King's Research Portal

Link to publication record in King's Research Portal

The role of chaperones in iron sulfur cluster biogenesis

Rita Puglisi[1,2], Annalisa Pastore[1,2,3*]

[1]UK Dementia Research Institute at King's College London, London, SE5 9RT, United Kingdom

[2]The Wohl Institute at King's College London, 5 Cutcombe Rd, SE59RT London (UK)

[3]Department of Molecular Medicine, University of Pavia, Pavia (Italy)

*To whom correspondence should be addressed

Tel. +44 20 78485659

Email: annalisa.pastore@crick.ac.uk

Running title: Chaperones in iron-sulfur cluster biogenesis

Abstract

Iron-sulfur cluster biogenesis is a complex process mediated by numerous proteins among which two chaperones, called HscB and HscA in bacteria. They are highly conserved up to eukaryotes and homologous to DnaJ and DnaK respectively but with specific differences. As compared to other chaperones, HscB and HscA have escaped attention and relatively little is known about their functions. After briefly introducing chaperones, we reviewed here the current structural and functional knowledge on these proteins and our understanding on their role in cluster formation. We critically evaluated the literature and highlighted the weak aspects which will require more attention in the future. We sincerely hope that this study will inspire new interest on this important and interesting system.

Abbreviations

AD: Alzheimer disease; ALS: amyotrophic lateral sclerosis; FeS: iron sulfur; HSP: heat shock proteins; ISC: iron-sulfur cluster, PD: Parkinson disease; polyQ: polyglutamine

Introduction

The amino acid sequence of a protein is thought to contain all the information required to determine a functional structure in a given environment (Anfinsen 1973). Nevertheless in the intracellular environment, the folding of polypeptides could easily undergo misfolding and aggregation triggered by mutations, stress conditions, crowding, confinement or other causes. Protein folding *in vivo* is thus not always spontaneous and the various organisms have evolved a family of highly conserved proteins called molecular chaperones that have essential roles in many cellular processes, including protein folding, targeting, transport, degradation and signal transduction (Hartl and Hayer-Hartl 2002). The function of chaperones is to mediate the correct folding of other proteins by targeting misfolded structures and preventing protein aggregation (Muchowski and Wacker 2005).

In recent years, molecular chaperones have assumed an increasing importance for health in view of the several neurodegenerative disorders characterized by conformational changes in proteins that experience misfolding, aggregation and intra- or extra-neuronal accumulation as amyloid fibrils. Examples of misfolding diseases are Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and related polyglutamine (polyQ) expansion diseases. Although the protein culprits that aggregate in these disorders are unrelated in size, function and primary sequence, the resulting aggregates typically contain fibrillar, amyloid-like structures which are detergent insoluble and protease resistant, with a high β-sheet content and a cross-β structure (Dobson 2003). In addition to the ubiquitin-proteasome system and the lysosomes, molecular chaperones provide an important line of defence against misfolded, aggregation-prone proteins and are among the most potent suppressors of neurodegeneration known in animal models of human diseases (Muchowski and Wacker 2005). Neurons and post-mitotic cells cannot dilute potentially toxic species through cell division so that the misfolded proteins accumulate, also as a consequence of a decreased proteasome activity and chaperone function. Toxic species initiate a cascade of pathogenic protein-protein interactions that culminates in neuronal dysfunction and death. Fibrillar aggregates are inert or possibly even protective rather than being directly pathogenic because they can sequester toxic proteins (Arrasate, Mitra et al. 2004). Plaques and inclusion

bodies that are characteristic of AD, PD, ALS and PolyQ diseases co-localize with chaperones and components of the ubiquitin-proteasome degradation system (Sherman and Goldberg 2001). This reflects an irreversible transition towards an aggregated state with consequent loss of function caused by a failed attempt to refold or degrade the aggregated proteins.

The scope of this mini-review is to summarize the information available on molecular chaperones with specific attention for those involved in iron sulfur (FeS) cluster biogenesis, an essential pathway which is still relatively underexplored (Rouault and Tong 2005, Lill 2009, Py and Barras 2010). Being aware that the large plethora of excellent review articles on chaperones, we remand the readers to other more complete reviews for a more general perspective on chaperones which may cover complementary aspects (Meriin and Sherman 2005, Muchowski and Wacker 2005, Saibil 2013, Finka, Mattoo et al. 2016 and the articles in the recent issue in J. Biol. Inorg. Chem, 2018, Vol 23, Issue 4). We apologise if some important papers have unintentionally been neglected.

Heat Shock proteins

Temperature increases activate the cellular heat shock response with consequent increase of heat shock protein (Hsp) production. Hsps act as chaperones and are essential for the recovery from stress-induced damage (Lindquist 1986). They are classified into six families, Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small Hsp (sHsp), according to their molecular weight. They are present in essentially all organisms, prokaryotes, archaea and eukaryotes, and in almost all compartments. Hsp100s belong to the AAA+ protein family (ATPase with diverse activities) with a typical ring shaped structure. Hsp90s reside in various cellular locations such as cytosol, mitochondria and the endoplasmic reticulum where they stabilise misfolded proteins and regulate the activity of various signalling proteins. Hsp70s assist folding of several substrates in combination with their Hsp40 co-chaperones and have a conserved ATPase domain and a C-terminal substrate domain. Hsp60 are homo-heptameric complexes with a double-ring structure that contains a large central cavity in which proteins bind and fold. Eukaryotic members of this family are chaperonines from groups I and II. The Hsp40 family contains co-chaperones that bind Hsp70s through a conserved J-domain. They promote ATP hydrolysis resulting in a conformation change that facilitates recognition of non-native protein substrates. Hsp40s also bind and chaperone protein substrates to Hsp70s enhancing the efficiency of the refolding cycle. Finally, the sHsp chaperones are small proteins (molecular mass around 40kDa) that assemble into large oligomeric structures. They

transiently interact and stabilise misfolded substrates until the Hsp70-Hsp40 system can actively refold them (Muchowski and Wacker 2005).

The Hsp70 Family

Hsp70 chaperones are activated by stress response and are involved in folding newly synthesized and misfolded proteins, preventing protein aggregation and cellular trafficking. They also help translocation of proteins across membranes as well as assembly/disassembly of oligomeric structures (Mayer, Brehmer et al. 2001). Both the sequences and the structures of Hsp70s are highly conserved among all species (Karlin and Brocchieri 1998). They contain two domains: the nucleotide binding domain (NBD) at the N-terminus and the substrate binding domain (SBD) at the C-terminus. The activity of this family of chaperones is correlated to their ability to bind and hydrolise ATP to ADP. The general view is that switching between the low-affinity ATP-bound and the high-affinity ADP-bound conformations results in the release of the substrate. In turn, ATPase activity is stimulated by binding of the substrate or the co-chaperone and further enhanced by the presence of both (Bukau and Horwich 1998, Mayer 2013). The co-chaperone thus controls the ATPase cycle by targeting the Hsp70 protein and the substrate (Mayer and Bukau 2005). Nonetheless, this hypothesis does not explain old results whereby Hsp70s with ATP, in the presence of co-chaperones, bind substrates better than with ADP. If the ADP state was the "high affinity one" and hydrolysis was just used to switch to the high affinity state, we would expect the binding to match the one of the high-affinity state. As an alternative, it was proposed that this observations could be explained only from a non-equilibrium perspective as suggested by Zuiderweg et al. (Zuiderweg, Bertelsen et al. 2013) and formally worked out by De Los Rios and Barducci (De Los Rios and Barducci 2014).

In prokaryotes, another Hsp70 homologue was identified in addition to the stress-related Hsp70 DnaK and called HscA. (Seaton and Vickery 1994). As other Hsp70 proteins, coupled with ATP binding and hydrolysis, HscA has general chaperone-like activities (prevention of aggregation and protein folding assistance) and acts with the co-expressed the J-protein co-chaperone, HscB, which stimulates the ATPase activity. Despite these similarities, HscA shares only 40% sequence identity with other Hsp70s (Vickery and Cupp-Vickery 2007). Also, differently from DnaK, HscA does not require a nucleotide exchange factor, GrpE for maximal activity (Silberg, Hoff et al. 1998). Its main role is supposed to be related to FeS cluster biogenesis as demonstrated by inactivation of genes encoding both HscA and HscB which reduces formation of FeS cluster proteins (Takahashi and Nakamura 1999, Tokumoto

and Takahashi 2001). Ssq1/Jac1 and Ssc1/Jac1 are Hsp70 and co-chaperones found in specific fungi and in other eukaryotes. Studies of the phylogenetic distribution demonstrated that Ssq1 is more closely related to DnaK than to HscA, suggesting that Ssq1 evolved before HscA. If this were true, this would imply that specialized Hsp70s with a role in FeS cluster biogenesis may have arisen twice in evolution (Schilke, Williams et al. 2006). Chaperone systems in bacteria and in mitochondria have significant biochemical differences: Ssq1 binds the mitochondrial nucleotide exchange factor Mge1 and has a higher affinity for ATP than HscA (Lutz, Westermann et al. 2001, Schmidt, Strub et al. 2001, Dutkiewicz, Schilke et al. 2003).

The structure of HscA and HscB and their complexes

Dysfunctions in FeS protein biogenesis and mitochondrial iron accumulation in heart and neurones are part of the phenotype of a genetic neurodegenerative disease called Friedreich's ataxia. This pathology is caused by the deficiency of a mitochondrial protein, frataxin, highly conserved throughout species (Pandolfo and Pastore 2009) and currently thought to be a regulator of FeS cluster biosynthesis (Prischi, Konarev et al. 2010, di Maio, Chandramouli et al. 2017). The biogenesis of FeS clusters is an essential process that involves a complex molecular machine with macromolecular structures containing multiple subunits with specific functions. The *isc* operon is one of the three machines responsible for FeS cluster biogenesis in bacteria. It presents a high level of conservation with the homologous eukaryotic system (Lill and Kispal 2000). Among the bacterial *isc* components are the desulfurase IscS, the scaffold protein IscU on which the FeS cluster is assembled, an alternative scaffold IscA, the two chaperones HscA and HscB, the transcriptor regulator IscR, ferredoxin (Fdx) and the modulator IscX. The names of these proteins are confusing because they can change completely in the close eukaryotic orthologues.

FeS cluster biosynthesis can be simplified in two steps: first the FeS cluster is assembled on IscU and is then transferred to the target apo-proteins (Ayala-Castro, Saini et al. 2008). *In vitro* studies have suggested that the two chaperones may assist FeS cluster formation by maintaining the scaffold protein in a conformation suitable for cluster assembly through interacting with the loaded scaffold protein IscU. Alternatively, it was suggested that HscA may facilitate cluster transfer from cluster-loaded (holo)-IscU to other acceptor cluster free (apo)-proteins (Hoff, Silberg et al. 2000).

A full understanding of the role of HscA/Ssq1 and HscB/Jac1 requires detailed structural information of the chaperone, co-chaperone and substrates. Several efforts have been spent to

determine the structures of HscA and of its complex with the scaffold protein IscU. The scaffold protein interacts with the chaperone through the recognition sequence LPPVK (Cupp-Vickery, Peterson et al. 2004, Tapley and Vickery 2004) and binding is stabilised by the presence of the co-chaperone which enhances ATPase activity (Hoff, Silberg et al. 2000, Silberg, Tapley et al. 2004). A crystal structure of the complex of the substrate binding domain fragment (SBD) of HscA and a IscU-derived peptide ([98]ELPPVKIHC[106]) is available (**Figure 1**) (PDB code 1U00 (Cupp-Vickery, Peterson et al. 2004). This structure has revealed that SBD consists of two distinct subdomains (the α- and β-subdomains) and is overall similar to the SBD of DnaK (Zhu, Zhao et al. 1996), although the α-helical subdomain is shifted relative to the β-subdomain. The IscU peptide binds in an extended conformation in a hydrophobic cleft of the β-subdomain through a nonpolar and hydrogen bond interactions which contribute to the binding affinity.

The crystal structure of the isolated co-chaperone HscB is also available (PDB code 1FPO (Cupp-Vickery and Vickery 2000)). It reveals the presence of two distinct domains: an N-terminal J-domain and a C-terminal domain that consists of a three-helix bundle (**Figure 2**). In contrast to DnaJ, HscB does not have intrinsic chaperone activity and lacks the C-terminal domain necessary for interaction with unfolded polypeptides (Hoff, Silberg et al. 2000). It has instead a C-terminal domain that is involved in binding to the substrate, the scaffold protein IscU, through residues L92, L96 and F153 (Walsh, Bursac et al. 2004, Fuzery, Tonelli et al. 2008, Fuzery, Oh et al. 2011). These features are not unique and are shared within the subclass of Class C J-domain chaperones (Kampinga and Craig 2010).

The J-domain of HscB is responsible for the interaction with HscA through residues H32, P33 and D34 (Kim, Alderson et al. 2014). The two domains make contacts with each other through an extensive hydrophobic interface, the presence of which suggests that their relative orientations are fixed. This observation could imply that HscB may function as a scaffold to facilitate positioning of the substrate on HscA in addition to having a role in the regulation of the ATPase activity (Cupp-Vickery and Vickery 2000).

More recently, it was described a previously undetected weak interaction between the HscB and the desulfurase IscS, one of the two main players of the *isc* machine (Puglisi, Yan et al. 2016). It was shown that the surface of interaction involves a region of HscB in the longer stem of the approximately L-shaped molecule which binds a cavity of IscS near the active site of the enzyme. Interestingly, this region overlaps with the surface of IscS involved also in binding to ferredoxin, frataxin and the small prokaryotic specific IscX (Pastore, Adinolfi et

al. 2006, Prischi, Konarev et al. 2010, Yan, Konarev et al. 2013).

The mechanism of the ATPase reaction cycle

The mechanisms involved in IscU binding and release are not well understood. HscA exhibits a low intrinsic ATPase activity (0.6 min^{-1} at 37°C and pH 7.5) but HscB was found to stimulate this activity up to 3.8-fold. To better understand the mechanism and regulation of HscA, Silberg and Vickery investigated the kinetics of ATP hydrolysis and proposed a model for the ATPase reaction cycle (Silberg and Vickery 2000). According to this model, HscA binds ATP by a two-step process in which HscA is converted from a high peptide affinity (R-state) to a low peptide affinity (T-state). The rates of binding and release are faster for the T-state than for the R-state complex (Silberg, Tapley et al. 2004). This implies that ATP hydrolysis is the rate limiting step subject to co-chaperone regulation (Silberg and Vickery 2000) and suggests that conformational changes involving the α-subdomain could be required depending on the nucleotide-bound state of the chaperone.

HscA interacts with the scaffold protein IscU and the interaction was shown to stimulate ATPase activity (Hoff, Silberg et al. 2000). In turn, ATP destabilises HscA-IscU complexes allowing their release (Silberg, Hoff et al. 2001). IscU also interacts with HscB which may serve to control the association of IscU with HscA since, in the presence of HscB, the affinity of IscU for HscA is increased >18-fold. HscB has also a synergistic effect on IscU stimulation of the ATPase activity of HscA, increasing the rate >50-fold over that found at saturating levels of IscU alone (Hoff, Silberg et al. 2000). To better understand the mechanism by which HscB and IscU regulate HscA, Silberg et al. examined their binding to the different conformational states of HscA and their effects on the kinetics of the individual steps of the ATPase reaction cycle (Silberg, Tapley et al. 2004). While IscU binds both ADP (R-state) and ATP (T-state) HscA complexes, HscB interacts only with an ATP-bound state. Both IscU and HscB modestly accelerate the rate-determining step in the HscA reaction cycle, i.e. the hydrolysis of ATP. When present together, an enhancement of the HscA(ADP)-IscU complex formation is observed. Following the ADP/ATP exchange, IscU seems also to stimulate the HscA transition from the R to the T-state thereby accelerating the rate at which the HscA-ATP is regenerated (Silberg, Tapley et al. 2004) (**Figure 3**).

Models of the interaction of the chaperones with IscU and FeS cluster transfer

Chandramouli et al. studied cluster transfer from IscU to target apo-Ferredoxin mediated by

HscA and HscB chaperones (Chandramouli and Johnson 2006). Since ATP hydrolysis and the presence of HscB accelerate this process, the authors suggested that conformational changes accompanying the ATP (T-state) to ADP (R-state) transition in the HscA chaperone could be required for catalysis. ATP binding to HscA would lead to a tense (T) state with decreased substrate-binding affinity. HscB would bind IscU and escort it to HscA. The presence of HscB would enhance IscU binding to HscA in the ATP-bound T-state and lead to a transient HscA-ATP-HscB-IscU complex. This would undergo ATP hydrolysis and loss of HscB to yield an ADP-bound relaxed (R) state with increased affinity for IscU. The IscU substrate would subsequently be released after ADP/ATP exchange and the R to T transition that occurs upon ATP binding to HscA. As a result, the authors suggested two alternative mechanistic schemes for a chaperone-catalysed FeS cluster transfer from FeS-IscU to apo-acceptor proteins (Chandramouli and Johnson 2006). The first involves direct coupling between ATP hydrolysis and cluster transfer and requires interaction between apo-Fdx and the T-state HscA-ATP-HscB-IscU complex. The second involves ATP hydrolysis preceding cluster transfer and interaction between apo-Fdx and the R-state HscA-ADP-IscU complex. The chaperones could cause changes in IscU conformation that facilitate cluster release or capture by the acceptor protein (Bonomi, Iametti et al. 2008).

These two mechanisms are further complicated by the hypothesis suggested by the Markley's group that IscU could exist as an equilibrium between a structured conformation (S) and a partially disordered one (D). If this were true, cluster formation could stabilise the S-state, which could have a lower affinity for IscS and detach from it (Markley, Kim et al. 2013). At this point, the S-state would be selectively recognised by HscB (Kim, Fuzery et al. 2009). The holo IscU-HscB complex would than represent a target for HscA in its ATP-bound form. After formation of HscA(ADP), the equilibrium between the IscU conformations would be shifted toward the D-state ensuring release of the cluster (Kim, Tonelli et al. 2012). This possibility would lead to two alternative schemes which are the direct consequences of the Chandramouli and Johnson's models (**Figure 4**).

HscA and HscB in FeS cluster biogenesis

Several studies have been performed investigating the effects of the HscA/HscB chaperones on specific steps of the FeS cluster biosynthesis leading to controversial results. Kinetic studies of FeS cluster transfer from holo-IscU to apo-Fdx in the presence of *T. maritima* chaperone DnaK-DnaJ demonstrated an inhibitory effect on the rate of FeS cluster transfer from IscU (Wu, Mansy et al. 2005). In contrast, Chandramouli et al. observed that the HscA-

HscB complex from *A. vinelandii* facilitates FeS cluster transfer *in vitro* from the holo-IscU scaffold protein to apo-Fdx and that cluster transfer is an ATP-dependent process (Chandramouli and Johnson 2006). A study of the chaperones from *E. coli* showed that the HscA-HscB-ATP complex, as well as isolated HscB, have an inhibitory effect when the cluster is formed on IscU and independently from the source of sulfur (chemical or enzymatic). In addition, in the presence of the only HscB, the cluster assembled on IscU is transferred to apo-Fdx at slower rate than in the absence of HscB unless ATP and HscA are also present (Bonomi, Iametti et al. 2008, Iametti, Barbiroli et al. 2015). A more recent investigation showed that HscB, in isolation or in co-presence of HscA with or without ATP, could slow down the rate of enzymatic cluster formation independently from the final acceptor (Puglisi, Yan et al. 2016). This was explained by an interaction between HscB with the desuflurase IscS near the catalytic site which would restrict the motion of the IscS catalytic loop from the catalytic site to IscU to transfer the persulfide (di Maio, Chandramouli et al. 2017).

Conclusions and open questions

Despite considerable progresses in understanding the function of the two chaperones HscA and HscB (and their homologues in eukaryotes) in FeS cluster biogenesis, several aspects remain to be clarified. First of all remains open why the chaperones are needed at all given that the scaffold protein readily transfers the cluster to other acceptors without the need of any assistance (Bonomi, Iametti et al. 2005). More attention should be paid to find cases in which the cluster transfer could be problematic and would thus require an active role of the chaperones. It is also rather obscure why the co-chaperone HscB should bind the desulfurase IscS (Puglisi, Yan et al. 2016): the significance of this interaction is unclear if we accept tout court the hypothesis that the chaperone function is only to assist the cluster transfer from IscU after its detachment from the enzyme. It should also be clarified the importance of ATP in restoring the native state of the substrates according to the De Los Rios and Goloubinoff's model (Goloubinoff and De Los Rios 2007). Full understanding of the molecular bases of the allosteric regulation of the IscU interaction would certainly require the structure of full-length HscA both in the ADP/ATP forms. Likewise, the existence *in vivo* of a D state of IscU is far from having been demonstrated. IscU is certainly a marginally unstable protein (Prischi, Pastore et al. 2010, Iannuzzi, Adrover et al. 2014) but it gets fully stabilised in the presence of zinc (Iannuzzi, Adrover et al. 2014), the cluster (Markley, Kim et al. 2013) and IscS (Yan, Kelly et al. 2014). Increasing evidence suggests that zinc could be not just an opportunistic

partner in the absence of the cluster but an important factor in the regulation of the machine (Selbach, Chung et al. 2014, Galeano, Ranatunga et al. 2017, Fox, Martelli et al. 2018). Further effort thus need to be directed to establish the state of folding of IscU in the cell. Finally, it should be clarified whether HscB is only a co-chaperone or has a more active role in the mechanism and how the HscA and HscB act in a concerted way. These open questions set the path for further studies focused on these two specific chaperones which may also, in turn, explain more general properties of this important family of proteins.

Acknowledgements

References

Anfinsen, C. B. (1973). "Principles that govern the folding of protein chains." Science **181**(4096): 223-230.

Arrasate, M., S. Mitra, E. S. Schweitzer, M. R. Segal and S. Finkbeiner (2004). "Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death." Nature **431**(7010): 805-810.

Ayala-Castro, C., A. Saini and F. W. Outten (2008). "Fe-S cluster assembly pathways in bacteria." Microbiol Mol Biol Rev **72**(1): 110-125, table of contents.

Bonomi, F., S. Iametti, A. Morleo, D. Ta and L. E. Vickery (2008). "Studies on the mechanism of catalysis of iron-sulfur cluster transfer from IscU[2Fe2S] by HscA/HscB chaperones." Biochemistry **47**(48): 12795-12801.

Bonomi, F., S. Iametti, D. Ta and L. E. Vickery (2005). "Multiple turnover transfer of [2Fe2S] clusters by the iron-sulfur cluster assembly scaffold proteins IscU and IscA." J Biol Chem **280**(33): 29513-29518.

Bukau, B. and A. L. Horwich (1998). "The Hsp70 and Hsp60 chaperone machines." Cell **92**(3): 351-366.

Chandramouli, K. and M. K. Johnson (2006). "HscA and HscB stimulate [2Fe-2S] cluster transfer from IscU to apoferredoxin in an ATP-dependent reaction." Biochemistry **45**(37): 11087-11095.

Cupp-Vickery, J. R., J. C. Peterson, D. T. Ta and L. E. Vickery (2004). "Crystal structure of the molecular chaperone HscA substrate binding domain complexed with the IscU recognition peptide ELPPVKIHC." J Mol Biol **342**(4): 1265-1278.

Cupp-Vickery, J. R. and L. E. Vickery (2000). "Crystal structure of Hsc20, a J-type Co-chaperone from Escherichia coli." J Mol Biol **304**(5): 835-845.

De Los Rios, P. and A. Barducci (2014). "Hsp70 chaperones are non-equilibrium machines that achieve ultra-affinity by energy consumption." Elife **3**: e02218.

di Maio, D., B. Chandramouli, R. Yan, G. Brancato and A. Pastore (2017). "Understanding the role of dynamics in the iron sulfur cluster molecular machine." Biochim Biophys Acta **1861**(1 Pt A): 3154-3163.

Dobson, C. M. (2003). "Protein folding and misfolding." Nature **426**(6968): 884-890.

Dutkiewicz, R. and M. Nowak (2018). "Molecular chaperones involved in mitochondrial iron-sulfur protein biogenesis." J Biol Inorg Chem **23**(4): 569-579.

Dutkiewicz, R., B. Schilke, H. Knieszner, W. Walter, E. A. Craig and J. Marszalek (2003). "Ssq1, a mitochondrial Hsp70 involved in iron-sulfur (Fe/S) center biogenesis. Similarities to and differences from its bacterial counterpart." J Biol Chem **278**(32): 29719-29727.

Finka, A., R. U. Mattoo and P. Goloubinoff (2016). "Experimental Milestones in the Discovery of Molecular Chaperones as Polypeptide Unfolding Enzymes." Annu Rev Biochem **85**: 715-742.

Fox, N. G., A. Martelli, J. F. Nabhan, J. Janz, O. Borkowska, C. Bulawa and W. W. Yue (2018). "Zinc(II) binding on human wild-type ISCU and Met140 variants modulates NFS1 desulfurase activity." Biochimie.

Fuzery, A. K., J. J. Oh, D. T. Ta, L. E. Vickery and J. L. Markley (2011). "Three hydrophobic amino acids in Escherichia coli HscB make the greatest contribution to the stability of the HscB-IscU complex." BMC Biochem **12**: 3.

Fuzery, A. K., M. Tonelli, D. T. Ta, G. Cornilescu, L. E. Vickery and J. L. Markley (2008). "Solution structure of the iron-sulfur cluster cochaperone HscB and its binding surface for the iron-sulfur assembly scaffold protein IscU." Biochemistry **47**(36): 9394-9404.

Galeano, B. K., W. Ranatunga, O. Gakh, D. Y. Smith, J. R. Thompson and G. Isaya (2017). "Zinc and the iron donor frataxin regulate oligomerization of the scaffold protein to form new Fe-S cluster assembly centers." Metallomics **9**(6): 773-801.

Goloubinoff, P. and P. De Los Rios (2007). "The mechanism of Hsp70 chaperones: (entropic) pulling the models together." Trends Biochem Sci **32**(8): 372-380.

Hartl, F. U. and M. Hayer-Hartl (2002). "Molecular chaperones in the cytosol: from nascent chain to folded protein." Science **295**(5561): 1852-1858.

Hoff, K. G., J. J. Silberg and L. E. Vickery (2000). "Interaction of the iron-sulfur cluster assembly protein IscU with the Hsc66/Hsc20 molecular chaperone system of Escherichia coli." Proc Natl Acad Sci U S A **97**(14): 7790-7795.

Iametti, S., A. Barbiroli and F. Bonomi (2015). "Functional implications of the interaction between HscB and IscU in the biosynthesis of FeS clusters." J Biol Inorg Chem **20**(6): 1039-1048.

Iannuzzi, C., M. Adrover, R. Puglisi, R. Yan, P. A. Temussi and A. Pastore (2014). "The role of zinc in the stability of the marginally stable IscU scaffold protein." Protein Sci **23**(9): 1208-1219.

Kampinga, H. H. and E. A. Craig (2010). "The HSP70 chaperone machinery: J proteins as drivers of functional specificity." Nat Rev Mol Cell Biol **11**(8): 579-592.

Karlin, S. and L. Brocchieri (1998). "Heat shock protein 70 family: multiple sequence comparisons, function, and evolution." J Mol Evol **47**(5): 565-577.

Kim, J. H., T. R. Alderson, R. O. Frederick and J. L. Markley (2014). "Nucleotide-dependent interactions within a specialized Hsp70/Hsp40 complex involved in Fe-S cluster biogenesis." J Am Chem Soc **136**(33): 11586-11589.

Kim, J. H., A. K. Fuzery, M. Tonelli, D. T. Ta, W. M. Westler, L. E. Vickery and J. L. Markley (2009). "Structure and dynamics of the iron-sulfur cluster assembly scaffold protein IscU and its interaction with the cochaperone HscB." Biochemistry **48**(26): 6062-6071.

Kim, J. H., M. Tonelli, R. O. Frederick, D. C. Chow and J. L. Markley (2012). "Specialized Hsp70 chaperone (HscA) binds preferentially to the disordered form, whereas J-protein (HscB) binds preferentially to the structured form of the iron-sulfur cluster scaffold protein (IscU)." J Biol Chem **287**(37): 31406-31413.

Lill, R. (2009). "Function and biogenesis of iron-sulphur proteins." Nature **460**(7257): 831-838.

Lill, R. and G. Kispal (2000). "Maturation of cellular Fe-S proteins: an essential function of mitochondria." Trends Biochem Sci **25**(8): 352-356.

Lindquist, S. (1986). "The heat-shock response." Annu Rev Biochem **55**: 1151-1191.

Lutz, T., B. Westermann, W. Neupert and J. M. Herrmann (2001). "The mitochondrial proteins Ssq1 and Jac1 are required for the assembly of iron sulfur clusters in mitochondria." J Mol Biol **307**(3): 815-825.

Markley, J. L., J. H. Kim, Z. Dai, J. R. Bothe, K. Cai, R. O. Frederick and M. Tonelli (2013). "Metamorphic protein IscU alternates conformations in the course of its role as the scaffold protein for iron-sulfur cluster biosynthesis and delivery." FEBS Lett **587**(8): 1172-1179.

Mayer, M. P. (2013). "Hsp70 chaperone dynamics and molecular mechanism." Trends Biochem Sci **38**(10): 507-514.

Mayer, M. P., D. Brehmer, C. S. Gassler and B. Bukau (2001). "Hsp70 chaperone machines." Adv Protein Chem **59**: 1-44.

Mayer, M. P. and B. Bukau (2005). "Hsp70 chaperones: cellular functions and molecular mechanism." Cell Mol Life Sci **62**(6): 670-684.

Meriin, A. B. and M. Y. Sherman (2005). "Role of molecular chaperones in neurodegenerative disorders." Int J Hyperthermia **21**(5): 403-419.

Muchowski, P. J. and J. L. Wacker (2005). "Modulation of neurodegeneration by molecular chaperones." Nat Rev Neurosci **6**(1): 11-22.

Pandolfo, M. and A. Pastore (2009). "The pathogenesis of Friedreich ataxia and the structure and function of frataxin." J Neurol **256 Suppl 1**: 9-17.

Pastore, C., S. Adinolfi, M. A. Huynen, V. Rybin, S. Martin, M. Mayer, B. Bukau and A. Pastore (2006). "YfhJ, a molecular adaptor in iron-sulfur cluster formation or a frataxin-like protein?" Structure **14**(5): 857-867.

Piccioli, M. (2018). "The biogenesis of iron-sulfur proteins: from cellular biology to molecular aspects." J Biol Inorg Chem **23**(4): 493-494.

Prischi, F., P. V. Konarev, C. Iannuzzi, C. Pastore, S. Adinolfi, S. R. Martin, D. I. Svergun and A. Pastore (2010). "Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly." Nat Commun **1**: 95.

Prischi, F., C. Pastore, M. Carroni, C. Iannuzzi, S. Adinolfi, P. Temussi and A. Pastore (2010). "Of the vulnerability of orphan complex proteins: the case study of the E. coli IscU and IscS proteins." Protein Expr Purif **73**(2): 161-166.

Puglisi, R., R. Yan, S. Adinolfi and A. Pastore (2016). "A New Tessera into the Interactome of the isc Operon: A Novel Interaction between HscB and IscS." Front Mol Biosci **3**: 48.

Py, B. and F. Barras (2010). "Building Fe-S proteins: bacterial strategies." Nat Rev Microbiol **8**(6): 436-446.

Rouault, T. A. and W. H. Tong (2005). "Iron-sulphur cluster biogenesis and mitochondrial iron homeostasis." Nat Rev Mol Cell Biol **6**(4): 345-351.

Saibil, H. (2013). "Chaperone machines for protein folding, unfolding and disaggregation." Nat Rev Mol Cell Biol **14**(10): 630-642.

Schilke, B., B. Williams, H. Knieszner, S. Pukszta, P. D'Silva, E. A. Craig and J. Marszalek (2006). "Evolution of mitochondrial chaperones utilized in Fe-S cluster biogenesis." Curr Biol **16**(16): 1660-1665.

Schmidt, S., A. Strub, K. Rottgers, N. Zufall and W. Voos (2001). "The two mitochondrial heat shock proteins 70, Ssc1 and Ssq1, compete for the cochaperone Mge1." J Mol Biol **313**(1): 13-26.

Seaton, B. L. and L. E. Vickery (1994). "A gene encoding a DnaK/hsp70 homolog in Escherichia coli." Proc Natl Acad Sci U S A **91**(6): 2066-2070.

Selbach, B. P., A. H. Chung, A. D. Scott, S. J. George, S. P. Cramer and P. C. Dos Santos (2014). "Fe-S cluster biogenesis in Gram-positive bacteria: SufU is a zinc-dependent sulfur transfer protein." Biochemistry **53**(1): 152-160.

Sherman, M. Y. and A. L. Goldberg (2001). "Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases." Neuron **29**(1): 15-32.

Silberg, J. J., K. G. Hoff, T. L. Tapley and L. E. Vickery (2001). "The Fe/S assembly protein IscU behaves as a substrate for the molecular chaperone Hsc66 from Escherichia coli." J Biol Chem **276**(3): 1696-1700.

Silberg, J. J., K. G. Hoff and L. E. Vickery (1998). "The Hsc66-Hsc20 chaperone system in Escherichia coli: chaperone activity and interactions with the DnaK-DnaJ-grpE system." J Bacteriol **180**(24): 6617-6624.

Silberg, J. J., T. L. Tapley, K. G. Hoff and L. E. Vickery (2004). "Regulation of the HscA ATPase reaction cycle by the co-chaperone HscB and the iron-sulfur cluster assembly protein IscU." J Biol Chem **279**(52): 53924-53931.

Silberg, J. J. and L. E. Vickery (2000). "Kinetic characterization of the ATPase cycle of the molecular chaperone Hsc66 from Escherichia coli." J Biol Chem **275**(11): 7779-7786.

Takahashi, Y. and M. Nakamura (1999). "Functional assignment of the ORF2-iscS-iscU-iscA-hscB-hscA-fdx-ORF3 gene cluster involved in the assembly of Fe-S clusters in Escherichia coli." J Biochem **126**(5): 917-926.

Tapley, T. L. and L. E. Vickery (2004). "Preferential substrate binding orientation by the molecular chaperone HscA." J Biol Chem **279**(27): 28435-28442.

Tokumoto, U. and Y. Takahashi (2001). "Genetic analysis of the isc operon in Escherichia coli involved in the biogenesis of cellular iron-sulfur proteins." J Biochem **130**(1): 63-71.

Vanlander, A. V. and R. Van Coster (2018). "Clinical and genetic aspects of defects in the mitochondrial iron-sulfur cluster synthesis pathway." J Biol Inorg Chem **23**(4): 495-506.

Vickery, L. E. and J. R. Cupp-Vickery (2007). "Molecular chaperones HscA/Ssq1 and HscB/Jac1 and their roles in iron-sulfur protein maturation." Crit Rev Biochem Mol Biol **42**(2): 95-111.

Walsh, P., D. Bursac, Y. C. Law, D. Cyr and T. Lithgow (2004). "The J-protein family: modulating protein assembly, disassembly and translocation." EMBO Rep **5**(6): 567-571.

Wu, S. P., S. S. Mansy and J. A. Cowan (2005). "Iron-sulfur cluster biosynthesis. Molecular chaperone DnaK promotes IscU-bound [2Fe-2S] cluster stability and inhibits cluster transfer activity." Biochemistry **44**(11): 4284-4293.

Yan, R., G. Kelly and A. Pastore (2014). "The scaffold protein IscU retains a structured conformation in the Fe-S cluster assembly complex." Chembiochem **15**(11): 1682-1686.

Yan, R., P. V. Konarev, C. Iannuzzi, S. Adinolfi, B. Roche, G. Kelly, L. Simon, S. R. Martin, B. Py, F. Barras, D. I. Svergun and A. Pastore (2013). "Ferredoxin competes with bacterial frataxin in binding to the desulfurase IscS." J Biol Chem **288**(34): 24777-24787.

Zhu, X., X. Zhao, W. F. Burkholder, A. Gragerov, C. M. Ogata, M. E. Gottesman and W. A. Hendrickson (1996). "Structural analysis of substrate binding by the molecular chaperone DnaK." Science **272**(5268): 1606-1614.

Zuiderweg, E. R., E. B. Bertelsen, A. Rousaki, M. P. Mayer, J. E. Gestwicki and A. Ahmad (2013). "Allostery in the Hsp70 chaperone proteins." Top Curr Chem **328**: 99-153.

Figure Legends

Figure 1. Crystal structure of the complex of the substrate binding domain fragment (SBD) of HscA and the IscU-derived peptide [98]ELPPVKIHC[106]. (PDB: 1U00).

Figure 2. Crystal structure of co-chaperone HscB shows two domains: an N-terminal J-domain and a C-terminal domain (PDB: 1FPO). Residues involved in the binding with HscA are coloured in red. In blue and yellow, residues responsible for the binding with IscU and IscS respectively.

Figure 3. Model of IscU binding cycle of HscA. The figure evidences HscA conformational changes associated with substrate binding and ATPase activity. The scheme was adapted from (Silberg, Tapley et al. 2004)

Figure 4. Two possible models of mechanism of chaperones mediated FeS cluster transfer. Proteins involved undergo conformational changes. IscU is supposed to switch from a disordered to a structured form. HscA exists in two conformations with a low and a high-affinity for ATP. The figure was adapted from (Chandramouli and Johnson 2006). The two models differ by the stage at which an acceptor intervene and the possible placement of the D and S states of IscU.
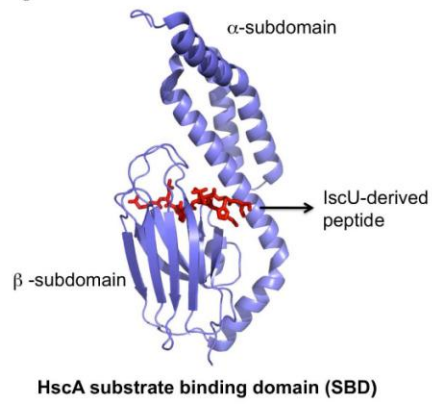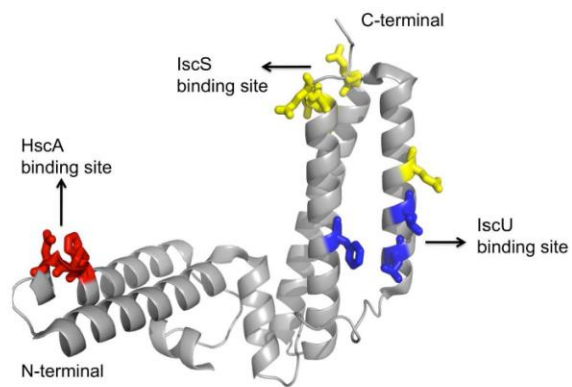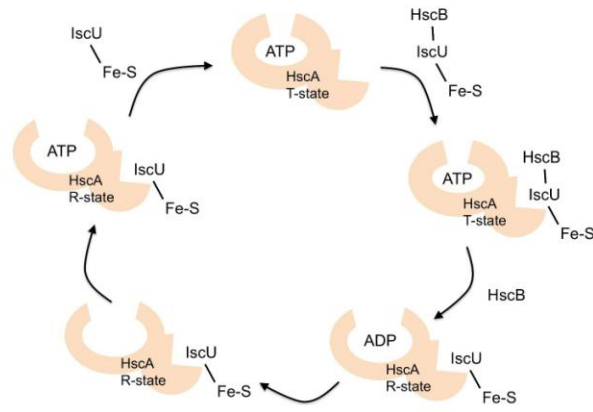
Figure 1

α-subdomain

IscU-derived peptide

β-subdomain

HscA substrate binding domain (SBD)

Figure 2

C-terminal

IscS binding site

HscA binding site

IscU binding site

N-terminal

Figure 3

Figure 4