Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 3349–3350, March 1998

Commentary

An important step forward in the genetic manipulation of mosquito vectors of human disease

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Mosquito-transmitted diseases are on the rise and have a significant impact on human morbidity and mortality on a global scale. A dramatic increase in the incidence of infections caused by the dengue virus places 2 billion people at risk each year (1). Despite the existence of an effective vaccine, yellow fever epidemics have reemerged in Africa and South America and pose a major threat for impoverished urban areas (2). With an annual mortality of at least 1.5 million people, malaria is the leading cause of childhood mortality in tropical Africa (3). Recent resurgence of the mosquito-borne Rift Valley Fever virus has resulted in hundreds of deaths in Ethiopia and Kenya.

In the 1950s there were high hopes that mosquito-transmitted diseases soon would be under control. Widespread use of pesticides had radically reduced both the numbers of mosquitoes and the incidence of infection, particularly in Asia. But subsequent mosquito insecticide resistance has resulted in a resurgence of infection in areas that had become free from disease. Drug-resistant forms of *Plasmodium*, the microorganism that causes malaria, have evolved that put not only endemic people at risk, but also travelers in the affected areas. Vaccines to mosquito-transmitted diseases have been slow in development, and vaccination programs often do not help those most affected by the problem because affected countries are often unable to afford to buy or deliver the vaccine to their impoverished citizens (2). Furthermore, estimates that global warming will increase the range and transmission season of vector mosquitoes place an increasing number of people at risk in the future (4).

In conjunction with control programs based on vaccination and mosquito elimination, a promising approach is to engineer mosquitoes to modulate vector competence genetically to reduce transmission of the pathogen (3, 5). However, a major barrier to the genetic engineering of mosquitoes has been the lack of a method for effective and reliable germ-line transformation. Now the important and long-awaited breakthrough has been achieved, as reported in two papers published in this issue of the *Proceedings* (6, 7). Two transformation vectors based on independent transposable element systems have been developed and have been shown to produce stable integration of foreign DNA into the genome of the primary vector of dengue and yellow fever, the *Aedes aegypti* mosquito. The integrated DNA is inherited according to Mendelian rules over at least 10 generations. This achievement opens up a wide vista of possibilities of introducing and testing foreign DNA sequences into the mosquito germ line for both basic research and the development of a wide array of biological control methods.

Fifteen years ago, the development of a successful germ-line transformation system allowed the development of a new generation of tools for the genetic manipulation and engineering of *Drosophila*. These tools have revolutionized *Drosophila* research through the introduction of powerful methodologies such as directed gene modification, analysis of *in vitro* mutagenized genes, gene cloning by transposon tagging, and enhancer trapping (8, 9). Spradling and Rubin (10, 11) first successfully transformed the *Drosophila* germ line by using a plasmid DNA construct containing a *P* transposable element and a marker gene. They microinjected this construct, along with a transposase, an enzyme required for germ-line mobility, into embryos undergoing the transition between syncitial and cellular blastoderm. At this stage, the *P* element, carrying the marker gene as cargo, is able to transpose from the plasmid to a random chromosomal site in the genome of the fly.

The development of similar transformation systems for mosquitoes and other pest insects has been a major goal of molecular insect science since the breakthrough in *Drosophila*. Achievement of transformation involves two complementary steps: 1) Development of a method for successfully integrating foreign DNA into the germ line, thus ensuring subsequent stable inheritance. 2) Identification of an appropriate marker gene to indicate that the foreign DNA fragment has been incorporated. Both of these steps have proved considerably more difficult to achieve in mosquitoes than in *Drosophila*. Endogenous transposable elements that occur naturally in mosquito species have been described (12), but so far none have been found to be suitable as gene carriers for transformation. For *Ae. aegypti*, this problem was solved by genetically engineering two different transposable elements originating in other insect species, the *Hermes* element from the house fly, *Musca domestica* (7), and the *mariner* element from *D. mauritiana* (6). Various aspects of mosquito biology have contributed to the relative difficulty of obtaining successful transformation, including the hard cuticles of mosquito eggs, which are considerably more refractory to penetration than the cuticles of *Drosophila* eggs.

Just as the successful development of the *D. melanogaster* genetic transformation system depended heavily on previous basic research on *P* transposable elements (13) so does the *Aedes* success build on many years of basic work by a number of research teams. Transposable elements are classified into two types, based on their mode of transposition. Class I elements that use reverse transcriptase as an intermediate in transposition have proved to be less suitable for modification as gene carriers than the class II elements that use DNA as a transposition intermediary (9). Following the discovery of the unsuitability of *P* elements for mosquito transformation because of the narrow host range of these elements (14), other class II elements came under close scrutiny for this purpose. The focus first was on plasmid mobility assays that tested whether a prospective element could mobilize (excise and integrate) in the mosquito embryonic environment. Only when this condition was met was it followed by efforts to integrate the element into the *Ae. aegypti* genome. In a number of key experiments, members of the *hAT* family of transposable elements were identified as the most likely candidates as general transformation vectors (e.g., ref. 14). Later it was shown that one member of this family, *Hermes*, derived from

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the house fly (15), was capable of mobility in *Ae. aegypti* embryos (16).

Another key feature of the successful transformation of *Ae. aegypti* was the identification of a suitable dominant or codominant allele of a marker gene that would provide an easily scored and unequivocal indication of transformation. Once again no suitable allele was available in the mosquito, but the wild-type allele of the *cinnabar* gene in *Drosophila* was found to complement a white-eyed mutation in an old laboratory strain of *Ae. aegypti* (17) and this allele possessed the attributes of an excellent marker.

The new ability to transform mosquitoes does not herald the potential for enactment of a new biological control strategy any time in the near future. Such a strategy will involve an ambitious program with a number of challenging steps, each of which must be achieved before the final goal is reached. In addition to developing an effective transformation system for genetic manipulation of target mosquito species, it is necessary to identify genetic mechanisms for making mosquitoes refractory to the relevant viruses. It then will be necessary to develop appropriate mechanisms to ensure the population spread of the engineered constructs in a safe and effective manner (18, 19). The latter step also will depend on a better understanding of the population genetics and transmission properties of target mosquitoes (3, 5).

A start has been made toward the identification of genes that render mosquitoes refractory to some important vector-borne diseases, but much still remains to be done. Studies have shown refractory-correlated genetic expression during parasitic challenge (20), or quantitative trait loci (QTL) that are associated with the refractory condition (21, 22), and even a QTL that is associated with reduced susceptibility (23). As yet however, there has not been any concrete identification of refractory genes. The achievement closest to this ultimate goal was accomplished by Olson and colleagues (24). They successfully prevented replication of the dengue virus in the salivary glands of previously susceptible mosquitoes when they transduced the mosquitoes with a recombinant virus that contained antisense RNA directed at a premembrane coding region of this virus. But this was not a refractory gene of mosquito origin. Instead, a viral gene directed against itself was used to prevent transmission. Although this approach involved only a single generation, it now may be possible, by using the new transformation technique, to incorporate the viral constructs permanently into the mosquito germ line.

Another major challenge on the horizon is to repeat for anopheline mosquitoes what has just been achieved with *Ae. aegypti*. *Anopheles gambiae*, an important malaria vector, is the focus of intense studies in a number of laboratories. An earlier attempt to transform this species initially produced promising results but fell short in failing to achieve stable integration of DNA in the germ line (25). Technical and biological problems posed by *Anopheles* transformation are even harder to solve than for *Ae. aegypti*, but given the continuing collaborative sustained efforts being exerted, and the seriousness of the growing worldwide health threat, there appear to be excellent prospects for a successful outcome.

In addition to scientific and technical aspects, the adoption of a strategic research plan (available on the Worldwide Web at: http://klab.agsci.colostate.edu/mfnet/mftacp.html#RTFToC1) and the support for this research provided by both government and nongovernment funding sources have made substantial contributions to the successes reported here. The technical difficulties and risky nature of long-range programs often preclude adequate sustained funding from government agencies in the absence of short-term results. In addition to the World Health Organization, the John D. and Catherine T. MacArthur Foundation has initiated and funded a seed grant for support of research on the biology of disease vectors by a network of investigators from a number of different universities. Much of the success of the present results can be ascribed to recent funding from this source, which has provided not only research grant funds, but also has promoted an unusual amount of interdisciplinary collaboration and collegial interactions.

1. Scott, T. W., Naksathit, A., Day, J. F., Kittaypong, P. & Edman, J. D. (1997.) *Am. J. Trop. Med. Hyg.* **57,** 235–239.
2. Robertson, S. E., Hull, B. P., Tomori, O., Bele, O., LeDuc, J. W. & Esteves, K. (1996) *J. Am. Med. Assoc.* **276,** 1157–1162.
3. Collins, F. H. & James, A. A. (1996) *Sci. Med.* **3,** 52–61.
4. Jetten, T. O. & Focks, D. A. (1997) *Am. J. Trop. Med. Hyg.* **57,** 285–297.
5. James, A. A. (1996) *Science* **272,** 829.
6. Coates, C. J., Jasinskiene, N., Miyashiro, L. & James, A. A. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3748–3751.
7. Jasinskiene, N., Coates, C. J., Benedict, M. Q., Cornel, A. J., Salazar Rafferty, C., James, A. A. & Collins, F. H. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3743–3747.
8. Engels, W. R. (1995) in *Transposable Elements*, eds. Saedler, H. & Gierl, A. (Springer, Berlin), pp. 103–123.
9. Kaiser, K., Sentry, J. W. & Finnegan, D. J. (1995) in *Mobile Genetic Elements*, ed. Sheratt, D. J. (IRL, Oxford), pp. 69–100.
10. Spradling, A. C. & Rubin, G. M. (1982) *Science* **218,** 341–347.
11. Rubin, G. M. & Spradling, A. C. (1982) *Science* **218,** 348–353.
12. Tu, J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7475–7480.
13. Bingham, P. M., Kidwell, M. G. & Rubin, G. M. (1982) *Cell* **29,** 995–1004.
14. O'Brochta, D. A., Gomez, S. P. & Handler, A. M. (1991) *Mol. Gen. Genet.* **225,** 387–394.
15. O'Brochta, D. A., Warren, W. D., Saville, K. J. & Atkinson, P. W. (1996) *Genetics* **142,** 907–914.
16. Sarkar, A., Yardley, K., Atkinson, P. W., James, A. A. & O'Brochta, D. A. (1997) *Insect Biochem. Mol. Biol.* **27,** 359–363.
17. Bhalla, S. C. (1968) *Mosquito News* **28,** 380–385.
18. Kidwell, M. G. & Ribeiro, J. M. C. (1992) *Parasitol. Today* **86,** 275–286.
19. Sinkins, S. P., Braig, H. R. & O'Neill, S. L. (1995) *Proc. R. Soc. London B* **261,** 325–330.
20. Wattam, A. R. & Christensen, B. M. (1992) *Am. J. Trop. Med. Hyg.* **47,** 702–707.
21. Zheng, L., Cornel, A. J., Wang, R., Erfle, H., Voss, H., Ansorge, W., Kafatos, F. C. & Collins, F. H. (1997) *Science* **276,** 425–428.
22. Gorman, J. J., Severson, D. W., Cornel, A. J., Collins, F. H. & Paskewitz, S. M. (1997) *Genetics* **146,** 965–971.
23. Beerntsen, B. T., Severson, D. W., Klinkhammer, J. A., Kassner, V. A. & Christensen, B. M. (1995) *Exp. Parasitol.* **81,** 355–362.
24. Olson, K. E., Higgs, S., Gaines, P. J., Powers, A. M., Davis, B. S., Kamrud, K. I., Carlson, J. O., Blair, C. D. & Beaty, B. J. (1996) *Science* **272,** 884–886.
25. Miller, L. H., Sakai, R. K., Romans, P., Gwadz, R. W., Kantoff, P. & Coon, H. G. (1987) *Science* **237,** 779–781.