

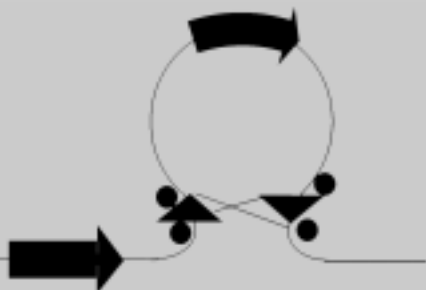
ISB NEWS REPORT

COVERING AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY DEVELOPMENTS

MAY 2002

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NEWS AND NOTES

TRANSGENE MOVEMENT, INTROGRESSION, AND PROMOTER HOPPING

Neal Stewart

Editorial Note:

In November 2001 the journal Nature published a "Letter to Nature" by David Quist and Ignacio Chapela in which they concluded that not only were transgenic DNA sequences present in traditional Mexican corn landraces but they had introgressed into native Mexican corn. The authors further asserted that promoters, and possibly genes, were reassorting throughout the genome.¹ Soon after the paper was published, many scientists were quick to offer criticisms of Quist and Chapela's conclusions and question Nature's decision to publish them.²⁻⁴

In April 2002 Nature published two brief communications that identified gaps in, and incorrect inferences drawn from, the Quist and Chapela data.^{5,6} Quist and Chapela's response to these criticisms,⁷ and an editorial note disavowing the paper by stating that the evidence provided by Quist and Chapela was incomplete and hence did not justify its original publication, were also included in the issue.⁸ The present article is written not to extend the debate concerning the Quist and Chapela report, but to offer readers a review of the molecular methodologies available to identify the purported adventitious presence of transgenes in corn landraces, as well as a critique of those used by Quist and Chapela, and to opine on the ramifications of illicit transgene flow into corn so that readers may better, as the Nature Editor suggests, "judge the science for themselves."

Based on the results of PCR, nested PCR, and inverted PCR, along with the subsequent analysis of DNA sequencing data, Quist and Chapela¹ conclude that unintended transgene movement in corn has occurred in a remote part of Mexico and that the transgenes have reassorted throughout the host plants' genome. Though PCR can be used to indirectly confirm the presence of transgenes in a host plant by amplifying genes of interest, the use of Southern blot analysis to look directly for integration of the transgenes is more accurate and less prone to misleading artifactual results. The experiments should be performed using a judicious selection of appropriate restriction enzymes and transgenes as probes. Quist and Chapela later reported the results of DNA dot blot,⁷ an analysis that

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gives no indication of molecular size of insert or copy number, or even if the signal observed is from a transgene integrated into its host's genome. PCR putatively confirms whether or not the sequence is present in a biological sample, but does not rule out the possibility that a signal is the result of a contaminant, in this case from the cauliflower mosaic virus itself. Southern blot analysis can confirm whether an intact transgene is integrated into the genome and if other associated gene sequences are present. Hence, direct identification of transgenes using Southern blot is considered more accurate than the method chosen by Quist and Chapela that indirectly identifies promoter sequences.

The two scientific communications in *Nature* rebutting the Quist and Chapela original paper indicated that at least two of the primary assertions are not tenable. The first claim, that transgenes have introgressed into Mexican corn landraces, is implausible if based solely on the original data. Quist and Chapela used an ultrasensitive nested PCR method to detect the signals of the 35S promoter in pooled samples of ears of corn, demonstrating that few kernels on any given ear (i.e., individuals in a population) were transgenic. As pointed out by Metz and Fütterer,⁵ the presence of a few transgenic kernels on ears of corn is more consistent with F1 hybridization than introgression. Introgression requires repeated backcrosses and stabilization of the gene in the new genome, and all or most of an ear would be transgenic. No one has yet found evidence that unintended transgenic DNA has been introgressed into corn landraces anywhere. If strong selection for the transgene was present, one would expect introgression to occur rapidly, which it apparently has not.

The second contested conclusion drawn by Quist and Chapela is that the 35S promoter is hopping around the genome. Their original data came from inverse PCR (i-PCR) in which host DNA is circularized and known sequences are used to construct primers designed for outward DNA amplification around the circle. Both Metz and Fütterer⁵ and Kaplinsky *et al.*⁶ propose that the original i-PCRs gave false results. One would expect transgene sequences from Bt or glyphosate tolerance genes to be found adjacent to putative 35S promoter sequences, but apparently they were not. In response, Quist and Chapela acknowledge misreading sequence similarity between a putative *adh1* intron often used in transgenic constructs and the *adh1* and *bronze1* gene sequences that are both endogenous to corn.⁷

Kaplinsky *et al.* also point out that the i-PCRs likely misprimed, thus leading to artifactual results, which then led to the allegedly erroneous conclusions.⁶ Quist and Chapela acknowledge that the i-PCR method was misprimed, but for only two of seven sequences. However, they stand by their findings that transgenes were randomly dispersed in the host and refute the assertion that their PCR-based results were artifactual or false positives, adding that follow-up DNA-DNA hybridization analyses supported their



original conclusions. Critics concede that it is possible that transgenic corn is illegally grown in Mexico and that transgenes are flowing. In fact, an April 19, 2002, newspaper report⁹ cites claims by Mexican government scientists that transgenic DNA is present in corn gene banks in Mexico, leaving less doubt that integration of transgenes into Mexican landraces has occurred.

Many people are concerned that transgenes in Mexico could pose a hazard to the genetic variation that is coveted for future genetic improvement of the crop. More research is needed to assess the consequences of gene flow into plants in geographic centers of diversity such as Mexico. In my opinion, one possible scenario would be that an initial degree of genetic variation would be maintained or, alternatively, that the transgene could actually increase genetic diversity by increasing the effective population size, allowing greater recombination. However, if the transgene was introgressed and highly selected upon by man, only the immediately-linked genes would accompany the transgene in progeny. If there is an effect (positive or negative), it will be confined to subsequent genetically-altered varieties of the crop, since corn is a human invention and is not found growing wild. Hence, as in Europe in recent years, the real effect of the Quist and Chapela study may be to focus attention on the politics and regulation of transgenic crops.

How much liability should the US and agro-companies assume for transgene escape? Should US regulatory agencies deregulate a transgenic crop in the US and assume that it will not be grown illegally in other countries? We have seen that illegal cultivation of transgenic crops can occur in many parts of the world, such as recently reported in Brazil and India.⁵ Unlike the scientific picture, which is rather clear, politics are muddy and emotional. Many international regulatory offices are similarly immobilized by policy and cannot respond scientifically—another opaque situation.

Finally, scientific solutions will likely be called to bear on transgene containment—politics and regulation by themselves are ineffective. Advanced “Terminator” technology may offer a quite plausible solution to the illegal growth of transgenic crops, at least in cases where seed meant for food can possibly germinate in unintended locations. While Terminator is viewed by some as a threat to advancing genetics and agronomy in developing countries, its application could, in this instance, have direct benefit. Work needs to proceed to develop safe plant seed toxin systems driven by benign inducible promoters, as the potential ecological advantage of such technology is enormous. Such gene flow control systems offer no new paradigm compared with

hybrid seed, which must be created each generation, and may help to alleviate the current precarious situation.

Sources

1. Quist D and Chapela IH. 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414: 541–543.
2. Stewart CN Jr. 2002. The future of transgenic plants. *ISB News Report*, January, 6-7.
3. Mann CC. 2002. Has GM corn ‘invaded’ Mexico? *Science* 295: 1617-1618.
4. Conko G and Prakash CS. 2002. Report of transgenes in Mexican corn called into question. *ISB News Report*, March, 3-5.
5. Metz M and Fütterer J. 2002. Suspect evidence of transgenic contamination. *Nature* 416: 600-601.
6. Kaplinsky N, Braun D, Lisch D, Hay A, Hake S, and Freeling M. 2002. Maize transgene results in Mexico are artefacts. *Nature* 416: 601.
7. Quist D and Chapela IH. 2002. Quist and Chapela reply. *Nature* 416: 602.
8. Editor, *Nature*. 2002. Editorial note. *Nature* 416: 601.
9. Brown P. 2002. Mexico’s vital gene reservoir polluted by modified maize. *Guardian*, April 19 2002. <http://www.guardian.co.uk/Archive/Article/0,4273,4397091,00.html>.

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TECHNOLOGY NEWS

PRUNING BACK TRANSGENES

Claire Granger

The introduction of a foreign gene into plant cells during transformation is only the first step in the creation of a transgenic plant. Only a few cells in the population targeted for transformation will actually take up and stably integrate the transgene, leaving the vast majority of cells untransformed. To ensure that only the cells carrying the transgene are regenerated into whole plants, scientists link the gene for the trait of interest to a marker gene that

usually confers some selective advantage to the plant cells. With this advantage, stably transformed cells survive or outgrow their untransformed neighbors under selective conditions. Once these transgenic cells have been regenerated into whole plants, the marker gene is no longer needed; however, it remains in the transgenic plant's genome and continues to be expressed.

This outcome is undesirable for a number of reasons. First, marker genes typically confer resistance to some type of negative selection, usually treatment with an antibiotic or herbicide. The continued presence of these genes in widely grown crop species has stimulated public concern that the genes may become transferred to other organisms, leading to an increase in antibiotic resistant bacterial strains (from antibiotic resistance markers) or the creation of new, aggressively weedy plant species (from herbicide resistance markers). Although, to date, there is little evidence that either of these scenarios should be considered genuine sources of concern, many researchers developing transgenic crops would prefer to avoid the issue entirely.

A second issue with the continued presence of marker genes is the risk that the ongoing, high-level expression of the marker will lead to a phenomenon known as "yield drag." Yield drag is a reduction in the yield of the crop due to diversion of amino acids and other resources away from the production of the crop's product (i.e., grain). Instead, these resources are used to maintain high levels of the protein encoded by the introduced marker gene.

Finally, the continued presence of the marker gene in a crop variety becomes a limiting factor for the further engineering of that variety. In the process of "trait stacking," a number of genes conferring different benefits are introduced into a plant through successive rounds of transformation. However, the number of marker genes available for each plant variety is limited, and, with each transgene requiring its own marker, the researcher soon runs out of options.

Several alternatives to negative selectable markers have been developed. Green fluorescent protein (GFP) or its derivatives have been successfully used to identify transformed tissues. The protein is readily visible and appears to be largely nontoxic to plants. However, GFP does not provide any selective benefit to the cell so transformed cells often quickly become overgrown by their untransformed neighbors. It is possible to dissect out the transformed cells after visualizing GFP; however, the clusters of transgenic cells are often so small that it is difficult to get a pure sample. The small size of the se-

lected cell sample also makes regeneration very difficult. Overall, GFP-selection is probably a marker system that is only appropriate for use with plant varieties that have proved incompatible with other selectable markers.

Another alternative is the positive selection marker PMI (phospho-mannose isomerase) sold by Syngenta under the brand name of "Positech." Expression of PMI in plants confers the ability to use mannose-6-phosphate as a sole carbon source. Since the vast majority of plant species lack this ability, growing cells on mannose-6-phosphate following transformation means that only transformed cells will continue to grow. Untransformed cells are slowly starved and eventually die off. The PMI enzyme is naturally found in mammalian species, including humans, so there is less of a perceived safety concern with the use of this marker. In addition, the enzyme appears to have a low allergenic potential. However, although PMI represents a useful new marker, the potential for yield drag and the limits of trait stacking remain.

A third approach to the marker gene issue consists of the physical removal of the gene once its function is complete, thus eliminating any concerns over the gene's safety and impact on yield. Furthermore, once the gene is removed, it can be used again to introduce a new trait in further rounds of transformation. Several methods have been proposed to accomplish physical removal. Cotransformation, used in conjunction with *Agrobacterium*-mediated transformation, introduces the gene of interest and the marker gene on separate T-DNAs (the segment of DNA that becomes integrated into the plant's genome during *Agrobacterium*-mediated transformation). Ideally, the two T-DNAs will integrate into different sites in the plant's genome (e.g., different chromosomes). Once selection has occurred, the transgenic plant is crossed with a non-transgenic plant of the same variety. Due to the normal segregation and recombination that occurs during sexual reproduction, a certain number of offspring will have the gene of interest but not the marker gene. In practice, however, four times the number of primary transformants must be generated to assure the isolation of a transgenic plant stably expressing the gene of interest but lacking the marker gene.¹ For most researchers, this requirement makes cotransformation too labor intensive for general application. In addition, due to the segregation step, this method is limited to crop species that are propagated sexually.

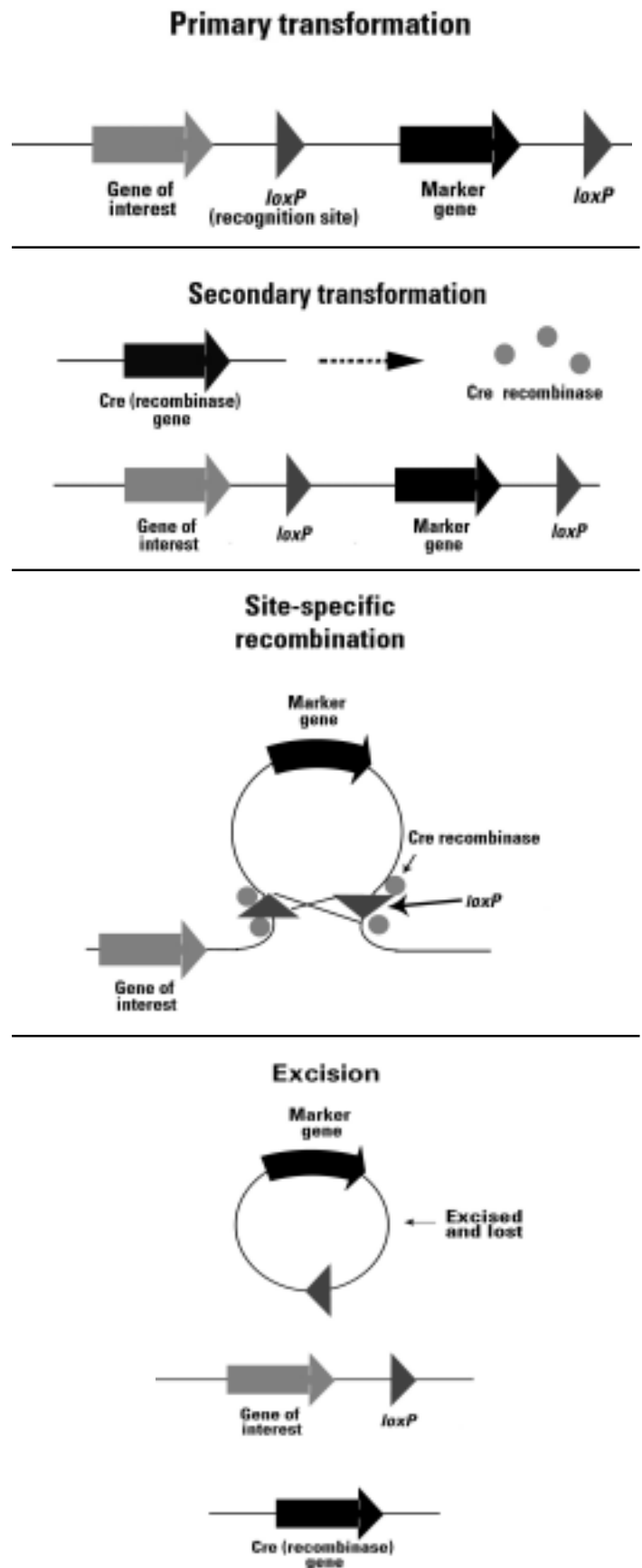
Other methods of marker gene removal include the use of homologous recombination and transposable elements. With homologous recombination, the marker gene is flanked by repeated sequences known to promote recombination. If

and when recombination occurs between the two repeats, the marker gene is excised and lost. Although this method has been shown to be highly effective when the plant's plastid genome is the target of transformation, it has been far less successful when used with transformation of the plant's nuclear genome. The use of transposable elements for marker gene removal involves the placement of the marker gene onto a transposon, a segment of DNA that "hops" around in the plant's genome, followed by segregation of the marker gene and the gene of interest as described for cotransformation. The drawbacks of this approach include variable rates of transposition in different plant varieties and the labor involved in outcrossing the transgenic plants and selection of the progeny.^{1,2}

The removal of marker genes through site-specific recombination is a method that has attracted a great deal of attention since it was first described in 1991 by David Ow and his colleagues at the Plant Gene Expression Center (<http://www.pgec.usda.gov>) in Albany, California. In this approach, the marker gene, flanked by specific sequences known as recognition sequences, is introduced along with the gene of interest into plant cells. Selection is then applied and transgenic plants are regenerated. These primary transgenics then undergo a second round of transformation to introduce the gene for a recombinase that is specific for the recognition sequences. Alternatively, the recombinase can be introduced by crossing the transgenics with a line already carrying the recombinase gene. Once expressed, the recombinase binds to the recognition sequences, promoting recombination, and the marker gene is excised (Fig. 1). The excised sequence is then permanently lost from the plant's genome.

A number of site-specific recombination systems have been tested for marker gene removal in plants: the Cre/lox system originally isolated from bacteriophage P1; the R-RS system from *Zygosaccharomyces rouxii*; and the FLP/(FRT) system of *Sachromyces cerevisiae* (in each pair, the first letter or group of letters represents the designation of the recombinase, while the second group of letters represents the designation of the recognition sequence). One of the main drawbacks of the method is the double round of transformation required to produce marker-free plants. The alternative, crossing the transgenics with a line already carrying recombinase gene, was found to produce unacceptably low rates of excision.^{1,2} Another problem with this approach is that the constitutively-expressed gene for encoding the recombinase remains in the plants. Plants continuing to express the Cre recombinase at high levels have been observed to have significantly altered morphology and/or fertility.² The Cre gene can be removed through

Figure 1.



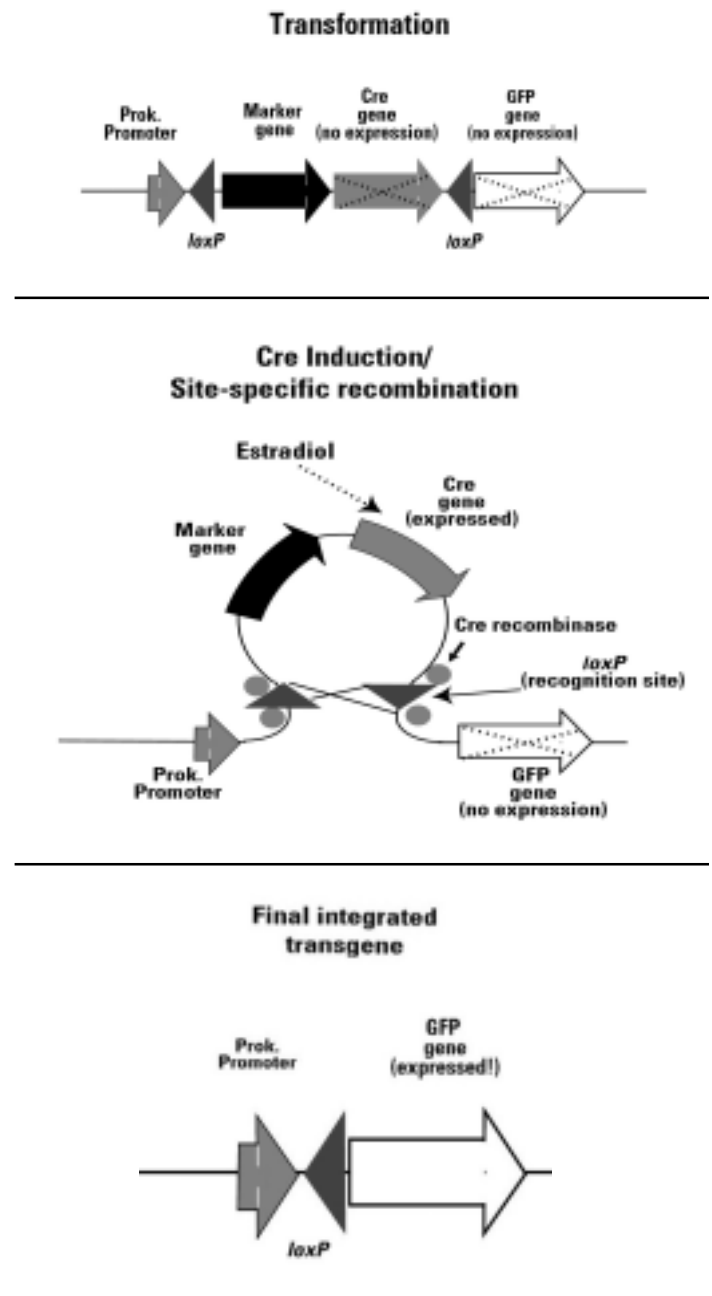
segregation by crossing the transgenic plant with non-transgenics of the same variety, but this option is not open to those crop species that are propagated asexually (i.e., potato).

Recent innovations, however, have made site-specific recombination the most appealing method for marker gene removal. In an article published in *Nature Biotechnology*, Nam-Hai Chua and other researchers based at the Rockefeller University in New York announced they had developed a method for using the Cre/lox system to remove marker genes with only a single round of transformation.³ They accomplished this by placing the gene encoding the recombinase, Cre, under the control of a chemically-inducible promoter containing the XVE hybrid transactivator. In the absence of the activator, estradiol, there is no expression of the gene encoding Cre, and thus no recombination. The construct also contained a marker gene encoding resistance to the antibiotic kanamycin and the gene for GFP, representing the gene of interest. The construct was cleverly designed such that GFP would only become linked to its promoter and expressed upon excision of the sequence encoding the Cre and kanamycin resistance genes and their respective promoters (Fig. 2).

The researchers introduced this construct into *Arabidopsis thaliana* using *Agrobacterium*-mediated transformation. After selecting transformant shoots on kanamycin-containing growth medium, a five shoots were transferred to medium containing estradiol to induce excision of the Cre/marker sequences during regeneration of intact plants. In two of the three lines recovered, excision of the Cre and marker genes appeared to be complete, as a total loss of kanamycin resistance was observed. In addition, plants of both lines exhibited expression of GFP, a trait that segregated in a Mendelian fashion in later generations. Interestingly, plants regenerated from shoots that had not undergone excision induction displayed no GFP expression. However, when the progeny of these plants were treated with estradiol, GFP expression was detected in individuals from all transgenic lines. In addition, all GFP-expressing plants were found to be kanamycin sensitive, confirming the successful excision of the marker gene.

This approach offers several benefits over earlier strategies. First, only a single round of transformation is required, reducing labor significantly. Second, no outcrossing is required to segregate the gene encoding the recombinase, so the system can be used with vegetatively-propagated crop species. Third, the recombinase gene is removed, eliminating the negative impact of long term, high-level expression of the recombinase. Finally, the timing of

Figure 2.



marker gene removal can be tightly controlled so researchers can perform selection and then remove the marker gene when it is no longer needed.

The potential of site-specific recombination for controlling transgene introduction, expression, and removal has been further explored in two recent publications in the journals *Nature Biotechnology*⁴ and *Plant Molecular Biology*.⁵ In a commentary in the March 2002 issue of *Nature Biotechnology*, Robert Keenan and Willem Stemmer of Maxygen, Inc. discussed the potential of using chemically-inducible, site-specific recombination in conjunction with tissue-



ANIMAL RESEARCH

SLEEPING BEAUTY AND THE MOUSE

Eric Wong

specific promoters to create plants in which the transgene is only present and expressed in the part of the plant in which it is needed (i.e., expression in the leaves and stems for pathogen resistance, but with the transgenes excised in the harvested seed). Even more ambitious is the application of site-specific recombination described by David Ow, who published the original article on this method of marker gene removal. In an article in *Plant Molecular Biology* published earlier this year, Ow describes how site-specific recombination could be used to make plant transformation a more precise and effective process.⁵ By using the system to insert a gene into a known location in the plant genome, he claims such problems as positional effects and widely-varying copy numbers, which currently plague plant transformation methods, could be reduced or eliminated.

The basic system is still far from perfect, however. Molecular analysis of the transgenic plants described in the *Nature Biotechnology* research revealed that some of the plants had failed to completely excise the marker and Cre genes.³ Therefore, although these plants exhibited all the visible signs of marker gene loss, at the molecular level they were found to contain some cells still carrying the marker and Cre genes. The authors believe once the technique has been properly optimized, this outcome can be avoided. Certainly the initial results look very promising and, considering the enormous potential of the technique, there is sure to be much more research on the application of site-specific recombination in transgenic plants.

Sources

1. Ebinuma H, Sugita K, Matsunaga E, Endo S, Yamada K, and Komamine A. 2001. Systems for the removal of a selection marker and their combination with a positive marker. *Plant Cell Reports* 20(5): 383-392.
2. Ow DW. 2001. The right chemistry for marker gene removal? *Nature Biotechnology* 19(2): 115-116.
3. Zuo JR, Niu Q-W, Moller SG, and Chua N-H. 2001. Chemical-regulated, site-specific DNA excision in transgenic plants. *Nature Biotechnology* 19(2): 157-161.
4. Keenan RJ and Stemmer WPC. 2002. Nontransgenic crops from transgenic plants. *Nature Biotechnology* 20(3): 215-216.
5. Ow DW. 2002. Recombinase-directed plant transformation for the post-genomic era. *Plant Molecular Biology* 48(1-2): 183-200.

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Methods for generating transgenic mammals include microinjection of DNA into fertilized eggs, use of recombinant viruses, and manipulation of embryonic stem cells. In lower organisms like the fruitfly (*Drosophila melanogaster*) and the worm (*C. elegans*), efficient transgenesis has been achieved using transposable elements. A comparable, efficient transposable element system for mammals has been lacking.

In 1997 an inactive transposon was identified and cloned from the genome of the Atlantic salmon. Using site-directed mutagenesis, an active transposon called Sleeping Beauty was reconstructed by selectively modifying inactivating mutations. Mobilization of the Sleeping Beauty transposon was mediated by the activity of the Sleeping Beauty transposase, SB10. This transposase is active in a wide range of cells including cultured human cells, mouse somatic tissues, and the mouse germ line.

In the April 2, 2002, issue of the *Proceedings of the National Academy of Sciences, USA*, researchers at the University of Minnesota and the Genomics Institute of the Novartis Research Foundation report the use of the Sleeping Beauty transposon to insert DNA into one-cell mouse embryos. Coinjection of a transposon-containing vector and mRNA expressing the SB10 transposase resulted in integration of the transposon into the genome of mouse embryos. In addition to the transposition events, random integration of the vector was also observed.

To determine if a transposon with a foreign gene can also be stably integrated into the genome, a transposon containing the agouti gene under the control of a keratin promoter was coinjected into one-cell mouse embryos along with mRNA expressing the SB10 transposase. Integration of the transposon into the genome was examined by Southern blot and expression of the agouti gene was detected as yellow coat color in non-agouti mice (e.g., in black C57BL/6 mice).

Of the 20 resulting offspring, nine were positive for the transposon, indicating a transgenesis frequency of 45%. Analysis of the nine transgenic mice revealed that three mice had only the transposon-mediated event, three mice had random integration of the transposon vector, and three

mice had both types of insertions. An average of three transposition events per embryo were observed. Six out of the twenty mice showed some yellow pigmentation in their coats, indicating that the agouti transgene was expressed. Surprisingly the three mice that had only the transposon-mediated insertions did not have yellow coats.

Segregation analysis of the multiple transposon insertions present in one founder mouse revealed variable phenotypes associated with the different transposition events. Some transposon integration events were associated with no phenotype (black coat color), whereas other integration events were associated with yellow pigmented skin with black hair or yellow pigmented skin and hair (see Fig. 1). These results indicate that a gene within the transposon can be expressed after transposition, but expression of the gene is variable presumably due to chromosomal position effects.

Figure 1.



Coat color phenotypes observed
 B=normal
 I=intermediate expression in the skin but not the coat
 Y=yellow coat color in the skin and coat

(Dupuy 2002; Copyright National Academy of Sciences, U.S.A.)

Previous transgenesis studies have shown that expression of a transgene was dependent upon its site of integration in the genome. Integration of a transgene (or transposon) into a chromosomally active region of the genome would likely result in expression of the transgene; whereas integration into a chromosomally silent region would likely lead to inactivation of the transgene.

The Sleeping Beauty transposon system generates a high frequency of transgenesis (45%) in mice and may represent an alternative strategy for transgenesis in other mammals. In agriculturally important animals such as cows, pigs, and sheep, transgenesis rates have been typically 10-fold lower compared to mice. Transposon-mediated transgenesis may represent a more efficient method for generating transgenic farm animals if the Sleeping Beauty transposase is active in one-cell embryos from these mammals.

Source

Dupuy AJ *et al.* 2002. Mammalian germ-line transgenesis by transposition. *PNAS* 99: 4495-4499.

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REGULATORY NEWS

INDIA GIVES NOD FOR BOLLGARD COTTON

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After much debate and extensive field trials, the Government of India has at last cleared the commercial cultivation of Bt cotton. The Genetic Engineering Approval Committee (GEAC), which met on March 26th, 2002, formally approved the commercial release of Bollgard cotton based on an application by MAHYCO, an Indian company that has a joint venture with Monsanto. MAHYCO has been conducting multi-location field trials on Bt cotton in India since 1998. Initially, the limited field trials created much controversy from the “green” activists. However, during the 2000 and 2001 planting seasons, expanded trials ran smoothly under the supervision of the Indian Council of Agricultural Research (ICAR) and the state agricultural universities.

The approval of Bt cotton was expected during the last planting season but was deferred at the last minute. At that time, GEAC reasoned that the results of trials conducted during 2000 were not conclusive and more data was needed, particularly from biosafety tests conducted within the Indian environment. However, Greenpeace International’s campaign against Bt cotton and the continued pressure of some anti-GM activists were also partly responsible for the deferral. Since then, two major developments perhaps hastened GEAC’s recent approval. One was the illegal cultivation of Bt cotton on nearly 10 000 hectares in Gujarat, Maharashtra, and Andhra Pradesh by farmers during the 2001 season, and the failure of State Governments to take effective remedial steps even after the unapproved cultivation came to its notice. The second was the counter campaign by some farmers’ organizations and industrial confederations in favor of Bt cotton. However, GEAC stated that the decision to permit the commercial release of Bt cotton this year was made after adequate data on field performance and biosafety assessments had been generated in accordance with country guidelines.



And, according to the GEAC Chairman, farmers could earn Rs.10 000 more per hectare by planting Bt cotton than they are currently earning with traditional varieties.

This year's release is not without riders. The most important rider, which is generating a lot of concern from the media and public, relates to 'refugia'. GEAC has required that every farmer who plants Bt cotton, irrespective of holding size, has to grow 20% non-Bt cotton as refugia in the form of border rows around the field. The second rider is that the approval is valid only for three seasons, from April 2002 to March 2005, at which time approval will again be reviewed based on collected results and farmers' reactions. Another condition stipulates that MAHYCO has to continuously conduct insect susceptibility tests and pollen transfer studies, and report the data back to GEAC. Under the seed act, MAHYCO has to submit details of the quantity of Bollgard seed produced and sold to farmers every year.

While MAHYCO sought approval for four hybrids, GEAC cleared only three: Mech 12; Mech 162; and Mech 184. These hybrids are primarily suitable for the central, western, and southern parts of the country. The fourth hybrid, Mech 915, was not approved, mainly because a full two years of data were not generated due to its late approval in 2000 for trials in northern India. This hybrid is mainly intended for the northern states of Punjab, Haryana, and parts of Rajasthan, where cotton is grown on a considerable area, mostly under irrigation.

Meanwhile, MAHYCO is preparing for commercial sales and, according to the company, can supply seeds for about one-lakh acres (1 lakh = 100 000) for the current planting season that begins in June to July. Though this is a relatively small area (total cotton area is nine million hectares), marketing of these seeds would still be significant considering that diffusion of new agricultural technologies is a slow process in India.

GEAC's formal approval of Bt cotton did not end the controversy surrounding GM crops, but rather increased it, with consumer organizations also joining the fray. Supporters of GM crops are pleased that at last the technology has been given a chance to prove its usefulness. Among the prominent persons who supported the release are Sharad Joshi of Shetkari Sanghatana, a noted economist and farmers leader who recently chaired the Government of India Task Force on Agriculture. It is Dr. Joshi's opinion that the progress of new technologies should not be stopped, and farmers will be the best judges of GM seeds. Representatives of the cotton industry also estimate that if

Bt cotton is adopted widely by farmers, cotton production in the country could reach up to 200 lakhs bales in the next few years, up from the current production of 156 lakhs bales. Industry sources also assert that one of the main reasons for the weak competitiveness of Indian cotton in world markets is low productivity, and add that Bt cotton might at least partially address this problem.

However, criticisms mount that approval of Bt cotton had been hastily made without fully assessing long-term impacts. Prominent among the critics are Dr. Vandana Shiva of the Research Foundation for Science, Technology and Natural Resource Policy and Dr. Suman Sahay of Gene Campaign (<http://www.cuts-india.org/Gene-Campaign.htm>). Their main argument is that the requirement for a 20% refugia set-aside will work against small and marginal farmers with holdings of between one to three hectares and who comprise 70 to 80% of farmers in India. Such small-scale farmers may not realize significant gains from growing Bt cotton, although companies originally claimed that GM technology would help the millions of small farmers in India. Another noted scientist, Dr. P. M. Bhargava, also believes that approval has been accorded prematurely and adequate rigor in biosafety protocols has not been followed. He even pleaded for a five-year moratorium on the release of GM crops until the country institutes a comprehensive mechanism for biosafety and food quality assessment. Even political leaders are treading cautiously on the issue. Mr. Vadde Shobhanadreshwar Rao, the Minister of Agriculture of Andhra Pradesh (a leading cotton growing state in the country and one that has witnessed the maximum number of cotton farmer suicides), stated, "let the farmers finally decide on the usefulness of Bt cotton," adding that farmers are wise enough to adopt anything good and discard things that do not work.

In the final analysis, approval of Bt cotton is considered by many proponents to be a step forward, despite many questions that remain on the feasibility of implementing the 20% refugia clause in a country like India where thousands of small farmers cultivate the crop in remote areas. This controversial but potentially powerful technology has at least been given a fair try, and the government's decision has encouraged other companies to accelerate research on GM crops. Though the government states that clearance will be made on a 'case by case' basis, companies are hopeful that approvals will be easier now that a precedent has been set. M/S Pro-agro, the Indian arm of Aventis, is planning to file an application with the Indian Department of Biotechnology for release of GM mustard, and they are working on other vegetable crops. Approval also puts pressure on the public sector R&D system to strengthen

GM research and develop useful products for the farmers. Most importantly, the issue is widely debated in the country and, for the first time, consumer organizations are becoming aware of the issue and beginning to air their views on labeling. Unlike in the West, consumer awareness in India is quite low and anything that heightens this awareness is a welcome development toward safer food and environments.

Sources

1. Bt cotton gets green signal from panel. *Times of India*, March 27, 2002. <http://www.timesofindia.com>
2. Nod for Mahyco Bt cotton places India in GM orbit. *Economic Times*, March 27, 2002. <http://www.economictimes.com>
3. Aventis to seek nod for GM mustard. *Economic Times*, March 29, 2002.
4. Happy pickings for cotton. *Economic Times*, April 1, 2002.
5. What is wrong with Bt cotton? *Economic Times*, April 2, 2002.
6. Insects too have a share of cotton crop with Bt cotton. *Eenadu* (Telugu Daily), April 8, 2002. <http://www.eenadu.net/>
7. The perils of mutant food. *Down to Earth*, April 15, 2002, p 49. <http://www.cseindia.org/html/dte/dte.htm>

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INDUSTRY NEWS

DON'T BANK ON ATCC DEPOSIT TO SUPPORT DNA PATENT CLAIMS

Phillip B. C. Jones

A recent decision of the Court of Appeals for the Federal Circuit shows that the court is taking a narrow view of DNA patent claims. The case began when Enzo Biochem Inc. sued Gen-Probe Inc. and others for patent infringement. Last year, a federal district court decided that Enzo's patent claims are invalid because they fail to meet the written description requirement. On April 2nd, the Federal Circuit announced that the lower court was correct (Docket No. 01-1230).

The patent at issue, Enzo's US patent No. 4,900,659, includes claims to nucleic acid probes that hybridize with

Neisseria gonorrhoeae DNA, but not to *N. meningitidis* DNA. According to claim 1, a DNA probe is covered within the scope of the claim if the ratio of the amount of the DNA probe that hybridizes to *N. gonorrhoeae* DNA, compared with the amount of the DNA probe that hybridizes to *N. meningitidis* DNA, is greater than about five. Claim 1 identifies certain *N. gonorrhoeae* and *N. meningitidis* DNA samples used as standards in the assay, details hybridization conditions, and provides instructions for calculating the ratio from hybridization data.

The Federal Circuit took the position that the hybridization assay distinguishes the claimed nucleotide sequences from unclaimed nucleotide sequences only by what they do, which is a purely functional distinction. The court warned that a "description of what genetic material does, rather than what it is, does not suffice." In the court's opinion, therefore, a nucleic acid molecule described only by its ability to hybridize with another DNA molecule fails to meet the written description requirement.

Certain Enzo patent claims specify probes deposited with American Type Culture Collection. For example, claim 4 refers to ATCC 53409, which contains an 850 base pair *N. gonorrhoeae* DNA probe. The court decided, however, that even these narrower claims were only defined by the function of the probes, and that a deposit is not sufficient to comply with the written description requirement. Again, the court indicated that the nucleotide sequences should have appeared in the patent specification.

Enzo may decide to appeal this decision to the US Supreme Court. In the meantime, many patent owners are probably wondering if a description of a protein binding target provides a sufficient written description of a claimed antibody. A copy of the Enzo decision can be found on the Federal Circuit's website (<http://www.fedcir.gov/dailylog.html>).

Other Bits of Biotech Law News

On April 2 DuPont, its subsidiary Pioneer Hi-Bred International Inc., Monsanto Company, and Monsanto's affiliates announced their creation of an agreement that gives the companies cross-licenses for technologies to genetically modify corn, canola, and soybean crops. Part of the deal is that Pioneer and Monsanto agreed to refrain from suing when one company uses certain proprietary technology owned by the other. For instance, Pioneer can use Monsanto's technology to work on a second generation of insect-protected corn products, while Monsanto can use Pioneer's corn transformation technology. DuPont and Monsanto also resolved all issues related to contested



germplasm, and the companies agreed to dismiss 11 pending lawsuits over genetically-modified seeds. According to their press release, one effect of the agreement will be to ensure that farmers have greater access to new agricultural technologies.

The DuPont-Monsanto cease-fire does not end the Bt patent wars. On April 10, the Federal Circuit published its opinion on several issues arising from an ongoing interference to determine who was the first to invent genetically-modified tomato plants that express *Bacillus thuringiensis* toxin. The parties are Monsanto and Mycogen Plant Science, now a part of DowAgroSciences LLC. The court sent the case back to the US Patent Office's Board of Appeals and Interferences, continuing the Bt patent battle between Monsanto and DowAgroSciences. Inventors should note that the case concerns research activities that occurred almost 20 years ago. Good record keeping is a must.

On March 8, the Federal Circuit heard arguments in a lawsuit originally filed by Monsanto Company against Mississippi soybean farmer Homan McFarling. At the center of the controversy is Monsanto's policy of prohibiting farmers from saving or reusing its RoundUp-resistant seeds once the initial genetically-modified crop is grown. Jim Waide, McFarling's attorney, has opined that the traditional farming practice of replanting saved seeds is at stake.

In a similar lawsuit, Monsanto sued Mississippi farmer Mitchell Scruggs for using seed from genetically-modified soybeans and cotton. According to Scruggs's attorney, there is a significant question about whether a utility patent that claims seed can extend to the progeny of that seed. This question may not be that difficult to answer. Although the Plant Variety Protection Act provides a farmer's exemption for using seed, US utility patent law lacks such an exemption. Under US patent law, therefore, the inquiry is whether the accused infringer is using an invention covered by the patent claim. Period. The question that the McFarling and Scruggs cases provokes is whether Congress will be motivated to create a special farmers exemption in the law that governs utility patents.

Speaking of patent infringement exemptions, Representative Lynn Rivers (D-Michigan) recently introduced legislation that would provide an exception for the infringement of patented genes (H.R. 3967; "Genomic Research and Diagnostic Accessibility Act of 2002"). The bill would amend patent law so that it would not be an act of infringement to use any patent for, or a patented use of, genetic

sequence information for purposes of research. This exemption does not apply, however, to any individual who is directly engaged in the commercialization of the patented gene. In her introductory remarks, Ms. Rivers emphasized that the research exemption provision would apply to all "genetic sequence patents," not just patents claiming human gene sequences. The impact of such an exemption is not clear, however. According to a 2001 National Academy of Sciences report ("The Operation of the Patent System: Insights from New Research"), university researchers feel free to use patented biotech inventions, and companies that own the patents generally refrain from asserting infringement actions against the researchers. The Rivers bill has been referred to the House Committee on the Judiciary, and a good source for information about the bill's progress is the Thomas Web site (<http://thomas.loc.gov>).

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