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Mobilization of quiet, endogenous Tc3 transposons of *Caenorhabditis elegans* by forced expression of Tc3 transposase

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The commonly studied *Caenorhabditis elegans* strain Bristol N2 contains ~15 copies per genome of the transposon Tc3. However, Tc3 is not active in Bristol N2. Tc3 contains one major open reading frame (*Tc3A*). We have fused this open reading frame to an inducible promoter and expressed it in a transgenic Bristol N2 line. Tc3A expression resulted in frequent excision and transposition of endogenous Tc3 elements. This shows that the Bristol N2 genome contains Tc3 transposons that are *cis* proficient for transposition, but are immobile because Tc3A is absent. We demonstrate that recombinant Tc3A binds specifically to the terminal nucleotides of the Tc3 inverted repeat, indicating that Tc3A is the Tc3 transposase. Activation of Tc3 transposition *in vivo* was accompanied by the appearance of extrachromosomal, linear copies of Tc3. These may be intermediates in Tc3 transposition.

Key words: Caenorhabditis elegans/Tc3/transposase/transposition

Introduction

The genome of the most commonly studied *C.elegans* strain, Bristol N2, contains ~15 copies of the Tc3 transposon per haploid genome (Collins *et al.*, 1989). Tc3 is 2335 bp long and has inverted repeats of 462 bp at its ends. All DNA transposons analyzed to date, or at least the autonomous versions of them, encode their own transposase, i.e. a DNA binding protein that carries out transposon specific steps in the spread of the element. From the sequence of Tc3 it is presumed that the transposase is encoded by a two-exon open reading frame of 987 nucleotides, found within Tc3 (positions 719-1135 and 1184-1753; P.Anderson, personal communication). This open reading frame is interrupted by a 48 bp intron which is removed in a fraction of the *Tc3A* mRNA (our unpublished results).

No excision or transposition of Tc3 has been detected in Bristol N2, either between generations (in the germ line) or in the soma. However, a 'high hopper' strain (Bergerac derivative TR679), selected for high transposition activity of the Tc1 transposon (Collins *et al.*, 1987), was shown to exhibit transposition of the Tc3 element (Collins *et al.*, 1989). The molecular basis for the activation of Tc1 and Tc3 transposition in this strain is not understood.

The presence of seemingly intact but nevertheless inactive copies of Tc3 in Bristol N2 is not unprecedented: it has previously been shown that the transposon most active in high hopper strains, Tc1, is also present in Bristol N2, but inactive in the germ line (Moerman and Waterston, 1984; Eide and Anderson, 1985). Possible explanations for the presence but inactivity of Tc3 in the Bristol N2 genome are: (i) the elements are *cis* deficient, i.e. they do not contain the sequences that are needed for proper recognition by the transposition machinery; (ii) the elements are *trans* deficient, i.e. they encode an inactive transposase; (iii) the elements are not properly transcribed at the right moment for transposase to be made and transposition to occur; (iv) the elements are inaccessible to transposase, e.g. as a result of chromatin structure, or as a result of regulatory proteins bound to the transposon; or (v) other proteins that are essential for the transposition reaction might be absent.

To discriminate between these possibilities we fused the presumed transposase gene (*Tc3A*) of a Tc3 element to a heat shock promoter and generated transgenic derivatives of Bristol N2. We found that *Tc3A* expression resulted in excision and transposition of endogenous Tc3 elements. From this we conclude that at least some of the resident Tc3 copies in Bristol N2 are *cis* proficient and accessible to transposase, and that Tc3 transposase is the limiting factor for transposition activity in this strain.

Results

Generation of transgenic C.elegans lines expressing Tc3A

We forced the expression of the putative Tc3 transposase in *C.elegans*. High expression of Tc3A might be detrimental for the animal due to the generation of many mutations. This has already been shown in the high hopper strain TR679 in which a high level of Tc1 transposition leads to fewer progeny and many mutant phenotypes (Collins *et al.*, 1987). *Tc3A* was therefore put under the control of an inducible promoter, the *hsp-16* promoter, which contains the necessary sequences for heat inducible gene expression in the soma (Stringham *et al.*, 1992). The *hsp-16/Tc3A* construct was injected into the gonads of Bristol N2, together with the visible marker *rol-6*^{D} (Kramer *et al.*, 1990). Transgenic lines were obtained which contain the *hsp-16/Tc3A* construct as part of an extrachromosomal array (as confirmed by Southern blot analysis, data not shown).

RNA isolated from transgenic lines contained an abundant 1.1 kb transcript which was Tc3 specific and only detectable after induction (Figure 1A). The level of expression differs between the transgenic lines because of differences in stability of the extrachromosomal DNA, and the contribution of the *hsp-16/Tc3A* construct to the extrachromosomal array. Tc3A polyclonal antisera (generated against synthetic peptides) did not detect the expression of the Tc3A protein (calculated molecular weight 38 kDa) before induction (Figure 1B, lane 1). After a 90 min heat shock (at 33°C) the Tc3A protein was detected; it rapidly increased during the

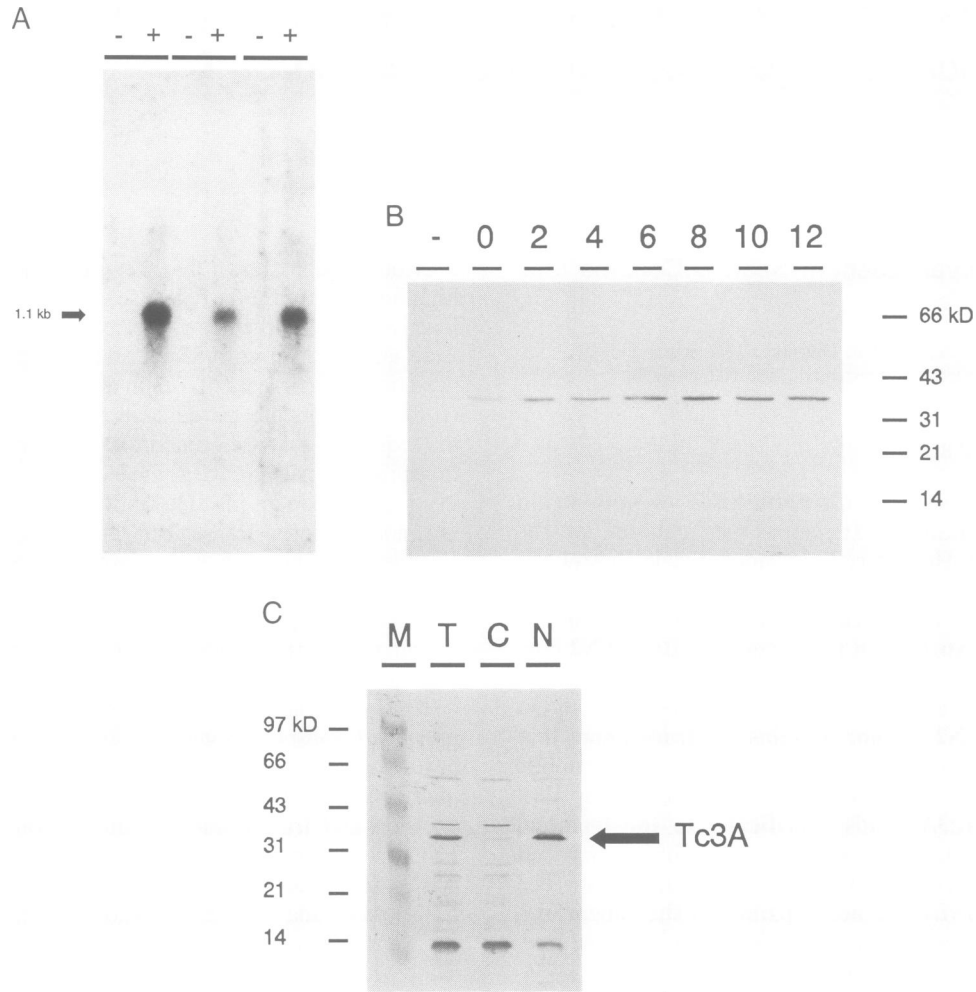

Fig. 1. Expression of Tc3A in transgenic animals after induction. (**A**) 2 μg total RNA from three independent *hsp-16/Tc3A* transgenic lines was analyzed on a Northern blot using a Tc3 specific probe (see Materials and methods). For each line RNA was isolated from heat shocked (+) and non-heat-shocked (−) cultures. (**B**) From one transgenic line several cultures were started. These were heat shocked for 2 h and subsequently kept at room temperature to allow them to recover. At the time-points indicated (in hours) above each lane, proteins were extracted and analyzed on a Western blot using a polyclonal antiserum directed against the C-terminal 13 amino acids of Tc3A. Similar results were obtained using an antiserum directed against the N-terminal peptide. Lane 1 contains an extract made before the heat shock and lane 2 an extract made directly after induction. (**C**) The cellular localization of Tc3A was determined. Transgenic animals were heat shocked for 2 h and allowed to recover at room temperature for 2 h. Animals were fractionated to give a cytoplasmic and a nuclear extract. These were analyzed on a Western blot using the polyclonal antiserum directed against the N-terminal peptide (T: total protein extract, C: cytoplasmic extract, N: nuclear extract, M: marker). The polyclonal antiserum directed against the N-terminal peptide has more cross-reactivity with other proteins than the polyclonal antiserum directed against the C-terminal peptide.

Table I. Survival of individual animals after expression of Tc3A

Strain	Adult		L2 larvae	
	No heat shock	With heat shock	No heat shock	With heat shock
Bristol N2	10	10	10	10
hsp-16/Tc3A transgenic	10	10	10	1
hsp-16/Tc3A transgenic	10	10	10	2

Ten adult animals and 10 L2 larvae from three cultures (the non-transgenic Bristol N2 and two independent transgenic lines) were placed on separate plates. The rest of the cultures were then incubated for 2 h at 33°C. After the induction 10 adults and 10 L2 larvae from each culture were also put on separate plates. The development of the heat shocked and non-heat-shocked animals and the production of offspring were monitored. Plates were scored positive for survival when the animals developed normally and produced offspring and negative when the animals did not develop normally and produced no or very few offspring (up to five larvae).

remaining 30 min of heat shock (Figure 1B, lane 2) and the subsequent 6 h of recovery at 20°C. The Tc3A protein level remained stable for at least 12 h after the heat shock. Tc3A protein was found in the nucleus and not in the cytoplasm, as expected for a transposase (Figure 1C).

Three functional assays were performed to determine

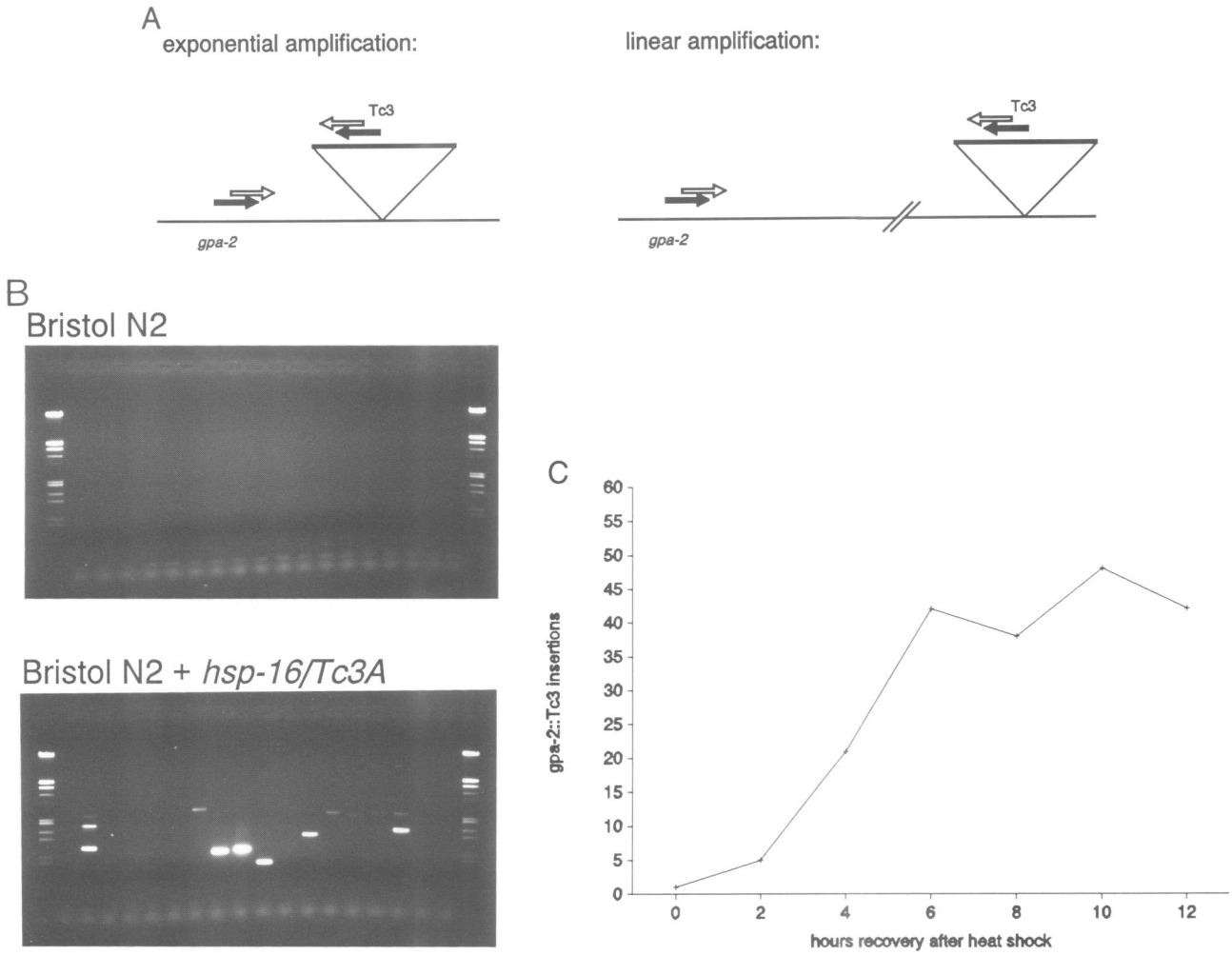

Fig. 2. Tc3 insertions in *gpa-2* after the induction of Tc3A. (**A**) The method of *gpa-2*::Tc3 detection is schematically depicted. When a Tc3 element inserts in the vicinity of *gpa-2* the region between the *gpa-2* primer (closed arrow) and the Tc3 primer (closed arrow) is exponentially amplified in a PCR. Only linear amplification occurs if Tc3 has not integrated in the vicinity of *gpa-2*. To increase the specificity and the sensitivity so that we could detect a single integration event, a second PCR was performed with nested primers (open arrows) using 1 μl of a 100-fold dilution of the first PCR. Tc3 insertions in both orientations are detected because the Tc3 primers are located in the inverted repeat. (**B**) DNA from the non-transgenic Bristol N2 and a *hsp-16/Tc3A* transgenic line was isolated after the cultures were heat shocked and allowed to recover for 2 h. In the first PCR 300 ng genomic DNA was used in 18 independent reactions. After the second PCR the samples were analyzed on a 1% agarose gel. The first and the last lane of each gel contain a DNA size marker (λ DNA digested with *Eco*RI and *Hin*dIII). (**C**) The number of insertions of Tc3 in *gpa-2* was determined after prolonged recovery at room temperature after a 2 h heat shock. For each time point 20 samples of 100 ng genomic DNA were analyzed as described in (A).

whether the Tc3A protein was active and led to Tc3 transposition: (i) animal survival, (ii) transposition of Tc3 and (iii) excision of Tc3.

Tc3A expression impairs larval development

Expression of active Tc3A protein could result in chromosomal breaks due to excision of Tc3 elements, and mutations due to new insertions of the Tc3 elements. This could reduce the survival of the animals. Three lines were analyzed for survival after Tc3A expression: non-transgenic Bristol N2, and two independent *hsp-16/Tc3A* transgenic lines. As shown in Table I, L2 larvae in particular were very sensitive to Tc3A production. The transgenic L2 larvae did not develop normally as a result of Tc3A expression. No phenotypic effect of Tc3A production was seen in adult animals.

Tc3A expression results in frequent somatic transposition

To study the effect of Tc3A production on Tc3 transposition more directly we determined the frequency of new Tc3 insertions in a randomly chosen gene. We used the *gpa-2* gene, which encodes the α subunit of a G protein (Fino Silva and Plasterk, 1990). Tc3 insertions in *gpa-2* were detected by PCR analysis. Two rounds of PCR, using nested primers,

Table II. The number of Tc3 insertions in *gpa-2* after induction of Tc3A

Strain	Treatment	*gpa-2*::*Tc3*[a]
Bristol N2	2 h heat shock + 2 h recovery	0
hsp-16/Tc3A	none	4
hsp-16/Tc3A	2 h heat shock	4
hsp-16/Tc3A	2 h heat shock + 2 h recovery	13
hsp-16/Tc3A transgenic segregants	2 h heat shock + 2 h recovery	11
hsp-16/Tc3A non-transgenic segregants	2 h heat shock + 2 h recovery	0

[a]The number of Tc3 insertions in *gpa-2* in 18 DNA samples each containing 300 ng genomic DNA in the first PCR.

allowed detection of single DNA molecules of *gpa-2* with a transposon insertion (Figure 2A). Following a 2 h heat shock (33°C) and a 2 h recovery period (20°C), DNA was isolated from both Bristol N2 and an *hsp-16/Tc3A* transgenic line. Figure 2B shows typical results after PCR detection of insertions. In 18 DNA samples from the Bristol N2 strain we found no insertions. This indicates that there is no detectable somatic Tc3 transposition activity in Bristol N2 [it has been reported previously that there is also no germ line transposition (Collins *et al.*, 1989)]. In 18 samples taken from the pooled DNA from the transgenic strain we found 13 insertions of Tc3 in *gpa-2*. Although the PCRs were performed on aliquots of the pooled DNA, the PCR products represent independent events because the *hsp-16/Tc3A* construct is only expressed in the soma upon induction and DNA was isolated within a few hours after the induction. In non-transgenic segregants (progeny of a transgenic animal in which the extrachromosomal transgenic DNA has been lost) of the *hsp-16/Tc3A* line, no insertions were found, whereas in transgenic segregants 11 insertions were detected after induction (Table II). This confirms that the presence of the *hsp-16/Tc3A* construct is necessary for Tc3 transposition and that the insertions are not a consequence of the heat shock.

Tc3 *gpa-2*

5'	tcccacactg**TA**TATCTAAACT	3'
	tcccacactg**TA**TAATAATGAA	
	tcccacactg**TA**TGACATTAAT	
	tcccacactg**TA**TTCTTATCGC	
	tcccacactg**TA**TTTGTCCTAT	
	tcccacactg**TA**CTAATATTAT	
	tcccacactg**TA**TTATCCTTTT	
	tcccacactg**TA**CTTTTCAATT	
	tcccacactg**TA**CGTGCACGAA	

Fig. 3. Sequence analysis of Tc3 insertions in the *gpa-2* gene. Nine insertions were randomly chosen and sequenced. The terminal nucleotides of the inverted repeat of Tc3 (lower case), the *gpa-2* sequence downstream of the insertion site (capitals) and the target site (TA) are indicated.

The transgenic line was also analyzed before induction of Tc3A, and four *gpa-2*::Tc3 insertions were found (Table II). This could be explained if there was a low level of expression of Tc3A from the *hsp-16* promoter without induction. Using a *hsp-16/lacZ* reporter construct we have observed some expression of *lacZ* without induction in early embryos (data not shown). Directly following a 2 h heat shock, no increase in *gpa-2*::Tc3 insertions was found (compared with the non-heat-shocked animals), although the Tc3A protein has been synthesized at this point (see Figure 1B, lane 2). An increase in Tc3 insertions (13 insertions) was detected only after an additional recovery period. Apparently some time is needed after the expression of Tc3A for new insertions to occur.

We extended the recovery period to study the time dependent increase of insertions after the heat shock. As shown in Figure 2C, the number of Tc3 insertions increased up to 6 h after the heat shock. No increase in the number of insertions was found in the period between 6 and 12 h after induction, even though the level of Tc3A protein remains high in this period (Figure 1B).

Nine PCR products were sequenced; they were all found to correspond to genuine Tc3 insertions in *gpa-2* (Figure 3). The complete terminal sequence of Tc3 was present and all of the insertions had taken place at the dinucleotide TA [the target site for Tc3 insertion (Collins *et al.*, 1989)]. We conclude from these results that Tc3A overexpression leads to correct Tc3 insertions. These insertions are probably restricted to the soma since the *hsp-16* promoter is only active somatically (Stringham *et al.*, 1992). We have found no evidence for Tc3 induced mutations in the progeny of heat shocked transgenic animals [as monitored by screening for *unc-22* mutants (Moerman and Baillie, 1978)].

Tc3A expression results in the appearance of extrachromosomal, linear Tc3 elements

Genetic analysis of Tc1 transposition has indicated that excision of Tc1 is initiated by double strand DNA breaks at the ends of Tc1 (Plasterk, 1991). Tc1 excision in the germ

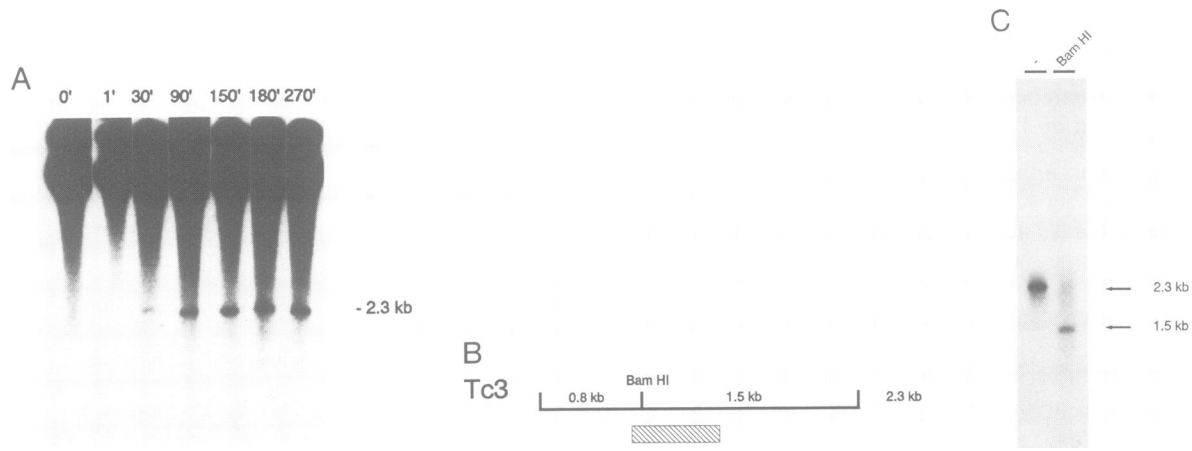

Fig. 4. Expression of Tc3A results in the appearance of linear, extrachromosomal Tc3 elements. (**A**) Several cultures were started from an *hsp-16/Tc3A* transgenic line. These were heat shocked for 2 h and allowed to recover at room temperature for various periods indicated above each lane in minutes. 2 μg genomic DNA isolated from these cultures was analyzed on a Southern blot using a Tc3 specific radiolabelled probe (see Materials and methods). (**B**) The Tc3 specific 2.3 kb band was isolated from the gel and digested with *Bam*HI, which has one recognition site within Tc3. The probe used to hybridize to the Southern blot depicted in panel C is indicated by the dashed box. (**C**) Undigested and *Bam*HI digested DNA was again analyzed on a Southern blot using the probe described in (B).

line is followed by template dependent double strand break repair. In accordance with this, linear and circular extrachromosomal Tc1 elements have been detected in strains with a high germ line transposition frequency (Rose and Snutch, 1984; Ruan and Emmons, 1984). We analyzed whether Tc3A expression results in excision of Tc3 and thus in the appearance of extrachromosomal, excised Tc3 elements. Extrachromosomal Tc3 has not been observed before in any strain.

Genomic DNA was isolated from an *hsp-16/Tc3A* transgenic line at various timepoints after a 2 h heat shock period. DNA was size fractionated on an agarose gel and transferred to nitrocellulose. With a Tc3 specific probe, strong hybridization was found at the position of high molecular weight DNA (Figure 4A). This represents Tc3 elements integrated in the genome and the *Tc3A* gene present on the extrachromosomal array. In DNA samples from heat shocked animals, a second band of ~2.3 kb appeared. The band increased in intensity upon prolonged recovery at 20°C. The size and the specific hybridization of the band with the Tc3 probe are consistent with it being linear, excised Tc3. After prolonged recovery (≥6 h) other extrachromosomal Tc3 specific bands were detected. These may represent other topological forms of the excised Tc3 element (data not shown).

Nuclear and cytoplasmic fractions were analyzed for the presence of excised copies of Tc3. The extrachromosomal Tc3 elements were found in the nucleus (where they are generated) and not in the cytoplasm (data not shown).

The amount of extrachromosomal Tc3 was estimated by comparison with genomic DNA that had been digested with *Bgl*II, which does not cut within the element (Figure 5).

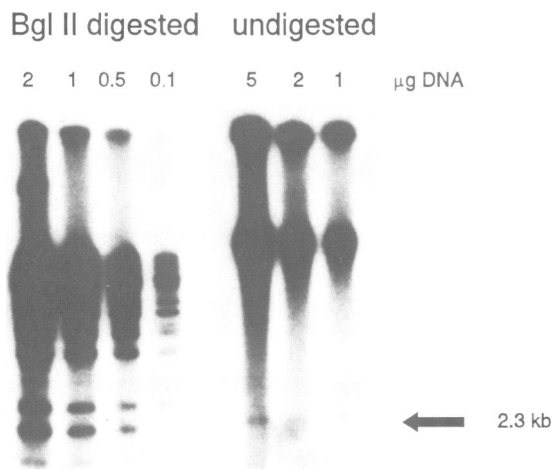

Fig. 5. Determination of the number of extrachromosomal Tc3 elements per cell. After 2 h heat shock and 2 h recovery, DNA from a transgenic line was isolated. Undigested and *Bgl*II digested DNA was analyzed on a Southern blot using a Tc3 specific probe (see Materials and methods). *Bgl*II has no recognition site within Tc3. The hybridization signal of the extrachromosomal Tc3 using 5 µg of DNA is comparable to a band of similar intensity and length representing two alleles (diploid genome) when 0.5 µg genomic DNA is digested with *Bgl*II. 50% of the animals from the culture analyzed were transgenic.

Taking into consideration the percentage of transgenic animals in the population, we estimated the average number of extrachromosomal Tc3 copies per transgenic cell to be ~0.4 after a 2 h recovery period. This is most probably an underestimation of the actual number of excised elements. The number of linear extrachromosomal Tc1 elements has been estimated to be between 0.1 and 1 element per cell in the strain Bergerac (Ruan and Emmons, 1984).

The structure of the predominant form of the excised Tc3 was analyzed by digestion with *Bam*HI, which has one recognition site within Tc3 (Figure 4B). A single band of 1.5 kb was detected using a fragment of Tc3 as a probe (Figure 4C). This indicated that the extrachromosomal Tc3 was present mainly as a linear fragment (if excised Tc3 was circular, we would have expected a band of 2.3 kb) and that the excised Tc3 element is co-linear with the integrated element. The restriction analysis indicates that Tc3 is excised by DNA double strand breaks at the border between Tc3 and the genomic sequence. This linear form of Tc3 appeared after only 30 min of recovery. Two other bands, which appeared much later, may be circular forms of Tc3 which could result from the ligation of the two ends of the linear Tc3 element or from intramolecular transposition.

Tc3A binds specifically to the terminal sequences of the inverted repeat of Tc3

To investigate whether Tc3A interacts directly with Tc3 we determined the binding characteristics of recombinant Tc3A. The *Tc3A* gene was fused to the maltose binding protein (MBP) gene and purified from *Escherichia coli* according to Guan *et al.* (1987). The MBP−Tc3A protein was

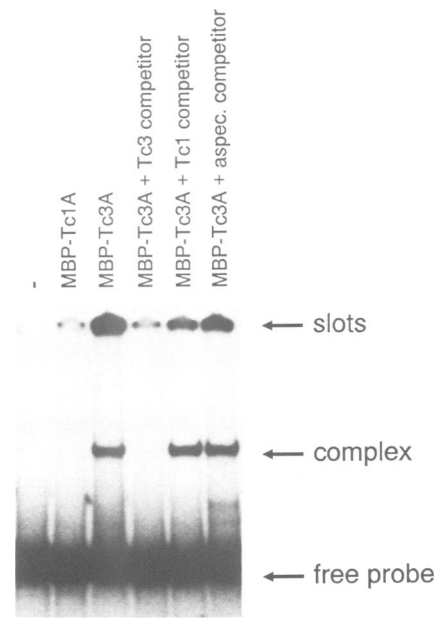

Fig. 6. Specific binding of Tc3 transposase to a DNA fragment containing Tc3 inverted repeat sequences. A 91 bp fragment containing the 40 terminal nucleotides of Tc3 was end-labelled with ^{32}P and used in band-shift experiments. The fragment was incubated in a volume of 10 µl with 1 µl of MBP−Tc1A (lane 2) or with 1 µl MBP−Tc3A (lanes 3−6). Lane 1 contains no added protein. Lanes 4, 5 and 6 contain a 5000-fold excess of unlabelled double stranded oligonucleotide as competitor DNA. Lane 4 contains a 40mer representing the 40 terminal nucleotides of Tc3. Lane 5 contains a 28mer representing the 28 terminal nucleotides of Tc1. Lane 6 contains a randomly chosen double stranded 28mer.

incubated with a radiolabelled fragment containing the terminal 40 nucleotides of the Tc3 inverted repeat. Protein–DNA complexes were resolved by gel electrophoresis. A specific retarded complex was detected (Figure 6). The formation of this complex could be competed by the addition of an excess of unlabelled Tc3 inverted repeat fragment, but not by an excess of unlabelled Tc1 inverted repeat fragment or a random sequence. An MBP–Tc1A fusion protein, purified in the same way, did not bind the Tc3 inverted repeat fragment. This shows that Tc3A binds specifically to the terminal part of the inverted repeat of Tc3. Similar results have been obtained for the Tc1 transposon for which it has been shown that the Tc1 transposase binds to the inverted repeat of Tc1 (J.C.Vos and R.H.A.Plasterk, manuscript in preparation).

Discussion

Tc3A is the limiting factor for Tc3 transposition in Bristol N2

We have shown here that expression of Tc3A *in vivo* results in the excision of Tc3 elements and in new Tc3 insertions. The Tc3A protein binds specifically to the Tc3 inverted repeats. Tc3A therefore has the properties expected for the Tc3 transposase.

The limiting factor for Tc3 transposition in Bristol N2 is Tc3 transposase, since transposition can be induced by expression of this protein. If other factors are needed for Tc3 transposition, they must already be present. However, RNase protection experiments indicate that Bristol N2 contains the same amount of *Tc3A* mRNA as is found in the transposition proficient strain TR679. The ratio of unspliced to spliced mRNA is also equal in TR679 and in Bristol N2 (unpublished results). Either the transposase protein is not produced in Bristol N2 (even though we found *Tc3A* transcripts in Bristol N2) or it is produced at the wrong time or the wrong place. We have not been able to detect the Tc3A protein in either Bristol N2 or TR679. This is not so surprising, since transposases are generally expressed at a very low level.

Regulation of Tc transposition

Tc1 transposition is subject to strict regulation. Transposition activity is tissue specific: Tc1 transposition in Bristol N2 is limited to the soma and does not occur in the germ line (Moerman and Waterston, 1984). Also, strains in which Tc1 is not actively transposing in the germ line (such as the Bristol N2 derivative CB30) can spontaneously become transposition proficient (CB4000, Babity *et al.*, 1990; J.Hodgkin, personal communication).

Germ line transposition of Tc1 has been correlated with the presence of a genetic locus termed *mut* (for mutator) (Collins *et al.*, 1987). One of these *mut* loci seems to coincide with a Tc1 element (Mori *et al.*, 1988). This is in agreement with the observed mobility of the *mut* locus itself. Our current model for the regulation of transposition assumes that a Tc element is inserted in the vicinity of an enhancer or promoter driving the expression of the transposase. If this promoter or enhancer is active in the germ line, germ line transposition will occur. If it is only active in somatic cells (which seems to be more common), only somatic activity follows (as for Tc1 in the Bristol N2). Spontaneous conversion from a transposition deficient strain to a transposition proficient strain could then be explained if a transposable element was brought into the vicinity of suitable regulatory sequences by a mutation, a rearrangement, or perhaps transposition after spurious transcription of a transposase gene in the germ line. A strain could also lose its transposition activity if the element that provides the transposase is lost after excision or is dissociated from the regulatory sequences required for activity. A similar model for the regulation of transposition has been suggested for the mariner element in *Drosophila* (Hartl, 1989). In our transgenic lines we force the expression of the Tc3A transposase from an extrachromosomal array, resulting in high levels of Tc3A. This circumvents the need for regulatory sequences in the vicinity of an endogenous insertion site of Tc3, and leads to high transposition activity of endogenous transposons.

For Tc3 transposition a second *mut* locus, *mut*-2, is important (Collins *et al.*, 1987). The nature of this locus is not known but *mut*-2 also leads to enhanced Tc1 transposition. High expression of Tc3A alone (which might have been induced by *mut*-2) cannot explain the elevated Tc1 activity in TR679 because Tc3A does not act on Tc1 elements. We could not detect extrachromosomal Tc1 elements or elevated Tc1 transposition after induction in the *hsp-16/Tc3A* transgenic lines (data not shown). This is not surprising since Tc3A does not bind to the inverted repeat of Tc1 (data not shown and Figure 6). Therefore we think that *mut*-2 is a mutated host factor: leading to a very high Tc1 activity and raising the Tc3 transposition activity above the detection limit.

Mechanism of Tc3 transposition

All well characterized transposons (including retroviruses and retrotransposons) appear to transpose by rather similar mechanisms (reviewed in Mizuuchi, 1992). Transposition occurs in three steps: first, cleavage occurs at the transposon ends to produce a 3′ OH terminus; then strand transfer occurs, where both 3′ OH groups produced in the first step are joined to 5′ phosphates of the target site; finally the process is finished by the host repair and synthesis machinery. Transposons differ in the first step described above. Some transposases, such as Mu transposase and retroviral IN proteins, make only a single strand cut at the transposon ends (Pato, 1989; Varmus and Brown, 1989; Mizuuchi, 1992). Others, such as the Tn*10* and Tn*7* transposases, cut both strands (Bainton *et al.*, 1991; Benjamin and Kleckner, 1992). These double strand cuts generate a linear transposition intermediate and leave a double strand break in the donor molecule. A similar mechanism has been proposed for P element transposition. Double strand breaks are left in the donor DNA *in vivo* (Engels *et al.*, 1990) and linear P elements can transpose *in vitro* (Kaufman and Rio, 1992), although no intermediate has been detected *in vivo*.

Excised transposons can also have a circular form. The excised Tec1 element from the ciliated protozoa *Euplotes* has a circular form (Tausta *et al.*, 1991). Similar structures have been found during transposition in maize (Sundaresan and Freeling, 1987). It is not known whether these excised elements can reintegrate.

Excised (linear and circular) Tc1 molecules are detected in strains in which Tc1 is actively transposing (Rose and Snutch, 1984; Ruan and Emmons, 1984). Genetic evidence

shows that double strand breaks are left in donor sequences when Tc1 excises. These breaks can be repaired by template directed repair using the sister or homologous chromosome (Plasterk, 1991). Here we have shown that excised, linear Tc3 molecules are produced when Tc3 transposition is induced. This is consistent with a model in which Tc3 transposase makes double strand breaks at the ends of Tc3 elements. The excised Tc3 elements would then be inserted into a target sequence somewhere in the genome.

It is striking that the increase in *gpa-2*::Tc3 insertions (Figure 2C) is seen only during the period in which Tc3A increases (the first 6 h of recovery after the heat shock; Figure 1B). Although the transposase is still present during the remaining recovery period we detect no increase in the number of *gpa-2*::Tc3 insertions. This could mean that either (i) the protein is present but only active for a short time after synthesis, (ii) the protein remains bound to the transposon after transposition preventing the transposase from mobilizing other elements, (iii) the transposase is not able to catalyze multiple transpositions or (iv) all accessible copies of Tc3 have been excised.

The assays that we have developed for the Tc3A transposase (specific binding to transposon DNA, excision and reintegration) can now be used for a structure−function analysis of the enzyme. The Tc1 and Tc3 integration target is essentially random, but always at a TA dinucleotide. It will be interesting to study how the transposase recognizes this short target sequence. Some other elements have been described which have the same short target sequence (e.g. the pogo element from *Drosophila*; Tudor *et al.*, 1992). Another unusual feature of Tc3 is the long stretch (462 bp) of almost perfect inverted repeats found at the transposon ends. With the *in vivo* and *in vitro* assays described here we can now study the importance of these inverted repeats for the transposition reaction.

Materials and methods

Generation of transgenic animals

Transgenic animals were obtained after co-injection (Mello *et al.*, 1991) of 200 μg/ml *rol-6^{D}* (pRF4) (Kramer *et al.*, 1990) and 5 μg/ml of the *hsp-16/Tc3A* construct into the gonads of Bristol N2 animals. The *hsp-16/Tc3A* construct was made by cloning the *Mae*I fragment from TR # 10 (Collins *et al.*, 1989) containing Tc3 except for the inverted repeats, into the *Sma*I site of pGEM-3Zf(+) (Promega) to give plasmid pRP714. The intergenic region between *hsp16-1* and *hsp16-48* (positions 1018−1398; Stringham *et al.*, 1992) was amplified by PCR. PCR primers (AB2096, 5′-ggctcgagcattcttgaagtttag; AB2097, 5′-ccgctcgaggctggacggaaatagtgg) were designed in such a way that an *Xho*I site was added at both ends of the intergenic region. The ATG of the *hsp16-48* was then fused in-frame to the open reading frame of Tc3 using an *Xho*I site within *Tc3A*, without the addition or deletion of amino acids of the Tc3A protein, to give pRP716. The sequence of Tc3 from TR # 10 (a clone from TR679) is identical to the sequence of the Tc3 element found on cosmid CELB0303 (derived from Bristol N2) (Sulston *et al.*, 1992; accession number M77692) except for the following point mutations in the coding region: T840 to A; G1354 to C; C1355 to G; A1439 to G and T1715 to A.

Transgenic and non-transgenic lines were maintained at 18°C. Expression of Tc3A in *hsp-16/Tc3A* transgenic lines was induced by incubating the plates for 2 h at 33°C in a humidified incubator, after which the plates were kept at room temperature (~20°C).

DNA analysis

Nematode DNA was isolated as described by Sulston and Hodgkin (1988). Digestion, Southern blotting and hybridization were performed as described by Sambrook *et al.* (1989). The probe used in the hybridization of Northern and Southern blots was the Tc3 internal *Pvu*II fragment isolated from TR # 10 (Collins *et al.*, 1989) unless specified differently in the figure legends. The probes were radiolabelled by random priming (Feinberg and Vogelstein, 1984).

Tc3 insertions in *gpa-2* were detected by two rounds of PCR. In the first reaction 100 or 300 ng genomic DNA was used in combination with a *gpa-2* specific primer (AB3172, 5′-gcaactgctcgaaacctccgcaac) and a Tc3 specific primer (AB2745, 5′-cttgaaacagacttgaacatcaaagatcgg). In the second PCR, 1 μl of a 100-fold dilution of the first PCR was used in combination with two nested primers: a *gpa-2* specific primer (AB3173, 5′-gtgctagtttcaatccaagatc) and a Tc3 specific primer (AB2730, 5′-cggaagttcctcaaaccttc). A 25 μl PCR reaction contained 1 μl DNA, 10 pmol of each primer, 0.05 M KCl, 0.01 M Tris−HCl pH 9, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.5 units *Taq* DNA polymerase (Gibco BRL). 30 cycles were performed, each consisting of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C.

The *gpa-2*::Tc3 insertions were sequenced according to Craxton (1991) using a Tc3 specific primer.

RNA analysis

RNA was isolated according to Rosenquist and Kimble (1988). Northern analysis was performed according to Sambrook *et al.* (1989).

Protein analysis

Protein samples were subjected to electrophoresis in 10% SDS−polyacrylamide minigels (Bio-Rad). For the detection of Tc3A the gels were blotted onto nitrocellulose using an electroblotter (Bio-Rad) and after washing with 1% BSA in TBST (10 mM Tris pH 8, 150 mM NaCl, 0.05% Tween 20) the blots were incubated with a 10 000-fold diluted antiserum. The bound primary antibodies were visualized using GAR/AP (Boehringer Mannheim) and NBT-BCIP (Promega).

Polyclonal antisera were obtained using synthetic peptides corresponding to the 13 most N-terminal (peptide HVL1: MPRGSALSDTERC) and the 13 most C-terminal (peptide HVL2: CEIIRTQGNPINY) amino acids of Tc3. These peptides were coupled to keyhole limpet hemocyanin and rabbits were injected according to Green *et al.* (1982).

Cellular fractionation

Worm pellets were mixed with 2 volumes of nuclear isolation buffer (NIB: 0.5 M sucrose, 50 mM Tris−HCl pH 7.4, 25 mM KCl, 0.5 mM EDTA, 0.15 mM spermine, 0.15 mM spermidine, 10 mM DTT, 5 mM $MgCl_2$, 0.1 mM PMSF). Frozen droplets of the worm suspension were ground in liquid nitrogen with a pestle in a ceramic mortar until a fine white powder was obtained. Four volumes of NIB were added and the solution was homogenized using a Dounce homogenizer (20 strokes). To remove empty carcasses and large particles the solution was centrifuged for 2 min at 200 *g* (4°C). The nuclei were then pelleted from the cleared supernatant by centrifugation for 10 min at 10 000 *g* (4°C). The supernatant contained the cytoplasmic fraction.

Transposase overexpression and purification

The Tc3 transposase gene with the intron removed by PCR was cloned in-frame in the *Eco*RI site of pMALc (New England Biolabs) to create pSDC310. The Tc1 transposase gene was similarly cloned into pMALc to create pSDC311. MBP fusion proteins were induced with IPTG for 3 h at 37°C and purified from *E.coli*, as described in the New England Biolabs protein fusion kit, using an amylose resin affinity column.

Gel retardation assay

The oligonucleotide AB3105 (5′-tcaaattaggggggtcctatagaactttcccacactgta; the 40 terminal nucleotides of Tc3 including the TA) and its complement (AB3104) were annealed and cloned into the *Hin*cII site of pUC18 to produce pSDC305. The 91 bp *Eco*RI−*Hin*dIII fragment of pSDC305 was end-labelled using DNA polymerase I (Klenow fragment) and used in band-shift experiments. The Tc3 40mer described above, annealed to its complement, was used as unlabelled specific competitor in the band-shift. The Tc1 oligo used as competitor was the 28mer 5′-agtggatatcttttggccagcactgta annealed to its complement. The non-specific oligo used was the 28mer 5′-aaaccgaggcaggaaaatccctagcagg and its complement.

Binding reactions were set up as follows: MBP−transposase fusion protein (~500 fmol) was added to the labelled DNA fragment (~2 fmol) in a 10 μl reaction, containing 25 mM HEPES−NaOH pH 7.6, 10% glycerol, 100 mM NaCl, 5 mM spermidine, 1 mM DTT, 0.4 mg/ml BSA and 0.2 mg/ml poly(dIdC). Reaction mixtures contained 10 pmol of unlabelled Tc3 or Tc1 or non-specific oligonucleotides as appropriate. The reaction was incubated at 25°C for 15 min and then loaded on a 6% polyacrylamide TAE gel. The gel was run for 3 h and protein−DNA complexes were visualized by autoradiography.

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