Université
franco-allemande
Deutsch-Französische
Hochschule

Summary

Thioredoxin reductase in *Anopheles gambiae* mosquitoes: role in redox homeostasis maintenance and manipulation for vector control.

Vector-borne diseases represent more than 17% of the total of infectious diseases and cause more than 800,000 deaths a year. Among the animals that can transmit human pathogens, blood-sucking mosquitoes are the most dangerous. For most of these diseases, avoiding human-mosquito interactions is the best way to hamper transmission. Among the strategies used for this purpose, individual protection measures and insecticides have been successfully used. The latter, however, lose their effectiveness as resistances against all currently available classes spread among mosquito populations. There is therefore an urgent need to develop new compounds with new modes of action. Most organisms possess two main redox buffers: glutathione and thioredoxins; however insects do not have the enzyme that recycles glutathione - glutathione reductase -. Instead, the thioredoxin / thioredoxin reductase system could compensate for this absence.

The objectives of this thesis were: (I) to better characterize the redox homeostasis of glutathione in mosquitoes, (II) to explore the role of the antioxidant thioredoxine reductase and (III) to assess its potential as a target for new insecticides.

To study the dynamics and the antioxidant response of glutathione, we established several lines of transgenic mosquitoes (*Anopheles gambiae* and *Aedes aegypti*) expressing a fluorescent protein sensitive to glutathione redox changes (roGFP). We thus found that, despite the absence of glutathione reductase, glutathione remained mostly reduced and controlled in these animals, especially in females. In addition, we observed an increase in its oxidation in intestinal cells during the pupal stage and after invasion by *Plasmodium* parasites - responsible for the symptoms of malaria - suggesting a role for this system in metamorphosis and the antiparasitic response.

To investigate the role of the enzyme thioredoxine reductase, we generated mutants for this gene in *Anopheles gambiae* - the malaria mosquito - using the CRISPR / Cas9 system. We have shown that this enzyme is essential during embryonic and post-embryonic development, but it is dispensable in intestinal cells at the adult stage. In this tissue, we have also shown that thioredoxine reductase is not essential for the maintenance of the redox homeostasis of glutathione. Transcriptomic studies comparing cells expressing or not this gene, have suggested that the loss of thioredoxine reductase in the intestine could be compensated for by the glutathione system, opening the question of how these animals regulate the latter.

Finally, *in vitro* inhibition studies led to the identification of several molecules that inhibit this enzyme in malaria mosquitoes. Oral exposure to one of these compounds proved to be toxic to these animals in a dose- and age-dependent manner.

In conclusion, we have shown that the redox homeostasis of glutathione is stably maintained and is closely regulated in mosquitoes, but this regulation is independent of thioredoxine reductase, at least in the intestine of adults. We have also shown that this enzyme is essential during development and that the inhibition of its activity in adults leads to the death of the animal, offering a promising target for the development of new insecticides. Finally, during this project, we also established several genetic tools for the study of redox processes in mosquitoes as well as for mutagenesis.