



Transgenesis of Bioluminescence (BL) Gene and Its Potential Use for Malaria Residual Transmission Issue and Other Vector Borne Diseases: New Considerations for Vector and Vector Borne Diseases (VBD) Control

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Authors' contributions

This work was carried out in collaboration among all authors. Author GC designed the study, performed the statistical analysis wrote the protocol and wrote the first draft. Author PC managed the literature searches and finalized the writing. All authors read and approved the final manuscript.

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ABSTRACT

The present article considers the potential of transgenesis of the bioluminescent gene for malaria and other vector borne diseases (VBD) control. Vector control is an important component of every vector control operations for the vector borne disease control. Actually the bioluminescence phenomena and the green fluorescent protein GFP open great field of researches and "On December 10, 2008 Osamu Shimomura, Martin Chalfie and Roger Tsien were awarded the Nobel Prize in Chemistry for "the discovery and development of the green fluorescent protein, GFP". Bio-molecular technologies and transgenesis open the field for getting BL mosquitoes such as *Plasmodium* (several references) for "Transmission Reducing Activity" (TRA) without any hazard on human beings and ecological level. For exemple BL mosquitoes would be of paramount importance for mark-release-recapture becoming easier to implement and giving more relevant and reliable

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data on relation between density (size of the population) and distance (and wind) as the vector population size decrease with increasing distance from the source of production (breeding site) or release point. BL appeared already of great interest to understand the relation vector/parasite and to assess transmission intensity. Our idea is to genetically produce "Bioluminescent Mosquitoes" allowing a better identification of their presence, behavior, densities, infected specimens, risk of transmission before/after vector control operations which could be greatly improved thanks to the targeting of mosquitoes resting site or flight range and any other biological component.

Keywords: *Transgenesis; vector borne disease; bioluminescent mosquitoes; bio-molecular technologies.*

1. INTRODUCTION

Vector control is an important component of every vector control operations for the vector borne disease control as it was recently published by Bhatt et al. [1] in malaria. Actually they estimated that between 2000 and 2015 some 663 million of malaria clinical cases were averted, insecticide treated nets (ITNs) were by far the most contributors (68% of cases averted); 25% resulting from drugs (Artemisin Combined Therapy "ACT") and 10% from Inside Residual Spraying ("IRS"). Therefore it appeared that more than 500 million of clinical malaria cases were averted thanks to vector control measures.

But remaining transmission remaining a matter of concern specially what is called "residual malaria transmission" and several recent international meeting have underlined these issues of residual malaria transmission [2,3,4,5,6] due to the change in the behavior of vector with implementation of vector control (such as deterrent effect from houses of permethrin treated nets or irritant effect of DDT) [7] or exophagic species [8,9,10] not impacted by operations done inside houses such as *An. dirus*, or insecticide resistant populations which remains in spite of vector control operations [11,12] or vector population still living during the long dry season in Sahel areas (hidden "somewhere") and new blow up of vector and malaria, when rains are coming again, or hibernation phenomena in "temperate" areas.

As underlined by Riehle et al. [13] "after implementing existing vector control approaches...it may be necessary to develop specialized tools to detect and control persistent sources of residual transmission; reservoirs of highly efficient vectors, o vectors that evade control, can contribute unevenly and cryptically to residual transmission".

2. METHODOLOGY

Presence and evaluation of "outside" mosquito population is of great concern and raises several issues in sampling methods [14] because everyone are well known for their bias, collecting actively or passively (by traps) mosquitoes at their resting sites or while feeding on human beings or on wild or domestic animals or ate their sexual phases (swarming) etc.

One of the issue is to evaluate this "residual" or natural amount of transmission [15] due to expophilic mosquitoes starting by knowing where are these anopheles outside and if they are still present in spite of "something was done" in term of chemical use (space spray) or environmental modification or else. Recent molecular biology technics opened new field of important research such as in depth genomic studies recently devoted to the exophilic exophagic malaria vector *Anopheles dirus* [16,17,18].

Our consideration is to promote studies and implementation of transgenesis in the targeted Anopheles population of the bioluminescent gene [19,20,21] which allows the well-known light of fireflies [22,23,24,25,26] or the GPF of jellyfish [27,28,29,30,31,32,33,34,35,36,37,38] and therefore to actually study the "outdoor population", in identifying their presence with the flight of such modified bioluminescent mosquitoes such as the nocturnal flight of fireflies.

Actually the bioluminescence phenomena and the green fluorescent protein GPF open great field of researches and "On December 10, 2008 Osamu Shimomura, Martin Chalfie and Roger Tsien were awarded the Nobel Prize in Chemistry for "the discovery and development of the green fluorescent protein, GFP" [39].



Fig. 1. Bioluminescent fireflies



Fig. 2. Picture of a flight of fireflies

The obvious presence of such bioluminescent vectors underlines thus the actual presence and therefore the risk of disease transmission, the needs for special adapted vector control and an reliable evaluation of their success (no more light = no more vectors = no more transmission) or failure.

Such bioluminescence can be expressed at 3 levels:

Larva with sampling larval population allowing better identification of positive breeding sites to be treated or still positive after larval control programme; this could be of paramount epidemiological for example with *Aedes* to determine the "house index" or the Breteau index procuring information on the risk of outbreak, or by mark-release recapture to have an estimate of the size of population. for the time being larva were marked with paints (different color) or radioactive material (such as P^{32} which emits beta particles and has a half-life of about 14 days and therefore cannot be used if the duration of larval stage extend for longer time (colder months in temperate regions) and the percentage of actually radioactive larva even after immersing them in P^{32} for 48h could be low [40];



Fig. 3. Example of marked larva

Males allowing identification of place and time of swarm [41] and it is known that control of swarm is a current developed approach in some African countries. One can easily envisage introducing BL gene with Sterile Males Technique allowing easy evaluation of the actual diffusion of such sterile ♂ in the population, or the needs for new mass release specimen etc

Females allowing identification of presence, flight, flight range, resting site close to human habitations or breeding site or resting sites among vegetation during the blood digestion and it is known that females take sugar feeding for

flight; bioluminescent could improve the determination of preferred plants for their focused treatment, an approach currently implemented. As *Plasmodium falciparum* strain expressing the firefly luciferase protein were prepared it should therefore be possible to easily identified infected specimen with both BL parasite and phenotype and make a quick evaluation of malaria risk with control operations (drugs, vaccines, vector control etc.).



Fig. 4. Foreseen of a bioluminescent Anopheles (GC drawing)

Mark-release-recapture of "BL mosquitoes" will procure important information on, among other indicators, their flight range/dispersal; the actual densities of the population, it is well known that currently used methods of captures inside and mainly outside gave biased samples.

Several technics were used to "mark" adults mosquitoes such as aniline dyes, dusts and powders, paints, radionuclides which could be not ecologically sound (alpha nuclides) or health hazard (gamma rays) and beta-emitting nuclides are used (such as P^{32} used by Gillies, [42] to study the dispersion of *Anopheles gambiae*) [42] but this method need technical equipment (to "make" radioactive mosquitoes, to detect them by Geiger-Müller counters; liquid scintillation counters, autoradiography) which make their use difficult in several field situation.

Bio-molecular technologies and transgenesis open the field for getting BL mosquitoes without any hazard on human beings and ecologically sound. They make the mark-release-recapture easier to implement and to get e relevant and reliable data on the relation between anopheles density and distance between human habitations and breeding site, natural or man-made (dams, rice-culture) and therefore on the risk (increasing or decreasing) of malaria transmission.

BL could be implemented for an evaluation of the presence of infected mosquitoes (with *Plasmodium* or virus) and their evolution with measures undertaken.

The bioluminescence phenomenon was already used to assess the Malaria Transmission Reducing Activity ("TRA").

Cevenini et al. [43] considered that "Multicolor Bioluminescence Boosts Malaria Research". They prepared "a panel of six ATP-dependent luciferases derived from different bioluminescent species (including the "LitRE6" from *Luciola italica*) or obtained by rational mutagenesis (which) were selected according to their enzymatic properties and expressed for the first time in the malaria parasite" to assess the efficacy of *P. falciparum* gametocyte drug treatments. They concluded that "the same approach could be easily applied to develop new screening assays for identifying antimalarial drugs targeting different parasite stages. Besides, this methodology can be used, for instance, to simultaneously compare expression of stage-specific gene products, measure distinct cellular pathways, or evaluate the activity of an inducible or treatment-responsive promoter as compared to a constitutive internal viability control, respectively driving the expression of the two luciferases in the same parasite.

Stone & Bousema [44] described "a novel method of assessing the transmission of a *Plasmodium falciparum* strain expressing the firefly luciferase protein in the SMFA (standard membrane feeding assay). They showed that "Measuring the mean luminescence intensity of groups of individual or pooled mosquitoes provides comparable estimates of transmission reducing activity at 5-10-fold the throughput capacity of the standard microscopy based SMFA. This high efficiency protocol may be of interest to groups screening novel drug compounds, vaccine candidates, or sera from malaria exposed individuals for transmission reducing activity (TRA)".

Stone et al. [45] "Using a *Plasmodium falciparum* strain expressing the firefly luciferase protein, we present a luminescence-based approach to SMFA evaluation that eliminates the requirement for mosquito dissections in favor of a simple approach in which whole mosquitoes are homogenized and examined directly for luciferase activity. Analysis of 6860 *Anopheles stephensi* mosquitoes across 68 experimental

feeds shows that the luminescence assay was as sensitive as microscopy for infection detection. The mean luminescence intensity of individual and pooled mosquitoes accurately quantifies mean oocyst intensity and generates comparable TRA estimates". Authors considered that "this new method of assessing *Plasmodium* infection and transmission intensity could expedite the screening of novel drug compounds, vaccine candidates, and sera from malaria-exposed individuals for TRA".

Moreover, the single-cell BL imaging of the human malaria parasite *P. falciparum* opens the possibility to monitor in real-time individual luciferase-expressing parasites in their stage-specific functional interactions with host tissues and cells and to assess how distinct cell types affect viability of specific parasite stages in *in vitro* and in *ex vivo* settings".

Siciliano & Alano [46] considered that "enlightening the malaria parasite life cycle: bioluminescent *Plasmodium* in fundamental and applied research", for them "mainly the human malaria parasite *Plasmodium falciparum* and the rodent parasite *P. berghei* have been engineered to express bioluminescent reporters in almost all the developmental stages of the parasite along its complex life cycle between the insect and the vertebrate hosts. *Plasmodium* lines expressing conventional and improved luciferase reporters are now gaining a central role to develop cell based assays in the much needed search of new antimalarial drugs and to open innovative approaches for both fundamental and applied research in malaria".

Miller et al. [47] reported that "bioluminescent parasites have previously been shown to be an effective and non-invasive alternative to monitoring liver stage burden"; they reported "the generation and characterization of a transgenic *P. yoelii* parasite expressing the reporter protein luciferase throughout the parasite life cycle. In vivo bioluminescent imaging of these parasites allows for quantitative analysis of *P. yoelii* liver stage burden and parasite development"; "Thus, this rapid, simple and noninvasive method for monitoring *P. yoelii* infection in the liver provides an efficient system to screen and evaluate the effects of anti-malarial interventions *in vivo* and in real-time".

Flores-Garcia et al. [48] used a "genetically engineered strain of *Plasmodium berghei* that expresses luciferase, GFP and the *Plasmodium*

falciparum orthologue of CSP, the effect of laboratory preparation, mosquito treatment and mouse factors on sporozoite infectivity was assessed using an in vivo bioluminescence assay on mice” they found that “Bioluminescence assay demonstrated similar detection levels of the quantity and kinetics of liver-stage infection, compared to PCR-based detection” and considered that “the PbGFP-Luc line and in vivo bioluminescence imaging provide highly sensitive read-outs of liver-stage infection in mice, and this method can be useful to reliably evaluate potency of pre-erythrocytic interventions”.

Azevedo et al. [49] described “a methodology that simplifies the *in vitro* screening of much-needed transmission-blocking (TB) compounds employing a bioluminescence-based method to monitor the *in vitro* development of sporogonic stages” and the most active compounds against the parasite’s sporogonic stages and TB compound screening.

Annoura et al. [50] described “simple and sensitive in vitro and in vivo assays to analyze *Plasmodium* liver stage development using transgenic *P. berghei* parasites (PbGFP-Luccon), which express the bioluminescent reporter protein, luciferase” and parasite development in hepatocytes is thus visualized and quantified by real time bioluminescence imaging both in culture and in live mice”.

Ploemen et al. [51] did some “evaluation of immunity against malaria using luciferase-expressing *Plasmodium berghei* parasites” using “a transgenic *Plasmodium berghei* parasite, PbGFP-Luccon, expressing the bioluminescent reporter luciferase” which appeared as “a straightforward and valuable tool for comprehension of the biological and immunological principles underlying protection against malaria”.

Matsuoka et al. [52] made an important breakthrough in producing “transgenic rodent malaria parasite (*Plasmodium berghei*) that contained the luciferase gene These transgenic (TG) parasites expressed luciferase in all stages of their life cycle, allowing observation of sporozoites in skin following their deposition by the probing infective mosquitoes. “Our transgenic parasites may have emitted stronger bioluminescence than previous TG parasites. Since luciferase activity diminished immediately

after the death of the parasites, luciferase activity could be an indicator of the existence of live parasites. Our results indicated that sporozoites survived at the probed site for more than 42 hours. We also detected sporozoites in the liver within 15 min of the intravenous injection. Bioluminescence was not observed in the lung, kidney or spleen. We confirmed the observation that the liver was the first organ in which malaria parasites entered and increased in number”.

Rocha et al. [53] considered that “paratransgenesis” could be a promising alternative for controlling malaria transmission by *Anopheles darlingi* in the Amazon region. “Paratransgenesis aims to inhibit the development of parasites within the vector through the action of genetically modified bacteria”. Symbiotic bacteria were isolated and transformed with a GFP expressing plasmid then reintroduced in mosquitoes by feeding. And it appeared that “their survival and persistence in the next generation was assessed by the isolation of fluorescent bacteria from eggs, larvae, pupae and adult homogenates”.

As well underlined by Irvin et al. [54] “Advances in insect biotechnology, in particular the development of genetic transformation methods, have led to renewed interest in genetic-based insect control strategies” and “*Anopheles gambiae*, the major vector of human malaria in Africa, is one high profile target of this new form of genetic insect control”. With their project, it is envisioned that the mosquito’s susceptibility to malaria parasites would be genetically altered; insects possessing this new genotype would be introduced into native susceptible mosquito populations in such a way as to lead to the replacement of endogenous susceptible mosquito populations, thereby reducing malaria transmission” but one of the key point should be to “recognized these genetically modified *Plasmodium* resistant specimen” among native susceptible one and get reliable data on the success of actions implemented (how fast a genotype must be disseminated among natural population) or the needs for more specimens. Actually Irvin et al (loc.cit.) underlined that “Other vector-borne diseases may also be targets of this form of genetic control. To implement these ideas, effective methods are needed to introduce novel genes into insect genomes, and transgenes must be identified that can lead to the elimination of pathogen transmission”.

3. RESULTS AND DISCUSSION

An important genetic and epidemiological breakthrough was recently done by Riehle et al. [12] who noticed that “the 2La inversion in the chromosome of *An.gambiae* populations of Burkina Faso, Guinea and Kenya is significantly associated with differential malaria infection. In an analysis of oocyst infection prevalence, *A.gambiae* complex mosquitoes with the homozygous 2L+/2L+ inversion genotype were significantly more likely to carry midgut oocysts than the 2La/2La homozygotes”. This is very interesting as it is well-known that inversions are physical rearrangements of a chromosome segment that suppress recombination and limit exchange and variation between two alternate suite of alleles, in other words a mutation inside a loop of inversion is maintained in further generation.

One can therefore envisage to insert the BL gene inside the 2La/2La inversion allowing the quick and easy direct “eye” phenotypical identification (“visualization”) of “infected BL Anopheles” among the wild population instead of the time consuming dissections and microscopical examination of polytene chromosomes of ovaries or dissection of salivary glands or Elisa tests checking CSP protein of sporozoites to evaluate the risk of transmission and the impact of malaria control measures. Inserted BL in 2La inversion being maintained in next generation thanks to the properties of chromosome inversion and could be a great progress for malaria control and elimination.

3.1 Bioluminescence in Fireflies. Some Background Data

There are more than 2000 species of fireflies, almost all of which produce light. This light comes from the abdomen of fireflies, which is lined with photophores. These photophores contain photocytes arranged in a ring around the trachea. Each photocyte consists of small organelles called peroxisomes, where the chemical reaction that produces light emission occurs.

This emission is controlled indirectly by the nervous system. Indeed, the brain sends a nerve signal to the muscles of the abdomen. The latter contract and expel reagents from the reaction on the surface of the epidermis of the firefly. The chemical reaction takes place and the light appears.

This light is then intensified by the epidermis of the firefly lantern. The serrations present on the cuticle of the abdomen of the fireflies allow indeed a better extraction of the light.

Bioluminescence produces a light called cold light. Indeed, almost all of the energy released is light energy. The amount of thermal energy produced is negligible.

3.2 Chemical Reaction

Two molecules are essential for this reaction: The luciferin substrate protein and the luciferase enzyme which bind to form a complex. Then in the presence of oxygen, this complex oxidizes and becomes unstable and excited. Finally, the complex becomes stable again and at the same time, releases carbon dioxide and energy in the form of a photon, that is, light energy.

The cycle of the reaction can continue because luciferase is present again and luciferin is synthesized by the animal body.

Fireflies emit on average a light of wavelength equal to 560 nanometers (nm), which corresponds to a light of color green / yellow but fireflies do not shine only a yellow or green color. The light of the bioluminescence of fireflies covers the entire domain of the visible spectrum.

According to species there are several variables of the reaction allowing the bioluminescence. This comes from the complexity of oxyluciferase (the essential element of the reaction) which exists in 6 different forms, varying with respect to the chemical environment.

Researchers succeeded in synthesizing and analyzing the 6 forms of oxyluciferase. Then they built a graph which compares their wavelength as well as their hydrogen potential (pH: Basicity or acidity) and gave the precise wavelengths of the emission as a function of each oxyluciferase used.

The gene coding for luciferase has been identified and even transplanted, a technic used in human cancer imaging. Indeed, the bioluminescence reaction generates a release of photons, which can be captured by sophisticated medical cameras sensitive to the presence of these photons through the material. To carry out this bioluminescence reaction and locate it at the level of the cancer cells or the found treatments, the researchers inject the living being studied

with a dose of luciferin then use a *gene coding for the luciferase* of which they affect the cancerous cells.

The luciferin-luciferase oxidation reaction which causes light emission thus allows the localization of the cells where the coding gene for luciferase has been transplanted.

One can then imagine anopheles with this bioluminescence which makes them visible outside during the night "explaining" the residual transmission and its maintenance, and its reduction, with the implementation of adapted vector control operations.

Transgenesis of encoding gene for bioluminescence such as GFP has already been done (see below) and gene coding for luciferase has already been identified owing the possibilities of developing such bioluminescent mosquitoes and better understanding field situation of vector borne diseases, therefore better targeting vector control without spraying useless and not ecologically friendly lot of insecticide outside.

3.3 Other Outcome

Transgenesis of bioluminescent gene can also be applied to *Aedes* in the framework of arbovirus control showing the presence of vectors, allowing the identification of their main nocturnal resting site and therefore better targeting focused control operations with their evaluation.

It should be possible to used genes coding for different wavelength transplanted in eggs of *Aedes aegypti* and *Aedes albopictus* to better recognize the presence of each species, their preferred (or common) breeding sites etc.

The field of use of this new approach of bioluminescent mosquitoes is "no limit". They can be used to improve vector control in some situations such as Martinique or Polynesia Islands or La Reunion island and several continental countries where dengue (and other arbovirus such as Zika, Chikungunya) outbreak occurred or are currently occurring and where ecological situation make difficult vector control such as space sprays not readily accepted by communities. The bioluminescence technic has already been used in studies of *Aedes aegypti* [55].



Fig. 5. Foreseen of a bioluminescent *Aedes albopictus* (GC drawing)

A great lot of genetical researches has been done for long time on *Aedes aegypti* with some particular strains (pink eyes, vestigial wings etc) and it could be relatively "easy" to prepare such BL specimens.

On the other hand Lu et al. [55] developed a "calcium bioluminescence assay for functional analysis of mosquito (*Aedes aegypti*) and *Tciks (Rhipicephalus microplus)* G Protein-coupled Receptors" with the protoprotein isolated from luminescent jellyfish to measure intracellular bioluminescence and determine calcium level (emitting luminescence) as tools for pest control.

As it was well underlined by Adelman et al. [56] "transgenesis technology has been developed for the yellow fever mosquito, *Aedes aegypti*. A number of marker genes, including the cinnabar(+) gene of *Drosophila melanogaster* and fluorescent protein genes, can be used to monitor the insertion of transposable elements. "The availability of multiple elements and marker genes provides a powerful set of tools to investigate basic biological properties of this vector insect, as well as the materials for developing novel, genetics-based, control strategies for the transmission of disease".

McGee et al. [57] studied the "infection, dissemination and transmission of a West Nile Virus Green Fluorescent Protein (GFP) Infectious Clone by *Culex pipiens quinquefasciatus* mosquitoes" and "focal expression of GFP was observed 3 days post-infection, with the majority of posterior midgut epithelial cells being positive by 7 days post -infections; GFP foci were observed in salivary glands 14 days post-infections. It was therefore possible to "see" the GFP virus inside the vector and get some

information on the dissemination of the West Nile Virus (WNV) with *Cx.p. quinquefasciatus*.

This could also be developed for *Culex tritaeniorhynchus* control according to Encephalitis Japanese risks of outbreak in SE Asian countries in spite of the already available vaccine.

3.4 Other Bioluminescence: The Green Fluorescent Protein: The GFP Gene in the Jellyfish

3.4.1 Chemical reaction of bioluminescence

In the jellyfish *Aequorea victoria*, there is no enzymatic reaction and oxygen is not necessary for Bioluminescence.

Indeed the jellyfish *A. victoria* is endowed with a photoprotein; Aequorin, derived from the crown of the jellyfish, which is composed of an apoprotein, coelenterazine, and a luciferin.

Aequorin to emit light needs to react with a calcium ion, which plays the role of luciferase.

The photon that it then emits has a wavelength of between 460 and 520 nm (in the blue domain) but with the presence of the green fluorescent protein, which is more commonly called GFP, this emission of light takes a Green wavelength when the jellyfish is illuminated by an ultraviolet lamp.

3.5 Green Fluorescent Protein: GFP

During the 1960s, Osamu Shimomura, Martin Chalfie and Roger Y. Tsien succeeded in isolating the GFP protein, which is the cause of

the green / blue bioluminescence of the jellyfish, during their research on jellyfish. A. discovery earned them the Nobel Prize in Chemistry in 2008.

GFP protein consists of 238 amino acids. Its internal structure (see three-dimensional structure) comprises a chromophore composed of three amino acids * 65 (glycine), 66 (tyrosine) and 67 (serine). Knowing that a chromophore is a group of atoms having one or more double bonds and forming with the rest of the molecule a double conjugated double bond sequence, it can create a delocalized electric cloud. It is a chemical group that absorbs or emits light.

3.6 The Use of the GFP Protein in Transgenesis

More than 5000 scientific articles dealing with the Green fluorescent protein were recently published [58,59,60,61,62] with detailed information on "structure and function of the GFP polypeptide, the mechanism of fluorescence emission, excited state protein transfer, the design of ratiometric fluorescent protein biosensors and an overview of the fluorescent proteins".

The bioluminescence in the jellyfish *Aequorea victoria* and more precisely the GFP protein, has allowed scientists to make some scientific advances that have become indispensable today thanks to transgenesis.

For research in biology, the goal is to express the GFP protein to certain cells in the body of a rat or mice for example [63]. Thus, if the GFP gene is inserted in a suitable place, it can confer green color to neurons, cancer cells, or even the entire

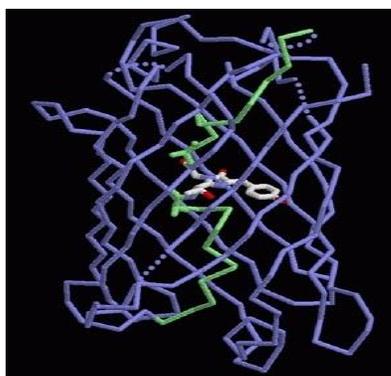


Fig. 6. Three-dimensional structure of GFP

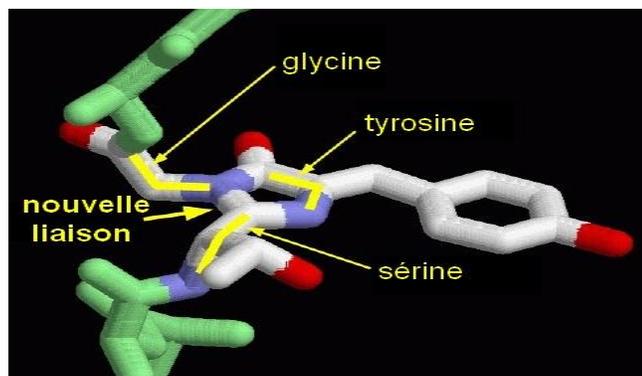


Fig. 7. Zoom on the chromophore of GFP

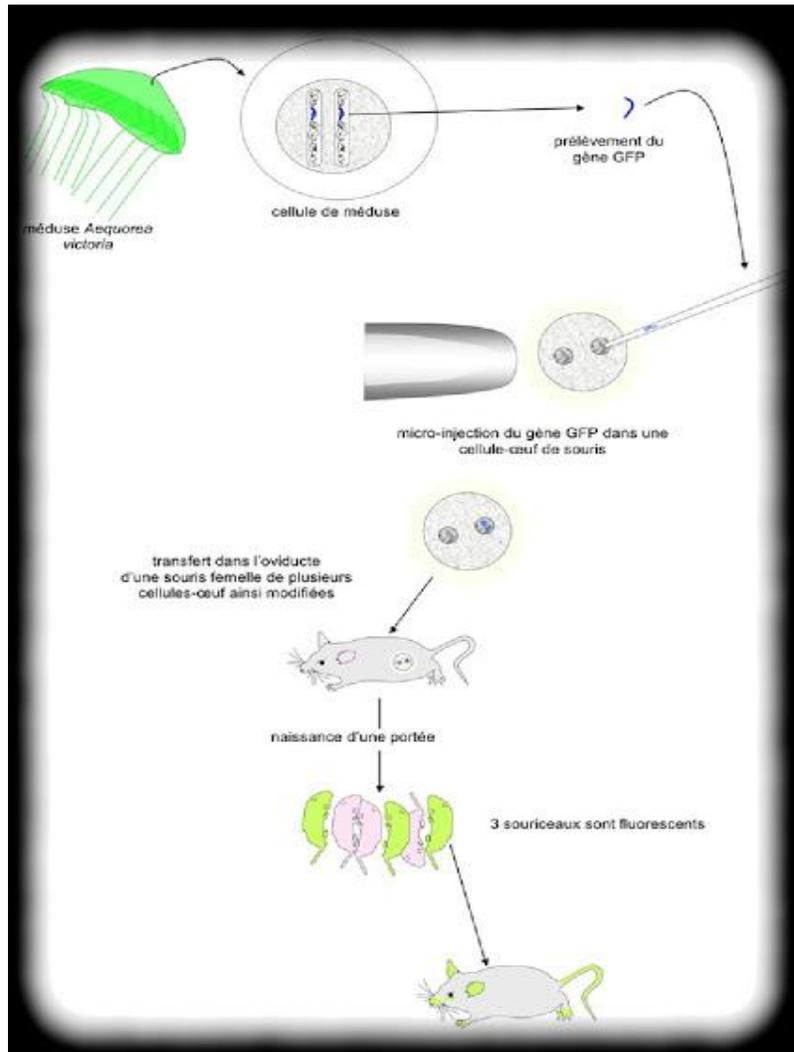


Fig. 8. The use of the GFP protein in transgenesis

pigmentation of the animal. This will help to understand how an organ or tissue develops, to analyze different parts of the genome, to follow the cellular locations of certain proteins, the axonal guidance of neurons or tissue transplants.

Before the discovery of the GFP protein, it was very difficult for scientists to study the production and development of a cell's proteins in a living organism.

This method consists of taking the GFP gene (using restriction enzymes) from the equator *Aequorea victoria*, then injecting it into a mouse egg cell and finally transferring it into the oviduct of the female mouse.

In order to achieve the fluorescent character of an organism, the DNA of a cell must be modified. For this purpose, the GFP gene is associated with another gene coding for another protein by means of plasmids (natural or artificially modified circular bacterial DNA molecule). Thus the GFP protein will be produced normally in the cell. It will be attached to the desired protein and we can follow its development.

Transgenesis in the medical field: Transgenesis also has many applications in the field of health. The GFP protein makes it possible in particular to understand how an organ or a tissue develops but also to follow the displacement of the desired protein.

4. CONCLUSION

A great lot of studies were devoted to “genetically modified mosquitoes” aiming at producing Sterile Males or *Plasmodium* resistant etc. But curiously the key point of improving the knowledge of their presence, mainly outside house where malaria transmission is still occurring, did not received the obviously needed attention. The same concern is devoted to other arbovirus with deadly outbreak of dengue, DHF, Zika etc where no vaccine is still available and vectors are exophilic, exophagic and breeding in both natural and anthropic sites.

It appeared that several insects and animals have developed some possibilities to produce some light with the phenomena of bioluminescence with different methods. The gene involved in these phenomena was already identified and transgenesis is already done, even for human health (cancer).

Our idea is to genetically produce such “Bioluminescent Mosquitoes” allowing a better identification of their presence, behavior, densities, risk of transmission before/after vector control operations which could be greatly improved thanks to the targeting of mosquitoes resting site or flight range and any other biological component.

The availability of multiple technics and marker genes provide a powerful set of new tools to investigate biological properties and infectivity of vectors as well as foreseeing new genetic based control strategies of vector borne diseases overcoming the current development of insecticide and drug resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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