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Review

The Hsp70 chaperones of the Tritryps are characterized by unusual features and novel members

Cassandra A. Louw [a], Michael H. Ludewig [a], Jens Mayer [b], Gregory L. Blatch [a,*]

[a] Biomedical and Biotechnology Research Unit, Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa
[b] Department of Human Genetics, Medical Faculty, University of the Saarland, Homburg 66421, Germany

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ABSTRACT

Proteins belonging to the Hsp70 class of molecular chaperones are highly conserved and ubiquitous, performing an essential role in the maintenance of cellular homeostasis in almost all known organisms. *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major* are human parasites collectively known as the Tritryps. The Tritryps undergo extensive morphological changes during their life cycles, largely triggered by the marked differences between conditions in their insect vector and human host. Hsp70s are synthesised in response to these marked changes in environment and are proposed to be required for these parasites to successfully transition between differentiation stages while remaining viable and infective. While the Tritryps Hsp70 complement consists of homologues of all the major eukaryotic Hsp70s, there are a number of novel members, and some unique structural features. This review critically evaluates the current knowledge on the Tritryps Hsp70 proteins with an emphasis on *T. brucei*, and highlights some novel and previously unstudied aspects of these multifaceted molecular chaperones.

Contents

1. Introduction

Proteins of the heat shock protein 70 (Hsp70) class of molecular chaperones comprise one of the most highly conserved protein families across species, and play a fundamental role in coordinating a number of essential cellular processes that include the folding and assembly of newly synthesised proteins, the refolding of misfolded and aggregated proteins, membrane translocation of secretory proteins, control of regulatory proteins, and the proteolytic degradation of denatured or unstable proteins [1,2]. The eukaryotic Hsp70 super-family consists of two major families, recently classified in

* Corresponding author. Tel: +27 46 603 8262; fax: +27 46 622 3984.
E-mail address: G.Blatch@ru.ac.za (G.L. Blatch).

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humans as HSPA (typical Hsp70 proteins; 13 members) and HSPH (Hsp110/Grp170 proteins; four members) [3,4]. Two well studied Hsp70 family members include HSPA1A that is highly inducible (also called inducible Hsp70 or Hsp72), and HSPA8 that is constitutively expressed (also called Hsc70 or Hsp73). Typical Hsp70 proteins consist of a 45 kDa N-terminal ATPase domain, a 15–18 kDa substrate binding domain and a 10 kDa C-terminal domain. The C-terminus of cytosolic Hsp70s ends with a conserved EEVD motif that is crucial for association with co-chaperones such as heat shock protein 40 (Hsp40) [5] and the Hsp70/Hsp90 organising protein (Hop) [6,7]. Hsp110/Grp170s are very similar to Hsp70s, with the main difference being the presence of large insertions (especially acidic insertions of 100–140 amino acids) in the substrate binding and C-terminal domains [2].

The ability of Hsp70s to interact with substrates, and enable them to refold into a functional native conformation is highly dependent on their nucleotide-bound state. In metabolically active cells in the absence of substrate and co-chaperones, Hsp70s are in an ATP-bound state, with low affinity for substrate, low basal ATPase activity, and thus disengaged with respect to chaperone activity [8]. In the presence of substrate and an Hsp40 partner protein, Hsp70 ATP hydrolysis is stimulated, resulting in Hsp70-ADP which has a high affinity for the substrate. Nucleotide exchange factors (NEFs) catalyze ADP release, resulting in regeneration of Hsp70-ATP, and release of the substrate to fold into its native state, or associate with Hsp70 in another cycle of assisted protein folding if necessary [9]. The binding of ATP to the N-terminal ATPase domain of Hsp70 results in a conformational change in the substrate binding domain, which triggers the release of substrate. Hsp70s associate with hydrophobic stretches on the substrate protein, thereby preventing illegitimate interactions that will lead to misfolding and aggregation, and facilitating appropriate interactions that lead to a folded functional state. As a general rule there are many more Hsp40s than Hsp70s for a particular species (e.g. 49 human Hsp40s) [3,4], and there is increasing evidence that the functional differentiation of Hsp70s occurs at the level of their specific Hsp40 partner proteins and the class of substrates processed (native, nascent, misfolded or aggregated protein types) [1]. Hsp40s are defined by the presence of a J domain that is essential for their interaction with an Hsp70 partner protein, and they have been categorized into four classes (Types I–IV) based on the presence or absence of functional domains in addition to the J domain (Types I–III) or J-like domain (Type IV). Many of the Types I and II Hsp40 proteins are capable of binding substrates and targeting them to an Hsp70, while the Type III proteins are highly specialized serving mainly to recruit an Hsp70 to a specific location [8]. The Type IV Hsp40s have a J-like domain in which the highly conserved HPD motif is corrupted [10].

There is currently a renewed interest in the Hsp70 protein family in a number of medically and economically significant parasites. Trypanosomal species are the cause of fatal disease in both man and animals. *Trypanosoma brucei* and *Trypanosoma cruzi* infections cause African sleeping sickness and Chagas disease, respectively, while *Leishmania major* infection results in debilitation and death from leishmaniasis [11]. *T. brucei*, *T. cruzi* and *L. major* are collectively known as the Tritryps [12]. The Tritryps endure marked changes in environmental conditions as they cycle between their insect vector and mammalian host systems. These changes in environmental conditions are accompanied by extensive morphological changes as the parasites differentiate from one form to another [13]. Certain heat shock proteins, in particular Hsp70, are synthesised at elevated levels in response to these life cycle transitions, and when these environmental changes are simulated in culture [14–16]. Proteomics analyses have shown increased synthesis of various heat shock proteins, including Hsp70s, during stage differentiation of *T. cruzi* and *Leishmania* species [17–21]. Proteomics analyses of developmental stages of *T. cruzi* have shown increased synthesis of Hsp90, Hsp70 and Hsp60 [19]. In particular, for the transition from trypomastigote to the amastigote stage (intracellular dividing form within the mammalian host), in addition to the increase in heat shock proteins, there was an almost exclusive increase in proteins involved in ER to Golgi trafficking [20]. Furthermore, proteomic studies on *T. brucei* indicated that most of the Hsp70s were expressed in both the procyclic (Pro; within the insect vector) and blood stream form (BSF; within the mammalian host) stages of the parasite life cycle [22,23]. It is very likely that the Tritryps Hsp70s play an important role in the adaptation of these parasites to the environmental changes associated with their life cycle stages; however, these proteins are possibly also important for parasite differentiation *per se*. The structural, functional and mechanistic features of the Tritryps Hsp70s that enable them to ensure protein homeostasis or augment differentiation processes are poorly understood, and there has been no comprehensive biochemical comparison of the Tritryps Hsp70 chaperone machinery to that of the human host system. While the heat shock proteins of the Tritryps have previously been categorized and reviewed elsewhere [24,25], the Hsp70 family has not been the subject of a comprehensive analysis. Therefore, this review aims to provide future perspectives for Tritryps Hsp70 research by highlighting some novel and previously unstudied aspects of these multifaceted molecular chaperones.

2. The Hsp70 protein family

The Hsp70s of the Tritryps were divided into groups based on their identity to well-characterized Hsp70s from other species (Table 1). A total of 12 Hsp70s were found in *T. brucei*, and included orthologues for all the subdivisions of the Hsp70 protein family [25] (Table 1). The sequenced and annotated *T. cruzi* genome contains a large number of sequences described as partial sequences which do not contain start and/or stop codons in their reading frames [12]. Previous investigations on the *T. cruzi* Hsp70 complement have indicated that, if partial sequences are included, the parasite possesses 28 Hsp70 proteins [25]. For the purpose of this review, these partial sequences were omitted, as it is possible that these sequences do not represent fully expressed proteins in the parasite, and would require further sequence validation to determine whether they represent sequencing artefacts. When these sequences are disregarded, *T. cruzi* is found to possess 11 full-length Hsp70 sequences (Table 1). The *L. major* parasite has a total of 14 Hsp70s, and is characterized by an unusually large mitochondrial Hsp70 complement (Table 1). The subcellular localisation for a number of the Hsp70s have been determined experimentally (as discussed in subsequent sections), or were predicted (WoLF PSORT; wolfpsort.org; [26]) so as to validate the groupings of the *T. brucei* Hsp70s (Fig. 1) and their orthologues in *T. cruzi* and *L. major* (Table 1).

3. Cytoplasmic Hsp70s

3.1. Hsp70

This class of proteins consist of the so-called typical cytosolic Hsp70s whose levels are enhanced under stress conditions. The gene encoding TbHsp70 is situated on chromosome XI of *T. brucei*, in close proximity to the gene encoding a novel cytosolic Hsp70, TbHsp70.c (Fig. 2). Proteomics studies indicated that TbHsp70 was expressed in both the BSF and Pro life cycle stages of *T. brucei* [22,23]. The culturing of *T. brucei* BSF parasites under conditions similar to that experienced during the infection of a mammalian host resulted in higher levels of TbHsp70 expression (steady state mRNA levels), regardless of whether or not heat shock had taken place. This suggested that TbHsp70 may be upregulated in response to stresses other than heat shock experienced by the *T. brucei* parasite [13]. TcHsp70 protein levels have been shown to increase in response to heat shock in culture, and its subcellular localization to change from cytoplasmic to largely nuclear [14]. TbHsp70 possesses a high sequence identity (Fig. S1) and a similar