Chapter 9
Chaperones and Proteases of *Plasmodium falciparum*

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Overview of Molecular Chaperones and Proteases

Proteins are responsible for carrying out the vast majority of biological functions in the cell. They are the most structurally complex macromolecules and their folding and degradation are tightly regulated processes mediated by chaperones and proteases. Thus maintaining proper protein homeostasis is extremely important for cell biology during normal and stress conditions.

In vitro, small proteins can fold in a short amount of time, within microseconds (Kubelka et al. 2004). Larger proteins may take considerably longer to fold, from minutes to hours (Herbst et al. 1997). *In vivo*, the folding of proteins is more difficult as the cytoplasm has an estimated protein concentration of 300–400 g/L (Hartl et al. 2011). This high concentration would drive self-assembly of polypeptide chains into harmful aggregates, the presence of which has been implicated in many diseases of aging such as Alzheimer's, Parkinson's, and Huntington's diseases, to name a few.

Molecular chaperones and proteases have evolved to ensure proper protein homeostasis (or proteostasis) in the crowded cellular environment. Chaperones are themselves proteins which interact with either nascent polypeptide chains as they emerge from the ribosome or with misfolded proteins to stabilize the native fold and prevent unwanted interactions. Chaperones are generally constitutively expressed but their levels can be further induced by heat shock or other cell stresses; thus many chaperones are named as Heat Shock Proteins or Hsp (Ritossa 1964). Most chaperones are highly conserved across species from prokaryotes to eukaryotes. Broadly, chaperones can be divided into a few classes depending on their molecular weight (prokaryotic ortholog in parenthesis): Hsp100 (ClpX and others), Hsp90 (HtpG), Hsp70 (DnaK), Hsp40 (DnaJ), Hsp60 (GroEL), Hsp10 (GroES), and small Hsps.

As part of maintaining proper protein homeostasis, proteins may be directed to proteases for degradation. To ensure that protein degradation proceeds in a specific regulated fashion, cells use multi-subunit proteases where the protease active sites are

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within a gated proteolytic chamber. As such, these proteases are termed chambered or self-compartmentalized proteases (Pickart and Cohen 2004). Similar to chaperones, chambered proteases also show significant similarities across kingdoms. In eukaryotes, the most well-studied system is the ubiquitin-proteasome system and its counterpart in prokaryotes are the HslV and ClpP systems. In prokaryotes, HslV forms dodecameric oligomers composed of identical stacked hexamers, while ClpP forms tetradecameric oligomers composed of identical stacked heptamers. Each subunit has a proteolytic active site. In eukaryotes, the 20S core particle (CP) of the proteasome is composed of 2 heptamers of 7 nonidentical β subunits sandwiched between 2 heptamers composed of 7 nonidentical α subunits. The protease activity in the proteasome is confined to 3 of the β subunits. Specificity for the eukaryotic proteasome is achieved through polyubiquitination of target proteins. For prokaryotes, specificity is achieved through an intrinsic motif in the polypeptide chain of the protein.

Since many substrates of chambered proteases are well folded and thus too large to fit into the proteolytic chamber, chaperone unfoldases are needed to unfold the substrate and thread it into the proteolytic chamber of the protease. These chaperones, which form the cap of the chambered protease, use ATP hydrolysis to unfold potential substrates and translocate them into the proteolytic chamber. In prokaryotes, this is accomplished by chaperones of the AAA+ (*A*TPases *A*ssociated with diverse cellular *A*ctivities) superfamily such as ClpX or ClpA. In eukaryotes, unfolding of protein substrates is done by the 19S regulatory particle (RP) cap composed of 17 subunits: 8 subunits in the 'base' complex of the cap and 9 in the 'lid' complex of the cap. Six of the subunits in the 'base' complex are AAA+ proteins.

Plasmodium falciparum, which is the causative agent in the most severe form of human malaria, has a unique life cycle which involves the poikilothermic female anopheles mosquito and the warm-blooded human host. This transition to the human host, results in a significant heat shock to the parasite. Furthermore, symptoms of malaria often include febrile episodes of the host leading to further heat shock of the parasite. As such, to maintain optimal proteostasis, the plasmodial genome encodes about 92 chaperones and 92 proteases (Wu et al. 2003; Acharya et al. 2007). Many of the chaperone and protease families are well represented in the *P. falciparum* genome. This review will attempt to summarize the available biochemical data on the chaperones and proteases involved in plasmodial proteostasis, namely: Hsp90, Hsp70, Hsp40, Hsp60, Hsp10, proteasome, and Clp proteases.

Molecular Chaperones of *P. falciparum*

Hsp90

Hsp90 is conserved across evolution. With the exception of archea, organisms of all kingdoms of life have one or multiple genes encoding Hsp90 isoforms. Hsp90 is highly expressed in the cell at 1–2 % of total cellular protein levels (Borkovich et al. 1989). Crystal structures of full length Hsp90 from bacteria (Shiau et al. 2006), yeast (Ali et al. 2006) and mammals (Dollins et al. 2007) have been solved. Not surprisingly, the structures reveal similar domain architectures. Hsp90 functions as

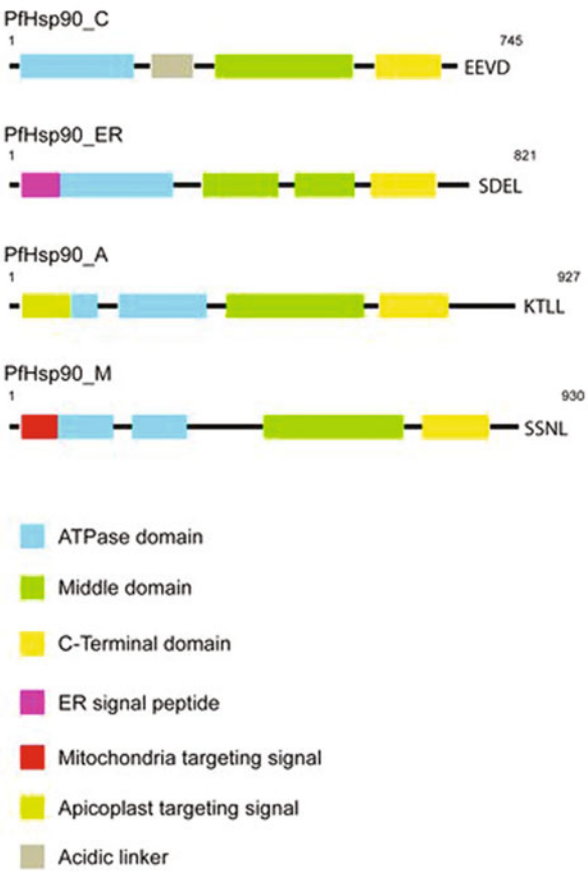

Fig. 9.1 Prediction of protein domains in PfHsp90 chaperones. Prediction was based on SUPERFAMILY (Gough et al. 2001). ER signal peptide and apicoplast targeting signal were predicted with PlasmoAP (Foth et al. 2003). Mitochondrial targeting signal was predicted with PlasMit (Bender et al. 2003)

a dimer and consists of a highly conserved N-terminal domain (NTD), responsible for ATP binding and hydrolysis. The middle domain of Hsp90, which is connected to the NTD by a charged acidic linker thought to be important for cochaperone regulation of Hsp90 function (Hainzl et al. 2009; Tsutsumi et al. 2009), contains a hydrophobic patch important for client-Hsp90 interactions (Meyer et al. 2003). The C-terminal domain (CTD) is responsible for protein dimerization (Minami et al. 1994). In some isoforms of Hsp90, the final 5 amino acids are MEEVD. This motif is usually characteristic of cytosolic Hsp90s and is the binding site for tetratricopeptide repeat (TPR) containing cochaperones. Hsp90 is involved in diverse biological pathways in the cell including: regulation of signaling pathways, antigen processing, protein trafficking and secretion, and RNA processing. The parasite encodes four Hsp90 genes (Fig. 9.1, Table 9.1) of which only one gene, Hsp90_C (PF3D7_0708400) is cytosolic and the other three genes encode putative organellar Hsp90s. Hsp90_ER (PF3D7_1222300, Grp94), Hsp90_A (PF3D7_1443900), and Hsp90_M (PF3D7_1118200) are predicted to be localized to the ER, apicoplast and mitochondria, respectively (Fig. 9.1). Like other eukaryotic Hsp90s, malaria Hsp90 is shown to be essential for the parasite (Banumathy et al. 2003).

Table 9.1 Hsp90 chaperones of *P. Falciparum*

	PfHsp90_C PF3D7_0708400	PfHsp90_ER PF3D7_1222300	PfHsp90_A PF3D7_1443900	PfHsp90_M PF3D7_1118200
MW (kDa)	86	95	107	108
Chromosome	7	12	14	11
Cellular localization	Cytoplasm[a, b]	ER[c] N.C.	Apicoplast[c] N.C.	Apicoplast[c] N.C.
Expression stage (RNA levels from PlasmoDB)	Schizont N.C.	Trophozoite, Schizont N.C.	Schizont, Ookinete N.C.	Gametocyte V N.C.
ATPase activity	[d]$K_M = 611\ \mu M$ $k_{cat} = 9.9 \times 10^{-2} min^{-1}$	N.D.	N.D.	N.D.

N.C. not experimentally confirmed, *N.D.* not experimentally established
[a] (Bonnefoy et al. 1994)
[b] (Banumathy et al. 2003)
[c] (Pallavi et al. 2010a)
[d] (Pallavi et al. 2010b)

PfHsp90_C is expressed as a 745 amino acid protein with a mass of 86 kDa (Bonnefoy et al. 1994, Su and Wellems 1994). Sequence comparison with human Hsp90 (hHsp90) revealed 59 % identity and 69 % similarity (Banumathy et al. 2003). Similar to eukaryotic Hsp90, PfHsp90 contains 3 major domains: N-terminal ATP binding domain, middle domain, and a C-terminal dimerization domain, containing the EEVD motif (Fig. 9.1). The most important difference between human cytoplasmic Hsp90 (hHsp90) and PfHsp90 is an additional 30 amino acids to the linker region, located between the N-terminal ATP binding domain and middle domain. This charged linker region has been shown to be important for the regulation of hHsp90 ATPase activity by cochaperones (Scheibel et al. 1999; Hainzl et al. 2009) and for hHsp90 secretion (Tsutsumi et al. 2009). PfHsp90 is not predicted to be exported out of the parasite due to lack of Plasmodium export element (PEXEL) and no study to date has reported PfHsp90 secretion.

The crystal structure of the N-terminal ATP binding domain has been solved for both hHsp90 and PfHsp90. Superposition of crystal structures show high similarity between the human and plasmodium proteins, with an overall r.m.s.d. of 0.79 Å (Corbett and Berger 2010). Biochemical characterization of Hsp90 however, showed that PfHsp90 is a hyperactive ATPase as it binds ATP with 30 % higher affinity and has six times higher ATPase activity than hHsp90 *in vitro* (Table 9.1) (Pallavi et al. 2010a). No studies have been done to elucidate the mechanism of the difference in ATPase activity between these two Hsp90 homologues. Despite this, higher ATPase activity could imply higher substrate turnover through the PfHsp90 chaperone cycle. This is consistent with the life cycle of the parasite, where the parasite experiences repeated episodes of heat shock among other stresses both upon entry into the host and during febrile episodes inside the host. Thus PfHsp90 may have been selected to have higher client turnover in the parasite.

PfHsp90 shares functional similarities with its mammalian counterpart. PfHsp90 has been found in a 450 kDa complex with PfHsp70 and two other proteins of 60 kDa and 50 kDa through a variety of immunoprecipitation experiments (Banumathy et al. 2003; Pavithra et al. 2004). Hsp90 and Hsp70 interaction in eukaryotes is known to be mediated by Hsp70-Hsp90 organizing protein (Hop) via TPR motifs in Hop (Nicolet and Craig 1989). Recently, a 66 kDa PfHop was shown to colocalize with PfHsp90 and PfHsp70 in the cytosol of trophoziote stage 3D7 parasites at 37 °C (Gitau et al. 2012). It was found that upon heat shock, PfHsp90 and PfHsp70 traffic into the nucleus, where they are thought to aid in the folding of transcription factors.

PfHsp90 interacts with parasite cochaperones such as PfPP5, Pfp23, and PfAha1 (Pavithra et al. 2004; Acharya et al. 2007; Chua et al. 2010; Chua et al. 2012). Based on a series of transcript expression studies done from patient derived samples, PfHsp90 has been proposed to be involved in many different processes important to the parasite biology. In addition to the aforementioned biochemical characterization of PfHsp90 interaction partners, PfHsp90 was shown to have concomitant expression with malarial signal transduction pathway proteins, other co-chaperones, and proteins important in ribosome function (Pallavi et al. 2010b). Although these studies are based solely on transcript levels, they present an important first analysis of the role of Hsp90 in malarial biology and spur further research to biochemically characterize relevant Hsp90-containing complexes.

PfHsp90 is a promising target for development of drugs to treat malaria. Geldanamycin (GA), a benzoquinone ansamycin antibiotic that inhibits Hsp90 ATPase activity, which is also in stage III clinical trials as anti-cancer drug, binds to PfHsp90 (Banumathy et al. 2003). *In vitro*, the $IC_{50(ATPase)}$ of GA for PfHsp90 was found to be 3 times lower than that for hHsp90, indicating PfHsp90 is more sensitive to GA inhibition. *In vivo*, GA was shown to inhibit Ring to Trophozoite stage progression of the parasite. However, the transition from Trophozoite to Schizont and from Schizont to Merozoite release was not affected (Banumathy et al. 2003). GA has been proven to be far too toxic in mammals (Dey and Cederbaum 2006, 2007; Kitson et al. 2013), but GA derivatives with less toxic profiles might be promising antimalarial drugs. Furthermore, novel Hsp90 inhibitors have recently been identified that may serve as new leads for antimalarial drugs including: (±)-2-amino-3-phosphonopropionic acid, harmine (harmaline), and acrisorcin (Shahinas et al. 2010; Shahinas et al. 2012).

Hsp70

70-kDa heat shock proteins (Hsp70s) are ATP-dependent molecular chaperones responsible for the execution of many diverse proteostasis maintenance activities including folding of nascent polypeptides (Pfund et al. 1998; Hartl and Hayer-Hartl 2002), refolding of misfolded and aggregated proteins, and protein translocation into organelles (Walton et al. 1994; Ryan and Pfanner 2002), among others. Hsp70 carries out its chaperone activity through ATP-dependent interaction with hydrophobic segments of client proteins. Canonical Hsp70 (bacterial DnaK) consists of a 45 kDa N-terminal ATPase domain (NBD) followed by a 15 kDa substrate binding domain (SBD), and a 10 kDa C-terminal subdomain (CTD).

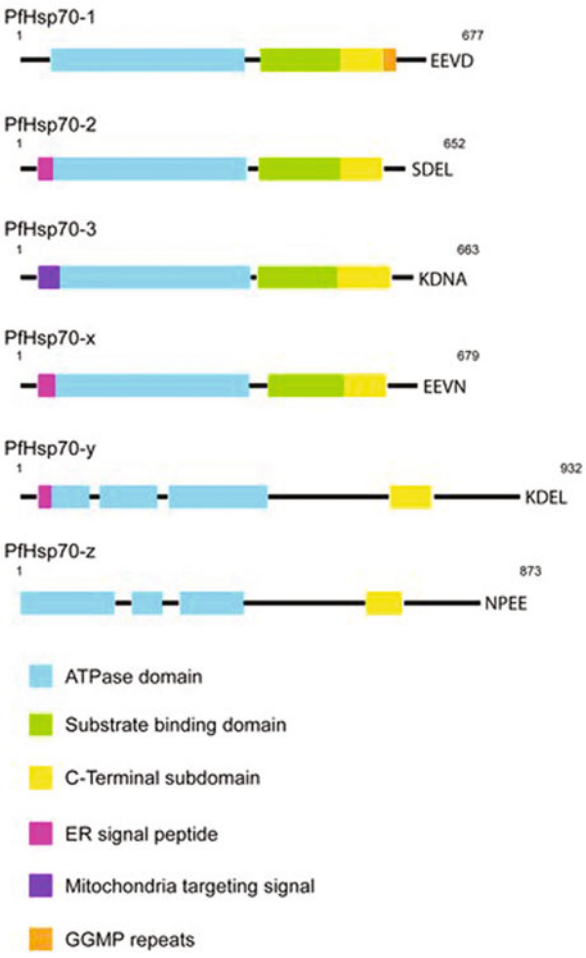

Fig. 9.2 Prediction of protein domains of all 6 PfHsp70 chaperones

The parasite encodes six genes of Hsp70 (Fig. 9.2, Table 9.2): PfHsp70-1 (PfHsp70_C, PF3D7_0818900), PfHsp70-2 (PfHsp70_ER, PF3D7_0917900), PfHsp70-3 (PfHsp70_M, PF3D7_1134000), PfHsp70-x (PF3D7_0831700), PfHsp70-y (PF3D7_1344200) and PfHsp70-z (PF3D7_0708800) (Table 9.2). The expression of PfHsp70-1 is confirmed in the cytoplasm and this protein translocates into the nucleus upon heat shock (Kumar et al. 1991). The protein contains a C-terminal EEVD motif, similar to other cytosolic eukaryotic Hsp70s. PfHsp70-1 interacts with PfHop (Gitau et al. 2012) and is thought to interact with other cochaperones via their TPR domains.

PfHsp70-2 is a homolog to the mammalian ER glucose-regulated protein (Grp78) (Kumar and Zheng 1992; Kappes et al. 1993). The protein codes for a C-terminal ER retention signal SDEL instead of the canonical KDEL, encoded by eukaryotic ER resident proteins (Pelham 1989). Through immuno-electron microscopy experiments, it was determined that PfHsp70-2 is largely localized to the parasite ER

Table 9.2 Hsp70 chaperones of *P. Falciparum*

	PfHsp70-1 PF3D7_0818900	PfHsp70-2 PF3D7_0917900	PfHsp70-3 PF3D7_1134000	PfHsp70-x PF3D7_0831700	PfHsp70-y PF3D7_1344200	PfHsp70-z PF3D7_0708800
MW (kDa)	74	73	73	76	108	100
Chromosome	8	9	11	7	13	7
Cellular localization	Nucleus + Cytoplasm[a]	ER[a]	Mitochondrion[b,c] N.C.	Exported to host cell, PV[d] and Maurer's clefts[g]	ER[b] N.C.	Cytoplasm[b] N.C.
Expression stage (RNA levels from PlasmoDB)	Trophozoite, Schizont N.C.	Schizont N.C.	Schizont N.C.	Schizont[g]	Schizont N.C.	Schizont N.C.
ATPase activity	[e]$K_M = 594.5\mu M$ $V_{max} = 8.9$ nmol min^{-1} mg^{-1} [f]$K_M = 616.5\ \mu M$ $V_{max} = 14.6$ nmol min^{-1} mg^{-1}	N.D.	N.D.	N.D.	N.D.	N.D.

N.C. not experimentally confirmed, *N.D.* not experimentally determined

[a] (Kumar et al. 1991)

[b] (Sargeant et al. 2006)

[c] (Slapeta and Keithly 2004)

[d] (Külzer et al. 2012)

[e] (Misra and Ramachandran 2009)

[f] (Matambo et al. 2004)

[g] (Grover et al. 2013)

(Kumar and Zheng 1992). However, PfHsp70-2 was not induced under glucose limiting conditions, which was shown to induce eukaryotic Grp78 expression (Kumar and Zheng 1992). Based on high-throughput yeast two-hybrid assays, PfHsp70-2 is expected to potentially interact with more proteins than PfHsp70-1 and PfHsp70-3 (LaCount et al. 2005), however, no data to date have shown the biochemical activity of this chaperone.

PfHsp70-3 is predicted to be targeted to the mitochondria of the parasite (Fig. 9.2). However, this protein was found to be exported to Mauer's clefts (Vincensini et al. 2005). Maurer's clefts are extra-parasitic membranous secretory organelles responsible for protein sorting and export to the RBC plasma membrane (Lanzer et al. 2006; Sam-Yellowe 2009). Very little is known about this PfHsp70 protein.

PfHsp70-x shares high sequence similarity (71 %) with PfHsp70-1. Although PfHsp70-x contains a C-terminal EEVN motif instead of the canonical EEVD motif (Fig. 9.2), this protein is predicted to be cytoplasmic. A recent study done by Külzer and coworkers (Külzer et al. 2012), surprisingly, observed PfHsp70-x to be exported into the host erythrocyte (Table 9.2). This finding was corroborated by an independent study done by Grover and coworkers (Grover et al. 2013). They found PfHsp70-x to be in the host cytoplasm, parasitophorous vacuole (Külzer et al. 2012; Grover et al. 2013) and partially with Maurer's clefts (Grover et al. 2013; Table 9.2). Külzer and coworkers found PfHsp70-x to interact with and form a complex with malarial PfHsp40 proteins in the host erythrocyte in structures called J-dots. Interestingly, PfHsp70-x does not contain PEXEL signal motif for export from the parasite (Külzer et al. 2012; Grover et al. 2013). Thus, PfHsp70-x may represent a new class of malarial proteins, containing an alternative export signal from the parasite. Additionally, it has been thought that exported PfHsp40 proteins primarily interact with host Hsp70 proteins to help proteins of malarial origin maintain correct folding in the host cell. Although a functional significance for the interaction between exported PfHsp70-x and exported PfHsp40 has not been established, the export of PfHsp70-x challenges the aforementioned idea and suggests that the parasite actively secretes chaperones to help remodel the host erythrocyte instead of borrowing the host chaperone machinery.

PfHsp70-y, which has a putative ER retention sequence KDEL (Sargeant et al. 2006), and PfHsp70-z, which is cytoplasmic, are thought to be members of the Hsp110/Grp170 protein families that are specialized Hsp70s possessing nucleotide exchange factor (NEF) activity for other Hsp70 proteins (Shonhai et al. 2007). Thus, PfHsp70-y and PfHsp70-z could act as NEFs for other PfHsp70s in the ER and cytoplasm, respectively (Fig. 9.2 and Table 9.2). PfHsp70-y and PfHsp70-z have the conserved ATPase domains but have a diverged substrate binding domain sequence (Shonhai et al. 2007). Unlike other members of the Hsp70 family, PfHsp70-y and PfHsp70-z lack a linker region responsible for allosteric control of Hsp70 function (Shonhai et al. 2007).

Of all of the Hsp70 proteins identified in the parasite, PfHsp70-1 is by far the best characterized (Table 9.2). Similar to eukaryotic Hsp70s, it contains an N-terminal nucleotide binding domain (NBD), followed by a substrate binding domain (SBD) and a C-terminal domain (CTD). Its expression was confirmed in the cytoplasm by

Kumar and coworkers (Kumar et al. 1991). The transcription of PfHsp70-1 gene as well as protein levels were found to be increased when parasites were grown at 39 °C (Joshi et al. 1992; Biswas and Sharma 1994). *In vitro* ATPase assays showed an optimal ATPase activity at 50 °C and the ATPase activity was maintained even at 80 °C (Misra and Ramachandran 2009). Matambo and coworkers purified recombinant PfHsp70-1 *in vitro* and found that it is mainly monomeric in solution with very few dimers and higher molecular weight oligomers formed (Matambo et al. 2004). Similar to PfHsp90, PfHsp70-1 had significantly higher ATPase activity compared to human Hsp70 by about 2.5 fold. However, the K_M for PfHsp70-1 was significantly higher than human Hsp70 by about 7.25 fold (Matambo et al. 2004). These data suggest that although PfHsp70-1 has higher ATP hydrolysis and turnover rate, its low affinity for ATP could make it susceptible to changes in ATP concentrations (Matambo et al. 2004). Misra and coworkers have also measured the ATPase activity of PfHsp70-1 and reported similar results (Misra and Ramachandran 2009). In the presence of the Hsp40 Pfj1, the ATPase activity of PfHsp70-1 was further enhanced (Misra and Ramachandran 2009).

The chaperone activity of PfHsp70-1 has been investigated. It was shown that PfHsp70-1 was able to refold glucose-6-phosphate dehydrogenase (G6PDH) and alpha glucosidase (Misra and Ramachandran 2009). *In vivo* experiments using a bacterial complement system showed that PfHsp70-1 and a chimeric version of PfHsp70-1, containing the ATPase domain of DnaK (*E. coli* Hsp70) and the substrate binding domain of PfHsp70-1 was able to rescue the thermosensitivity of *E. coli* dnaK756 strain at 40°C. This strain expresses a mutant form of DnaK and this mutant is partially functional at 37°C and non-functional at 40°C (Lopes Ferreira and Alix 2002). However, PfHsp70-1 was not able to rescue the thermosensitivity of *E. coli* dnaK103 strain, encoding a truncated DnaK (Shonhai et al. 2005). Another complementation study was done in yeast *S. cerevisae*. PfHsp70-1 was shown to be able to rescue deletions of yeast Hsp70s, SSA1 and SSA2, as well as a temperature sensitive strain expressing SSA1 (Bell et al. 2011). The rescue was shown to be dependent on the ATPase activity of PfHsp70-1 since a PfHsp70-1 G214D ATPase deficient mutant did not rescue thermosensitivity in the aforementioned strains. In addition, PfHsp70-1 was shown to ameliorate Hsp70 chaperone dependent processes such as protein translocation and ER associated degradation (ERAD) and reduced the toxic effects of oxidative stress (Bell et al. 2011).

PfHsp70-1 has been implicated in aiding protein translocation into the apicoplast. It is estimated that a major proportion of malarial proteins (94.4 %) destined for the apicoplast have predicted Hsp70 binding sites (Misra and Ramachandran 2010). Using previously determined rules for predicting transit peptide sequences into the apicoplast (Foth et al. 2003), Misra and coworkers were able to show that synthetic apicoplast targeted peptides were able to bind to PfHsp70-1.

Hence, based on the currently available data, there is strong evidence of the global chaperone activity of PfHsp70-1.

Hsp40

Hsp40 chaperones are generally thought to serve as co-chaperones for Hsp70 proteins, but also have functions independent from Hsp70s. Hsp40s are not ATPases, but rather act to enhance the ATPase activity of Hsp70s. Hsp40 proteins contain a conserved region of 70 amino acids called the J-domain usually located at their N-terminus. Within the J-domain is the HPD motif implicated in the interaction with Hsp70 chaperone. Domains required for substrate binding and dimerization are generally at the C-terminus of a canonical Hsp40 protein (Borges et al. 2005; Wu et al. 2005). When Hsp40 bound with client protein binds to Hsp70, client protein is transferred to the latter. Thus, Hsp40 expands the repertoire of client proteins that can interact with Hsp70. Hsp40 proteins are well studied and are involved in many different cellular processes, including: protein folding, prevention of protein aggregation, transcription, protein complex assembly, and trafficking of proteins into subcellular organelles.

P. falciparum encodes 43 genes for Hsp40 proteins. Broadly, Hsp40s can be divided into four distinct types based on the classification scheme proposed by Botha et al. (Botha et al. 2007). Type I Hsp40 contains four canonical domains: N-terminal J-domain having an HPD motif required for Hsp70 binding, followed by a GF rich region, important in the regulation of substrate binding (Yan and Craig 1999). Following the GF region, is the zinc-binding domain, which contains CXXCXGXG motifs that coordinate binding of two zinc atoms. At the C-terminus is the substrate binding domain. Type II Hsp40 lacks the zinc-binding domain. Type III Hsp40 contains only the J-domain and this domain may reside anywhere in the protein. Type IV Hsp40 contains variations in the conserved HPD motif in the J-domain and is thus not expected to interact with Hsp70 chaperones. 19 of the 43 PfHsp40 proteins contain PEXEL motifs and are predicted to be exported into the host cytoplasm. 11 out of the 12 Type IV Hsp40s are expected to be exported. These proteins are thought to aid protein export into the cytoplasm or in the remodeling of the host erythrocyte.

P. falciparum encodes two type I Hsp40 proteins [Pfj1 (PF3D7_0409400) and PfHsp40 (PF3D7_1437900), Fig. 9.3, Table 9.3] both proteins do not contain an export sequence and are shown to be intracellular. Pfj1 mRNA level was found to increase after heat shock at 43 °C (Watanabe 1997). Whether the increase in transcript level after heat shock corresponds to an increase in protein level is not known. This protein has been shown to be localized to the parasite apicoplast where it is proposed to aid in apicoplast DNA replication (Kumar et al. 2010). The J-domain of Pfj1 was shown to be interchangeable with bacterial J-domain when chimera of this protein was used in *E. coli* complementation studies in thermosensitive strain OD259 (Nicoll et al. 2007). The experiments show that plasmodial J-domain are capable of interacting with bacterial DnaK. However, since there are no recognized Hsp70 predicted to localize to the apicoplast of the parasite, this study seems to suggest that Pfj1 could be a functional chaperone in the cell, but its PfHsp70 partner still remains enigmatic.

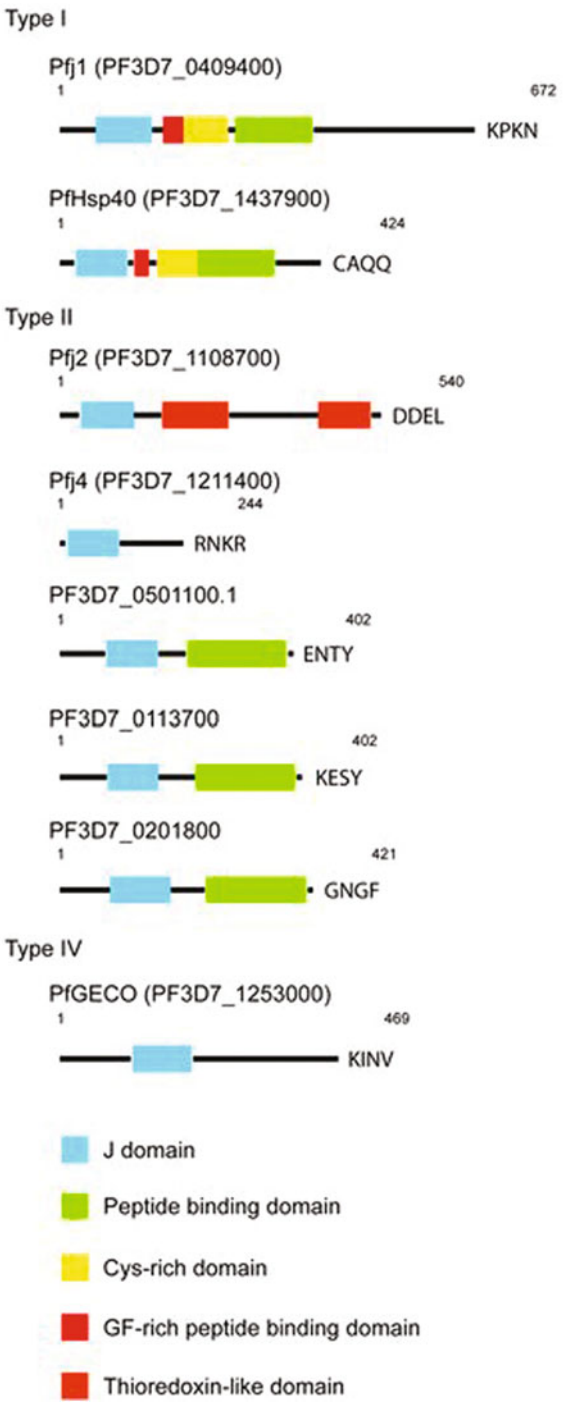

Fig. 9.3 Prediction of protein domains in PfHsp40 chaperones. PfHsp40 proteins of type I, II and IV discussed in the text are shown. Type III PfHsp40 chaperones are not shown since no members have been experimentally studied. Note that for PF3D7_0501100.1 there is another splice variant, PF3D7_0501100.2, which is 390 amino acids long

Table 9.3 Studied Hsp40 chaperones of *P. Falciparum*

	Type I		Type II					Type IV
	Pfj1 PF3D7_ 0409400	PfHsp40 PF3D7_ 1437900	Pfj2 PF3D7_ 1108700	Pfj4 PF3D7_ 1211400	PF3D7_ 0501100.1[e]	PF3D7_ 0113700	PF3D7_ 0201800[f]	PfGECO PF3D7_1253000
MW (kDa)	76	48	62	28	46	47	48	56
Chromosome	4	14	11	12	5	1	2	12
Cellular localization	Apicoplast[a]	Cytoplasm[c]	Could be exported Could be ER resident chaperone N.C.	Nucleus + cytoplasm[d]	J-dots[e]	J-dots[e]	Exported to Knob associated structures[f]	Exported to RBC cytoplasm[g]
Expression stage (RNA levels from PlasmoDB)	Trophozoite Detected expression throughout intraerythrocytic stages[a] Merozoite[i]	Trophozoite[c] Merozoite[i]	Constitutively transcribed[j]	Trophozoite, Schizont[d]	Trophozoite, Schizont[e]	Stable throughout cell cycle[e]	Synthesis begins after invasion[f]	Gametocyte I-IV[g]

Table 9.3 (continued)

	Type I		Type II					Type IV
	Pfj1 PF3D7_ 0409400	PfHsp40 PF3D7_ 1437900	Pfj2 PF3D7_ 1108700	Pfj4 PF3D7_ 1211400	PF3D7_ 0501100.1[e]	PF3D7_ 0113700	PF3D7_ 0201800[f]	PfGECO PF3D7_1253000
Additional Information	J-domain chimera reverses thermosensitivity of E. Coli OD259 strain[b] Involved in apicoplast DNA replication[a] Enhance activity of Hsp70-1[h]	Enhance ATPase activity of Hsp70-1[c] Chaperone activity in MDH aggregation assays[c]	Transcript levels decrease upon heat shock[j]	Forms complex with Hsp70-1[d] J-domain chimera reverses thermosensitivity of E. Coli OD259 strain[b]	Found in structures called J-dots. Interacts with Hsp70-x[e]	Found in structures called J-dots. Interacts with Hsp70-x[e]	Exported mainly in Trophozoite stages[f] Contains PEXEL motif and associates with PEXEL translocon[f]	Not required for gametogenesis[g]

N.C. not experimentally confirmed
[a] (Kumar et al. 2010)
[b] (Nicoll et al. 2007)
[c] (Botha et al. 2011)
[d] (Pesce et al. 2008)
[e] (Külzer et al. 2010). Note that there is another splice variant PF3D7_0501100.2 of 45 kDa.
[f] (Acharya et al. 2012). The protein is also named KAHsp40.
[g] (Morahan et al. 2011)
[h] (Misra and Ramachandran 2009)
[i] (Pesce and Blatch 2009)
[j] (Watanabe 1997)

The other type I Hsp40 (PfHsp40) was shown to be a primarily cytosolic protein (Fig. 9.3, Table 9.3). Its expression was found to be up-regulated by heat shock, in a similar fashion as PfHsp70-1 (Botha et al. 2011). Purified PfHsp40 increases the ATPase activity of PfHsp70-1, indicating direct interaction between the proteins. PfHsp40 chaperone activity was also assessed using malate dehydrogenase (MDH) aggregation assays. PfHsp40 alone had moderate chaperone activity in preventing MDH aggregation, however, in the presence of PfHsp70-1, this PfHsp70-PfHsp40 complex was 6 times more effective in preventing MDH aggregation as compared to PfHsp40 alone (Botha et al. 2011). The localization and chaperone activity of PfHsp40 suggests that it likely functions with PfHsp70-1 in the parasite cytoplasm.

P. falciparum encodes nine type II Hsp40 proteins, four of which are predicted to be exported out of the parasite (Fig. 9.3, Table 9.3). Five type II Hsp40 proteins have been characterized so far. Pfj4 (PF3D7_1211400), whose mRNA expression level increases after heat shock (Watanabe 1997), was also shown to have increased protein expression after heat shock (Pesce et al. 2008). Pfj4 was found to be localized primarily to the cytoplasm and nucleus. Reciprocal immunoprecipitation experiments and size exclusion chromatography established that Pfj4 forms a complex with PfHsp70-1 *in vivo* (Pesce et al. 2008). Pfj4 J-domain chimera with *E. coli* DnaJ was able to reverse the thermosensitivity of *E. coli* OD259 strain, indicating that the J-domain of Pfj4 can interact with bacterial DnaK (Nicoll et al. 2007).

PF3D7_0501100.1 and PF3D7_0113700 (Fig. 9.3, Table 9.3) were elegantly determined to be part of novel structures within the host erythrocyte which the authors termed 'J-dots' as they contain a high amount of J proteins. J-dots were shown to be distinct from Maurer's clefts (Külzer et al. 2010). These structures are membranous and may function in transport of proteins through the host cytosol. PF3D7_0501100.1 can form a complex with PfHsp70-x, the exported homolog of cytosolic PfHsp70-1 (Külzer et al. 2012). Protein PF3D7_0201800 (also named as KAHsp40 for *K*nob *A*ssociated *Hsp40*), was shown to be exported to the host cytoplasm. Immunofluorescence analysis has indicated that this protein associates with parasite encoded knob component proteins such as PfEMP3 and KAHRP. Knob structure is a result of remodeling of the host erythrocyte by the parasite and is involved in cytoadherence of remodeled RBC to the vasculature to evade splenic clearance (Acharya et al. 2012). PF3D7_1108700 (Pfj2) is constitutively expressed but its transcript levels decrease during heat shock (Watanabe 1997). The presence of DDEL motif at the C-terminus of Pfj2 seems to suggest that this protein may be retained in the ER and function in ER associated proteostasis.

Type III Hsp40 proteins of *P. falciparum* constitute the largest family of Hsp40 proteins, with 20 members, of which four are predicted to be exported. This family is, however, the least characterized of the four families. Currently, we could not find literature documenting the biochemistry or chaperone activity of Type III Hsp40 family members.

Type IV Hsp40 proteins consist of 12 proteins, of which 11 are predicted to be exported. Protein PF3D7_1253000 (PfGECO for *P. falciparum g*ametocyte *e*rythrocyte *cyto*solic protein; Fig. 9.3, Table 9.3) is expressed during gametocyte stages of the parasite, but is not essential for gametocytogenesis *in vitro*. The protein is exported to the RBC cytoplasm, however, its expression is not increased by heat shock (Morahan et al. 2011).

Hsp60

Chaperones of the Hsp60 class form 'test tube' like structures and function by sequestering the substrate from its environment within this test tube where it can fold in isolation. Thus, this class of chaperones is often referred to as chaperonins (Cpn). Cpn60 (also called Hsp60) type I chaperonins exemplified by GroEL in prokaryotes typically oligomerize into tetradecameric rings composed of two stacked heptamers forming two large cavities (Braig et al. 1994) inside which protein folding can occur. GroEL exerts its chaperone activity by forming a complex with its cochaperonin GroES (Cpn10 or Hsp10), which acts as a cap for the GroEL cavity. Typical Cpn60 have molecular weights of around 60 kDa and are found in the cytoplasm of prokaryotes and in the mitochondria of eukaryotes. Cpn10 usually has a molecular weight of about 10 kDa and forms a heptameric ring that interacts with the apical domain of Cpn60 to cap it (Xu et al. 1997). Cpn10 proteins consist of around 100 conserved amino acids forming the Cpn10 domain. Residues within the Cpn10 domain are thought to interact with Cpn60 (Landry et al. 1993). Plant plastids contain proteins with duplicated Cpn10 domains arranged in tandem, these proteins are known as Cpn20 (Bertsch et al. 1992). Cpn20 in *Arabidopsis thaliana* forms tetramers upon binding to Cpn60 (Koumoto et al. 1999).

P. falciparum has two members of the Hsp60 class of molecular chaperones, encoded by genes PF3D7_1232100 and PF3D7_1015600 in the parasite genome (Fig. 9.4, Table 9.4). *P. falciparum* chaperonin 60 (PfCpn60) is encoded by the former and produces a protein of ~ 81.2 kDa with a 25–30 % sequence identity to proteins in the Hsp60 family (Holloway et al. 1994). *P. falciparum* Hsp60 (PfHsp60) is encoded by the latter and produces a protein of ~ 61.2 kDa with a 54 % sequence identity to human Hsp60 (Syin and Goldman 1996; Das et al. 1997). Surprisingly, these parasite chaperones only share 29 % sequence identity with each other (Sanchez et al. 1999), which could indicate that they play different roles in the cell (Syin and Goldman 1996). Despite differences across species, Hsp60 is highly conserved within *Plasmodium spp.* where PfHsp60 shares 93 % identity with *Plasmodium yoelii* Hsp60 (PyHsp60), the causative agent of murine malaria (Sanchez et al. 1999).

PfCpn60 is poorly characterized. Although PfCpn60 contains a putative mitochondrial signal peptide (Holloway et al. 1994) and is predicted to be localized in the mitochondria, PfCpn60 was found by fluorescence tagging experiments to localize to the apicoplast (Sato and Wilson 2004; Sato and Wilson 2005). No chaperone activity has been reported in the literature for this protein.

Compared to PfCpn60, PfHsp60 is better characterized. Its expression and localization has been confirmed in the mitochondria of parasites (Das et al. 1997; Sato et al. 2003; Sato and Wilson 2005). PfHsp60 transcript levels increased 2–3 fold during asexual stage as compared to the sexual stage, indicating a developmentally regulated expression pattern (Syin and Goldman 1996). Not surprisingly, PfHsp60 transcript levels increased 3–4 fold when cells were heat shocked (Syin and Goldman 1996). Unexpectedly, immunoprecipitated PfHsp60 protein amounts did not show concomitant increase upon heat shock (Das et al. 1997). This may indicate regulation at the level of translation or protein stability (Das et al. 1997). Currently, no chaperone activity has been described in the literature for PfHsp60.

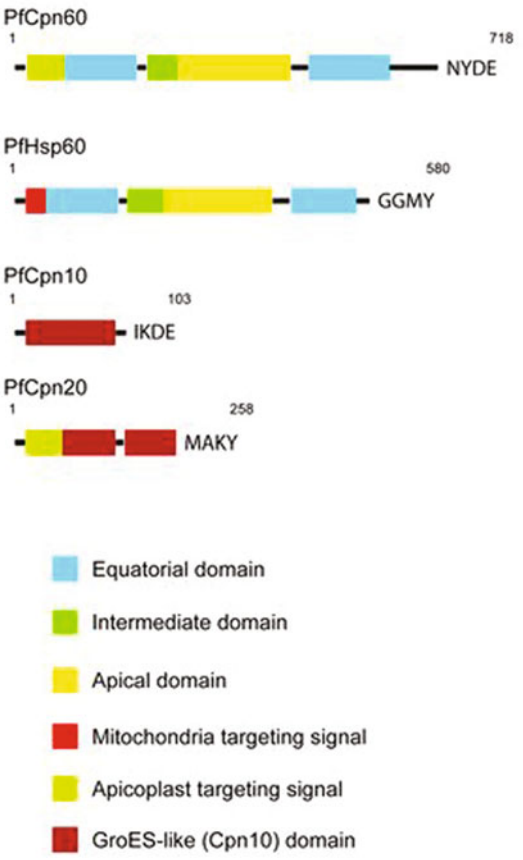

Fig. 9.4 Prediction of protein domains in the chaperonins and cochaperonins of *P. falciparum*

Table 9.4 Hsp60 chaperonins and Hsp10 cochaperonins of *P. Falciparum*

	PfCpn60 PF3D7_1232100	PfHsp60 PF3D7_1015600	PfCpn10 PF3D7_1215300	PfCpn20 PF3D7_1333000
MW (kDa)	81	62	11	29
Chromosome	12	10	12	13
Cellular localization	Apicoplast[a, b]	Mitochondria[b, c, d]	Mitochondria[b]	Apicoplast[b]
Expression stage (RNA levels from PlasmoDB)	Ring, Trophozoite, Schizont[a]	Ring, Schizont[a]	Ring, Schizont[b]	Ring, Schizont[b]

[a] (Sato and Wilson 2004)
[b] (Sato and Wilson 2005)
[c] (Sato et al. 2003)
[d] (Das et al. 1997)

P. falciparum encodes one PfCpn10 (PF3D7_1215300) and one PfCpn20 (PF3D7_1333000) (Fig. 9.4, Table 9.4). It was determined, through fluorescent protein tagging experiments that PfCpn10 localized to the mitochondria, whereas PfCpn20 localized to the apicoplast (Sato and Wilson 2005). This localization pattern seems to suggest the existence of a PfHsp60-PfCpn10 complex in the mitochondria and PfCpn60-PfCpn20 complex in the apicoplast. Further experiments are needed to determine the biological function of these distinct complexes.

Proteases of *P. falciparum*

Through bioinformatic analysis using exhaustive homology searches and comparative sequence analyses, 92 putative proteases were found in the *P. falciparum* genome (Wu et al. 2003). The putative proteases were classified into 5 distinct classes: aspartic, cysteine, metallo, serine and threonine based on their catalytic mechanisms. For this review, only the proteasome and the Clp ATP-dependent proteases will be discussed.

Proteasome

The proteasome is a threonine protease. The *P. falciparum* proteasome shares several features with the human proteasome as the *P. falciparum* genome encodes 14 genes for the proteasome: 7 α and 7 β subunits (Paugam et al. 2003; Mordmuller et al. 2006; Aminake et al. 2011). The proteasome was found to be expressed throughout the cell cycle (Mordmuller et al. 2006). Bioinformatic analysis has shown high sequence similarity between the *P. falciparum* and human proteasomes (Sessler et al. 2012). The subunits of the *P. falciparum* proteasome are expected to form a 28 subunit complex (analogous to the 20S component of human proteasome) composed of 4 rings stacked together, with the outer rings composed of the α1–α7 subunits and the inner rings composed of the β1–β7 subunits (Paugam et al. 2003). The plasmodial proteasome has a predicted size of ∼764 kDa. There is some debate about the experimentally determined size of the plasmodial proteasome. On native gels, the complex migrates at a molecular mass of 550 kDa with two other bands corresponding to sizes of 700 kDa and 300 kDa (Sessler et al. 2012). However, gel filtration of 3D7 infected erythrocyte free lysate probed with commercially available antibody against plasmodial proteasome showed a size of 670 kDa (Mordmuller et al. 2006), closer to the theoretical size of the complex. Despite this size difference, mass spectrometry of the band from native gel analysis showed the presence of all 14 subunits of plasmodial proteasome, indicating that there could be binding of possible regulatory subunits of the proteasome that affected its migration on the native gel (Sessler et al. 2012).

Many studies have shown that protease inhibitors such as lactacystin, GTX, thiostrepton, and epoxomicin affect many aspects of plasmodial biology and exhibit plasmocidal effect (Gantt et al. 1998; Hatabu et al. 2005; Lindenthal et al. 2005;

Czesny et al. 2009; Schoof et al. 2010; Aminake et al. 2011). Due to the multifaceted mechanism of action of these proteasome inhibitors, they present themselves as potent antimalarials. Biochemically, epoxomicin was shown to covalently bind to subunits β2 and β5, thus leading to inhibition of proteasome activity (Mordmuller et al. 2006). However, the mechanism of inhibition of many of the other inhibitors remains unclear.

Clp Proteases

The parasite has five Clp ATPases (Fig. 9.5, Table 9.5) that belong to the AAA+ superfamily: PfClpB1, PfClpB2, PfClpC, PfClpM (El Bakkouri et al. 2010) as well as PfHslU (also known as PfClpY) (Rathore et al. 2011). In addition, the parasite encodes three proteases (or protease-like) proteins: PfClpP, PfClpR, and PfHslV (also known as PfClpQ) (Fig. 9.6, Table 9.6). The Clp ATPases with the exception of PfClpM are nuclear encoded. PfClpM is encoded by the apicoplast genome of malaria and this protein is localized to the apicoplast (El Bakkouri et al. 2010). PfClpB2 was shown to be localized to the parasitophorous vacuole (PV) (de Koning-Ward et al. 2009) and has been proposed to be involved in the secretion of malarial proteins into the PV (de Koning-Ward et al. 2009). No experimental data is available on the function of PfClpB1, PfClpC, and PfClpM. However, a PfClpCRP is proposed to form in the apicoplast (El Bakkouri et al. 2010). PfClpB1, PfClpB2, and PfClpM do not bind a proteolytic component. PfHslU is predicted to localize to the mitochondria of the parasite because it interacts with the protease PfHslV that was shown to have mitochondrial localization to form the PfHslUV complex (Tschan et al. 2010).

a. PfClpCRP PfClpP is a serine protease that is nuclear encoded and is found to localize to the apicoplast (El Bakkouri et al. 2010; Rathore et al. 2010). PfClpP contains an extra 150 amino acids at the N-terminus as compared to *E. coli* ClpP. It is proposed that this stretch encodes signal sequences to target the protease into the apicoplast. Expression of PfClpP in blood stage parasites was confirmed (El Bakkouri et al. 2010; Rathore et al. 2010). Recombinant PfClpP with N-terminal truncation was purified in *E. coli* and was shown to form heptamers through a variety of different *in vitro* biophysical techniques (El Bakkouri et al. 2010). However, size exclusion chromatography analysis of parasite lysate showed that PfClpP eluted at ~660 kDa, indicating that PfClpP may be part of a larger complex *in vivo* (Rathore et al. 2010). The X-ray crystal structure of PfClpP has been solved as a tetradecamer (El Bakkouri et al. 2010; Fig. 9.6). In comparison to the *E. coli* ClpP structure, the PfClpP structure shows a more compact tetradecamer (Fig. 9.6). The protease has been shown to have weak activity against model peptides Suc-LLVY-AMC, and Suc-LY-AMC, indicating that this protease is active and has chymotrypsin-like serine protease activity (El Bakkouri et al. 2010; Rathore et al. 2010). The protease is proposed to have an important role in parasite growth and development. Specifically, inhibition of this protease with U1-lactone showed disruption of apicoplast development that leads to death of the parasite (Rathore et al. 2010).

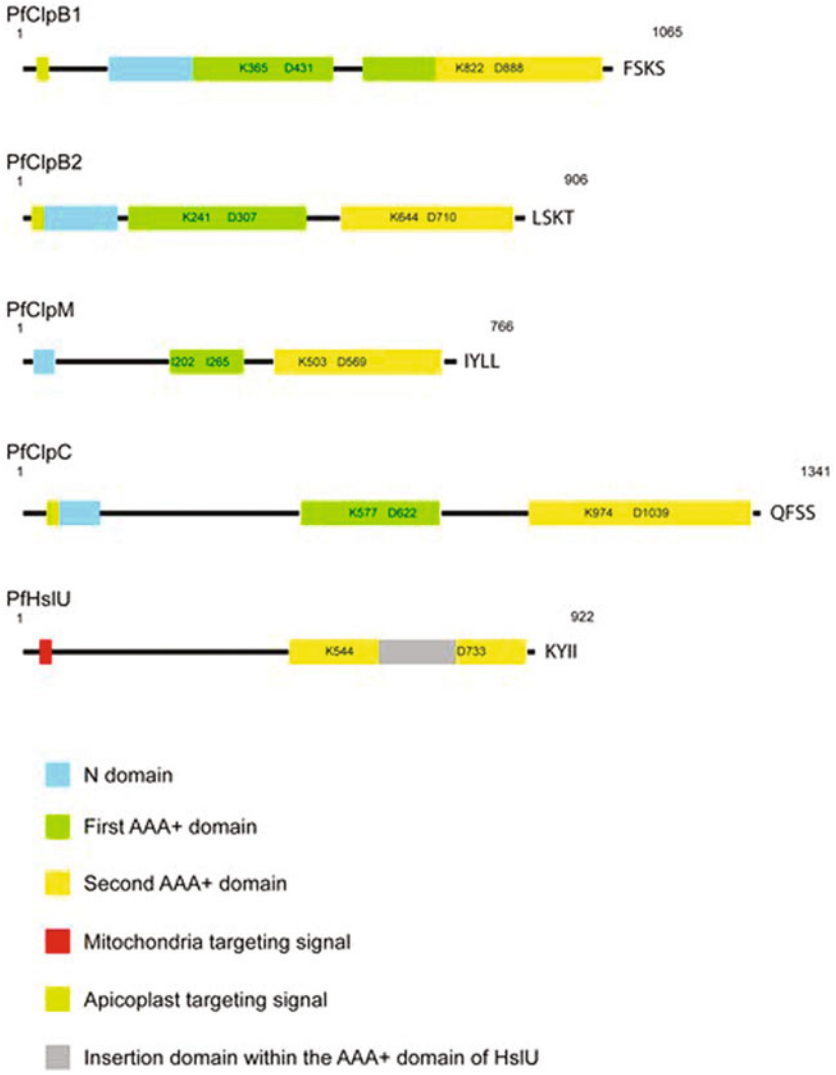

Fig. 9.5 Prediction of protein domains in PfClp ATPases. Residue numbers of the lysine residue in the Walker A motif and of the aspartic acid residue in the Walker B motif are indicated. Note that the N-terminal AAA+ domain of PfClpM, unlike the C-terminal AAA+ domain, does not contain the conserved Walker A and Walker B residues, and, thus, the N-terminal domain is not predicted to be an active ATPase. The AAA+ domain of PfHslU contains an insertion domain (*shown in grey*)

PfClpR is nuclear encoded and is found to also localize to the apicoplast (El Bakkouri et al. 2013). Western blot analysis of parasite lysate shows that the mature form of the inactive protease approximately corresponds to the size of PfClpR(49–244) (El Bakkouri et al. 2013), indicating that this protease undergoes cleavage upon trafficking into the apicoplast. Expression of PfClpR in blood stage

Table 9.5 Clp AAA+ chaperones of *P. Falciparum*

	PfClpB1 PF3D7_ 0816600	PfClpB2 PF3D7_ 1116800	PfClpM PFC10_ API0060	PfClpC PF3D7_ 1406600	PfHslU (PfClpY) PF3D7_0907400
MW (kDa)	123	103	91	156	106
Chromosome	8	11	Apicoplast	14	9
Cellular localization	Apicoplast[a]	PV[b]	Apicoplast[a]	Apicoplast[a]	Mitochondria N.C.
Expression stage (RNA levels from PlasmoDB)	Asexual blood stage[a]	Asexual blood stage[a]	Ring stage[a]	Asexual blood stage[a]	Schizont N.C.
AAA+ Type	Type I	Type I	Type I	Type I	Type II

N.C. not experimentally confirmed
[a] (El Bakkouri et al. 2010)
[b] (de Koning-Ward et al. 2009)

parasites has been confirmed (El Bakkouri et al. 2013). PfClpR(61–244) was successfully crystallized and this structure represents the first structure of a ClpR subunit (El Bakkouri et al. 2013). The structure shows the active site catalytic triad Ser-His-Asp in ClpP replaced by Gly-Asn-Asp, which the authors termed the inactive catalytic triad (Fig. 9.6). In addition, PfClpR has a conserved stretch of amino acids within the Plasmodium genus that was named the R-motif (El Bakkouri et al. 2013); the motif is not present in PfClpP. The R-motif is in close spacial proximity to the inactive catalytic triad (Fig. 9.6). Size exclusion chromatography of parasite lysate showed that PfClpR migrates at ~450 kDa (El Bakkouri et al. 2013) indicating the presence of PfClpR in a larger oligomer.

In *A. thaliana*, which encodes 6 ClpP paralogs and four ClpR paralogs (Peltier et al. 2004), there is evidence of formation of a ClpRP complex (Rudella et al. 2006). The possibility of forming a PfClpRP complex was explored with purified proteins using Surface Plasmon Resonance. The results seem to point to the formation of a PfClpRP complex via interaction of PfClpP heptamer with PfClpR heptamer (El Bakkouri et al. 2010). However analysis of parasite lysate did not show formation of PfClpRP complex (El Bakkouri et al. 2013). As well, the compact crystal structure of PfClpR (Fig. 9.6) is not favorable for formation of a $PfClpR_7$ and $PfClpP_7$ complex (El Bakkouri et al. 2013). Despite this, one cannot rule out the possibility that substrate binding or AAA+ chaperone binding to the protease might induce conformational changes that may allow for the formation of a PfClpRP complex. PfClpC has been proposed to be the corresponding chaperone cap for a potential PfClpCRP complex (El Bakkouri et al. 2010).

b. PfHslUV PfHslV is a threonine protease that has a highly conserved sequence across the Plasmodium species (Mordmuller et al. 2006). It has a 37 amino acid pro-sequence that is cleaved to form the mature enzyme (Mordmuller et al. 2006). Consequently, the active site threonine becomes residue number 1. The protein shows high similarity to *E. coli* HslV. In *E. coli*, HslV forms a ~200 kDa homododecamer

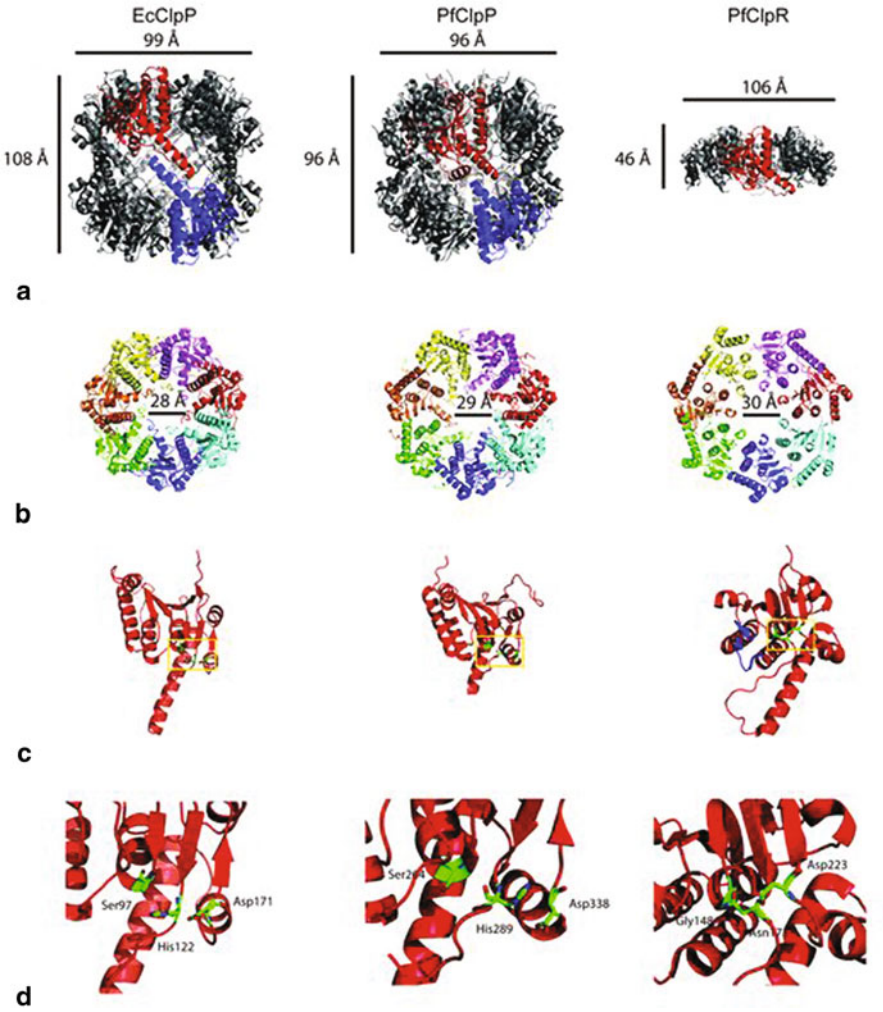

Fig. 9.6 Crystal structures of the serine proteases EcClpP (1TYF) (Wang et al. 1997), PfClpP (2F6I) (El Bakkouri et al. 2010), and PfClpR (4GM2) (El Bakkouri et al. 2013). (**a**) Side views of the oligomeric structures of the proteases are shown. EcClpP and PfClpP are tetradecamers in the crystal, while PfClpR is a heptamer. One monomer in a heptamer is colored red or blue. The rest of the subunits in the heptamer are in grey. The dimensions of the structures are shown for comparison. (**b**) Top views are shown with each monomer colored differently. The dimensions of the axial pores are indicated. (**c**) One subunit is shown for each protease with the active site boxed in yellow and active site residues colored in green. The R motif in PfClpR subunit is colored in blue. (**d**) Close-up view of the active site residues. Active site residues of each protease are labeled. PfClpR does not have a catalytic active site. Structures were drawn using PyMol (www.pymol.org)

(Kessel et al. 1996). Gel filtration of parasite lysate revealed that PfHslV forms a complex of approximately 230 kDa size (Mordmuller et al. 2006; Table 9.6). It was

Table 9.6 Clp proteases of *P. Falciparum*

	PfClpP PF3D7_0307400	PfClpR PF3D7_1436800	PfHslV (PfClpQ) PF3D7_1230400
MW (kDa)	43	28	23
Chromosome	3	14	12
Cellular localization	Apicoplast[a]	Apicoplast[b]	Mitochondria[c]
Expression stage (RNA levels from PlasmoDB)	Early Trophozoite[a] Trophozoite, Schizont[d]	Trophozoite, Schizont[b]	Merozoite[f], Trophozoite[e], Schizont[e, f]
Oligomeric State	Heptamer (gel filtration)[a] Tetradecamer (crystal)[a]	Heptamer[a, b]	about 230 kDa complex[f] - could be dodecamers
Protease Activity	Serine protease-like[a, d] Chymotrypsin-like[a, d]	No catalytic activity[b] Lacks Ser-His-Asp triad[b]	Threonine protease-like[e] Chymotrypsin-like[e] Peptidyl glutamyl[e]

[a] (El Bakkouri et al. 2010)
[b] (El Bakkouri et al. 2013)
[c] (Tschan et al. 2010)
[d] (Rathore et al. 2010)
[e] (Ramasamy et al. 2007)
[f] (Mordmuller et al. 2006)

shown that Schizonts expressed similar levels of pro- and mature PfHslV whereas the Merozoites expressed predominantly mature PfHslV (Mordmuller et al. 2006). This indicates stage dependence for enzyme maturation. Further, an independent study found PfHslV transcript levels to be lowest during Ring stage and highest during Schizont stage (Ramasamy et al. 2007). Through a series of elegant experiments, PfHslV was shown to be localized to the mitochondria (Tschan et al. 2010). PfHslV was able to cleave different fluorogenic peptide substrates: Cbz-GGL-AMC, Suc-LLVY-AMC and Z-LLE-AMC, demonstrating that this protease has threonine, chymotrypsin-like, and peptidyl glutamyl peptide hydrolase activities, respectively (Ramasamy et al. 2007). Since the human genome does not code for an HslV ortholog, this protease is gaining much attention as a novel potential anti-malarial drug target. *In silico*, the PfHslU ATPase forms a complex with PfHslV protease (Subramaniam et al. 2009). However, the exact oligomerization state and ATPase activity of PfHslU have not been characterized and the physiological function of PfHslUV remains undetermined.

Concluding Remarks

P. falciparum expresses a wide variety of different chaperone and protease systems to maintain proteostasis. Many of these chaperones and proteases are similar to those in prokaryotes and eukaryotes. However, several of these systems have novel

activities and are potential drug targets. Research about proteostasis in *P. falciparum* is still at its infancy and a concerted effort is needed to understand the biochemistry and cell biology of *P. falciparum* chaperones and proteases. Manipulating protein homeostasis in this parasite can be a novel approach to treat malaria.

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