



Research article

Identification of *Plasmodium falciparum* HSP70-2 as a resident of the *Plasmodium* export compartmentGladys T. Cortés^{a,*}, Mark F. Wiser^b, Claudio J. Gómez-Alegría^c^a Departamento de Salud Pública, Facultad de Medicina, Laboratorio de Equipos Comunes, Universidad Nacional de Colombia, Calle 45 No. 30-03, Edificio 471, Bogotá, Colombia^b Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA^c Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia, Grupo UNIMOL, Colombia

ARTICLE INFO

Keywords:

Biochemistry
 Biological sciences
 Cell biology
 Medical microbiology
 Microbiology
 Molecular biology
 Parasitology
 Plasmodium falciparum
 HSP70-2
 Endoplasmic reticulum
 Protein export
 Chaperone
 Secretory pathway

ABSTRACT

The malarial parasite remodels the host erythrocyte following invasion. Well-known examples are adhesive proteins inserted into the host erythrocyte membrane, which function as virulence factors. The modification of the host erythrocyte may be mediated by a specialized domain of the endoplasmic reticulum, or *Plasmodium* export compartment (PEC). Previously, monoclonal antibodies recognizing the PEC were generated and one of these monoclonal antibodies recognize a 68 kDa parasite protein. In this study, the 68 kDa protein was affinity purified and analyzed by peptide mapping using mass spectrometry. The results demonstrate that the 68 kDa protein is the *P. falciparum* homolog of the endoplasmic reticulum resident HSP70 called PfHSP70-2. This finding is consistent with the PEC being a domain of the endoplasmic reticulum and suggests a role for PfHSP70-2 in the export of *Plasmodium* proteins into the host erythrocyte.

1. Introduction

As part of its life cycle, the malaria parasite invades erythrocytes and this intraerythrocytic stage is responsible for the disease. During its intraerythrocytic stage, the parasite extensively modifies the host erythrocyte (De Koning-Ward, Dixon, Tilley and Gilson, 2016). These modifications include the formation of multiple membranous compartments within the erythrocyte cytoplasm, such as exomembranes systems, the parasitophorous vacuole, the tubovesicular network (Prajapati and Singh, 2013), Maurer's' clefts, the caveole-vesicle complex, J dots and other mobile compartments. Changes in the host erythrocyte cell membrane are primarily in its rigidity, adhesiveness, and permeability to nutrients. These changes promote the survival of the parasite and are often virulence factors. For example, the adhesiveness of *Plasmodium falciparum*-infected erythrocytes promotes survival of the parasite by sequestering infected erythrocytes to the capillary endothelium in the deep vasculature and thereby preventing clearance in the spleen (Elsworth et al., 2014). Cytoadherence and sequestration are responsible

for increased severity associated with *P. falciparum* infections (Bernabeu and Smith, 2017). This cytoadherence is mediated by a parasite protein (PfEMP1) that is expressed on the surface of the infected erythrocyte and serves as a ligand to bind to receptors on endothelial cells (Pasternak and Dzikowski, 2009). The modifications of the host erythrocyte are mediated by the export of parasite proteins into the host erythrocyte and the specific targeting of these exported proteins to distinct locations in the infected cell. In fact, the number of proteins exported into the host erythrocyte may account for 8–10% of the parasite's genome (Matthews et al., 2019). In regards to how the parasite exports proteins into the host erythrocyte, a *Plasmodium* export compartment (PEC), that is an early step in the targeting of proteins to the host erythrocyte, has been proposed (Wiser, 2007). This compartment is present throughout the erythrocytic stage of the parasite and is involved in the export of several different proteins that are targeted to extraparasite locations such as the parasitophorous vacuole, inclusions within the infected erythrocyte, or the host erythrocyte membrane. It is not clear if the PEC is a distinct compartment from the ER or if the PEC is a domain of the ER that

* Corresponding author.

E-mail address: gtcortesc@unal.edu.co (G.T. Cortés).

specializes in the export of proteins into the host erythrocyte. Consistent with the PEC being a domain of the ER (Marapana et al., 2018) have recently suggested that proteins with the host targeting sequence called PEXEL are sorted to a distinct location on the ER membrane. This implies that the *Plasmodium* ER of the blood stage parasite has distinct domains. Once passing through the PEC, exported proteins are then moved into the parasitophorous vacuole and translocated into the host erythrocyte and then directed to their final locations by various mechanisms (De Koning-Ward et al., 2016).

The exact nature of PEC is unknown and possible resident proteins include a SERCA-like ATPase unique to the Apicomplexa and COPII components (Wiser, 2007). In addition, several monoclonal antibodies that may recognize proteins of the PEC have been previously described (Cortés et al., 2003). These antibodies were generated by immunizing mice with a purified and concentrated membrane fraction released during the *in vitro* culture of *P. falciparum* (Winograd et al., 1999). The previously described monoclonal antibodies include Mab4F8, which recognizes a protein of 45 kDa, Mab134 that recognizes a protein doublet of 44/22 kDa, and Mab7 and MabIG2 that both recognize a protein of 68 kDa. These proteins are conserved in *Plasmodium* species in that the antibodies also recognize homologues in rodent *Plasmodium* species. However, the identities of these potential PEC resident proteins are currently unknown.

As a continuation of this previous study we determined the identity of the 68 kDa protein recognized by Mab7 (*Pf*68 kDa) following affinity purification and peptide fingerprinting using mass spectrometry. The results conclusively show that *Pf*68 kDa is equivalent to the *P. falciparum* homolog of the ER-resident HSP70, or *Pf*HSP70-2.

2. Methods

2.1. Culture of *P. falciparum*

P. falciparum-infected erythrocytes from the FCB-1 strain was maintained in continuous culture as previously described (Trager and Jensen, 1976). Parasites were synchronized using 5% sorbitol to eliminate mature forms according to (Lambros and Vanderberg, 1979). The culture was maintained until reaching a 5–15% parasitemia. After the rings matured, trophozoite and schizont-infected erythrocytes were concentrated by gelatin flotation (Jensen, 1979).

2.2. Preparation of affinity column

Ascitic fluid containing Mab7 was produced in Balb/c mice (10–12 weeks old) following immunosuppression with Pristan® (Sigma) and purified by ion exchange chromatography using monoQ sepharose (BioRad). The pooled fractions were concentrated with an Amicon filter with a <30 kDa cutoff and then dialyzed with a 0.1 M carbonate buffer, pH 8.6. Total protein concentration was determined by the bicinchoninic acid method (Walker, 1994) Cyanogen bromide activated resin was obtained from Pierce (Rockford IL, USA) and prepared according to their recommendations. Mab7 was conjugated to the resin and washed with phosphate-buffered saline (PBS) 10mM sodium phosphate, 0.145 M NaCl, pH 7.4, blocked with 0.1 M glycine, pH8.0, and washed again with PBS pH 7.4.

2.3. Protein purification and MS/MS analysis

Total protein extracts were prepared from enriched and intact *P. falciparum*-infected erythrocytes prepared by sorbitol synchronization and gelatin flotation as described above. Infected erythrocytes were hemolyzed with 20 volumes of hypotonic 5mM sodium phosphate, pH 8.0, containing protease inhibitors (Martínez et al., 1998). The lysate was centrifuged at 10,000Xg for 20 min. The pellet fractions were suspended in 10 volumes of 1% Triton X-100 in PBS plus protease inhibitors and were incubated for 30 min at 4 °C. The extracts were centrifuged at 45,

000 x g for 1 h. The supernatant containing the Triton X-100 solubilized proteins was subjected to affinity chromatography.

The Triton X-100 extract was passed over the Mab7 affinity column and then washed with PBS containing 0.1% Triton X-100 to remove non-specific proteins. The purified protein was eluted with 0.1 M glycine, pH 2.7, containing 0.1% Triton X-100. The eluted protein was precipitated with 10% trichloroacetic acid and preserved at -20 °C. This affinity purified 68 kDa protein was analyzed by SDS-PAGE and immunoblotting using Mab7 as previously described (Cortés et al., 2003).

The band corresponding to the 68 kDa protein was excised and shipped to the Proteome Factory AG (<https://www.proteome-factory.com>). At the Proteome Factory, *Pf*68 kDa was digested with trypsin and analyzed by tandem high-performance liquid chromatography (HPLC) and mass spectrometry (MS) utilizing electro spray ionization Fourier transform ion cyclotron resonance (ESI-FTICR). The experimental molecular masses of the peptides as determined by MS were then compared to theoretical molecular masses of peptides produced via an *in silico* digestion of proteins from the *P. falciparum* genome database using the MASCOT protein identification program (Matrix Science Ltd).

The Mab7 for purify *Pf*68 kDa used in the column was from ascitic fluids. This ascitic fluid was prepared before the doctoral Thesis and was previously approved by Comité Técnico de Investigaciones CT-152-94 from National Institute of Health, Bogotá-Colombia.

3. Results and discussion

The 68 kDa protein was affinity purified using Mab7. A total of 260 µg of a *P. falciparum* extract was passed over an affinity column, and following elution, 0.156 µg of protein was recovered. The eluted protein consisted of a single polypeptide of approximately 68 kDa that was recognized by Mab7 (Figure 1). Contaminating bands were quite minor in this purified preparation. The purified protein was then subjected to immunoblotting using Mab7 (Figure 1). Mab7 only recognized the 68 kDa protein. These results demonstrated that *Pf*68 kDa was successfully purified.

The band corresponding to the 68 kDa protein was excised from a gel and shipped to the Proteome Factory AG (<https://www.proteome-factory.com>). At the Proteome Factory, *Pf*68 kDa was digested with trypsin

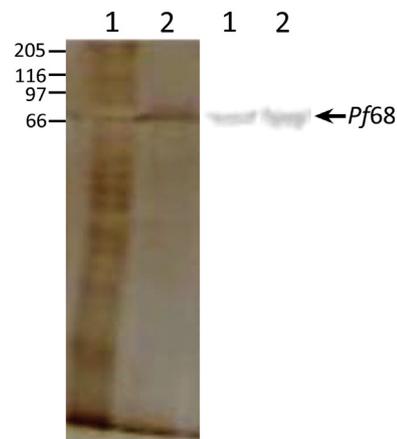


Figure 1. Analysis of the affinity-purified *Pf*68 kDa. The purified *Pf*68 kDa was analyzed by SDS-PAGE and silver staining (left panel). The same samples were also analyzed by immunoblotting with Mab7 (right panel). Lanes 1 correspond to the crude extract before affinity chromatography, and lanes 2 correspond to the purified product after affinity chromatography. A single band corresponding to *Pf*68 kDa (denoted with arrow) is recognized by Mab7 after affinity chromatography. The complete images of SDS-PAGE silver-stained gel correspond to the left panel of Figure 1, and the immunoblotting membrane corresponds to the right panel of Figure 1; both figures included as supplementary material (SM-1 and SM-2).

and the masses of the peptides were determined by mass spectrophotometry (MS). The experimental molecular masses of the peptides as determined by MS were then compared to theoretical molecular masses of peptides produced via an *in silico* digestion of proteins from the *P. falciparum* genome database.

The proteomic analysis of the purified 68 kDa protein was carried out twice. In both cases a single high-scoring match corresponding to PfHSP70-2 (PF3D7_0917900) was identified. In one analysis the score for PfHSP70-2 was 854 and the next highest scoring *P. falciparum* protein was 34. In the other analysis, the score for PfHSP70-2 was 910 and the next highest scoring *P. falciparum* protein was 62. Many of the peptides exhibited highly significant matches with expect scores <0.01 and 26 out of 27 peptides were considered as best matches (Figure 2). Furthermore, all of the peptides align with the PfHSP70-2 sequence (Supplemental data). In addition, the fact that all of the peptides are associated with PfHSP70-2 further indicated that the 68 kDa protein is highly purified and there are no co-migrating proteins in the sample. These results clearly demonstrate that the 68 kDa protein is equivalent to PfHSP70-2.

HSP70 is a family of highly conserved and ubiquitous molecular chaperones that function as protein folding catalysts (Mayer and Bukau, 2005). Six paralogs of HSP70 have been identified in the *Plasmodium* genome (Shonhai et al., 2007). Three of these paralogs are homologous to the organelle specific HSP70 proteins found in eukaryotes. Namely, PfHSP70-1 corresponds to the cytosolic HSP70, PfHSP70-2 corresponds to the ER HSP70, and PfHSP70-3 corresponds to the mitochondrial

HSP70. The ER-resident HSP70 is also known as glucose-regulated protein (Grp78) or as immunoglobulin-binding protein (BiP) (Daugaard et al., 2007). Furthermore, PfHSP70-2 has been previously localized to the ER in *P. falciparum* (Kumar et al., 1991). The PfHSP70-2 gene is transcribed throughout the blood stage cycle of the parasite (Le Roch et al., 2003). This is consistent with our previous observations that the PEC can be detected from the beginning of the ring stage throughout schizogony and fragments during the segmenter stage (Cortés et al., 2003). This indicates, that like the ER, the PEC fragments during segmentation and the budding merozoites contain the PEC.

Previously we published that there was an overlap between the compartments recognized by Mab7 and a polyclonal antibody against PfHSP70-2 (Cortés et al., 2003). In particular, PfHSP70-2 appears to be more extensively distributed in the parasite than the then described 68 kDa protein. This minor discrepancy may be due to differences between the epitopes recognized by the monoclonal and polyclonal antibodies. For example, the polyclonal anti-sera was raised against the last 11 amino acids of PfHSP70-2 (Kumar et al., 1991) and the epitope of Mab7 is unknown. It could also be that the epitope recognized by Mab7 is more accessible in the PEC than in the entire ER. Or perhaps PfHSP70-2 is more concentrated in the PEC than the remainder of the ER. In any event, this overlapping pattern of immunofluorescence is consistent with the PEC being a domain of the ER, or an ER-like organelle. In other words, PfHSP70-2 is found in both the ER and PEC and the differences between the labeling with polyclonal antibodies and Mab7 could reflect

gi1124506906		Mass: 72343	Score: 910	Queries matched: 50	emPAI: 1.43			
Heat shock protein 70 (HSP70) homologue [Plasmodium falciparum 3D7]								
Observed	Mr (expt)	Mr (calc)	ppm	Miss	Score	Expect	Rank	Peptide
466.2329	930.4512	930.4521	-0.95	0	27	0.023	1	R.VMDYFIK.M
554.2869	1106.5593	1106.5608	-1.31	1	26	0.067	1	R.KFDDQEVVK.D
607.8287	1213.6428	1213.6455	-2.27	0	10	2.3	1	K.FELSGIPPAQR.G
619.7934	1237.5723	1237.5727	-0.37	1	23	0.15	1	K.EFFNGKEPNR.G
629.8519	1257.6893	1257.6929	-2.85	0	20	0.17	1	K.IDEIVLVGGSTR.I
635.8404	1269.6663	1269.6677	-1.06	0	8	2.2	1	R.VEILNDELGNR.I
649.3057	1296.5967	1296.5986	-1.44	0	35	0.0064	1	K.FEELNDDLFR.E
658.8823	1315.7501	1315.7500	0.06	1	10	0.89	1	K.EIAQSFLGKPKV.N
678.3152	1354.6158	1354.6187	-2.14	0	56	5.5e-005	1	K.NNLDNYIQSMK.A
678.8083	1355.6021	1355.6027	-0.46	0	(11)	1.5	1	K.NNLDNYIQSMK.A
460.2365	1377.6876	1377.6888	-0.90	2	32	0.022	1	R.KFDDQEVVKDR.S
735.3665	1468.7184	1468.7198	-0.98	0	28	0.042	1	R.ITPSYVSEFVDGER.K
491.9468	1472.8186	1472.8199	-0.85	1	5	3.2	2	K.SKIDEIVLVGGSTR.I
748.8702	1495.7259	1495.7307	-3.17	1	74	1.1e-006	1	R.AKFEELNDDLFR.E
531.3006	1590.8799	1590.8803	-0.29	2	21	0.078	1	K.MKEIAQSFLGKPKV.N
830.4506	1658.8867	1658.8879	-0.69	0	67	5.4e-006	1	R.IINEPTAAALAYGLDK.K
833.4173	1664.8201	1664.8158	2.59	0	(57)	7.8e-005	1	K.NAVVTVPAYFNDAQR.Q
849.9567	1697.8988	1697.8988	-0.02	0	34	0.0081	1	K.LEATLHPTQTVFVVK.R
596.6677	1786.9812	1786.9828	-0.90	1	31	0.012	1	R.IINEPTAAALAYGLDKK.E
619.0060	1853.9962	1854.0000	-2.04	1	28	0.019	1	K.LEATLHPTQTVFVVKR.L
964.0348	1926.0551	1926.0574	-1.19	1	36	0.0024	1	R.SLLPYEIVNNQKPNIK.V
1003.9396	2005.8646	2005.8501	7.27	0	102	1.7e-009	1	K.DAEDWLNNSNADSEALK.Q
675.0066	2021.9979	2021.9979	-0.03	1	4	11	1	K.DKDTTFAPEQISAMVLEK.M
1180.0125	2358.0104	2358.0136	-1.34	0	35	0.012	1	K.LYGQPGGSPQPSGDEDVDSDEL.-
886.4336	2656.2789	2656.2769	0.74	0	(52)	0.00022	1	K.SQTFSTYQDNQPAVLIQVFEGE.R.A
886.7684	2657.2833	2657.2609	8.41	0	76	8.7e-007	1	K.SQTFSTYQDNQPAVLIQVFEGE.R.A
1023.1825	3066.5256	3066.5258	-0.07	0	66	1.1e-005	1	R.NLSVHVHSTQIEIEDIVEGHNFSETLTR.A

Figure 2. Peptide mapping of Pf68 kDa. Peptide masses following a trypsin digestion of Pf68 kDa were determined by mass spectrometry (Mr expt) and compared to theoretical peptide masses of proteins found in the *P. falciparum* genome (Mr calc). Twenty-six peptides from the 68 kDa protein exhibited top-ranked matches from PfHSP70-2 peptides in the *P. falciparum* genome database (denoted in red). Only one peptide from the 68 kDa protein did not have a top-ranked match associated with PfHSP70-2 (denoted in black). However, this peptide is found in the PfHSP70-2 sequence (Supplemental Data) meaning all 27 peptides generated by the trypsin cleavage are from PfHSP70-2. In addition, expect scores for many of the peptides were <0.01 and therefore are highly significant. This analysis is a representative of two independent analyses which were equivalent.

differences in those two compartments whether they are two separate compartments or the PEC is a domain of the ER. Furthermore, a partial overlap between Mab7 and polyclonal antibodies against the COPII components *PfSar1p* and *PfSec31p* was previously described (Cortés et al., 2003).

The location of *PfHSP70-2* in the ER and PEC implies that it has a role in protein trafficking and secretion. Indeed, it is known that chaperones help to refold proteins moving between subcellular compartments or to maintain exported proteins in an unfolded state until they reach their final destination (Kleizen and Braakman, 2004). In regards to the malaria parasite, this function would be needed whether proteins are being transported to locations within the parasite, or to locations within the host erythrocyte. However, exporting and targeting proteins into the host erythrocyte entails more complexity (Daniyan et al., 2019). From the PEC the exported proteins move into the parasitophorous vacuole and need to cross the parasitophorous vacuolar membrane (PVM). There is a protein complex in the vacuolar membrane called PTEX that translocates proteins into the erythrocyte cytoplasm (De Koning-Ward et al., 2016). After traversing the PVM, some of the exported proteins remain in the cytosol, but many proteins are trafficked through membranous structures established by the parasite, such as Maurers' clefts, to their final destinations in the host erythrocyte. Throughout this pathway proteins need to be maintained in an unfolded state and then ultimately exported proteins need to be correctly folded. Thus, chaperones are obviously important in the export of proteins into the host erythrocyte (Külzer et al., 2012).

An interactome of *PfHSP70-2* was analyzed in order to gain some further insight into the biology of *PfHSP70-2*. Previously, several proteins interacting with *PfHSP70-2* were identified in a yeast two-hybrid screen (LaCount et al., 2005), and we carried out an evaluation of these proteins in regards to potential interactions with *PfHSP70-2*. *PfHSP70-2* interacts with 16 proteins including itself (Table 1). Interaction of *PfHSP70-2* with itself is not unexpected since HSP70 is known to exist in various oligomer states (Takakuwa et al., 2019). Of particular relevance to this study, are

two proteins involved in secretion and two exported proteins. Other interacting proteins included several chaperones as well as six proteins with various functions and three conserved *Plasmodium* proteins of unknown function. In that the yeast two-hybrid system can be prone to promiscuous interactions, some of the interactions may need to be further evaluated experimentally. Nonetheless, some of these interactions are consistent with the presumed functions of *PfHSP70-2*.

For example, the interaction of *PfHSP70-2* with proteins involved in secretion and exported proteins is consistent with *PfHSP70-2* localization to the ER and PEC. For example, Sec16 plays a role in the assembly of COPII coats at ER exit sites (Sprangers and Rabouille, 2015). The overlap between COPII components, Sec31 and Sar1, and *PfHSP70-2* have been previously demonstrated (Cortés et al., 2003). Phosphatidylinositol-4-phosphate 5-kinase (PIP5K) is also involved in the regulation of secretion and is activated by ARF1. ARF1 is the target of Brefeldin A and Brefeldin A was originally used to identify the PEC (Wiser et al., 1997). Although further work is needed to conclusively demonstrate an interaction between *PfHSP70-2* between Sec16 and PIP5K, these results are consistent a role for *PfHSP70-2* in protein trafficking.

Interactions between chaperones and cargo proteins are well known (Kleizen and Braakman, 2004). And in this regard, *PfHSP70-2* also interacts with two proteins that are exported from the parasite: *PfEMP1* and an aminopeptidase of the M1 family (M1AAP). *PfEMP1* is expressed on the surface of infected erythrocytes and mediates cytoadherence to endothelial cells. Substantial work has been done on the trafficking of *PfEMP1* from the parasitophorous vacuole to the erythrocyte membrane (Kneuper et al., 2005) but little is known about the initial steps of *PfEMP1* export. Presumably, this involves passage through either the ER or PEC before export into the parasitophorous vacuole. The M1AAP is also exported into the parasitophorous vacuole in route to its transport to the food vacuole – a lysosomal-like organelle (Azimzadeh et al., 2010).

Table 1. Interactome: *Plasmodium falciparum* proteins interacting with *PfHSP70-2* as detected by yeast two-hybrid system.

Protein or Gene ID	Domain(s) ^a	Interactions ^b	Function
Secretory pathway and exported proteins			
SEC16	3	1	vesicle coat protein required for ER transport vesicle budding
PIP5K	3	4	regulator of motility, secretion and signaling, activated by ARF1
<i>PfEMP-1</i>	2 + 3	8	exported protein, ligand mediating cytoadherence of infected erythrocytes to endothelial cells
M1AAP	2	4	neutral aminopeptidase, trafficked through parasitophorous vacuole
Chaperones and protein-protein interactions			
<i>PfHSP70-2</i>	1 + 2+3 (B) ^c		
2 + 3 (P)	17	chaperone, ER	
<i>PfHSP70-1</i>	3	12	chaperone, cytoplasm
HEP1	1	4	maintain structure and function of HSP70-3 (mitochondrial)
KELCH10	1	5	Kelch-repeat β -propellers are generally involved in protein-protein interactions
<i>Plasmodium</i> proteins of presumed functions			
HUEL	1	36	protein degradation via proteasome
RAD23	1	22	DNA repair
HMGB3	1 + 2	7	DNA binding, chromatin structure, transcription
CCT	1	7	Lipid synthesis
GLYRS	3	2	glycine-tRNA ligase, protein synthesis
CEN2	3	6	cytoskeleton protein with key roles in cell division, including centrosome duplication
Conserved <i>Plasmodium</i> proteins of unknown function			
PF3D7_0606000	2 + 3	7	
PF3D7_0817300	1	17	
PF3D7_1464500	1	5	

^aThe domain of *PfHSP70-2* that binds to the identified protein (1 = N-terminal ATPase domain; 2 = Substrate binding domain; 3 = C-terminal domain). ^bNumber of other proteins interacting with identified protein as detected by yeast two hybrid system including *PfHSP70-2*. This is a potential measure of promiscuity. ^cB = bait and P = prey for interaction of *PfHSP70-2* with itself. Abbreviations: PIP5K (phosphatidylinositol-4-phosphate 5-kinase), ARF1 (ADP-ribosylation factor-1), EMP-1 (erythrocyte membrane protein-1), HSP (heat shock protein), HEP1 (HSP70-escort protein 1), HUEL (HECT-domain E3 ubiquitin ligase), HMGB3 (high mobility group protein B3), CCT (CTP: Phosphocholine cytidyltransferase), GLYRS (glycyl-tRNA synthetase), CEN (centrin).

Interactions with other chaperones is not surprising in that chaperones form many functional networks in *Plasmodium* (Daniyan et al., 2019). In addition, molecular chaperones account for approximately two percent of the *Plasmodium* genome and approximately five percent of the *Plasmodium* exportome. Interestingly, PfEMP1 interacts with another HSP70 protein, called PfHSP70-x. PfHSP70-x is unique to *Plasmodium* and is exported into the host erythrocyte cytoplasm where it presumably functions as a chaperone (Külzer et al., 2012). Furthermore, deletion of the PfHSP70-x gene interferes with the trafficking of PfEMP1 to the host erythrocyte membrane and reduces cytoadherence (Charnaud et al., 2017).

This study implicates PfHSP70-2 in the export of parasite proteins into the host erythrocyte. Clearly more information is needed on the precise role of PfHSP70-2 in this process as well as the role of other chaperones and co-chaperones. Furthermore, the uniqueness of this process in *Plasmodium* may provide useful therapeutic targets.

Author contribution statement

Mark F. Wiser: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gladys T. Cortés: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Claudio J. Gómez-Alegría: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by the Universidad Nacional de Colombia, Vicerrectoría de Investigación y extensión (Strategic Research Project-Hermes 42917), Medicine Faculty (Public Health Department and Laboratorio de equipos comunes), Programa de doctorado del Instituto de Biotecnología, Departamento de Farmacia, Facultad de Ciencias and Doctorado en Medicina Tropical, Universidad de Cartagena-SUE-Caribe. This project was also funded in part by a grant from the Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología (Colciencias-project no. 110140820398). Acknowledgments also, to Instituto Nacional de Salud, Bogotá, D.C., where the original work was initiated.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2020.e04037>.

Acknowledgements

This work was supported by the Universidad Nacional de Colombia, Vicerrectoría de Investigación y extensión (Strategic Research Project-Hermes 42917), Medicine Faculty (Public Health Department, Laboratorio de equipos comunes and Vicedecanatura de Investigación y Extensión (project No. 201010020832)), Programa de doctorado del Instituto de Biotecnología, Departamento de Farmacia, Facultad de Ciencias and Doctorado en Medicina Tropical, Universidad de Cartagena-SUE-Caribe. This project was also funded in part by a grant from the Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología (Colciencias-project no. 110140820398). Acknowledgments also, to Dr. Enrique Winograd who proposed the original work related with Pf68 kDa at Instituto Nacional de Salud, Bogotá, D.C., where the original work was initiated.

References

- Azimzadeh, O., Sow, C., Gèze, M., Nyalwidhe, J., Florent, I., 2010. Plasmodium falciparum PfA-M1 aminopeptidase is trafficked via the parasitophorous vacuole and marginally delivered to the food vacuole. *Malar. J.* 9, 1–16.
- Bernabeu, M., Smith, J.D., 2017. EPCR and malaria severity: the center of a perfect storm. *Trends Parasitol.* 33, 295–308.
- Cortés, G.T., Winograd, E., Wiser, M.F., 2003. Characterization of proteins localized to a subcellular compartment associated with an alternate secretory pathway of the malaria parasite. *Mol. Biochem. Parasitol.* 129, 127–135.
- Charnaud, S.C., Dixon, M.W.A., Nie, C.Q., Chappell, L., Sanders, P.R., Nebl, T., Hanssen, E., Berriman, M., Chan, J.A., Blanch, A.J., Beeson, J.G., Rayner, J.C., Przyborski, J.M., Tilley, L., Crabb, B.S., Gilson, P.R., 2017. The exported chaperone Hsp70-x supports virulence functions for Plasmodium falciparum blood stage parasites. *PLoS One* 12, 1–22.
- Daniyan, M.O., Przyborski, J.M., Shonhai, A., 2019. Partners in mischief: functional networks of heat shock proteins of Plasmodium falciparum and their influence on parasite virulence. *Biomolecules* 9, 2–17.
- Daugaard, M., Rohde, M., Jäättelä, M., 2007. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* 581, 3702–3710.
- De Koning-Ward, T.F., Dixon, M.W.A., Tilley, L., Gilson, P.R., 2016. Plasmodium species: master renovators of their host cells. *Nat. Rev. Microbiol.* 14, 494–507.
- Elsworth, B., Crabb, B.S., Gilson, P.R., 2014. Protein export in malaria parasites: an update. *Cell Microbiol.* 16, 355–363.
- Jensen, J.B., 1979. Some aspects of serum requirements for continuous cultivation of Plasmodium falciparum. *Bull. World Health Organ.* 57 (suppl. 1), 27–31.
- Kleizen, B., Braakman, L., 2004. Protein folding and quality control in the endoplasmic reticulum. *Curr. Opin. Cell Biol.* 16, 343–349.
- Kneuepfer, E., Rug, M., Klonis, N., Tilley, L., Cowman, A.F., 2005. Trafficking of the major virulence factor to the surface of transfected P falciparum-infected erythrocytes. *Blood* 105, 4078–4087.
- Külzer, S., Charnaud, S., Dagan, T., Riedel, J., Mandal, P., Pesce, E.R., Blatch, G.L., Crabb, B.S., Gilson, P.R., Przyborski, J.M., 2012. Plasmodium falciparum-encoded exported hsp70/hsp40 chaperone/co-chaperone complexes within the host erythrocyte. *Cell Microbiol.* 14, 1784–1795.
- Kumar, N., Koski, G., Harada, M., Aikawa, M., Zheng, H., 1991. Induction and localization of Plasmodium falciparum stress proteins related to the heat shock protein 70 family. *Mol. Biochem. Parasitol.* 48, 47–58.
- LaCount, D.J., Vignali, M., Chettier, R., Phansalkar, A., Bell, R., Hesselberth, J.R., Schoenfeld, L.W., Ota, I., Sahasrabudhe, S., Kurschner, C., Fields, S., Hughes, R.E., 2005. A protein interaction network of the malaria parasite Plasmodium falciparum. *Nature* 438, 103–107.
- Lambros, C., Vanderberg, J.P., 1979. Synchronization of Plasmodium falciparum erythrocytic stages in culture. *J. Parasitol.* 65, 418–420.
- Le Roch, K.G., Zhou, Y., Blair, P.L., Grainger, M., Moch, J.K., Haynes, J.D., De La Vega, P., Holder, A.A., Batalov, S., Carucci, D.J., Winzeler, E.A., 2003. Discovery of gene function by expression profiling of the malaria parasite life cycle. *Science* 301, 1503–1508.
- Marapana, D.S., Dagley, L.F., Sandow, J.J., Nebl, T., Triglia, T., Pasternak, M., Dickerman, B.K., Crabb, B.S., Gilson, P.R., Webb, A.I., Boddey, J.A., Cowman, A.F., 2018. Plasmeprin V cleaves malaria effector proteins in a distinct endoplasmic reticulum translocation interactome for export to the erythrocyte. *Nat. Microbiol.* 3, 1010–1022.
- Martinez, S.L., Clavijo, C.A., Winograd, E., 1998. Identification of peripheral membrane proteins associated with the tubo-vesicular network of Plasmodium falciparum infected erythrocytes. *Mol. Biochem. Parasitol.* 91, 273–280.
- Matthews, K.M., Pitman, E.L., de Koning-Ward, T.F., 2019. Illuminating how malaria parasites export proteins into host erythrocytes. *Cell Microbiol.* 21, 1–10.
- Mayer, M.P., Bukau, B., 2005. Hsp70 chaperones: cellular functions and molecular mechanism. *Cell. Mol. Life Sci.* 62, 670–684.
- Pasternak, N.D., Dzikowski, R., 2009. PfEMP1: an antigen that plays a key role in the pathogenicity and immune evasion of the malaria parasite Plasmodium falciparum. *Int. J. Biochem. Cell Biol.* 41, 1463–1466.
- Prajapati, S., Singh, O.P., 2013. Remodeling of human red cells infected with Plasmodium falciparum and the impact of PfHIST proteins. *Blood Cells Mol. Dis.* 51, 195–202.
- Shonhai, A., Boshoff, A., Blatch, G.L., 2007. The structural and functional diversity of Hsp70 proteins from Plasmodium falciparum. *Protein Sci.* 16, 1803–1818.
- Sprangers, J., Rabouille, C., 2015. SEC16 in COPII coat dynamics at ER exit sites. *Biochem. Soc. Trans.* 43, 97–103.
- Takakuwa, J.E., Nitika, Knighton, L.E., Truman, A.W., 2019. Oligomerization of Hsp70: current perspectives on regulation and function. *Front. Mol. Biosci.* 6, 1–7.
- Trager, W., Jensen, J., 1976. Human malaria parasites in continuous culture. *Science* 193, 673–675, 80.
- Walker, J.M., 1994. The bicinchoninic acid (BCA) assay for protein quantitation. *Methods Mol. Biol.* 32, 5–8.
- Winograd, E., Clavijo, C.A., Bustamante, L.Y., Jaramillo, M., 1999. Release of merozoites from Plasmodium falciparum-infected erythrocytes could be mediated by a non-explosive event. *Parasitol. Res.* 85, 621–624.
- Wiser, M.F., Lanners, H.N., Bafford, R.A., Favaloro, J.M., 1997. A novel alternate secretory pathway for the export of Plasmodium proteins into the host erythrocyte. *Proc. Natl. Acad. Sci. U.S.A.* 94, 9108–9113.
- Wiser, M.F., 2007. Export and trafficking of Plasmodium proteins within the host erythrocyte. *Acta Biol. Colomb.* 12, 3–18. ISSN electrónico 1900-1649.