International Journal of Medical Microbiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

The N-terminal extension of the *P. falciparum* GBP130 signal peptide is irrelevant for signal sequence function

Corinna Meyer[a], Luis Barniol[a], Jan A. Hiss[b], Jude M. Przyborski[a,*]

[a] Department of Parasitology, Faculty of Biology, Philipps University Marburg, Marburg, Germany
[b] Swiss Federal Institute of Technology (ETH) Zürich, Department of Chemistry and Applied Biosciences, Vladimir-Prelog-Weg 4, 8093 Zürich, Switzerland

ARTICLE INFO

Keywords:
Malaria
Plasmodium
Secretion
Protein export
PEXEL
Signal peptide

ABSTRACT

The malaria parasite *P. falciparum* exports a large number of proteins to its host cell, the mature human erythrocyte. Although the function of the majority of these proteins is not well understood, many exported proteins appear to play a role in modification of the erythrocyte following invasion. Protein export to the erythrocyte is a secretory process that begins with entry to the endoplasmic reticulum. For most exported proteins, this step is mediated by hydrophobic signal peptides found towards the N-terminal end of proteins. The signal peptides present on *P. falciparum* exported proteins often differ in length from those found in other systems, and generally contain a highly extended N-terminal region. Here we have investigated the function of these extended N-terminal regions, using the exported parasite protein GBP130 as a model. Surprisingly, several deletions of the extended N-terminal regions of the GBP130 signal peptide have no effect on the ability of the signal peptide to direct a fluorescent reporter to the secretory pathway. Addition of the same N-terminal extension to a canonical signal peptide does not affect transport of either soluble or membrane proteins to their correct respective subcellular localisations. Finally, we show that extended signal peptides are able to complement canonical signal peptides in driving protein traffic to the apicoplast of the parasite, and are also functional in a mammalian cell system. Our study is the first detailed analysis of an extended *P. falciparum* signal peptide and suggests that N-terminal extensions of exported *Plasmodium falciparum* proteins are not required for entry to the secretory system, and are likely to be involved in other, so far unknown, processes.

1. Introduction

In almost all eukaryotic cells, entry to the secretory pathway of most proteins is governed by N-terminal sequences present pre-proteins, referred to as signal peptides (SP) (Devillers-Thiery et al., 1975; von Heijne, 1990). Following initiation of translation, these hydrophobic, 15–30 amino acid regions emerge from the ribosome and are bound by the signal recognition particle. This leads to a pause in translation, and directs the nascent polypeptide chain to the endoplasmic reticulum (ER) where protein translocation resumes, with the mature protein entering either completely into the ER lumen, or being inserted into the ER membrane. In many cases the SP is then cleaved from the pre-protein by a signal peptide peptidase (Walter and Blobel, 1981a,b; Walter et al., 1981). At this point, if proteins lack any further targeting information, they generally follow the default secretory pathway, travelling through the Golgi apparatus to either the external media (soluble proteins), or the plasma membrane (membrane bound proteins). The early secretory system of the human malaria parasite *P. falciparum* appears to be similar to that studied in higher eukaryotes, however the final destination of secretory proteins is not the extracellular environment, but either the lumen of the parasitophorous vacuole, a compartment which separates the parasite from the host cell cytosol, or the cytosol and plasma membrane of the host erythrocyte (Lingelbach and Przyborski, 2006). Strikingly, many proteins exported from the parasite to the host cell contain atypical N-terminal SPs. These sequences share the hydrophobic characteristics of canonical SPs, but are generally recessed from the N-terminal end of the protein by between 10 and 50 amino acids (Sargeant et al., 2006; Hiss et al., 2008a,b). Earlier studies have shown that these unusual SPs are able to mediate secretion of reporter proteins such as the green fluorescent protein (GFP) (Wickham et al., 2001; Adisa et al., 2003; Marti et al., 2005; Gehde et al., 2009). Additionally, canonical signal peptides derived from either parasite, or even human proteins, are also capable of mediating secretion from the parasite (Rug et al., 2004; Marti et al., 2005; Przyborski et al., 2005). Thus, the biological relevance of the extended N-terminal regions of many *P. falciparum* signal peptides remains elusive. Logic would suggest that there should be a selective pressure on parasites to lose “excess” amino acids from proteins, and these regions are thus predicted to fulfil

* Corresponding author at: Parasitology, FB17, Philipps University Marburg, Karl von Frisch Strasse 8, 35043 Marburg, Germany.
E-mail address: przybors@staff.uni-marburg.de (J.M. Przyborski).

http://dx.doi.org/10.1016/j.ijmm.2017.07.003
Received 20 April 2017; Received in revised form 19 June 2017; Accepted 10 July 2017

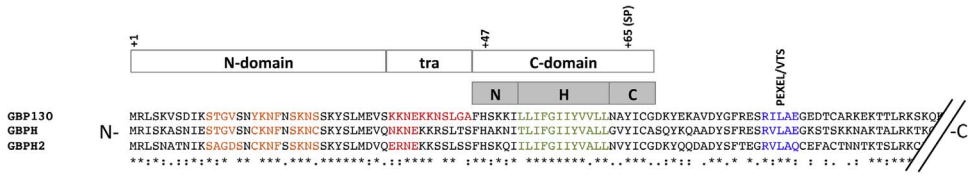

Fig. 1. Alignment of the N-terminal region of GBP130 with its homologues. NTraC organization is shown above in white according to Hiss et al. The N-, H-, and C-domain organization of the "canonical" signal sequence is shown in grey. Number refer to amino acids. SP, predicted signal peptide cleavage site. Orange, alternative transition areas; red: tested transition areas; green, hydrophobic core; blue, PEXEL/VTS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

an important biological function in the parasite's lifecycle. Here we have investigated the functionality of various artificial signal peptides based on the extended signal peptide of the exported parasite protein GBP130, with regard to their role in protein secretion. Our study is the first detailed analysis of an extended *P. falciparum* signal peptide, and reveals that the N-terminal extension present on this signal peptide is not required for protein entry to the secretory system, but is free to fulfil other functional roles.

2. Materials and methods

2.1. In silico analysis

Proteins for the analysis in Fig. 1 were retrieved from PlasmoDB (Aurrecoechea et al., 2009) using combinations of PlasmoDB queries (strategies) using the following settings: ExportPred score 5; SignalP 3.0 NN conclusion score 3, NN D-score 0.5, HMM Signal probability 0.5. Further analysis of the N-terminal region of GBP130 was carried out using the NtraC predictor (Hiss et al., 2008a,b).

2.2. Plasmid constructs

All primers used to generate constructs are listed in Table S2. For PCR amplification, the following templates were used: for PfGBP 130 derivates: PfGBP$^{1\text{-}150}$ (Gehde et al., 2009); for PfSTEVOR derivates: PfSTEVOR$^{1\text{-}296}$ (Przyborski et al., 2005); for PfACP^{TP}: PfACP DsRed (Sato and Wilson, 2004) for GST derivates: pGEX (Amersham). GBP$^{1\text{-}47}$: the coding sequence of PfGBP$^{1\text{-}47}$ was amplified by PCR using the primer pair GBP-*XhoI*-F/GBP47-*A*-Rev (restriction sites in italics; see Table below). The product was restricted with *XhoI/AvrII* and inserted into similarly digested pARL2-GFP vector (Przyborski et al., 2005). GBP$^{1\text{-}47}$ STEVOR$^{1\text{-}80}$: the coding sequence of PfGBP$^{1\text{-}47}$ was amplified and inserted as described above. The coding sequence of PfSTEVOR$^{1\text{-}80}$ was amplified by PCR using the primer pair STEV-FL_*AvrII*-F/STEV + 80_*KpnI*_R. The second product was restricted with *AvrII/KpnI* and inserted into similarly digested pARL2-GFP vector containing the first product. GBP$^{1\text{-}47}$ STEVOR$^{1\text{-}296}$: the coding sequence of PfGBP$^{1\text{-}47}$ was amplified and inserted as described above. The coding sequence of PfSTEVOR$^{1\text{-}296}$ was amplified by PCR using the primer pair STEV-FL_*AvrII*-F/STEVOR-FL-*KpnI*-R. The second product was restricted with *AvrII/KpnI* and inserted into similarly digested pARL2-GFP vector containing the first product. GBP$^{1\text{-}65}$ ACP^{TP}: the coding sequence of PfGBP$^{1\text{-}65}$ was amplified by PCR using the primer pair GBP-*XhoI*-F/GBP_ACP_SEW_R. The coding sequence of PfACP^{TP} was amplified by PCR using the primer pair GBP_ACP_SEW_F/ACP_TP_*K*_R. The coding sequence for GBP$^{1\text{-}65}$ ACP^{TP} was then amplified by overlapping extension PCR using the primer pair GBP-*XhoI*-F/ACP_TP_*K*_R. The product was restricted with *XhoI/KpnI* and inserted into similarly digested pARL2-GFP vector. GBP$^{48\text{-}65}$ ACP^{TP}: the coding sequence of PfGBP$^{48\text{-}65}$ was amplified by PCR using the primer pair GBP + 48-*X*-F/GBP_ACP_SEW_R. The coding sequence of PfACP^{TP} was amplified as described above. The coding sequence for GBP$^{48\text{-}65}$ ACP^{TP} was then amplified by overlapping extension PCR using the primer pair GBP + 48-*X*-F/ACP_TP_*K*_R. The product was inserted into pARL2-GFP vector with *XhoI/KpnI*. GBP$^{1\text{-}65}$ STEVOR$^{26\text{-}296}$: the coding sequence of PfGBP$^{1\text{-}65}$ was amplified by PCR using the primer pair GBP-*XhoI*-F/GBP_STEV + 26_SEW_R. The coding sequence of PfSTEVOR$^{26\text{-}296}$ was amplified by PCR using the primer pair GBP_STEV + 26_SEW_F/STEVOR-FL-*KpnI*-R. The coding sequence for GBP$^{1\text{-}65}$ STEVOR$^{26\text{-}296}$ was then amplified by overlapping extension PCR using the primer pair GBP-*XhoI*-F/STEVOR-FL-*KpnI*-R. The product was inserted into pARL2-GFP vector with *XhoI/KpnI*. GBP$^{48\text{-}65}$ STEVOR$^{26\text{-}296}$: the coding sequence of PfGBP$^{48\text{-}65}$ was amplified by PCR using the primer pair GBP + 48-*X*-F/GBP_STEV + 26_SEW_R. The coding sequence of PfSTEVOR$^{26\text{-}296}$ was amplified as described above. The coding sequence for GBP$^{48\text{-}65}$ STEVOR$^{26\text{-}296}$ was then amplified by overlapping extension PCR using the primer pair GBP + 48-*X*-F/STEVOR-FL-*KpnI*-R. The product was inserted into pARL2-GFP vector with *XhoI/KpnI*. GST$^{1\text{-}47}$ STEVOR$^{1\text{-}80}$: the coding sequence of GST$^{1\text{-}47}$ was amplified by PCR using the primer pair GST-*X*-F/GST + 47_*Avr*_R. The product was restricted with *XhoI/AvrII* and inserted into similarly digested pARL2-GFP vector. The coding sequence of PfSTEVOR$^{1\text{-}80}$ was amplified and inserted in the same manner as described above. GST$^{1\text{-}47}$ STEVOR$^{1\text{-}296}$: both coding sequences of GST$^{1\text{-}47}$ and PfSTEVOR$^{1\text{-}296}$ were amplified and inserted as described above. GST$^{1\text{-}47}$ GBP$^{48\text{-}150}$: the coding sequence of GST$^{1\text{-}47}$ was amplified and inserted as described above. The coding sequence of PfGBP$^{48\text{-}150}$ was amplified by PCR using the primer pair GBP + 48_*AvrII*_F/GBP + 150_*KpnI*_R. The second product was restricted with *AvrII/KpnI* and inserted into similarly digested pARL2-GFP vector containing the first product. GBP$^{40/1AA}$: alanine replacement at the position 40/41 of PfGBP$^{1\text{-}150}$ was introduced by PCR using the primers pairs GBP-xhoI-F/GBP-KAAS-R and GBP-KAAS-F/GBP150-B-R. The final product was amplified by overlapping extension PCR using the primer pair GBP-xhoI-F/GBP150-B-R. The product was restricted with *XhoI/BssHII* and inserted into similarly digested pARL2-GFP vector. GBP$^{43/4AA}$: alanine replacement at the position 43/44 of PfGBP$^{1\text{-}150}$ was introduced by PCR using the primers pairs GBP-xhoI-F/GBP-SAAA-R and GBP-SAAA-F/GBP150-B-R. The final product was amplified and inserted as described above. GBP$^{\Delta 36-46}$: the deletion of aa 36-46 of PfGBP$^{1\text{-}150}$ was introduced by PCR using the pairs GBP-xhoI-F/GBP-DeltaK-A-R and GBP-DeltaK-A-F/GBP150-B-R. The final product was amplified and inserted as described above. GBP$^{18/9AA}$: alanine replacement at the position 18/19 of PfGBP$^{1\text{-}150}$ was introduced by PCR using the primers pairs GBP-xhoI-F/GBP-YAAF-R and GBP-YAAF-F/GBP150-B-R. The final product was amplified and inserted as described above. GBP$^{\Delta 17\text{-}25}$: the deletion of aa 17-25 of PfGBP$^{1\text{-}150}$ was introduced by PCR using the pairs GBP-xhoI-F/GBP-DeltaY-S-R and GBP-DeltaY-S-F/GBP150-B-R. The final product was amplified and inserted as described above. GBP$^{1\text{-}150}$: the coding sequence of PfGBP$^{1\text{-}150}$ was amplified by PCR using the primer pair GBP-*XhoI*-F/GBP150-B-R. The product was restricted with *XhoI/BssHII* and inserted into similarly digested pARL2-GFP vector.

2.3. Parasite culture and transfection

Maintenance of *P. falciparum* (clone 3D7) was carried out in A+ human erythrocytes as previously described (Trager and Jensen, 1976). For transfection *P. falciparum* (3D7) were cultured in fresh 0+ human erythrocytes and were transfected at ring stage with approximately 100 µg plasmid DNA. Transfectants were selected with 5 nM WR99210 (Fidock and Wellems, 1997) (Jacobus Pharmaceuticals). As soon as transfectant parasites were detected, they were transferred to A+ human erythrocytes for normal culture. Parasites were synchronized using gelafundin flotation (late-stage parasites) or sorbitol (ring stages) as previously described (Pasvol et al., 1978; Lambros and Vanderberg,