Rats were trained to perform ICSS in a behavioural chamber with a lever which, when pressed, immediately triggered delivery of a single stimulus train (100 Hz, 0.5 ms biphasic pulses, 500 ms train duration) to the substantia nigra electrode. Priming stimulus trains were administered by the experimenter when the animal was close to the lever, in order to facilitate approach and chance contact with the lever. All training was performed using the same training techniques for each animal. The training time was the total period of time that the rat was in the chamber with access to the lever, until lever-pressing commenced (at least 20 self-initiated presses in two consecutive minutes). Two animals did not reach the criterion within five days and were not used in further experimental procedures.

In the remaining ICSS responders ($n = 18$), response rate versus current intensity data was collected daily until the currents required to support minimum and maximum lever-pressing rates (optimal current) were stable to within $\pm 10\%$ for three consecutive sessions. Each animal then underwent two days of extinction where the animals were allowed free access to the lever with the self-stimulation circuit disabled. In addition, non-contingent stimulus trains were applied by the experimenter at 5-min intervals when the animal was away from the lever, in order to dissociate lever-pressing from the delivery of stimulus trains. Successful extinction was defined as a failure to approach the lever within the interval between two successive primes. This was successfully achieved in all animals.

Electrophysiological recording methods

Electrophysiological data was obtained from 12 of the 18 ICSS-experienced rats and 5 additional control animals. Each rat was anaesthetized with urethane (1.4–1.6 g kg^{-1} intraperitoneally), supplemented with ketamine (10 mg kg^{-1} intramuscularly) and xylazine (2 mg kg^{-1} intramuscularly). An electrode was implanted in all animals in the medial agranular cortex contralateral to the recording site (anteroposterior +10.6 to 12.7 mm, mediolateral −2.0 mm relative to interaural line; dorsoventral 1.6 to 2.0 mm from brain surface). In control rats, a bipolar stimulating electrode was positioned in the substantia nigra at the same coordinates as the permanently implanted electrode in the ICSS-experienced rats but this electrode was not used for ICSS-like stimulation.

Intracellular records were made from striatal neurons with microelectrodes pulled from 3.0-mm diameter glass and filled with 1 M K-acetate containing 4% biocytin. Post-synaptic potentials evoked by cortical test stimuli (0.1 Hz, 0.1 ms biphasic pulses, 300 to 990 μA) were routinely recorded for 20 min before and 20 min after the application of a treatment protocol. In ICSS-experienced rats, this consisted of six ICSS-like stimulus trains administered at ten-second intervals at the animal's optimal current intensity (Table 1).

In five experiments, the dopamine D1/D5 receptor antagonist SCH 23390 (40 μg kg^{-1} intraperitoneally in 0.1 ml 0.9% NaCl) was administered approximately 30 min before the application of ICSS-like stimulus trains. This dose was based on our preliminary behavioural experiments (data not shown) and the reports of others[29], which show it is sufficient to attenuate the rewarding effect of ICSS. Each ICSS-like stimulus train was paired with a current pulse just above the threshold for action potential firing (1.3 ± 0.5 nA) of similar intensity to control rats that received only current injection (1.1 ± 0.2 nA). Cellular properties of the recorded neurons were measured as previously described[15]. Post-synaptic potentials were compared between treatment groups by a repeated measures analysis of variance (ANOVA) (treatment effect: degrees of freedom, d.f. $= 2$, $F = 5.1$, $P < 0.05$; no time effect or interaction). Post-hoc tests between individual groups were performed using a Bonferroni analysis (overall level of significance $P < 0.05$). Substantia nigra electrode positions were verified in unstained vibratome sections. There were no systematic differences in electrode positions between groups.

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Mobilization of a *Drosophila* transposon in the *Caenorhabditis elegans* germ line

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Transposons have been enormously useful for genetic analysis in both *Drosophila* and bacteria. Mutagenic insertions constitute molecular tags that are used to rapidly clone the mutated gene. Such techniques would be especially advantageous in the nematode *Caenorhabditis elegans*, as the entire sequence of the genome has been determined. Several different types of endogenous transposons are present in *C. elegans*, and these can be mobilized in mutator strains (reviewed in ref. 1). Unfortunately, use of these native transposons for regulated transposition in *C. elegans* is limited. First, all strains contain multiple copies of these transposons and thus new insertions do not provide unique tags. Second, mutator strains tend to activate the transposition of several classes of transposons, so that the type of transposon associated with a particular mutation is not known. Here we demonstrate that the *Drosophila* mariner element *Mos1* can be mobilized in *C. elegans*. First, efficient mobilization of *Mos1* is

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possible in somatic cells. Second, heritable insertions of the transposon can be generated in the germ line. Third, genes that have been mutated by insertion can be rapidly identified using inverse polymerase chain reaction. Fourth, these insertions can subsequently be remobilized to generate deletion and frameshift mutations by imperfect excision.

Mos1 is a member of the *mariner/Tc1* family, which was initially identified in the fruitfly *Drosophila mauritiana*[2]. Like the other members of the *mariner/Tc1* family, *Mos1* contains a single open reading frame (ORF), which encodes the transposase. The transposase binds to and cleaves at the inverted terminal repeats that are present at each end of the transposon (reviewed in refs 3 and 4). The *Mos1* transposase is the only protein necessary for transposition *in vitro*[5]. Because no additional factors are required for transposition, the *Mos1* transposon is capable of transposition in heterologous species *in vivo*[6,7].

To determine whether the *Mos1* element could be mobilized in *C. elegans* cells we analysed *Mos1* transposition in somatic cells. We generated an extrachromosomal array expressing the *Mos1* transposase under the control of a heat-shock promoter (Fig. 1a). Another extrachromosomal array was generated using the *Mos1* transposon; this array also contained the dominant genetic marker *rol-6(sd)*, which causes animals to roll. These two strains were crossed and progeny carrying both arrays were subjected to heat shock as young adults. After 12 h the heat-shocked animals were collected, and *Mos1* insertions into arbitrary genes (*gpa-2* and *unc-49*) were assayed using polymerase chain reaction (PCR)[8]. For all insertions, the *Mos1* inverted terminal repeats were complete, the *Drosophila* sequences that flanked *Mos1* in the donor plasmid were no longer present, and the insertions all took place at a TA di-nucleotide. Transposon insertions were distributed uniformly in exons, introns and 3′ non-coding sequences of *gpa-2* and *unc-49* (Fig. 1b). Thus, Mos transposase was sufficient to catalyse insertion of *Mos1* into *C. elegans* chromosomes without *Drosophila* host factors.

To express the Mos transposase in the germ line, we generated an extrachromosomal array expressing the transposase under the control of the *glh-2* promoter, which is expressed in the germ line[9]. Transposase expression in the germ line was determined by assaying for excision of transposons from a defined chromosomal location. Specifically, the *Mos1*-containing array was integrated on chromosome V to generate *ox*Is*25[mos1; rol-6(sd)]*. Heterozygous *ox*Is*25/dpy-11* worms segregated Dpy and Rol progeny as expected for these closely linked markers (Fig. 2). However, when the *glh-2::Transposase* transgene was crossed into this strain, 21% of the transposon arrays experienced loss of the transposons and interspersed copies of *rol-6* (see Methods for calculation). Presumably, this catastrophic loss of the transposons and *rol-6* was caused by excision of the Mos elements from the chromosome. A small fraction of these broken chromosomes recombined with the intact homologue. Specifically, 3% of the *dpy*-marked chromosomes (6 out of 198) also contained the integrated array. This hotspot for recombination is likely to arise as a result of double-strand breaks caused by *Mos1* excision[10]. Most visible excisions, however, were generated by intramolecular healing of the chromosome, which deleted the Mos array. This conclusion was confirmed by analysing excision from a homozygous array: 37% of chromosomes from an *ox*Is*24[Mos1; rol-6(sd)]* homozygote lost the array when exposed to the transposase. In the homozygote, no wild-type chromosome was available for gene conversion or recombination.

To determine if excision of *Mos1* from the array was associated with insertion in the genome, we screened for *de novo* insertions in animals that lacked the source array (Dpy progeny) or in individuals that had excised the array (non-Rol progeny). Insertions were identified in 1% of non-Rol progeny (2 out of 227) and in 10% of Dpy progeny (11 out of 116, Table 1). These results demonstrate that transposition of *Mos1* could be achieved in the *C. elegans* germ line. However, we observed that high rates of excision were not accompanied by high rates of insertion; these *in vivo* results are in agreement with previous biochemical data suggesting that these two processes are not mechanistically coupled[11]. We also tested whether *Mos1* could be mobilized from an extrachromosomal array into the chromosomes. The non-Rol progeny from double transgenic animals (*ox*Ex*167[glh-2::Transposase]; ox*Ex*164[Mos1; rol-6(sd)]*) were analysed for transposition events using PCR. Again, we detected an insertion frequency of 1% (3 insertions out of 302 progeny). Thus, these results closely match those obtained for integrated arrays.

We also determined whether the heat-shock promoter could mobilize the transposon from the integrated array in the chromosome. Animals bearing the transposase construct and the integrated transposon (*ox*Is*25/dpy-11; ox*Ex*166[hsp::Transposase]*) were subjected to heat-shock treatment and the Dpy progeny were analysed for transposition events. The heat-shock construct caused the efficient insertion of *Mos1* into new locations in the genome. Approximately 27% (25 out of 94) of F_1 or F_2 Dpy worms carried new transposon insertions (Table 1). F_1 progeny were also analysed for catastrophic excision, that is, for the appearance of non-Rol, non-Dpy progeny (Fig. 2). In contrast to results using the *glh-2* expression vector, catastrophic excision was not observed (Table 1). The absence of catastrophic excision is probably due to efficient

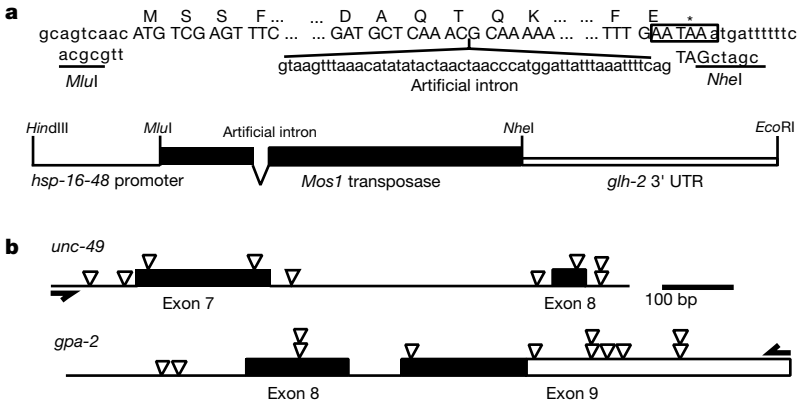


Figure 1 Mobilization of *Mos1* in *C. elegans* somatic cells. **a**, Engineering of the *Mos* transposase encoding sequence. Restriction sites were generated at the 5′ and 3′ ends of the coding sequence (new sequence is indicated under the original sequence). The endogenous polyadenylation signal (boxed) was disrupted; an artificial intron was introduced in the coding sequence to improve transposase expression[24]. Upper case denotes the coding region. **b**, Localization of *Mos1* insertions in *unc-49* and *gpa-2* genes after induction of Mos transposase expression in somatic cells. White triangles, insertion sites; black boxes, coding exons; white boxes, non-coding exonic sequence. Arrows indicate genomic primers used to amplify the insertions.

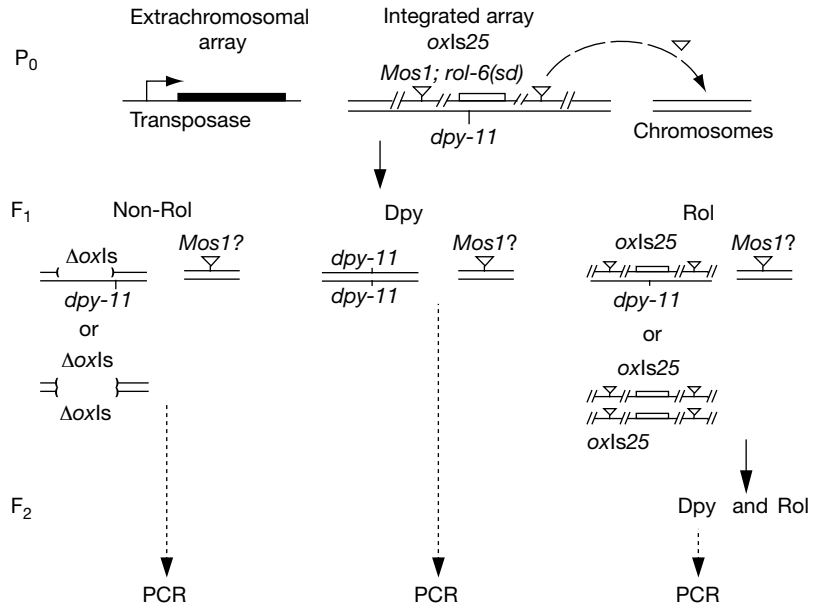


Figure 2 Germline mobilization of *Mos1*. Mos transposase was expressed from an extrachromosomal array using either a *glh-2* or a heat-shock promoter. The *Mos1* transposon was contained in an array integrated on chromosome V (*ox*Is*25[Mos1; rol-6(sd)]*). The array-containing chromosome was balanced by the *dpy-11(e224)* mutation. In the next generation, catastrophic excision of the transgene was observed (indicated as Δ*ox*Is) among the progeny.

template-dependent repair, in which the double-strand break is repaired by copying the original sequence from a sister chromatid. Catastrophic excision might be observed when using the *glh-2* promoter because of the timing of the excision event. For example, if excision precedes replication of the DNA then in the absence of a sister strand the break might be repaired by joining of the broken ends.

We next tested whether transposition could occur from an extrachromosomal array using the heat-shock promoter to express the transposase. Hermaphrodites bearing both arrays (*ox*Ex*166 [hsp::Transposase]; ox*Ex*164[Mos1; rol-6(sd)]*) were subjected to heat-shock treatment as young adults. The non-Rol progeny were analysed by PCR for transposition events. New insertions were observed in 8.9% of the F_1 progeny (33 out of 369). The frequency of transposition was low but one of the principal limiting factors may have been the stability of the extrachromosomal array that was used as a transposon source. The initial experiments were performed when the array was only 20% stable and transposition frequencies were in the range of 5%; after many generations the array became more stable (75%) and transposition frequency reached 44%.

What is the distribution of insertions? To determine the location of the mobilized transposons, we cloned the left junctions of 58 insertions using inverse PCR. Insertion sites were distributed on all six chromosomes (Fig. 3a), 11 were located in predicted exons and 10 in predicted introns. Sequences flanking both sides of the transposon were determined for ten of the localized insertions. In each case, the inverted terminal repeats were complete and flanked by a TA dinucleotide that arose from the duplication of the original TA found in the genomic sequence (Fig. 3b). Comparison of the 58 insertion-site sequences did not reveal a strong consensus motif for the target DNA, except for a TA-rich bias (72%) compared with the overall TA content of the genome (64%). Although exonic DNA comprises 27% of the genome[12], only 19% of the insertions were found in predicted exons. Insertions into non-coding regions are probably enhanced by the higher TA content of these regions[13,14].

The transposition events documented above were all excisions from an array of transposons residing in *Drosophila* DNA. To determine whether the transposase acts on a single *Mos1* transposon in a *C. elegans* chromosome, we remobilized *ox*Ti*4* (Transposon insertion) using the *glh-2::Transposase* construct. A variety of excision events were detected, including the three-nucleotide excision footprint previously characterized for *Mos1* excisions[15], as well as smaller footprints, deletions and even incomplete excisions (Fig. 3c). As these products could arise from excision events in somatic cells, we identified a strain in which the transposon had excised in the

Table 1 Comparison of excision and insertion frequencies

		F_1 non-Rol				F_1 Dpy				F_2 Dpy			
Expression vector	Experiment	Recombinants or excisions (non-Rols/Rols + non-Rols)		Insertions/ non-Rol		Recombinants (*dpy–11 ox*Is)		Insertions/ F_1 Dpys		Recombinants (*dpy-11 ox*Is)		Insertions/ F_2 Dpys	
None		3/691	0.43%	ND		0/92	0%	ND		ND		ND	
glh-2	1	541/4,078	13.3%	2/188	1.1%			ND				ND	
	2	138/651	21.2%	ND		0/39	0%	2/39	5%	2/19	10%	2/17	11%
	3	250/1,191	20.8%	0/39	0%	2/37	5%	0/35	0%	2/27	7%	7/25	28%
	Total	929/5,920	15.7%	2/227	0.9%	2/76	3%	2/74	3%	4/46	9%	9/42	21%
hsp	1	4/1,048	0.38%*	ND				ND		4/24	17%	6/24	25%
	2	0/41	0%*	ND		0/13	0%	0/13	0%	0/17	0%	4/17	23%
	3	1/140	0.71%*	ND		1/20	5%	7/20	35%	3/20	15%	8/20	40%
	Total	5/1,229	0.41%*	ND		1/33	3%	7/33	21%	7/61	11%	18/61	30%

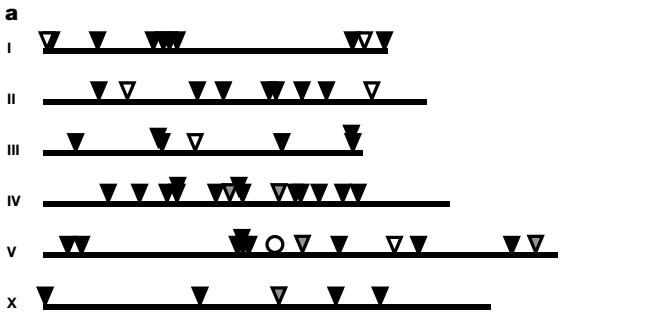
glh-2 and heat-shock (*hsp*) promoters were used to drive Mos transposase expression in the germ line. New *Mos1* insertions were identified by PCR. Recombinants between *dpy-11* and the insertion appeared as non-Rol progeny or as Dpy worms also containing *Mos1* flanked by original *Drosophila* genomic sequences (compare with row marked 'none'). Excisions were recognized as the number of non-Rol progeny appearing in the F_1 greater than expected for recombination. ND, not done.
* Number on non-Rol progeny does not significantly differ from control lacking a transposase-expressing construct (3/691, 0.43%).

germ line. The excision left a 3-base pair (bp) footprint and the duplicated TA dinucleotide, which together resulted in a +2 frameshift. These data indicate that single copies of the *Mos1 Drosophila* transposon can excise from *C. elegans* DNA in the germ line to introduce frameshift or deletion mutations at the transposon insertion site.

To determine the mutagenic rate of *Mos1*, we performed an F_2 selection for animals defective in the detection of high osmolarity (Osm). Early adult worms, which contained both the *Mos1* transposon and transposase arrays (*ox*Ex*166[hsp::Transposase]; ox*Ex*229[Mos1]*), were subjected to heat-shock treatment, and F_2 worms were tested for the Osm phenotype. A total of 161,064 haploid genomes were screened and ten Osm mutants were identified. To compare the *Mos1* mutagenic rate to that of a chemical mutagen, we screened for Osm mutants using the mutagen *N*-nitroso-*N*-ethylurea (ENU). In this screen 1,936 haploid genomes were screened and eight strong Osm mutants were identified. From this data *Mos1* seems to be approximately 60-fold less efficient as a mutagen than ENU. Six out of the ten Osm mutants were associated with a *Mos1* insertion. After out-crossing the strains many times, the relevant gene containing the *Mos1* insertion was identified using inverse PCR and the mutation confirmed by complementation or mapping experiments. The other four mutants seem to be 'hit and run' events in which a transposon was no longer detected in the mutant strain. It is possible that the hit and run events were caused by the mobilization of other transposons in the genome. There are at least 55 copies of a Mariner-like element (MLE), which is closely related to *Mos1* (refs 16, 17) in the *C. elegans* haploid genome. However, no changes in MLE distribution were detected in eight strains in which *Mos1* insertions had occurred (Southern blot not shown).

All of the insertion mutations were caused by insertions into exons. Insertions into introns are not likely to be mutagenic, as they can be spliced out during transcription. To improve mutagenicity we constructed a transposon that would disrupt transcription. A 378-bp fragment containing a consensus splice acceptor site followed by the *unc-54* polyadenylation signal sequence was inserted into the *Sal*I site of *Mos1* (*MosPolyA*), and extrachromosomal arrays were generated. The recombinant transposon was mobilized using the integrated heat-shock construct (*ox*Is*31*). Progeny were assayed for insertions by single-worm PCR and the location of the insert was determined by inverse PCR. Insertions were generated from the wild-type and *MosPolyA* elements at identical rates (6 out of 108 F_1 progeny for each, Fig. 3a). These data also show that short, single-copy DNA can be introduced into the *C. elegans* genome using the *Mos1* transposon. However, a 2-kb insertion decreased *Mos* transposition frequency by at least 10-fold (0 insertions from 212 progeny).

These experiments demonstrate the feasibility of using a heterologous transposon as a mutagen in *C. elegans*. The use of *Mos* should significantly accelerate forward genetic strategies. Furthermore, mobilization of an engineered transposon containing, for example, recombinase or meganuclease sites should enable the development of new tools to manipulate the *C. elegans* genome. □



b

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oxTi1   GTTTAGCGACGAGTGACATAccaggtgtac.........gtacacctgaTAATTCTCCGAAAGCTTC
oxTi2   TCGATAAATAAATTATTTTAccaggtgtac.........gtacacctgaTAATTCTATCCAAAAATC
oxTi3   AAAGTAGTGGATGCGATATAccaggtgtac.........gtacacctgaTAATAAGAGAGGCGAAGG
oxTi4   TCCTCTTTTCCAGACGAGTAccaggtgtac.........gtacacctgaTATATCCTTTTGTTCCTT
oxTi5   GTCGGACAATCAGAAGTGTAccaggtgtac.........gtacacctgaTAAGAACTAAAAGGACAC
oxTi6   TTGAACAATAAATACTAATAccaggtgtac.........gtacacctgaTATTGTTGTCCTCAAGAT
oxTi8   GACGCAATAAATCCACAATAccaggtgtac.........gtacacctgaTAATTTTCCCGACTCTTA
oxTi9   CCCTCTCCAATAGTCTAGTAccaggtgtac.........gtacacctgaTAAATGTCATCAGAATTC
oxTi11  ACCAAAAGCAAAAACACTTAccaggtgtac.........gtacacctgaTAACCAAATGATGGGTGG
oxTi18  AGTCAAAAACCTGAGGTATAccaggt...unc-54polyA...acctgaTAGTTGAACATGTTCCAA
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c

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oxTi4   TCCTCTTTTCCAGACGAGTAccaggtgtac..........tacacctgaTATATCCTTTTGTTCCT
        TCCTCTTTTCCAGACGAGTA----------------------------TATATCCTTTTGTTCCT
        TCCTCTTTTCCAGACGAGTA----------------------------aTATATCCTTTTGTTCCT
        TCCTCTTTTCCAGACGAGTA--------------------------tgaTATATCCTTTTGTTCCT
        TCCTCTTTTCCAGACGAGTAc--------------------------TATATCCTTTTGTTCCT
        ---------------------------249-bp deletion----tgaTATATCCTTTTGTTCCT
        TCCTCTTTTCCAGACGAGa---------143-bp deletion-------------------------
        TCCTCTTTTCCAGACGAGTA--------188-bp deletion-------------------------
        ---------------------------463-bp deletion-------------------------
        TCCTCTTTTCCAGACGAGTAattgtttactctcagtgcagtcaacatgtcgaTATCCTTTTGTTCCT
```

Figure 3 *Mos1* genomic insertions. **a**, Distribution of *Mos1* inserts on the physical map of the *C. elegans* genome. Black triangles, insertions from an extrachromosomal array, white triangles, insertions from the integrated array *ox*Is*25*; grey triangles, insertions of *MosPolyA*; white circle, position of *ox*Is*25,* the integrated array of *Mos1* transposons. Insertion sites can be obtained from Wormbase (http://www.wormbase.org/). **b**, DNA sequence of *Mos1 de novo* insertions. Genomic fragments that flank the transposon left end were isolated by inverse PCR and sequenced. A primer was designed in the genomic region to the right of the insert and used with a *Mos1*-specific primer to amplify and sequence the right end flanking the fragment. The insertion *ox*Ti*18* was amplified using worm-specific primers and the entire element was sequenced. At insertion sites TA, dinucleotides (bold) were duplicated during the process of transposon integration. Lower case, *Mos1* sequence; upper case, genomic sequence. **c**, Lesions generated by excision of the *ox*Ti*4* insert. The extrachromosomal transgene *[glh-2::Transposase]* was crossed into animals that were homozygous for the *ox*Ti*4* insertion. PCR was used to analyse the *ox*Ti4 insertion site after the loss of *Mos1*. Top line is the sequence of *ox*Ti*4*. Lower case, *Mos1* sequence; upper case, genomic sequence; bold, TA dinucleotide duplicated during *Mos1* insertion; dash, deleted base pairs. The bottom lines are the excision products. The fourth line represents the footprint obtained from the germline excision event. The insertion (bottom line, italic letters) corresponds to an internal fragment of *Mos1* (residues 147–178).

Methods

Construction of transgenic strains

To build the *hsp::Transposase* plasmid, the transposase-encoding sequence was amplified from pBluescribe M13+/*Mos1* (ref. 18), modified as described in Fig. 1 and subcloned between the *hsp-16-48* promoter[8,19] and the *glh-2* 3′ untranslated region (residues 35,383–36,190 of cosmid C55B7)[9]. In the *glh-2::Transposase* construct, the transposase ORF was preceded by a 2.2-kb *glh-2* promoter fragment (residues 29,882–32,095 of cosmid C55B7). To generate the *ox*Ex*166[hsp::Transposase]* array, the gonads of *lin-15(n765)* hermaphrodites were injected with the gel-purified fragments of the following contructs: *hsp::Transposase* (injection concentration of 10 ng μl^{-1}); the *unc-122::gfp* construct (pPD97/98) that expresses green fluorescent protein (GFP) in the coelomocytes (gift of P. Sengupta) (5 ng μl^{-1}); and EKL15(*lin-15+*)[20] (10 ng μl^{-1}). *ox*Ex*167[glh-2::Transposase]* was generated by injecting the *glh-2::Transposase* construct (10 ng μl^{-1}) with *lin-15(+)* and *unc-122::gfp* fragments. The *Mos1*-containing array *ox*Ex*164[Mos1; rol-6(sd)]* was built by injecting a fragment containing the 1.3-kb *Mos1* element flanked by *Drosophila simulans* sequences[18] (10 ng μl^{-1}) and a fragment of pRF4 (ref. 21) (*rol-6(sd)*) (10 ng μl^{-1}). In each case, plasmid backbones were removed from purified fragments and wild-type worm genomic DNA that was digested with *Eco*RV was co-injected to optimize germline expression (final DNA concentration of 100 ng μl^{-1})[22]. *ox*Ex*229[Mos1]* was generated by injecting uncut *Mos1* plasmid[18] at 10 ng ul^{-1}, *myo-2*::GFP marker (pPD118.33) at 2 ng ul^{-1} and pBluescriptKII at 88 ng ul^{-1}.

Detection of *Mos1* transposition

For detection of somatic transposition, DNA purification and PCR were performed as described[12]. *Mos1* insertions were detected at high frequency (2.5 ± 1.0 inserts in 10 ng genomic DNA, mean ± s.d.; $n = 5$ experiments). Given that the maximum distance of the inserts from our gene primers was approximately 1 kb, we estimated that an average of ten insertions occurred per cell in heat-shocked animals.

The integrated array *ox*Is*25[Mos1; rol-6(sd)]* contained 15–20 *Mos1* elements as determined by Southern blot analysis. Catastrophic excision of the array was analysed by PCR using one primer complementary to *Mos1* (oJL102: 5′-CAACCTTGACTGTCGAACCACCATAG-3′) and one primer complementary to *D. simulans* flanking DNA (oJL104: 5′-ACAAAGAGCGAACGCAGACGAGT-3′). The probability of a single chromosome

containing the array of *Mos1* elements experiencing catastrophic excision could be derived from a Punnett square where the ratio, *R*, of non-Rol worms over the probability of recombination, *p*, is $R = 1/4p + 1/4p + (1/2p)^2$.

To detect *de novo* insertions, the presence of *Mos1* was detected by PCR using two primers located in the transposon (oJL102 and oJL103: 5′-TCTGCGAGTTGTTTTT GCGTTTGAG-3′). The absence of *D. simulans* flanking sequence was checked using oJL103 and oJL104. Furthermore, we performed a positive control for PCR on each DNA sample using primers from the *cha-1* gene.

Insertions of *Mos1* were localized in the genome by inverse PCR: 100 ng total genomic DNA was digested with *Sau3A*, self-ligated under dilute conditions, and then subjected to two rounds of nested PCR using the following primers: oJL103/oJL114 5′-AAAGATTCA GAAGGTCGGTAGATGGG-3′ (first PCR), oJL115 5′-GCTCAATTCGCGCCAAACT ATG-3′ / oJL116 5′-GAACGAGAGGCAGATGGAGAGG-3′ (second PCR). PCR products were sequenced using oJL115 as a primer.

To build the recombinant element *MosPolyA*, the *unc-54* polyadenylation site was amplified by PCR using oJL125 (5-′AATATTTAAATTTTCAGCATC-3′) and oJL126 (5′-CGTAACATATGATAAGGTATTT-3′) and the resulting 378-bp fragment was ligated into the *Sal*I site of *Mos1*. Transposons from multiple arrays of wild-type (*ox*Ex*338, ox*Ex*339, ox*Ex*340*) and recombinant Mos elements (*ox*Ex*344, ox*Ex*345, ox*Ex*346*) were mobilized in parallel. The presence of *MosPolyA* in strains was detected using oJL102 and oJL103 in single-worm PCR reactions. Insertion sites of *MosPolyA* were determined by inverse single-worm PCR. Five worms were lysed in 20 μl lysis buffer[23] and 0.625 worm equivalents were digested with *Hha*I or *Mse*I, then ligated under dilute conditions. Nested PCR reactions were performed using primer 1A (5′-GACCTTGTGA AGTGT-CAACCTTGACTG-3′)/1B (5′-GACAATCGATAAATATTTACGTTTGCGAGAC-3′) in the first reaction and oJL102/2B (5′-CATCTATATGTTCGAACCGACATTCCC-3′) in the second reaction. Amplified products were gel-purified and sequenced using primer 2B.

Characterization of *Mos1* re-excision events

Mos1 was remobilized from *ox*Ti*4* (insertion in chromosome II at position 4,948 in cosmid T13C25). DNA from the progeny of *ox*Ti*4; ox*Ex*167[glh-2::Transposase]* hermaphrodites was prepared. A first PCR round was performed with primers located 1,671 nucleotides upstream and 3,144 bp downstream to *ox*Ti*4* (oJL149 5′-AAGTATGGCCAAACGACCCG ACAC-3′ and oJL150 5′-GCATTGGCACCTTTCTCCCTTCT-3′, respectively). Short PCR fragments were detected in 18 out of 27 cultures. A second round was performed using nested primers (oJL145 5′-ACAGGCAGCATTTTGTAGTCT-3′ and oJL148 5′-AGGCTGCCTCGTAAGTTCCTACAG-3′, respectively). Short PCR products were gel-purified, subcloned and sequenced.

To clone worms that lost the *ox*Ti*4* insertion, pools of 15 individuals from *ox*Ti*4; ox*Ex*167[glh-2::Transposase]* progeny that lost the transposase array were transferred to fresh plates and allowed to lay eggs for 24 h. We then analysed adult worms by a single round of PCR using the primers oJL145–oJL148. Sixty individuals were cloned from the progeny of the pool exhibiting short PCR product, and analysed at the next generation to identify clones that lost *ox*Ti*4*. One inherited excision was identified from among 954 progeny tested.

Osm screen

Early adult worms, which contained both the *Mos1* transposon and transposase arrays (*ox*Ex*166[hsp::transposase]; ox*Ex*229[Mos1]*), were subjected to heat-shock treatment at 35 °C for 1 h. F_2 progeny were placed in a ring of 4 M fructose. Wild-type worms remain in the ring of fructose, whereas mutants that are unable to detect the high osmolarity cross the border.

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Role of thrombin signalling in platelets in haemostasis and thrombosis

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Platelets are critical in haemostasis and in arterial thrombosis, which causes heart attacks and other events triggered by abnormal clotting[1–5]. The coagulation protease thrombin is a potent activator of platelets *ex vivo*[6]. However, because thrombin also mediates fibrin deposition and because multiple agonists can trigger platelet activation[7], the relative importance of platelet activation by thrombin in haemostasis and thrombosis is unknown. Thrombin triggers cellular responses at least in part through protease-activated receptors (PARs)[8]. Mouse platelets express PAR3 and PAR4 (ref. 9). Here we show that platelets from PAR4-deficient mice failed to change shape, mobilize calcium, secrete ATP or aggregate in response to thrombin. This result demonstrates that PAR signalling is necessary for mouse platelet activation by thrombin and supports the model that mouse PAR3 (mPAR3) does not by itself mediate transmembrane signalling but instead acts as a cofactor for thrombin cleavage and activation of mPAR4 (ref. 10). Importantly, PAR4-deficient mice had markedly prolonged bleeding times and were protected in a model of arteriolar thrombosis. Thus platelet activation by thrombin is necessary for normal haemostasis and may be an important target in the treatment of thrombosis.

We disrupted the *Par4* gene in embryonic stem cells by inserting the *lacZ* coding sequence into exon 1 such that β-galactosidase