Structural Analysis of Molecular Chaperones: Nucleotide Exchange Factors of Hsp70 and the Assembly Chaperone RbcX

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Contents

1 Summary

Protein folding and complex assembly are crucial processes for cell survival. Molecular chaperones accomplish these tasks by recognizing folding intermediates and by prevention of protein aggregation. In this work, two chaperone systems were studied: The regulation of the general purpose molecular chaperone Hsp70 by nucleotide exchange factors (NEF) and the assembly of the RuBisCO enzyme complex by the specific chaperone RbcX.

In order to function efficiently, Hsp70 requires two groups of cochaperones: J-domain proteins, which stimulate ATP hydrolysis by Hsp70s, and NEFs, which trigger the release of ADP from Hsp70. In the present work, the structure and function of two evolutionary distinct groups of NEFs were characterized, HspBP1 and Hsp110 homologs. The human NEF HspBP1 is an Armadillo repeat protein that tightly binds to subdomain IIb in the nucleotide-binding domain (NBD) of human Hsp70, thereby destabilizing the other parts of the NBD and releasing the bound nucleotide. Hsp110, which is structurally similar to Hsp70, embraces the Hsp70 NBD and stabilizes it in an open conformation with low nucleotide affinity. Direct interactions of the substrate protein with Hsp110 may support Hsp70-assisted folding further in a cooperative process.

The cyanobacterial protein RbcX is an assembly chaperone specific for the RuBisCO enzyme complex. By association with the C-terminus of the large RuBisCO subunit, RbcX guides the controlled formation of the RuBisCO core complex. RbcX is subsequently displaced by the small RuBisCO subunits.

Zusammenfassung

Die Faltung von Proteinen und der Zusammenbau von Proteinkomplexen sind entscheidende Prozesse für das Überleben von Zellen. Molekulare Chaperone helfen dabei, indem sie Faltungsintermediate erkennen und die Proteinaggregation verhindern. In der vorliegenden Arbeit wurden zwei Systeme von Chaperonen untersucht: Die Regulation von Hsp70, einem molekularen Chaperon mit breiter Substratspezifität, durch Nukleotidaustauschfaktoren (NEFs) und der Zusammenbau des RuBisCO-Enzymkomplex durch das spezifische Chaperon RbcX.

Um effizient zu arbeiten, benötigt Hsp70 zwei Familien von Cochaperonen: J-Domänenproteine, welche die Hsp70 ATP-Hydrolyse anregen, und NEFs, welche die Freisetzung von ADP aus Hsp70 beschleunigen. Es wurden die Struktur und Funktion zweier NEFs, HspBP1 und Hsp110, untersucht. Der menschliche NEF HspBP1 ist ein Armadillo-Repeat-Protein, welches mit hoher Affinität an die Subdomäne IIb der Nukleotidbindungsdomäne (NBD) von Hsp70 bindet, den Rest der NBD aber destabilisiert

und so das Nukleotid freisetzt. Hsp110, welches strukturelle Ähnlichkeit zu Hsp70 aufweist, umfasst die NBD von Hsp70 und stabilisiert so eine offene Konformation mit niedriger Nukleotidaffinität. Die direkte Wechselwirkung des Substratproteins mit Hsp110 könnte die Hsp70-vermittelte Faltung zusätzlich in einem kooperativen Prozess unterstützen.

Das cyanobakterielle Protein RbcX ist ein spezifisches Assemblierungschaperon des RuBisCO-Enzymkomplexes. Durch Anlagerung an das C-terminale Ende der großen Untereinheit von RuBisCO bewirkt RbcX den kontrollierten Zusammenbau eines RuBisCO-Rumpfkomplexes. Danach wird RbcX von den kleinen RuBisCO-Untereinheiten verdrängt.

2 Molecular chaperones and their functions in the cell

Proteins consist of linear chains of amino acids linked by peptide bonds. Since only the amino acid sequence is inherited by progeny, the three-dimensional structure - the fold - of a natural protein must in principle be determined by its amino acid sequence. In aqueous solution, the folding process of a protein is driven by the tendencies to bury hydrophobic sidechains within the interior of the protein structure and to compact the structure by formation of secondary structure elements such as α-helices and β-sheets, resulting in the essential exclusion of water from the core of the protein structure. An artificial protein with a random amino acid sequence would probably spontaneously undergo a similar process, yet the compaction would most likely result in an ensemble of distinct structures or an aggregate of multiple chains clustering together. Evolution selects natural proteins for function, which typically requires a certain surface shape of the protein structure exposing a pattern of appropriate functional groups, for example in the substrate binding pocket of a biocatalyst. Typically, amino acid sequences should thus be optimized to yield proteins, which robustly fold into a defined, functionally active conformation. This native fold is expected to represent the global energy minimum of the conformational space of a given protein; otherwise the outcome of the folding process would depend critically on the starting conformation. Local minima might exist however, representing metastable conformational states.

Since proteins typically do evolve in complex environments containing many different macromolecules such as the cytoplasm of a cell, (modern) natural protein sequences are not necessarily optimized for independent folding. Instead, biological macromolecules called molecular chaperones exist that generally facilitate the folding process of proteins, apparently by undergoing (nucleotide-dependent) conformational transitions between states of high and low affinity for partially folded proteins. In addition, molecular chaperones support the assembly of protein complexes in the cell. The process of co-evolution of molecular chaperones with their substrates frequently resulted in strong dependencies: The substrate spectra of different families of molecular chaperones were found to be clearly distinct (Dekker et al., 2008; McClellan et al., 2007; Zhao et al., 2005a). Some proteins strictly require a specific chaperone for folding: For example, the folding of actin is absolutely dependent on the chaperone complex TRiC (TCP-1 ring complex), while the functionally homologous prokaryotic protein MreB, which has essentially the same overall structure, folds

in absence of TRiC. The requirement of molecular chaperones in cellular environments might have resulted from the frequent occurance of high concentrations of partially folded proteins, which promote the unspecific association and aggregation of non-native proteins during the folding process. Because of the crowding effect exerted by the macromolecules in the cell, the effective concentrations in the cytosol are even higher (reviewed in (Zhou et al., 2008)). Once large aggregates have formed, proteins are difficult to be re-solubilised, probably because of the presumed low surface to volume ratio of the aggregate particles.

The control of the concentrations of its active protein components is crucial for the functioning of every cell and thus for the survival of biological organisms. Molecular chaperones form a vital part of the protein synthesis and maintenance machinery, and contribute to protein homeostasis in addition to factors which control protein expression and degradation. Which principal categories of molecular chaperones exist and how they function in mechanistic terms has been worked out during the past 20 years (reviewed in (Bukau et al., 2000; Hartl and Hayer-Hartl, 2002; Young et al., 2004)). Initially, molecular chaperones were found to be expressed at high concentrations upon exposure of cells to thermal stress and were consequently termed "heat shock proteins" (nomenclature, Hsp followed by the apparent molecular weight). This finding reflects the requirement to restore the function of stress-denatured proteins in the cell by re-folding. The importance of the very same or homologous molecular chaperones for constitutive protein folding emerged only later. Today, five families of general molecular chaperones found in most genome sequences have been characterized, (i) Hsp70s, with mammalian Hsp70 and the bacterial DnaK as best-studied family members, (ii) the chaperonin folding cages (Hsp60, GroEL, thermosome, TRiC), (iii) Hsp90 homologs, (iv) AAA+ proteins (AAA, ATPases Associated with various cellular Activities), and (v) the so-called small heat shock proteins. All but the last category are ATPases, which (directly or indirectly) use the energy released upon ATP hydrolysis to prevent aggregation and drive the folding reaction forward. Together the different systems of molecular chaperones form a protein folding network, in which substrates are distributed to the appropriate folding helper. Each of these chaperone systems is further regulated by cochaperones, which control the nucleotide cycle of the respective chaperone. In case of Hsp70, J-domain proteins trigger the ATP hydrolysis activity of the chaperone and nucleotide exchange factors (NEF) promote ADP release, enabling a new folding cycle. In the first part of this work, the functional interaction between Hsp70 and specific NEFs was studied in detail.

A large fraction of the proteins in the cell execute their cellular function by forming part of protein-protein complexes. For example, an abundance of 491 different, often multi-subunit protein-protein complexes were found in *Saccharomyces cerevisiae*, which has 6466 open reading frames in total (Gavin et al., 2006). Taking into account the importance of protein complexes for the functioning of the cell, knowledge about assembly and disassembly processes of such cellular entities is sparse (Ellis, 2006). Interface regions within many complexes are largely hydrophobic, and the respective folded subunits are thus assumed to be unstable in isolation. While general purpose molecular chaperones certainly play an important role in stabilizing these complex subunits, dedicated chaperones might be required to guide the assembly of specific protein complexes. Interestingly, the term "molecular chaperone" was first used for such a specific assembly chaperone, nucleoplasmin, that is required for the assembly of the proteinaceous core particle of nucleosomes from four histone subunits *in vitro* (Laskey et al., 1978). Nucleoplasmin is thought to shield the positive surface areas of histones, which normally contact the phosphate backbone of DNA, during assembly. Another well-studied example for chaperone-mediated assembly is the formation of the 20S proteasome core particle, a complex containing 14 different subunits in eukaryotes (Rosenzweig and Glickman, 2008). In the second part of this work, a specific assembly chaperone of the cyanobacterial RuBisCO enzyme complex, RbcX, was structurally and functionally characterized.

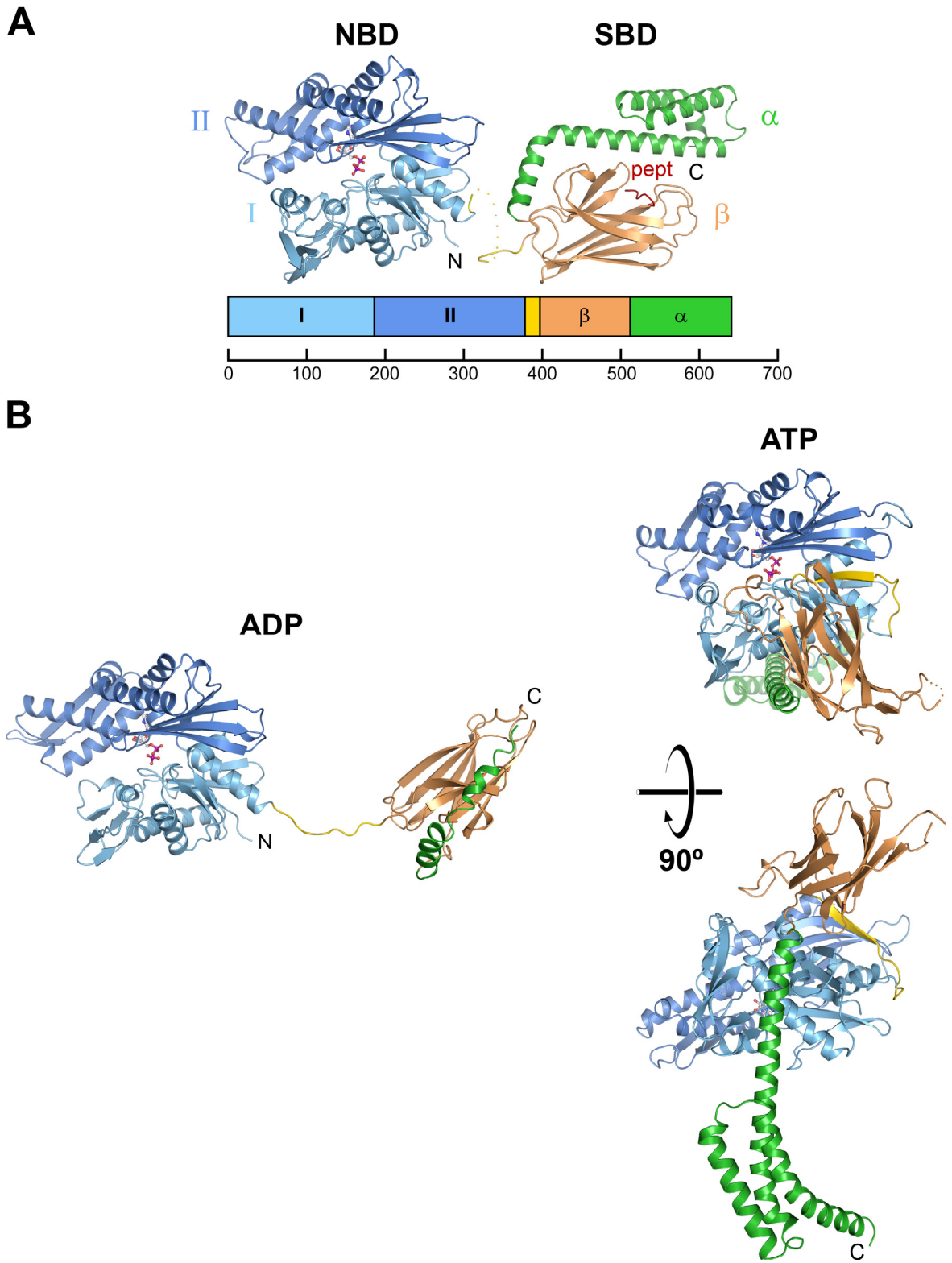
3 Structure and Function of Nucleotide Exchange Factors of Hsp70

3.1 The Hsp70 system

Arguably, the Hsp70 system of molecular chaperones is the central protein folding machinery of bacterial and eukaryotic cells (reviewed in (Mayer and Bukau, 2005)). These chaperones were shown to participate in the *de novo* synthesis of proteins at the ribosome, the assembly and disassembly of protein complexes, the transport of proteins across biological membranes, the refolding of stress-denatured proteins, and in the ubiquitylation and degradation of mis-folded proteins. Furthermore, Hsp70 is thought to hand over client proteins to the Hsp90 system and to the chaperonin TRiC in eukaryotic cells.

Hsp70 proteins themselves are characterized by a highly conserved N-terminal nucleotide binding domain (NBD) with similarity to actin that binds and hydrolyzes ATP and a bipartite peptide binding domain (PBD) comprising a β-sandwich and an α-helix bundle subdomain (Figure 1A). The PBD recognizes short stretches of amino acids with hydrophobic sidechains, which are normally buried in the interior of native protein structures (Rüdiger et al., 1997). The interaction with the substrate peptide sequences is allosterically controlled by the nucleotide binding to the NBD. In the ATP-bound state, attached peptides are rapidly exchanged by Hsp70, while in the ADP-bound form, peptides are tightly bound.

Figure 1 (following page): Structural states of Hsp70 proteins
(A) Domain composition of Hsp70 family proteins. The structures of the bovine Hsc70 NBD in complex with ADP (Flaherty et al., 1994) and of the PBD of *E. coli* DnaK in complex with the peptide NRLLLTG (shown in red) (Zhu et al., 1996) are shown in ribbon representation together with a schematic representation of the domain structure of Hsp70 proteins. The highly conserved inter-domain linker is shown in yellow.
(B) Crystal structures of conformational states of Hsp70. The crystal structures of a C-terminally truncated fragment of DnaK from *Geobacillus kaustophilus* in the ADP state (Chang et al., 2008) and of the Hsp70 family protein Sse1p in complex with ATP (Liu and Hendrickson, 2007) are shown. The nucleotide-free state is believed to resemble the ADP-state.


A
NBD
SBD
II
I
N
α
pept
C
β
I
II
β
α
0
100
200
300
400
500
600
700
B
ATP
ADP
C
N
90º
C

3.1.1 Conformational cycle of Hsp70 proteins

The allosteric mechanisms by which the bound nucleotide controls the substrate binding affinity of Hsp70 are not yet clear. The crystal structures of the isolated Hsc70 NBD in complex with ADP and of the DnaK PBD in complex with the peptide NRLLLTG are thought to best represent the ADP state of Hsp70 (Flaherty et al., 1994; Zhu et al., 1996) (Figure 1A). The bound peptide was found enclosed between the β-sandwich and α-helix bundle subdomains of the PBD. In this conformation, the domains appear to be loosely attached to each other with the highly conserved inter-domain linker accessible to protease digestion (Buchberger et al., 1995; Revington et al., 2005; Rist et al., 2006; Swain et al., 2007). In a recent crystal structure of C-terminally truncated DnaK in its ADP state, the linker region was found bound in a substrate-like manner by an adjoining DnaK molecule (Figure 1B); in the structure of the corresponding Hsc70 fragment, the PBD was disordered altogether in line with the proposed flexibility (Chang et al., 2008). The nucleotide-free state of Hsp70 is assumed to resemble the ADP-bound state. At the high cellular adenine nucleotide concentrations, this state should however be short-lived.

In the ATP-bound conformation, the linker appears to be firmly bound and protected from solvent and the structures of the individual domains seem less dynamic (Rist et al., 2006; Swain et al., 2007). The crystal structure of the distant Hsp70 homolog Sse1p in complex with ATP might serve as a working model for this conformational state (Liu and Hendrickson, 2007). In this structure, the inter-domain linker is buried at the bottom of the NBD, leading to an intimate contact between NBD and PBD (Figure 1). Both domains were found dramatically rearranged compared to the conformations of the isolated domains. The two lobes of the NBD exhibit a twisted arrangement in comparison with the ADP-bound conformation of the Hsc70 NBD, and the α-helix bundle subdomain is lifted from the β-sandwich domain and contacts the NBD instead. In this conformational state of Sse1p, the residues catalyzing ATP hydrolysis appear misplaced and the peptide binding pocket is buried in the hydrophobic core of the β-sandwich domain. Binding of the peptide substrate stimulates the ATPase activity of Hsp70 by an allosteric mechanism. The highly conserved inter-domain linker appears to play an important role in this process, since constructs comprising only the NBD and the linker segment have constitutively stimulated ATPase activity (Swain et al., 2007; Vogel et al., 2006).

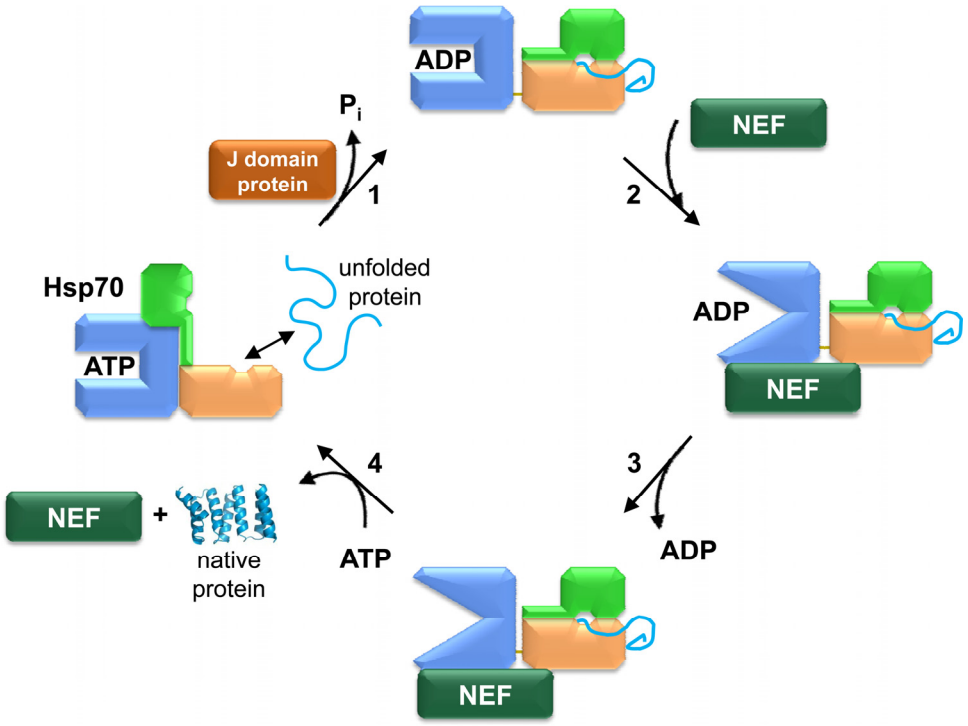
3.1.2 Mechanism of action: Paradigms of Hsp70 function in eukaryotic cells

How exactly Hsp70 promotes the folding of substrate proteins is currently largely unclear. Simple binding and dissociation of unfolded proteins should suppress aggregation side reactions and thus increase folding efficiency (kinetic partitioning model), but an actual acceleration of folding should only occur when the substrate is somehow remodelled in complex with Hsp70. Large proteins with complicated fold topology, requiring a distinct pattern of long range contacts, might be prone to become kinetically trapped as metastable intermediates during folding. Hsp70 might help to disentangle these partially folded precursors and thus accelerate folding (local unfolding model). During the import of proteins into the matrix of mitochondria, the mitochondrial Hsp70 machinery is able to actively unfold structured model proteins on the cytosolic side prior to threading the chain through the membrane pore. Several models have been proposed to explain this activity (reviewed by (Goloubinoff and De Los Rios, 2007)): Hsp70 might act as an ATP-driven motor exerting a power stroke or as a Brownian ratchet, respectively. In the motor model, defined conformational changes in mitochondrial Hsp70 exert a pulling force on the substrate chain and thus trigger unfolding; in the Brownian ratchet model, binding of mitochondrial Hsp70 to emerging recognition motifs in the peptide chain prevents retro-transposition and refolding of the substrate protein. In the entropic pulling model, which combines aspects of both propositions, the pulling force results from Brownian motion of Hsp70. Another aspect of Hsp70 function under debate is whether and how multiple Hsp70 molecules might cooperate in the folding of a protein chain.

3.1.3 Components of the Hsp70 system

For proper function, regular Hsp70s largely depend on cochaperones, which regulate the transitions between the nucleotide states (Figure 2).

Figure 2 (following page): Function of cochaperones in the folding cycle of Hsp70
In the ATP-bound state shown on the left, Hsp70 rapidly exchanges peptide substrate (indicated as blue line). Transient interaction with J-domain proteins (amber) triggers ATP hydrolysis by Hsp70, resulting in a conformation that tightly binds the substrate. The dissociation of ADP is accelerated by complex formation of Hsp70 with a nucleotide exchange factor (NEF, dark green). Binding of ATP triggers dissociation of the NEF and the release of the substrate protein for folding.



J-domain proteins, named after the bacterial Hsp40 protein DnaJ, are able to trigger the ATP hydrolysis by Hsp70. Hsp40s are also thought to associate with unfolded proteins and to recruit these to Hsp70. In the genomes of eukaryotes, a large number of J-domain proteins were found, which combine the J-domain segment with various adaptor domains that are thought to recruit Hsp70 for special functions (Young et al., 2003). For example, the J-domain protein auxilin recruits Hsc70 for the removal of clathrin triskelions from clathrin-coated vesicles. Another type of co-chaperone, the nucleotide exchange factors (NEF) of Hsp70, facilitates the dissociation of ADP from Hsp70, enabling the binding of ATP, which triggers substrate dissociation. Eukaryotes employ at least four structurally distinct classes of NEFs, GrpE homologs, BAG domain proteins, HspBP1 homologs and members of the Hsp110/Grp170 family (see Figure 3 for an overview). These factors will be discussed in detail in the following paragraphs. In combination, J-domain proteins and NEFs accelerate Hsp70 ATP hydrolysis and conformational cycling. Interestingly, the protein HIP/p48 (HIP, Hsp70-interacting protein), which was reported to interact with the NBD of Hsc70 and to antagonize the NEF Bag-1 (Höhfeld and Jentsch, 1997; Höhfeld et al., 1995), might act as nucleotide dissociation inhibitor of Hsp70 in analogy to GDIs (GDP dissociation inhibitor), which stabilize the GDP-bound conformation of small GTPases (these regulatory proteins also employ GTPase activators (GAPs) and NEFs (GEFs)).

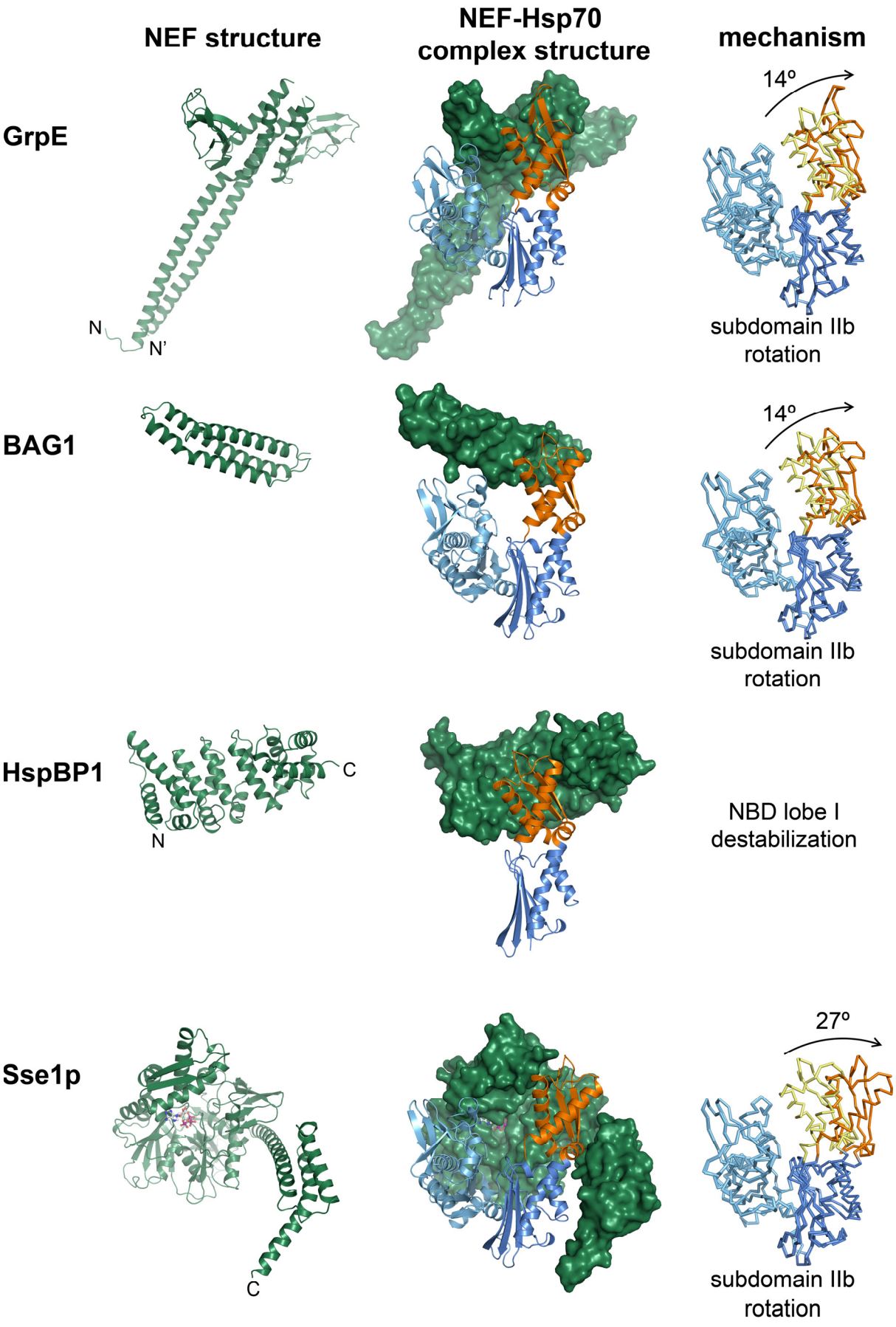

NEF structure
NEF-Hsp70 complex structure
mechanism
GrpE
N
N'
14º
subdomain IIb rotation
BAG1
14º
subdomain IIb rotation
HspBP1
N
C
NBD lobe I destabilization
Sse1p
C
27º
subdomain IIb rotation

Figure 3 (previous page): Nucleotide exchange factors of Hsp70
For better comparison, the Hsp70 NBDs of the different NEF-Hsp70 crystal structures (Harrison et al., 1997; Polier et al., 2008; Shomura et al., 2005; Sondermann et al., 2001) were superposed on the coordinates for the ADP state of bovine Hsc70 (Flaherty et al., 1994). A ribbon representation of the NEF is shown followed by the complex structures in which the NEF is shown in surface representation. On the right, the structural transitions of the Hsp70 NBD upon complex formation with the respective NEF in comparison to the ADP-bound conformation of the Hsc70 NBD are shown.

Another group of Hsp70 cochaperones with modular architecture is characterized by the presence of a so-called TPR-clamp domain (TPR, tetratrico peptide repeat) (Young et al., 2003). This domain specifically interacts with the conserved EEVD motifs at the ultimate C-termini of both eukaryotic Hsp70 and Hsp90. Among these, the adaptor protein HOP/p60 (HOP, Hsp70/Hsp90 organizing protein) links Hsp70 and Hsp90 and facilitates the transfer of Hsp90 client proteins (Scheufler et al., 2000). The yeast homolog Sti1p was also reported to trigger ATP hydrolysis by Hsp70 (Wegele et al., 2003). The ubiquitin ligase CHIP (C-terminus of Hsp70- interacting protein), another TPR-clamp domain protein, is thought to target Hsp70 substrates for proteasomal degradation (Höhfeld et al., 2001).

3.1.4 Diversity of the Hsp70 machinery

Most organisms contain multiple isoforms of Hsp70 proteins. For example, *E. coli* contains three isoforms, DnaK, HscA and HscC (reviewed in (Genevaux et al., 2007)). DnaK constitutes the general purpose Hsp70, working together with DnaJ and the nucleotide exchange factor GrpE. DnaK is constitutively expressed and further induced upon heat shock. HscA appears to be more or less specialized for the assembly of iron-sulphur cluster proteins (together with the J-domain protein HscB).

Eukaryotes have an even greater diversity of Hsp70 isoforms. Some of the cytosolic isoforms are constitutively expressed (Hsc70, heat-shock protein 70 cognate, HspA8 in humans), the expression of others is induced during and after stress conditions (Hsp70, HspA1). Organelles of prokaryotic origin like mitochondria and chloroplasts contain DnaK-like Hsp70s and consequently homologs to the prokaryotic NEF GrpE. The Hsp70 homolog BiP/Grp78 in the ER lumen (ER, endoplasmic reticulum) is essential for the co-translational import of proteins into this compartment (Vogel et al., 1990).

In addition, remote homologs exist in eukaryotes that have significantly diverged from canonical Hsp70 in sequence. Together with the J-domain protein zuotin, the yeast Hsp70 homolog Ssz1p forms the so-called ribosome-associated complex (RAC) (Gautschi et al.,

2001). This complex appears to be conserved among eukaryotes (Otto et al., 2005). The mammalian homologs are named Hsp70L1 and MPP11/Zrf-1, respectively. RAC is thought to recruit canonical Hsp70 proteins to the emerging nascent chain on translating ribosomes and might guide the folding of nascent chains (Gautschi et al., 2002). Ssz1p has very poor ATPase activity and appears to be an ancillary protein that supports the function of the ribosome-associated J-domain protein in combination with canonical Hsp70s (Huang et al., 2005). Another clearly distinct variant of eukaryotic Hsp70 are the Hsp110/Grp170 homologs (Easton et al., 2000). These comprise a subfamily from which several isoforms are found in the cytosol (Hsp110) and the ER lumen (Grp170). Hsp110 family proteins have PBD sequences distantly related to canonical Hsp70 proteins, comprising a large insertion into the β-sandwich domain and a C-terminal extension to the 3-helix bundle domain (Easton et al., 2000; Liu and Hendrickson, 2007). Interestingly, Hsp110 homologs act as NEFs for regular Hsp70s and appear to be versions of Hsp70 specialized for nucleotide exchange. Hsp110s will be discussed in detail in chapter 3.2.4.

3.2 Nucleotide exchange factors of Hsp70

3.2.1 GrpE homologs

GrpE was the first NEF of Hsp70s to be characterized. Initially, mutations in the genes for GrpE as well as DnaK and DnaJ were observed to inhibit the replication of the λ-bacteriophage genome in *E. coli*, indicating a functional cooperation of the three gene products (reviewed in (Georgopoulos, 2006)). Only later, the importance of DnaK for general protein folding in *E. coli* and the roles of the individual gene products in the folding process became apparent. DnaK by itself exhibits a low intrinsic ATP hydrolysis rate (~0.04 min^{-1}, (Karzai and McMacken, 1996)). Unfolded substrate proteins can accelerate the hydrolysis step but not sufficiently for effective folding. In additional presence of DnaJ however, the rate of hydrolysis can increase by >1000-fold. Under these conditions however, the ADP dissociation step becomes rate-limiting for the overall DnaK reaction despite the high concentrations of ATP in the cytosol. Addition of the NEF GrpE thus significantly accelerates nucleotide cycling and concomitantly protein folding by DnaK (Liberek et al., 1991). In fact, the presence of a functional GrpE is essential for the survival of *E. coli* cells (Ang et al., 1986). The crystal structure of GrpE in complex with the NBD of its associated Hsp70, DnaK of *E .coli* revealed how nucleotide exchange is mediated by GrpE (Harrison et

al., 1997) (Figure 3). In the crystal structure and in solution, GrpE forms tight dimers that asymmetrically contact only one DnaK molecule (Schönfeld et al., 1995; Wu et al., 1996). GrpE has a bipartite structure composed of an α-helical N-terminal part and a small β-sheet domain at the C-terminus. The alpha-helical fragment forming the dimer interface extends far beyond the measures of the NBD, and might contact the substrate binding region of DnaK. Indeed, while full-length GrpE interferes with substrate binding to DnaK, GrpE missing 33 residues at the N-terminus does not (Harrison et al., 1997). The interaction with DnaK is mainly mediated by the beta-sheet region of one GrpE molecule inserting into the central cleft of the NBD. Figure 4 shows the commonly used definition of the subdomain structure in the Hsp70 NBD, with subdomains Ia and Ib forming lobe I, and IIa and IIb lobe II, respectively (Flaherty et al., 1990). The nucleotide binding site is located at the bottom of the central cleft between subdomains Ib and IIb close to the center of the domain. In structures of ADP-bound Hsc70 NBDs, residues from all four subdomains contact the nucleotide.

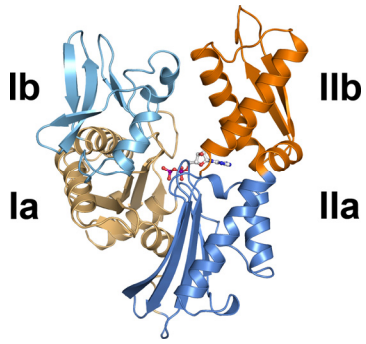


Figure 4: Subdomain structure of the NBD of Hsp70
The NBD of bovine Hsc70 is shown in its ADP-bound conformation as ribbon representation (Flaherty et al., 1994). The subdomains are indicated by different colors. ADP is shown in ball-and-stick representation.

Comparison of the GrpE-DnaK complex with the structure of mammalian Hsc70-ADP complex indicated that binding of GrpE induces a 14º rotation of subdomain IIB resulting in an opening of the nucleotide binding cleft incompatible with nucleotide binding (Figure 3).

3.2.2 BAG domain proteins

In eukaryotes, homologs to GrpE were found only in mitochondria and chloroplasts, i.e. organelles of bacterial origin that preserve the bacterial DnaK chaperone system within. The cytosolic and ER-lumenal Hsp70s have a higher spontaneous ADP release rates than their bacterial counterparts, and thus were thought to have evolved such that assisted release of ADP from the nucleotide binding cleft is dispensable. Consequently, the finding that BAG-1 (Bcl2-associated athanogene) can accelerate nucleotide exchange by mammalian Hsp70 came as a surprise (Höhfeld and Jentsch, 1997). BAG-1 was initially described as a cellular partner of Bcl-2, a negative regulator of apoptosis. The binding to Hsp70 is mediated by a ~110 amino acid long domain, which is present in a group of eukaryotic proteins with modular domain structure, the so-called BAG domain family (Takayama and Reed, 2001). For example, BAG-1L contains an ubiquitin-like domain and a nuclear localization sequence (NLS) in addition to the BAG domain. Structurally, the BAG domain of BAG-1 is unrelated to GrpE and consists of a ~60 Å long bundle of three α-helices (Briknarova et al., 2001; Sondermann et al., 2001) (Figure 3). The mechanism of nucleotide exchange is conserved with GrpE, however: In the crystal structure of the complex with the NBD of Hsc70, the BAG domain binds to subdomains Ib and IIb, stabilizing an open conformation of the NBD very similar to DnaK in complex with GrpE (Sondermann et al., 2001). In both structures, subdomain IIb is rotated outward by 14 º, suggesting that it acts as a molecular switch to modulate nucleotide affinity. Thus GrpE and BAG-1 represent thus an example of convergent evolution. Though being mechanistically similar, GrpE and BAG-1 exhibit functional differences: In contrast to GrpE, which triggers the dissociation of both ADP and ATP from DnaK, BAG-1 was found to be selective for ADP (Brehmer et al., 2001). In addition, BAG-1 and GrpE do accelerate nucleotide exchange exclusively on their respective binding partners Hsc70/Hsp70 and DnaK (Brehmer et al., 2001).

While BAG domains were clearly shown to accelerate the dissociation of ADP from Hsc70 and consequently enhanced the ATPase rate of Hsp70 in presence of Hsp40, contradictory results were reported on the effect of BAG-1 on protein folding by Hsp70. Depending on the model systems used, both inhibitory and stimulating effects of BAG-1 were observed. A plausible explanation for these conflicting results is that the effect of BAG-1 on protein folding is concentration-dependent and also strongly influenced by the presence or absence of specific cochaperones (reviewed in (Alberti et al., 2003; Takayama

and Reed, 2001)). BAG domain proteins might thus just not be general NEFs like GrpE in *E. coli*, where no other NEF is present. Indeed, the diverse BAG domain proteins are expressed at low levels in the cytosol of mammals, indicating more specialized functions. Furthermore, BAG domain proteins are generally absent from the eukaryotic ER lumen, a compartment with high demand for Hsp70-mediated protein folding activity.

3.2.3 HspBP1/Fes1p homologs

The human protein HspBP1 (Hsp70 binding protein 1) was first identified in a yeast-two-hybrid screen for Hsp70-interacting proteins (Raynes and Guerriero, 1998). Similar to BAG-1, HspBP1 was initially described as an inhibitor of Hsp70-mediated protein folding, but later found to accelerate nucleotide exchange of Hsp70 (Kabani et al., 2002b; Raynes and Guerriero, 1998). In contrast to BAG domain proteins, HspBP1 is a relatively abundant cytosolic protein. Sequence homologs to HspBP1 were identified throughout the eukaryotic domain. For example, the *S. cerevisiae* homolog Fes1p was shown to act as NEF for the yeast Hsc70 Ssa1p and for the ribosome-associated Hsp70-homolog Ssb1p (Dragovic et al., 2006b; Kabani et al., 2002a; Shomura et al., 2005). The ER lumen of eukaryotic cells contains a homolog of HspBP1 named BAP/Sil1 (BAP, BiP-associated protein, the *S. cerevisiae* homolog is named Sil1p or Sls1p) that cooperates with the ER-resident Hsp70, BiP/Kar2p. All HspBP1 homologs are compact proteins of ~35 kDa with no discernible additional domains other than the conserved Hsp70 binding domain. For example, only 80 amino acid residues are removed from the N-terminus of HspBP1 by limited proteolysis (McLellan et al., 2003). The remaining core domain preserves the full NEF function (Shomura et al., 2005).

Deletion of *FES1* in *S. cerevisiae* results in a temperature-sensitive phenotype, suggesting a role of Fes1p in the stress response (Kabani et al., 2000; Shomura et al., 2005). Using firefly luciferase as a reporter in *S. cerevisiae* cells, we could demonstrate that the absence of Fes1p causes a folding defect at 37 °C. An increased amount of unfolded, enzymatically inactive luciferase was present and found associated with Ssa1p and Ydj1p, the homologs of Hsc70 and Hsp40 in yeast, respectively. Expression of a rationally designed mutant of Fes1p, Fes1p(A79R,R195A), which is unable to bind to Ssa1p and Ssb1p, cannot complement the genomic deletion of the *fes1* gene. Together, these findings clearly indicate an important role for Fes1p in folding.

By solving the crystal structure of the core domain of human HspBP1, we could show that it forms an all-α-helical structure containing four Armadillo repeats in the central segment and flanking α-helix bundles at the chain termini, a structure distinct from both GrpE and the BAG domain (Figure 3). Similar to other Armadillo repeat proteins, the overall structure is slightly curved. The concave face of HspBP1 forms the protein-protein interface to Hsp70. In complex with the lobe II fragment of the Hsp70 NBD, the curved NEF appears to embrace subdomain IIb (Figure 3).

In comparison with BAG-1, which binds on top of the NBD, HspBP1 binds rather sideways onto subdomain IIb in the crystal structure. This would cause a severe steric conflict with subdomain Ib assuming that it occupies the same position as in the ADP-bound conformation. Probing the conformation of the entire NBD in complex with HspBP1 by limited proteolysis and by tryptophan fluorescence spectroscopy indicated indeed a less compact conformation for the NBD as compared to the complex with BAG or in absence of nucleotide exchange factors. These data are consistent with partial unfolding of lobe I or a substantial opening of the lobes in the Hsp70 NBD. HspBP1 and its homologs thus trigger nucleotide exchange by a mechanism distinct from BAG domain proteins and GrpE. The distortion of the Hsp70 NBD structure might be sufficient to dissociate bound ADP, however rotation of subdomain IIb as observed in the BAG-Hsc70 complex might occur in addition. The different conformations imposed on the NBD of Hsp70/Hsc70 by HspBP1 and BAG-1 may also have differential effects on the crosstalk with the substrate binding domain and with downstream effectors like CHIP (Alberti et al., 2004; Connell et al., 2001; Meacham et al., 2001).

3.2.4 Hsp110 family proteins

A Hsp110 family protein was first reported to act as NEF for Hsp70 in a landmark paper by Stirling and colleagues in 2004 (Steel et al., 2004). The ER-lumenal Hsp110 protein (i.e. Grp170 homolog) Lhs1p of yeast was shown to trigger the release of ADP from Kar2p, the equivalent to BiP in yeast. In addition, Hsp110s were characterized as “holdases”, chaperones that are able to keep stress-denatured proteins soluble, but are unable to actively refold them (Goeckeler et al., 2002; Oh et al., 1997; Oh et al., 1999). The association of Hsp110 and Hsp70 family members has been reported for several eukaryotic organisms, among them the cytosolic homologs in *S. cerevisiae*, the Hsp110 protein Sse1p and the

regular Hsp70s Ssa1p and Ssb1p (Shaner et al., 2005; Steel et al., 2004; Wang et al., 2000; Yam et al., 2005; Yamagishi et al., 2004; Zhang and Guy, 2005). In addition to Sse1p, which is constitutively expressed, a closely related stress-inducible isoform, Sse2p, exists (Mukai et al., 1993). Together, Sse1p and Sse2p, were reported to be essential (Raviol et al., 2006b; Shaner et al., 2004). Humans have three genes for cytosolic Hsp110 isoforms, Hsp105 (subsequently named Hsp110), Apg-1 and Apg-2 (Ishihara et al., 1999; Kaneko et al., 1997; Nonoguchi et al., 1999). The non-redundant ER homolog Grp170 (glucose-regulated protein, also Orp-150, oxygen-regulated protein) is essential in mice and its deletion results in embryonic lethality (Kitao et al., 2001).

Using the fluorescence of the nucleotide homolog MABA-ADP (N8-(4-N'-methylanthraniloylaminobutyl)-8-aminoadenosine 5'-diphosphate) as a reporter (Packschies et al., 1997; Theyssen et al., 1996), we could demonstrate that cytosolic Hsp110 homologs act as NEFs for cognate Hsp70s and trigger the dissociation of ADP (Dragovic et al., 2006a). Sse1p accelerates ADP release from Ssa1p and Ssb1p. Hsp110 has a similar effect on mammalian Hsp70. Independently, Bukau and colleagues reported nucleotide exchange activity of Sse1p on Ssa1p and Ssb1p, confirming our findings (Raviol et al., 2006b). Dissection of the domain structure of Sse1p showed that both the NBD and the SBD are required for NEF activity and that this function is sensitive to C-terminal truncations in Sse1p (Dragovic et al., 2006a). In combination with cognate Hsc70 and Hsp40, the cytosolic Hsp110 homologs were able to increase the folding efficiency of firefly luciferase *in vitro* compared to folding reactions in the absence of these factors. The bioluminescence generated during the reaction catalyzed by firefly luciferase is commonly used as a sensitive test for Hsp70 activity, and the folding of this enzyme is believed to be a model reaction for the biogenesis of multi-domain proteins. Both the initial rate and the final yield of the luciferase folding reaction were increased by addition of Sse1p, Ssa1p and Ydj1p (yeast Hsp40).

By determining the crystal structure of Sse1p in complex with the NBD of human Hsp70, we were able to define the mechanistic framework for the cooperation of Hsp110 and Hsp70 molecular chaperones (Polier et al., 2008). In the complex, the NBDs of Sse1p and Hsp70 are facing each other, forming an extensive, quasi-symmetrical dimer interface (Figure 3). In addition, the α-helix bundle subdomain (3HBD, 3-helix bundle domain) of Sse1p interacts with the flank of Hsp70 lobe II, stabilizing an open conformation of the Hsp70 NBD. In comparison with the NBD complexes of GrpE and BAG-1, the NBD opening is more pronounced with an effective sideways rotation of subdomain IIb of 27 º (GrpE and

BAG-1 induce a 14 º rotation (Figure 3) (Harrison et al., 1997; Sondermann et al., 2001)). The SBD of Hsp70, which is not present in our crystals, appears not to contribute substantially to the interaction with Sse1p, as its presence neither alters complex formation nor NEF activity significantly compared to the NBD alone. An independent structural study on the Hsp110-Hsp70 complex confirmed this view (Schuermann et al., 2008).

In the crystal structure, the nucleotide binding pocket of Sse1p is occupied by ATP and the overall conformation of Sse1p is virtually unaltered in comparison to the crystal structure of the Sse1p·ATP complex (Liu and Hendrickson, 2007), explaining the previously reported ATP requirement for complex formation (Andreasson et al., 2008; Shaner et al., 2006). Interestingly, a loop in the Hsp70 NBD directly contacts the Sse1p-bound ATP, "sensing" the nucleotide state of the Hsp110 binding partner.

Sse1p and the Hsp70 NBD form extensive contacts in the crystal structure, burying ~1760 $Å^2$ of surface on each binding partner. The contact interface is characterized by close surface shape complementarity with a large number of polar interactions. Sequence analysis showed that key contact residues are highly conserved in the Hsp110 protein subfamily, indicating that the Sse1p-Hsp70 NBD complex structure is representative for all Hsp110 homologs. The contact residues on the NBD of human Hsp70 are almost identical to the corresponding residues in the cognate yeast Hsp70, explaining the remarkable efficiency of nucleotide exchange on mammalian orthologs by Sse1p (Polier et al., 2008; Shaner et al., 2006). Mutational analysis of the key contact regions in Sse1p indicated that the interface of the Sse1p 3HBD with the Hsp70 NBD is critical for nucleotide exchange activity, while the NBD-NBD interface seemed less sensitive towards amino acid alterations. Virtually the same effects on NEF activity were observed upon mutation of complementary contact residues on Ssa1p, the *S. cerevisiae* ortholog to Hsc70.

In order to better understand how Sse1p contributes to protein folding by the Hsp70 machinery, we subjected our Sse1p mutant proteins to a series of functional assays. Using a fluorescent peptide probe bound to Ssa1p for sensitive detection, we could show that nucleotide exchange can become rate-limiting for substrate release, i.e. for the Hsp70 conformational cycle, despite the substantial off-rate of ADP from eukaryotic Hsp70s. While unmodified Sse1p accelerated peptide release by approximately one order of magnitude, Sse1p mutant proteins that were virtually unable to trigger ADP dissociation had only very little effect on peptide release. Similarly, the presence of these Sse1p mutants did not increase the efficiency of Hsp70-mediated luciferase folding *in vitro* in contrast to wildtype Sse1p protein, suggesting that its NEF activity is most important for Sse1p function.

Interestingly, additional mutations targeting the β-sandwich domain of Sse1p, which does not directly contribute to Ssa1p binding, aggravated the effect of mutations at the protein-protein interface impairing NEF activity, indicating that Sse1p might support protein folding by other means than nucleotide exchange as well. To assess the functionality of the Sse1p mutants *in vivo*, we tested the ability of the mutant proteins to complement the lethal phenotype of the combined deletion of *SSE1* and *SSE2* in *S. cerevisiae*. Only the *sse1* mutants that were virtually NEF-inactive were unable to support the growth of yeast in absence of functional Sse1p, confirming our notion that NEF activity is most important for *SSE1* function.

The ATPase activity of Hsp110 homologs Lhs1p and mouse Hsp105α was reported to be stimulated in complex with their cognate Hsp70 partners, Kar2p and Hsc70, respectively (Steel et al., 2004; Yamagishi et al., 2004), opening interesting possibilities for the functional cooperation of the two proteins. Sse1p has been reported to have intrinsic ATPase activity similar to canonical Hsp70 proteins (Raviol et al., 2006a) , while other Hsp110 homologs appeared to have no ATPase activity (Oh et al., 1999; Yamagishi et al., 2004). Unambiguous electron density for intact ATP has been found in the binding pocket of Sse1p in the crystal structures of the complex with the NBD of Hsp70 (Polier et al., 2008), indicating that at least under the crystallization conditions and in absence of the Hsp70 PBD Sse1p-bound ATP is not hydrolyzed. Similarly, mutations that are predicted to abolish ATPase activity and conformational cycling did not impair Sse1p function *in vitro* and *in vivo* (Polier et al., 2008; Raviol et al., 2006b; Shaner et al., 2004), demonstrating that ATP hydrolysis and conformational cycling are not critical for Sse1p function. The conformational state of Sse1p is nevertheless strongly dependent on its nucleotide state (Andreasson et al., 2008; Raviol et al., 2006a).

3.2.5 The role of the nucleotide exchange factors in eukaryotic cells

Why do eukaryotic cells have such a plethora of Hsp70 NEFs? There is evidence that the different NEFs are functionally only partially redundant: While - judged by their modular domain structure - BAG domain proteins might be highly specialized, HspBP1 and Hsp110 homologs both appear to serve as general NEFs. Over-expression of the HspBP1 homolog Fes1p can however only partially complement the combined deletion of *SSE1* and *SSE2* in *S. cerevisiae*, resulting in a thermo-sensitive phenotype (Raviol et al., 2006b). Mutations in the mammalian Sil1 gene that potentially inactivate its NEF function cause Marinesco-

Sjögren syndrome, an autosomal recessive neurodegenerative disorder in humans, and the ataxia phenotype of woozy mice, despite the presence of a functional Grp170 gene (Anttonen et al., 2005; Senderek et al., 2005; Zhao et al., 2005a). In both cases, Purkinje cells selectively degenerate while other tissues are much less affected. Interestingly, upregulation of mouse Grp170, ORP150, was reported to enhance Purkinje cell survival during embryonal development (Kitao et al., 2004), suggesting that this cell type might be especially sensitive to a lack in ER folding capacity. In this respect it is worth noting that the knock-out of Grp170 in mice is inviable, and thus functional Sil1 appears not to complement the loss of Grp170 (Kitao et al., 2001). It is unclear however, how the expression levels of Grp170 and Sil1 vary across different cell types and during development. In canine pancreatic microsomes, the concentrations of BiP, Grp170, and Sil1 were 5.00, 0.60, and 0.005 μM, respectively (Weitzmann et al., 2007). In *S. cerevisiae*, deletion of *LHS1* results in a constitutively induced UPR (unfolded protein response, upregulation of ER chaperones in response to stress in this compartment), and the growth defect resulting from the combined deletion of *LHS1* and *IRE1*, an effector in UPR induction, was suppressed by overexpression of *SIL1*, suggesting that Lhs1p and Sil1p are functionally redundant in yeast (Tyson and Stirling, 2000). Sse1p was found associated with various Hsp90-Hsp70 substrate complexes in yeast, while Fes1p was notably absent in these complexes (Zhao et al., 2005b). In contrast to Sse1p and human Hsp110, Fes1p and human HspBP1 were furthermore unable to enhance Hsp70/Hsp40-mediated refolding of firefly luciferase *in vitro* (Dragovic et al., 2006a; Polier et al., 2008; Tzankov et al., 2008). The functional difference between Hsp110 and HspBP1 NEFs might be caused by their different interaction modes with Hsp70 and by Hsp110s ability to interact with the substrate. As a potent holdase, Hsp110s might be directed preferentially towards certain Hsp70-substrate complexes. Interactions of these substrates with both Hsp70 and Hsp110 might aid the remodelling of the client protein before its release and thus improve the folding efficiency. On the other hand, the distinct binding modes of HspBP1 and Hsp110 in the Hsp70-NEF complexes might be differentiated by other Hsp70 cochaperones. We found that the J-domain containing factor RAC and Fes1p competed for binding to the ribosome-associated Hsp70 of yeast, Ssb1p (Dragovic et al., 2006b). Comparison of the crystal structures of the complexes of the Hsp70 NBD with a J-domain and with Sse1p indicated that the interaction sites are far apart and no direct competition between these cochaperones would be expected (Jiang et al., 2007; Polier et al., 2008). Another aspect might be the timing of the folding reaction. Different substrates might require

unique Hsp70-interaction intervals for efficient folding and the average period between binding and release of the NEF may act as general timer for this.

3.2.6 Evolution of Hsp70 nucleotide exchange factors

Both regular Hsp70 and Hsp40 homologs share remarkable sequence similarity between eubacteria and eukaryotes, indicating a common origin. In contrast, the absence of GrpE homologs in the cytosol and the ER lumen of eukaryotes suggests that either NEFs of Hsp70 were not present in the last common ancestor of bacteria and eukaryotes or that Hsp70 was acquired later by the archaeal precursor of eukaryotes through horizontal gene transfer. The latter hypothesis is in line with the absence of Hsp70 homologous genes in most archaeal genomes. Furthermore, a simultaneous transfer of DnaK and DnaJ seems likely, considering that these genes are encoded as one operon in many prokaryotic genomes.

Sequence homology to Hsp70 and absence of Hsp110 homologs in prokaryotes suggest that the Hsp110 family emerged by divergent evolution after the juncture, i.e. Hsp110 proteins presumably derive from canonical eukaryotic Hsp70s. It is tempting to speculate that primordial Hsp70 proteins were able to catalyze nucleotide exchange via homo-dimer formation in a way resembling our hetero-dimeric complex structure. Dimer formation might have enabled tight coordination between Hsp70 molecules during collaborative folding of larger, possibly multi-domain protein substrates. A peptide-charged Hsp70 molecule would have been able to recruit another ATP-bound Hsp70 molecule to the substrate, while itself being primed for a new folding cycle by concomitant dissociation of ADP. Thus, transition through alternate conformational states would have been coupled in the hypothetical homo-dimeric Hsp70 complex similar to a two-stroke engine. Intriguingly, most key residues for the interactions observed in the Sse1p-Hsp70N complex are conserved among all sequences of canonical Hsp70s. Presumably, a specialization on nucleotide exchange of Hsp110 proteins enabled tighter control and increased efficiency of the Hsp70 folding cycle. BAG domain proteins and HspBP1/Fes1p homologs might have evolved subsequently from unrelated proteins that had increased affinity for the NBD of Hsp70. Both proteins have generic folds, 3-helix bundle and successions of Armadillo repeats, which are employed as structural scaffold in many different functional contexts in eukaryotes, indicating their great adaptability for protein-protein interactions.

4 The role of RbcX in the folding of RuBisCO

Subunit folding and enzyme complex assembly of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) by molecular chaperones has been employed as an important model system in the study of molecular chaperones. The most common quarternary structure of RuBisCO, the enzyme responsible for the fixation of atmospheric CO_2 in form of organic compounds by plants and cyanobacteria, is a hexadecamer of 8 large RbcL subunits forming the core complex and 8 small RbcL subunits at the periphery (reviewed in (Andersson, 2008)). A simpler type of RuBisCO found in certain cyanobacteria consists only of RbcL dimers, which is believed to be the minimal active unit. RuBisCO is assumed to be the most abundant protein on earth. The disparately huge amounts are presumably necessary to compensate for the enzyme's low activity and its poor selectivity of CO_2 over O_2 (Andersson, 2008). The benefits of an engineered form of RuBisCO with improved kinetic characteristics would be enormous; however progress in this effort is hampered by difficulties to produce active plant RuBisCO in prokaryotic expression hosts. It was recognized early that the large subunits are associated with the plant homolog of GroEL in chloroplasts, Cpn60 (Barraclough and Ellis, 1980; Roy, 1989). Later it was shown that the GroEL/GroES chaperonin complex of *E. coli* folds cyanobacterial RbcL and produces active RuBisCO, yet with modest efficiency (Goloubinoff et al., 1989). The presence of the gene RbcX, which is often found in the same operon as the genes for the RuBisCO subunits in cyanobacteria, enhances the yield of active RuBisCO, suggesting that it might act as a chaperone in the genesis of the complex (Li and Tabita, 1997). Interestingly, gene sequences homologous to cyanobacterial RbcX were discovered in plant genomes. Our studies showed that co-expression of cyanobacterial RbcX with RbcL improves the efficiency of $RbcL_8$ core complex assembly (Saschenbrecker et al., 2007). In the crystal structure, the 15 kDa small RbcX protein forms an arc-shaped dimer with conserved surface patches at the central crevice and at the outer edges (Figure 5). Our mutational analysis indicated that both surface areas are critical for $RbcL_8$ core complex assembly. The central crevice recognizes the highly conserved peptide motif EIKFEFD at the C-terminus of RbcL, an interaction, which presumably promotes the recruitment of RbcX to RbcL monomers or dimers. The other conserved surface area might help organizing RbcL dimers into higher oligomers. Competition experiments with a cognate RbcS indicate that RbcX is rapidly displaced by RbcS upon completion of the RbcL core particle, yielding the enzymatically active RuBisCO

complex. In remarkable contrast to the general molecular chaperones, RbcX thus specifically recognizes its unique substrate protein RbcL and guides its assembly to a larger complex by an unknown mechanism, preventing irreversible aggregation of RbcL. Given the abundance of macromolecular complexes in eukaryotic cells, many more specialized chaperones like RbcX might exist, which assist efficient complex assembly. Combining two structurally independent, individually weakly binding interaction motifs might be a general strategy to balance initial substrate recognition and subsequent displacement of the chaperone from the assembled complex.

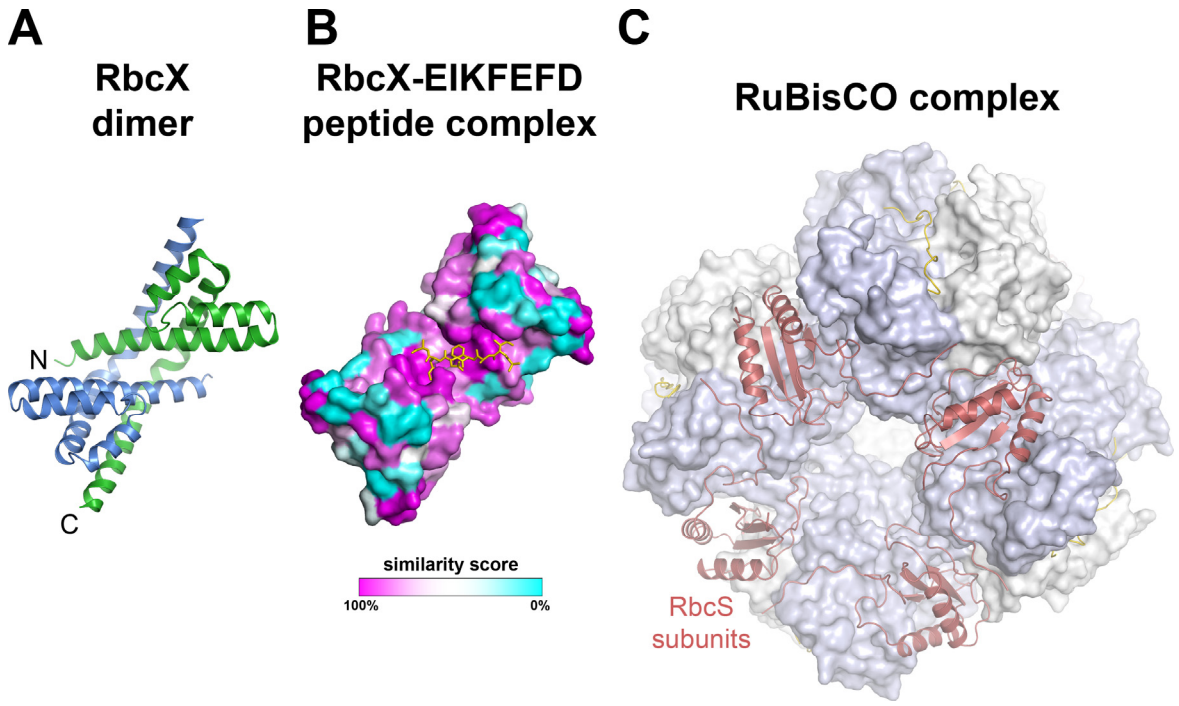


Figure 5: Crystal structures of RbcX and of the RuBisCO enzyme complex
(A) Crystal structure of the RbcX dimer. The crystal structure of the RbcX dimer from *Synechococcus* PCC 7002 is shown in ribbon representation.
(B) Structure of the RbcX-EIKFEFD peptide complex. The similarity score for all available RbcX sequences is plotted onto the surface of RbcX. The bound RuBisCO signature peptide is shown in yellow at the center.
(C) For comparison, the structure of the RuBisCO complex of *Synechococcus* PCC 6301 is shown to scale (Newman et al., 1993). The RbcL subunits are shown in surface representation except for the C-terminal signature peptide (shown in yellow). The RbcS subunits are indicated as red ribbons.

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7 Curriculum vitae

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Education

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1994 - 1998	Dissertation at the TUM Supervisor, Prof. Dr. Dr. Adelbert Bacher Title Structure and Reaction Mechanism of GTP cyclohydrolase I Grade Summa cum laude
1988 - 1994	Study of Chemistry (Diploma) at the TUM Grade Summa cum laude
1977 - 1986	Max-Born Gymnasium Germering

Employment

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1999 – 2002	Postdoctorate in the group of Dr. Winfried Weissenhorn at the EMBL Outstation in Grenoble, France
1994 - 1999	Co-worker of Prof. Dr. Dr. Adelbert Bacher at the Institute of Organic Chemistry and Biochemistry of the TUM

Awards

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List of publications[1]

Peer-reviewed publications

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Patent applications

2007 European patent: Means and methods for providing ribulose bisphosphate-carboxylase-oxygenase with improved properties (EP000001988168A1)

8 Appendix: Selected publications

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