Control of Vector Borne Diseases Through Microbial Paratransgenesis

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Abstract: **Advances in vector genomics offer new promise for the control of arthropod vectors of disease. The diseases that are transmitted by arthropods cause severe morbidity and mortality throughout the world. The burden of many of these diseases is borne largely by developing countries. Radical changes in vector-biology research are required if scientists are to exploit genomic data and implement changes in public health. Use of chemicals to control vector borne diseases often exert influence associated with environmental toxicity and affects adversely on human health. Moreover, this method leads to potentiate the vector for resistance. The strategy for the control of vector borne diseases should be paratransgenic. Symbiotic or commensal microbial pathogens should transformed to express products of gene that interfere with the transmission of pathogen. That is to say the genetically modified microbes are to be re-introduced back to the insect (vector). This method definitely, decrease the ability of the vector to transmit the pathogen into the human body. The paratransgenic method if utilized efficiently, it may reduce rate of for example Trypanosoma cruzi, the causative agent of Chagas disease, the triatomine bug, Rhodnius prolixus, and Leishmania donovani by the sand fly Phlebotomus argentipes. There are many effector biomolecules (example: antimicrobial peptides and specific single chain antibodies), currently being explored for the activities of "anti-parasite" in the systems to control vector borne diseases. This method deserve environmental protection and exert positive influence on the health of human being. However, the field application of laboratory-based evidence of paratransgenesis imposes the use of more realistic confined semi-field environments.**

Keywords: **Paratransgenesis, Chagas disease, triatomine bugs, visceral leishmaniasis, sand flies, microbiology, risk assessment, horizontal gene transfer**

I. INTRODUCTION

The emergence of insecticide resistance in many disease vectors highlights the necessity to develop new strategies to control these insects. Insect-borne diseases cause significant human morbidity and mortality. Insect-borne diseases are responsible for severely affecting human life around the world, causing significant morbidity and mortality. Malaria alone is responsible for 1-2 million deaths annually, and approximately 300 million are at risk of becoming infected. The lack of effective vaccines to control the spread of a significant number of vector-borne diseases makes control mainly dependent on insecticides. However, the appearance of insecticide resistance requires the development of new strategies to reduce pathogen transmission in the field (Hill, *et al*, 2005). Current control and preventive methods against vector-borne diseases rely mainly on insecticides. Novel approaches against vector borne diseases include transgenesis and paratransgenesis to reduce vector competence. Vector transgenesis and paratransgenesis are novel strategies that aim at reducing insect vectorial capacity, or seek to eliminate transmission of pathogens such as *Plasmodium sp.*, *Trypanosoma sp.*, and Dengue virus currently being developed. Vector transgenesis relies on direct genetic manipulation of disease vectors making them incapable of functioning as vectors of a given pathogen. Paratransgenesis focuses on utilizing genetically modified insect symbionts to express molecules within the vector that are deleterious to pathogens they transmit. Despite the many successes achieved in developing such techniques in the last several years, many significant barriers remain and need to be overcome prior to any of these approaches become a reality. In the process of transgenesis, exogenous gene (may be called as transgene) is introduced into a living organism. This get results into change in the genetic makeup of organism and it will exhibit a new property and transmit that property to its offspring. The process of transgenesis can be facilitated by liposomes, enzymes, plasmid vectors, viral vectors, pronuclear injection, protoplast fusion, and ballistic DNA injection. Transgenesis can occur in nature (DiCarlo, *et al*, 2013). This transgenic organism achieve the genetic code and it is able to express the foreigin genes. Paratransgenesis is the process that attempts to eliminate a pathogen from vector populations through the transgenesis of a symbiont of the vector. The goal of this technique is to control vector-borne diseases. The very first step in paratrangenesis is to identify proteins that prevent the vector species from transmitting the pathogen. Then, genes that are coding for these proteins are introduced into the symbiont. This enable the vector to achieve the expected protein sequence. And the last step in the

paratransgenic approach is to introduce these transgenic symbionts into vector populations in the wild. This strategy is as good as establishment of Trojan Horse. The Trojan horse appears harmless, but is, in fact, malicious. In the paratransgenic strategy, the native microbial flora in the body of vectors are isolated and used for *in vitro* genetic transformation.The first example of this technique used *Rhodnius prolixus* which is associated with the symbiont *Rhodococcus rhodnii*. *R. prolixus* is an important insect vector of Chagas disease that is caused by *Trypanosoma cruzi*. The strategy was to engineer *R. rhodnii* to express proteins such as Cecropin A that are toxic to *T. cruzi* or that block the transmission of *T. cruzi* (Durvasula, *et al* , 1997). Attempts are also made in Tse-tse flies using bacteria (Aksoy, *et al* , 2008 and De Vooght, *et al* , 2012) and in malaria mosquitoes using fungi (Fang, *et al* , 2011), viruses (Ren, *et al* , 2008), and bacteria (Rodrigues, *et al* , 2008). For the successful control of vector borne diseases, through the paratransgenesis, there need to handle the matter carefully. The Symbiotic bacteria can be grown *in vitro* easily. They can be genetically modified, such as through transformation with a plasmid containing the desired gene. The engineered symbiont is stable and safe. The association between vector and symbiont cannot be attenuated. Field delivery should be easily handled.

II. ENDOSYMBIONTS FOR THE VECTOR CONTROL :

The endosymbiont or endobiont is any organism that lives within the body or cells of another organism, i.e. forming an endosymbiosis. Examples are nitrogen-fixing bacteria (called rhizobia), which live in root nodules on legume roots, single-cell algae inside reef-building corals, and bacterial endosymbionts that provide essential nutrients to about 10–15% of insects (Margulis and Chapman,2009). Many instances of endosymbiosis are obligate; that is, either the endosymbiont or the host cannot survive without the other, such as the gutless marine worms of the genus *Riftia*, which get nutrition from their endosymbiotic bacteria. The most common examples of obligate endosymbioses are mitochondria and chloroplasts. Some human parasites, e.g. *Wuchereria bancrofti* and *Mansonella perstans*, thrive in their intermediate insect hosts because of an obligate endosymbiosis with *Wolbachia spp.* They can both be eliminated from said hosts by treatments that target this bacterium. However, not all endosymbioses are obligate. Also, some endosymbioses can be harmful to either of the organisms involved. Two major types of organelle in eukaryotic cells, mitochondria and plastids such as chloroplasts, originated as bacterial endosymbionts. Such symbiogenesis was first suggested in 1905, and articulated by the Russian botanist Konstantin Mereschkowski in 1910. It was reintroduced by Lynn Margulis in the 1960s.

The young ones of tsetse flies get some of the symbiontic organisms the secretion of milk glands during their intrauterine life (Aksoy and Hypsa, 1997). There are number of symbionts in the various arthropods are transmitted to their young ones by various ways and means. The significantly present *Wolbachia* organisms of *Aedes* and *Culex spp.* mosquitoes are transmitted by vertical manner via gametes to their next generations (Townson H., 2002). The young ones of tsetse flies get some of the symbiontic organisms the secretion of milk glands during their intrauterine life (Aksoy and Hypsa, 1997). The *Rhodococcus* is a genus of aerobic, nonsporulating, nonmotile Gram-positive bacteria closely related to *Mycobacterium* and *Corynebacterium* (van der Geize & Dijkhuizen, 2004 and Burkovski, 2008). While a few species are pathogenic, most are benign, and have been found to thrive in a broad range of environments, including soil, water, and eukaryotic cells. Fully sequenced in October 2006, the genome is known to be 9.7 megabasepairs long and 67% G/C (McLeod, *et al*, 2006). The triatomine bug (*Rhodinus prolixus*) is the vector for Chaga's disease. The mid gut of this triatomine bug is with the *Rhodococcus rhodhini,* symbionts. These symbionts get transmitted from adult to the progenies through coprophagy (Beard, *et al* , 1998). Understanding of the biology of the transmission of symbionts from the adult vectors to progenies is imperative for the symbiont based control approach to be effectively used in the field conditions. Perturbation of the interaction between endosymbiont, vector and parasite is the ultimate idea of the symbiont based approach to control VVBDs. Arthropod vectors benefit from the symbiosis and augment their functional capabilities to facilitate their expansion in to novel niches. The best examble is *Wigglesworthia glossinidia* an intracellular endosymbiont of *Glossina* fly which synthesises plethora of vitamin biosynthetic products that may promote host reproduction. *Wolbachia* organism's biosynthesis of riboflavin and FAD, are essential for the *Brugia malayi* coenzymes and cytochromes. The presence of symbionts and the pathogenic organisms together in the vector and the possible role of the interaction between the two in favour of disease transmission had been reported Amongst the bacterial gut flora the *S. marcescens* chitinase has the ability to digest the peritrophic membrane of mosquito (Huber, *et al* , 1991). *Plasmodium* ookinetes produce chitinase to invade the midgut epithelium of mosquito. The competence of the vector to transmit a parasite/pathogen requires a molecule(s) within the vector that interacts with the parasite/pathogen. Proteolytic lectin present in the midgut of tsetse fly stated to have trypanosome transforming activity (Amin, *et al* , 2006). Through the Creation of paratransgenic tsetse fly with the phenotype to check the activity of proteolytic lectin of the fly would limit the transmission of African trypanosmiosis.

III. BACTERIAL ENDOSYMBIONTS IN INSECTS:

As with endosymbiosis in other insects, the symbiosis is obligate in that neither the bacteria nor the insect is viable without the other. Scientists classify insect endosymbionts in two broad categories, 'Primary' and 'Secondary'. Primary endosymbionts (sometimes referred to as P-endosymbionts) have been associated with their insect hosts for many millions of years (from 10 to several hundred million years in some cases). They form obligate associations (see below), and display cospeciation with their insect hosts. Secondary endosymbionts exhibit a more recently developed association, are sometimes horizontally transferred between hosts, live in the hemolymph of the insects (not specialized bacteriocytes, see below), and are not obligate. Among primary endosymbionts of insects, the best-studied are the pea aphid (*Acyrthosiphon pisum*) and its endosymbiont *Buchnera sp.* APS (Douglas, 1998), the tsetse fly *Glossina morsitans morsitans* and its endosymbiont *Wigglesworthia glossinidia brevipalpis* and the endosymbiotic protists in lower termites. As with endosymbiosis in other insects, the symbiosis is obligate in that neither the bacteria nor the insect is viable without the other. Scientists have been unable to cultivate the bacteria in lab conditions outside of the insect. With special nutritionally-enhanced diets, the insects can survive, but are unhealthy, and at best survive only a few generations. In some insect groups, these endosymbionts live in specialized insect cells called bacteriocytes (also called *mycetocytes*), and are maternally-transmitted, i.e. the mother transmits her endosymbionts to her offspring. In some cases, the bacteria are transmitted in the egg, as in *Buchnera*; in others like *Wigglesworthia*, they are transmitted via milk to the developing insect embryo. In termites, the endosymbionts reside within the hindguts and are transmitted through trophallaxis among colony members. The primary endosymbionts are thought to help the host either by providing nutrients that the host cannot obtain itself or by metabolizing insect waste products into safer forms. For example, the putative primary role of *Buchnera* is to synthesize essential amino acids that the aphid cannot acquire from its natural diet of plant sap. Likewise, the primary role of *Wigglesworthia*, it is presumed, is to synthesize vitamins that the tsetse fly does not get from the blood that it eats. In lower termites, the endosymbiotic protists play a major role in the digestion of lignocellulosic materials that constitute a bulk of the termites' diet. Bacteria benefit from the reduced exposure to predators and competition from other bacterial species, the ample supply of nutrients and relative environmental stability inside the host. Genome sequencing reveals that obligate bacterial endosymbionts of insects have among the smallest of known bacterial genomes and have lost many genes that are commonly found in closely related bacteria. Several theories have been put forth to explain the loss of genes. It is presumed that some of these genes are not needed in the environment of the host insect cell. A complementary theory suggests that the relatively small numbers of bacteria inside each insect decrease the efficiency of natural selection in 'purging' deleterious mutations and small mutations from the population, resulting in a loss of genes over many millions of years. Research in which a parallel phylogeny of bacteria and insects was inferred supports the belief that the primary endosymbionts are transferred only vertically (i.e., from the mother), and not horizontally (i.e., by escaping the host and entering a new host). Attacking obligate bacterial endosymbionts may present a way to control their insect hosts, many of which pests or carriers of human disease. For example, aphids are crop pests and the tsetse fly carries the organism *Trypanosoma brucei* that causes African sleeping sickness. Other motivations for their study is to understand symbiosis, and to understand how bacteria with severely depleted genomes are able to survive, thus improving our knowledge of genetics and molecular biology.Less is known about secondary endosymbionts. The pea aphid (*Acyrthosiphon pisum*) is known to contain at least three secondary endosymbionts, *Hamiltonella defensa*, *Regiella insecticola*, and *Serratia symbiotica*. *H. defensa* aids in defending the insect from parasitoids. *Sodalis glossinidius* is a secondary endosymbiont of tsetse flies that lives inter- and intracellularly in various host tissues, including the midgut and hemolymph. Phylogenetic studies have not indicated a correlation between evolution of *Sodalis* and tsetse (Aksoy, *et al* ,1995). Unlike tsetse's P-symbiont *Wigglesworthia*, though, *Sodalis* has been cultured *in vitro* (Welburn, *et al*, 1987).

IV. STRATEGIES OF CONTROLLING VECTOR BORNE DISEASES THROUGH THE MICROBIALS

The endosymbionts of arthropod vectors can be targeted and or manipulated in different ways to control the VVBDs of man and animals. The attempt of vector control can be achieved in three different ways by

utilizing *Wolbachia* by Chemotherapeutic, Immuno-logical and *Wolbachia* cytoplasmic incompatibility (CI) based approach. The Chemotherapeutic approach exploits the endosymbionts of arthropods vectors as a chemotherapeutic target with the aim to disturb the symbiosis Sterility was observed in healthy tsetse flies fed with tetracycline (2500µg/ml) due to damage to the mycetome bacterial endosymbionts (Nogge, 1076). Antibiotic (Tetracycline group) against the endosymbionts of nematode parasites when administered to the mammalian host disrupt the symbiosis and lead to growth retardation and/or infertility of the parasite. The immunization of animals with the whole killed endosymbionts or purified antigens or recombinant antigens of the endosymbionts would render them immune to tick vectors. The concealed antigens or the mid gut antigens of the blood feeding arthropods like ticks, glossina, mosquitoes are the potential vaccine targets being exploited by the researchers for the control of vectors (Willadsen, 1995). Instead of targeting the host (vector) antigens, the endosymbionts could be targeted to disturb the symbiotic relationship between the vector and the symbiont. Following ingestion of the blood from immunized animals, these antibodies together with other components of the immune system such as complement, will destroy the symbionts inside the vector, leading either to death or to disruption of normal gut physiology of the tick and reduce growth and egg-laying ability. In Wolbachia cytoplasmic incompatibility (CI) based approach, Wolbachia infections in arthropods can manipulate reproduction of their hosts in a variety of ways e.g., induced parthenogenesis, male killing, parthenogenesis, and cytoplasmic incompatibility (Cl) (Huber, *et al*, 1991). The Wolbachia of mosquito species is the extensively studied symbiont of mosquitoes and the same is the widely explored Wolbachia of arthropod vectors. cytoplasmic incompatibility phenomenon of the Wolbachia of mosquito species is presently being explored as a means to control the mosquito population. The Wolbachia cytoplasmic incompatibility (CI) is a phenomenon in which mating between Wolbachia infected male insect and female insect of the same species without Wolbachia infection (Unidirectional CI) and mating between insects of the same species with different Wolbachia strain infection (Bidirectional CI), result in embryonic mortality (Bourtzis, 1998). The reciprocal mating (infected female x uninfected male) and mating between infected individuals are fully compatible. Although the mechanism of CI has not been elucidated in the molecular level, presently it is explained with two terminologies, modification and rescue. Modification is the process in which the Wolbachia modifies the sperm of the infected male during spermatogenesis by an unknown process. The modified mature sperm is devoid of Wolbachia. If a modified sperm enters an incompatible egg (uninfected or infected with different strain) a delay in break down of nuclear membrane of pronuclei of sperm resulting in mitotic asynchrony (Tram and Sullivan, 2002). Amongst the incompatible crosses, bidirectional CI could be exploited to control the mosquito population which in turn limits the transmission of mosquito borne diseases. The genetic transformation, of commensal or symbiotic bacteria of the arthropod vector is to alter the vector's ability to transmit pathogen. It is an alternative means of blocking the transmission of VBDs. The mid gut bacteria of arthropod vectors can be engineered to express and secrete effector proteins which block the parasite invasion or kill the parasite in the mid gut or hemolymph or reproductive tract. The arthropod vectors that harbor the genetically transformed endosymbionts are called as paratransgenic vector. For this strategy to be used in vector borne disease control, bacteria that survive inside the vector's body have to be identified. The endosymbionts of arthropod vectors can be cultured and genetically transformed to express the effector gene inside the vector in such a way the gene product kills the parasite/pathogen that the vector transmits. Isolation and characterization of the endosymbionts present in midgut, hemolymph and reproductive tracts is the very first step towards the paratransgenic apparoach. In this strategy, the normal symbiont population of the arthropod vector can be replaced with genetically modified symbionts, resulting in population of arthropod vectors refractory to the particular vector borne parasite/pathogen. This strategy has shown promise in controlling the transmission of *Trypanosoma cruzi* by *Rhodnius prolixus.* The genetically transformed *Rhodococcus rhodnii* was delivered into the asymbiotic first instar nymph orally in such a manner to express an antimicrobial peptide L-cecropin A, inside the gut lumen which conferred resistance status to the paratransgenics (Durvasula, *et al* , 1997). The wolbachia might be used to prevent the transovarian transmission of arboviruses viz., dengue virus, La crosse virus, rift valley fever virus. With the paratransgenic approach, African trypanosomosis can be controlled using the tsetse symbiont. Here the genes are not inserted to tsetse chromosome, but instead to the tsetse symbiont, Sodalis. The sodalis is well suited for paratransgenesis. This is because, it resides in the gut in close proximity to pathogenic trypanosomes; a system for

culturing Sodalis in vitro has been developed (Welburn, *et al* , 1987) and can be used in conjunction with standard molecular biology techniques to insert and express foreign genes of interest in this bacterium (Beard, 1993) ; Sodalis is highly resistant to many trypanocidal peptides, (Hao, *et al* , 2001; Hu and Aksoy, 2005); recombinant Sodalis (recSodalis) can be reintroduced into tsetse by thoracic microinjection and passed on to future progeny where they successfully express the marker gene product. Lastly, its genome is completely sequenced and annotated, and this information will serve as a valuable resource that can be exploited to improve the efficiency of this expression system. Because these commensal bacteria live naturally in close proximity to where trypanosomes develop and replicate in the tsetse midgut, expression of trypanocidal products in Sodalis has the potential to block parasite development in the fly. Though this technology is useful in many aspects, it has some demerits viz., socio-ethical issues, fitness of the genetically modified (GM) symbiont, alteration of ecosystem, horizontal gene transfer among compatible bacteria, transfer of GM symbiont to non-target arthropods and subsequently getting into the food chain, it can not be kept aside of our list of arsenals to fight against VBDs. The endosymbionts of arthropod are having small genome and/or more psuedogenes which indicates the long term co-evolution with loss of considerable size of their genome and functional genes by conserving the genes essential to adapt to the arthropod microenvironment. This genome erosion and adaptive degeneration phenomenon questions the stability of the transgene/ effector gene in long run. The advancements in the biotechnology will find safer solutions to the aforementioned problems in near future.

V. CONCLUSION

Whether transformed symbionts, can replace non-transformed in natural insect populations, can't be said with certainty, despite of the early success with the transformation of insect vector symbionts. Symbionts perhaps have no fitness load on insect hosts and are capable of being transmitted via transovarian transmission or lateral transmission. Thus, a strong gene drive system can potentiate the effectiveness of paratransgenesis. An example of such gene drive system can be *Wolbachia* Endosymbionts . Although there has been a great advances in the development of stable lines of genetically modified disease vectors (Perera, et al , 2002 ; Ito , 2002 and Lobo, 2002). There are many challenges in to the application of transgenesis to control vector-borne diseases outside the laboratory. Future studies mimicking field conditions likely will uncover the importance of fitness to the establishment of transgenic mosquitoes in natural habitats. India which suffers lot due to dengue, chikungunya, malaria and filariosis need a programme on *Wolbachia* CI and paratransgenic based approach to encounter afore-mentioned VBDs. The main constraints to the establishment of an efficient transgenic vector approach are the scarcity of a transgenes that effectively reduce pathogen load. Again further studies utilizing QTL mapping, reverse genetics, gene knockdown, or other techniques to identify traits associated with vector competence can reveal candidate genes that, when targeted, may effectively block pathogen development and transmission.

Conclusively enough, A variety of very effective methods have been employed for suppressing arthropod vector populations, including the application of biological control agents and the elimination of breeding sites, with a continuing and heavy reliance on the use of chemical insecticides. However, the development of insecticide resistance by vector insects, the cost of developing and registering new insecticidal compounds, and the increase in legislation to combat the detrimental effects of insecticidal residues on the environment, have emphasized the need to assess alternative strategies for vector control which are cost effective and safer. Endosymbionts of the arthropod vector are identified as a potential source for the control of VVBDs and symbiont based approaches are considered as cost effective. Bioprospecting of molecules involved. The strategy for the control of vector borne diseases should be paratransgenic. Symbiotic or commensal microbial pathogens should transformed to express products of gene that interfere with the transmission of pathogen. That is to say the genetically modified microbes are to be re-introduced back to the insect (vector). This method definitely, decrease the ability of the vector to transmit the pathogen into the human body. The paratransgenic method if utilized efficiently, it may reduce rate of for example *Trypanosoma cruzi*, the causative agent of Chagas disease, the triatomine bug, *Rhodnius prolixus*, and *Leishmania donovani* by the sand fly *Phlebotomus argentipes*. There are many effector biomolecules (example: antimicrobial peptides and specific single chain antibodies), currently being explored for the activities of “anti-parasite” in the systems to control vector borne diseases.

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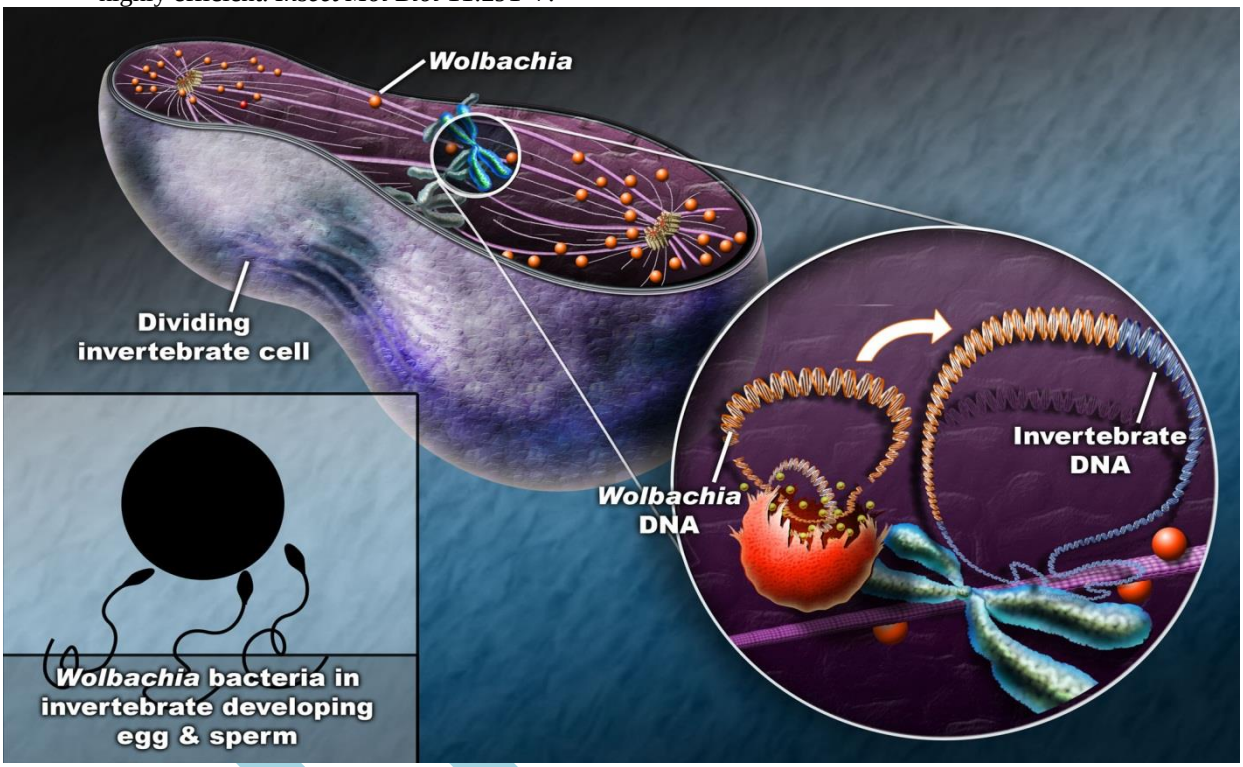

Fig. 1: Wolbachia enters the host cell, and then Wolbachia DNA is incorporated into DNA of host cell.

(Source of Image: http://www.science20.com/news_account/wolbachia_genome_discovered_inside_drosophila_genome).

