

A prince for Sleeping Beauty

A new transposon vector can deliver large DNA cargos to vertebrate cells, making it a useful tool for genetic applications.

It sounds like a modern fairy tale: Sleeping Beauty finds a companion—not one to kiss her awake, but one who perfectly complements her. Stephen Ekker from the University of Minnesota had a significant role in finding just such a companion for the transposon system Sleeping Beauty (*SB*). Transposons, mobile DNA elements flanked on either side by inverted repeats, are excised by a transposase at these terminal repeat sequences and inserted again almost at random elsewhere in the genome. *SB* was the first transposon vector developed with the ability to deliver cargo to any vertebrate cell, but it had its limitations. Ekker had been looking to find another transposon to expand the toolkit and to complement *SB*'s shortcomings.

His team used the *Tol2* transposon from the medaka fish as a basis for constructing a vector composed of the minimum sequences required for transposition and tested it *in vitro* and *in vivo* (Balciunas *et al.*, 2006). One big advantage over *SB* became apparent right away: whereas *SB* is most effective in mobilizing DNA stretches of up to 2 kb, *Tol2* does not lose efficacy with loads of 10 kb. A possible explanation for this difference in cargo capacity lies in the biochemistry of the transposases; whereas *SB* requires a tetramer to mobilize its DNA, *Tol2* likely only needs a dimer.

At present, the Ekker team delivers the transposase via a second plasmid, but they are developing several other options. One is to administer RNA or protein to the cells, and another to use a *cis* vector with the transposase and the terminal repeats on the same plasmid. Ekker expects the design of such *cis* vectors for *Tol2* to be easier than it was for *SB* because, unlike *SB*, *Tol2* is not hampered by overexpression inhibition. When *SB*'s transposase is expressed at too-high levels, DNA mobilization is inhibited, forcing scientists to hunt for a promoter that drives transposase expression at just the right levels. This difficulty limited vector design to researchers whom Ekker refers to as “transposon connoisseurs”. Not so with *Tol2*; as Ekker predicts: “When you

are making a *cis* vector with *Tol2*, you can say ‘I am just going to take the most powerful promoter I know in the tissue I want to target’ and park it upstream [of the terminal repeats] and you know it is going to work.”

Ekker is convinced that the ease of design will disseminate the use of transposon vectors to a larger audience. The fact that transposons integrate at random makes them ideally suited for applications such as gene trapping and other genome-wide studies.

Ekker also sees a rosy future for *Tol2* in gene therapy, as it can deliver larger DNA inserts than viral vectors that cap their cargo at 8 kb. As far as safety concerns with a transposon vector go, Ekker sees random integration as an advantage over viruses that clearly prefer transcriptional units. But he also says, “We don’t really know what the safety concerns are going to be from a truly random vector. We know from our mouse studies that it is not an oncogenic scenario, unless you make the cargo oncogenic.”

To test the *Tol2* transposon *in vivo*, Ekker’s team chose a mouse model of hereditary tyrosinemia type 1—a disease caused by the deficiency of an enzyme that hydrolyzes fumarylacetoacetate, the accumulation of which is toxic for hepatocytes. The researchers injected the mice with the *Tol2* transposon encoding the enzyme and another plasmid carrying the *Tol2* transposase and showed that this protocol corrected the metabolic deficiency in the animals.

In gene therapy, as in other genetic applications, *SB* and *Tol2* can show their combined strength: they can deliver any two genes, each only activated by its own transposase, or one can carry a gene and the other the transposase under a tissue-specific promoter, giving researchers a precise targeting tool.

It looks like *SB* and *Tol2* have a lot of work ahead of them—and a good shot at living happily ever after.

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Balciunas, D. *et al.* Harnessing a high cargo-capacity transposon for genetic applications in vertebrates. *PLoS Genetics* 2, e169 (2006).