Transgenic Res (2006) 15:751–760
DOI 10.1007/s11248-006-9014-6

BRIEF COMMUNICATION

Generation of trangenic *Xenopus laevis* using the *Sleeping Beauty* transposon system

L. Sinzelle · J. Vallin · L. Coen · A. Chesneau · D. Du Pasquier · N. Pollet · B. Demeneix · A. Mazabraud

Received: 22 December 2005 / Accepted: 15 May 2006 / Published online: 7 September 2006

Abstract Using the *Sleeping Beauty* (*SB*) transposon system, we have developed a simple method for the generation of *Xenopus laevis* transgenic lines. The transgenesis protocol is based on the co-injection of the *SB* transposase mRNA and a GFP-reporter transposon into one-cell stage embryos. Transposase-dependent reporter gene expression was observed in cell clones and in hemi-transgenic animals. We determined an optimal ratio of transposase mRNA versus transposon-carrying plasmid DNA that enhanced the proportion of hemi-transgenic tadpoles. The transgene is integrated into the genome and may be transmitted to the F1 offspring depending on the germline mosaicism. Although the transposase is necessary for efficient generation of transgenic *Xenopus*, the integration of the transgene occurred by an non-canonical transposition process. This was observed for two transgenic lines analysed. The transposon-based technique leads to a high transgenesis rate and is simple to handle. For these reasons, it could present an attractive alternative to the classical Restriction Enzyme Mediated Integration (REMI) procedure.

Keywords *Xenopus laevis* · Transgenesis · Transposon · *Sleeping Beauty*

Electronic Supplementary Material Supplementary material is available to authorised users in the online version of this article at http://dx.doi.org/10.1007/s11248-006-9014-6.

L. Sinzelle · J. Vallin · A. Chesneau · D. D. Pasquier · N. Pollet · A. Mazabraud (✉)
Transgenèse et Génétique des Amphibiens, CNRS UMR 8080, IBAIC, Université Paris-Sud, 91405 Orsay Cedex, France
e-mail: andre.mazabraud@ibaic.u-psud.fr

L. Coen · B. Demeneix
Département Régulation, Développement et Diversité Moléculaire, CNRS UMR 5166, MNHN, 7 rue Cuvier, 75231 Paris Cedex 5, France

Introduction

Xenopus amphibians have provided useful models for cell biology and vertebrate development studies for decades. Molecular genetic techniques such as injection of DNA, mRNA, antibodies or morpholino antisense oligonucleotides can be successfully applied in *Xenopus* embryos. All these methods are essentially transient since the injected molecules are progressively degraded by cellular processes.

An efficient germinal transgenesis method in *Xenopus* was created using nuclear transplantation allowing late development and organogenesis studies (Kroll and Amaya 1996; Sparrow et al. 2000). This method called ''Restriction Enzyme Mediated Insertion'' (REMI) produces non-mosaic and stable transgenic animals, but suffers from its technical complexity. In addition, chromosomal

insertion occurs randomly at one or several genomic positions into concatemers creating damage or insertional mutations into cellular genes.

Recently Pan et al. (2005) have used the *I-SceI* meganuclease for the creation of transgenic *Xenopus laevis* with high-frequency transgenesis and efficient germline transmission, although the mechanism of the transgene integration was unknown. More recently reported data have shown that the phiC31 integrase can mediate integration of plasmid DNA at a high enough efficiency to be able to study the F0 generation of transgenes (Allen and Weeks 2005).

Transposon technology has been widely used as a molecular genetic tool in plants, bacteria, fungi, insects and vertebrates (Ivics and Izsvak 2004). The members of the *Tc1-mariner* superfamily of DNA transposons constitute the most attractive candidates for gene delivery in vertebrates. Indeed, they do not require specific host factors to move within the genome and provide stable gene expression from low copy integration events. Their structures are simple, with a single open reading frame encoding a transposase, flanked by ITRs (Plasterk and Van Luenen 2002). They move via a cut-and-paste mechanism whereby the tranposase binds at precise sites in each of the ITRs, cuts out the transposon and inserts it into a new DNA locus.

The *Sleeping Beauty* (*SB*) transposon, which belongs to this superfamily, has been artificially reconstructed from sequences of fish *Tc1* transposons inactivated throughout evolution (Ivics et al. 1997). The transposon gene transfer system is composed of the transposase enzyme *SB*, able to mobilize in *trans* a non-autonomous version of a transposon carried in a plasmid vector. This transposon contains the Inverted Repeat/Direct Repeat sequences (IR/DR) of *SB* transposon, flanking a promoter/reporter gene sequence.

SB has been shown to transform a variety of vertebrate cell lines from fish to human, including *Xenopus laevis* A6 kidney cells, although efficiency and precision of transposition varied significantly among cell lines, suggesting potential involvement of host factors (Izsvak et al. 2000). *SB* has been developed as a gene delivery system in the germline of mice and fishes (Dupuy et al. 2002; Davidson et al. 2003; Grabher et al. 2003) and has been successfully used as gene therapy vector in animal models of human genetic diseases (for review, Hackett et al. 2005). This transposon system can also act as an insertional mutagenesis vector for the identification of cancer genes in tumours (Collier et al. 2005; Dupuy et al. 2005).

Here we report the use of the *SB* transposon system as a genetic tool for germinal transgenesis in the amphibian model *Xenopus laevis.* The method consists of the co-injection into fertilized eggs of a *SB* transposon encoding the green fluorescent protein (GFP) driven by an ubiquitous promoter and *SB* transposase mRNA. We demonstrate that this transposon-based transgenesis approach present major advantages such as a high transgenesis rate of injected embryos and the simplicity of the injection procedure as compared with the classical REMI procedure.

Materials and methods

Injection and transgenesis techniques

Xenopus eggs were collected, fertilized and dejellied as described by Sive et al. (2000). Embryos were reared in 0.1× MMR at 18°C until the desired stage. Using a nanoject injector (Drummond Scientific Company, Broomall, PA, USA), 100 pg of plasmid DNA with or without *SB* mRNA in a 9.2 nl total volume were microinjected into one-cell stage embryos. Embryos were staged according to Nieuwkoop and Faber (1994) and raised to sexual maturity. The transgenic carriers were identified by out-crossing to wild type animals. REMI transgenics lines and somatic gene transfer (SGT) in tadpole dorsal muscle and brain were made as described previously (de Luze et al. 1993; Ouatas et al. 1998; Sparrow et al. 2000).

Plasmid vectors

The *Pst*I–*Sna*BI CAG-EGFP cassette from the pCAG-EGFP plasmid, containing the cytoskeletal-β-actin promoter driving the GFP cDNA (gift of J.I. Miyazaki and K. Sakamaki) was cloned into a *Stu*I–*Msc*I digested pT plasmid (Ivics et al. 1997) to create the pT[βactin-GFP]. The T[βactin-GFP] cassette was isolated by a *Kpn*I–*Bam*HI

digestion and inserted into a *Kpn*I–*Bam*H1 digested ISceI-pBSSK vector (Thermes et al. 2002), resulting in the pST[βactin-GFP] vector. The transposon unit pT[CMV-GFP] was cloned as a *Sal*I–*Sac*I fragment into the pDSRed2-NI vector (Clontech) at the same sites, giving the pRed-pTGFP construct. The pCMVSB (also called pSB10) and the pBSSK/*SB10* plasmids provided by Z. Ivics were used as the source of transposase (Ivics et al. 1997; Grabher et al. 2003). *SB10* mRNA was transcribed in vitro from the plasmid pBSSK/SB10, using the mMessage mMachine kit (Ambion).

Western blotting

Proteins were extracted in EB buffer (50 mM βglycero-phosphate, 20 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, pH 7.3, leupeptine 10 μg/ml, aprotinine 20 μg/ml) supplemented with a protease inhibitor cocktail (Roche). Proteins were separated on SDS-PAGE and blot was tested against a mouse polyclonal *SB* transposase primary antiboby (a kind gift of Z. Ivics), followed by an anti-rabbit peroxidase coupled secondary antibody (Novocastra, USA). Finally, blots were developped using the chemiluminescence kit (Amersham) and the reactivity was visualized on hyperfilm ECL (Amersham).

Genomic DNA extraction and Southern blot analysis

To extract genomic DNA (gDNA), embryos were sacrified 3 weeks after injection, lysed in 300 μl of lysis buffer (0.4 M NaCl, 10 mM Na_2EDTA, 20 mM Tris pH 7.5, 0.5% SDS, 100 μg/ml Proteinase K) and incubated overnight at 37°C with occasional agitation. After two phenol–chloroform extractions, DNA was precipitated by adding 2.5 vol of ethanol. The DNA pellets were washed in 70% ethanol, air dried and resuspended in a solution of 10 mM Tris HCl and 0.1 mM EDTA (pH = 7.6). Ten micrograms of genomic DNA isolated from wild type animals and transgenic lines were digested with *Bam*HI, *Eco*RI and *Pvu*II restriction enzymes. Probes were obtained by PCR amplification. The pST[βactin-GFP] plasmid was used as a template for PCR reactions to obtain the 3′IR/DR probe (primers ins3′+: 5′-AAGCTCTGACCTCAATCC-TATA-3′ and ins5′+/3′–: 5′-TTGAAGTCG-GAAGTTTACATACACYT-3′) and the GFP probe (primers 5′GFP: 5′-ACCATGTGA-TCGCGCTTC-3′ and 3′GFP: 5′-AAGTTCAG-CGTGTCCGGC-3′). The PCR reactions contain 1 μM of primers, 200 μM of dNTP and 1 unit *Taq* DNA polymerase in a total volume of 50 μl. The cycling procedure was 94°C for 5 min, then 30 amplification cycles under the following conditions: 94°C for 1 min, 54°C for 1 min and 72°C for 1 min with a final extension step at 72°C for 10 min. The probes were labelled with α^{32}P-dCTP by random priming using the NEblot™ kit (New Englands Biolabs). Southern blot analysis, were performed at high stringency as described (Sinzelle et al. 2005). One hundred picograms of pST[βactin-GFP] plasmid was digested with the same restriction enzymes and prepared for Southern blot as positive control.

Photography

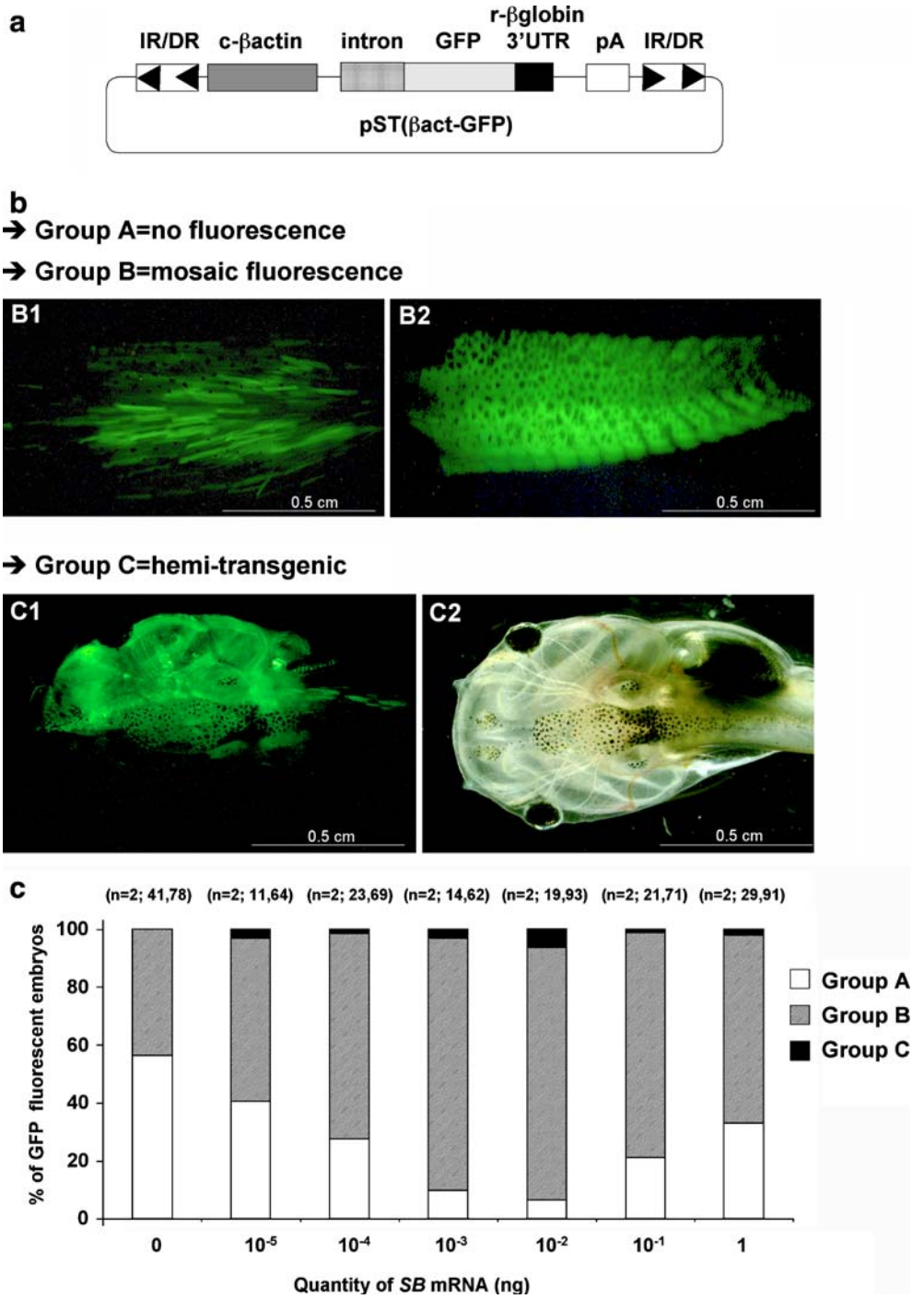
Animals were observed and scored using a Olympus SZX12 microscope with fluorescent illumination. Brightfield and GFP images were captured using a colour video camera (Spot insight color, Diagnostic instrument) with a 460- to 490-nm excitation filter and a 510-nm emission filter.

Results and discussion

Production of hemi-transgenic embryos using the *SB* transposon system

To determine whether the *SB* transposon system could be suitable for *Xenopus* transgenesis, we co-injected one-cell stage embryos with the pST[βactin-GFP] reporter vector containing a non-autonomous version of a transposon and in vitro synthesized mRNA encoding the *SB* transposase. The transposon was composed of the GFP reporter gene under the control of the chicken βactin ubiquitous promoter (βactin), flanked by the *SB* IR/DRs (Fig. 1a).

We first checked the efficiency of *SB* mRNA translation in the context of early *X. laevis* embryos. One-cell stage embryos were co-injected with 1 ng of *SB* mRNA and 100 pg of the plasmid reporter pT[CMV-GFP]. *SB* transposase was detectable by Western blotting as soon as 1 h after injection (2-cell stage) and lasted at least 1 week (Stage 48). Using these conditions, no toxic effect on embryonic survival and development were observed. In three experiments, the



number of surviving embryos at stage 30 varied from 25% to 60% when injected with the plasmid only and from 31% to 47% when co-injected with 1 ng of *SB* mRNA.

We then monitored GFP expression in embryos injected with plasmid alone or with *SB* transposase mRNA. Embryos were scored at a late stage, 3 weeks after injection to reduce confusion of signals arising from free plasmids with that from integrated transgenes. Once injected, plasmid molecules may be maintained several months in the tissues as extrachromosomal episomes (Etkin and Pearman 1987). GFP expression persist when the transgene is integrated, whereas the fluorescence attributable to free plasmids is gradually lost at later stages of development. Embryos were grouped according to the level of fluorescence (Fig. 1b): (A) no fluorescence; (B) mosaic fluorescence either in a few dispersed cells (B1) or in large clones of cells reflecting an early integration event (B2); (C) hemi-fluorescent embryos, either on the right or left side of the body (C1 and C2). The addition of mRNA transposase increased the number of embryos with mosaic fluorescence. With *SB* transposase, 64% of embryos (40/62) exhibited a mosaic fluorescence (group B) compare to 43% (17/40) without the *SB* transposase. In our study, the most remarkable result was the finding of hemi-fluorescent embryos using the pST[βbact GFP] plasmid, in the presence of *SB* transposase only. Since no uniformly fluorescent embryo was generated, the integration event might have occurred either in one of both blastomeres at the 2-cell stage or at the 1-cell stage in a chromosomal locus repressed in one side of the animal.

◀ **Fig. 1** Generation of hemi-transgenic tadpoles using *SB* (**a**) Schema of the pST[βact-GFP] transposon-carrying construct used in this study. The GFP cDNA is driven by the chicken cytoskeletal-β-actin promoter (c-βactin), flanked with a chimeric intron from the chicken βactin gene and the rabbit βglobin gene (intron). On its 3′ end, the GFP cDNA is flanked with the rabbit βglobin (r-βglobin) 3′UTR. (**b**) GFP fluorescence patterns observed in embryos injected with pST[βact-GFP] and transposase mRNA. Group A: no fluorescence; Group B: mosaic fluorescence at various levels (B1 and B2 lateral views of tadpole tails showing fluorescence in dispersed cells and in large clone of cells, respectively); Group C: hemi-fluorescent tadpole (C1 and C2: dorsal view of the head of a hemi-fluorescent tadpole illuminated with GFP filter and visible light, respectively). (**c**) Variation of the hemi-fluorescence pattern depends on the quantity of injected *SB* transposase mRNA. Three weeks after co-injection of the pST[βact-GFP] with various amount of transposase mRNA, embryos were scored according to the fluorescent groups (A–C). Injection of plasmid alone was used as controls. The number of experiments (*n*) and of embryos in each experiment are indicated within brackets

Determination of an optimal quantity of transposase increasing the hemi-transgenic tadpoles numbers

Previously reported data for the *Sleeping Beauty* transposon have highlighted the impact of the transposase amount on transposition efficiency in vivo (Geurts et al. 2003). When the transposase concentration exceeds an optimal concentration in a given cell, an inhibitory effect known as ''overproduction-inhibition'' appears. To determine the optimal ratio of *SB* mRNA versus plasmid DNA in our approach, amounts of transposase mRNA, ranging from 10^{-5} ng to one 1 ng were tested. Embryos were co-injected with 100 pg of the pST[βact-GFP], and classified according to the level of fluorescence 3 weeks after injection (Fig. 1c). We found that 10^{-2} ng of injected transposase mRNA gave the greatest number of hemi-fluorescent tadpoles (6.5%) in two independent series of injection. No hemi-fluorescent embryos were obtained without transposase, in agreement with our previous analysis. We observed hemi-transgenic tadpoles whatever the amount of transposase mRNA injected, and hence an inhibitory effect is unlikely. However, the number of hemi-transgenic tadpoles decreased when the *SB* mRNA exceeded 10^{-2} ng suggesting a regulatory effect of the transposase quantity. With the optimal transposon/transposase ratio, a high proportion of surviving hemi-transgenic animals was obtained. Hemi-transgenics are helpful as the wild-type side of the animal provides an internal control when studying *in vivo* gene expression at late stages of development.

Using the meganuclease *I-SceI*, Pan et al. (2005) observed an overall trangenesis frequency of 10%. With the REMI procedure, the percentage of trangenic animals measured at stage 40 represented 5–10% of the number of injected

oocytes under optimal conditions (Offield et al. 2000).

In vivo detection of excision events

With the aim to directly visualizing the putative excision events of the transposon from the donor plasmid (Liu et al. 2004), we developed an excision assay based on the fluorescence emission properties of the pRed-pTGFP plasmid (Fig. 2a). This construct contains the transposon [CMV-GFP] inserted backwards between another CMV promoter and the Red Fluorescent Protein (RFP) gene. Indeed, in the absence of any excision event, only green fluorescence is emitted. In presence of transposase, if excision of the [CMV-GFP] transposon occurs, the RFP gene is placed under the control of the CMV promoter and a red fluorescence can be visualized together with the green fluorescence (Fig. 2b). Because of the dilution of the donor plasmid during tadpole development, we used somatic gene transfer technique to analyse the excision process (de Luze et al. 1993; Ouatas et al. 1998). The pRed-pTGFP molecule was injected in tadpole tail muscle and brain in presence or in absence of a *SB*-coding plasmid (pCMVSB). The results showed that red fluorescence is clearly seen in muscle only when the transposase was present, demonstrating that excision events can be followed *in vivo* (Fig. 2c). Similar results were obtained in the brain after transfection of the pRed-pTGFP molecule (Fig. 2d). These results provide evidence that the injected mRNA encodes an active transposase able to catalyse the excision from the donor plasmid corresponding to the first step of the *SB* transposition mechanism.

Germline transmission rates and molecular analysis of integration

To correlate between the level of fluorescence in F0 animals and germline transmission of the transgene, the F1 offsprings of 16 founders belonging to each fluorescence phenotype group B and C were analysed for their ability to establish transgenic lines. F0 animals were mated to wild type individuals and the F1 offsprings were screened for fluorescence (Table 1). For each founder, the germline mosaicism rate is determined as the percentage of fluorescent F1 offspring from a founder outcross. Transgenesis frequency is defined as the percentage of F0 animals producing a fluorescent offspring.

The offspring of four animals injected with pST[βact-GFP] alone showed no fluorescence. When the pST[βact-GFP] plasmid was co-injected with *SB* mRNA, transgenesis frequency reached 40% (5/12), and F0 germline mosaicism rates varied from 0.3% to 34%. All the F1 animals expressing the GFP showed ubiquitous fluorescence (Figure I, supplementary materials). Three animals out of 10, in the mosaic fluorescence group B, transmitted the transgene to their offspring. The two half-fluorescent animals (S1 and T1) transmitted the transgene in the progeny with germline transmission rates varying from 2.5% to 34 %.

In order to assess the stablility of the transgene in germline, GFP positive F1 animals from lines T5, R1 (group of fluorescence B) and S1 (group of fluorescence C) were mated to wild type animals and the F2 offsprings were screened for fluorescence (Table 1). For the T5 and R1 lines from the mosaic fluorescence group, 30 and 102 embryos out of 59 and 201 were ubiquitously GFP positive, respectively (Table 1 and Figure II, supplementary materials). For S1 line belonging to hemi-fluorescence group, out of 246 embryos, 124 were ubiquitously GFP positive (Table 1). These results clearly demonstrated that the transgene was stably transmitted in F2 generation by a Mendelian fashion.

We investigated the molecular nature of integrations by Southern blot analysis on two F0 animals, from lines T1 and T5, exhibiting hemi-fluorescence and mosaic fluorescence, respectively, and on their offsprings (Fig. 3). In addition, the analysis was performed on sibling embryos, which did not exhibit detectable fluorescence. Both lines T1 and T5 showed plasmid insertions, revealed by the hybridization of two probes, respectively recognising the GFP gene, and the transposon inner part of the 3′IR/DR extending into the plasmid vector. These results proved that integration of the transgene occured, although not through a canonical cut-and-paste

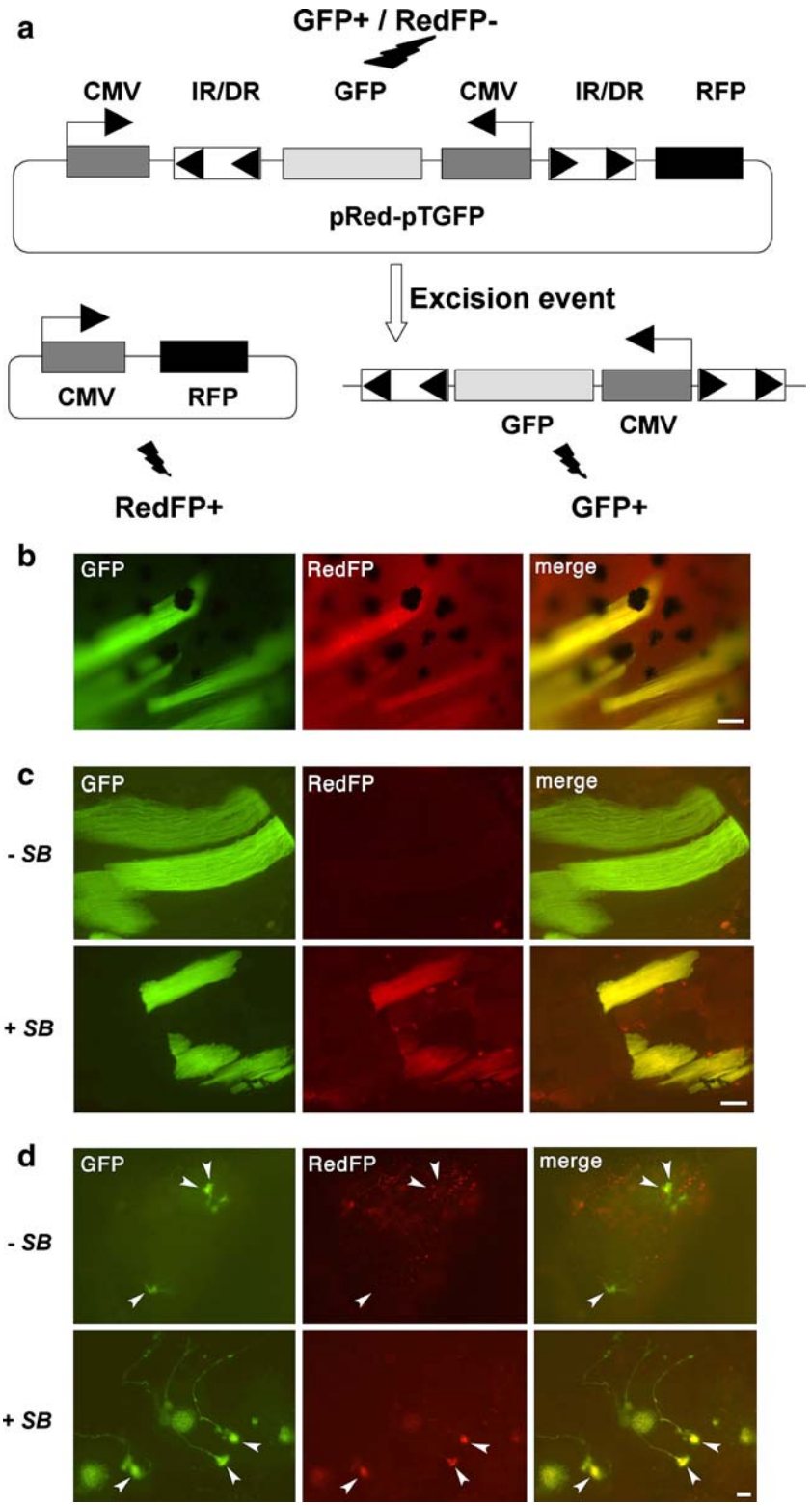


Fig. 2 In vivo detection of excision after co-transfection of pRed-pTGFP and pCMV-SB using somatic gene transfer. (**a**) The pRed-pTGFP plasmid was used to detect the excision events. The transposon containing the transgene CMV-GFP was inserted between the CMV promoter and the Red fluorescent protein (RFP) sequence. In the absence of transposase, the transcription of the pRed-pTGFP produced green fluorescence and no red fluorescence signal (GFP+/RedFP–). The excision of the transposon results in transcription of the RFP gene (RedFP+). (**b**) Observation of green (GFP) and red (RedFP) fluorescence expression, or the two fluorescence together (merge) in muscle of living embryos 7 days after transfection in presence of transposase activity. (**c**) Histological sections prepared from transfected muscle show that RFP expression was only obtained in presence of transposase activity *(+SB)* and never seen in the absence of co-transfected transposase (*–SB*). (**d**) In vivo expression 7 days after somatic gene transfer in brain showed only green fluorescence in the absence of transposase activity (*–SB*), whereas green and red fluorescence were observed in co-transfection with pRed-pTGFP and pCMV-SB constructs (*+SB*). Positive cells are indicated with arrowheads. Scale bars = 100 μm (**b**), 50 μm (**c**) and 20 μm (**d**)

Table 1 Analysis of the germline transmission of the inserted transgene in the F1 and F2 offsprings. The F0 founders belonging to the phenotypical groups of fluorescence B and C are listed. Four F0 animals were obtained upon injection of the pST[βact-GFP] alone (control) and 12 were obtained upon co-injection of the transposase mRNA. For each F0 founder, the F0 germline mosaicism rate is determined as the percentage of fluorescent F1 offspring from a founder outcross. The total number of analysed embryos is indicated. Transgenesis frequency is defined as the percentage of F0 animals producing a F1 fluorescent offspring. The total number and the percentage of fluorescent F2 embryos analysed from F1 outcrosses (T5, R1, S1 lines) are indicated

F0		F1			F2	
Individual founder	Group of fluorescence	Total number of F1 embryos analysed	F0 Germline mosaicism %	Transgenesis frequency %	Total number of F2 embryos analysed	Percentage of F2 embryos GFP positif
Control						
AE1	B	34	0	$n = 0/4 = 0\%$	–	–
J7	B	137	0			
M6	B	257	0			
W14	B	101	0			
+SB10 mRNA						
01	B	28	0	$n = 5/12 = 40\%$	–	–
T5	B	611	0.3		59	50.8
AC4	B	602	0		–	–
K20	B	470	0		–	–
R1	B	40	15		201	50.7
R21	B	459	0		–	–
AD1	B	47	21		–	–
AD2	B	317	0		–	–
AG4	B	81	0		–	–
S2	B	63	0		–	–
S1	C	80	2.5		246	50.4
T1	C	12	34		–	–

transposition mechanism, carrying plasmid sequences adjacent to the transposon into *Xenopus* genome. Surprisingly, non-expressing F1 siblings were positive for hybridization with both probes. These data suggest that several integrations of the transgene occurred in F0 germline, but that some of them are silenced by position effects. From these results, we can conclude that the germline rates obtained previously were probably underestimated and represented consequently a minimal rate of transgenesis. Recently, Allen and Weeks (2005) have highlighted the importance of preventing chromatin position effect. In their transgenesis study, the use of the phiC31 integrase required insulated gene sequences to obtain transgenic embryos with expected expression pattern.

From the present study, we conclude that *SB* transposase can be used with some advantages to obtain numerous transgenics, and specifically hemi-transgenic animals using an ubiquitous promoter coupled to the GFP in our assay. This *SB* transfer system provides a simple method using classical injection of genetic material into fertilized eggs and could be a useful complement to the REMI technique. Nevertheless, thorough studies are needed to investigate if the *SB* transposon system could drive accurately the spatial and temporal expression of a given tissue-specific promoter or enhancer of interest.

As other transgenic techniques based on plasmids injection into one-cell stage embryos (Pan et al. 2005), the *SB* method has a limitation in F0 studies due to the background of fluorescence resulting from unintegrated plasmids. Therefore, any study on early development has to be done on the F1 generation. For this type of work, the sibling species *Xenopus tropicalis* is preferential to *X. laevis* as it has the advantage of a shorter generation time for the analysis of expression in

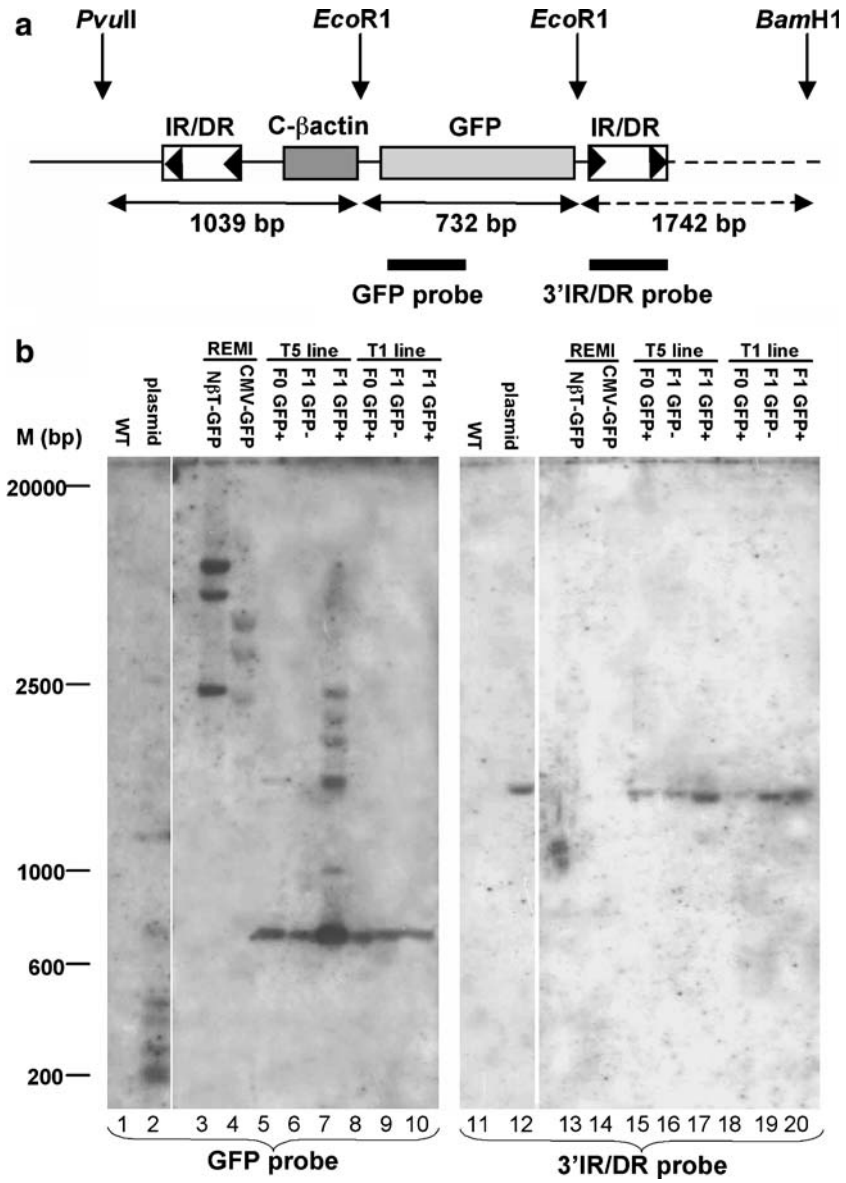


Fig. 3 Southern blot analysis of genomic insertion of the transposon in transgenic *Xenopus* lines. (**a**) Structure of the pST[βact-GFP] plasmid depicting the relative position of both probes, detecting GFP and the 3′IR/DR, respectively. *Eco*R1 cuts twice in the transposon and *Pvu*II and *Bam*H1 cut once in the plasmid. Genomic DNA (gDNA) was digested by these three restriction enzymes. (**b**) Southern blot analysis of two *Xenopus* transgenic lines. Lanes 1–10 correspond to GFP hybridization and lanes 11–20 to 3′IR/DR hybridization. Lanes 1 and 11 correspond to gDNA extracted from wild type embyos (WT). Probed gDNA, extracted from the tail of transgenic F0 animals of line T1 (T1 line, F0 GFP+, lanes 8 and 18) and T5 (T5 line, F0 GFP+, lanes 5 and 15), gDNA from single F1 not fluorescent siblings from line T1 (T1 line, F1 GFP–, lanes 9 and 19) and T5 (T5 line, F1 GFP–, lanes 6 and 16) and gDNA from a single fluorescent F1 embryo of line T1 (T1 line, F1 GFP+, lanes 10 and 20) and line T5 (T5 line, F1 GFP+, lanes 7 and 17). Lanes 3 and 13 correspond to gDNA from NβT-GFP REMI transgenics. Lanes 4 and 14 correspond to gDNA from CMV-GFP REMI transgenics. Lanes 2 and 12 correspond to 100 pg of pST[βactin-GFP] plasmid, used as hybridization control (plasmid). The multiple bands in lane 7 probably correspond to incomplete cutting of genomic integrants, some of which corresponding to plasmid DNA. Bands of 1771 bp and 2474 bp are obtained if the first and second *Eco*RI sites shown in the Fig. 3a are not cut, respectively

F1. This species with a diploid genome has become the genomic reference in amphibians and is therefore better suited for genetic approaches and transgenic studies.

Acknowledgements The authors thank Z. Ivics and Z. Izsvak for the hepful comments of the manuscript and the gift of the three plasmids pT[CMV-GFP], pCMVSB and pBSSK/SB10, and *SB* transposase antibodies. This research was funded by grants from ARC, CNRS, the 5th PCRDT EC program and the Paris-Sud University.

References

Allen BG, Weeks DL (2005) Transgenic *Xenopus laevis* embryos can be generated using phiC31 integrase. Nat Method 2:975–979

Collier LS, Carlson CM, Ravimohan S, Dupuy AJ, Largaespada DA (2005) Cancer gene discovery in solid tumours using transposon-based somatic mutagenesis in the mouse. Nature 436:272–276

Davidson AE, Balciunas D, Mohn D, Shaffer J, Hermanson S, Sivasubbu S, Cliff MP, Hackett PB, Ekker SC (2003) Efficient gene delivery and gene expression in zebrafish using the *Sleeping Beauty* transposon. Dev Biol 263:191–202

de Luze A, Sachs L, Demeneix B (1993) Thyroid hormone-dependent transcriptional regulation of exogenous genes transferred into *Xenopus* tadpole muscle *in vivo*. Proc Natl Acad Sci USA 90:7322–7326

Dupuy AJ, Clark K, Carlson CM, Fritz S, Davidson AE, Markley KM, Finley K, Fletcher CF, Ekker SC, Hackett PB, Horn S, Largaespada DA (2002) Mammalian germ-line transgenesis by transposition. Proc Natl Acad Sci USA 99:4495–4499

Dupuy AJ, Akagi K, Largaespada DA, Copeland NG, Jenkins NA (2005) Mammalian mutagenesis using a highly mobile somatic *Sleeping Beauty* transposon system. Nature 436:221–226

Etkin LD, Pearman B (1987) Distribution, expression and germ line transmission of exogenous DNA injected into fertilized eggs of *Xenopus laevis*. Development 99:15–23

Geurts AM, Yang Y, Clark KJ, Liu G, Cui Z, Dupuy AJ, Bell JB, Largaespada DA, Hackett PB (2003) Gene transfer into genomes of human cells by the *Sleeping Beauty* transposon system. Mol Ther 8:108–117

Grabher C, Henrich T, Sasado T, Arenz A, Wittbrodt J, Furutani-Seiki M (2003) Transposon-mediated enhancer trapping in medaka. Gene 322:57–66

Hackett PB, Ekker SC, Largaespada DA, McIvor RS (2005) *Sleeping Beauty* transposon-mediated gene therapy for prolonged expression. Adv Genet 54:189–232

Ivics Z, Izsvak Z (2004) Transposable elements for transgenesis and insertional mutagenesis in vertebrates: a contemporary review of experimental strategies. Methods Mol Biol 260:255–276

Ivics Z, Hackett PB, Plasterk RH, Izsvak Z (1997) Molecular reconstruction of *Sleeping Beauty*, a *Tc1*-like transposon from fish, and its transposition in human cells. Cell 91:501–510

Izsvak Z, Ivics Z, Plasterk RH (2000) *Sleeping Beauty*, a wide host-range transposon vector for genetic transformation in vertebrates. J Mol Biol 302:93–102

Kroll KL, Amaya E (1996) Transgenic *Xenopus* embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. Development 122:3173–3183

Liu G, Cui Z, Aronovich EL, Whitley CB, Hackett PB (2004) Excision of *Sleeping Beauty* transposons: parameters and applications to gene therapy. J Gene Med 6:574–583

Nieuwkoop PD, Faber J (1994) Normal table of *Xenopus laevis* (Daudin). Elsevier North Holland Publishing Company, Amsterdam

Offield MF, Hirsch N, Grainger RM (2000) The development of *Xenopus tropicalis* transgenic lines and their use in studying lens developmental timing in living embryos. Development 127:1789–1795

Ouatas T, Le Mevel S, Demeneix BA, de Luze A (1998) T3-dependent physiological regulation of transcription in the *Xenopus* tadpole brain studied by polyethylenimine based in vivo gene transfer. Int J Dev Biol 42:1159–1164

Pan FC, Chen Y, Loeber J, Henningfeld K, Pieler T (2005) *I-SceI* meganuclease-mediated transgenesis in *Xenopus*. Dev Dyn 235:247–252

Plasterk RHA, Van Luenen HGAM (2002) The *Tc1/mariner* family of transposable elements. In: Craig NL et al (eds) Mobile DNA II. ASM Press, Washington, USA, pp 519–531

Sinzelle L, Pollet N, Bigot Y, Mazabraud A (2005) Characterization of multiple lineages of *Tc1*-like elements within the genome of the amphibian *Xenopus tropicalis*. Gene 349:187–196

Sive HL, Grainger R, Harland R (2000) Early development of *Xenopus laevis*: a laboratory manual. Cold Spring Harbor Laboratory Press

Sparrow DB, Latinkic B, Mohun TJ (2000) A simplified method of generating transgenic *Xenopus*. Nucleic Acids Res 28:E12

Thermes V, Grabher C, Ristoratore F, Bourrat F, Choulika A, Wittbrodt J, Joly JS (2002) *I-SceI* meganuclease mediates highly efficient transgenesis in fish. Mech Dev 118:91–98