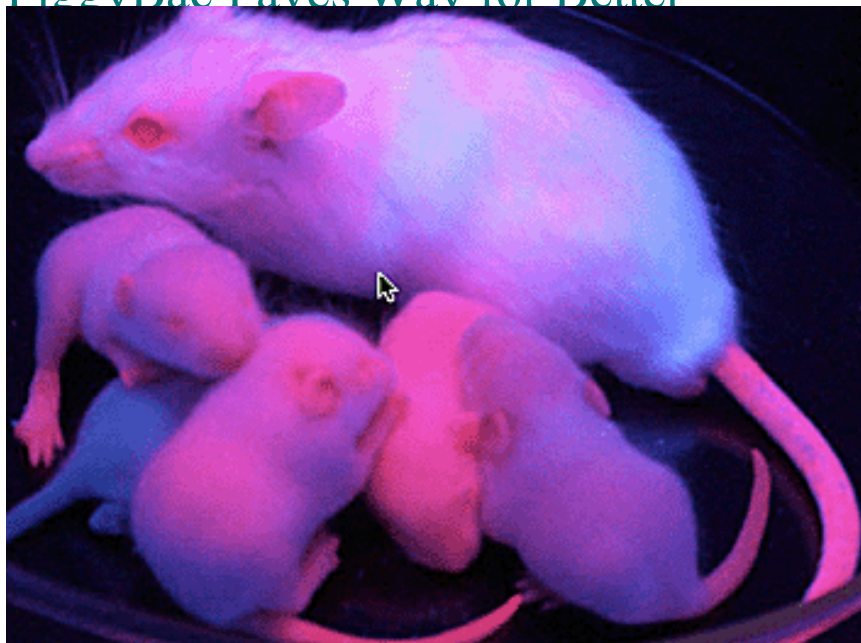


RESEARCH NEWS

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## PiggyBac Paves Way for Better



**Image Title:** The image shows transgenic mice that carry the piggyBac transposon that has caused their cells to express red fluorescent protein. - Courtesy of Tian Xu, HHMI at Yale University School of Medicine.

Howard Hughes Medical Institute researchers have harnessed a mobile gene from the cabbage looper moth and modified it for routine use to determine the function of genes in mice and other vertebrates. If the new tool works as they expect, it will speed understanding of genes involved in human biology and disease and accelerate the search for effective new therapies.

The researchers report their study in the August 12, 2005, issue of the journal *Cell*.

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Certain genes or genetic elements, called transposons, can hop from one place to another in the genomes of various organisms. In people, this genetic shuffling ensures that the immune system can generate a huge assortment of protective antibodies. Bacteria use the mechanism to swap antibiotic-resistance genes among themselves. And scientists have “borrowed” and adapted the same handy technique to insert genes and mutate genes in fruit flies and simpler organisms to learn the function of individual genes.

“We know how many genes are in the genome, but that does not tell us how they carry out their jobs,” said senior author Tian Xu, a Howard Hughes Medical Institute (HHMI) investigator at Yale University School of Medicine. “We have found a way to systematically inactivate genes in the mouse genome so we understand the functions of these genes.”

With a large inventory of genome sequences in hand, over the last few years many scientists have shifted their attention to determining the function of all of those genes. The strategy is as systematic as the genome sequencing projects—mutate each gene, observe the consequences, and investigate the molecular mechanisms. In the past two decades, only about 3,000 of the estimated 25,000 genes shared by mice and humans have been analyzed in detail, Xu said. A reliable gene-transposing tool could make that job much easier and quicker.

Xu and his colleagues at Fudan University in Shanghai, China, began their studies with a transposon called *piggyBac*. With the help of a partner enzyme, *piggyBac* can reliably and efficiently insert itself into the genomes of human and mouse cell lines and in mouse embryos, even while carrying a couple of extra genes, the study shows.

“This paper could be a very important advance,” said Allan Spradling, an HHMI investigator at Carnegie Institution in Baltimore. “It really comes down to its application in practice—how well it works, day in and day out. It’s fairly rare to get a transposon that works on a large scale in an effective way. This looks very promising.”

Transposons have been used in mice before. But one such active transposon, *Sleeping Beauty*, does not appear to travel widely among the chromosomes and cannot carry larger fragments of DNA. As a result, scientists have been searching for stable, versatile transposons that can insert randomly in many different mammalian chromosomes and also carry genes into mice and other organisms that are more closely related to people.

*PiggyBac* was originally identified in the cabbage looper moth. Unlike many mobile genetic units that work only in their native hosts, *piggyBac* can flit around the genomes of other insects. For that reason, it has been used experimentally as a tool to control pest insects from moths to flies and mosquitoes. The transposon has also seen heavy use in the genetic workhorse, *Drosophila*, where it is perhaps second only to the P element, whose talent to engineer changes in the fly’s genome was co-discovered by Spradling 23 years ago. The P element has since become a backbone of

modern fruit fly genetics.

Researchers in Xu's group first tried to adapt the P element for use in mice and to improve the efficiency of the *Sleeping Beauty* transposon system. When those efforts failed, they chose *piggyBac* because its enzyme looked and acted differently and it had a good track record in a range of insects.

In their experiments, *piggyBac* incorporated itself into many chromosomes in human and mouse cells. It also carried other genes effectively. Inserted into mouse embryos, the transposon and its cargo was inherited through five generations. *PiggyBac* can also be removed from its place in the family tree by breeding a mouse with another that carries the enzyme necessary to excise the jumping gene.

“We do not know why the P element did not work in mammalian organisms, and we do not know why this particular one works,” Xu said, “but this system is a dream tool for geneticists working with vertebrates and mammals.”

*PiggyBac* inserts randomly into the genome, with a clear preference for genes. This bodes well for its use in mutating genes and for identifying unrecognized genes in places of the genome that have been especially difficult to sequence. Furthermore, the markers it carries make it easy to find. In Xu's lab, the jumping gene carried red fluorescent protein and an enzyme that changes the coat color of a white mouse to grey or black. The transposon acts as a genetic beacon, so researchers can easily track its location without having to sequence the entire genome, as can happen with the chemical mutagen technique.

In three months, the two graduate students who led the project generated knockout mice for each of 75 different genes. Xu, Min Han, an HHMI investigator at the University of Colorado, Boulder, and Yuan Zhuang of Duke University, and their colleagues at Fudan University are in the process of scaling up *piggyBac* for the Mouse Functional Genome Project, which is aiming to mutate the majority of mouse genes at a state-of-the-art research facility in China.

Xu expects the technique to be particularly useful for animal models of genetically complex diseases, such as diabetes, where many genes contribute to the disease process.