3B.4 ABSTRACT

GENETIC MODIFICATION OF INSECTS OF MEDICAL IMPORTANCE: PAST, PRESENT AND FUTURE

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Together with AIDS and tuberculosis, malaria is among the deadliest infectious diseases in the world and responsible for an estimated 2 million deaths, mostly of African children, every year. Also worrisome, is that the number of cases has been increasing, not decreasing. This suggests that the weapons available to fight malaria is not sufficient and underscores the need to develop new approaches to complement the existing ones.

Unlike AIDS and tuberculosis, transmission of *Plasmodium*, the causative agent of malaria, strictly depends on an intermediate host, the mosquito vector. Consequently, the mosquito stages of parasite development are potential week points that can be targeted for disease control. Several laboratories, including ours, are exploring the possibility of using genetic modification of the mosquito to render it incapable to sustain parasite development. The presentation will review past efforts to develop transgenic mosquito technology, the present status of the field and future prospects.

Insertion into the mosquito germ line of genes that render the mosquito refractory to the parasite has three essential requirements: the ability to introduce genes into its germ line, the characterization of promoters that can drive foreign genes in the appropriate mosquito tissue and at the appropriate time, and the identification of "effector genes" whose products interfere with parasite development without affecting mosquito fitness.

Germ line transformation. The first multicellular organism ever to be genetically transformed is an insect, *Drosophila melanogaster*. Although this was reported in 1982, genetic transformation of *Ae. aegypti* was reported only 16 years later (1998) and that of an anopheline mosquito (*An. stephensi*) in 2000. These advances made feasible the introduction of foreign genes into the malaria vector.

Promoters. Development of *Plasmodium*, the causative agent of malaria, occurs in three mosquito compartments: midgut, hemocoel and salivary glands. Characterization of promoters (and associated regulatory sequences) that drive expression of proteins in the first two compartments occurred in 2000 while a promoter for salivary gland expression was just reported.

Effector genes. There have been several effector genes characterized, including the SM1 peptide that blocks parasite invasion of the midgut and salivary gland, phospholipase A2 that interferes with midgut invasion by unknown mechanisms, and monoclonal antibodies that recognize parasite surface proteins.

In conclusion, experiments conducted in the past decade demonstrate that it is feasible to genetically modify mosquitoes to render them refractory to the malaria parasite. The next

big challenge is to devise means to introduce the transgenes into mosquito populations in the field. One factor that will influence the success of such endeavor is the fitness of the genetically modified mosquitoes. In contrast to reports from other laboratories, we found that a transgene by itself does not impose a fitness load to the mosquito. Moreover, we found that when mosquitoes are fed with *Plasmodium*-infected blood, transgenic mosquitoes may actually have a fitness advantage over their non-transgenic counterparts.

Several approaches have been suggested for driving genes into populations, including the use of transposable elements, Wolbachia and meiotic drive. Implementation of either of these will require major technical hurdles to be overcome and these are unlikely to be solved in the near future. For the short term, we feel that an alternate approach – paratransgenesis - offers considerable hope. The mosquito, as all higher organisms, carries bacteria in its gut and the number of these bacteria increases dramatically after a blood meal (nutrients stimulate bacteria multiplication). Moreover, the bacteria are in the same compartment - the midgut lumen - where the most vulnerable stages of *Plasmodium* development take place. Instead of genetically modifying the mosquito, we propose to modify the mosquito's midgut bacteria to produce the anti-*Plasmodium* proteins (paratransgenesis). Initial experiments indicate that this approach may be feasible. The advantages of such paratransgenesis approach include its low tech nature which is important for use in disease endemic countries, ease of expression of any combination of effector proteins, ease of changing effector genes if resistance or other problems develop, and the ability to concomitantly target multiple mosquito species with the same recombinant bacteria. One major technical problem that needs to be solved is to devise a method to introduce the recombinant bacteria into wild mosquito populations. Possible solutions for this potential obstacle will be discussed.