PROSPECTS FOR GENETIC MANIPULATION OF INSECT VECTORS AS A STRATEGY FOR THE CONTROL OF VECTOR-BORNE DISEASE

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Abstract—Insect vector control has proved to be an effective method for reducing the transmission of disease-causing organisms to human populations in many tropical countries. A variety of methods have been employed to suppress vector populations, including the application of biological control agents and the elimination of breeding sites, with a continuing and heavy reliance on the use of chemical insecticides. However, the development of insecticide resistance by vector insects, the cost of developing and registering new insecticidal compounds, and the increase in legislation to combat the detrimental effects of insecticidal residues on the environment, have emphasised the need to assess a variety of alternatives to vector control. What is required is a completely novel approach either to suppress vector populations, or to alter their ability to transmit disease-causing organisms in such a way as to have a profound and long-lasting effect on disease transmission. Genetic manipulation of insect vectors may provide just such an approach. The major requirements for being able to manipulate the genomes of insects will be reviewed, together with the progress which has been made to create transgenic vector insects. The potential of this methodology will then be explored, emphasising how its most immediate use will be as an analytical tool. Finally, the feasibility of creating refractory vector strains by genetic manipulation and releasing them into the environment will be assessed in relation to its future use as a disease control strategy.

INTRODUCTION

Molecular biology has been used in a variety of ways to extend our understanding of insect vectors and their ability to transmit disease to human populations. It is hoped that in the longer term novel vector control strategies might be developed through the application of these techniques, possibly by creating transgenic insect vectors, either to suppress the vector population or to reduce their ability to transmit disease-causing organisms (Crampton *et al.*, 1990a).

It also is important to consider what we are trying to achieve by applying genetic manipulation techniques to insect vector populations. This technology can be used as an analytical tool to provide a greater understanding of insect vector biology and ecology and the interactions between vectors, the disease-causing organisms they transmit and the human host. The expectation is that this knowledge will allow the development of new approaches to the control and management of these diseases by an increased ability to interrupt or disrupt disease transmission.. This might be achieved through the identification of new targets for attack, the development of novel methods for insect vector population suppression, the ability to alter insect vector behaviour and hence disrupt transmission, and finally to alter the ability or efficiency of insects to transmit pathogenic organisms (Crampton, 1994).

In any consideration of transgenic technology and how it may be applied to medically important insects, the following factors need to be considered: i) the practical requirements for creating transgenic insects; ii) how best to apply the technology and thus what gene systems from insects or other organisms need to be defined in order to undertake the desired manipulations. Our recent research has focused on developing methods for introducing DNA constructs into cells and embryos, the identification of endogenous transposable elements which could form the core of transformation vectors, and the characterisation of promoters which are induced in the mosquito gut when it takes a blood meal. In the longer term we wish to introduce genes coding for anti-parasitic agents into mosquitoes in such a way that parasite transmission is blocked.

THE REQUIREMENTS FOR GENETIC MANIPULATION

Despite the dramatic advances made recently with respect to genome manipulation in *Drosophila melanogaster* there is an urgent need for a much greater understanding of the molecular biology of

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mosquito disease carriers. This must include an analysis of the complexity and organisation of their genomes and an understanding of the distribution of coding and repetitive sequences. These details form an integral part of the design and interpretation of cloning and hybridisation experiments. In addition, the exploitation of transgenic technology requires methods for the introduction of DNA both into living mosquitoes and into cultured cells. Ideally, this will involve a transformation vector which is capable of directing efficient and stable integration into the chromosomes of the recipient. The introduced DNA has not only to be expressed but should also carry a selectable marker for the identification of transformed individuals or cells. Ultimately, there will be a need to study alternative promoter and enhancer sequences so that the spatial and temporal expression of the introduced DNA may be controlled. Finally, none of this will be of any relevance unless appropriate genetic target systems can be identified, cloned and characterised at the molecular level.

Methods for introducing DNA into mosquito embryos

In relation to embryo microinjection, the early development of all insects is relatively similar in general terms to that of *Drosophila*. There may, however, be significant differences in developmental rate and embryo physiology. For example, mosquito embryos differ from those of *Drosophila* by having opaque, rigid chorions which cannot be removed without loss of viability. In addition, the mosquito embryo is extremely susceptible to desiccation during the period at which injection must take place.

Here, we describe the system which we have developed in our own laboratory for transformation of the mosquito *Aedes aegypti* (Morris *et al.*, 1989). However, similar techniques have been used elsewhere both for *Anopheles* and for other *Aedes* species, and all are based in general on the methods developed for *Drosophila melanogaster*. As indicated above, the rigid, opaque endochorion of the mosquito embryo cannot be removed, and the embryos are extremely sensitive to desiccation. However, glass capillaries with tips of 100–300 μm×4–10 μm can be used to puncture the rigid endochorion without tearing it and deliver the DNA solution without damage to the embryo. The slightly viscous DNA solution can not be expelled manually from such a fine needle and is therefore injected by means of a two phase nitrogen supply. The lower pressure prevents backflow and the higher pressure delivers 160–800pl of DNA solution (corresponding to 1–5% of the embryo volume) into the posterior pole of the embryo at the syncytial blastoderm stage, before cell partitioning occurs. This is where the pole cells, which are the germ line primordia, develop. Injection of DNA close to the site of pole cell formation is not critical to germ line incorporation, but the timing is clearly important if the DNA is to be taken up by the developing germ line cells. All of our injections are normally completed within 2 hours of oviposition. After injection, the embryos are covered with a water-saturated halocarbon oil, which permits the normal uptake of water until they are returned to standard insectary conditions. In this way DNA has been introduced into mosquito embryos (Miller *et al.*, 1987; McGrane *et al.*, 1988; Morris *et al.*, 1989), with survival rates comparable to those obtained with *Drosophila* (Spradling and Rubin, 1982). Similar methodologies have also been employed to introduce DNA into the embryos of silk moth (Mahalingam *et al.*, 1992), medfly (Malacrida *et al.*, 1992), the sheep blowfly and the housefly (Atkinson *et al.*, 1992).

DNA vector systems used in non-drosophilid transformation

The experimental design for the creation of transgenic insects, including mosquitoes, is based on that developed for *Drosophila melanogaster*. G_0 individuals which survive micro-injection with the P element vector/helper DNA, pUChsneo/pUChsπ(Δ2–3), are mated *inter-se* and allowed to produce progeny. These G_1 individuals are the first which might be expected to express antibiotic resistance throughout all tissues and larvae are therefore subjected to selection with the neomycin derivative, G418. The molecular nature of any transformation events is determined by DNA analysis using radioactively labelled transformation vector DNA to probe Southern blots of genomic DNA extracted from the putative transformants and their progeny (Miller *et al.*, 1987; Morris *et al.*, 1989). Intact vector P elements have been detected in 5–10% of adults that have

developed from injected embryos (G_0), confirming that the introduced DNA is not immediately broken down by the mosquito. Furthermore, we have detected the chromosomal integration of vector DNA in several G_0 individuals. This probably reflects direct incorporation into a proportion of the somatic cell nuclei since it is only in the following generation (G_1) that we might expect a germ line integration event to have been transmitted to every nucleus. More promisingly, vector DNA has been identified in the chromosomes of the G_1 and G_2 progeny of injected embryos, suggesting that integration has occurred in the germ line of the mosquito and that this DNA shows normal Mendelian inheritance. Some of these events, however, appear to be unstable from one generation to the next and this phenomenon, together with the molecular basis of the transformation events, awaits further investigation.

As indicated above, chromosomal integration of the introduced P element DNA has been observed in both *Anopheles* and *Aedes* mosquitoes and the integration events appear, in some cases, to be heritable and clearly involve the germ line of the transgenic mosquitoes (Miller *et al.*, 1987; McGrane *et al.*, 1988; Morris *et al.*, 1989). Although these events did not result from normal P element transposition, some functional role of the P sequences can not be excluded. This is particularly true since similar experiments in *Lucilia cuprina* (Atkinson *et al.*, 1992) and *Ceratitis capitata* Malacrida *et al.*, 1992) have failed to produce any integration of vector sequences. Research in other laboratories is now being directed towards the identification of these accessory *Drosophila* proteins and the cloning of the genes involved may facilitate high efficiency P transposition in non-drosophilids. It is clear, however, from this work and from other experiments involving the transfection of the same DNA into cultured mosquito cells (Lycett *et al.*, 1989) that the P element system in its present form is not suitable for routine use in the mosquito. Thus, whilst the means are currently available for introducing DNA into both mosquito embryonic germ lines and cultured cells, a major stumbling block is the lack of an appropriate, high efficiency, DNA transformation vector system for manipulating the mosquito genome.

In addition to the use of the P element derived DNA vector systems, experiments to assess the mobility of a number of other transposable elements in non-drosophilid systems have been attempted. These experiments have included the use of fully processed cDNA copies of both the *Ac* and *Spm* elements from *Zea mays* (Comley *et al.*, 1992) which have been shown to transpose actively in a number of evolutionary disparate organisms and may prove to act autonomously in mosquitoes (Kunze and Starlinger, 1989). In addition, experiments involving the introduction of *hobo*, *gypsy* and *mariner* into *Anopheles gambiae* embryos are currently underway.

The search for mobile genetic elements in mosquito genomes

It is clear that the germ line integration events so far observed in mosquitoes do not involve normal P element transposition. The absence of this controlled mobility poses certain limitations, for example with respect to transposon tagging for functional cloning. Research elsewhere is concentrating on the precise mechanism of P transposition and attempts are being made to modify the P element system for more general use (O'Brochta, 1990). Such research may yet lead to the "universal vectors" originally envisaged. At the same time, there remains the possibility that P elements may never function as efficient transposition mediated transformation vectors in non-drosophilids. We, and others, are therefore actively searching for endogenous transposable elements which may yet prove to be the most suitable transformation vectors.

The isolation of endogenous transposable genetic elements may ultimately prove central to the development of efficient transformation and transposon tagging systems in mosquitoes. A number of approaches have been taken to identify such mobile elements and one of these was to analyse specific gene systems, such as the ribosomal DNA of mosquitoes, in an attempt to isolate variants of these genes which may have arisen from the insertion of transposons. No such insertions have, as yet, been identified in *Aedes aegypti* DNA (Gale and Crampton, 1989) but insertion events have been detected in the rDNA of *Anopheles gambiae* and these elements are being fully defined (Paskewitz and Collins, 1989). The elements appear to resemble a particular class of mobile element known as non-viral retroposons. It is unlikely, however, that these elements will prove useful as transformation vectors because of the ill defined nature of their mode of transposition. More recently, the polymerase chain reaction has been employed to identify sequences related to the

mariner transposable element from *Drosophila mauritiana* in the genomes of a wide range of insect vector species (Robertson, 1993). It appears that *mariner* is of ancient origin and is widely distributed within insects. Active elements have been identified in *Drosophila* which can transpose into the genome of different *Drosophila* species. One can readily postulate the existence of active elements in other species, which can likewise cross species-specific boundaries. Currently, five principal subfamilies of *mariner* have been identified, although more may follow. As such, there may be restrictions on the transposition activity of these elements, but it seems that *mariner* has a far wider scope as a transformation vector than does the P element.

A major difficulty arises in the identification of active elements in species of insect, including *An. gambiae*, that carry a large number of copies of *mariner*. Another consequence of this may be that any *mariner*-based vector may be redundant in such an insect due to the presence of host/element repressor systems. However, a *mariner*-based vector should have great potential for the genetic manipulation of species with no, or very few, copies of the element present. This possibility seems unlikely, at least in the case of *An. gambiae*, where all strains studied so far possess large numbers of *mariner*-like elements.

Mariner could yet prove to be a potent tool for studying insect systems. Many in the field of transgenic mosquitoes are interested in the use of transposable elements to incorporate specific genes, such as those for refractoriness to *Plasmodium falciparum*, into wild populations of *An. gambiae* (Warren and Crampton, 1994). As Kidwell and Ribeiro (1992) point out, once a transposable element has become fixed in a population it is unlikely that a second opportunity would be available for using the same transposable element as a DNA vector in an autonomous system. In their review, they further explore the problems associated with the use of loaded transposons and the mechanisms that could be used to drive specific gene constructs through populations. These problems relate not only to *mariner*, but probably to any other potential transposable element-based transformation system. While there are high hopes and great expectations for *mariner* as a future transformation vector for the genetic manipulation of insect vector species, it would seem prudent to continue our search for other endogenous transposable elements in an attempt to develop a number of transformation systems and DNA vectors for the insect species of interest.

We originally adopted an alternative strategy to identify directly a specific class of mobile elements, known as retrotransposons, in the mosquito DNA. The approach relies on utilising the characteristic biochemical and structural properties of these elements. This work has led to the successful isolation of several retrotransposon-like elements from the *Aedes aegypti* genome (Crampton *et al.*, 1990a and b). We have also used PCR as a particularly rapid methodology to identify endogenous Class I, retrotransposon-like elements and Class II transposable elements in mosquito DNA (Warren and Crampton, 1991). Using PCR technology, together with oligonucleotide primers corresponding to highly conserved amino acid motifs in the *copia/Ty1* group LTR retrotransposon reverse transcriptases, we have amplified DNA sequences from an *Ae.aegypti* total genomic template. The major PCR product is a DNA fragment of approximately 250bp. One would expect to observe a product of this size following amplification of autonomous elements from the *copia/Ty1* group using these same primers. Following DNA sequencing, the deduced amino acid sequences were aligned with the amino acid sequences of reverse transcriptases of well characterised LTR retrotransposons including *copia*, *Ty912*, *Tal-3* and *Tnt-1*, and the *Ae.aegypti* sequence clearly exhibits considerable homology to these counterparts.

The cloned PCR product was used to identify and isolate homologous genomic clones from a representative EMBL4 total genomic library. Interestingly, when the entire sequence of one of these genomic clones was determined, the clone contained two incomplete non-LTR retrotransposons in opposite orientation in the DNA. One element contains an open reading frame showing considerable homology to *copia*-like elements (including protease, integrase and reverse transcriptase motifs), and the other homology to the second open reading frame of LINE-like elements. Neither element seems to be complete, and neither contains LTRs. We also have evidence that sequences homologous to the LINE-like ORF are actively transcribed in *Ae.aegypti* cells in culture, whereas the *copia*-like sequences are not. We are currently analysing other representatives of both these elements in an attempt to find more complete elements which may have retained their ability to mediate their own transposition in mosquitoes.

Class II transposable elements are characterised by their mode of transposition through a DNA intermediate and short inverted terminal repeat sequences flanking a transposase encoding sequence required for transposition. Class II elements have proved to be powerful tools for gene cloning through transposon tagging, as markers for genome elucidation and as DNA transformation vectors. Several elements have fulfilled these roles, the most notable being the P-element of *Drosophila melanogaster* (Engels, 1989). However, as with the P element these transposons appear to be seemingly ineffective outside closely related species and endogenous elements may be required to carry out similar functions in a species or genus of interest. The *Tc1* element was first recognised genetically in the nematode *Caenorhabditis elegans* (Emmons *et al.*, 1983). It is 1610bp long with 54bp perfect terminal repeats (Rosenzweig *et al.*, 1983). The *Tc1* transposase has been recently elucidated by Vos *et al.* (1993). *Tc1* has proved invaluable for transposon tagging, leading to the isolation of genes and genome elucidation of *C. elegans* (Moerman and Waterson, 1989). Given the broad spectrum of animals in which *Tc1*-like elements have been discovered it seems highly probable that members of this family of transposons will be identified in many more diverse organisms. We have now identified *Tc1*-like sequences in the *An. gambiae* genome using PCR and oligonucleotide primers corresponding to conserved amino acid motifs within the transposase encoding region of *Tc1*-like elements. The *An. gambiae Tc1*-like PCR clone, TRANG, exhibited homology to the transposase region of several *Tc1*-like elements from a variety of organisms and shares 78% amino acid sequence identity with the corresponding region of the active *Tc1* element from the nematode *C. elegans*. Southern blot analysis of genomic DNA from several strains of *An. gambiae* probed with TRANG indicates a very low copy number of its counterparts within this mosquito genome. Variations in the position of TRANG in the genome further suggests that it is, or was, capable of transposition. Genomic clones containing this sequence have been obtained and sequence analysis is nearing completion.

The search for stage and tissue specific promoters for use in mosquito transformation vectors

At some stage it will be desirable to express defined genes in mosquitoes and other insects in a tissue or stage specific fashion. For this to be envisaged, stage and tissue specific promoters have to be defined. None are, as yet, available but attempts to characterise the DNA sequences responsible for expressing certain genes, particularly in mosquito systems, are underway by identifying genes which are expressed in a tissue specific fashion and then defining the upstream, putative tissue-specific promoter sequence. One example of this approach, has been the identification of an *Ae. aegypti* sequence which is only expressed in the female salivary gland (James *et al.*, 1989). The expectation is, therefore, that this will allow the definition of a salivary gland-specific promoter sequence which may eventually allow the controlled expression of an introduced gene sequence in this tissue. More recently, trypsin genes have been cloned and characterised from *Ae. aegypti* (Barillas-Mury *et al.*, 1991), the Blackfly, *Simulium vittatum* (Ramos *et al.*, 1993) and *An. gambiae* (Muller *et al.*, 1993). In each case, the expression of one or more of these trypsin genes has been shown to be induced in the insect midgut by a blood meal. It is therefore likely that gut-specific, blood meal inducible promoters will shortly be available for each of these insects. Such promoters are clearly of interest as they will allow the expression of antiparasitic agents in the insect gut when it takes a blood meal, that is, when the insect first comes in contact with the organisms which it can transmit to the human population. However, it will be necessary to develop methods for establishing the functionality of putative promoter sequences. To this end, we and others have begun to develop the methodologies for transfecting mosquito cells in culture.

The simple, controlled environment of cultured cells allows one to follow the expression of cloned genes, and so delineate promoter and enhancer sequences. Genetically manipulated cell cultures are also able to over-produce specific proteins which facilitates their isolation and purification. Cultured mosquito cells have been used to examine different transfection techniques and vectors and to help establish a suitable system for germline transformation of *Ae. aegypti* (Lycett, 1990). Initially, experiments involved introduction of the P element vector and helper constructs into several cell lines by a variety of techniques devised to generate transient cell membrane pores, including calcium phosphate precipitation (Wigler *et al.*, 1977), dextran sulphate (Lopata *et al.*, 1984), polybrene (Durbin and Fallon, 1985), electroporation (Chu *et al.*, 1987) and

lipofection (Felgner *et al.*, 1987). Much of this work has concentrated on the immortal Mos20 fibroblast cell line which was derived from minced, trypsinised, neonate larvae of the *Aedes aegypti* London strain in 1969. Polybrene and electroporation mediated transfection have proved to be most successful for these cells, producing approximately 30 and 4000 transformants per 10^6 cells, respectively. Subsequently, constructs incorporating the chloramphenicol acetyl transferase (CAT) reporter gene system have been utilised to optimise expression of the CAT gene under the control of the *Drosophila* heat shock promoter, hsp70, in the Mos20 mosquito cultured cells (Lycett and Crampton, 1993).

Subsequently, in collaboration with A. Crisanti and M. Muller (Rome) and R. Sinden (Imperial, London), we have used these transfection techniques to introduce reporter gene constructs into *An. gambiae* gut cells in culture as a means to begin defining functional blood meal inducible promoters derived from the *An. gambiae* trypsin and chymotrypsin-like genes. Genomic clones have been isolated encompassing both these loci (Muller *et al.*, 1993), and constructs derived utilising the 5' and 3' regions from trypsins 1, 2 and 4, and chymotrypsins 1 and 2. In one set of constructs, the CAT reporter gene together with the trypsin-derived signal sequence has been placed downstream from the 5' region of each gene; in a second set of constructs the same signal/reporter has been placed between the 5' and 3' region from each gene. The Act/CAT/SV40 construct has been employed as a positive control, and each of these constructs has been transfected into cultured primary gut cells derived from blood-fed and non-blood-fed female mosquitoes. 2.2kb of the 5' region of trypsin 2 was sufficient to induce CAT expression in transfected gut cells from blood fed females, but not from non-blood fed females. In the case of Trypsin 1, both the 5' region (2kb) and 3' region (1kb) were necessary to induce CAT expression in transfected gut cells from blood fed females. Deletion constructs of these regions are being assessed to determine the minimum region of DNA necessary to induce expression in similar cells. Preliminary gel shift experiments are underway to define whether there is any evidence of factors binding to these regions. Similar experiments are being carried out with the chymotrypsin genes from *An. gambiae.*

THE POTENTIAL APPLICATION OF TRANSGENIC TECHNIQUES IN MOSQUITO VECTORS

Once the systems necessary to create transgenic insects have been developed, how may this technology be applied? Three possible uses of transgenic technology in insects include: i) its use as an analytical tool to increase our understanding of insect biology (including ecology, behaviour, population genetics, evolution, insect/pathogen interactions, etc); ii) to introduce anti-parasite genes into mosquitoes so as to eliminate parasites, or disrupt their onward transmission to vertebrate hosts (this requires the 'useful' genes to be driven through insect population with high efficiency); iii) the technology could be used to improve the SIT so as to suppress insect populations more effectively. Our recent studies have focused on the second of these applications.

Potential target genes for manipulation

There are a number of obvious targets for manipulation including the genes involved in the insect immune system, developmental control genes and insecticide resistance genes. Genes influencing all of these factors have now been characterised at the molecular level for a number of different insects and it is now feasible to consider manipulating them in the germ line of these insects. In addition, a number of genes are of particular interest because they are directly implicated in the ability of insects to transmit disease causing organisms. Examples include the filarial susceptibility (f^m) and *Plasmodium* susceptibility (*pls*) loci of the mosquito, *Aedes aegypti.* The f^m locus is genetically well defined and there is good data on its linkage relationships. Refractoriness to infection is due to a partially sex-linked, dominant gene (Macdonald and Ramachandran, 1965). There is marked variation in the susceptibility of this mosquito to different filarial worms, although all of the alleles concerned map at about the same place on the sex chromosome. Also of particular interest is a strain of *Anopheles gambiae* which has been selected for refractoriness to the malaria parasite and

characterised genetically (Collins *et al.*, 1986). Attempts are currently underway to clone these genes but it is difficult to undertake such a cloning exercise in the absence of any knowledge of the gene product. Considerable progress has now been made towards the molecular characterisation of this gene, with the development of a linkage map for the X chromosome of *Anopheles gambiae* (Zheng *et al.*, 1991; 1993). Once fully refined, this map should allow the genes influencing refractoriness to be cloned and fully characterised. Clearly, the use of transgenic technology through transposon tagging will assist in the characterisation of refractory genes and their products.

An important genotypic characteristic not met by the majority of genes encoding refractoriness is that any such gene introduced into the insect would have to be capable of altering the phenotype through the expression of a single gene copy. Unfortunately, at present, there is no gene or gene product defined at the molecular level which is known to directly affect phenotype in relation to pathogen development in, or transmission by, any insect. However, in the mosquito system a number of molecules are known to affect the transmission of malaria by anophelines. Foremost among these are the so called transmission blocking vaccines, which can achieve a total transmission blockade (Winger *et al.*, 1987). These vaccines attack antigens present on the gametes and ookinetes of the malaria parasite and antibodies which recognise these antigens are able to block the development of the parasite in the mosquito midgut. A very exciting possibility therefore, is to introduce the genes coding for such antibodies into the mosquito genome thus directly conferring the transmission blocking phenotype to the insect. In this case, a transgenic mosquito would be created incorporating an antibody gene expressed in the insect midgut in response to a blood meal and which therefore blocks transmission of malaria. In its simplest form, this approach would involve introducing what is, essentially, a monoclonal antibody into the mosquito. This would not be ideal for a number of reasons, and some way of introducing polyclonal transmission blocking antibody coding genes would be required. However, for the present, the introduction of a single transmission blocking antibody gene under the control of a gut specific, blood meal inducible promoter is being attempted as a model to assess the system.

This type of approach is an attractive one for a number of reasons: it eliminates the need for the detailed molecular analysis of refractory mechanisms in mosquitoes and it would be a 'dominant' gene system (i.e. one gene copy only would be needed in each cell of the mosquito). The antigen target on the stage of the malaria parasite present in the mosquito is highly conserved, suggesting that the parasite may be less able to avoid this type of transmission control mechanism. Finally, the use of transgenic insects incorporating an antibody gene could be applied to any vector transmitted pathogen (parasite or viral) where a target antigen can be identified as being inhibited by the expressed molecule.

To date, mouse antibody genes have been cloned and introduced into mosquito cells in culture and mouse Fab molecules have been expressed and detected using immunohistochemical staining techniques. The transmission blocking antibody genes coding for Pbs21 have now been cloned and are being expressed in mosquito cells to ensure that they produce functional antibody. As indicated above, we have available DNA sequences which will induce gene expression in blood fed mosquito gut cells. It may therefore be possible in the near future to link the transmission blocking antibody gene to these promoter sequences and create a mosquito expressing the transmission blocking antibody genes in such a way as to block or disrupt the transmission of malaria. If successful, transgenic mosquitoes expressing antimalarial antibodies may represent a potential strategy for controlling malaria and may establish a precedent for a wide range of new anti-disease strategies.

TRANSGENIC INSECTS: THE FUTURE

The questions posed by considering the release of transgenic insects emphasise the need to assess the biological consequences of such a release. It is, however, difficult to gauge the possible hazards of such a release in the absence of experimental evidence and these ethical and safety considerations need to be faced at an early stage. In order to undertake an informed appraisal where the possible net benefits may be balanced against the potential hazards, considerable effort will have to be devoted to utilising caged populations and the controlled release of molecularly

tagged individuals together with mathematical modelling of these populations. One particular problem of releasing transgenic insects into natural populations is the concern that the transgenes may 'escape', i.e. move through both the target insect population as well as other organisms with which they have contact. Such organisms may include symbionts, or closely related species with which the target population may (rarely) interbreed. Clearly, this poses a problem, particularly where a major aim will be to drive beneficial genes through target populations, obviating the necessity to release large numbers of transgenics. Regulatory bodies may require assurances that gene constructs will never be able to spill over into closely related species. Whilst the probability of such events may be extremely rare, concern about the possibility may well stop the application of transgenic insect technology. In the case of direct release of transgenics to a defined target population in a limited locality, such a problem could be overcome by pre-sterilising the insects. This would be similar to the current SIT, and would necessitate repeated, large-scale release of the relevant transgenic insects. These possibilities are for the future, and await the development of reliable and efficient DNA vectors systems to construct the desired transgenic insects.

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