transfer because transposable elements are not capable of moving from one species to another directly. There are reports of viruses carrying transposable elements (e.g., Miller and Miller 1982), and viruses might therefore act as shuttles of transposable elements for horizontal transfer (Fraser 1986). It is known that viruses carried by parasitoids can play an important role in suppression of the host’s immune response, thereby preventing encapsulation of the parasitoid egg (Strand and Pech 1995). It is possible that viruses...
are (or were) present in the Ascogaster-Adoxophyes system and therefore could act (or could have acted) as vectors to carry transposable elements between the species.

The second question to be addressed is that of the direction of horizontal transfer. We performed Southern blot analysis (fig. 6a) and PCR assays (fig. 6b) on two other sibling species of Adoxophyes moths and another species of the wasp genus Ascogaster. MLEs were detected in the summer fruit tortrix Adoxophyes orana fasciata (Yoshiyama et al. 2000) and the tea tortrix Homona magnanima, but no MLEs were detected in the parasitoid wasp Ascogaster quadridenata. This suggests that transfer was from the host to the parasitoid. This direction is more reasonable than the reverse for another reason: infection of a host by a parasitoid usually leads to the death of the host, thereby precluding successful transfer from the parasitoid to the host. We conclude that the pathway of horizontal transfer was probably from the moth to the wasp.

If this scenario is true, we can infer the sequence of the MLE at the time of transfer. Our analysis of the consensus sequence of the wasp MLEs, along with the comparison with the moth-derived M4 and the lacewing sequences, allows us to infer that at the time of horizontal transfer, the MLE that was transferred must have had the amino acid sequence of the wasp consensus sequence.

The third question to be addressed is whether or not horizontal transfer is common in host-parasite systems. There are few relevant data. P elements of Drosophila are thought to have been introduced into Drosophila melanogaster by a horizontal transfer from Drosophila willistoni (Daniels et al. 1990; Kidwell 1992b). That transfer has been postulated to have been effected by physical transfer of P elements from an egg of one species to an egg of the other by the egg predatory mite Proctolaelaps regalis (Houck et al. 1991). In this case, the P element is not present in the genome of the mite, and the ecological relationship between the mite and the Drosophila species is not particularly close. To our knowledge, there are no other reports suggesting that an especially intimate ecological proximity, such as a host-parasitoid or host-parasite relationship, contributed to horizontal transfer of a transposable element.

In an effort to extend our results, we tested four other pairs of hosts and parasitoids for MLEs (table 2). Among these, only one host species (Mythimna separata) and no parasitoids had MLEs. The attractive hy-
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Fig. 5.—PCR amplification from genomic DNA of moth and wasp using primers designed from flanking regions of MLE genomic copies. Lane 1: BRL 1-kb ladder; lane 2: the smaller tea tortrix Adoxophyes honmai, using M1F and M1R primers; lane 3: the smaller tea tortrix A. honmai, using W5F and W5R primers; lane 4: the parasitoid wasp Ascogaster reticulatus, using M1F and M1R primers; lane 5: the parasitoid wasp A. reticulatus, using W5F and W5R primers. Sizes of DNA standards are given in base pairs at left. Preparation of genomic DNA samples and PCR primers and reactions were as described for figure 1.

Fig. 6.—a, Southern blot hybridization of wasp and moth genomic DNAs with an MLE probe. Lane 1: parasitoid wasp Ascogaster reticulatus; lane 2: parasitoid wasp Ascogaster quadridentata; lane 3: smaller tea tortrix Adoxophyes honmai; lane 4: summer fruit tortrix Adoxophyes orana fasciata Walsingham; lane 5: tea tortrix Homona magnanima Diaknomoff. Sizes of DNA standards are given in base pairs at left. b, PCR amplification of genomic DNA using MAR 124F–MAR 276 primers. Lane 1: BRL 1-kb ladder; lane 2: parasitoid wasp A. reticulatus; lane 3: A. quadridentata; lane 4: smaller tea tortrix A. honmai; lane 5: summer fruit tortrix A. orana fasciata Walsingham; lane 6: tea tortrix H. magnanima Diaknomoff; lane 7: Drosophila mauritiana; lane 8: Drosophila melanogaster (Canton-S); lane 9: distilled water. Sizes of DNA standards are given in base pairs at the left.

Hypothesis that the special intimacy of the host-parasitoid relationship could facilitate horizontal transfer of transposable elements is thus far demonstrated only in our single example.

Conclusions

Many examples show that horizontal transfers of transposable elements have occurred in a wide range of organisms (Syvanen 1994). However, mechanisms of these horizontal transfers remain unknown. Our results suggest that the intimate host-parasitoid relationship may sometimes facilitate horizontal transfer.

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