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Precise and imprecise somatic excision of the transposon Tc1 in the nematode *C.elegans*

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ABSTRACT

Eleven chromosomal products of somatic excision of Tc1 transposable elements have been cloned and sequenced. The cloning method did not involve genetic reversion; therefore the products analyzed should be representative. Six empty religated target sites were from excision of one Tc1 element inserted near actin genes on linkage group V; five were from a second Tc1 element inserted elsewhere on the same linkage group. All six products from the first element were identical in sequence to an empty target site from a second strain, indicating excision had been precise. Two of the products from the second element were also precise, whereas the other three contained four extra nucleotides at the point of excision, indicating an imprecise excision. The four nucleotides are the same in all cases and could represent two terminal nucleotides of the transposon plus a two-nucleotide target site duplication. The difference in the ratio of precise to imprecise excision at the two insertion sites suggests a possible chromosomal position effect on the pathway of Tc1 somatic excision.

INTRODUCTION

Certain eukaryotic transposable elements undergo excision from the chromosomal DNA at significant frequencies. For such elements excision may be the first step of a conservative transposition pathway. Excision can be followed by ligation of the chromosomal target site. The nucleotide sequence of the religated site has been determined for the plant transposable elements Taml of Antirrhinum majus, and Ac/Ds and Spm-I8 of Zea mays (1), and for the FB and P elements of Drosophila (2,3). Religated sites have generally been recovered from revertants of transposon-induced mutations. Nevertheless, excision has usually been found to have been imprecise, and to have left behind or deleted a small and variable number of nucleotides. Religated sites have been obtained without genetic selection for the Spm-I8 element, which undergoes frequent excision in somatic cells. Their structure was found to be similar to products isolated from genetic revertants (1,4).

Tc1 transposable elements are highly-conserved DNA segments of 1610 nucleotides, bounded by perfect 54 nucleotide inverted repeats (5). They are

found in all C. elegans strains examined so far. Tc1 elements undergo excision at low frequency in the germline (6,7), and at much higher frequency in somatic cells (8). Extrachromosomal linear and circular monomer copies of the transposon can be detected that are presumably products of somatic excision (9,10). Religated target sites arising from somatic excision can be detected in preparations of genomic DNA by Southern hybridization using sequences flanking Tc1 elements as probes. By this method, somatic excision has been demonstrated to occur at each of several genomic sites where Tc1 elements have been examined (6-8,11). We report here that excision of Tc1 elements in somatic cells of C. elegans can be both precise and imprecise. The ratio of precise to imprecise events appears to be different for two elements at different chromosomal locations, suggesting that flanking sequences at the insertion site may influence the excision pathway.

MATERIALS AND METHODS

Isolation of Chromosomal Empty Sites

Isolation of the 3.1 kb EcoRI/KpnI fragment containing the excision site of Tc1.1 was accomplished as follows. Twenty micrograms of Bergerac DNA double digested with EcoRI and KpnI was applied across the top of a 0.7% agarose gel and fractionated together with standards as shown in Figure 1. The standards were cut from the gel and stained with ethidium bromide for photographing. The appropriate region of the gel containing unstained Bergerac DNA was cut out and the DNA isolated as follows: electroelution into 1X Tris-borate buffer in a dialysis bag at 10 V/cm for 1-2 hr (12), purification and concentration on a NACS Bio-60 column (Bethesda Research laboratories) or Elutip D (Schleicher & Schuell), ethanol precipitation, and resuspension in 40 microliters of TE buffer (1 mM Tris-HCl, pH 7.5; 1 mM EDTA). The isolated Bergerac restriction fragments were ligated to the sequencing vector pUC18 (13) which was linearized by double digestion with EcoRI and KpnI. The ligation reaction was performed in a solution containing 300 ng of nematode DNA, 50 ng of vector DNA, 50 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol, and 1-5 units of T4 ligase (International Biotechnologies, Inc.). The reaction was allowed to proceed for 2-12 hr at room temperature. E. coli strain JM109 (14), made competent by the standard $CaCl_2$ method (12), was transformed with the ligation mixture (50 ng DNA/1.5 x 10^8 competent cells). Transformed cells were selected by plating on agar containing ampicillin at 50 ng/ml, and colonies containing religated target sites were identified by colony hybridization (15), using

as probe the 3.5 kb EcoRI fragment from Bristol. The probe was gel-purified to eliminate vector sequences, and labeled by nick-translation.

An identical procedure was used to isolate the 155 bp SstI fragment from the Tc1.2 religated site, except that a 1.5% agarose gel was used to fractionate the genomic DNA, and the pUC18 vector was linearized by digestion with SstI. Transformed colonies were screened with gel-purified 7.0 kb BamHI fragment of pCe1001 cloned from Bristol.

DNA Sequencing

Nucleotide sequences of cloned religated target sites were determined by the dideoxy chain termination method (16), using primers homologous to the pUC18 vector. In every case the DNA sequence was determined at least twice; in some cases the sequence of both strands was determined.

RESULTS

Cloning of Chromosomal Empty Sites

We have directly isolated without genetic selection religated target sites from two genomic locations of Tc1 elements in the Bergerac strain, and determined their nucleotide sequence. The two Tc1 elements involved are denoted Tc1.1 and Tc1.2 (11). Both elements are located on linkage group V in the Bergerac strain, and were isolated as DNA polymorphisms between Bergerac and Bristol, which lacks elements at these locations (17,18). The sequence of the Tc1.1 element and its target site, and the sequence of the Tc1.2 target site have been reported (5,19). The sequence of the ends of the Tc1.2 element has been determined starting from 17 nucleotides within one end and 21 nucleotides within the other end of the element (this work), and is identical to that of Tc1.1. Otherwise Tc1.2 has not been sequenced, but it is the same length as Tc1.1 and does not differ from Tc1.1 with respect to any of a number of restriction sites that have been examined.

Somatic excision of Tc1.1 and Tc1.2 is demonstrated by the faint bands on the lanes of Bergerac DNA in Figure 1A and B, where Southern hybridizations of genomic DNA probed with sequences flanking Tc1.1 and Tc1.2 are shown. The flanking probes reveal that the DNA is polymorphic, consisting of a mixture of filled and empty sites. This is due to spontaneous excision of the Tc1 element, which occurs generally in somatic tissues at a frequency of about 5% per generation, and throughout the life cycle (8,11). Restriction fragments containing empty sites were cloned by cutting out the appropriate region of an agarose gel, electroeluting the DNA, ligating the eluted DNA to a sequencing vector, pUC18 (13), and screening the

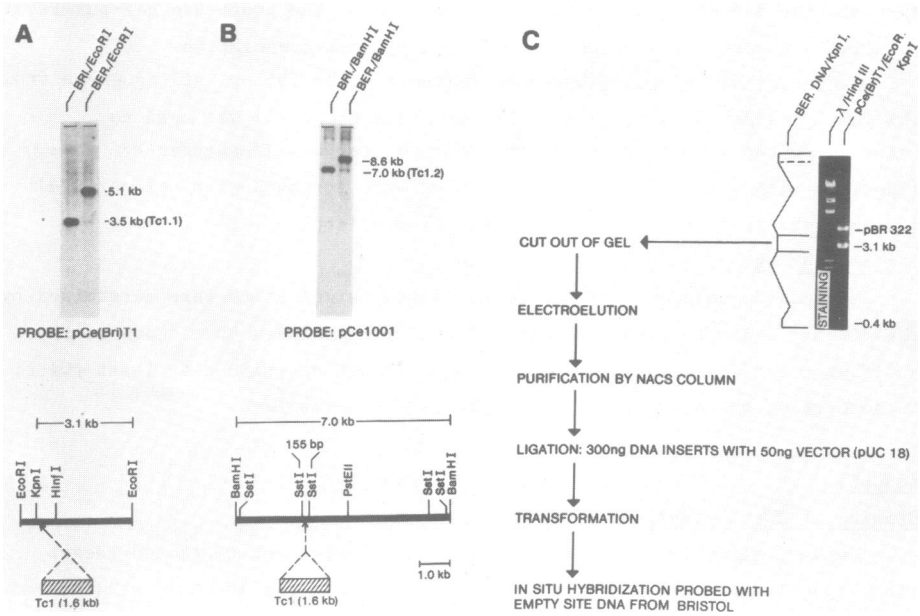

Figure 1. Method of cloning empty target sites. (A) Somatic excision of Tc1.1. Plasmid pCe(Bri)T1 containing a 3.5 kb EcoRI fragment cloned from the Bristol strain (17) was used as a probe in a Southern hybridization of EcoRI-digested genomic DNA from Bristol and Bergerac. Genomic DNA was fractionated on a 0.7% agarose gel and transferred to nitrocellulose for hybridization to the nick-translated probe (12). The 3.5 kb probe hybridizes primarily to a fragment of 5.1 kb in Bergerac, due to the presence of a 1.6 kb Tc1 element. A small amount of 3.5 kb fragment is also observable in Bergerac, due to somatic excision of the element. A restriction map of the 3.5 kb EcoRI fragment is shown. The fragment isolated for sequencing was a 3.1 kb KpnI/EcoRI fragment spanning the target site. (B) Somatic excision of Tc1.2. A similar Southern hybridization is shown for Tc1.2, using as a probe plasmid pCe1001 containing a 7.0 kb BamHI Bristol fragment (18). The 155 bp SstI fragment spanning the insertion site and shown on the restriction map was isolated from Bergerac for sequencing. (C) Outline of the strategy for cloning chromosomal empty sites described under MATERIALS AND METHODS.

partial genomic library with the flanking sequence probe (Figure 1C).

DNA Sequence Comparison of Empty Sites with Corresponding Filled Sites

The nucleotide sequence of 11 religated excision sites isolated in this way is shown in Figure 2. Each site was isolated from DNA of a separate population of Bergerac worms grown from a single self-fertilizing hermaphrodite, to ensure that independent excision events were analyzed. In view of the low frequency of germline excision in this strain (10^{-3} to 10^{-5}) (6,7), the overwhelming likelihood is that these DNA fragments arose from

A. TC1.1

FILLED SITE BERGERAC	AAATTTTTCAAATACAGTGC GCACTGTATATGTGCTACA
EMPTY SITE BERGERAC CLONE 1	AAATTTTTCAAATA---------- TATGTGCTACA
2	AAATTTTTCAAATA---------- TATGTGCTACA
3	AAATTTTTCAAATA---------- TATGTGCTACA
4	AAATTTTTCAAATA---------- TATGTGCTACA
5	AAATTTTTCAAATA---------- TATGTGCTACA
6	AAATTTTTCAAATA---------- TATGTGCTACA
CORRESPONDING SITE BRISTOL	AAATTTTTCAAATA---------- TATGTGCTACA

B. Tc1.2

FILLED SITE BERGERAC	ATAGTACATATTTACAGTGC GCACTGTATGTACTTACAG
EMPTY SITE BERGERAC CLONE 1	ATAGTACATATTTA--------- TGTATGTACTTACAG
2	ATAGTACATATTTA---------- TGTACTTACAG
3	ATAGTACATATTTA---------- TGTACTTACAG
4	ATAGTACATATTTA--------- TGTATGTACTTACAG
5	ATAGTACATATTTA--------- TGTATGTACTTACAG
CORRESPONDING SITE BRISTOL	ATAGTACATATTTA---------- TGTACTTACAG

Figure 2. DNA sequence comparisons. (A) Comparison of religated target sites from Bergerac, formed by excision of Tc1.1, to the corresponding filled site from Bergerac and empty site from Bristol (5). Transposon sequences are boxed; the putative target site duplication is double underlined. The alignment of the single TA dinucleotide in the empty sites with the left-hand TA of the filled site is arbitrary. (B) Similar comparison for religated target sites of Tc1.2. Extra nucleotides in three of these sequences are aligned to show their identity to the two terminal nucleotides of the 3' end of the transposon (TG) and the two bases of the target site duplication (TA).

somatic excision events. The sequences of the empty sites from Bergerac are compared to the sequence of the corresponding filled site from Bergerac and the empty site from Bristol (5,19).

Six empty sites produced by excision of Tc1.1 in Bergerac are identical in sequence to the corresponding empty site from Bristol, and therefore resulted from precise excision. One copy of a pair of TA dinucleotides flanking the Tc1 element in the filled site is absent from the excision products. The origin of these TA dinucleotides is ambiguous. All Tc1 elements so far examined at the nucleotide level have inserted at a TA dinucleotide and are flanked by a pair of TA dinucleotides. One interpretation of the flanking TA dinucleotides is that they represent a duplication of the target site TA. A second interpretation is that one T and A are part of the element, and Tc1 duplicates no target site sequences. The ambiguity can

only be resolved if a Tc1 element inserted at a sequence other than TA is found.

Two empty sites from Tc1.2 excision also have the same sequence as the corresponding empty site from Bristol, and are therefore the products of precise excision. The remaining three, however, have four extra nucleotides left at the site of excision, indicating an imprecise excision. The four extra nucleotides, TGTA, are identical in each case and are the same as the last two nucleotides of the 3' end of the Tc1 element (TG) plus the putative target duplication (TA).

DISCUSSION

Both precise and imprecise excision products have also been isolated for P elements of *Drosophila* (3). In this case as well, the structure of the products of imprecise excision events could be accounted for by assuming that nucleotides from the target site duplication (8 nucleotides in length) and from the ends of the transposon are variably left behind at the excision site. A somewhat different interpretation was required for the results obtained with plant transposable elements, where extra nucleotides found in religated target sites could only be accounted for by assuming that sequences in the target site had been duplicated upon excision of the element (1).

The ratio of precise to imprecise excision of Tc1 appears to be different at the two genomic locations we have examined. Sequence analysis of additional excision products is required to confirm this conclusion. Possibly the nucleotides at the insertion site influence the detailed pathway of excision and the resolution or repair of its products. Apparent position effects on the behavior of transposable elements in maize have long been known from genetic observations (20). Muller-Neumann *et al.* (21) have proposed that the differences in the timing and frequency of excision of two Ac elements in maize is caused by differences in flanking sequences. Tc1-induced mutations in the *unc-54* and *unc-22* loci of the same strain of *C. elegans* undergo germline reversion at very different frequencies (6,22). Although the frequencies of somatic excision of Tc1 at five examined genomic sites showed no significant differences (11,23), a position effect on the pattern of excision products is suggested here.

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