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ISIS Report - March 19 2001

Terminator insects give wings to genome invaders

The United States Department of Agriculture has approved field release of GM pink bollworm this summer, which are made with a mobile genetic element that can jump many species. This is tantamount to giving wings to the most aggressive genome invaders. **Dr. Mae-Wan Ho** exposes evidence of instability in these GM insects, and warns of rampant horizontal gene transfer and recombination, should such GM insects become released.

Geneticists have created a particularly hazardous class of gene transfer vectors for engineering insects. These are transposons, or mobile genetic elements, which, as the name implies, are genetic units that can move from one site to another in the same genome or move between genomes belonging to unrelated species. Transposons are related to viruses and proviral sequences that, like transposons, are found in the genomes of all species.

A transposon consists of several genes flanked by terminal repeat sequences. One of the genes will code for the enzyme transposase, which is necessary for moving the element. However, elements that have lost the transposase gene can nevertheless get help from the enzyme coded in other transposons. Transposons come in groups, or superfamilies, many of which have members distributed widely across species belonging to different phyla of both

animals and plants. These 'promiscuous' transposons have found special favour with genetic engineers, whose goal is to create 'universal' systems for transferring genes into any and every species on earth. Almost none of the geneticists has considered the hazards involved.

A group in Boston created a vector from *mariner*, a superfamily of transposons found across genomes of diverse species from insects to plants and vertebrates, including human beings. One element belonging to this superfamily, *Hirmar1*, isolated from the horn fly, was used to make 'minitransposons' consisting of the short inverted terminal repeats, between which any gene expression cassette(s) can be inserted. The researchers constructed a minitransposon with a kanamycin antibiotic resistance marker gene driven by a bacterial promoter. This minitransposon was found to jump easily into the *E. coli* and the *Mycobacterium* chromosome. It is known to recognize the dinucleotide TA. The probability of this dinucleotide occurring in any stretch of DNA is 0.25^2 or 6.25%. Within the 500 base pairs of the bacterial chromosome analysed, 21 of the 23 possible TA dinucleotide insertion sites were occupied [1].

The experiment shows that the transposon can be stripped down to the bare minimum of the flanking repeats, and it can still jump into genomes. The reason, as mentioned earlier, is that the transposase function can be supplied by a 'helper' transposon. Such helper transposons are ubiquitous. So, it would seem obvious that integrated transposon vectors may easily jump out again, to another site in the same genome, or to the genome of unrelated species. There are already signs of that in the transposon, *piggyBac*, used in the GM bollworms to be released by the USDA this summer.

The *piggyBac* transposon was discovered in cell cultures of the moth *Trichopulsia*, the cabbage looper, where it caused high rates of mutations in the baculovirus infecting the cells by jumping into its genes [2]. The *piggyBac* is 2.5kb long with 13 bp inverted terminal repeats. It has a specificity for sites with the base sequence TTAA. (The probability of this sequence occurring is 0.25^4 or 0.4%.) This transposon was later found to be active in a wide range of species, including the fruitfly *Drosophila*, the mosquito transmitting yellow fever, *Aedes aegypti*, the medfly, *Ceratitis capitata*, and the original host, the cabbage looper [3]. The *piggyBac* vector gave high frequencies of transpositions, 37 times higher than *mariner* and nearly four times higher than *Hirmar*.

In another experiment, the integrative *piggyBac* vector, with its transposase gene disabled and carrying the green fluorescent protein gene cassette, was used to transform the silkworm, *Bombyx mori L* [4]. Transposase function was provided by a helper-plasmid containing a *piggyBac* transposon also disabled by having one of its terminal repeats removed. The integrative vector and helper plasmids were both injected into silkworm embryos. The adult fertile moths (G0) resulting from the injected embryos were mated in single pairs among themselves or backcrossed to the unmodified parent, and the resultant broods (G1) were analysed.

A total of 2498 embryos from two strains of silk worms were injected, 1164 (46.6%) of the embryos hatched resulting in 654 (26.7%) fertile adults, single-pair matings among which 12 broods (0.5%) expressing green fluorescent protein were found.

The genomic DNA of the broods were analysed with Southern blot (a technique that gives information on the inserts). Here is how the authors reported their results.

"Southern blot analyses of the DNA of transformed G1 insects showed that one to three different inserts were present in a single animal and that larvae from the same progeny [ie, brood] had different insertions. These insertions were inherited independently at the G2 generation . . .

"The presence of multiple independent inserts in many G1 larvae indicates that a single gamete from the G0 parent can harbor several insertions and that different gametes can have different insertions. Eighteen insertions were observed in 12 G1 individuals issued from three transformed parents. It is likely that this result underestimates the total number of insertion events that occurred in the G0 moths." (p.82)

What was the explanation for such a large number of different inserts? There were two possible explanations.

"Either the integration events [of the *piggyBac* vector] in the germ line occurred late during development [of the injected embryo]", so that the same adult carries a population of germ cells each with different insertions, "or successive rounds of transposition took place after an initial insertion event". The latter hypothesis, considered more likely, "would explain why -- despite the low frequency of insertion in the parental population [0.5%] -- the number of inserts is high in the transformed insects. . . . A similar situation was also observed in transgenic *C. capitata*, and it was also attributed to secondary mobilizations of an initial single insert." (p.82)

In other words, there is evidence that the inserts had moved between the G0 and the G1 generations, and possibly, again between the G1 and G2 generations. The "stable germ line transformation" claimed (p.83) is based on a dangerous instability of the insert, which is prone to secondary mobilization.

These artificial transposons are already aggressive genome invaders, and putting them into insects is to give them wings, as well as sharp mouthparts for efficient delivery to all plants and animals and their viruses. The predictable result is rampant horizontal gene transfer and recombination across species barriers. The unpredictable unknown is what kinds of new deadly viruses might be generated [5], and how many new cases of insertion mutagenesis and carcinogenesis they may bring [6]. It is the height of folly and irresponsibility to release such GM insects, let alone GM insects carrying female-killing genes [7].

References

1. Rubin EJ, Akerley BJ, Novik VN, Lampe DJ, Husson RN, and Mekalanos JJ. In vivo transpostion of mariner-based elements in enteric bacteria and mycobacteria. *Proc. Natl. Acad.Sci USA* 1999: 96: 164-1650. See also "Can such rampant gene shuffling be safe?" Mae-Wan Ho and Angela Ryan, *ISIS News 4*, March 2000

2. See "Terminator insects -- a primer" by Joe Cummins, ISIS Report, March 2001

3. Lobo N, Li X and Fraser Jr. MJ. Transposition of the *piggyBac* element in embryos of *Drosophila melanogaster*, *Aedes aegypti* and *Trichoplusia ni*. *Mol Gen Genet* 1999: 261: 803-10.

4. Toshiki T, Chantal T, Corinne R, Toshio K, et al. Germline transformation of the silkworm *Bombyx mori L.* using a *piggyBac* transposon-derived vector. *Nature Biotechnology* 2000: 18: 81-84.

5. See "Genetic engineering superviruses" by Mae-Wan Ho, ISIS Report, March 2001

6. See "Unregulated hazards, 'naked' and 'free' nucleic acids" ISIS Report, Jan. 2000

7. Thomas DD, Donnelly CA, Wood RJ and Alphey LS. Insect population control using a dominant, repressible, lethal genetic system. *Science* 2000: 287: 2474-6.

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