

# ScienceWatch

New developments from the LSU AgCenter

## Transgenic Animals and Plants use Vectors to Improve Production and Disease Resistance

**B**oth Dr. Richard Cooper and Dr. James Oard are transferring genes into species in which they do not normally occur. Dr. Cooper's research involves inserting genes into target cells of animals and, in doing so, transforming the recipient host in ways that would benefit the organism. An example is introducing into channel catfish a lytic peptide that, while not occurring normally in the species, will allow the fish to defend itself against a viral or bacterial infection without the use of antibiotics that would prevent the fish from being consumed and would otherwise decimate fish populations.

Dr. Oard's research involves inserting foreign genes into plants, mainly to express a desirable trait in the target host. An example of these transgenic plants, as the altered plants are called, is a new rice that has better herbicide tolerance and resistance, better insect resistance and other traits that will benefit producers and the rice industry.

### Transposon-Based Transformation Vectors

One of the major problems in inserting a desired gene sequence into a recipient cell or animal is the stable incorporation of that gene into the recipient's genome. Current methods rely on electroporation, micromanipulation or lipofection coupled with homologous recombination events to transform target cells. These procedures have a low success rate for transformation and typically are inaccurate in their placement. Dr. Cooper has described a method in which a mini-transposon is used as the transforming vector.



A transposon is a mobile genetic element capable of inserting at random into a DNA sequence. A wild-type transposon typically includes a transposase (an enzyme causing transposition), flanked by two insertion sequences. Generally, transposons are used to create mutations and to study pathogenesis. Previously used methods have left many undesirable conse-

quences resulting from spontaneous transposition and expression of unwanted phenotypic traits. However, Dr. Cooper has described a way in which a modified transposon, or mini-transposon, can be used to introduce novel genetic elements into a target cell or organism. Mini-transposons are modified in three ways:

(1) The transposase gene has been removed from its native site between the insertion sequences and instead placed upstream from the transposon so as to make the transposable element more stable after insertion in the target genome.

(2) The insertion sequences are shortened to reduce the frequency of homologous recombination.

(3) The entire transposon "cassette" has been placed under the control of an inducible promoter.

The vector created by Dr. Cooper has a complete native cecropin gene (promoter, signal sequence, and gene for cecropin B), a lytic peptide, downstream from an inducible promoter. The construct is then introduced into *E. coli* and selected for in the appropriate media. The vector can be maintained in this way indefinitely without any harm to the

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host cell because the promoter must be induced before active transformation of the cecropin gene can occur .

Once the promoter is induced, the transposase causes the gene between the insertion sequences to “jump” to a specific site in the target cells genome where, once it is inserted, it will remain stable since the vector carrying the transposase gene, and hence the transposase, is lost. Therefore the recipient host has the cecropin gene, or gene of choice, integrated into its genome where it can be expressed either constitutively or by induction.

The possible applications of the cecropin gene insertion, specifically, could be in introducing this lytic peptide into fish such as the channel catfish that would allow the fish to defend itself against a bacterial or viral infection without the use of antibiotics. This vector construct would also enable the insertion of cecropin into tumor-infiltrating lymphocytes (TIL cells) to deliver the cecropin peptide to the site of tumor proliferation for destruction of that tumor .

Dr. Cooper’s invention allows for the rapid and efficient transformation of a eukaryotic genome. Its use does not require the high level of skill needed for microinjections. Nor does it rely on homologous recombination events for a successful transformation.

### Isolation and Characterization of Rice Ubiquitin Promoters for Plant Transformation

Dr. Oard’s goal is similar to Dr. Cooper’s. However, Dr. Oard is concerned with the efficient transformation of a plant genome. Foreign genes are transferred into plants, named “transgenic plants,” primarily to express proteins that will confer a beneficial trait to that target organism. The success of gene transfers into many crop plants depends on the optimization of physical and biological parameters, availability of suitable promoters, selectable marker genes and a sensitive agent to identify those cells that have been transformed from those cells that have not. In general, a strong and constitutive promoter is desirable to ensure successful expression of a foreign gene into a new system.

Toward that end, Dr. Oard has found a rice promoter that will regulate specific gene expression in the rice system after its delivery by a transforming vector .



Ubiquitin is one of the most conserved proteins throughout eukaryotes. Dr. Oard found that rice actively expressed ubiquitin but that no genomic rice ubiquitin genes or corresponding promoters had ever been isolated. The rice ubiquitin promoters should drive strong and constitutive expression of transgenes in rice and other monocot crop plants. Therefore, Dr. Oard isolated and characterized these promoters and evaluated their activity.

Dr. Oard found four different ubiquitin promoters within the rice genome. Based on promoter analysis by computer software, several common transcriptional factors are predicted to interact with the rice ubiquitin promoter, indicating that this region would serve as an excellent regulatory element for trans gene expression in rice and other monocots. Research then showed that the promoter did indeed successfully regulate gene expression.

It is believed that this promoter system will allow for the creation of genetically engineered plants for herbicide tolerance/resistance, insect resistance, and other traits that will enhance the productivity and economic well-being of the rice industry.

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