Mitochondrial Hsp70 - function and evolution

ABSTRACT

Hsp70 molecular chaperones function in variety of critical cellular processes, including protein folding, translocation of proteins across membranes and assembly/disassembly of protein complexes. Hsp70 systems consist of a core Hsp70 protein and its co-chaperones: J-protein and nucleotide release factor NRF. These co-chaperones regulate the cycle of interaction with protein substrate via stimulating the ATPase activity of Hsp70 (J-protein) and promoting nucleotide exchange (NRF). Compartments within the eukaryotic cell often contain multiple Hsp70s, J-proteins and NRFs. The capabilities of these systems to carry out diverse cellular functions results from either specialization of an Hsp70 or by interaction of multifunctional Hsp70 with an array of specialized J-proteins. The well-studied Hsp70 systems of yeast mitochondria provide an excellent example of functional divergence and evolution of Hsp70 machineries.

INTRODUCTION

Hsp70 chaperone systems may be found in all compartments of the eukaryotic cell, where they function in many vital processes. Hsp70 mechanism is based on a reversible binding of short hydrophobic sequences of amino acid residues, exposed on the surface of proteins, called substrates. Such a universal mechanism allows Hsp70 chaperones to perform many important functions [1-3], e.g., folding the polypeptide chain of newly synthesized proteins, modulating the interactions between proteins by affecting their conformation, or promoting polypeptide transport through cellular membranes. Hsp70 systems also facilitate the refolding of polypeptides that lost their native conformation under stress conditions, and, if the refolding is unfeasible, redirect the polypeptides to intracellular proteolytic systems.

The involvement of Hsp70 proteins in such a wide variety of cellular processes is surprising and requires explanation, while their fundamental biochemical activity is limited to, a regulated by the ATP binding and hydrolysis, cyclic interaction with short polypeptide fragments of substrate proteins. The paper discusses molecular mechanisms and the evolution of a functional differentiation of Hsp70 systems in mitochondria. These processes can be illustrated by two major strategies: (1) the multiplication and specialization of genes encoding Hsp70 proteins (2) the multiplication and specialization of genes encoding co-chaperones, J-proteins. I will focus on the mitochondrial Hsp70 in yeast, as the genomic and experimental data have been obtained on such model organisms as *Saccharomyces cerevisiae*, *Sichzosaccharomyces pombe*, or *Neurospora crassa*. Yeasts are also the main object of study for our team. But before turning to mitochondrial systems, I will discuss the chaperon cycle of substrate binding and mechanisms responsible for multiplication of a number of genes encoding proteins of Hsp70 systems.

THE CYCLE OF BINDING A PROTEIN SUBSTRATE

Domain structure of Hsp70 proteins family is very similar in different organisms (Fig. 1). Each of them consists of two domains connected by a flexible linker. N-terminal ATPase domain (~ 44 kDa) is composed of two parts forming a pocket with the ATP binding site. The C-terminal protein, the substrate binding domain (~ 26 kDa) [3], consists of two parts: a two-layered twisted β-sheet that forms a binding pocket for several hydrophobic amino acids on the exposed substrate surface, and a domain of α-helix structure which forms a lid over the substrate binding site. The linker between the ATPase domain and substrate binding domain plays an important role in the allosteric communication between domains [4].

A fundamental biochemical activity of Hsp70 is the cyclic binding of a protein substrate. It is regulated by ATP hydrolysis which changes the conformation of

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Received: April 27, 2016
Accepted: May 18, 2016

Key words: J-protein co-chaperones, mitochondrial DNA, mitochondrial protein import, iron-sulfur clusters, protein evolution

Acknowledgments: I would like to thank Dr. Rafał Dutkiewicz, Prof. Elizabeth Craig and Dr. Brenda Schilke (University of Wisconsin-Madison) for a long-term cooperation. Results described in the paper were obtained by PhD students: Marlena Duchniewicz, Aleksandra Germaniuk, Helena Kniesznerr, Małgorzta Macierzanka, Sebastian Pukszta, Bogusława Paterkiewicz, Magdalena Płotka, Grzegorz Ciesielski, Szymon Ciesielski, Jacek Kominek, Julia Majewska, Mateusz Manicki, Michał Rogaczewski, Wojciech Delewski, Bartłomiej Tomiczek and Małgorzata Nowak.

The research was supported by the Professor Academic Grant Master/Mistrz (6/2014) awarded by the Foundation for Polish Science..

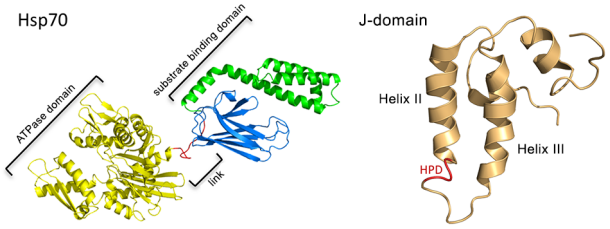


Figure 1. The structure of Hsp70-J domain. Hsp70 structure has been preserved in evolution. ATPase domain (yellow) contains a deep slot with the ATP binding site. The substrate binding domain consists of a β-sheet slot (blue), interacting with a group of hydrophobic amino acids, and covered by α-helical lid (green). Flexible link (red) allows the domains to an interact allosterically (Figure by PDB id: 2KHO). Each functional J-domain contains the HPD tripeptide, that is necessary to stimulate the ATPase activity of a partner Hsp70 (Figure by PDB id: 1XBL).

both domains (Fig. 2). When ATP is bound, the ATP domain gains a conformation that allows it to directly interact with both the linker sequence and the substrate binding domain. Furthermore, the α-helical lid opens due to the interaction with the ATPase domain, which, in turn, exposes the polypeptide binding site [5]. This conformation allows for both rapid binding and releasing a substrate protein. Upon the ATP to ADP hydrolysis, the conformation of the two domains changes significantly. The substrate binding domain detaches from the ATPase domain and the helical lid closes the polypeptide binding site, thus it stabilizes the interaction with the substrate protein [6]. In turn, a replacement of ADP ~~on~~ by ATP induces a conformational change enabling the polypeptide to detach and, therefore, the new cycle of substrate binding may be initiated again.

Hsp70 proteins do not function independently. The hydrolysis of ATP and the exchange of ADP into ATP are two

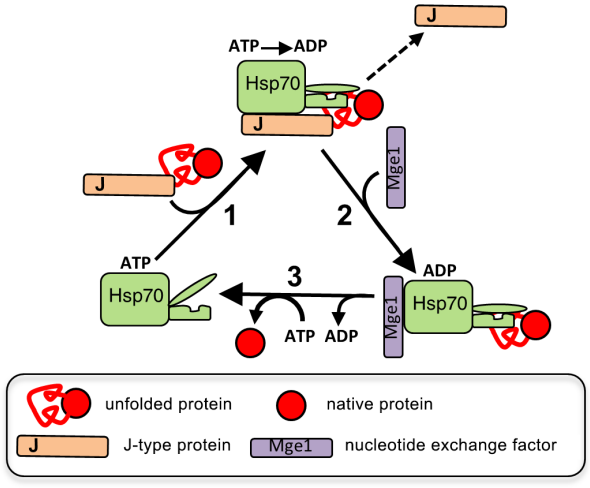


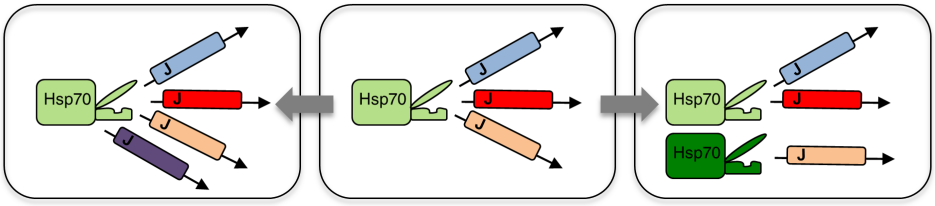
Figure 3. Evolution of the functional differentiation of Hsp70 systems. In many compartments of the eukaryotic cell a single Hsp70 protein functions with a number of J-proteins. Each of them determines the level of contribution of the partner Hsp70 in different cellular process (middle panel). The process of Hsp70 functional differentiation systems results in the engagement of a new J- protein (left panel) and/or duplication and specialization of Hsp70 protein (right panel).

key activities (Fig. 2), stimulated by J-protein and the nucleotide exchange factor [7,8]. A characteristic feature of all J-proteins is the J-domain (Fig. 1B). A typical J-domain is composed of four α-helices, two of which, helix II and III, form a finger like structure with anti-parallel chains linked by a flexible loop. The loop contains three amino acid residues (histidine, proline and aspartic acid, HPD) that occur in all J-domains [9]. The J-domain stimulates the ATPase activity of Hsp70, which requires a direct protein-protein interaction and the HPD motif. J-proteins also contain additional domains supporting their interaction with Hsp70, although these domains may be structurally divergent. In some cases, the additional domains allow J-proteins to bind to their substrate protein and transfer it to the Hsp70 partner (Fig. 2). Other J-proteins, comprise of domains that tether them to particular locations within a cell, where Hsp70 activity is required [1].

Nucleotide exchange factors are evolutionarily diverse group of proteins of a common ability to interact with Hsp70, which leads to conformational changes facilitating a dissociation of ADP, formed as a result of ATP hydrolysis [8]. Thus, nucleotide exchange factors initiate binding of another ATP molecule, which results in dissociation of a substrate protein bound to Hsp70. The interaction of Hsp70 with the auxiliary proteins regulates the cycle of a substrate protein binding. On the one hand, J-proteins promote the formation of the stable Hsp70-substrate complex by stimulation of the ATPase activity, and on the other hand, the nucleotide exchange factors regulate the frequency of the cycle of substrate binding.

MULTIPLICATION OF THE NUMBER OF GENES ENCODING HSP70 PROTEINS AND J-PROTEINS

Hsp70 and J-proteins are the exception among chaperones because they are encoded by multigene families in organisms belonging to each of three domains of life (Fig. 3). For example, the genome of *S. cerevisiae* encodes 14 Hsp70 chaperones and 23 J-proteins, whereas in human genome as many as 17 genes of Hsp70 have been identified that co-interact with J-proteins encoded by 41 genes [1,10,11]. Also several viral genomes harbor genes encoding Hsp70 chaperones and J-proteins [12]. This genetic variation distinguishes Hsp70 chaperones from other chaperon systems. Why is this happening? One of the factors promoting ~~of~~ such diversity may be that the reversible polypeptide binding is a mechanism that may be used in a variety of cellular processes. Moreover, the cycle of binding and dissociation of the substrate protein from Hsp70 is strictly regulated on several levels. Firstly, Hsp70 can discriminate particular substrate proteins by modifying the polypeptide binding site. Also, the J-protein determines which proteins are to be bound and delivered to its Hsp70 partner. Secondly, the speed and frequency of the protein substrate binding cycle can be regulated by a specific nucleotide exchange factor. Functional diversity of Hsp70 proteins is also supported by the fact that they function as a monomers, while other chaperones are oligomers composed of several subunits [3]. In this way their features, such as binding of cellular proteins other than the typical substrate proteins, are not limited by the structural requirements, necessary for the formation of stable oligo-



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mers. These Hsp70 properties expand their range of action to many important cellular functions that are not associated only with the process of polypeptides folding, which before was believed to be the main function of chaperone proteins.

FUNCTION AND EVOLUTION OF MITOCHONDRIAL HSP70 SYSTEMS

FUNDAMENTAL MITOCHONDRIAL HSP70 SYSTEM

Mitochondria were formed in the process of endosymbiosis of a cell that was probably a representative of α-proteobacteria [13]. It is not surprising though, that the fundamental mitochondrial Hsp70 system is closely related to bacterial chaperones: Hsp70 DnaK, J-protein DnaJ and nucleotide exchange factor GrpE [14]. In *S. cerevisiae* equivalents of these proteins are known as Ssc1 Hsp70, Mdj1 J-protein and Mge1 nucleotide exchange factor (Fig. 4). Bacterial and yeast proteins reveal a significant degree of similarity in terms of their structure and sequence. In some cases, bacterial proteins from *E. coli* can replace mitochondrial proteins in biochemical experiments or in living yeast cells [15,16]. The Ssc1/Mdj1/Mge1 protein system is located in the mitochondrial matrix, where it functions similarly to its bacterial equivalent (DnaK/DnaJ/GrpE). For example, both biochemical studies and studies on yeast cells have shown that the fundamental Hsp70 mitochondrial system is involved in the folding of polypeptide chains of mitochondrial proteins in both physiological conditions or under thermal stress [17-19]. After lowering the temperature, the Ssc1/Mdj1/Mge1 system refolded and reactivated the aggregated proteins [19].

Evidence suggest that these processes are consistent with the described above mechanism of the cyclic binding and dissociation of the substrate protein regulated by ATP hydrolysis. The reaction is triggered by Mdj1 that binds the denatured protein and targets it to Ssc1. At the same time Mdj1 J-domain stimulates the Hsp70 ATPase by stabilizing the substrate binding. Mge1 releases ADP and enables the next ATP molecule to bind and the protein substrate to dissociate, which initiates a new cycle of substrate binding. Although, most studies have been conducted using model substrate proteins, several native substrates of Ssc1/Mdj1/Mge1 system have been also identified. These include Mip1, mitochondrial DNA polymerase, and Var1, mitochondrial ribosomal subunit [17,19,20].

Despite the obvious similarities of bacterial and mitochondrial systems, the latter is also involved in functions typical of mitochondria. This system is responsible for the maintenance and propagation of mitochondrial DNA [20,21]. This fact may indicate that the Ssc1/Mdj1/Mge1 system has evolved under the influence of a selection pressure caused by the specificity of mitochondrial function. Mitochondria have their own genome (mtDNA) encoding a limited number of mitochondrial proteins, mitochondrial tRNA and subunits of mitochondrial ribosomes [22,23]. Maintenance and replication of mtDNA is prerequisite for a proper functioning of both the mitochondria themselves and a eukaryotic cell. These processes are managed by a complex of proteins associated with mtDNA, known as the mitochondrial nucleoid [22,24]. The components of Ssc1/Mdj1/Mge1 system occur in nucleoid complex as many other mitochondrial proteins whose main activity is not evidently related to DNA metabolism [22,25]. So far, little is known about the role of these proteins in the maintenance and replication of mtDNA.

However, the results of research on Mdj1 are unambiguous. Both deletion of the gene encoding Mdj1 and the substitution of the HPD sequence in J-domain lead to a rapid loss of mtDNA, even under optimal conditions in yeast culture [21], in which the mitochondrial DNA polymerase is fully active [20]. Moreover, most of Mdj1 is located in the nucleoid complex probably by a direct interaction of Mdj1 with mtDNA [21]. Although, the molecular mechanism of Mdj1 in maintenance and replication of mtDNA has not been known yet, the results suggest that Mdj1 has to be located in the vicinity to the nucleoid complex and that it has to interact with its partner Hsp70 Ssc1 [21]. It can be speculated that

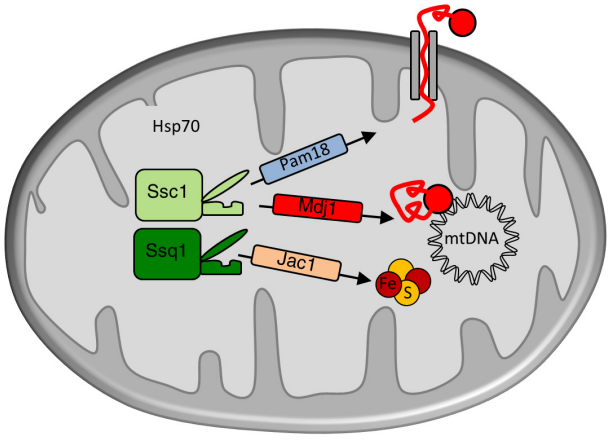

Figure 4. The Hsp70 systems found in the mitochondrial matrix in *S. cerevisiae*. Multifunctional Hsp70 Ssc1 interacts with J-proteins; with Mdj1 in the polypeptide folding and in the maintenance and propagation of mtDNA; with Pam18 in protein import. Specialized Hsp70 Ssq1, which has been formed as a result of *SSC1* gene duplication, interacts with Jac1 during biogenesis of iron-sulfur centers (Fe/S).

this mechanism modifies the stability of the protein complexes involved in the mtDNA transactions. Similar activity exhibit homologous bacterial proteins (DnaK/DnaJ/GrpE) which modify the stability of the protein complex involved in the initiation of DNA replication of a phage e.g., DNA replication of bacteriophage λ that is *E. coli* parasite [26,27]. This activity is essential for a virus propagation in a bacterial cell. Proteins of the Hsp70 system are also involved in the viral replication in eukaryotic cells [12].

SPECIALIZED HSP70 SYSTEM OF PROTEIN IMPORT INTO MITOCHONDRIA

Although the fundamental Ssc1/Mdj1/Mge1 system supports the folding of polypeptide chains of a protein substrate which is typical for chaperones in physiological conditions or under stress, the components of the system: Ssc1 and Mge1 are also involved in other processes in which interact with specialized J-proteins (Fig. 4). One of such processes is the import of proteins into the mitochondrial matrix [28,29].

In the process of evolution, many genes have been translocated from mitochondrial genome of an endosymbiont into the nuclear genome of a host cell. Although, the genes have changed the location, their protein products still function in mitochondria. It was possible due to a developed protein transport from the cytoplasm to mitochondria [30,31]. Upon evolution of this system the mitochondrial Hsp70 (Ssc1 in *S. cerevisiae*) has become a factor directly responsible for the transport of polypeptides through the channel of the inner mitochondrial membrane. In the process of mitochondrial evolution, the Hsp70 activity of reversible binding to substrate protein is utilized. Ssc1 binds to the hydrophobic sequences that become exposed as the unfolded polypeptide is moving through the transport channel driven by the difference in membrane potential between the outer and inner mitochondrial membranes. The Ssc1 reversible binding prevents the polypeptide chain withdrawing from the channel, thereby enabling it to slide into the mitochondria [29]. In this process, the ATPase activity of Ssc1 is supported by the specialized J-protein in *S. cerevisiae*, Pam18 or Tim14 [32,33], and the initiation of the next polypeptide binding cycle is promoted by Ssc1 molecule, stimulated by Mge1 nucleotide exchange factor [34].

Evolutionary origin of Pam18 remains unknown. Though, it is been already reported that its homologs occur in all eukaryotic cells and their unique and evolutionary conserved domain structure distinguishes Pam18 from other J-protein, with which they share only the J-domain [35,36]. In contrast to Mdj1 or DnaJ, Pam18 does not bind the protein substrate [32,33]. Therefore, its only function in the process of polypeptide translocation is to stimulate the ATPase activity of mtHsp70 Ssc1. Domain structure of Pam18 enables it to function effectively in the process of polypeptide import. Its J-domain is located in the mitochondrial matrix and is connected to a transmembrane domain, which locates Pam18 in the inner mitochondrial membrane [35]. The main mechanism of Pam18 localization in the proximity to the transport channel, involves its interaction with another protein, termed Pam16 or Tim16 [37]. The similarity of the structure and sequence of Pam 18 and 16 proteins points at their common origin. However, Pam 16 has a degenerate J-domain, which lacks the HPD sequence, thus the protein does not have the ability to stimulate Ssc1 ATPase [38]. Instead, PAM16 provides a structural role. N-terminal domain of PAM16 interacts directly with the proteins of the transport channel and the J-domain binds to the J-domain of Pam18, locating it in the vicinity to Ssc1 [37]. Evolutionary origin of PAM16 is not known. However, its occurrence in all eukaryotic cells, suggests that the mechanism of the location of Pam18 through its interaction with Pam16 is ancient [39]. Perhaps, when the original Pam18/16 gene was duplicated, its copies have been functionally differentiated. Pam18 has retained its ability to stimulate the ATPase, while Pam16 has specialized in the interaction with proteins of the transport channel. In this way, the process of evolution has shaped the complicated mechanism of their co-interaction that allows the polypeptides to be efficiently transport through the channel of the inner mitochondrial membrane.

Gene duplication of Pam18 resulted in the formation of a new domain in *S. cerevisiae* and other closely related yeast species [35]. In addition to the above-described J-domain and the transmembrane domain, Pam18 in yeasts contains an additional domain located in the intermembrane space of a mitochondrion. This domain allows for a direct interaction between Pam18 and the protein of the transport channel located in the intermembrane space, thereby stabilizing the Pam18 localization in a direct vicinity of the transport channel [40]. The origin of the additional intermembrane domain remains the object of speculation. One of the hypotheses assumes that the domain has been formed following the Pam18 gene duplication [35]. The second copy of the gene, encoding Mdj2 protein, differs from Pam18 in two ways: it lacks the intermembrane domain, and a deletion of MdJ2 gene is not lethal but hinders yeast growth in anaerobic conditions [32,33,35,40]. Perhaps, following gene duplication, a mutation could occur that resulted in the shift of a reading frame thus forming the primary version of the Pam18 intermembrane domain. This domain has diverged gradually to gain the ability to interact with the protein of transport channel, which has provided Pam18 the leading role in the protein import. This evolutionary scenario required the Mdj2 functional protein that at the early stages of differentiation of the intermembrane domain protected the cell against a possible decrease in Pam18 activity.

The location of Pam18 in the vicinity of the transport channel may suggest that the protein itself recruits Ssc1 to the site of action. However, the location of Ssc1 depends on its direct interaction with Tim44 protein which is one of the structural components of the transport channel [28,29]. As the Ssc1-Tim44 interaction does not utilize the typical mechanism of a substrate protein binding by Hsp70, it can be assumed that it is a derived feature of mitochondrial Hsp70, which has evolved under the selective pressure for the effective mitochondrial protein import.

In conclusion, two changes led to the transformation of the mitochondrial Hsp70 in a key element of the system responsible for the import of mitochondrial proteins: (1) new specialized J-protein, and 2) new interaction between the

mitochondrial Hsp70 and Tim44. Tim44 protein is an evolutionarily conserved protein that is a component of the protein complex associated with the protein import channel of the inner mitochondrial membrane.

HSP70 SYSTEM INVOLVED IN THE MITOCHONDRIAL BIOGENESIS OF IRON-SULFUR CENTRES

Given that the mitochondria inherited from their bacterial ancestors many biochemical pathways, it is not surprising that they constitute the metabolic center of an eukaryotic cell. One of the key functions of mitochondria is the synthesis of iron-sulfur centers (Fe/S), which are *prosthetic* group of numerous cellular proteins [41]. Fe/S centers occur, among others, in proteins involved in mitochondrial oxidative phosphorylation and in many other proteins located in all compartments of eukaryotic cell. In humans, a disruption of mitochondrial Fe/S biogenesis leads to many diseases [42].

Only one among the three alternative pathways of Fe/S biogenesis in bacteria has been inherited by the mitochondria. It is called ISC, *iron-sulfur-cluster.* In this pathway, the Fe/S center is first synthesized within a IscU scaffold protein and then transferred from IscU on the recipient proteins. In bacteria, the Fe/S transfer is promoted by a specialized Hsp70 system consisting of Hsp70 HscA and J-protein HscB [43]. The evolutionary origin of this system remains unknown, but it is present in all species of bacteria harboring the ISC pathway [44]. The feature that distinguishes the HscA/HscB system from the DnaK/DnaJ/GrpE system described above, is its high specialization in terms of the substrate protein, here only IscU, and the co-chaperone J-protein, here only HscB. When HscA/HscB is involved, the substrate-binding cycle does not require any nucleotide exchange factor [43], because HscA binds ATP with lower affinity than DnaK, and thus the nucleotide exchange occurs spontaneously.

Although many bacteria and all eukaryotes utilize the Hsp70 system for Fe/S centers transfer from a scaffold protein to recipient proteins, the changes that took place within the system upon mitochondrial evolution illustrate both the plasticity and specialization of its components. Mitochondria have inherited most of the protein components of the ISC pathway from their bacterial ancestors. Both the scaffold protein (Isu in *S. cerevisiae*) and J-protein (Jac1 in *S. cerevisiae*) are present in all eukaryotes, though, the gene encoding specialized Hsp70 (HscA) has been lost in the course of mitochondrial evolution. So far, its (ortholog) has been not identified in any of the studied eukaryotic genomes [45]. The majority of eukaryotic genomes, including human, encodes only one copy of the mitochondrial Hsp70, which is closely related to bacterial DnaK [11]. Evidence suggest that multifunctional mitochondrial Hsp70 has replaced the specialized bacterial protein in the biogenesis of Fe/S centers [46] cooperating in this process with the specialized J-protein Jac1, thus enabling the transfer of Fe/S centers from the Isu scaffold protein on to the recipient proteins.

However, the evolutionary history does not end here. A group of yeast species, closely related to *S. cerevisiae*, possess mitochondrial Hsp70 (Ssq1), which functions exclusively in the biogenesis of Fe/S centers! How is it possible? The evolutionary analysis has shown that Ssq1 emerged in the common ancestor *Candida albicans* and *S. cerevisiae* as a result of the duplication of a gene encoding mitochondrial Hsp70 approx. 400 million years ago [45]. After the duplication, one copy retained activities typical of mitochondrial Hsp70, supporting the polypeptide folding, protecting the proteins against stress, importing the mitochondrial proteins and maintaining the mtDNA. The second copy has been specialized and functions in the biogenesis of the Fe/S centers exclusively [11].

The specialized protein, Ssq1, reveals the high affinity for both the J-protein, Jac1, and for the substrate protein, Isu [47-50]. Though, Ssq1 does not interact with other J-proteins (Mdj1 or Pam18) and does not bind a variety of protein substrates interacting with the multifunctional mitochondrial Hsp70 [45,47]. Thus, Ssq1 has resembled itself biochemically to bacterial HscA that specializes in the biogenesis of Fe/S centers. However, in contrast to HscA, but just like DnaK and mitochondrial Hsp70, the cycle of substrate binding by Ssq1 depends on the Mge1 nucleotide exchange factor [47]. Ssq1 specialization is an example of evolutionary convergence at the biochemical level. A duplication of the gene encoding mitochondrial Hsp70 has restored the specialized system of Hsp70 protein that had functioned in bacterial ancestors of mitochondria.

The presence of Ssq1 in fungal species correlates with structural changes that has occurred in its interacting partner J-protein, Jac1, [51]. In species, in which Jac1 co-occurs with Ssq1, alternations have been observed in the structure of J- domain. In particular, the loop linking α-helix II and III, and α -helix III are shortened (Fig. 1). Biochemical studies have shown that these structural changes and numerous amino acid substitutions within the α-helix II are responsible for high affinity interactions of Jac1 and Ssq1 proteins [46]. It is a unique example of a co-evolution of Hsp70 system proteins that leads to their specialization. An altered Jac1 protein does not effectively cooperate with multifunctional mitochondrial Hsp70.

Both the specialized and multifunctional Hsp70 proteins in order to transfer Fe/S centers use a typical mechanism of the cyclic interaction with substrate protein. In the mitochondria of *S. cerevisiae* the process is initiated by Jac1 protein, which, in addition to the N-terminal J-domain, contains a unique C-terminal domain specialized in the binding of Isu scaffold protein [52,53]. Molecular mechanism of this interaction is the same in bacterial and mitochondrial orthologs of Jac1. Jac1-Isu complex interacts with Ssq1-ATP which promotes the ATPase activity and stabilizes the Isu binding by Ssq1 [47]. Although the molecular mechanism of the transfer of Fe/S center is not known yet, it is believed that conformational changes within the Isu scaffold protein, caused by its interaction with Ssq1, destabilize the Fe/S center binding thus supporting its transfer of to a recipient protein [54]. In the mitochondrial system, Mge1 nucleotide exchange factor promotes the release of Isu and the initiation of a new binding cycle [47]. Studies on *S. cerevisiae* have also shown that Jac1 not only binds Isu, but also competes for the binding with a protein complex responsible for the

Fe/S synthesis within Isu [55,56]. Moreover, Isu in the complex with Jac1, is protected from proteolysis, catalyzed by mitochondrial Pim1 protease [57]. In turn, Ssq1 is able to directly interact with one of the target proteins, Grx5 [58]. It suggests that proteins of the Hsp70 system, involved in the biogenesis of Fe/S centers, reveal the biochemical activity extending beyond the typical mechanism of the cyclic binding of substrate protein.

So far, it been difficult to answer the question whether Hsp70 protein specialization involved in the biogenesis of Fe/S centers in yeast is of adaptive significance. On the one hand, none of the species that have diverged after the duplication of mitochondrial Hsp70, has lost the gene encoding Ssq1 [11]. On the other hand, the majority of eukaryotes functions without this protein. Further studies on this system may shed a new light on the functional importance of Hsp70 systems divergence.

SUMMARY – VERSATILITY OF HSP70 SYSTEMS ENABLES THEIR USAGE IN NUMEROUS BIOLOGICAL PROCESSES

Evolutionary versatility of Hsp70 systems is based on two basic adaptive mechanisms (1) a highly specific interaction with the substrate protein which depends on a direct specialization of the Hsp70-substrate interaction (Ssq1-Isu interaction), and/or on the specific interaction of J-proteins with the particular substrates that are targeted then to the partner Hsp70 (Jac1-Isu interaction), (2) „recruitment" of Hsp70 protein to the site of action, or by a direct interaction of Hsp70 with proteins, which are not their typical substrates (mitochondrial Hsp70 - Tim44 interaction), or by locating the corresponding J-protein at a site of Hsp70 action (Mdj1 complexed with mtDNA). The above-mentioned evolutionary strategies are not limited to mitochondrial Hsp70, but this system clearly illustrates the Hsp70 differentiation mechanisms used in all compartments of a eukaryotic cell.

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Mitochondrialne Hsp70 - funkcja i ewolucja

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Słowa kluczowe: białka typu-J, mitochondrialne DNA, import białek mitochondrialnych, centra żelazo-siarkowe, ewolucja białek

STRESZCZENIE

Zdolność do cyklicznego oddziaływania z substratami białkowymi umożliwiła białkom opiekuńczym Hsp70 udział w różnorodnych procesach komórkowych takich jak: fałdowanie łańcucha polipeptydowego białek, transport polipeptydów przez błony, modulowanie odziaływań pomiędzy białkami, zapobieganie agregacji polipeptydów oraz rozbijanie agregatów białkowych. Aktywności te wymagają współpracy białka Hsp70 z białkami pomocniczymi typu-J oraz czynnikami wymiany nukleotydów. Białka pomocnicze regulują cykl hydrolizy ATP katalizowanej przez Hsp70, który umożliwia odwracalne wiązanie substratu białkowego. W poszczególnych przedziałach komórki eukariotycznej może współwystępować kilka różnych Hsp70 oraz białek typu-J. Dlatego ich funkcjonowanie w różnorodnych procesach wymaga specjalizacji albo samego Hsp70 albo też zdolności wielofunkcyjnego Hsp70 do oddziaływania z zestawem wyspecjalizowanych białek typu-J. Badane przez nas systemy Hsp70 funkcjonujące w mitochondriach dostarczają przykładów obydwu typów specjalizacji. Tym samym stanowią dogodny model badawczy umożliwiający poznanie zarówno molekularnych jak też ewolucyjnych podstaw funkcjonalnego różnicowania systemów Hsp70.