Biochimica et Biophysica Acta 1865 (2017) 208–219

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Toxoplasma gondii immune mapped protein 1 is anchored to the inner leaflet of the plasma membrane and adopts a novel protein fold

Yonggen Jia [a,1], Stefi Benjamin [b,1], Qun Liu [c], Yingqi Xu [b], Sunil Kumar Dogga [a], Jing Liu [c], Stephen Matthews [b,*], Dominique Soldati-Favre [a,*]

[a] Department of Microbiology and Molecular Medicine, CMU, University of Geneva, Rue Michel-Servet 1, CH-1211 Geneva, Switzerland
[b] Department of Life Sciences, Imperial College London, South Kensington, London SW7 2AZ, UK
[c] Key Laboratory of Animal Epidemiology and Zoonosis, Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China

ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form 14 November 2016
Accepted 21 November 2016
Available online 22 November 2016

Keywords:
Toxoplasma gondii
Acylation
Invasion
Vaccine
NMR
Protein structure

ABSTRACT

The immune mapped protein 1 (IMP1) was first identified as a protective antigen in *Eimeria maxima* and described as vaccine candidate and invasion factor in *Toxoplasma gondii*. We show here that TgIMP1 localizes to the inner leaflet of plasma membrane (PM) via dual acylation. Mutations either in the N-terminal myristoylation or palmitoylation sites (G2 and C5) cause relocalization of TgIMP1 to the cytosol. The first 11 amino acids are sufficient for PM targeting and the presence of lysine (K7) is critical. Disruption of *TgIMP1* gene by double homologous recombination revealed no invasion defect or any measurable alteration in the lytic cycle of tachyzoites. Following immunization with TgIMP1 DNA vaccine, mice challenged with either wild type or IMP1-ko parasites showed no significant difference in protection. The sequence analysis identified a structured C-terminal domain that is present in a broader family of IMP1-like proteins conserved across the members of Apicomplexa. We present the solution structure of this domain determined from NMR data and describe a new protein fold not seen before.

1. Introduction

The phylum of Apicomplexa comprises important medical and veterinary protozoan parasites including *Plasmodium*, *Toxoplasma*, *Eimeria*, *Neospora*, *Scarcocystis*, *Babesia*, *Theileria* and *Cryptosporidium* species. The limited repertoire of efficacious drugs and the emergence of drug resistance have considerably hampered the control of these parasites and boosted the research towards the development of vaccines [1]. In this context, an immune mapped protein 1 (IMP1) was identified as an antigen eliciting protective immunity against chicken coccidian *E. maxima*, by combining parasite genetics and selective barriers with population-based genetic fingerprinting [2]. More recent studies in *Toxoplasma gondii* and *Neospora caninum* reported that TgIMP1 and NcIMP1 provided protection following immunization with DNA vaccine in the mouse model [3,4]. Antibodies raised against NcIMP1 localized it to the plasma membrane (PM) and were shown to have inhibitory effects on host cell invasion [4].

IMP1 lacks conserved domains with known function except for the predicted myristoylation and palmitoylation sites at the N-terminus, which are presumed to confer membrane association to the protein. Protein myristoylation is a process of co-translational attachment of myristic acid through an amide bond (C14:0) to N-terminal glycine residues and palmitoylation is the reversible posttranslational attachment of palmitate (C16:0) via a thioester linkage to cysteine. The lipid acylation of proteins, especially those implicated in invasion or as structural components of inner membrane complexes, contribute to pathogenesis in *T. gondii* and *Plasmodium* species [5–7].

In this study, we show that the acylation sites within the N-terminus of TgIMP1 are involved in anchoring the protein to the cytoplasmic face of the parasite plasma membrane (PM). Given this internal localization, we have revisited the reported roles of IMP1 in invasion and as a vaccine candidate by generating a mutant parasite lacking *TgIMP1*. Furthermore, we have identified a conserved globular domain at the C-terminus of TgIMP2.1, which represents a broader protein family present in all Apicomplexa. We also elucidate the high resolution structure of the *Plasmodium falciparum* IMP1-like homologue (PfIMP2) by solution state NMR and reveal a new protein fold.

2. Results

2.1. IMP1 possesses a globular C-terminal domain conserved across the Apicomplexa

Bioinformatic analysis indicate that IMP1 is restricted to the members of the coccidian-subgroup of the Apicomplexa. It also reveals that

* Corresponding authors.
E-mail addresses: s.j.matthews@imperial.ac.uk (S. Matthews), dominique.soldati-Favre@unige.ch (D. Soldati-Favre).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.bbapap.2016.11.010
1570-9639/

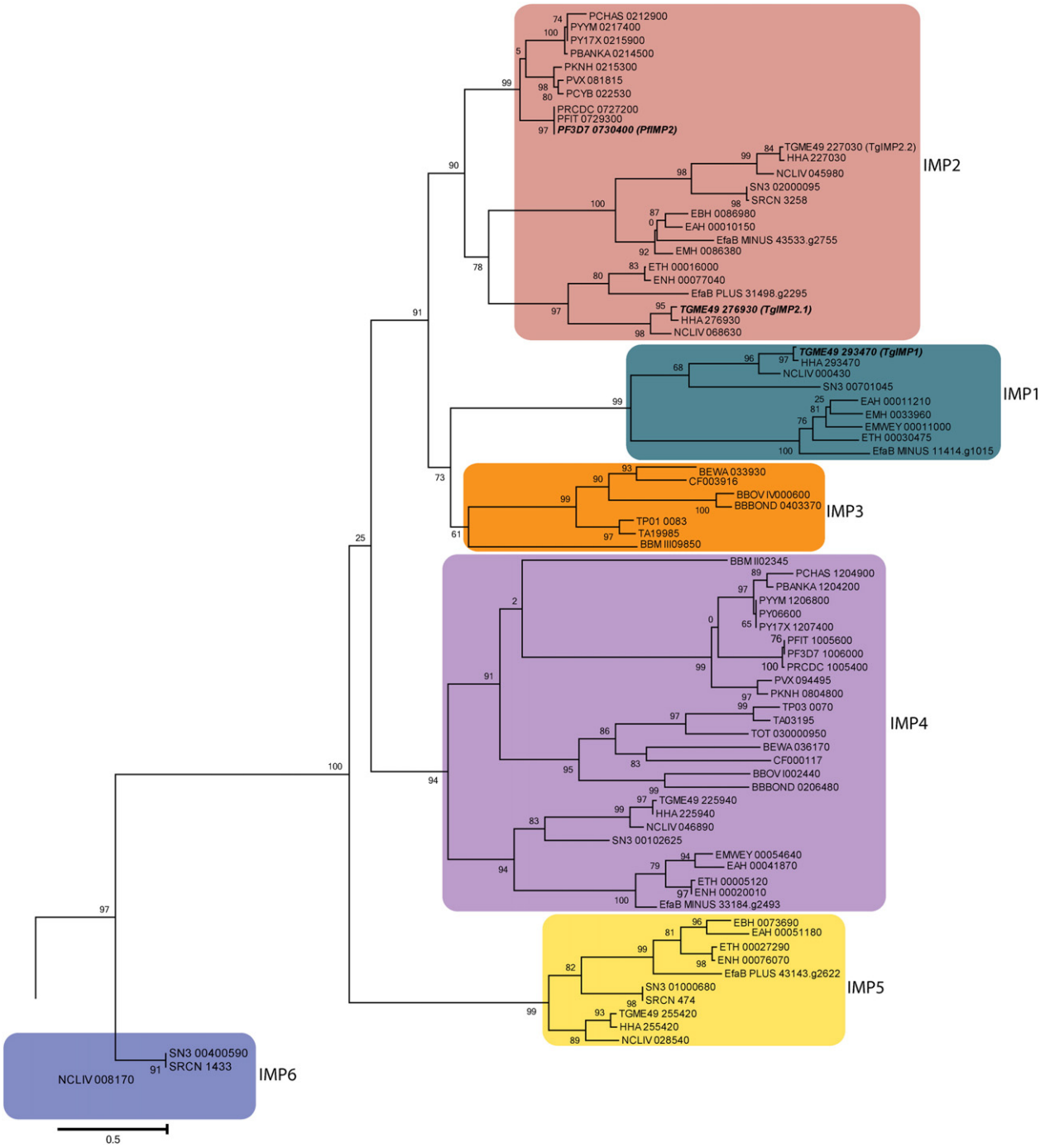

Fig. 1. Phylogenetic analysis of IMP1 homologues across all apicomplexan parasites. Phylogenetic tree based on sequences of Apicomplexan IMP related proteins cluster into six different groups, named IMP1-6. The tree was constructed using PhyML using LG model of amino acids substitution with NNI topology search, based on an amino acid alignment by MUSCLE. Values at tree nodes represent confidence values supporting nodal placement. The sequences are labeled with their accession numbers in EuPathDB.

IMP1 belongs to a broader family of proteins, including closely related IMP1-like proteins that group into six distinct phylogenetic clusters (IMP1 to IMP6 Fig. 1). The protein sequence alignment of all IMP1 and IMP1-like proteins used for the phylogeny is presented in Supplemental Fig. S1. TgIMP1 and other members of the IMP1 cluster possess putative N-terminal myristoylation and palmitoylation sites followed by an extended linker region and a conserved C-terminal domain (termed the IMP1-like domain). In contrast, members in other groups lack the residues predicted for N-terminal acylation. While the proteins clustering in IMP1, IMP5 and IMP6 are found restricted to the coccidians, the members of IMP4 are present in all Apicomplexa except *Cryptosporidium species*. The conservation of this globular domain across the phylum defines this broader family of IMP1-like proteins that may share conserved or similar functions.

2.2. IMP1 is localized to the inner leaflet of the plasma membrane in T. gondii

In eukaryotic cells, protein post-translational modification by dual acylation such as myristoylation and palmitoylation, confers membrane association for those proteins lacking a transmembrane domain. The conserved glycine followed by cysteine residues (MGXXCS/T) at the N-terminus of IMP1 implies that this protein is membrane-bound through such dual acylation. To determine the subcellular distribution