

LOCALIZATION AND FUNCTIONAL ANALYSIS OF
AN AQUAPORIN GENE (AQP 4886_gp) FROM
TSETSE FLY, *GLOSSINA PALLIDIPES*

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ABSTRACT

Tsetse flies are vectors of African trypanosomes, the protozoan agent of devastating disease, trypanosomiasis that afflicts both humans and animals. Currently, there is no promising vaccine in the horizon and treatment efforts are further constrained by the rapid increase in parasite drug resistance observed in patients. In addition, little effort is being made to develop new and effective drugs. Alternative methods to control trypanosomiasis and its transmission are therefore required. The trypanosome parasite develops into its infective metacyclic stage in the salivary glands of the tsetse fly, where the saliva provides a specific medium for its maturation and also becomes the fluid vehicle for the transfer of the parasites to the host through a blood meal. Water exchange across the salivary gland membrane occurs through aquaporin (AQP) water channels in brown dog tick, *Rhipicephalus sanguineus*. This study focused on the role(s) played by a putative water channel protein identified in the salivary glands of tsetse fly, *Glossina pallidipes*, in relation to feeding and survival. The salivary gland AQP gene (herein named AQP 4886_gp), a homolog of GMsg 4886 gene from the transcriptome of *Glossina morsitans morsitans*, was PCR-amplified and cloned from *G. pallidipes*. The AQP 4886_gp protein has a predicted molecular mass of 25.222 KDa. Topographic analysis suggested that AQP 4886_gp has the general aquaporin topology and possesses two conserved 'NPA' signature motifs (Asn-Pro-Ala) found in aquaporins. Multiple sequence alignment and protein distant tree plotted using Neighbour-Joining method shows that AQP 4886_gp is more closely related to many insect AQPs than vertebrates'. The AQP 4886_gp transcript was localized to the salivary glands, malpighian tubules, and midgut. These tissues are involved in high rates of water exchange in insects. The gene was detected in different life-cycle stages of the fly; larva, pupa, unfed teneral fly and adult

tsetse fly using semi-quantitative reverse transcription (RT)-PCR. Functional studies of AQP 4886_gp were carried out using RNA interference (RNAi) technique, where gene-specific double stranded RNA (dsRNA) was injected into experimental flies. The control group was injected with nuclease-free water (NFW). The effects of transient gene silencing were monitored by semi-quantitative RT-PCR, and relevant bioassays (survival, feeding success). AQP 4886_gp gene knockdown was not lethal to the flies as they continued to survive and feed. The survival rates of 83% were achieved in both injected test and control groups. Binomial test of proportions showed no significant differences in the feeding success between the test (dsRNA-injected) and control (NFW-injected) flies at $p < 0.05$.