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Genetics

Transposon tools for recombinant DNA manipulation: Characterization of transcriptional regulators from yeast, *Xenopus*, and mouse

(tagged transposons/two-hybrid test/enhancer trap)

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ABSTRACT **Transposon Tn*1000* has been adapted to deliver novel DNA sequences for manipulating recombinant DNA. The transposition procedure for these "tagged" Tn*1000*s is simple and applicable to most plasmids in current use. For yeast molecular biology, tagged Tn*1000*s introduce a variety of yeast selective markers and replication origins into plasmids and cosmids. In addition, the β-globin minimal promoter and *lacZ* gene of Tn*βlac* serve as a mobile reporter of eukaryotic enhancer activity. In this paper, Tn*βlac* was used to localize a mouse HoxB-complex enhancer in transgenic mice. Other tagged transposons create Gal4 DNA-binding-domain fusions, in either *Escherichia coli* or yeast plasmids, for use in one- and two-hybrid tests of transcriptional activation and protein–protein interaction, respectively. With such fusions, the *Saccharomyces cerevisiae* Swi6 G_1/S-phase transcription factor and the *Xenopus laevis* Pintallavis developmental regulator are shown to activate transcription. Furthermore, the same transposon insertions also facilitated mapping of the Swi6 and Pintallavis domains responsible for transcriptional activation. Thus, as well as introducing novel sequences, tagged transposons share the numerous other applications of transposition such as producing insertional mutations, creating deletion series, or serving as mobile primer sites for DNA sequencing.**

Recombinant DNA can be manipulated *in vivo* by tagged transposons carrying novel sequences (1–3). This approach exploits the genetic mobility of transposons to juxtapose two sequences of DNA as an alternative to conventional *in vitro* methods. With no constraints of restriction-site availability, considerable savings in time and cost of DNA manipulation are possible. Tagged transposons can also localize a gene by loss of function and permit DNA sequencing from transposon-specific primer sites (4–6). Mapping insertion sites in turn allows one to generate truncation series for mapping functional domains or epitopes (7). Thus, tagged transposons have multiple applications in addition to the insertion of novel genetic elements.

Tn*1000* has several advantages for recombinant DNA manipulation. It will transpose a wide range of vectors, inserting only once per plasmid and with less insertion-site bias than other transposons (8). Tn*1000* contains a nonessential gene that can be replaced with foreign DNA. Short, 35-bp inverted terminal repeats of Tn*1000* allow tagged sequences to be placed close to the point of insertion and, if required, to form translational fusions between novel sequences carried by the transposon and target sequences. The short termini also permit transposon-primed nucleotide sequencing of adjacent target DNA (4–6). Finally, Tn*1000* has the major practical advantage of having a simple, one-step delivery system to produce a population of entirely transposed plasmids (9).

Here, derivatives of Tn*1000* are described which introduce a variety of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* genetic markers and replication origins. Gal4 DNA-binding-domain fusions can also be made with tagged transposons for two-hybrid tests of protein–protein interaction (10, 11) and "one-hybrid" assays of transcriptional activation (12). Finally, the wider potential of transposons is illustrated by the detection of eukaryotic enhancer activity in transgenic mice.

Table 1. Bacteria and yeast

Species	Strain	Relevant genotype	Ref.
E. coli	DH1	*recA1*	13
	MH1592	DH1 R388	*
	MH1595	DH1 R388::Tn*TRP1ARS1*	*
	MH1598	DH1 R388::Tn*HIS3*	*
	MH1599	DH1 R388::Tn*URA3*	*
	MH1601	DH1 R388::Tn*TRP1*	*
	MH1638	DH1 R388::TnXR	*
	MH1798	DH1 R388::Tn2μ*HIS3*	*
	MH1799	DH1 R388::Tn_p*ADH–GAL4*$_{1–147}$	*
	MH1846	DH1 R388::Tn2μ *HIS3*$_p$ *ADH–GAL4*$_{1–147}$	*
	MH1844	DH1 R388::Tn_pβ-globin–*lacZ*	*
	MH1827	DH1 R388::Tn*his7*	*
	MH1829	DH1 R388::Tn*ura4*	*
	MH1832	DH1 R388::Tn*his7ars1*	*
	MH1831	DH1 R388::Tn*ura4ars1*	*
	HB101	*recA1 rpsL*	14
Sch. pombe	CHP429	*his7-366 leu1-32 ura4-D18 ade6-M216 h*$^-$	15
S. cerevisiae	PCY2	*MATα Δgal4 Δgal80 URA3::GAL1-10-lacZ lys-801 his3-Δ200 trp1-Δ63 leu2 ade2-101*	16

*This work.

MATERIALS AND METHODS

Bacterial, Plasmid, and Yeast Stocks. Bacterial, *Sch. pombe*, and *S. cerevisiae* stocks are detailed in Table 1. R388 is a 32.3-kb IncW conjugative plasmid conferring trimethoprim and sulfanilamide resistance (17). pTR44 comprises a PCR-derived, 2.3-kb *SWI6* structural gene cloned into YEp13 (18). pFC1 is pCR11 carrying the 1196-bp Pintallavis gene, corre-

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sponding to amino acids 2–399 (19, 20). pFC2 encodes a $Gal4_{1-147}$–Pintallavis fusion in pGBT9 (21). An 11-kb *Hin*dIII fragment of mouse *Hox-b4–Hox-b5* intergenic sequences was cloned into pPolyIII (22) to form pB4Δ2. pTR46 is pBR322 with a filled-in *Eco*RI site.

Derivation of Tagged Tn*1000*s. Tagged Tn*1000*s (Fig. 1) were constructed in a transposed derivative of pTR46. The *S. cerevisiae* genes in Tn*HIS3*, Tn*TRP1*, and Tn*URA3* were from the YDp plasmids (23). The 2μ origin of Tn2μ*HIS3* was from YEp24. The *TRP1–ARS1* fragment of Tn*TRP1ARS1* was from YRp12 (24). TnXR has the *Eco*RI/*Sac* I/*Kpn* I/*Sma* I/*Bam*HI/*Xba* I fragment of pUC18 polylinker between the *Eco*RI and *Xba* I sites of Tn*1000*. The *Sch. pombe* genes in Tn*ura4* and Tn*his7* were from pTZ19U-*ura4* (P. Russell, personal communication) and pEA2 (15), respectively. Subsequent insertions of *ars1* from pIRT2 (25) produced Tn*ura4ars1* and Tn*his7ars1*. $Tn_pADH–GAL4_{1-147}$ has the $_pADH–GAL4_{1-147}$ sequence of pAS1 (26) and a PCR-derived transposon terminus with an open reading frame continuing out from $GAL4_{1-147}$ to the end of the transposon. Tn2μ$HIS3_pADH–GAL_{41-147}$ also contains the 2μ replication origin from YEp24 and the *HIS3* gene of YDpH (23). $Tn_pβ$-globin–*lacZ* has a PCR-derived γ terminus, and the β-globin promoter, *lacZ* coding sequences, and the simian virus 40 polyadenylation signal from BGZ40 (27) and the *Bam*HI/*Spe* I/*Xba* I/*Not* I/*Eag* I/*Sac* II/*Sac* I/*Eco*RI fragment of pBluescript polylinker. Tagged Tn*1000*s were transferred to the conjugative plasmid R388 in cointegrate transfer matings (9).

DNA Manipulations. DNA preparations and manipulations used conventional methods (28). "δ" primer, 5′-AGGGGAACTGAGAGCTCTA-3′, and "γ" primer, 5′-CCTGAAAAGGGACCTTTGTATACTG-3′, were used for transposon-primed nucleotide sequencing (4–6, 29).

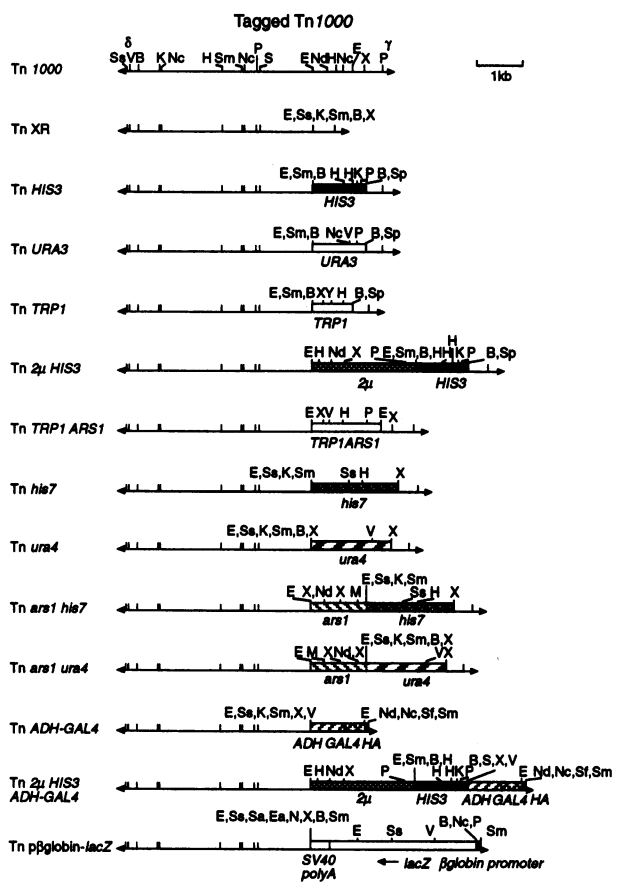

FIG. 1. Structural diagrams of tagged Tn*1000*s. Restriction enzyme sites: B, *Bam*HI; H, *Hin*dIII; E, *Eco*RI; Ea, *Eag* I; M, *Mlu* I; N, *Not* I; Nc, *Nco* I; Nd, *Nde* I; P, *Pst* I; S, *Sal* I; Sa, *Sac* II; Sf, *Sfi* I; Sm, *Sma* I; Ss, *Sst* I, V, *Eco*RV; X, *Xba* I.

Tn*1000* Transposition. The procedure for transposition is described in Fig. 2. A detailed protocol will be supplied upon request.

Transgenesis. Transgenic production and staining reactions for expression of the *lacZ* reporter gene were performed as described (30, 31).

RESULTS

Overview of Transposon Tagging. For transposition, a target plasmid is introduced into the appropriate transposon donor strain (Fig. 1; Table 1). The transformed donor strain is then conjugated with *E. coli* HB101. By selection for the drug resistance of the target plasmid and the streptomycin resistance of HB101, every colony arising from the mating contains an independently transposed plasmid (Fig. 2). A full understanding of the underlying *in vivo* events is not central to the application of this technique, but further details of this process can be found elsewhere (1). Applications of tagged Tn*1000*s are summarized in Table 2 and exemplified below.

Applications of Tagged Transposons Carrying Yeast Selective Markers and Replication Origins. Tn*1000* was modified to deliver the *S. cerevisiae HIS3, URA3*, or *TRP1* genes or *Sch.*

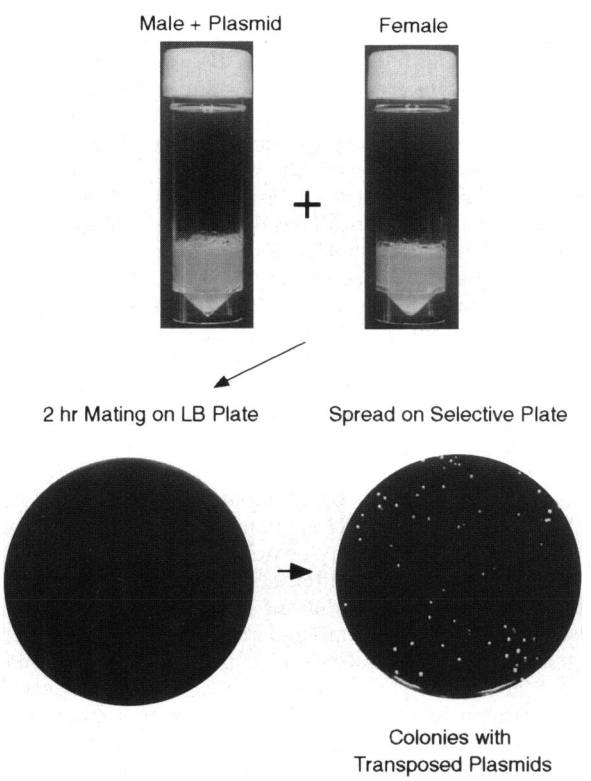

FIG. 2. Tn*1000* transposition. Target plasmids are introduced into the appropriate tagged-transposon male donor strain (Table 1). Two milliliters of a logarithmic-phase culture of the transformed male donor cells are washed twice with drug-free Luria (L) broth to remove the selective antibiotic. The cell pellet is resuspended in 1 ml of a logarithmic-phase culture of the female strain, HB101. The mixture of cells is poured onto an L agar plate and incubated at 37°C for 2 hr to allow mating. Cells are recovered by washing twice in 25 ml of L broth and resuspended in 1 ml of L broth with no drugs. Between 10 and 100 μl of cell suspension is spread on selective plates and incubated overnight at 37°C. For transposition of an ampicillin-resistance plasmid, selective agar contains streptomycin at 100 μg/ml, ampicillin at 50 μg/ml, and methicillin at 50 μg/ml. Methicillin enforces ampicillin selection and without it the method is unlikely to succeed. For transposition of a tetracycline-resistance plasmid, selective plates contain streptomycin at 100 μg/ml and tetracycline at 20 μg/ml.

Table 2. Applications of tagged Tn*1000*

Area of use	Transposon(s)	Applications
S. cerevisiae	Tn*TRP1*, Tn*URA3*, Tn*HIS3*	Introducing selective marker, gene localization, DNA sequencing, domain mapping
	Tn*TRP1ARS1*, Tn2μ*HIS3*	Creation of yeast plasmid, gene localization, DNA sequencing, domain mapping
Sch. pombe	Tn*ura4*, Tn*his7*	Introducing selective marker, gene localization, DNA sequencing, domain mapping
	Tn*ura4ars1*, Tn*his7ars1*	Creation of yeast plasmid, gene localization, DNA sequencing, domain mapping
One- and two-hybrid tests	Tn$_p$*ADH–GAL4*$_{1-147}$	Creation of Gal4 DNA-binding-domain fusions, gene localization, DNA sequencing, domain mapping
	Tn2μ*HIS3*$_p$*ADH–GAL4*$_{1-147}$	Creation of yeast plasmid and Gal4 DNA-binding-domain fusions, gene localization, DNA sequencing, domain mapping
Enhancer trap	Tn$_p\beta$-globin–*lacZ*	Introduction of β-globin minimal promoter–*lacZ* reporter for enhancer activity, introduction of *Not* I site for cosmid dissection, gene localization, DNA sequencing, domain mapping
General purpose	TnXR	Gene disruption, mobile primer site for DNA sequencing, introduction of *Eco*RI–*Xba* I segment of pUC18 polylinker, intermediate for transposon construction

pombe his7 or *ura4* sequences (Fig. 1). Depending on the point of insertion, such tagged transposons either introduce an additional selective marker into a yeast plasmid or disrupt a preexisting marker and replace it with another (Table 2).

The related transposons Tn*TRP1ARS1*, Tn2μ*HIS3*, Tn*his7ars1*, and Tn*ura4ars1* introduce *S. cerevisiae* and *Sch. pombe* replication origins and a selective marker for the conversion of *E. coli* vectors into yeast plasmids. As a simple example, pBluescript was converted into a *Sch. pombe* plasmid by transposition with Tn*ars1his7* (Fig. 3). In practice, the insertion of additional sequences by transposition also allows gene localization in large primary inserts and provides primer sites for DNA sequencing. With the impending conclusion of the yeast DNA sequencing program, transposition of large genomic library plasmids or cosmids offers a rapid, one-step means of gene identification and functional analysis. In addition, multiple hits in cloned DNA from other species can serve as primer sites for complete sequencing.

A Tagged-Transposon Approach to One- and Two-Hybrid Assays. Fusions of the Gal4 DNA-binding domain are employed in one- and two-hybrid tests of transcriptional activation and protein–protein interaction, respectively (10, 11). As an alternative to standard *in vitro* methods, Tn$_p$*ADH–GAL4*$_{1-147}$ (TnAG) (Fig. 1) was constructed for creating Gal4 DNA-binding-domain fusions of proteins encoded by yeast plasmids. As a demonstration of fusions produced by transposition, the *S. cerevisiae* Swi6 protein was tested in one-hybrid assays for transcriptional activity. Swi6 is a component of the *S. cerevisiae* transcription factors SBF and MBF/DSC1, although its precise role is unknown (32). Three of forty-eight random insertions of TnAG into *SWI6* of pTR44 activated expression of the *GAL1–10*$_{uas}$ → *lacZ* reporter gene of *S. cerevisiae* PCY2 (Fig. 4*A*), a standard strain used in one- and two-hybrid assays (16). The three positive clones, Ins1, Ins2, and Ins3, are in-frame fusions of the *GAL4*$_{1-147}$ DNA-binding domain with *SWI6* sequences 3′ to the point of insertion as confirmed by transposon-primed sequencing (Fig. 4*B*). Quantitative analysis of β-galactosidase activity associated with Ins1, Ins2, and Ins3 (Fig. 4*C*) confirmed that the Swi6 protein was able to activate transcription. In addition, Ins4, an in-frame insertion of TnAG with no transcriptional activation was identified, indicating that the region of Swi6 between Ins3 and Ins4 is required for transcriptional activation. Gal4 fusions produced by TnAG were next tested for their suitability in two-hybrid assays. The transcriptionally inactive Gal4–Swi6 fusion, Ins4, was found to interact with the S1–Gal4 activation fusion but not with the Gal4 activation domain alone (Fig. 4*C*). S1 was previously shown to interact with Swi6 in conventional two-hybrid assays and will be described elsewhere (R. Fagan, B.A.M., and S.G.S., unpublished results). Thus, Gal4 fusions can be created by transposition as a rapid alternative to conventional *in vitro* methods. These fusions can be used in one- and two-hybrid assays and also permit domain mapping of the activation domain and the site of interaction.

A Tagged-Transposon Approach to One- and Two-Hybrid Assays in Non-Yeast Plasmids. A further development was to construct a transposon which simultaneously creates Gal4 fusions and converts *E. coli* plasmids into yeast vectors. The objective was to bypass the usual subcloning of DNA into specialized plasmids for one- and two-hybrid assays. Tn2μ*HIS3*$_p$*ADH–GAL4*$_{1-147}$ (Tn2HAG) (Fig. 1) carries the *ADH* promoter and *GAL4* DNA-binding domain for express-

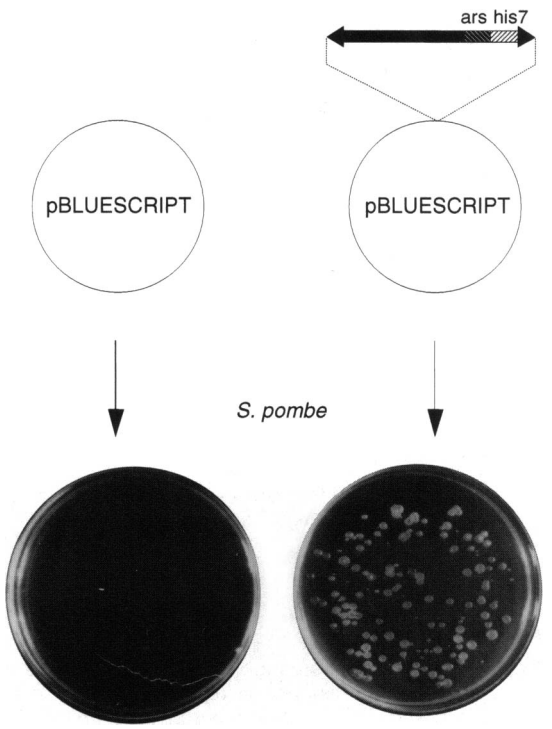

FIG. 3. Construction of a *Sch. pombe* plasmid by transposition with Tn*his7ars1*. Histidine prototrophic colonies of *Sch. pombe* CHP429 were selected after transformation with pBluescript (*Left*) or DNA from a pool of pBluescript randomly transposed with Tn*his7ars1* (*Right*).

ing fusions, and the *HIS3* selectable marker and the 2μ replication origin to convert *E. coli* plasmids into yeast vectors.

As an example of the use of Tn2HAG, the *X. laevis* Pintallavis protein was shown to have transcriptional activation ability in a yeast one-hybrid assay. In *Xenopus*, the *Pintallavis* gene is involved in the induction and patterning of early mesodermal tissue and is thought to be a transcriptional activator based on homology with the HNF-3β protein (33). pFC1, carrying the Pintallavis gene (19, 20), is a simple *E. coli* plasmid unable to replicate in yeast. pFC1 was transposed with Tn2HAG and a pool of 500 independent insertions produced transformant colonies of *S. cerevisiae* PCY2. This demonstrates that the 2μ and *HIS3* elements of Tn2HAG could convert an *E. coli* plasmid into a yeast vector (Fig. 5*A*). In addition, ≈5% of these colonies activated expression of the $GAL1\text{–}10_{uas} \rightarrow lacZ$ reporter gene (Fig. 5*A*). Transposon-primed sequencing of such positive clones confirmed that in-frame fusions had occurred between $GAL4_{1\text{–}147}$ of Tn2HAG and Pintallavis sequences (Fig. 5*B*). Thus, in this system, Pintallavis acts as a transcriptional activator. The positions of Tn2HAG insertions indicate that at least 208 N-terminal residues can be deleted without loss of transcriptional activation (Fig. 5*B*). This indicates that the transcriptional activation domain lies in the C-terminal portion of

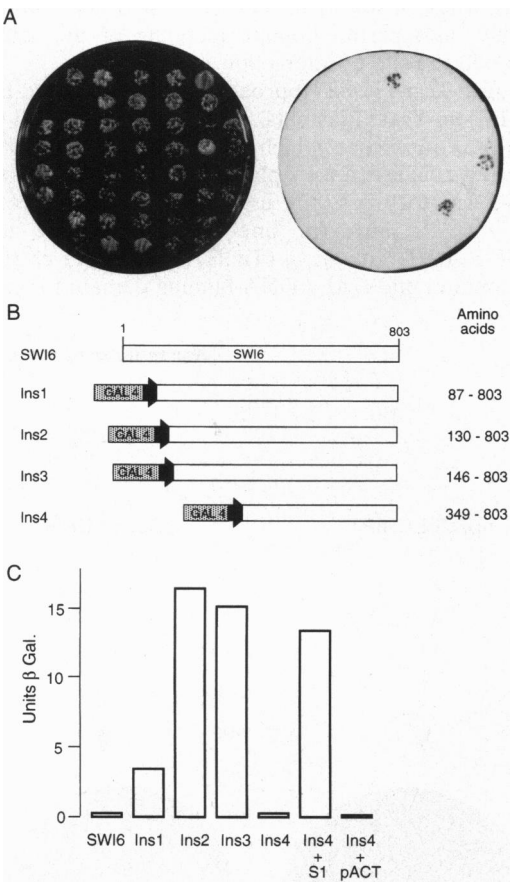

FIG. 4. Construction of $Gal4_{1\text{–}147}$ – Swi6 fusions for one- and two-hybrid assays by transposition. (*A*) Transcriptional activation screen of 48 insertions of TnAG into the *SWI6* gene carried by pTR44. The 48 transposed plasmids were introduced into *S. cerevisiae* PCY2. Transformants (*Left*) were assayed on replica filters (*Right*) for expression of the $GAL1\text{–}10_{uas} \rightarrow lacZ$ reporter gene. (*B*) Fusions of $Gal4_{1\text{–}147}$ with truncated C-terminal fragments of Swi6. Remaining amino acids from the point of gene disruption were determined by transposon-primed DNA sequencing. (*C*) Quantification of transcriptional activation by $Gal4_{1\text{–}147}$ – Swi6 fusions in one- and two-hybrid assays. S1, fusion of *S1* gene and *GAL4* activation domain in pACT (26); pACT, empty vector.

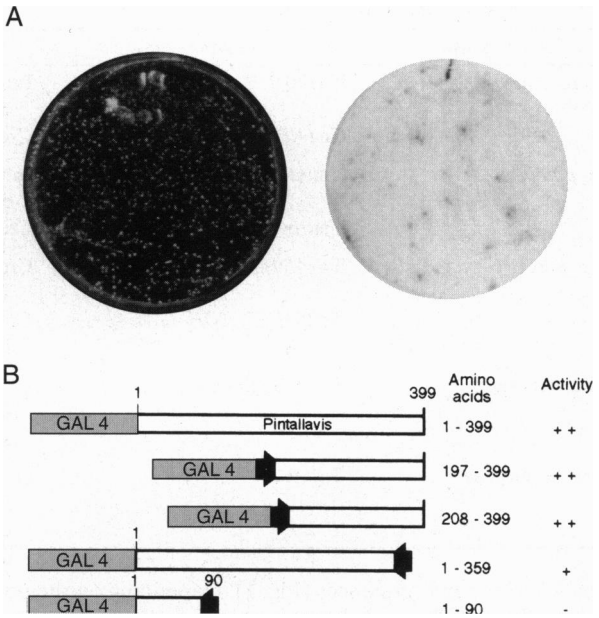

FIG. 5. Analysis of *X. laevis* Pintallavis transcriptional activation by transposition. (*A*) One-step construction of yeast plasmids expressing $Gal4_{1\text{–}147}$ fusions using Tn2HAG. A pool of ≈500 random insertions of Tn2HAG into pFC1 carrying the *Pintallavis* gene was introduced into *S. cerevisiae* PCY2. Transformants to histidine prototrophy were selected (*Left*) and screened for expression of a $GAL1\text{–}10_{uas} \rightarrow lacZ$ reporter gene on a replica filter (*Right*). (*B*) Localization of Pintallavis transcription activation domain. Pintallavis deletion derivatives fused with the $Gal4_{1\text{–}147}$ DNA-binding domain were assayed for β-galactosidase activity as an indicator of transcriptional activation activity: ++, strong activation; +, weak activation; −, no activation.

Pintallavis distal to residue 208. To map this domain further, plasmid pFC2, encoding a fusion of the Gal4 DNA-binding domain to the Pintallavis protein, was transposed with TnXR (Fig. 1) to create transposon-derived C-terminal truncations. When tested in a one-hybrid assay, deletions between residues 90 and 359 eliminated transcriptional activation (Fig. 5*B*), confirming the C-terminal localization of this domain.

Thus, starting with a simple *E. coli* plasmid clone of a higher eukaryotic gene, transposition was used for the one-step construction of yeast plasmids expressing Gal4 DNA-binding-domain fusions.

A Transposon Enhancer Trap. A transposon was designed to localize and detect mammalian enhancer activity (Table 2). The aim was to avoid the time-consuming subcloning normally needed to dissect large primary clones and join subfragments with a basal reporter gene. The enhancer trap transposon $Tn_p\beta$-globin–*lacZ* (Tn*βlac*) contains an expression cassette comprising 80 bp of human minimal β-globin promoter, the *E. coli lacZ* structural sequences, and the simian virus 40 polyadenylation signal (Fig. 1). These elements were derived from BGZ40, a plasmid extensively used for detecting enhancer activity in vertebrates (27, 34, 35). Finally, a *Not* I site, included in a short polylinker, was located 3′ to the polyadenylation signal to allow isolation of segments of linear DNA for microinjection (Figs. 1 and 6*A*).

Tn*βlac* was used to map regulatory components in the 11-kb intergenic region between the *Hox-b4* and *Hox-b5* genes. *Hox* genes are grouped in four complexes in vertebrates (36) and their regulation during development has been extensively studied in transgenic mice via detection of *lacZ* reporter activity (31, 34, 37, 38). After transposition of plasmid pB4Δ2 carrying the *Hox-b4–Hox-b5* intergenic region with Tn*βlac*, a derivative with 6 kb of genomic sequence upstream of a Tn*βlac* insertion was identified. Digestion with *Not* I released a 10-kb fragment which comprised 6 kb of test sequences 5′ to the

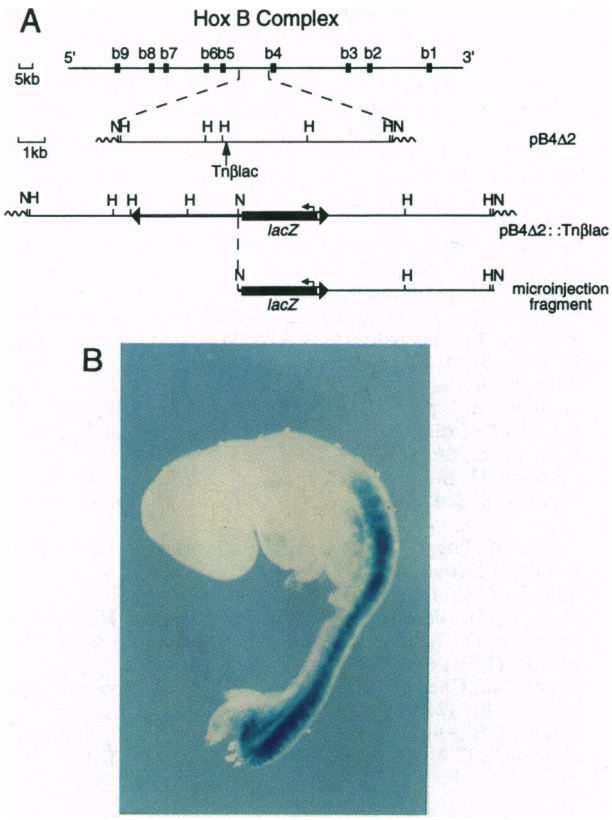

FIG. 6. Detection of a murine *Hox-b* enhancer activity with Tn*βlac* in transgenic mice. (*A*) pB4Δ2 was transposed with Tn*βlac*. By utilizing the *Not* I site of the transposon, a *Not* I fragment encompassing *lacZ* and 5′ *Hox-b* DNA was purified from the transposed plasmid and microinjected into fertilized mouse eggs. (*B*) Transgenic embryo at 9.5 days postcoitum showing characteristic sites of expression of *Hox* genes in the posterior part of the neural tube and in the somitic mesoderm.

reporter gene with an intervening 35 bp of the Tn*1000* γ terminus (Fig. 6*A*). Transgenic mice were then generated by microinjection of fertilized ova. Embryos carrying the transgene showed expression of the *lacZ* reporter in the posterior part of the neural tube and in the somitic mesoderm with a clear-cut anterior limit (Fig. 6*B*), characteristic of *Hox* enhancers (31, 39). In particular, the location of the anterior limit of expression in both neural tube and somitic mesoderm correlates well with *Hox-b5*, as would be expected from the position of the transposed genomic fragment in the *HoxB* complex. Thus, the expression of the transposon-derived reporter was driven by a authentic *Hox* gene enhancer mapping to the *Hox-b4–Hox-b5* intergenic region.

Hence, a simple transposition procedure revealed the activity and position of eukaryotic enhancer activity in a large clone. In addition, the mobile primer sites of Tn*1000* allowed the potential activators to be sequenced and further characterized (data not shown). The use of the enhancer-trap transposon is not limited to transgenic mice but should be applicable to similar experiments in cultured cells.

DISCUSSION

The genetic mobility of transposon Tn*1000* has been harnessed to deliver novel DNA sequences and manipulate recombinant DNA from yeasts, *Xenopus*, and mouse. Furthermore, in developing tagged Tn*1000*, we have produced a simple system for DNA manipulation which is "user friendly" and applicable to most plasmids in current use.

The simpler tagged transposons deliver selective markers and replication origins for the manipulation of budding- and fission-yeast plasmids. Even in this guise, they prove surprisingly useful. For example, transposition of an *E. coli* plasmid with Tn2*μHIS3* bypasses the usual *in vitro* manipulations to create a yeast vector and simultaneously localizes a target gene by functional knockout, provides sites for transposon-primed DNA sequencing, and generates a 3′ truncation series for mapping domains or epitopes. Multiple uses of transposon insertions, summarized in Table 2, are a common feature of the transposon approach and substantially reduce the DNA manipulations required for the analysis of sequence function and structure.

The applications of tagged transposons in yeast one- and two-hybrid assays of eukaryotic protein activity are also demonstrated. Sequences already cloned in yeast plasmids are transposed with TnAG, while Tn2HAG enables *E. coli* plasmids to be transposed for immediate use in yeast. With these transposons, the *S. cerevisiae* Swi6 protein and the *Xenopus* Pintallavis protein were shown to have transcriptional activation activity in yeast one-hybrid assays. In addition, in-frame fusions with no transactivation activity were identified for use in two-hybrid tests. These transcriptionally active and inactive fusions also permitted domain mapping.

The joining of two sequences by an alternative method to *in vitro* enzymatic methods has many potential applications. As an example of future possibilities, a mouse *Hox* gene enhancer activity was detected with transposon Tn*βlac*. To date, identifying enhancer regions has required the movement of fragments, predetermined by restriction-site availability, next to a $_{p}$β-globin–*lacZ* reporter. The transposition approach avoids subcloning by the reverse strategy of moving the reporter gene to the test sequences.

The "toolbox" of transposons is designed primarily as an easy-to-use, "off the peg" delivery system for a central core of genetic elements. The transposon approach is particularly valuable when a sequence has to be repeatedly introduced in multiple sites. As cosmids and P1 plasmids with few unique restriction enzyme sites can be tagged by transposition (P. le Tissier, P. Fantes, and I. Samejima, personal communications), the difficult task of manipulating large plasmids can be greatly simplified. Clearly, similar tools with other elements such as promoters, selective markers or reporter sequences could be tailored to suit other genetic systems or assays. To simplify these future constructions, a simple "cassette" system could be developed for introducing other sequence tags into Tn*1000*. Thus, the applications of tagged transposons are limited only by the imagination of the user.

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