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The *piggyBac* transposon holds promise for human gene therapy

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Ever since their molecular isolation in eukaryotic organisms, transposons have been precious implements of the geneticist's toolkit. The properties that make transposons so useful are their ability to move from one chromosomal position to another and the relatively minimal requirements for transposition to occur in the test tube and in living cells (1). In particular, transposons can be harnessed to stably integrate sizeable pieces of DNA into a host's chromosome (2, 3). This quality provides a tremendous potential for transgenesis and large-scale insertional mutagenesis. Transposon-mediated DNA delivery, although first limited to a small number of invertebrate species, has become progressively more applicable to vertebrates, including mammalian cells (3–5). This progress has opened the door to the development of a new generation of vectors for human gene therapy and mammalian forward genetics that are potentially more easily controlled, more versatile, and safer than viral vectors (5). However, applications of transposon vectors for clinical trials in gene therapy, and the predictable manipulation of mammalian genomes, have been hindered by their low integration efficiency relative to viral vectors. In this issue of PNAS, Wu *et al.* (6) move one step further in the quest for a superior gene delivery tool in mammals. They identified *piggyBac* (*PB*) as the most active and flexible transposon system yet tested for transformation of mammalian cells.

Mammalian Transposon Toolbox

DNA transposons are the most frequently used mobile elements for manipulating and transforming genomes of prokaryotic and eukaryotic organisms. They transpose through a “cut-and-paste” mechanism whereby the transposase (generally encoded by the element) catalyzes the excision of the transposon from one chromosomal site and its reinsertion elsewhere in the genome. This process has two key properties that make DNA transposons very attractive as genetic tools. First, most DNA transposons require only a single protein, the transposase, to transpose both *in vitro* and *in vivo* (1). Thus, there seems to be little dependence on host-specific factors, although host factors may modu-

late the frequency and outcome of DNA transposition (3, 7). This property allows many DNA transposons to be active in a broad range of host species and cell types. Second, the transposase can act efficiently in trans on virtually any DNA substrate that contains the cis sequences required for transposase recognition and cleavage (1). These cis sequences range from just a few dozen to hundreds of base pairs at each end of the transposon. Consequently, the gene responsible for mobility can be physically dissociated from the DNA to be delivered, providing a means to induce and control the source of transposase and thereby enhancing the subsequent chromosomal stability of the transgene. Thus, a typical DNA transposon delivery system is composed of two components introduced into the host cells (e.g., by microinjection or cotransfection): (i) an expression plasmid providing the transposase and (ii) a donor plasmid containing the DNA to be integrated (which includes a transformation marker) flanked by the transposase cis-acting sequences.

These properties have been exploited to design a series of relatively simple yet powerful technologies that are widely used for the transformation, manipulation, and large-scale mutagenesis of genomes of several invertebrate and plant model organisms. Several transposons from the *Tc1/mariner* superfamily isolated from insects and nematodes also turned out to be active in vertebrate cells (3). However, none of these elements jumped at high frequencies in mammalian cells, hampering their development into powerful genetic tools (8). This situation began to change with the resurrection of *Sleeping Beauty* (*SB*), a synthetic transposon assembled from bits and pieces of defective *Tc1/mariner* elements gathered from salmonid fishes (4). The demonstration that the reconstructed element could transpose at appreciable frequency in human cells (4) was followed by several improvements, which established *SB* as the premier nonviral vector for mammalian transgenesis (3, 5). In human cells, *SB* transposition efficiency is at least an order of magnitude greater than that of other *Tc1/mariner* transposons so far examined. Furthermore, *SB* has been successfully used to mediate stable chromosomal integration of functional

genes in somatic cells of adult mice and to transform the mouse germ line, albeit with success rates that remained too low for routine applications (3, 8). Efforts to enhance the efficiency and range of applications of the *SB* system are ongoing, and recent developments indicate that *SB* can be harnessed into an effective tool for forward genetic screens and cancer gene discovery in the mouse (9). Meanwhile, the recent studies by Ding *et al.* (10) and Wu *et al.* (6) reveal that *PB* has also earned its place in the mammalian transposon toolbox.

piggyBac Leads the Pack

The *PB* transposon was first identified when it jumped from its insect host, the cabbage looper moth *Trichoplusia ni*, into the genome of a baculovirus (11). This horizontal movement was a first hint at *PB*'s potential as a highly active and versatile DNA vehicle. Indeed, *PB* has been used to transform the germ line of more than a dozen species of insects spanning five different orders (12), the planarian *Girardia tigrina* (13), and the malaria parasite *Plasmodium falciparum* (14). Recently, it was shown that *PB* also efficiently transposes in human and mouse cell lines and could readily mediate the introduction of foreign genes (up to 14 kb) in the mouse germ line (10). To further evaluate the capability of *PB* as a vector for applications in human gene therapy and mammalian mutagenesis, Wu *et al.* (6) performed a “side-by-side” comparison of *PB* transposition efficiency with those of a hyperactive version of *SB* (*SB11*), the *mariner* element *Mos1* from *Drosophila mauritiana*, and *Tol2*, the only transposon isolated from a vertebrate (Japanese medaka fish) known to be currently active in nature (3).

Three (*SB*, *Tol2*, and *PB*) of the four transposon systems examined by Wu *et al.* (6) have been shown to mediate gene transfer in human cell lines (3, 10), but there has been no direct comparison of their relative transformation efficiency. Using a well established protocol (akin

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to the two-plasmid system described above), the authors measured the activity of each transposon system in three common human cell lines and the Chinese hamster ovarian (CHO) cell line. *PB* and *Tol2* displayed transposition activity in all cell lines, *SB11* was active only in three lines, and *Mos1* was not active in any. For each line and all conditions assayed (i.e., varying amount of transposase expression plasmid), *PB* consistently displayed the highest transposition activity among transposons tested. The transposition rate of *PB* was from 1.5- to 3.5-fold higher than that of the second-most-active transposon in any given cell line (either *SB11* or *Tol2* depending on the cell line). The percentage of *PB* transposition reached 10% of the 10^5 CHO cells seeded and averaged 7% in three separate experiments with this cell line. This activity level is the highest ever reported for any DNA transposon in mammalian cells, a remarkable finding considering that the *PB* system used by Wu *et al.* is directly derived from the naturally occurring, WT form of the *PB* transposon. Because active DNA transposons tend to be “average jumpers” (i.e., suboptimal) in nature (15), there is likely room for further enhancement of *PB* activity.

An important limitation of current transposon- and viral-based gene transfer technologies is the inability to control the target specificity of insertion, which may result in poor or haphazard expression of the transgene and in adverse side effects on host genes, including insertional disruption or deregulation. This problem is exemplified by recent retroviral vector gene therapy trials in which three patients developed leukemia because of recurring integration of the vector near the *LMO2* protooncogene (16). Thus, a crucial prospect for human gene therapy with DNA transposons is development of vectors with high integration specificity. In principle, it could be achieved by engineering chimeric transposases fused to heterologous site-specific DNA-

binding domains (DBD) (17, 18). Wu *et al.* (6) examined the amenability of the *PB* transposase to this type of molecular modification. They tested whether the fusion of a GAL4 DBD to the N terminus of *PB*, *SB11*, or *Tol2* transposase affected their activity in CHO cells. The GAL4-*PB* transposase fusion retained a level of activity comparable with the WT transposase, whereas the GAL4-*SB11* and GAL4-*Tol2* chimeras essentially lost their transposition activity. These findings are congruent with previous studies showing a drastic reduction of *SB* activity when the transposase was fused to two different heterologous zinc finger DBDs (19). Thus, it appears that the *PB* transposase possesses a higher level of intrinsic flexibility than other transposases functioning in vertebrate cells. The next logical step would be to engineer a chimera of the *PB* transposase with an endogenous or “designed” DBD specific to one “safe haven” in the human genome.

Future Directions

Much remains to be done to verify that *PB* is the “dream tool” of mammalian geneticists and gene therapists. In particular, little is known about the behavior of *PB* in different mammalian cell types and tissues. It is known that *SB* transposes with very different efficiencies in cells from different species, types, and tissues (3). Such variability may be explained by the peculiar requirements and interaction of the transposase with host factors. For example, the human proteins HMGB1 and Miz-1 each interact directly with the *SB* transposase and modulate *SB* transposition efficiency in cultured cells (20, 21). Apparently, these human proteins can still interact with *SB* despite its distant origin (from fishes) because of the high degree of conservation of HMGB-1 and Miz-1 proteins among vertebrates. The natural host of *PB* is a lepidopteran insect; thus, one might expect a reduced possibility of cross-interaction and interference with mammalian proteins, including those that

could limit or repress transposition. This wide evolutionary gap could, in part, explain the higher transposition efficiency of *PB* in mammalian cells compared with the fish elements *SB* and *Tol2* (6) and also supports the idea that *PB* transposition depends less on cellular factors than these elements. Thus, *PB* might be less sensitive to intrinsic proteomic variations occurring among different mammalian cell types and tissues or throughout development.

One concern that applies specifically to *PB* deserves to be addressed in future experiments. In contrast to *SB* (a *Tc1*-like element), which has no close relatives in the human genome, *PB* is a member of a superfamily that is well represented in the human genome. There are $\approx 2,000$ *PB*-like elements dispersed throughout human chromosomes. Two major families (MER85 and MER75) are among the most recently amplified DNA transposon families recognizable in the human genome (ref. 22 and J. Pace and C.F., unpublished data). Thus, a large fraction of these *PB*-like transposons have likely preserved the cis sequences required for transposition. However, these two families are almost entirely represented by short copies with no transposase ORF, and there is no evidence that any endogenous *PB*-like element has transposed within the last ≈ 40 million years (J. Pace and C.F., unpublished data). Nonetheless, a handful of seemingly intact sources of *PB*-like transposase remain in the human genome (23). Despite the vast evolutionary distance between human and moth, it cannot be excluded that these endogenous sources of *PB*-like transposase could excise and/or propagate a transgene flanked by *PB* termini or, conversely, that an exogenous source of *PB* transposase could mobilize some of the human *PB*-like transposons. This conjecture poses a potential safety issue that will need to be addressed if one would like to see *PB* leap forward to clinical applications.

- Craig NL, Craigie R, Gellert M, Lambowitz AM (2002) *Mobile DNA II* (Am Soc Microbiol, Washington, DC).
- O'Brochta DA, Atkinson PW (2004) *Methods Mol Biol* 260:227–254.
- Miskey C, Izsvak Z, Kawakami K, Ivics Z (2005) *Cell Mol Life Sci* 62:629–641.
- Ivics Z, Hackett PB, Plasterk RH, Izsvak Z (1997) *Cell* 91:501–510.
- Hackett PB, Ekker SC, Largaespada DA, McIvor RS (2005) *Adv Genet* 54:189–232.
- Wu SC-Y, Meir Y-JJ, Coates CJ, Handler AM, Pelczar P, Moivadi S, Kaminski JM (2006) *Proc Natl Acad Sci USA* 103:15008–15013.
- Lozovskaya ER, Hartl DL, Petrov DA (1995) *Curr Opin Genet Dev* 5:768–773.
- Fischer SE, Wienholds E, Plasterk RH (2001) *Proc Natl Acad Sci USA* 98:6759–6764.
- Dupuy AJ, Jenkins NA, Copeland NG (2006) *Hum Mol Genet* 15:R75–R79.
- Ding S, Wu X, Li G, Han M, Zhuang Y, Xu T (2005) *Cell* 122:473–483.
- Fraser MJ, Smith GE, Summers MD (1983) *J Virol* 47:287–300.
- Handler AM (2002) *Insect Biochem Mol Biol* 32:1211–1220.
- Gonzalez-Estevéz C, Momose T, Gehring WJ, Salo E (2003) *Proc Natl Acad Sci USA* 100:14046–14051.
- Balu B, Shoue DA, Fraser MJ, Jr, Adams JH (2005) *Proc Natl Acad Sci USA* 102:16391–16396.
- Barry EG, Witherspoon DJ, Lampe DJ (2004) *Genetics* 166:823–833.
- Check E (2005) *Nature* 433:561.
- Coates CJ, Kaminski JM, Summers JB, Segal DJ, Miller AD, Kolb AF (2005) *Trends Biotechnol* 23:407–419.
- Maragathavally KJ, Kaminski JM, Coates CJ (2006) *FASEB J* 20:1880–1882.
- Wilson MH, Kaminski JM, George AL, Jr (2005) *FEBS Lett* 579:6205–6209.
- Zayed H, Izsvak Z, Khare D, Heinemann U, Ivics Z (2003) *Nucleic Acids Res* 31:2313–2322.
- Walisko O, Izsvak Z, Szabo K, Kaufman CD, Herold S, Ivics Z (2006) *Proc Natl Acad Sci USA* 103:4062–4067.
- Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, *et al.* (2001) *Nature* 409:860–921.
- Sarkar A, Sim C, Hong YS, Hogan JR, Fraser MJ, Robertson HM, Collins FH (2003) *Mol Genet Genomics* 270:173–180.