A genetically tagged, defective I element can be complemented by actively transposing I factors in the germline of I-R dysgenic females in *Drosophila melanogaster*

Abstract

Non-LTR retrotransposons, also known as LINEs, transpose by reverse transcription of an RNA intermediate. Their mechanism of transposition is apparently different from that of retrotransposons and similar to that of proviruses of retroviruses. The I factor is responsible for the I-R system of hybrid dysgenesis in *Drosophila melanogaster*. Inducer strains contain several functional I factors whereas reactive strains do not. Transposition of I factors can be experimentally induced: they are stable in inducer strains, but transpose at high frequency in the germline of females, known as SF females, produced by crossing reactive females and inducer males. We have constructed an I element, called *IviP2*, marked with the *vermilion* gene, the coding sequence of which was interrupted by an intron. Splicing of the intron can only occur in the transcript initiated from the I element promoter. Transposed copies expressing a wild-type *vermilion* phenotype were recovered in the germline of SF females in which I factors were actively transposing. This indicates that trans-complementation of a defective I element, deficient for the second open reading frame, by functional I factors can occur in the germline of dysgenic females.

Key words *Drosophila melanogaster* • Hybrid dysgenesis • I factor • Transposition • Complementation

Introduction

LINEs (Long Interspersed Nucleotidic Elements) comprise a middle repetitive DNA family (Singer 1982) corresponding to the most abundant class of eucaryotic transposable elements (Hutchison et al. 1989), which have been found in a wide range of organisms (Eickbush 1992). One of these elements, the I factor of *Drosophila melanogaster*, controls the I-R system of hybrid dysgenesis. This species can be divided into two types of strains based on their response in this system: inducer strains contain about 10 copies of active I factors, while reactive strains are devoid of functional I elements (Finnegan 1989; Bucheton 1990). I factors only transpose at high frequency in the germline of the female progeny, called SF females, resulting from crosses between inducer males and reactive females (Picard 1976). SF females are more or less sterile, and mutations and chromosomal rearrangements occur at high frequency in their germline (Picard et al. 1978). These abnormalities are not observed in the germline of the RSF females that are obtained by crossing inducer females and reactive males. However, I factors transpose in the germline of RSF females albeit about 5 times less frequently than in the germ-line of SF females (Picard 1976). Transposition does not occur in males. The frequency of transposition I factors correlates with a particular state characteristic of the reactive strains and known as reactivity. Reactive mothers can range from strong to weak, as revealed by the proportion of the eggs laid by their SF daughters (obtained by crossing these females with standard inducer males) that remain unhatched. The hatching percentage of the eggs laid by SF females is very low (approximately 0%) if they are descended from strongly reactive mothers, whereas it is high (nearly normal), when they are progeny of weakly reactive mothers (Bucheton et al. 1976; Bucheton and Picard 1978).

Complete, functional I factors are 5.4 kb long and have two large open reading frames (ORFs; Fawcett
et al. 1986; Abad et al. 1989). The protein encoded by ORF1 has three cysteine motifs that would allow nucleic acid binding, suggesting that it could be involved in the formation of a ribonucleoprotein complex required for transposition and/or regulation of the I factor. Potential reverse transcriptase and RNAse H activities are encoded by ORF2.

It has been demonstrated that I factors transpose by reverse transcription of an RNA intermediate (Jensen and Heidmann 1991; Pélisson et al. 1991). A full-length RNA which presumably represents the transposition intermediate, is synthesized specifically during transposition, in amounts that correlate with the frequency of transposition (Chaboisier et al. 1990). It is produced under the control of an internal RNA polymerase II promoter (McLean et al. 1993). The study of the transposition mechanism and its control in flies is difficult because quite a few elements have the potential to transpose during IR hybrid dysgenesis. More detailed studies require I elements tagged by genes giving easily detectable phenotypes. We have constructed an element marked with a reporter gene, the expression of which is dependent on splicing of an intron during retrotransposition, as first described by Heidmann et al. (1988) for retroviruses. The behaviour of this element, in which part of ORF2 was replaced by the vermilion gene, has been studied in transgenic lines. It has been shown to transpose when complemented by actively transposing I factors in the germ-line of SF females.

Materials and methods

Drosophila melanogaster stocks

All strains used in the experiments behave as M strains in the PM system of hybrid dysgenesis (see Engels 1989). The genetic nomenclature follows Lindsley and Zimm (1992). Binscy is a strongly reactive stock, homozygous for the complex inversion In(1)sc11sc85k + d49, marked by the y^a and B mutations. O/O was obtained from crosses between the v e si and ros566 reactive strains. The O/O line contains v and ry566 mutations and is weakly reactive. Cha is a wild-type strongly reactive stock. Cy/Pm is strongly reactive, y^9T, ry566 and v567; Cy0/Sco; ry566 are inducer strains and were constructed by Fridell and Searles (1991).

Construction of the pIviP2 plasmid

The 53-nucleotide sequence of the second intron of the P factor was synthesized in vitro and inserted into the EcoRV site of the vermilion gene cloned in plasmid pYC1.8 (Fridell and Searles 1991). This intron is spliced in all Drosophila melanogaster tissues (Laski et al. 1987) and no external sequences are required for precise splicing. The I element tagged by this gene, called IviP2, was constructed by replacing the SpeI-NcoI fragment of the I factor ORF2 by this artificial vermilion gene interrupted by the intron. In this construct (Fig. 1), the transcriptional orientation of the I factor is opposite to that of the vermilion gene, so that the P intron can be spliced from the transcript initiated from the I factor promoter. The SalI fragment containing the IviP2 element was introduced in the SalI site of the pDm23 transformation vector.

Construction of transgenic lines

P element transformation was performed according to Spradling and Rubin (1982) using the helper plasmid puchsA23. Microinjections were done into embryos of the O/O reactive stock containing ros56 and vermilion mutations. Transgenic lines were established from [v, ry56] G1 flies and maintained by sibling crosses.

Southern blot experiments

Southern blot experiments were carried out as described by Maniatis et al. (1989). Probes were labelled with ^32P according to the procedures described by these authors.

PCR amplification experiments

To study the sequence of the IviP2 element before and after transposition, PCR amplification was carried out on genomic DNAs, using an oligonucleotide (TTGCCACCAACAGATTTGGG) located between positions 1804 and 1823 in the I element sequence reported by Fawcett et al. (1986), and another oligonucleotide (ATAC-TTCTATTCCCCGC) located in the vermilion gene sequence between positions 2356 and 2375 (Searles et al. 1990). These oligonucleotides are therefore located on both sides of the P element intron that interrupts the vermilion gene (see Figs. 1 and 4). PCR amplification of IviP2 produces a 570-bp fragment and should produce a 520-bp fragment containing an EcoRV restriction site after transposition (see below).

Results

A tagged I element was constructed as described in Materials and methods (Fig. 1). This element, called