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Genetic transformation of arthropod vectors for control of vector borne diseases

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The vector borne diseases cause heavy loss to mankind and livestock industry throughout the world. Global climate changes reported to contribute to the recurrence and new epidemics of vector borne diseases. Unfortunately, the available strategies to control vector borne diseases are insufficient and public health burden of the major vector borne diseases is on increasing trend. Complete eradication of a vector population is not realistic and desirable for many arthropod vector species due to biological constraints. Genetic transformation of vectors offers the way to control the diseases transmitted by the arthropod vectors without killing them and is also an economically viable option. Genetic transformation of arthropod vectors is a new strategy in the genomic era in which the synthetic effector gene is introduced into the genome of vectors to block the developmental stages of parasite/pathogen inside the vector and subsequent driving of the effector gene into the wild vector population in a geographical area. However, the knowledge on the putative risk factors associated with the release of transgenic arthropod vectors in the field is lacking and considerable work is needed before the deployment of transgenic arthropod vectors in nature.

Keywords: arthropod vectors, vector borne diseases, genetic transformation, effctor genes, transformation marker genes, viral vectors, transposons

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Introduction

Arthropod vectors inflict heavy loss to mankind and livestock industry, directly by biting and sucking blood, and indirectly by transmitting vector borne diseases. Vector borne diseases claim millions of lives of human and livestock every year. Among them malaria itself claims more than one million human lives every year[1]. The economic loss in the livestock industry due to ticks and tick borne diseases in Australia alone was estimated as $150 million every year[2]. The important vectors and vector borne diseases are listed in Table 1. The traditional control methods like chemotherapy and vaccination against vector borne diseases and use of insecticide, sterile insect release, biological control, immunological control and pheromone trapping against insect vectors are not sufficient enough to control the vector borne diseases[3]. Moreover, the antigenic variation present in most of the vector borne parasites/pathogens poses a challenge in the development of effective vaccine against vector borne diseases[4,5]. The global warming has been facilitating breeding of arthropod vectors and their entry into new geographical areas and is likely to disturb the delicate equilibrium and contribute to new epidemics of vector borne diseases[6]. The development of resistance against insecticides and chemotherapeutic agents increased the reoccurrence of these diseases. Dam construction, irrigation and other developmental projects, urbanization, increased human travel and deforestation have all resulted in changes in vector population densities that appear to have enabled the emergence of new vector borne diseases and resurgence of old diseases[7]. So it is the time to explore newer strategies to combat vector borne diseases. Although eradication is a common arthropod pest control strategy, it is not realistic for many pests due to biological constraints, and it may even be undesirable if it merely produced an empty ecological niche that might be readily filled by new immigrants[8]. Advances in molecular and cell biology, and genomics have allowed us to identify weak links in the diseases transmission of vectors, and tremendous advancements in the field of genetic engineering have paved the way to explore newer approaches. Although ongoing efforts to develop and improve conventional methods for the control of vector borne diseases have not been abandoned, novel strategies are needed.

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Table1—Important vector and vector borne diseases of man and livestock

Vector	Vector borne diseases	Parasite/pathogen
	Viral diseases	
Ae.aegypti	Yellow fever	Flaviviridae (yellow fever virus)
	Dengue fever	Flavidiridae (DEN 1-4)
	Chikungunya	Togaviridae (Chikungunya virus)
Culex spp	Japanese encephalitis	Flavidiridae (Japanese encephalitis virus)
	West Nile encephalitis	Flavidiridae (West Nile virus)
Haemophysalis spinigera	Kyasanur forest disease	Flavidiridae (KFD virus)
Culex & Aedes spp	Equine encepalitis	Togaviridae (Equine encephalitis virus)
Hyalomma spp	Crimean-Congo Haemorragic fever	Bunyaviridae (CCHF virus)
Culicoides spp	Bovine ephemeral fever	Rhabdoviridae (Bovine ephemeral fever virus)
	Bluetonge	Reoviridae (Bluetonge virus)
Hyalomma dromedarii	African horse sickness	Reoviridae (African horse sickness virus)
Ornithodorus mobuta	African swine fever	African swine fever virus
Rhipicephalus appendiculatus	Nairobi sheep disease	Bunyaviridae
	Rickettsial diseases	
R. sanguines	Ehrlichiosis	*Ehrlichia canis, E. equi*
	Human monocytic ehrlichiosis	*E. senetsu, E. chaffeensis, E. phgocytophilia*
Amblyoma variegatum	Cowdriosis	*Cowdria ruminantium*
	Anaplasmosis	*Anaplasma marginale*
R. sanguines, Dermacenter andersoni, Boophilus decoloratus		
Pediculus humanus	Epidemic typhus	*Rickettsia prowazekii*
Xenopsylla cheopis	Murine typhus	*R. typhi*
Ixodid ticks	Tick borne spotted fever	*R. rickettsii, R. conorii, R. sibirica, R. japonica, R. australis*
	Spirochete diseases	
Ixodes ricinus	Lyme disease	*Borrelia burgdoferi*
P. humanus	Epidemic relapsing fever	*B. recurrentis*
	Bacterial diseases	
Xenopsylla cheopis	Plague	*Yersinia pestis*
Dermacentor spp	Tularemia	*Francisella tularensis*
	Protozoan diseases	
Anopheles spp	Malaria	*Plasmodium falciparum, P. vivax, P. ovale*
	African trypanosomosis	*Trypanosoma brucei rhodesiense and T. b. gambiense*
Glossina spp	Nagana	*T. congolanse, T. vivax, T. evansi*
Triatomine bugs	Chagas' diseases	*Trypanosoma cruzi*
Phlebotomus spp, *Lutzomyia* spp	Leishmaniosis	*Leishmania donovani, L . tropica, L. braziliensis*
H. a. anatolicum, R. appenticulatus	Theileriosis	*Theileria annulata, T. parva, T. hirci*
Boophilus spp	Babesiosis	*B. bigemina, B. bovis, B. ovis, B. motasi, B. equi,*
Ixodes spp	Human babesiosis	*B. microti*
	Helminth diseases	
Culex spp, *Anopheles* spp, *Aedes* spp	Lymphatic filariosis	*Wuchereria bancrofti, Brugia malayi, B. timori*
	Onchocercosis	*Onchocerca volvulus*
Simulium spp	Dirofilariosis	*Dirofilaria immitis*
Culex spp, *Aedes* spp	Stephanofilariosis	*Stephanofilaria assamensis*
Musca *spp*	Parafilariosis	*Parafilaria bovicola*
	Habronemiosis	*Habronema megastoma, H. muscae*
	Setariosis	*Setaria digitata, S. cervi*
Anopheles spp, *Aedes* spp	Diphilidiosis	*Diphylidium caninum*
Ctenocephalides spp, *Chrysops* sp	Loaosis	*Loa loa*

Using genetic transformation technique the vector competence of an arthropod vector could be modified. Genetically modified vectors are unable to transmit pathogen/parasite and can replace their susceptible counterparts in nature. Transgenic arthropod vector approach is attractive because the mass rearing of disarmed vectors in disease endemic countries is likely to be economically viable and relatively low cost technology[9]. Steps involved in the genetic transformation of arthropod vectors and limitations of this application are discussed in this review.

Genetic Transformation of Arthropod Vector

The discovery of Rubin and Spradling that the transposable P elements can be manipulated to introduce the heterologous gene into *Drosophila* genome was a milestone in the transgenic arthropod technology[10]. In the last 5 years, the insect molecular geneticists witnessed the realization of long sought goal of genetically transforming arthropod vectors of medical and veterinary importance to manipulate their vectorial capacity. The first successful transgenic anopheline mosquitoes that are impaired in transmitting the malaria parasite were generated by Ito and co-workers[11]. This indicates that we are moving beyond the technology development phase of vector transformation to the technology application phase. Genetic transformation of arthropod vectors to change their vector competence[12, 13], involves the following steps:

Selection of effector gene

⇩

Selection of a suitable promoter to drive the expression of effector gene

⇩

Selection of transformation marker with suitable promoter

⇩

Selection of appropriate vector

⇦⇨

Virus Transposons

Introduction of recombinant virus/construct into insect genome

⇩

Selection of transformed insects

⇩

Assessment of refractoriness of transgenic insects

⇩

Assessment of vertical transmission and post integration behaviour of transposons

⇩

Controlled study & field release

I. Selection of Effector Genes

The knowledge of vector-pathogen/parasite interaction at molecular level is imperative in the selection of an effector gene (gene capable of interfering with parasite/pathogen development inside the vector, without imposing fitness load). The information on sexual stage in the life cycle of the parasite, host (arthropod vector) factors required by the parasite/pathogen to develop inside the vector, the parasite antigens essential for their establishment inside the arthropod vector and organs/tissues of vector where the parasite does develop, are essential for the selection of effector gene. The effector gene product expressed inside the transgenic arthropod vector interferes with the pathogen/parasite stages present inside the vector and impairs the disease transmission potential of the vector.

Anti-malarial Effector Genes

Anti-CSP single chain antibody gene: A single chain antibody against *Plasmodium gallinaceum* circumsporozoite protein inhibits sporozoite invasion of mosquito salivary glands[14].

SM1 gene (Synthetic molecule 1): 12 amino acid peptide-inhibits *P. berghei* ookinete invasion into mid gut epithelium of *An. stephensi* and sporozoite invasion into salivary glands[15].

PLA2 gene: Inhibits *P. berghi* ookinete invasion[16].

II. Selection of Suitable Promoter to Drive Expression of Effector Gene

Expression of the anti-parasitic effector gene in the arthropod vector requires the use of appropriate promoter. The promoter has to be selected to express the anti-parasitic effector molecules in the tissues/organs where the development of parasite occurs and also relative to the time of the arrival of parasite. Ubiquitous promoters that are active in all tissues at all times can also be useful. But, generalized and constitutive expression of a foreign gene in the vector possibly would affect the fitness of the transgenic arthropod vector.

Spatial Consideration in Promoter Selection

Development of parasite/pathogen stages in the arthropod vector usually occurs in the mid-gut lumen, hemocoel, salivary glands and ovary. In mosquitoes, the anti-parasitic genes under the control of carpoxypeptidase and AgAper1 promoters (peritrophic membrane protein promoter) are expressed into midgut lumen. The genes under the

control of vitellogenin promoter are expressed into fat body after blood meal[17]. Maltase-I and Apyrase promoters are used to express the effector molecules in salivary glands.

Temporal Consideration in Promoter Selection

The time of effector gene expression should be relative to the arrival of the parasite. Carboxypeptidase and AgAper1 promoters drive the expression of the effector gene in the mid-gut lumen in response to blood meal[18].

III. Selection of Transformation Marker Gene with Suitable Promoter

Selection of genetically transformed arthropod vector is an important step in the production of transgenic arthropod vector. The transformed vectors are selected based upon the expression of marker gene. Attempts to transform the non-drosophilid insects using insecticide resistance gene as marker turned out to be problematic because of the selection of too many false-positives due to the non-DNA vector related insecticide resistance selection. Insecticide resistance gene in the insect vector intended for deployment in nature pose the problem of transferring the resistance gene to the non-target insects. Green fluorescent protein (GFP) gene from the jellyfish, *Aequorea victoria* could serve as a marker for germ line transformation[19]. The transgenic vectors can be detected at early larval stages, saving time and effort of rearing the non-transgenic vectors. Ubiquitous promoters like actin promoter drive the expression of marker gene in all the tissues and at all the times. Generalized expression of marker gene reduces the fitness of the transformed arthropod vectors in terms of survivability. Tissue or organ specific promoters like 3XP3, eye specific promoter would be the right choice to express the marker gene[20].

IV. Selection of Appropriate Vector/Delivery System

Several gene vectors have been developed to deliver the transgene in non-drosophilid insects. These include four transposons and two viruses (Sindbis alpha viruses and Pantropic retroviruses). The four transposons are proving useful as gene vectors for transgenic arthropod production and for stable germ line transformation of several arthropod vectors. The two viral vectors are currently limited to the transient expression of transgenes in insects that are infected with engineered virus.

Viral Vectors

Sindbis Alpha Viruses

Sindbis viruses are alpha viruses in the family of Togaviridae. They are enveloped positive-sense, single stranded RNA viruses naturally harbored by most of the insects. The 5′ end of two-third of the 11.7 kb genome of this virus codes the non-structural proteins, like RNA-dependent RNA polymerase (RDRP). The 3′ one-third of the RNA genome, which is referred to as 26S sub genomic RNA, codes for the capsid and envelope proteins[21] . The basic design of the expression system rests on the ability of this virus to carry and package genomic RNA longer than the canonical 11.7 kb. The anti-parasitic effector gene can be added to the 3′ end of virus genome under the transcriptional control of a copy of the promoter that drives the expression of 26S sub genomic RNA. These recombinant viruses contain two regions that are transcribed from the negative sense RNA, are referred to as double sub genomic Sindbis (dsSIN) viruses. Addition of the effector gene and marker gene to the 26S sub genomic region of the Sindbis viral vector in DNA form and subsequent *in vitro* transcription, packaging with capsid and envelope proteins yields recombinant virus. After infecting the insect cell the recombinant virus expresses the effector gene and marker gene along with its structural proteins.

Limitations

Despite the proven utility, Sindbis virus gene expression system suffered from limitations like production of this virus requires some expertise in virus culture techniques. This virus also shows strong neurotropism and very limited ability to infect the midgut epithelium.

Pantropic Retroviruses

Retroviruses have been extensively developed as vertebrate gene expression tools. Their specific tissue tropism constitutes a very serious limitation to their use. The tropic characteristics of retroviruses are determined by the envelope proteins and so modification of these proteins may result with altered tropisms. This system has not been successfully used to create transgenic arthropod vectors.

Transposons

Transposons are class II mobile genetic elements that move directly from DNA to DNA by a cut-and-paste process, which is independent of host specific factors. Transposons range in size from 1.3 to 3.0 kb

and are bounded by inverted terminal repeats (ITR), which are recognized by the transposase enzyme, the only gene encoded by the transposon, allowing excision of the transposon and its insertion into the host genome[10]. They are vertically transmitted to progeny and can probably move between individuals of different species in a process known as horizontal gene transfer[22]. Transposable elements have dramatic transmission advantages and have been observed to spread through natural populations. The behaviour of transposable elements is central to their success as transformation vectors but is also the source of many scientific questions that must be answered before deployment of an insect population transformed with a transposable element. The effector gene and the marker gene can be inserted into the transposon and used for the transformation of target arthropod vector. P elements were determined not to work outside the genus *Drosophila*, due to either endogenous repression or missing host-encoded factors[23]. Four transposons unrelated to P elements representing four different families of eukaryotic transposable elements can be used to genetically transform non-drosophilid insects. These are the Minos, Mos1, Hermes and piggyBac elements.

Minos Element

A transposable element derived from *D. hydei*, a member of Tc1 family of transposable elements. It is approximately 1.8 kb in size, possesses 255 bp inverted terminal repeats (ITR) and contains two long open reading frames separated by a 60 bp intron. It inserts at TA residues and creates 2 bp target site duplication. It is capable of transforming different *A. gambiae* cell lines and in developing *A. stephensi* embryos[24].

Mos1 Element

A transposable element derived from *D. mauritiana,* a member of mariner family of transposable element[25]. Up to date this is the only naturally occurring mariner element isolated from an insect. This element can be used to transform a wide range of organisms from microbes to vertebrates. Mos1 element could accurately transpose in *Ae. aegypti* and *Lucilia cuprina*[26] and it has been used in the transformation of *Ae. aegypti*[27]. This transposon, as that of Minos element, inserts only at TA residues and creates 2 bp target site duplication. Magnesium ions are very essential for the activity of Mos1 transposase (Insertional specificity).

Hermes Element

The Hermes element from *Musca domestica* is a member of the hAT family of transposable elements. It is 2749 bp in length, contains 17 bp inverted terminal repeats and codes a transposase of 70 kDa size. This element is found in house fly population throughout the world[28]. Hermes element has been used to generate stable transgenic insect lines like, *Ae. Aegypti*[29], *Stomoxys calcitrans* and *Culex quinquefasciatus*. Hermes element creates 8 bp duplications at the target site. This target site duplication of 8 bp length at the site of insertion, conformed the consensus sequence of 5′-GTnnnnAC3′ observed for other hAT elements.

piggyBac Element

piggyBac was identified as an insertion sequence that caused a plaque morphology mutation in *Galleri melonellea* nucleopolyhedrosis virus that was being passaged through cells of the cabbage looper, *Tricoplusia ni*. It is 2.5 kb in size and contains 13 bp inverted terminal repeats. It contains a 2.1 kb long open reading frame that encodes a transposase with little or no structural similarity with the other eukaryotic transposases. The piggyBac element inserts at TTAA sequences in the genome and creates a duplication of this sequence at site on insertion[30,31]. Unlike other insect transposons so far characterized, piggyBac is excised absolutely precisely from the donor site. It can transform a range of insect species and this element enjoys wider use. It was used in the genetic transformation of *M. domestica, Ae. aegypt* [17], *Anopheles albimanus , A. stephensi, A. gambiae*[32] with transformation efficiencies ranging from 1% in *A. gambiae* to 40% in *A. albimanus*.

Mode of Transposition of Transposable Elements

The initial step in the transposition reaction and in retroviral integration is the creation of a single strand nick that exposes the 3′OH ends of the transposable element. The 5′ end of the element can be processed in one of the two ways leading to either replicative or cut-and-paste transposition. If no cut occurs at the 5′ end of the element, it is inserted into the target site while still remaining attached to the donor site. Replication then forms a “cointegrate” that contains two copies of the transposable element together with the donor backbone joined to the target site. This type of transposition is called replicative transposition. For transposable elements that move only by cut-and-paste transposition, a cut always occurs at the 5′ end

of the element, and the resulting double strand break enables only the transposable element to be inserted at the target site. All the class II mobile genetic elements move by cut-and-paste transposition. The process of genetic transformation of arthropod vectors to alter their vector competence is explained here by designing a hypothetical plasmid construct. Fig. 1 displays a hypothetical construct consisting of a plasmid sequence and a transposon. The anti-parasitic effector and transformation marker genes (GFP) are inserted into the transposon sequence. The transposase gene is under the control of hsp70 promoter. The effector and transformation marker genes are under the control of carpoxypeptidase and eye specific 3XP3 promoters, respectively. This construct can be used for stable germ line transformation of arthropod vectors.

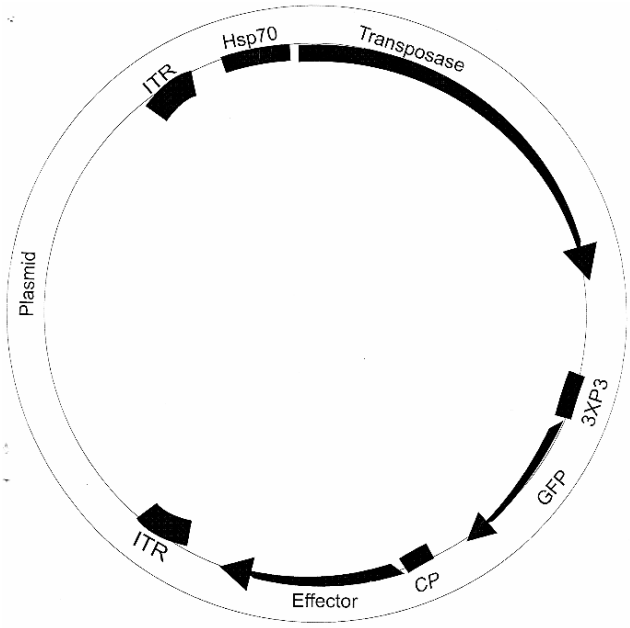

Fig. 1—Hypothetical plasmid-transposon construct inserted with marker gene and effector gene; ITR, Inverted terminal repeat; Transposases, Gene coding for transposase; 3XP3, Eye specific promoter; GFP, Green fluorescent protein; CP, Carpoxypeptidase promoter; & Effector, Gene coding for effector molecule

Introduction of Construct into Germline/Egg

Genetic transformation technologies depend on viable physical and biological methods for delivering transgene to the cells and ultimately to the nuclei of cells of interest. For stable germline transformation, the germ cells are the targets of all efforts at DNA introduction. A number of methods for delivering DNA to developing insect germ cells have been used, including microinjection[33], biolistics[34] and electroporation[35]. Among these methods, microinjection remains the best option for penetrating insect chorions and delivering vector DNA to germ cells. The widely accepted technique for microinjection of vector DNA into *Ae. aegypti* embryos has been described by Morris[33]. Mosquito embryos need to be slightly desiccated to allow small volume of DNA to be introduced. Back flow of cellular contents from punctured embryos into the needle can be a problem in embryos that are under-desiccated. The back flow can lower the survival rate. Use of pressure control to maintain the holding pressure of DNA injected into embryos reduces the back flow and allows the deposition of a controlled volume of DNA into the posterior pole of embryo. Survival rate (the number of embryos that survive microinjection as a percentage of the total number injected) is only 10-25% in *Ae. aegypti*. The optimal age for microinjection of *Ae. aegypti* embryos is before melanization i.e., between 90 and 120 min after oviposition.

Mode of Action of Construct

It is hypothesized that the construct, after delivered into the germ cells, would enter the nucleus through nuclear pore complex actively and the transposase mRNA would be transcribed first, after getting a heat shock. The transposase translated in the cytoplasm would enter the nucleus actively. The structure of transposase includes an N-terminal DNA binding domain, a central nuclear localization signal and a C-terminal catalytic domain. The transposase binds to the inverted terminal repeat sequences (ITR is the substrate for transposase) of both the ends and excise the transposon from the plasmid sequence. The transposon along with effector gene and marker gene excised from the plasmid sequence gets integrated into the insect genome by the catalytic activity of transposase. Integration of transposon into the arthropod vector genome occurs randomly or at preferred target sequences depends upon the type of transposon. After integration, the anti-parasitic effector gene and transformation marker gene (GFP) along with the transposon would become part of the arthropod vector genome. As the GFP gene is under the control of eye specific promoter (3XP3), the expression of green fluorescent protein occurs at early stages of the vector development. So, selection of the transgenic arthropod vectors could be done in the larval stage itself by seeing the GFP fluorescence in the eyes of the larvae. The carboxypeptidase promoter is expected to drive the expression of anti-parasitic effector protein into the

midgut lumen of adult insect, after getting the stimulation of blood meal. The effector molecules secreted into the midgut lumen would interfere with development of parasite stages in midgut and subsequent transmission (Fig. 2).

Transposable elements, as a part of arthropod genome, are inherited in a Mendelian fashion. When an individual with a transposable element mates with one lacking the element, it may multiply and eventually spread to all the chromosomes in the hybrid genome even if the transposable element carries a selective disadvantage[36] .When all the gametes of a hybrid carry transposable elements, they could theoretically be driven to fixation in the population even if they reduced fitness as long as the reduction was less than 50%[22]. The population dynamics of the transposable elements with the marker and effector gene is presumed to be like that of the transposable elements as such[37].

Assessment of Refractoriness of Transgenic Arthropod Vectors

Transgenic arthropod vector expressing the anti-parasitic effector molecule has to be assessed for the development of parasitic stages inside it. Disease transmission potential of the transformed vectors can be assessed by controlled study. The transgenic vector is to be allowed to feed either the infected blood or on the infected animal and after some period, to be allowed to feed on the naive susceptible animals. The non-transmission of pathogen/parasite to the naive susceptible animal is the indicator of the refractoriness of the transgenic arthropod vector. Subsequently, the developmental stages of the pathogen/parasite inside the transgenic vector have to be studied. Non-development of the pathogen/parasite stages inside the transgenic vector is the indication of refractoriness.

Assessment of Vertical Transmission of Transposon

The transmission of transposon along with the effector gene to the progenies of the transformed arthropod vectors has to be assessed in a controlled study. Polymerase Chain Reaction and/or Southern hybridization techniques could be used to detect the transgene in the genome of progenies of the transformed arthropod vectors.

Assessment of Post Integration Behaviour of Transposable Elements

The post-integration behaviour of gene vectors (remobilization, replication and spreading potential) is

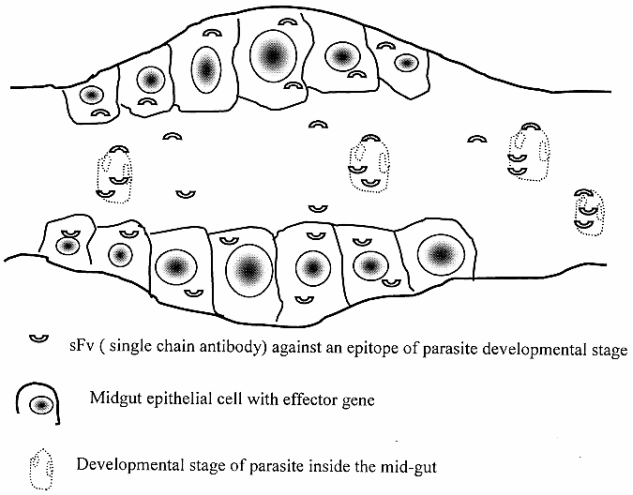

Fig. 2—Mid-gut of transgenic arthropod vector showing refractoriness to parasite infection.

particularly relevant to a number of applications of this technology. Replication of class II transposable elements by gap repair mechanism and/or jumping ahead of replication fork and the extent at which this occurs will determine the potential of the element as a genetic drive system. Stability of the integrated gene vectors is of concern to some researchers because genotypes created, using these vectors, may need to be maintained without change over time and would be of limited use in the laboratory or in the field if they were changing position within the insect genome or being lost from it. The transposase can function *in trans*, meaning that it can recognize the inverted terminal repeats of a transposable element other than the one in which it is located to catalyze its transposition. The role of endogenous transposases is also central to predicting the behaviour of introduced transposable elements, but, as with the activity of transposable elements, this aspect is insufficiently understood. To those responsible for assessing the risks associated with releasing transgenic arthropod vectors into the environment, the issue of stability of the transposons has additional significance. Transposable element display (TE display) analysis can be done to determine the post integration mobility of the gene vectors/transposons[38].

Field Release of Transgenic Arthropod Vectors

Till date nowhere in the world, field release of the transgenic arthropod vectors has been done because of the putative risk factors. Successful creation of *Anopheles* mosquito refractory to *Plasmodium* infection has generated lot of enthusiasm among the researchers to control vector borne diseases with

population replacement strategy[11]. Research on fitness cost of different effector genes, use of different class II transposable elements as genetic drive system, and risk evaluation are underway. The release of transgenic arthropod vectors in the environment should be planned and executed with appropriate oversight.

Limitations of the Technology

Socio-ethical Issues

Perceived complexity has also been a barrier to progress in regulations involving environmental release of genetically modified arthropod vectors. While there are few intrinsic ethical concerns about killing pests, eco-centric approaches to ethics do raise some objections to modification of ecosystem components. The methods used for monitoring field trials are argued to be inadequate by those campaigning against genetically modified organism[39].

Genetic Fitness of Transgenic Arthropod Vectors

The idea of population replacement is predicted on the ability of transgenic arthropod vectors to have a genetic fitness that is at least equal to wild population. But fitness load imposed by the foreign genes introduced into the arthropods may decrease their survivability and/or reproductive ability. Genetic fitness of the transgenic arthropod vector is essential to drive the transgene in the wild vector population. In case of malaria, fixation of the anti-malarial gene must be ensured because more than 99% of the mosquitoes in an area must be refractory for a reduction in malaria transmission to actually occur[40]. Releases that fall short of this goal are likely to make no impact and could even increase the rate.

Difficult to Get Back Once Released

It is difficult to control or eradicate the transgenic arthropod vectors after their release into the environment. This potential will make any consideration of environmental and human health risk assessment more complex.

Transfer of Insecticide Resistance to Target & Non-target Insects

The selection of transgenic arthropod vectors based on the insecticide resistance marker (coded by the gene vector), pose the problem of transferring the resistance gene to the target and non-target insects.

Prior Presence of the Same Transposon

Transposable element based gene vectors are not only to introduce genes into arthropod vector genome but also to promote the spread of transgenes through gene pool of wild population of the vector species. Prior presence of the same transposons within the genome can lead to the development of regulatory systems that limit the movement of this type of element. The inadvertent contamination of wild population with transposable element targeted for use in future gene spreading efforts could pose a risk to the future success of such efforts.

Horizontal Transfer of Transposons

The risk of interspecific exchange of a transgene remains a serious concern to those deploying certain plants in the field. This question will also emerge when the risks of transgenic arthropod vectors are fully considered. The transfer of genes across species boundaries indicates that there is risk of any transgene being transferred to non-target species.

Lack of Guidelines for Containment & Risk Assessment of Transgenic Arthropod Vectors

Risk in transgenic arthropod research has the probability that a transgene will escape into nature and the hazard that it would cause[41]. Currently, no established procedures exist for how a risk assessment for transgenic insects would be conducted. Moreover, uniform international containment guidelines for research involving transgenic arthropods are lacking.

Instability of Introduced Gene

The members of the same transposable element family have been reported to be capable of cross-mobilization. Cross-mobilization could jeopardize the stability of any introduced gene in a host with a transposable element[42]. Loss of transgene after few generations under field conditions is also reported[43].

Problem in Secondary Use of the Same Transposable Element

When two P element-containing individuals mate, repressors (partially deleted transposable elements) function in each individual to prevent transposition. Only when one individual lacks any element at all (intact or deleted), is the element able to transpose to a new individual[22]. So, a given transposable element may only be an effective agent for spreading a gene through a population a single time. If a gene were intentionally or accidentally introduced into the population using a transposable element, the introduction of additional genes using the same element may not be possible.

Human Health Hazard

An arthropod vector transformed not to transmit a disease is capable of transmitting other diseases. For

example: transgenic *Ae. aegypti* refractory to dengue virus infection can be able to transmit yellow fever and chikungunya virus. Any introduced population of this species could thus potentially transmit several human diseases unless specifically engineered to be refractory to all of them. The liability and risk assessment issues with such a scenario are extremely complicated.

Bioterrorism

The transgenic arthropod technology is a double edged sword. The technology is proposed to develop arthropod vectors not to transmit parasite/pathogen. But this technology in the hands of terrorists can also be used to develop arthropod vectors to secrete some toxic proteins from their salivary glands.

Conclusion

With considerable advances in molecular genetics and biotechnology, it seems feasible that the technical tools will soon be available to overcome the limitations and to create transgenic insect vectors refractory to parasite/pathogen infection that can drive the effector genes into the wild arthropod vector populations. Now, the goal of insect molecular geneticists is to construct a system of insect gene transformation with a set of reagents and protocols that will simplify the genetic transformation of insects. At the same time a set of guidelines and risk analysis protocol should also be developed simultaneously to speed up field release of the transgenic arthropod vectors. The mode of horizontal transfer should be explored and ultimately managed by designing gene transfer system that can avoid them. The current research on transgenic insects should be broadened to include the evolutionary ecology of refractoriness. Considerable challenges lay ahead but there are reasons to be optimistic that we will be able to add genetic transformation of arthropod vectors to our arsenal in the fight against vector borne diseases.

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