Expression and Localization of Serpentine Receptor-like Proteins in Toxoplasma gondii Tachyzoites.

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INTRODUCTION: Serpentine Receptors are the largest family of stimuli-sensing proteins described to date, comprising around 800 highly druggable members. These membrane proteins are capable of translating a plethora of different signals, such as peptides, light, ions and hormones, into intracellular responses. Despite displaying high sequence divergence, these receptors have, as a common feature, seven transmembrane domains. An in silico analysis of the genome of the malaria-causative parasite, Plasmodium falciparum, identified four candidates for serpentine receptors. The proteins encoded by these genes have homology to predicted proteins in another Apicomplexan parasite, Toxoplasma gondii. As the latter organism allows for easier genetic manipulation, it was used to shed light on the expression and localization of two of the most promising serpentine receptor candidates, Sr10 and Sr25.

MATERIALS AND METHODS: The knock-in strategy with plic-3HA-CAT plasmid was used to introduce a hemmaglutinin tag on the 3' translated region of the genomic loci of Sr10 and Sr25 of T. gondii. Selected clones were analysed by PCR, western and immunofluorescence.

RESULTS AND DISCUSSION: Surprisingly, Sr10 and Sr25 displayed an intracellular localization. Sr25 colocalized with a recently described T. gondii organelle, the plant-like vacuole, as revealed by co-localization with the vacuolar-H\textsuperscript+-pyrophosphatase (TgVP1). SR25 ran at ~60 kDa. Sr10 is supposed to be a processed protein, as western blot analysis revealed two bands at ~80 kDa and ~60 kDa. Co-localization experiments are ongoing. Knockdown experiments of the serpentine receptor-like candidates could allow the assessment of the functions of these membrane proteins. Both knock-in parasite cell lines express a transactivator that could regulate gene activity, provided the gene of interest is placed under the appropriate promoter with tet operator elements.

CONCLUSIONS: While intracellular serpentine receptors are not unheard of, such as the ocular albinism receptor 1, which is located to melanosomes, it is also possible that these serpentine receptor-like proteins evolved novel functions.

Keywords: knock-in, serpentine receptors, Toxoplasma.