Transgenesis and the Management of Vector-Borne Disease

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TRANSGENESIS AND THE MANAGEMENT OF VECTOR-BORNE DISEASE
Edited by Serap Aksoy

Transgenesis and the Management of Vector-Borne Disease

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PREFACE

Parasitic, bacterial and viral agents continue to challenge the welfare of humans, livestock, wild life and plants worldwide. The public health impact and financial consequences of these diseases are particularly hard on the already overburdened economies of developing countries—especially in the tropics. Many of these disease agents utilize insect hosts (vectors) to achieve their transmission to mammals. In the past, these diseases were largely controlled by insecticide-based vector reduction strategies. Now, many of these diseases have reemerged in the tropics, recolonizing their previous range, and expanding into new territories previously not considered to be endemic. Habitat change, irrigation practices, atmospheric and climate change, insecticide and drug resistance as well as increases in global tourism, human traffic and commercial activities, have driven the reemergence and spread of vector-borne diseases. While these diseases can be controlled through interventions aimed at both their vertebrate and invertebrate hosts, no effective vaccines exist, and only limited therapeutic prospects are available for their control in mammalian hosts. Molecular technologies such as transgenesis, which is the subject of this book, stand to increase the toolbox and benefit disease management strategies.

The debate on the feasibility and acceptability of the genetically modified insect technology continues. There is no doubt that the technology has revolutionized the study of insect sciences and promises to improve the reproduction and viability of beneficial insects and the use of insects as bioreactors—but will it meet the expectations as a disease control strategy? In the contributed chapters in this book, we begin by providing a historical perspective to transgenic technology applied with the goal of modification of vector competence (Chapter 1). Recent advances utilizing viral transducing systems (Chapter 2) as well as symbionts (Chapters 3 and 4) are described in more detail for the introduction and expression of anti-pathogen products in several vector systems. New applications for use of transposons are presented in Chapter 5. With expression systems well underway, effector molecules must now be chosen that will be toxic to the pathogen of interest when expressed in insects. In this regard, advances made in basic insect physiology and immunology, coupled with genomics information and gene mapping strategies (Chapter 6), are most relevant. Of importance for the success of genetically modified insects is the identification of gene drive systems to deliver desirable phenotypes into natural populations such that the modified disease resistant insects

can establish and lead to a reduction in disease transmission. For this purpose, *Wolbachia*-mediated drive systems continue to be promising candidates, and Chapters 9 and 10 address the recent developments in these fields. In addition, certain strains of *Wolbachia* are currently being used to modify insect age structures, a highly promising and innovative approach to reducing disease transmission (Chapter 11). Transgenic applications can also advance biological control methods such as sterile insect technique (Chapter 7) and genetic sexing and sterilization approaches (Chapter 8).

It is likely that fine-tuning the remaining technological challenges will make these systems safer for eventual field applications. Building on laboratory successes, the challenge now is to plan field-based investigations to test the ability of genetically modified insects to interfere with disease transmission cycle. While the integration of transgenic approaches for human disease management may face significant cultural and ethical challenges, a strong economic engine may drive the application of this technology against agricultural diseases as discussed in Chapter 12. Experience from the agricultural systems may provide important data that can guide the future applications in the context of human disease systems. Interdisciplinary field-based investigations are now necessary to describe the interactions between insects, pathogens and symbionts in natural populations to predict the feasibility of using modified insects to reduce the spread of harmful zoonotic diseases. We must gain a better understanding of the ecological factors, which influence disease epidemiology and insect population dynamics (Chapter 13). This information is essential to better predict how and where transgenic technologies, possibly in combination with other control approaches, may best be implemented.

A most important area not addressed in this book concerns the social and political implications of transgenic application. In this regard, it would be important for the scientific evidence to inform and steer the development of public health policy guidelines. The scientific discoveries and their potential benefits as well as limitations need to be shared with the general public through continued education and advocacy, especially in disease endemic countries where these technologies can have the highest impact on public health. Ultimately, acceptability and applicability will depend on the choices made by the populations where these diseases take their greatest toll.

Serap Aksoy, PhD

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CONTENTS

CHAPTER 1

Perspectives on the State of Insect Transgenics

David A. O'Brochta* and Alfred M. Handler

Abstract

Genetic transformation is a critical component to the fundamental genetic analysis of insect species and holds great promise for establishing strains that improve population control and behavior for practical application. This is especially so for insects that are disease vectors, many of which are currently subject to genomic sequence analysis, and intensive population control measures that must be improved for better efficacy and cost-effectiveness. Transposon-mediated germ-line transformation has been the ultimate goal for most fundamental and practical studies, and impressive strides have been made in recent development of transgene vector and marker systems for several mosquito species. This has resulted in rapid advances in functional genomic sequence analysis and new strategies for biological control based on conditional lethality. Importantly, advances have also been made in our ability to use these systems more effectively in terms of enhanced stability and targeting to specific genomic loci. Nevertheless, not all insects are currently amenable to germ-line transformation techniques, and thus advances in transient somatic expression and paratransgenesis have also been critical, if not preferable for some applications. Of particular importance is how this technology will be used for practical application. Early ideas for population replacement of indigenous pests with innocuous transgenic siblings by transposon-vector spread, may require reevaluation in terms of our current knowledge of the behavior of transposons currently available for transformation. The effective implementation of any control program using released transgenics, will also benefit from broadening the perspective of these control measures as being more mainstream than exotic.

Introduction

The goal of this introductory chapter is to give an overview of the state of the art of insect transgenesis, with emphasis on recent advancements in methodology and applications and especially as they relate to vectors of disease. Many of these topics are reviewed in much greater detail in the forthcoming chapters.

The ability to genetically transform the germ-line of non-drosophilid insects was first achieved, as a routine process, only within the last decade and impressive strides have been made in the methodology, practical applications and the types of insects amenable to the process. Some of these advances include defining vector host ranges, vector-stabilization methods, "marker" gene development and paratransgenesis. Significant progress has been made as well, in the somatic or non-heritable forms of transgenesis that preceded development of germ-line transformation. Somatic transformation is particularly useful in the laboratory and has allowed rapid determinations of promoter function and sequence-function relationships. Paratransgenesis, achieved by expressing genes that alter insect phenotypes in microbial symbionts, holds great promise as an insect

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control tool. In addition, certain microbial symbionts provide unique opportunities to preferentially spread transgenes through insect populations. These advances come at a particularly propitious time, coinciding with the availability of genomic sequence data from some of the most important species in terms of human health, agriculture, and fundamental research. Transgenesis fulfills the need of insect molecular biologists to understand sequence function as part of ongoing functional genomic analyses, and to develop new approaches for the control of pest populations, their behavior, and/or their competence to transmit pathogens. Less explored, but of enormous significance is the potential of transgenic insect technology to improve the reproduction and viability of beneficial insects, and to use insects as bioreactors.

The first great challenge for germ-line transformations was the development of functional vector systems for non-drosophilid species. Initial hope that the *P* element system widely used in Drosophila could be similarly applied to other insects met with great frustration, with the inability of many researchers to find true *P*-mediated transformants.[1-4] We know now that *P* has a highly restricted host range although we do not understand the basis of this limitation.[5] Interestingly initial and more recent successes in insect transgenesis have resulted equally from advancements in marker gene development as well as vector system development. Indeed, the first insect transformations utilized both existing and newly discovered transposable elements as vectors, and markers created from newly isolated genes that could complement eye color mutations, as was done routinely in Drosophila. The yellow fever mosquito *Aedes aegypti* was transformed with *Hermes*[6] and *mariner*[7] transformation vectors containing the *kynurenine hydroxylase* gene that complemented the *kh*^{w} allele.[8,9] At about the same time, the tephritid fruit fly pest, *Ceratitis capitata*, was transformed with *Minos*[10] and *piggyBac*[11] vectors using the *white* (or *white eye*) gene cDNA (under *hsp70* promoter control) isolated from the same species.[12] In these experiments, successful transformants were identified by a mutant-rescue effect that restored the wild type eye color phenotype in the mutant host. The utility of these markers was limited to species with appropriate mutant strains and clones of the complementing wild-type allele. In the past decade these four transposon vectors remain the primary vehicle for all insect transformations, as most have a broad range of transpositional activity. Yet the ability to use these vectors in hosts not amenable to mutant-rescue selection depended on more broadly applicable marker genes and promoters. This has been achieved by use of a variety of dominant-acting fluorescent protein genes,[13] first tested using a viral-based somatic transformation system,[14] under the regulation of functionally conserved transcriptional promoters and regulatory systems.

Despite the significant progress in transformation of disease vectors and agricultural pests, some species have yet to be transformed or have been so only with great difficulty. Suboptimal performance of insect transformation may be a function of technical expertise or effort, but certainly for Anopheles, species such as *An. stephensi* are transformed routinely,[15-20] while the highly important species *An. gambiae* requires much more effort.[21,22] Similarly, some lepidopteran species, such as *Bombyx mori*, are transformed routinely,[23-25] while others such as *Spodoptera frugiperda* and *Heliothis virescens* have yet to be transformed. To expand the number of species amenable to transformation, significant challenges remain in improving gene vector delivery to germ cells, increasing integration rates, extending host ranges, understanding post-integration behavior of gene vectors and developing transposable element-based gene spreading technologies.

Transposon Vectors and Insect Transformation

All transformation systems of insect germ-lines utilize Class II transposable elements as vectors that transpose by a DNA-mediated "cut-and-paste" process.[26] These mobile genetic elements are typically 1 to 5 kb in length and have terminal sequences that are inverted repeats of one another. The terminal inverted repeats (TIR) are usually 30 base pairs or less, but some are several hundred base pairs and some terminal regions also have requisite sub-terminal inverted repeat sequences. The TIRs and the DNA that lies between them are

excised and reinserted into new chromosomal positions as part of the transposition process. In between the TIR regions are DNA sequences that encode for a transposase enzyme that catalyzes both the cut and paste processes. Elements with TIRs and a transposase coding region are said to be self-mobilizing or autonomous, although they may require other host-encoded nuclear proteins. Importantly, while the TIRs and transposase gene are normally linked, the TIRs and any intervening DNA can be mobilized by an unlinked source of transposase. This feature has allowed the development of non-autonomous vectors that include the TIRs, marker genes, and genes-of-interest but with the transposase gene either mutated or deleted.[27,28] Non-autonomous vectors are mobilized when transposase is provided in trans - either from a plasmid-encoded transposase gene, transposase RNA or transposase protein. When vectors and transposase sources are coinjected into preblastoderm embryos, transposase catalyzes vector transposition and integration, but because the source of transposase is not contained on a vector it does not become integrated and is diluted and degraded over time. In the absence of transposase, vectors that have chromosomally integrated cannot undergo further transposition and are expected to be stable.

The first transposable element developed for use as a gene vector in *Drosophila melanogaster*, *P*,[29] was not effective in other species.[30] Interestingly most of the functional transposable elements that have been used as gene vectors in non-drosophilids were discovered fortuitously. Only recently have directed searches for functional transposable elements been fruitful as a source of new gene vectors. For example, the *Minos* element was discovered in *D. hydei* during the analysis of ribosomal RNA genes, and it was found to be closely related to *Tc* elements from nematodes.[31] The *Mos1 mariner* element from *D. mauritiana* was discovered during the analysis of an unstable allele of the *white* gene.[32] *Hermes*, a member of the *hobo, Ac, Tam3* (*hAT*) family was discovered in the housefly, *Musca domestica* during the evaluation of the *D. melanogaster hobo* element as a gene vector in that species.[33] *Hermes* is widely functional, but quite importantly, it has been shown to functionally interact with *hobo*,[34] providing some of the strongest experimental evidence to support the need for methods to stabilize transgene integrations. Other *hAT* elements have been found in tephritids that also functionally interact with *hobo*[35] and the *hopper* element from *Bactrocera dorsalis* has the potential for vector development.[36] Currently, the most widely used insect transposable element gene vector is the *piggyBac* element. This element was discovered during the analysis of mutations in baculoviruses and was subsequently found to have originated in the genome of the cabbage looper moth, *Trichoplusia ni*, which had served as a host for the virus.[37,38] Recently, bioinformatic analysis of public genomic DNA sequence databases aimed at finding functional transposable elements have resulted in the development of a number of new insect gene vectors e.g., *Herves*[39] and *Buster* (P.W. Atkinson and D.A. O'Brochta, unpublished). Non-insect transposable elements have also been used as gene vectors in insects. The bacterial transposable element *Tn5* has been used as a gene vector in *Ae. aegypti* although with very limited effectiveness.[40]

Enormous progress has been made in the study of the mechanism of action of at least two insect transposable elements that are used as gene vectors. Successful efforts to express and purify the transposases of the *Mos 1* and *Hermes* elements have enabled a wealth of biochemical information to be obtained regarding how these proteins perform the reactions involved in element excision and transposition. The successful determination of transposase crystal structures has provided unique insights into the mechanism of transposition and the relationship of these elements to other 'integrase' proteins.[41] In vitro assays have aided in assessing the biochemical requirements for transposable element mobility, and embryonic- and cell-line-mobility assays continue to be highly informative for assessing transposon mobility in a specific cellular environment. The development of powerful assay systems has allowed the testing of numerous mutations on both transposase enyzymatic activity and their binding specificities.[42] These advancements in functional analysis should not only result in enhancement of vector function in the near future but also the improved ability to modulate transposition for particular applications.

Early during the study of transposable element systems it was realized that most were members of larger families of elements that shared structural and functional similarities. The study of the evolutionary and natural history of transposable elements has been extended to the elements currently used for insect transgenesis. The extensive analysis of the *mariner/Tc* family has led to a greater understanding of the role of horizontal transfer in the life history of transposable elements and the role of this phenomenon on the preservation of functional elements despite their negative influence on the survival of their hosts.[43,44] The *Minos* element, which is widely distributed among drosophilids,[45,46] is strongly related to *Tc* elements originally discovered in nematodes,[31] and has exhibited function in invertebrate and vertebrate systems.[47,48] The *hAT* elements *Hermes* and *Herves* were discovered by virtue of their relationship to other *hAT* elements, and so their structural and functional similarities to *hAT*s is understood. However, *Hermes* was discovered using a degenerate-PCR approach using discrete amino acid homologies,[49] while *Herves* used a sophisticated algorithm to discover existing database sequences fitting specific criteria.[39] While the *piggyBac* element has been used as a vector in the widest range of insect species, analysis of its distribution or the existence of a related family of elements is most recent relative to the other systems. Unlike the other vector transposons, elements nearly identical to the original *piggyBac* discovered in *T. ni*, have been found in other noctuid moths[50] in addition to distantly related tephritid fruit flies in the Bactrocerid family.[51] Yet similar to the other vectors, somewhat distantly related *piggyBac*-like elements have been found in *Bombyx*[52] and *Heliothis*,[53] with more distantly related sequences found in a large range of organisms.[54]

The continued discovery of transposable elements related to current insect gene vectors in many other organisms has important implications for the use of these vectors. First, the broad commonalities of structure and function undoubtedly led to our ability to effectively harness these elements as vectors for use in a wide range of host species. These commonalities, however, now become a challenge for practical application of transgenic insects, since this also increases the risk of vector remobilization by the same or related systems that might preexist in a host species. Such remobilizations would undoubtedly negatively impact desired strain characteristics or the ability to safely release such strains into the environment.

Transformation Markers

Reliable phenotypic markers for identifying transformed insects have been critical to the development of insect transformation technology and will also be essential for monitoring transgenic insects intentionally released into the field as part of future programmatic operations. The first genes used as transformation markers complemented eye-color mutations in mutant hosts, and while these were effective, their use was limited to species for which appropriate mutant strains were available.[55] Furthermore, these eye-color complementation markers were typically sensitive to chromosomal position effects.

Chemical-based selections have not been adopted for routine production of transgenic insects although two systems have been tested.[56] The first attempts at non-drosophilid transformations used the *neomycin phosphotransferase II* gene that confers resistance to the neomycin analog, Geneticin® (or G418). This system was first developed as an effective screen in Drosophila; yet in non-drosophilids false positives were common making it highly unreliable. It is possible that for some insects, such as tephritid flies, bacterial populations in the insect gut that were already neomycin resistant and may have allowed some non-transgenic insects to survive Geneticin® treatment (H. Genc and A.M. Handler, unpublished). A similar system based on *organophosphorus dehydrogenase* (*opd*) was developed using the insecticide paroxon for selection, but it also proved unreliable and further complicated by chemical exposure and waste disposal issues.[56] Nevertheless, a visible marker in conjunction with a reliable chemical selection system that allows mass selection could greatly simplify the creation of transgenic insects, particularly in insects where existing gene vectors are highly inefficient and require many progeny to be produced before a transgenic is found.