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The *Himar1 mariner* transposase cloned in a recombinant adenovirus vector is functional in mammalian cells

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ABSTRACT

***Mariner* transposons belong to the *mariner*/Tc1 superfamily of class II, DNA-mediated elements. One of these transposons, *Himar1*, isolated from the horn fly, is independent of host-specific factors that would limit transfer between different species, making it an ideal candidate for gene transfer technology development. To determine the activity of *Himar1* transposase in mammalian cells, we introduced the *Himar1* transposase gene into an adenovirus (Ad) vector under control of the phage T7 RNA polymerase promoter. Mammalian cells infected with the Ad vector carrying the *Himar1* gene efficiently expressed the *Himar1* transposase in the presence of T7 polymerase. In *in vitro* inter-plasmid transposition reactions, *Himar1* transposase expressed by the Ad vector mediated precise cut-and-paste transposition and resulted in a characteristic duplication of TA at the integration site of the target plasmid. Further studies showed that this transposase was capable of catalyzing transposition between two plasmids co-transfected into 293T7pol cells, which express T7 RNA polymerase. Combining the integration capability of *mariner* transposons with the transduction efficiency of Ad vectors is expected to provide a powerful tool for introducing transgenes into the host chromosome.**

INTRODUCTION

Mariner transposons are members of the *mariner*/Tc1 superfamily of transposable elements which are widely distributed among animals, including insects, nematodes, flatworms and humans (1–6). They are among the simplest eukaryotic transposons (~1.3 kb), consisting of little more than a single open reading frame (ORF) encoding the transposase protein flanked by inverted terminal repeats (ITRs) of ~30 bp. Only two *mariner* family elements are currently known to be active. The first is *Mos1*, an autonomous version of the original *mariner* element isolated from *Drosophila mauritiana*. The other is *Himar1*, a reconstructed element isolated from the horn fly, *Haematobia irritans* (7–9). Many other *mariner*/Tc1 elements are known from sequence data, but most of these contain multiple inactivating mutations (2).

Mariner transposons move through a DNA intermediate during transposition using a 'cut-and-paste' mechanism, resulting in excision of the transposon from the original location and insertion at novel sites in the genome (10). Two essential components are necessary in this cut-and-paste process: an active transposase functioning *in trans* and the ITRs that flank the elements and are recognized and mobilized by the transposase. *Mariner* family transposons always integrate into a TA target dinucleotide, which is duplicated upon insertion. It has been recently demonstrated that purified *Himar1* transposase is sufficient to mediate transposition in an *in vitro* assay, consistent with the hypothesis, based on the presence of this element in very divergent species, that no host-specific factors are needed for transposase function (9). Given these facts, *mariner* transposons could potentially be exploited for gene delivery to mammalian cells by inserting a foreign gene between the ITRs and transiently producing the transposase, thus inducing integration into the host chromosome DNA. As a preliminary step towards this goal, it is important to determine whether the *mariner* family transposases can be expressed in mammalian cells and can function as predicted by *in vitro* experiments.

Adenovirus (Ad) vectors have been used for heterologous gene expression in mammalian cells, for vaccine development and for gene therapy (11–18). A number of properties of Ad vectors facilitate their use as expression vectors in mammalian cells. Ad type 5 (Ad5) can easily be grown to high titers and can infect both replicating and non-replicating cells of many types. Using standard recombinant DNA techniques, the 36 kb double-stranded DNA genome is relatively easy to manipulate. Ad virions are physically and genetically stable if the vectors are constructed and propagated appropriately. Viruses with a deletion of E1 can be propagated in 293 cells and can infect other mammalian cell lines, but are defective for replication. However, despite these significant attributes, there are several disadvantages of

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Ad as a gene transfer vector, one of which is its failure to integrate, with a resultant inability to sustain long-term transgene expression, at least in proliferating cells, as might be required for certain applications. A combination of the highly efficient transduction capability of Ad together with persistent expression resulting from transposase-induced integration of the transgene into the chromosome would produce an extremely powerful vector for a variety of applications, perhaps including gene therapy.

In this article, we describe the construction and characterization of an Ad vector expressing *Himar1* transposase. Because *mariner*-like sequences are found in the DNA of many species, including humans (3–6), and because Ad vectors efficiently transduce many human cell types, the vector was designed to express transposase only in the presence of T7 RNA polymerase. Thus the *Himar1* transposase gene was introduced into Ad under control of the phage T7 promoter. We show that 293T7pol cells infected with an Ad vector carrying the *Himar1* gene efficiently expressed the transposase and demonstrate that the *Himar1* transposase was capable of catalyzing transposition both *in vitro* and in mammalian cells.

MATERIALS AND METHODS

Construction of recombinant plasmids

Plasmids constructed by standard protocols (19) were used to transform *Escherichia coli* DH5α by the $CaCl_2$ method. Plasmid DNA was prepared by the alkaline lysis method (20) and purified by CsCl/ethidium bromide density gradient centrifugation. Restriction enzymes were purchased from Boehringer Mannheim Canada, Gibco BRL and New England Biolabs. The coding sequence for the *Himar1* transposase was obtained from plasmid pMarNde 18 (9).

Cells and viruses

293 cells (Ad5 E1-transformed human embryonic kidney cells; 21,22) were grown as a monolayer in F11 minimal essential medium (MEM) (Gibco BRL) with 10% fetal bovine serum and antibiotics. 293T7pol cells expressing T7 polymerase obtained from L.Prevec (McMaster University, Hamilton, Canada; manuscript in preparation) were grown in F11 MEM supplemented with 10% fetal bovine serum, antibiotics and 400 µg/ml G418. Recombinant Ad vectors were rescued and propagated using the 293 cell line as described by Hitt *et al*. (23). High titer recombinant viral stocks were prepared as crude lysates of infected 293 cells. Viral stocks were titered on 293 cell monolayers and all titers are expressed as plaque forming units (p.f.u.)/ml.

Expression of *Himar1* transposase in cells infected with recombinant Ad vector

293T7pol cells in 60 mm dishes were infected at a multiplicity of infection (m.o.i.) of 5 p.f.u./cell in 200 µl phosphate-buffered saline (PBS) supplemented with 0.65 mM Mg^{2+} and 0.5 mM Ca^{2+} per dish. After 30 min adsorption at room temperature 5 ml culture medium was added and the cells were incubated at 37°C. Twenty four hours post-infection, medium was removed from the dishes and the cells were rinsed with 5 ml ice-cold PBS. Cells were scraped into 400 µl SDS–PAGE loading buffer [50 mM Tris–HCl, pH 6.8, 100 mM dithiothreitol (DTT), 2% SDS, 0.1% bromophenol blue, 10% glycerol] and the DNA sheared by passage through a 25 gauge needle several times and transferred into 1.5 ml Eppendorf tubes. An extract of *Drosophila melanogaster* with the *Himar1* transposase gene under control of the *D.melanogaster* heat shock promoter *hsp70* (D.J.Lampe, unpublished data) was used as a positive control for transposase expression. To heat shock flies, four flies were transferred to a vial and incubated at 35°C for 1 h. Either 1 or 20 h after heat shock as noted, the flies were killed and ground in 200 µl SDS–PAGE loading buffer.

Western blot analysis

SDS–PAGE was performed following the standard procedures of Laemmli (24). Proteins were transferred from SDS–10% polyacrylamide to Millipore Immobilon P polyvinylidene difluoride membranes (Millipore, Mississauga, Canada) at 80 V for 1 h, using a Bio-Rad transblot cell (Bio-Rad Laboratories, Richmond, CA). The transposase protein was detected by western blotting using a mouse polyclonal anti-*Himar1* transposase antibody at a dilution of 1:500 in Tris-buffered skimmed milk powder (5%) and a goat anti-mouse immunoglobulin G labelled with horseradish peroxidase (HRP) (Pierce, Rockford, IL) as the secondary antibody at a dilution of 1:2000 in the same solution as the first antibody. The HRP reaction was monitored using enhanced chemiluminescence reagents for western blots from Amersham (Oakville, Canada) and Kodak XAR5 film (Eastman Kodak Co., Rochester, NY).

Preparation of nuclear cell extracts for *in vitro* transposition assay

293T7pol cells in 60 mm dishes were infected with virus at a m.o.i. of 5 p.f.u./cell and 24 h post-infection cells were washed twice with cold PBS. Ice-cold TEN solution (0.1 M Tris–HCl, pH 8.0, 0.01 M EDTA, pH 8.0, 1 M NaCl; 1.5 ml) was added and incubated for 5 min. Cells were resuspended, transferred into 15 ml tubes, then centrifuged at 2000 r.p.m. for 10 min at 4°C. Fifty microliters of buffer A [0.1 M HEPES, pH 7.9, 0.1 M $MgCl_2$, 0.3 M KCl, 0.5 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM benzamidine, 1 µg/ml pepstatin A and 1 µg/ml leupeptin] were added to the cell pellet and the cells were lysed by passage through a 25 gauge needle several times. Cell lysates were centrifuged at 12 000 r.p.m. for 20 s and the nuclear pellets were separated from the cytosolic (supernatant) fractions. The nuclear pellets were resuspended in buffer C (20 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 400 mM NaCl, 0.2 mM EDTA, 25% glycerol, 1 mM DTT, 1 mM PMSF, 0.1 mM benzamidine, 1 µg/ml pepstatin A and 1 µg/ml leupeptin) and incubated for 30 min on ice with gentle agitation. The nuclear fractions were centrifuged for 20 min to remove any debris.

An *in vitro* transposition assay for *Himar1* transposase expressed by Ad vector

The donor construct pMinimariner-Kan has been described previously (25). The target plasmid was a naturally occurring tetramer of pBluescript KS+ (pBSKS+) (9). The assays were performed in transposition buffer (10% v/v glycerol, 25 mM HEPES, pH 7.9, 250 µg acetylated BSA, 2 mM DTT, 100 mM NaCl and 5 mM $MgCl_2$) and contained 1 µl cell extract in a final volume of 20 µl. One hundred nanograms of target plasmid pBSKS+ tetramer and 32 ng donor plasmid pMinimariner-Kan were used for each 20 µl reaction. The reaction mix was incubated

for 2 h at 30°C and stopped by addition of 80 µl stop solution (50 mM Tris–HCl, pH 7.6, 0.5 mg/ml proteinase K, 10 mM EDTA, 250 µg/ml yeast tRNA) and, after a further incubation at 37°C for 30 min, DNA was extracted with phenol/chloroform and precipitated with ethanol. The DNA pellet was dissolved in 10 µl TE and 1 µl DNA was used to transform TOP 10 F′ *E.coli* cells (Invitrogen) by electroporation (Bio-Rad). One milliliter of SOC medium was added to the transformed cells and the suspension incubated at 37°C with vigorous shaking for 30 min. An aliquot corresponding to 1 µl undiluted cell culture was plated on LB-Amp (100 µg/ml) agar plates and 500 µl were plated on LB-Amp (100 µg/ml)-Kan (30 µg/ml) agar plates. The oligonucleotides 76r (5′-AATCATTTGAAGGTTGGTAC-3′) and 1218f (5′-TCGCTCTTGAAGGGAA CTATG-3′) were used to sequence the potential transposition products.

Activity of the Ad transposase in cultured cells

293T7pol cells in 60 mm dishes were mock infected or infected with AdT7Mariner at a m.o.i. of 5 p.f.u./cell and 4 h later the cells were co-transfected with 5 µg each pMinimariner-Kan and pBSKS+ tetramer. After 4 h the medium was replaced. Eighteen hours after transfection, cellular DNA was extracted as described (12). The DNA pellet was dissolved in 50 µl TE and 10 µl was used to transform *E.coli* DH5α cells by the $CaCl_2$ method. One milliliter of LB medium was added to the transformed cells and the suspension incubated at 37°C with vigorous shaking for 30 min. The cells were concentrated 10-fold by centrifugation. An aliquot corresponding to 1 µl undiluted cell culture was plated on LB-Amp (100 µg/ml) agar plates and the remaining 100 µl were plated on LB-Amp (100 µg/ml)-Kan (30 µg/ml) agar plates for colony isolation and plasmid DNA analysis.

RESULTS

Construction of a recombinant Ad vector encoding *Himar1* transposase

AdT7Mariner encoding *Himar1* transposase was constructed as follows. The 1.3 kb *Xba*I–*Eco*RI fragment from pMarNde 18 (9) containing the coding sequence for *Himar1* transposase was cloned into pUC18, generating pUCMAR. The coding sequence for *Himar1* was excised from pUCMAR by digestion with *Sal*I and *Eco*RI and inserted into *Sal*I and *Eco*RI-digested shuttle plasmid pT7ProR (Tomanin *et al.*, manuscript in preparation), which contains the T7 promoter, the encephalomyocarditis virus internal ribosomal entry site (IRES) element and T7 terminator sequences, to produce pT7Mariner. The *Sal*I–*Nde*I fragment was removed from pT7Mariner, resulting in pT7MarNde, in which the coding region for *Himar1* is immediately downstream of the T7 promoter. Further details on the cloning steps can be obtained from the authors. The recombinant virus AdT7Mariner was generated by *in vivo* recombination between pT7MarNde and pBHG10 (26) following co-transfection of 293 cells (Fig. 1). Candidate AdT7Mariner plaques were screened by restriction enzyme digestion of viral DNA and gel electrophoresis. Recombinants with the predicted DNA structure were plaque purified and grown to high titer for subsequent studies.

Detection of *Himar1* transposase expression in 293T7pol cells by western blot assay

The level of *Himar1* transposase expression was measured in infected 293T7pol cells, which express T7 RNA polymerase. Cells were either mock infected or infected at a m.o.i. of 10 p.f.u./cell with AdT7Mariner or AdBHG10T7βgal (27), the latter being a negative control. Cell extracts were prepared 24 h post-infection and analyzed by western blot analysis. AdT7Mariner expressed readily detectable amounts of *Himar1* transposase in infected 293T7pol cells (Fig. 2A). The major species (~41 kDa) detected in AdT7Mariner-infected 293T7pol cell extracts co-migrated with the transposase produced by heat shock-treated *D.melanogaster* carrying the *Himar1* gene under *hsp70* control. One other band migrating at ~47 kDa was detected specifically in extracts from AdT7Mariner-infected cells and may be the result of translation from aberrantly spliced mRNAs containing transposase sequences. Other species with molecular weights of ~38 and 44 kDa were present in control extracts (e.g. 293T7pol cells infected with AdBHG10T7βgal) and presumedly resulted from cross-reactivity in the polyclonal antibody preparation. Western blot analysis did not detect any transposase expression in 293 cells infected with AdT7Mariner (Fig. 2B). This tightly regulated expression from the T7 promoter is consistent with previous results obtained using the T7 system in Ad vectors (27).

An *in vitro* assay for *Himar1* transposase expressed by AdT7Mariner in 293T7pol cells

An overview of the *in vitro* assay for transposition is shown in Figure 3. The assay measures transfer of the kanamycin resistance gene (Kan^R) from a donor plasmid to a site on the target ampicillin resistance (Amp^R) plasmid, conferring double resistance on the resulting recombinant. The target plasmid is a tetramer of pBSKS+, which is used in order to increase the chance of recovering transposition products, since insertions into any given Amp^R gene or origin of replication in one of the monomers will produce a viable plasmid product (9). The donor plasmid is pMinimariner-Kan, in which the kanamycin resistance gene is flanked by minimariner sequences, including *Himar1* ITR sequences. The donor and target plasmids were mixed and incubated with nuclear extracts from either AdT7Mariner-infected 293T7pol cells or mock-infected 293T7pol cells as a control. While performing the transposition assays, we attempted to ensure that the colonies picked were independent by incubating *E.coli* transformed with DNA extracted from the *in vitro* reaction mixture for no more than 30 min before plating. Kan^R-Amp^R transposition products were selected on agar plates containing both kanamycin and ampicillin. Selection on agar plates containing ampicillin alone was used as a control for the efficiency of overall DNA recovery and transformation. A significant number of Kan^R-Amp^R colonies were generated in reactions that used AdT7Mariner-infected cell extracts, while no Kan^R-Amp^R bacteria were recovered from reactions with mock-infected cell extracts (Table 1). The average relative efficiency of transposition catalyzed by AdT7Mariner-infected cell extracts was 6.2 ± 2.2 per 10^3 Amp^R colonies.

Four doubly resistant colonies were analyzed in detail by restriction digestion as well as sequence analysis. An *Nco*I site is present only in the Kan^R gene of pMinimariner-Kan, whereas *Eco*RI sites are located outside the minimariner segment in the plasmid backbone sequences of pMinimariner-Kan and at the

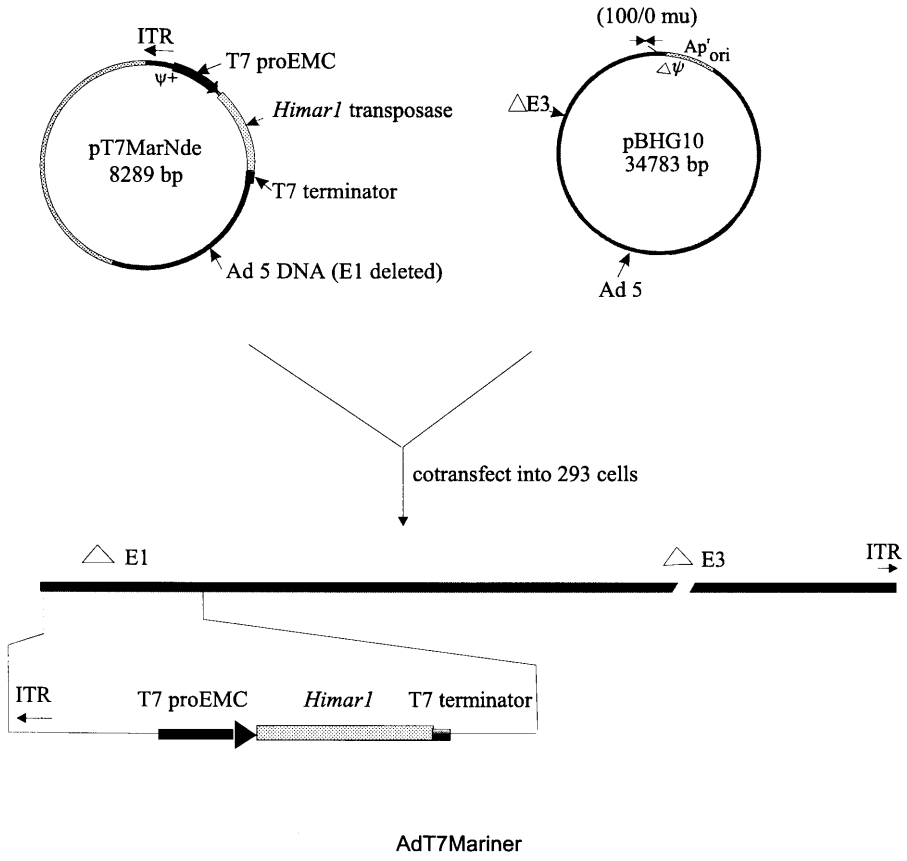


Figure 1. Schematic representation of the final step in the generation of AdT7Mariner. The cloning strategy is described in detail in Results. The *Himar1* transposase gene was inserted into pT7ProR (not shown), placing it under control of the T7 promoter (pT7MarNde). To obtain AdT7Mariner, pT7MarNde was co-transfected into 293 cells with pBHG10 to produce the illustrated vector by homologous recombination. mu, map unit; Δψ, packaging signal deleted; ψ^+, presence of packaging signal; Ap^r, ampicillin resistance gene; ori, bacterial origin of replication; ΔE3, E3 deleted.

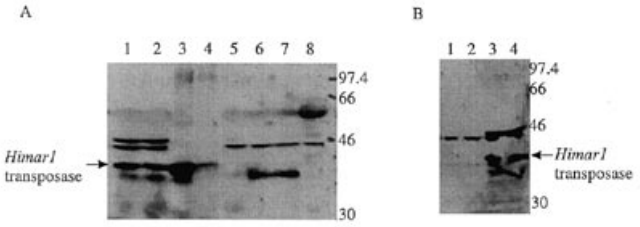


Figure 2. Western blot analysis of cell extracts from 293 or 293T7pol cells infected with AdT7Mariner or control virus as described in Materials and Methods. Molecular weight in kDa is indicated on the right and the *Himar1* transposase band (~41 kDa) is indicated on the left (A) or right (B). (**A**) Lanes 1 and 2, extract of 293T7pol cells infected with AdT7Mariner; lane 3, extract of *Drosophila* with the *Himar1* gene after 20 h heat shock; lane 4, extract of *Drosophila* with the *Himar1* gene after 1 h heat shock; lane 5, extract of mock-infected 293T7pol cells; lanes 6 and 7, extract of 293T7pol cells infected with AdBHG10T7βgal; lane 8, extract of mock-infected 293 cells. (**B**) Lanes 1–2, extract of 293 cells infected with AdT7Mariner; lanes 3 and 4, extract of 293T7pol cells infected with AdT7Mariner.

junctions of the pBSKS+ tetrameric repeats (see Fig. 3). Therefore, insertion of the minimariner-Kan^R sequence into a target plasmid would increase the size of a pBSKS+ monomer by 1.3 kb. Isolation of DNA from four doubly resistant plasmids generated by the *in vitro* transposition assays described above, followed by digestion with *Eco*RI and *Nco*I produced a 2.9 kb band (corresponding to three target monomers) and two novel bands with a combined size totalling ~4.2 kb (a 2.9 kb monomer target with a 1.3 kb minimariner-Kan^R insertion; Fig. 4A).

To verify that these plasmids derived from true transposition events, we analyzed the sequences of these four transposition products at the junctions between the target and donor sequences. The results indicate that in each doubly resistant plasmid the transposon had inserted at a single TA dinucleotide, duplicating the target TA as predicted by the known mechanism for *Himar1* transposition (Fig. 4B). Two of the four transposition products, M1-2 and M1-4, were identical (Fig. 4B).

Himar1-mediated integration in human cells

In order to determine whether *Himar1* transposase is active in AdT7Mariner-infected cells, 293T7pol cells were infected with AdT7Mariner and then the cells were co-transfected with pMinimariner-Kan and pBSKS+ tetramer. In the absence of any selection for transposition, the DNA was extracted from these cells and used to transform *E.coli* DH5α cells. The structures and sequences of the four recombinant products (i.e. Kan^R-Amp^R plasmids) detected in two independent experiments (two for each assay) were consistent with accurate transposition from the donor

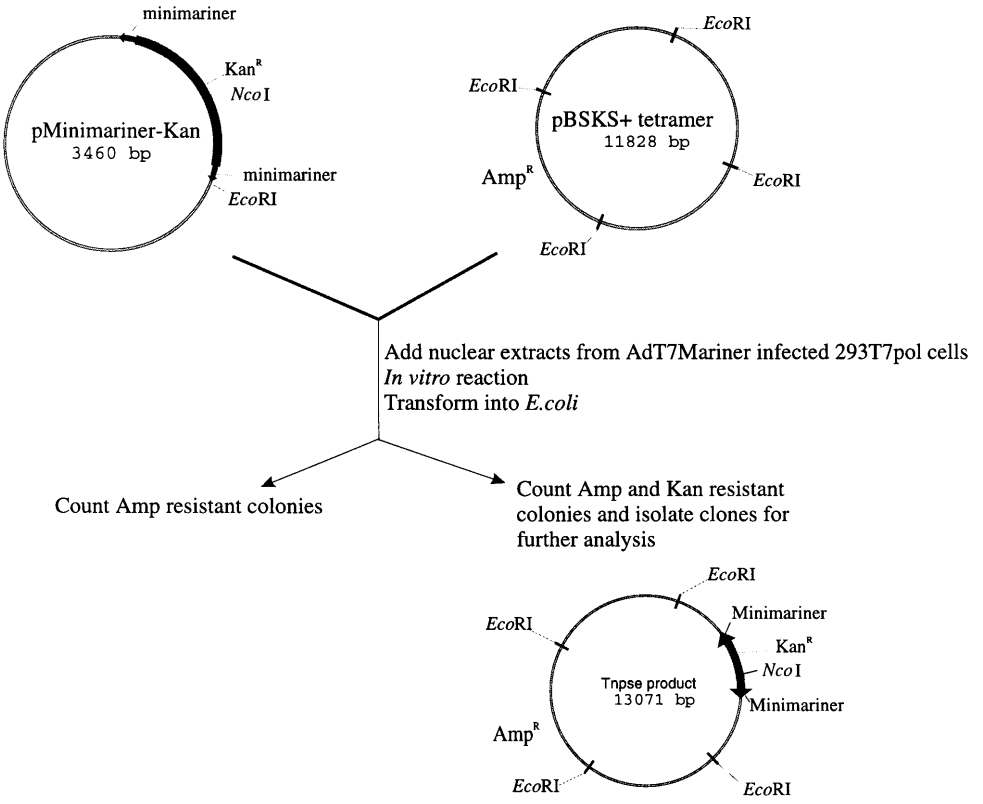


Figure 3. An overview of the *in vitro* assay for transposition. The assay is described in Materials and Methods.

to the target plasmids (Fig. 5). One of the doubly resistant plasmids, C9, was identical to the *in vitro* transposition products M1-2 and M1-4. No doubly resistant plasmids were recovered from cells co-transfected with pMinimariner-Kan and pBSKS+ tetramer in the absence of AdT7Mariner.

Table 1. *In vitro* transposition assays[a]

	Amp^R (colonies/ml bacteria)	Amp^RKan^R (colonies/ml bacteria)	Efficiency
Control[b]	5×10^3	0	
Experiment 1	3×10^3	28	$9.3/10^3$
Experiment 2	5×10^3	30	$6/10^3$
Experiment 3	4×10^3	20	$5/10^3$
Experiment 4	4×10^3	18	$4.5/10^3$
Average rate			$6.2 \pm 2.2/10^3$

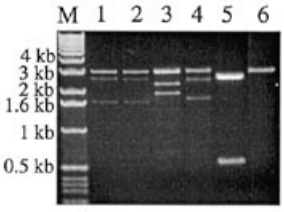
[a]A nuclear extract prepared from 293T7pol cells infected with AdT7Mariner was incubated *in vitro* with target plus donor plasmids in four separate *in vitro* assays as described in Materials and Methods.
[b]A nuclear extract from mock-infected 293Tpol cells was added to donor and target plasmid mixtures for the *in vitro* assay.

DISCUSSION

An Ad vector encoding *Himar1* transposase has been constructed

Mariner is a widespread and diverse family of transposons. Because *mariner* family transposition relies solely on the *mariner* transposase and requires no host cell factors, we predicted that *Himar1* transposase should be capable of catalyzing transposition in mammalian cells. We are interested in combining the high cell transduction efficiencies attainable with Ad vectors with transposable element components that might allow integration of transgenes at high frequency. As a first step in the program we cloned the *Himar1* gene in an Ad vector to test for expression and function of the transposase in infected mammalian cells. Because *mariner*-like elements are found in the DNA of many species, including the human genome (3–6), we wished to ensure that the transposase gene cloned in Ad could be expressed only under very tight control. It has already been shown that in the absence of T7 polymerase, expression from the T7 promoter in Ad vectors is extremely low (27). Therefore, we used the T7 RNA polymerase system and 293T7pol cells expressing T7 RNA polymerase to obtain transposase expression, avoiding the possibility of expression in other cell types which might possibly promote rearrangements of the infected cell genome due to induction of transposition by endogenous elements. *Himar1* transposase could be expressed in AdT7Mariner-infected 293T7pol cells, which express T7 RNA polymerase as well as allow virus replication. In 293 cells infected with AdT7Mariner,

A



B

	Target	5'ITR	3'ITR	dupl	Target
Donor	GGATTATA-	ACAGGTTGG.....CCAACCTGT-	**TA**	-TAATCTGC	
M1-2	ACCAAATA-	ACAGGTTGG.....CCAACCTGT-	**TA**	-CTGTCCTT	
M1-4	ACCAAATA-	ACAGGTTGG.....CCAACCTGT-	**TA**	-CTGTCCTT	
M2-13	AGGGCTTA-	ACAGGTTGG.....CCAACCTGT-	**TA**	-CCATCTGG	
M2-14	AAACTTTA-	ACAGGTTGG.....CCAACCTGT-	**TA**	-AAAGTGCT	

Figure 4. (**A**) Restriction analysis of *in vitro* transposition products. DNA was isolated from four independent doubly resistant colonies (M1-2, M1-4, M2-13 and M2-14) generated by *in vitro* transposition assays using extracts from AdT7Mariner-infected 293 T7 cells. The DNA was digested with *Nco*I and *Eco*RI and fragments were separated by agarose gel electrophoresis and stained with ethidium bromide. Lane M, DNA molecular marker; lane 1, M1-2; lane 2, M1-4; lane 3, M2-13; lane 4, M2-14; lane 5, donor plasmid pMinimariner-Kan; lane 6, target plasmid pBSKS+ tetramer. A 2.9 kb fragment is indicative of a pBSKS monomer without an insert and the presence of two fragments totalling 4.2 kb is diagnostic for insertion of the 1.3 kb minimariner-Kan sequence into a pBSKS+ monomer. (**B**) Sequence analysis of the transposon termini and the insertion site of these Kan^R-Amp^R products.

there was no detectable transposase expression, confirming that in the AdT7 system transposase can only be expressed in the presence of T7 polymerase.

Himar1 transposase expressed by Ad vector is active both in a cell-free system and in human cells

Recently it was reported that the *D.mauritiana mariner* element is active in the human parasite *Leishmania major* (1). Furthermore, a synthetic salmonid transposon, *Sleeping Beauty*, was shown to mediate precise cut-and-paste transposition in fish as well as in mouse and human cells (28). These data suggest that *mariner*/TC1-type elements are autonomous and may be able to cross distant species boundaries *in vivo*. Our data indicate not only that *Himar1* transposase could be expressed in mammalian cells infected with AdT7Mariner, but also that this transposase was functional. *In vitro* assays of infected cell extracts showed clearly that cut-and-paste transposition occurred as predicted. In addition, we detected no inhibitors of transposase present in uninfected cell extracts when they were added to purified *Himar1* transposase for the *in vitro* assay (data not shown). Neither did we detect any transposition in the absence of transposase expression, although mammalian proteins HMG1 and HMG2 have been reported to stimulate prokaryotic recombination (29).

Furthermore, upon co-transfection of donor and target plasmids into 293T7pol cells infected with AdT7Mariner, the transposase could remove a Kan^R gene flanked by minimariner sequences from the donor plasmid and paste it into the target plasmid. It also has been shown that *Mos1*, one of only two known active *mariner*

	Target	5'ITR	3'ITR	dupl	Target
Donor	GGATTATA-	ACAGGTTGG...CCAACCTGT-	**TA**	-TAATCTGC	
C9	ACCAAATA-	ACAGGTTGG...CCAACCTGT-	**TA**	-CTGTCCTT	
C12	GACAGTTA-	ACAGGTTGG...CCAACCTGT-	**TA**	-CCAATGCT	
A-1	CCGAGATA-	ACAGGTTGG...CCAACCTGT-	**TA**	-GGGTTGAG	
A-2	TTCTGCTA-	ACAGGTTGG...CCAACCTGT-	**TA**	-TGTGGCGC	

Figure 5. Sequence analysis of the transposon termini and the insertion site of the four Kan^R-Amp^R products (C9, C12, A-1 and A-2) from two intracellular transposition experiments.

elements, could catalyze inter-plasmid transposition reactions (1). We assume that the minimariner-Kan^R cassette was inserted into the host chromosome as well, but such an event would be difficult to detect in these experiments, since these cells are permissive for productive Ad infection, resulting in lysis of the host cells. Based on the finding that two transposition products were recovered from each of the cell culture transposition assays and assuming that the frequency of transposition between plasmids (in events per megabase DNA sequence) is identical to that between a plasmid and chromosome DNA, we estimate that minimariner element transposition into the human genome would occur with a frequency of ~1/10 cells.

Ad *Himar1* transposase activity confirms a model of the transposition of the Tc1/*mariner* family

Transposition events can be of three general types: non-replicative transposition, replicative transposition or retrotransposition. The members of Tc1/*mariner* family belong to the non-replicative transposition elements, as are the bacterial transposons Tn*7* (30,31) and Tn*10* (32) as well as the *Drosophila* P element (33). A hallmark of transposition catalyzed by *mariner* transposases is the presence of a duplicated TA dinucleotide at the integration site. Sequence analysis of our transposition products confirmed that all junction sequences contained the predicted ends of the element and TA target site duplications flanking the insertion. *Himar1* transposase was originally isolated from insect cells and the activity of this transposase in mammalian cells further indicates that the members of the Tc1/*mariner* family are independent of species-specific factors, in contrast to P elements, which are restricted to *Drosophila* species (34).

Of the eight transposition products sequenced, three are identical (two from *in vitro* assays, one from intracellular events), suggesting that there might be some 'hotspots' present in the target sequence, as observed previously (25). Several factors could affect regional preferences for integration, such as the primary DNA sequence flanking the TA, DNA binding proteins and bending of the DNA, although further studies are required to determine which if any of these are important.

Potential applications of the AdT7Mariner vector

One goal of gene therapy is to introduce transgenes efficiently into target cells in which the corresponding genes are defective or missing in such a way that expression of the transgene product is long lasting. A variety of recombinant viruses have been explored for the application of gene therapy. Of these, recombinant retroviruses were among the first and most extensively utilized vector systems. The major problems of retroviruses are their inability to infect non-dividing cells and difficulty in preparing high titers of virus stocks. Adeno-associated virus (AAV) is a

more recently developed virus-based system. In the absence of helper virus, wild-type AAV is known to stably integrate into a specific site in human chromosome 19 and remain latent (35–37). However, it has been difficult to develop a packaging cell line with this system because many of the AAV and adenoviral genes necessary for recombinant AAV production are toxic to cells when expressed constitutively (38).

Many researchers have suggested that *mariner* transposons could be adapted for use as transformation vectors that might be employed in a broad range of animals (39–41). If appropriate delivery of *mariner* transposases into mammalian cells could be attained, it would be very useful for introducing transgenes into the host chromosome. As mentioned in the introduction, Ad vectors possess many attractive features, with the result that they have emerged as vehicles of choice for many gene transfer applications. The data presented here suggest that the integration capability of *mariner* transposons can be combined with the transduction efficiency of Ad vectors for the transfer of specific genes to the genome of transduced cells. This is an important step towards enlarging the application of Ad vectors for gene therapy. In addition, we predict that this system could also be very useful in the genetic analysis of human and other mammalian genomes. For example, the Ad-*mariner* system can be applied to identify specific genes within mammals through transposon tagging or 'trapping'. All these interesting aspects of the Ad-*mariner* system will require further study.

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REFERENCES

1 Gueiros-Filho,F.J. and Beverley,S.M. (1997) *Science*, **276**, 1716–1718.
2 Robertson,H.M. (1993) *Nature*, **362**, 241–245.
3 Morgan,G.T. (1995) *J. Mol. Biol.*, **254**, 1–5.
4 Auge-Gouillou,C., Bigot,Y., Pollet,N., Hamelin,M.H., Meunier-Rotival,M. and Periquet,G. (1995) *FEBS Lett.*, **368**, 541–546.
5 Robertson,H.M. and Zumpano,K.L. (1997) *Gene*, **205**, 203–217.
6 Robertson,H.M. and Martos,R. (1997) *Gene*, **205**, 219–228.
7 Hartl,D.L., Lohe,A.R. and Lozovskaya,E.R. (1997) *Annu. Rev. Genet.*, **31**, 337–358.
8 Robertson,H.M. and Lampe,D.J. (1995) *Mol. Biol. Evol.*, **12**, 850–862.
9 Lampe,D.J., Churchill,M.E.A. and Robertson,H.M. (1996) *EMBO J.*, **15**, 5470–5479.
10 Plasterk,R.H. (1996) *Curr. Topics Microbiol. Immnuol.*, **204**, 125–143.
11 Berkner,K.L. (1992) *Curr. Topics Microbiol. Immnuol.*, **158**, 39–66.
12 Graham,F.L. and Prevec,L. (1991) *Methods Mol. Biol.*, **7**, 109–128.
13 Crystal,R.G., McElvaney,N.G., Rosenfeld,M.A., Chu,C.S., Mastrangeli,A., Hay,J.G., Brody,S.L., Jaffe,H.A., Eissa,N.T. and Danel,C. (1994) *Nature Genet.*, **8**, 42–51.
14 Le Gal La Salle,G., Robert,J.J., Berrard,S., Ridoux,V., Stratford Perricaudet,L.D., Perricaudet,M. and Mallet,J. (1993) *Science*, **259**, 988–990.
15 Prevec,L., Campbell,J.B., Christie,B.S., Belbeck,L. and Graham,F.L. (1990) *J. Infect. Dis.*, **161**, 27–30.
16 Ragot,T., Vincent,N., Chafey,P., Vigne,E., Gilgenkrantz,H., Couton,D., Cartaud,J., Briand,P., Kaplan,J.C., Perricaudet,M. *et al*. (1993) *Nature (Lond.)*, **361**, 647–650.
17 Stratford-Perricaudet,L.D., Makeh,I., Perricaudet,M. and Briand,P. (1992) *J. Clin. Invest.*, **90**, 626–630.
18 Zabner,J., Couture,L.A., Gregory,R.J., Graham,S.M., Smith,A.E. and Welsh,M.J. (1993) *Cell*, **75**, 207–216.
19 Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
20 Birnboim,H.C. and Doly,J. (1978) *Nucleic Acids Res.*, **7**, 1513–1523.
21 Graham,F.L., Smiley,J., Russell,W.C. and Nairn,R. (1977) *J. Gen. Virol.*, **36**, 59–72.
22 Louis,N., Evelegh,C. and Graham,F.L. (1997) *Virology*, **233**, 423–429.
23 Hitt,M., Bett,A.J., Addison,C., Prevec,L. and Graham,F.L. (1995) In Adolph,K.W. (ed.), *Methods in Molecular Genetics*. Academic Press, Orlando, FL, Vol. 7B, pp. 13–30.
24 Laemmli,U.K. (1970) *Nature (Lond.)*, **227**, 680–685.
25 Lampe,D.J., Grant,T.E. and Robertson,H.M. (1998) *Genetics*, **149**, 179–187.
26 Bett,A.J., Haddara,W., Prevec,L. and Graham,F.L. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 8802–8806.
27 Tomanin.R., Bett,A.J., Picci,L., Scarpa,M. and Graham,F.L. (1997) *Gene*, **193**, 129–140.
28 Ivics.Z., Hackett,P.B., Plasterk,R.H. and Izsvak,Z. (1997) *Cell*, **91**, 501–510.
29 Paull,T.T., Haykinson,M.J. and Johnson,R.C. (1993) *Genes Dev.*, **7**, 1521–1534.
30 Bainton,R.J., Gamas,P. and Craig,N.L. (1991) *Cell*, **65**, 805–816.
31 Bainton,R.J., Kubo,K.M., Feng,J.N. and Craig,N.L. (1993) *Cell*, **72**, 931–943.
32 Bender,J. and Kleckner,N. (1986) *Cell*, **45**, 801–815.
33 Kaufman,P.D. and Rio,D.C. (1992) *Cell*, **69**, 27–39.
34 Rio,D.C., Barnes,G., Laski,F.A., Rine,J. and Rubin,G.M. (1988) *J. Mol. Biol.*, **200**, 411–415.
35 Kotin,R.M. (1994) *Hum. Gene Ther.*, **5**, 793–801.
36 Kotin,R.M., Siniscalco,M., Samulski,R.J., Zhu,X., Hunter,L., Laughlin,K.A., Mclaughlin,S.K., Muzyczka,N., Rocchi,M. and Berns,K.I. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 2211–2215.
37 Samulski,R.J., Zhu,X., Xiao,X., Brook,J.D., Housman,E., Epstein,N. and Hunter,L. A. (1991) *EMBO J.*, **10**, 3941–3950.
38 Carter,B.J. (1990) In Tijssen,P. (ed.), *Handbook of Parvoviruses*. CRC Press, Boca Raton, FL, Vol. II, pp. 247–284.
39 Robertson,H.M. (1995) *J. Insect Physiol.*, **41**, 99–105.
40 Kidwell,M.G. (1993) *Nature*, **362**, 202.
41 Lidholm,D.A., Lohe,A.R. and Hartl,D.L. (1993) *Genetics*, **134**, 859–868.