Characterization of Gandalf, a new inverted-repeat transposable element of Drosophila koepferae

Abstract The cloning and characterization of Gandalf, a new DNA-transposing mobile element obtained from the Drosophila koepferae (repleta group) genome is described. A fragment of Gandalf was found in a middle repetitive clone that shows variable chromosomal localization. Restriction, Southern blot, PCR and sequencing analyses have shown that most Gandalf copies are about 1 kb long, are flanked by 12 bp inverted terminal repeats and contain subterminal repetitive regions on both sides of the element. As with other elements of the DNA-transposing type (known as the ‘Ac family’), the Gandalf element generates 8 bp direct duplications at the insertion point. Coding region analysis has shown that the longer open reading frame found in Gandalf copies could encode part of a protein. However, whether or not the 1 kb copies of the element are actually the active transposons remains to be elucidated. Gandalf shows a very low copy number in D. buzzatii, a sibling species of D. koepferae. An attempt to induce interspecific hybrid dysgenesis in hybrids of these two species has been unsuccessful.

Key words Drosophila • Transposable elements • repleta group • Ac family • Hybrid instability

Introduction

Finnegan (1989) classified eukaryotic transposable elements into two classes depending on whether they do or do not code for a reverse transcriptase (Class I or Class II respectively). Class II transposable elements, also known as inverted-repeat transposable elements or DNA-transposing elements, are characterized by the presence of terminal inverted repeats, usually of less than 100 bp; exceptions include the foldback elements and the Minos element of Drosophila hydei (Templeton and Potter 1989; Franz and Savakis 1991). The best characterized elements belonging to Class II code for at least one protein, which is usually known as transposase and is involved in the transposition of the element (Jacobson et al. 1986; Rio 1990; Frey et al. 1990). Although in some cases it has been found that the transposases of different elements are related (Harris et al. 1988; Hehl et al. 1991; Calvi et al. 1991; Doak et al. 1994), no close relatives have been found for some of the best-known element proteins, such as the P transposase. Moreover, because defective elements are often predominant, the products of several elements have not been defined (Ueda et al. 1986; Wobus et al. 1990).

In a previous study (Marin et al. 1992), over 100 DNA clones from D. koepferae (repleta group) and a similar number of clones from its sibling species D. buzzatii were analysed for their content of repetitive DNA. An unexpected number of clones contained non-satellite repetitive sequences. Around 80% of the clones carried highly repetitive DNA, probably simple sequence DNA (Marin et al. 1992). However, only a few clones were obtained that showed the characteristics of mobile elements, that is, middle repetitive sequences with a dispersed and variable pattern of in situ hybridization of polytene chromosomes. When these latter clones were studied, it was found that most of them carried Class I elements. Homologies with the well-known D. melanogaster retrotransposon Gypsy, as well as with the Anopheles gambiae non-viral retroposon TIAg, have been found in D. koepferae clones (Marin and Fontdevila, submitted). Moreover, Labrador and Fontdevila (1994) have characterized a new retrotransposon, Osvaldo, first found in a D. buzzatii clone.
Additionally, sequences related to the D. melanogaster Copia retrotransposon have been found in both species (Francisco et al. 1994). Only one clone of D. koepferae (cDk210) was shown to carry a fragment of a short inverted-repeat mobile element, which we have named Gandalf (Tolkien 1954). In this work, we describe the molecular characterization of this new Class II element.

Research on the mobile elements of D. koepferae and D. buzzatii was stimulated by the finding of high rates of chromosomal instability in hybrids of these two species (Naveira and Fontdevila 1985). Under the simplest assumptions, this phenomenon could be due to a hybrid dysgenesis syndrome, similar to those found intraspecifically in D. melanogaster. These syndromes are related to the high rates of transposition of one of several mobile elements (P. hobo and I: Rubin et al. 1982; Bucheton et al. 1984; Blackman et al. 1987) in the genome of peculiar strains normally devoid of elements or lacking active ones (Engels 1989). Some time ago, we discovered that the mobile sequence included in the cDk210 clone revealed a very small number of bands when hybridized with genomic DNA of D. buzzatii, while in its sibling D. koepferae and in other closely related species, a characteristic middle repetitive pattern was found. This result suggested that this mobile sequence could be related to the interspecific phenomenon previously described by Naveira and Fontdevila (1985), a possibility that is investigated in the present work. Although we have been unsuccessful in detecting transposition in our hybrids, this could be because the introgressed Gandalf copies were inactive or gave transposition rates of less than $3 \times 10^{-3}$ transpositions/gamete per generation.

**Materials and methods**

**Drosophila stocks**

Various stocks of 22 different species of the repleta group were used. 


Basic molecular biology techniques

DNA extraction from Drosophila stocks, Southern blot and in situ hybridizations and DNA sequencing were performed as described in Marin et al. (1992).

**Sequence analysis**

GCC (Genetics Computer Group 1991; see Devereux et al. 1984) programs FASTA, TFASTA, BESTFIT and GAP were used to analyse the Gandalf sequences in the GenBank, EMBL, PIR and Swiss-Prot databases.

**Polymerase chain reaction (PCR) primers and methods**

Four different primers were used for PCR amplifications. 210.1 (5'-GCTGACAATCTCTCAAGCACA-3') and 210.4 (5'-GCAAATGGCAATTTAGGGG-3') correspond to nucleotides 975–952 and 8–28 of Gandalf 1 (see below). Adh1 (5'-AAGAATATCATCTCTTGCGCTTGGG-3') and Adh2 (5'-CCCGATGCTTGCTTGGTCATTCACT-3') were selected from the sequence of the Adh2 gene of D. buzzatii (EMBL accession number M62743) based on their G–C content and conservation in species of the repleta group. The Adh2 sequence of D. buzzatii was aligned with those of eight other Drosophila species of the repleta group, and the more conserved zones at the extremes of the D. buzzatii sequence were selected. The Adh primers were used to obtain a fragment of this gene, which was used as probe in the introgression experiments detailed in the next section.

**Design of the introgression experiment**

The stocks KO2 (D. koepferae from San Luis, Argentina) and BSL (D. buzzatii from the same locality) were selected because they interbreed easily (Marin et al. 1993), producing abundant F₁ and some, albeit not very abundant, offspring when F₁ females are backcrossed with D. buzzatii males (we designate the offspring of the successive backcrosses B₁, B₂, and so on). Sixty virgin KO2 females were crossed with 60 BSL males to generate 200 F₁ offspring. F₁ females were individually backcrossed with BSL males and 26 of these crosses produced B₂ offspring. The same scheme of individual crosses was applied for three further generations, selecting as progenitors those individuals that still carried D. koepferae chromosomal fragments. These individuals can be selected by detecting asynapsis between the homologous polytene chromosomes in the salivary glands of their offspring (Naveira et al. 1986). After analysis of 25 larvae of the KO2 stock, which showed a very low degree of polymorphism for Gandalf positions, suggesting high endogamy, we were able to select, by cytogenetic analysis, those individuals that carried Gandalf elements in the introgressed D. koepferae chromosomal fragments.