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A specialized mitochondrial molecular chaperone system: A role in formation of Fe/S centers

E. A. Craig[a,*] **and J. Marszalek**[b]

[a] Department of Biomolecular Chemistry, 1300 University Avenue, University of Wisconsin, Madison, Wisconsin 53706 (USA), Fax +1 608 262 5253; e-mail: ecraig@facstaff.wisc.edu
[b] Department of Molecular and Cellular Biology, Faculty of Biotechnology, University of Gdansk, 80-822 Gdansk, Kladki 24 (Poland)

Abstract. Mitochondria contain a specialized system of molecular chaperones that plays a critical role in the biogenesis of Fe/S centers. This Hsp70:J-protein system shows many similarities to the system found in bacteria, but the precise role of neither chaperone system has been defined. However, evidence to date suggests an interaction with the scaffold protein on which a transient Fe/S center is assembled, and thus implies a role in either assembly of the center or its transfer to recipient proteins.

Key words. Mitochondria; Hsp70; Fe/S center; iron; Jac1; Ssq1; molecular chaperone.

Hsp70s of yeast mitochondria

Molecular chaperones of the hsp70 class are found in virtually every compartment of the eukaryotic cell. In the yeast *Saccharomyces cerevisiae,* 3 out of the 16 hsp70s are present in the mitochondrial matrix [1–3]. Ssc1, which is essential for viability and is the most abundant of the three, making up 1–3% of mitochondrial protein, plays multiple roles in mitochondrial metabolism (for reviews see [4–6]). About 10% of Ssc1is tethered to the inner membrane at the site of the translocation channel and is critical for translocation of proteins from the cytosol into the matrix. Ssc1 also facilitates the folding of these imported proteins, as well as those synthesized on mitochondrial ribosomes [7]. Consistent with the fact that expression of Ssc1 increases upon a heat shock, its function is important under stress conditions. Ssc1 protects mitochondrial proteins from heat inactivation, and facilitates degradation of mitochondrial proteins that either fold improperly or become permanently damaged after exposure to stress [8].

A second hsp70 protein of mitochondria, Ecm10, shares 82% amino acid identity with Ssc1, but in contrast to Ssc1 is of low abundance. The high level of sequence identity between these two hsp70s suggests similar biochemical properties and physiological functions. These predictions were borne out by experimental analysis [3]. Although no phenotype was observed upon deletion of *ECM10*, synthetic growth defects were observed with a conditional allele of *SSC1*, *ssc1-3*. In addition, overexpression of Ecm10 was able to overcome defects of *ssc1-3* in in vitro protein import experiments, suggesting that functions of Ssc1 and Ecm10 do overlap.

The third member of the yeast mitochondrial hsp70 family, and the focus of this review, Ssq1, is more distantly related to Ssc1, having 52% amino acid identity [2]. Neither this low level of sequence conservation, nor the early analysis of the *ssq1* deletion strain, provided substantive clues to Ssq1's physiological function. Ssq1 is not essential; the Δ*ssq1* strain grows very slowly at low temperatures, but nearly normally at 37 °C. The absence of Ssq1 resulted in no defects for the translocation of many precursor proteins into mitochondria, suggesting a role other than protein translocation. Ssq1, which is not induced by heat shock, is present in mitochondria at very low concentrations, 500–1000-fold lower than Ssc1 [2, 9]. This low abundance pointed to the possibility that Ssq1 plays a very specific function in mitochondrial metabolism. As discussed extensively below, recent results indicate that Ssq1 is specifically involved in the assembly of Fe/S centers.

* Corresponding author.

Basic biochemical properties of hsp70

While different hsp70s may be involved in a wide variety of physiological roles, all hsp70s that have been studied have the same basic core biochemical properties activities: (i) binding of short hydrophobic peptide sequences and (ii) a weak ATPase activity, which is stimulated by the binding of peptides, unfolded polypeptides or, rarely, native protein substrates [10]. The stability of the hsp70-protein substrate interaction depends on the conformation of the chaperone, which is regulated by the bound nucleotide. When an ADP molecule is bound to the nucleotide binding site, hsp70 exhibits stable peptide binding; when ATP is bound, binding of peptide is relatively unstable. Therefore ATP hydrolysis converts hsp70 to the form which has a relatively stable interaction with peptide. Exchange of ADP for ATP results in dissociation of the bound peptide. The transient nature of the hsp70-protein substrate interaction, allowing cycles of binding and release of hsp70, is critical for function in a variety of cellular processes.

Co-chaperone of mitochondrial hsp70s

Hsp70s do not function alone. Rather, they act with co-chaperones that modulate the ATPase/polypeptide-binding cycle of hsp70s. These co-chaperones include J-type proteins and nucleotide release factors [10]. The defining feature of J-type co-chaperones is a J domain, a conserved region named after the canonical J protein of *Escherichia coli*, DnaJ. A hallmark of the J domain is a conserved tripeptide, histidine, proline, aspartic acid (HPD) [11, 12]. J-type proteins interact directly with hsp70 and increase the stability of the hsp70-protein substrate interaction by stimulating the ATPase activity of hsp70. Many J-type proteins also interact with protein substrates, and are thus thought to target them to hsp70s, effectively increasing the substrate specificity of particular hsp70 proteins.

In yeast mitochondria, Mdj1, which has a domain structure very similar to that of DnaJ, is a co-chaperone for Ssc1 [13]. Jac1, a second J-type protein of the mitochondrial matrix that is absolutely essential in most strain backgrounds, is the focus of this review [14–16]. It is small compared with most J-type proteins. The mature form in the matrix is only 20.4 kDa, having only the J domain in common with larger J-type proteins. However, it too is capable of binding protein substrates [J. Marszalek and E. Craig, unpublished]. The J domain and the C terminus are critical, suggesting that both interaction with a hsp70 and binding of protein substrates are critical for Jac1 function. Substitution of three alanines for the HPD is very deleterious, resulting in cells that grow very slowly and are thermosensitive [15]. C-terminal truncations do not support growth ([16]; Y. Cheng and E. Craig, unpublished). As described below, Jac1 is a J-type cochaperone for Ssq1.

Nucleotide release factors are essential components of at least some hsp70 chaperone machines. GrpE of *E. coli*, the first nucleotide release factor identified, releases ADP and protein substrate from DnaK [17]. As a consequence, a new cycle of nucleotide and protein substrate binding ensues. However, in some cases, when the off-rate of nucleotide from hsp70 is rapid, release of ADP and protein substrate does not require assistance of nucleotide release factors [18]. In yeast mitochondria Mge1, an essential protein, acts as a nucleotide release factor for Ssc1, and as described below, probably Ssq1 as well [19–22].

Ssq1 and Jac1: molecular chaperones involved in iron metabolism in mitochondria

As the result of a variety of genetic screens, a connection between the function of Ssq1 and mitochondrial iron metabolism was recently made. An *SSQ1* mutant allele was identified in a genetic screen designed to find genes responsible for regulation of iron homeostasis in yeast cells [23]. This mutant, as well as a deletion of *SSQ1,* accumulated large quantities of iron in mitochondria [24]. In addition, a search for a gene whose inactivation would lead to a synthetic lethal interaction with Δ*ssq1* identified the gene *NFU1*, which is related to genes known to be involved in Fe/S center formation in nitrogen-fixing bacteria [24]. An unrelated genetic selection identified both *SSQ1* and *JAC1*, as well as *NFS1*, which encodes a core component of the Fe/S assembly system [14]. In this screen mutants of *SSQ1, JAC1* and *NFS1* were found as suppressors of the defect in methionine and lysine biosynthesis caused by endogenous oxygen toxicity in yeast lacking the copper/zinc superoxide dismutase. While the physiological details of the cause of all of these genetic interactions remain to be elucidated, the take-home message was clear: Ssq1 and Jac1 are in some way involved in mitochondrial iron metabolism and assembly of Fe/S centers [25].

Identification of both *SSQ1* and *JAC1* mutants in the same genetic screen suggested that Jac1 is the J partner for Ssq1[14]. Extensive evidence now supports the idea that Ssq1p and Jac1 are partners involved in the biogenesis of Fe/S centers and iron homeostasis in mitochondria. Both proteins are localized to the mitochondrial matrix [2, 15, 26], and mutation of either gene results in a 10-fold increase of mitochondrial iron levels and significant decreases in the enzymatic activities of mitochondrial proteins containing Fe/S centers, including aconitase, the cytochrome bc_1 complex and succinate dehydrogenase [9, 14–16, 24, 26]. Because increased levels of iron result in increased production of oxygen radicals within mito-

chondria [27] that can damage Fe/S centers, the decrease in the activity of Fe/S-containing enzymes could be attributed to the indirect effect of iron accumulation. However, this simple explanation was ruled out. Activities of Fe/S-containing enzymes were also reduced in Δ*ssq1* and *jac1* strains grown on media containing low levels of iron [15, 16]. Such growth conditions result in normal levels of iron in mitochondria, thus indicating that Ssq1 and Jac1 play more direct roles in biogenesis of enzymes containing Fe/S centers. An additional indication that Ssq1 and Jac1 involvement in the process of incorporation of Fe/S centers is direct comes from in organello assays of Fe/S center assembly [26]. Apo-ferredoxin synthesized in vitro and imported into isolated mitochondria is converted into the holo-form upon incorporation of Fe/S center, as monitored by altered mobility in native gels. Formation of holo-ferredoxin was compromised in *jac1* and *ssq1* mitochondria, indicating that both proteins play a role in this process.

An additional connection to iron metabolism and an indication that Ssq1 and Jac1 function together comes from analysis of the maturation of a nuclear-encoded protein of the mitochondrial matrix, Yfh1 [23]. Yfh1 is a homologue of the human protein frataxin, which is associated with the disease Freidrich's ataxia that is characterized by decreased activity of Fe/S-containing enzymes and an increase in mitochondrial iron levels ([28]; for review see [29]). The phenotype of a *yfh1* deletion strain is remarkably similar to that of *ssq1* and *jac1* mutants.

Following the import of Yfh1, its presequence is cleaved twice by the mitochondrial processing peptidase (MPP) [30, 31]. The second cleavage reaction is inhibited in mitochondria isolated from both *ssq1* and *jac1* mutants [9, 23]. Neither Ssq1 nor Jac1 is required for the import or the initial processing event of Yfh1, or any other proteins tested, for that matter [2, 16, 26]. However, Ssc1 is required for these initial events of Yfh1 maturation [9]. As discussed below, the exact role of Ssq1 in Yfh1 maturation remains unresolved.

Fe/S center assembly system

Recent studies indicate a remarkable conservation during evolution of the basic mechanism of assembly of Fe/S centers and their insertion into recipient proteins (for review see [25, 32]). While much of the groundbreaking work involved the analysis of the specialized system of assembly of Fe/S centers into nitrogenase of nitrogen-fixing bacteria, it is now established that all bacteria have a related system used more generally for many Fe/S center-containing proteins. This system is very similar to that of mitochondria. While much remains to be learned, according to current thinking, an Fe/S center is transiently assembled on a scaffold protein prior to transfer to the recipient protein (fig. 1). NifU of nitrogen-fixing bacteria serves this function for nitrogenase [33]; the related proteins, IscU or Isu from bacteria and mitochondria, respectively, serve this role for other Fe/S proteins [34, 35]. Additional proteins act as sulfur or iron donors. NifS has been identified as the sulfur donor for nitrogenase [33]; IscS and Nfs1, the related proteins from bacteria and mitochondria, respectively, serve this role for the general assembly system [35]. The identity of the iron donor remains an open question.

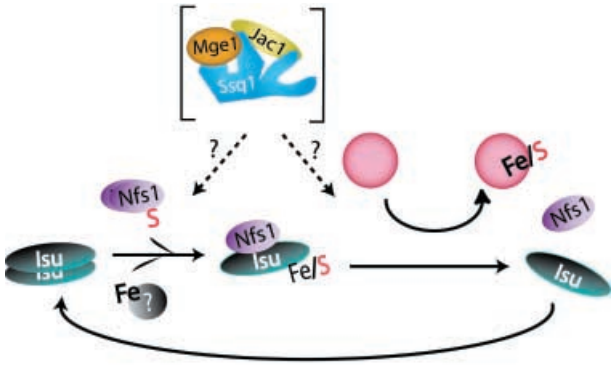

Figure 1. Model of pathway of assembly of Fe/S centers into recipient proteins. As described in the text, an Fe/S center is transiently assembled on Isu (IscU in bacteria)) and then transferred to the recipient protein. The names for protein are those for the yeast mitochondrial system. The molecular chaperones Ssq1, Jac1 and Mge1 (Hsc66 and Hsc20 in bacteria) are proposed to facilitate either the assembly of the Fe/S center or its transfer to the recipient protein.

The biochemical functions of the mitochondrial proteins have not yet been studied in mechanistic detail, but physiological studies leave little doubt that they are involved in Fe/S assembly, as decrease in the activity of these proteins in cells results in lowered activity of many Fe/S center-containing enzymes. In addition, reduction in activity of these proteins resulted in increased levels of iron in mitochondria, as described for cells having a reduced amount of Ssq1 and Jac1 function [9, 14–16, 23, 24]. Increased mitochondrial iron levels have become a hallmark of disruption of the function of proteins involved in the biogenesis of Fe/S centers in mitochondria. The physiological mechanism behind this disruption of iron homeostasis is not understood.

Bacterial chaperones involved in biogenesis of Fe/S centers

In bacteria the genes known to be involved in Fe/S cluster assembly, such as IscU and IscS, are clustered in the genome [36]. Interestingly, the cluster also contains two genes encoding molecular chaperones, *hscA* and *hscB* (fig. 2). *hscA* encodes Hsc66 protein, which belongs to hsp70 family [37, 38]. *hscB* encodes Hsc20 protein, a

representative of the J-type family of proteins [38]. The presence of the two chaperone genes within the *isc* cluster suggests that as in yeast mitochondria, chaperone proteins play a role in biogenesis of Fe/S centers, and that Hsc66 and Hsc20 form a functional Hsp70:J-protein pair. While the physiological data are less extensive than those from yeast, the fact that deletion of *hscA* results in cells that grew more slowly than wild type and showed a fourfold reduction in activity of the Fe/S center-containing protein FNR indicates an involvement in Fe/S center biogenesis in vivo [38, 39]. In addition, as described below, the biochemical analysis of Hsc66 and Hsc20 is more extensive than that presently available for Ssq1 and Jac1.

As in yeast mitochondria, more than one Hsp70 is present in the cytosol of *E. coli*. Hsc66, encoded in the *isc* gene cluster, and DnaK share 41% sequence identity [37]. Both are abundant proteins. DnaK comprises about 1.2% of cellular protein under normal growth conditions; upon induction by stress such as a temperature, the amount can increase to 3% [40]. In contrast to DnaK, Hsc66 expression is not induced by elevated temperature. Its cellular concentrations were estimated to be either the same as DnaK under normal conditions [41] or approximately fivefold lower than DnaK [40]. Regardless of which number is correct, hsc66 is present at vastly higher concentrations in the *E. coli* cytosol than Ssq1 is in the mitochondrial matrix, where it makes up about 0.003% of the protein.

Like other hsp70s, Hsc66 binds and hydrolyses ATP [41]. However, unlike DnaK, release of the reaction products, ADP and Pi, is not the rate-limiting step of the ATPase cycle. Rather ATP hydrolysis itself is rate limiting [42]. These results suggested that Hsc66 belongs to the group of hsp70 proteins that do not require a nucleotide release factor. Indeed, GrpE did not stimulate the ATPase activity of Hsc66, nor was binding of GrpE to Hsc66 detected [43].

It is well established that DnaJ is the J-protein partner of DnaK. Recent data from the Vickery laboratory established that Hsc20 is the J-protein partner of Hsc66, as Hsc20 stimulates its intrinsic ATPase activity. The DnaK:DnaJ and Hsc66:Hsc20 partnerships seem to be exclusive, as the ATPase activity of Hsc66 is stimulated very inefficiently by DnaJ even at very high concentrations. No stimulation of the ATPase activity of DnaK by Hsc20 has been detected [43].

Although hsp70s and J-proteins most often bind unfolded or partially folded polypeptides as substrates, a few native proteins have been identified as hsp70 substrates. For example, the transcription factor σ^{32} and phage λ DNA replication proteins λO and λP are substrates of DnaK and DnaJ [44]. Recent evidence indicates that IscU, which provides a scaffold for Fe/S center assembly in bacteria, is a native substrate of the Hsc66-Hsc20 pair [42, 45]: (i) the apparent affinity of Hsc66 for IscU is higher in the presence of ADP compared with ATP; (ii) IscU stimulates the ATPase activity of Hsc66, a stimulation that is enhanced to several hundredfold in the presence of Hsc20 and (iii) the ability of Hsc66 to suppress the aggregation of denatured rhodanase, a standard assay for monitoring binding to the peptide-binding cleft of hsp70, is competed by IscU. In addition, IscU binds to Hsc20 [45]. The presence of Hsc20 increases the efficiency of the IscU-Hsc66 interaction, indicating that Hsc20 might play a role in substrate 'targeting', similar to the 'targeting' of σ^{32} to DnaK by DnaJ [45].

The finding that IscU behaves as a native substrate for the Hsc66-Hsc20 system raises the question whether this system evolved specifically to interact with IscU in the Fe/S center assembly pathway or whether it also serves more general chaperone functions. Like other hsp70s, Hsc66 can bind unfolded proteins (e.g. rhodanese, citric synthase) [46]. However, the physiological significance of these interactions is not known. In vivo studies of mutants lacking DnaK or Hsc66 indicate that DnaK, but not Hsc66, plays a role in preventing protein aggregation and refolding of partially denatured proteins upon a heat shock [40].

In conclusion, two distinct hsp70 machineries function in *E. coli*: (i) DnaK, functioning with co-chaperones DnaJ and GrpE in multiple important processes under both normal and stress conditions and interacting with a wide variety of substrates, and (ii) the Hsc66-Hsc20 system, which may function in a single metabolic pathway, Fe/S center biogenesis, interacting with a very limited number of substrates. Available data indicate that there is no exchange of individual components between these systems.

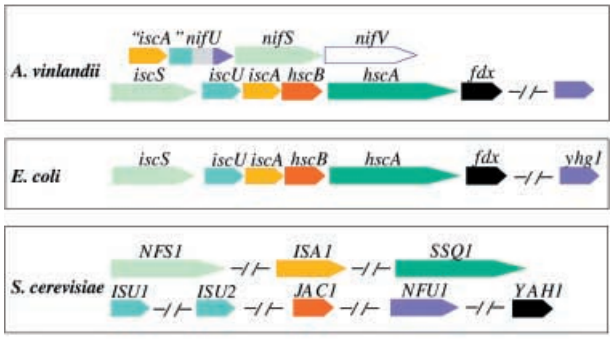

Figure 2. Organization of genes encoding components of the Fe/S center assembly machinery. Genes shown in the same color have sequence similarity. Genes separated by hatch marks are not in close proximity in the genome. *Azotobacter vinelandi* has two operons; the upper one in the figure is involved in nitrogen fixation. Adapted from [25, 36].

Comparison of the mitochondrial Ssq1-Jac1 and the bacterial Hsc66-Hsc20 chaperone system

The presence of chaperone systems involved in assembly of Fe/S centers in both mitochondria and the bacterial cy-

tosol raises questions as to whether these two systems are functionally the same, and whether the strict separation found for the DnaK:DnaJ and Hsc66:Hsc20 systems in bacteria is also true for the Ssc1:Mdj1 and Ssq1:Jac1 systems in mitochondria. Although the presence of a chaperone machinery involved in Fe/S assembly has been conserved from bacteria to the mitochondria of eukaryotes, a recent phylogenetic analysis indicated that the situation may be more complicated, with the homology between bacteria and mitochondrial systems being limited [47]. The focus of the reported analysis was Yfh1/frataxin. In 56 completely sequenced genomes, two genes were identified with identical phylogenetic distribution to the Yfh1/frataxin gene: *hscA* and *hscB*. This analysis also reveals that *hscB* is a true ortholog of mitochondrial *JAC1*, meaning that the two genes diverged from a common ancestor after a speciation event. The case of *hscA* is more complicated. In many bacteria orthologs of both frataxin and *hscA* are present. However, the topology of the hsp70 phylogenetic tree indicates that the mitochondrial Hsp70s Ssc1and Ssq1 are more closely related to DnaK than HscA [47]. Therefore, it was proposed that during evolution of mitochondria the bacterial *hscA* gene was replaced by its paralog *SSQ1*.

This phylogenetic analysis leads to the prediction that the biochemical properties of Ssq1 might resemble those of DnaK more than those of Hsc66, while Hsc20 and Jac1 would be quite similar. As mentioned above, one of the most important differences between DnaK and Hsc66 is the requirement for the nucleotide release factor (GrpE) in the cycle of binding of protein substrates to DnaK, which is due to the tight binding of nucleotide compared with Hsc66. Recent analysis of Ssq1 has revealed some biochemical similarities between DnaK and Ssq1. Ssq1 binds nucleotide tightly, and the mitochondrial nucleotide release factor Mge1 stimulates its release. In addition, the interaction between purified Mge1 and Ssq1 is ATP sensitive, as is the GrpE:DnaK interaction [J. Marszalek and E. Craig, unpublished results]. The Mge1:Ssq1 complex has been observed in mitochondria as well [26, 48]. Using co-immunoprecipitation analysis, ATP-sensitive interactions between Mge1 and both Ssq1 and Ssq1 were detected. Therefore, while the Hsc66:Hsc20 system in bacteria appears to be completely separated from the DnaK:DnaJ:Mge1system, in mitochondria the nucleotide exchange factor Mge1 is shared between Ssc1 and Ssq1.

The biochemical differences between Ssq1 and HscA, and the similarities between DnaK and Ssq1, are consistent with the results of phylogenetic analysis, but they give rise to questions of whether these two hsp70s can substitute for one another. Evidence indicates that, in fact, Ssc1 can in part substitute for Ssq1. Twofold over expression of Ssc1 partially suppresses the cold-sensitive growth phenotype of Δ*ssq1* cells, as well as the accumulation of mitochondrial iron and the defects in Fe/S enzyme activities normally found in Δ*ssq1* mitochondria [9]. Moreover, Δ*ssq1* mitochondria containing twofold more Ssc1 efficiently converted the intermediate form of Yfh1 to the mature form. These results indicate that the nonspecific Ssc1 chaperone is able to replace functions of Ssq1, when present at very high concentration in comparison with the cellular concentration of Ssq1. On the other hand, Ssq1 does not appear able to replace Ssc1 in its function in protein import [2], nor its ability to refold thermally inactivated proteins [J. Krzewska, K. Liberek and J. Marszalek, unpublished], indicating that this specialized chaperone is not able to function in place of its nonspecific counterpart.

In contrast, the function of the J proteins of the mitochondria appear to be restricted, with Jac1 likely playing a specific role in Fe/S center biogenesis. Consistent with this idea, incorporation of Fe/S centers into apo-ferredoxin in organello was not affected in the *mdj1* mutant, while depletion of Jac1 had significant effects [26]. However, open questions remain. The ability of increased amounts of Ssc1 to suppress the defects of a Δ*ssq1*indicates that Ssc1 can facilitate Fe/S center formation. Does Ssc1 functionally interact with Jac1 in this process? Although Ssc1 can suppress the Δ*ssq1,* it needs to be in 1000–2000-fold excess to do so. What are the characteristics of Ssq1 that allow it to function so much more efficiently in Fe/S center biogenesis? Further biochemical and genetic analysis is needed to answer these questions.

Possible roles of the chaperone system in Fe/S center biogenesis

The analyses described above indicate that both bacteria and mitochondria have chaperone systems intimately involved in the assembly of Fe/S centers, but that their hsp70 components were derived from different ancestors and have some different biochemical properties. These findings raise the obvious question of whether the mitochondrial and bacterial chaperones perform the same function in Fe/S assembly. As described in the previous section, a major clue to the function of Hsc66 and Hsc20 in the bacterial system was the identification of IscU, the proposed scaffold for Fe/S center assembly, as a native substrate for both proteins [35, 45, 46].

Isu1 and Isu2 are mitochondrial orthologs of IscU protein, which is >70% identical to the mitochondrial proteins [24, 49]. Presence of one of the *ISU* proteins is required for cell viability, as an *ISU1 ISU2* double deletion is inviable. However, deletion of the gene encoding Isu1, which is present at higher concentration than Isu2, results in a stronger phenotype than a deletion of the gene encoding Isu2. Δ*isu1* cells grow poorly on nonfermentable carbon sources and have significantly lower activities of

enzymes containing Fe/S centers such as aconitase and succinate dehydrogenase [24, 49].

The presence of these proteins in mitochondria and the indications of their involvement in Fe/S assembly raises the obvious question of whether they are substrates for Ssq1 and Jac1. Preliminary results indicate that they are. Purified Isu1 interacts with Ssq1, and this stable interaction is disrupted by ATP. In addition, Jac1 is able to catalytically stimulate functional interactions between Isu1 and Ssq1, as low concentrations of Isu1 and Jac1 cooperatively stimulate Ssq1 ATPase activity [J. Marszalek and E. Craig, unpublished results]. As mentioned above, J-type cochaperone activation of substrate binding by hsp70 was previously established for the DnaK-DnaJ chaperone system, and can be taken as a supporting evidence that Isu1 is a true native substrate of the Ssq1p-Jac1 chaperone system [50, 51].

Therefore, the data available at this time suggest that both bacterial Hsc66 and mitochondrial Ssq1 bind the protein on which the Fe/S center is transiently assembled prior to transfer to the apo-form of the recipient protein. What is the role of interaction between Isu/IscU and chaperones? Based on this interaction, one could hypothesize that chaperones may be involved in either the formation of the center, or its transfer (fig. 2). Possibly chaperones are important for interactions between Isu/IscU and the donors of either sulfur or iron, facilitating their transfer. Alternatively, since hsp70 and J-proteins have been shown to facilitate dissociation of protein complexes [52], and IscS and IscU are known to form a heterodimer [34, 35], the chaperones might be involved in dissociation of this complex. On the other hand, they might interact with the Isu/IscU and acceptor apo-protein, facilitating the transfer of Fe/S centers. Obviously, further biochemical analysis is needed to test these hypotheses.

Possible additional roles of Ssq1:Jac1

The fact that mutations in either *SSQ1* or *JAC1* affect processing of Yfh1 upon import into mitochondrial matrix suggests that this protein may be a specific substrate of Ssq1-Jac1 system. However, so far the processing defect has only been observed in isolated mitochondria. In vivo the steady-state level of mature Yfh1 in mitochondria lacking Ssq1p is 75% of that found in wild-type mitochondria, raising the question of whether this effect on Yfh1 processing is physiologically important [9]. Also, the defect of Yfh1 processing observed for *jac1* mutants was not as severe as for *ssq1* mutants [16]. Thus, it is possible that the defect of Yfh1 processing is a secondary effect of *ssq1* and *jac1* mutation, and that Yfh1 is not a true substrate of the chaperone system. On the other hand, Ssq1 might be involved in processing and subsequent folding events. Since folding of Yfh1 has not been monitored, it is not known whether the mature Yfh1 in *ssq1* mitochondria is functional. Thus, it remains an open question whether Ssq1 has substrates in addition to the *ISU* proteins and functions outside the Fe/S center assembly pathway.

Conclusion: specialized function of chaperones in Fe/S center biogenesis

Chaperone proteins involved in biogenesis of Fe/S centers both in mitochondria and in bacteria have evolved into highly specialized systems. Up to this point, only the orthologous proteins IscU/Isu1 meet the criteria of substrates for these specialized Hsp70s, joining the small number of bona fide native protein substrates of hsp70 and J-type proteins. This substrate specificity suggests that chaperones engage in either the assembly of the Fe/S center on this Isu/IscU scaffold, or the transfer of the center to a recipient protein.

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