Addmore Shonhai · Gregory Blatch
Editors

Heat Shock Proteins of Malaria

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Chapter 1
The Importance of Molecular Chaperones in Survival and Pathogenesis of the Malaria Parasite *Plasmodium falciparum*

Jude Przyborski

It has been estimated that one child dies of malaria every minute. Although massive research efforts have been directed towards the fight against this deadly disease, still over 0.6 million people fall victim to malaria every year, and approximately half of the world's population live in malaria risk areas (World Health Organization 2011). Malaria impacts not just the fate of individuals, but also the countries in which they live. Countries with high rates of malaria have an annual growth rate lower than those which are free of, or have eradicated the disease (Gallup and Sachs 2001). Thus, malaria in the modern world is part of a vicious circle of poverty and disease, with those most at need of help concomitantly being those least economically able to help themselves. In addition to the socioeconomic challenges involved in reduction of malaria occurrence across the world, the parasite itself is fighting back.

Chloroquine, once seen as the "magic bullet" against malaria first became ineffective in the 1950s due to rapidly spreading resistance in the parasite population. Indeed the parasite has become resistant to all but the latest artemisinins, dramatically limiting the options available to clinicians (White 2004). Although several experimental malaria vaccines are currently under trial, so far none has shown the potential to be used successfully on a global scale (Vaughan and Kappe 2012).

While improvements in the prevention and management of malaria will always be on the global agenda, new therapeutic targets are also desperately needed for the treatment of malaria. To this end, a concerted research effort has been directed towards understanding the basic biology of malaria parasites, with a view to identifying targets and strategies with potential to roll back the burden of malaria on individuals and communities.

This book concentrates on our current knowledge on the role of heat shock proteins in the survival of malaria parasites, and their interaction with the host. Malaria parasites, in common with most other organisms, possess a large complement of proteins designed to protect the cell against changing environmental and intracellular

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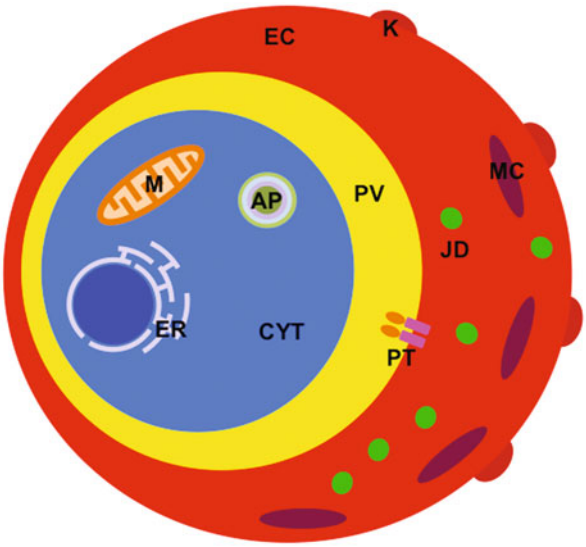

Fig. 1.1 Localisation and function of heat shock proteins in the malaria-infected cell. Parasite, *blue*; parasitophorous vacuole (*PV*), *yellow*; erythrocyte, *red*. Heat shock proteins have been shown, or are predicted, to play a role in processes taking place in the parasite cytosol (*CYT*), endoplasmic reticulum (*ER*), apicoplast (*AP*), mitochondria (*M*), parasitophorous vacuole (*PV*), in association with the PTEX complex (*PT*), within the cytosol of the erythrocyte (*EC*), associated with knobs (*K*), J-dots (*JD*) and Maurer's clefts

conditions. Many of these proteins belong to the class of heat shock proteins. Since their initial discovery in *Drosophila*, many different members of this family have been identified and characterized in detail. Although originally implicated in cellular protection against thermal insult, we now know that members of the heat shock class of proteins are involved in numerous and varied cellular processes including folding of nascent proteins, protein quality control and degradation, protein trafficking and protein refolding following cellular stress. Due to their involvement in helping proteins fold (or re-fold) into their correct three dimensional structures, some members of the Hsp class are also referred to as molecular chaperones.

Parasites, by definition, survive and multiply within a host organism or cell. Although the parasitic way of life comes with benefits such as a ready supply of sufficient nutrients, it also entails the parasite giving up a certain level of independence. Thus, parasites must endure whichever conditions their host experiences, but over which they have no direct influence. Additionally, many parasites require passage through several different hosts and possibly "free-living" or egg stages to complete their life-cycle, further increasing the stresses endured. To enable the parasite to survive such changing and unpredictable times, it has been noted that, against a background of general genomic reduction, many parasites still contain a large complement of heat shock proteins. This fact suggests that many parasites depend heavily on heat shock proteins to survive, making them a potentially attractive drug target.

Recent studies have revealed that *Plasmodium* encodes a wide variety of heat shock proteins, which are involved in many essential and novel cellular processes. Within the parasite itself, heat shock proteins have been found in both the cytosol, apicoplast, ER and mitochondria (Fig. 1.1). These proteins generally carry out housekeeping functions or are involved in protein trafficking, akin to processes found in other systems. Upon invading the host erythrocyte, the parasite massively modifies

its host cell. This "cellular renovation" is thought to be mediated by parasite-encoded proteins which are transported from the parasite to the host cell. Trafficking of these proteins to their respective cellular localisation involves the action of a wide range of heat shock proteins, themselves with diverse localisation (Fig. 1.1). In the following chapters world experts in malaria heat shock proteins give a detailed overview of our current state of knowledge, detailing their role in both typical, but also atypical processes within the infected cell. These articles highlight that the malaria parasite, as in so many things, obeys the adage "The same. But different".

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Chapter 2
General Structural and Functional Features of Molecular Chaperones

Adrienne L. Edkins and Aileen Boshoff

Introduction to Molecular Chaperones and Stress at a Cellular Level

Molecular chaperones are the guardians of protein homeostasis. Proteins require a particular three dimensional structure in order to fulfil their function, despite being synthesised as a linear string of amino acids joined by peptide bonds. These amino acids must subsequently fold to achieve the appropriate spatial arrangement of these residues in order to arrive at the final three dimensional structure of the protein. Sequence determines structure; the information required to adopt a native three-dimensional conformation is encoded in the primary amino acid sequence, although the number of possible theoretical conformations of even a small protein is tremendously large (Anfinsen 1973). Protein folding occurs spontaneously, often in a co-translational manner, whereby the N terminus of the protein begins to fold while the C terminal regions are still being translated. The folding process is driven largely by hydrophobic amino acids within the protein as they avoid the aqueous cellular environment. Once folded, certain proteins may also associate non-covalently with other proteins into higher order functional complexes. Proteins undergo this process in a crowded intracellular environment that should favour protein aggregation and misfolding (Ellis and Hartl 1999; Ellis 2001). Protein folding is assisted by a group of proteins known as molecular chaperones (Mayer 2010). Molecular chaperones are catalysts in the physiological folding process, which, through transient non-covalent associations with proteins, prevent aggregation and misfolding during de novo folding, as well as regulating subsequent stages of protein translocation and complex formation. The importance of molecular chaperones to protein folding is enhanced

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under non-physiological or stressful conditions. Cellular stress could include a wide range of stimuli, including heat, oxidation and chemicals. The main biological consequence of cellular stress is the loss of protein function due to stress induced protein unfolding and aggregation. This loss is potentially disastrous for any cell that cannot overcome it. Molecular chaperones prevent aggregation and promote refolding after stress and hence promote cell survival. This so-called stress response is ubiquitous and conserved across all organisms. Chaperone assisted protein folding in cells is largely controlled by a group of proteins known as heat shock proteins (HSP) (Bukau et al. 2006).

Classification of Heat Shock Proteins as Molecular Chaperones

Heat shock proteins are a group of proteins that form a significant share of the molecular chaperone protein class. These proteins are required for preserving the appropriate folding and conformation of other proteins in the cell and are consequently called molecular chaperones. As a result of the discovery of heat responsive genes by Ritossa (Ritossa 1962) after heat shock of Drosophila salivary glands, the products of these genes were isolated and subsequently called heat shock proteins (Tissieres et al. 1974). Under conditions of stress, heat shock proteins accumulate in the cell and control the potentially deleterious consequences associated with stress by preventing protein misfolding and actively refolding proteins; inhibiting protein aggregation or self-association; if proteins are irreversibly denatured, they are handed over to the proteasome for degradation (Hendrick and Hartl 1993; Becker and Craig 1994). Stressful circumstances induce the synthesis of over twenty heat shock proteins that enable cells to adapt to environmental and metabolic changes and to survive stress conditions (Arsene et al. 2000). However other than heat stress, these proteins are induced by many types of cellular stressors including hyperthermia, exposure to heavy metals, UV radiation, oxidative stress, nutrient deficiencies, dehydration, osmotic pressures and viral infections. As a consequence, heat shock proteins have been used to study the stress response of numerous organisms and their application as biomarkers continues to receive attention, particularly for marine organisms experiencing environmental stress (Clark and Peck 2009).

Heat shock proteins are evolutionarily conserved, abundant and ubiquitous proteins in all cells and play similar roles in organisms from bacteria to humans. They are amongst the most highly expressed and can account for 1–2 % of the total protein in unstressed cells, and this can increase up to 4–6 % after heat shock (Garrido et al. 2001). Heat shock proteins are localised to different compartments in the cell, and despite being highly conserved, carry out tasks specific to their environment. Not all heat shock proteins are expressed during cellular stress; under normal growth conditions, heat shock cognate (Hsc) proteins are constitutively expressed in the absence of stress and perform critical “housekeeping” functions to maintain cellular homeostasis (Ingolia and Craig 1982; Hartl and Hayer-Hartl 2002). The large and varied heat shock protein class is grouped into several subfamilies based on their

sizes in kDa namely, small Hsps, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100. In 2009, new guidelines for the nomenclature of the human heat shock protein families were proposed, this had arisen as a result of increasing numbers of proteins and discrepancies in the existing nomenclature (Kampinga et al. 2009). In this classification, the human heat shock proteins have been renamed to the following: HSPH (former name HSP110), HSPC (HSP90), HSPA (HSP70), HSPD/E (HSP60/HSP10) and CCT (TRiC), DNAJ (HSP40), and HSPB (small HSP or sHSP) (Kampinga et al. 2009).

Heat shock proteins are integral components of the chaperone network in the cell and many of their functional cycles work in concert with a group of co-chaperones and cofactors that function as regulators (Hendrick and Hartl 1993, 1995). Molecular co-chaperones regulate the activity of selected chaperones and most can be classified according to the presence of particular domains: the bcl-2 associated athanogene (BAG) domain, the tetratricopeptide repeat (TPR) domain, or the DnaJ or J domain. Despite the fact that heat shock proteins differ in their size, structures and activity, they all bind non-native proteins (some bind native proteins as well); and some exert their functions co-translationally by interacting with nascent polypeptides, while others act post-translationally by providing an environment that enhances folding (Bhutani and Udgaonkar 2002). Most heat shock proteins are ATP-dependent and require ATP to control binding and dissociation of substrate polypeptides, while some use an ATP-independent mechanism.

The Hsp100 class of chaperones forms large hexameric structures and uses energy generated by the hydrolysis of ATP for protein remodelling (Bukau et al. 2006). The disaggregation activity of hexameric Hsp104 requires the collaboration of the Hsp70 system (Glover and Lindquist 1998). Hsp90 (HSPC) has more specialised roles in the cell. The ATP-dependent molecular chaperone Hsp90 is required for the activation and regulation of an ever growing list of client proteins involved in diverse biological processes, and unlike Hsp70 (HSPA), many of these client proteins are not in an extended conformation but are almost completely folded (Zuehlke and Johnson 2010). Many client proteins first interact with the Hsp70-Hsp40 chaperone system before being transferred to Hsp90 via the TPR-containing Hop (Hsp70-Hsp90 organising protein) (Wegele et al. 2004). The ATP-dependant chaperone activity of Hsp70 is based on the ability to bind short hydrophobic segments of proteins and this is regulated by Hsp40 (DNAJ), which functions as a co-chaperone of Hsp70 (Flynn et al. 1991; Gragerov and Gottesman 1994). The Hsp110 (HSPH) family, a subgroup of the Hsp70s, are essential nucleotide exchange factors for Hsp70 (Dragovic et al. 2006a). The highly diverse Hsp40 proteins provide specificity to the chaperone reaction by targeting substrates to Hsp70; the influence of this functional specificity of Hsp40 was the subject of a recent review by Kampinga and Craig (2010). In addition, Hsp40 proteins function as chaperones in their own right and are able to suppress protein aggregation in an ATP-independent manner (Lu and Cyr 1998).

Hsp60 (HSPD) proteins form large ring-shaped complexes composed of 14 subunits arranged in two stacked 7-membered rings, and ATP binding triggers conformational changes that result in the co-chaperone Hsp10 (HSPE) forming a lid over the structure (Braig et al. 1994). The folding of nascent polypeptides often

requires the cooperation of both the Hsp70 and Hsp60 families and these families are also responsible for most of the general folding events in the cell (Hartl et al. 1992; Fink 1999). The small heat shock proteins (HSPB) are the least conserved (Narberhaus 2002) and the least studied, due in part to the lack of a consistent model of oligomerization and substrate binding (Eyles and Gierasch 2010). The largely stress inducible small heat shock proteins function as ATP-independent chaperones to prevent protein aggregation and assist protein renaturation in cooperation with ATP-dependent chaperones (Jakob et al. 1993).

Certain heat shock proteins are essential for the maintenance of viability, eukaryotic cytoplasmic Hsp90 is essential for viability under all growth conditions (Borkovich et al. 1989). This observation has led to the emergence of Hsp90 as an anti-cancer drug target, including breast cancer (Beliakoff and Whitesell 2004). In addition to their critical role in cellular homeostasis, heat shock proteins have been implicated in the induction and propagation of human disease. New roles of heat shock proteins in human physiology and disease are rapidly emerging. This has lead to the concept of chaperonopathy to indicate a pathologic condition resulting from defective chaperones (Brodsky and Chiosis 2006; Macario and Conway de Macario 2007a, b). In addition, the use of heat shock proteins as therapeutic tools and potential cancer vaccines are also being investigated (Lee et al. 2006). Heat shock proteins also play important roles in immunity and protection as well as pathogenesis of infectious diseases as both the host and pathogen increase heat shock protein production (Zugel and Kaufmann 1999). Heat shock proteins also exert their function outside of the cell and form the extracellular heat shock protein complement; these proteins reach the extracellular space via a variety of mechanisms including cell lysis and participate in processes such as cell signalling and immunity (Calderwood et al. 2007; Tsan and Gao 2009). Several extracellular heat shock proteins play a role in the migration of cancer cells and thus this population of proteins also needs to be considered in the fight against cancer (Schmitt et al. 2007; Sims et al. 2011).

Foldases: Molecular Chaperones Involved in Protein Folding

The Hsp60/Chaperonin Family of Molecular Chaperones

The Hsp60 (HSPD) family is well characterised and highly conserved. Members of the Hsp60 protein family, also referred to as chaperonins, are represented by GroEL in prokaryotes, and mitochondrial Hsp60, plastid Rubisco subunit binding protein, archaea group II chaperonins and TRiC/CCT in eukaryotes (Hartl et al. 1992). Chaperonins assist in the folding of nascent and misfolded proteins in an ATP-dependent manner (Houry et al. 1999) but their mechanism of action is different to that of Hsp70. It is estimated that under normal growth conditions, 10–15 % of all cytoplasmic proteins rely on GroEL in order to fold correctly, and this increases to 30 % under conditions of stress (Ewalt et al. 1997). Many of the cytoplasmic proteins that interact with GroEL have been identified (Houry et al. 1999) and GroEL acts