Comparative analysis of apicoplast-targeted proteins in *Toxoplasma gondii* and other Apicomplexa species

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Toxoplasma gondii is an important medical and veterinary Apicomplexa pathogen and induces cerebral toxoplasmosis, highly common cause of morbidity in HIV patients. It was reported earlier that *T. gondii* apicoplast is involved in pathogen stage conversion and proliferation; however the role of different apicoplast proteins involved in these processes was not established. Elucidation of molecular mechanisms underlying the role of apicoplast in control of parasite proliferation is highly desirable for development of novel therapeutics that control infection and reactivation of this parasite.

In this study we have analyzed nucleus encoded proteins with either confirmed or anticipated apicoplast-targeting like for housekeeping proteins: bacterial type RNA polymerase sigma subunit (RpoD), DNA ligase, cysteinyl-tRNA synthetase (CysRS), cell-cycle-associated protein kinase PRP4, enzymes IspA, IspB, IspE, IspF, IspG (GpcE), and IspH (LytB) of the mevalonate-independent pathway for isoprenoid biosynthesis, sulphur mobilisation protein SufC for an iron-sulphur cluster biogenesis pathway, enzymes LipA and LipB for lipoic acid biosynthesis. These proteins are encoded in nuclei of *T. gondii* or related species such as *Eimeria tenella*, *Neospora caninum* and *Plasmodium* spp. GpcE, LytB, and LipA proteins contain iron-sulphur clusters. SufC (with SufB, encoded in apicoplast) is necessary for iron-sulphur cluster biogenesis. These proteins in *T. gondii* have extended N-terminus compared to other Apicomplexa species and no N-terminal signal peptide typical for *Plasmodium* spp apicoplast-targeted proteins [1-4]. N-terminus in *T. gondii* apicoplast-targeted proteins can be extended from 40 aa (LipB) and up to 408 aa (DNA ligase). Conservative domains, common for Apicomplexa and cyanobacterium *Synechocystis* sp. PCC 6803 proteins are located at C-

terminus. Significant additional extension of *T. gondii* proteins N-terminus possibly suggests that these proteins need processing.

Significant N-terminus extension was also found for *T. gondii* protein kinase (ToxoDB:TGME49_209050) and some other proteins with no established connection to apicoplast. In contrast, rhoptry proteins secreted in response to pathogen-host interaction don't have additional N-terminus extension. For example *T. gondii* ROP22 (ToxoDB:TGME49_207700) is even 12 aa shorter than *P. vivax* protein (PlasmoDB:PVX_111320).

The unique features of *T. gondii* apicoplast proteins structure described above can be exploited for either genetic or drug-induced manipulations that may help to elucidate the role of these proteins in vital stages of pathogen development and control of its proliferation.

T. gondii, *N. caninum* and *E. tenella* proteins are available from ToxoDB database, release 8.0; *Plasmodium* spp. proteins – from PlasmoDB database, release 9.2.

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