Subcellular Biochemistry 78

Gregory Lloyd Blatch
Adrienne Lesley Edkins *Editors*

The Networking of Chaperones by Co-chaperones

Control of Cellular Protein Homeostasis

Springer

Subcellular Biochemistry

Volume 78

Series Editor
J. Robin Harris
University of Mainz, Mainz, Germany

The book series SUBCELLULAR BIOCHEMISTRY is a renowned and well recognized forum for disseminating advances of emerging topics in Cell Biology and related subjects. All volumes are edited by established scientists and the individual chapters are written by experts on the relevant topic. The individual chapters of each volume are fully citable and indexed in Medline/Pubmed to ensure maximum visibility of the work.

More information about this series at http://www.springer.com/series/6515

Gregory Lloyd Blatch • Adrienne Lesley Edkins
Editors

The Networking of Chaperones by Co-chaperones

Control of Cellular Protein Homeostasis

Springer

Editors
Gregory Lloyd Blatch
Victoria University, College of Health and Biomedicine
Melbourne
Victoria
Australia

Adrienne Lesley Edkins
Department of Biochemistry and Microbiology
Rhodes University, Biomedical Biotechnology Research Unit
Grahamstown
South Africa

ISSN 0306-0225
Subcellular Biochemistry
ISBN 978-3-319-11730-0 ISBN 978-3-319-11731-7 (eBook)
DOI 10.1007/978-3-319-11731-7

Library of Congress Control Number: 2014956684

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Cellular protein homeostasis is vital for cellular survival and requires a balance between the integrated processes of protein folding, degradation and translocation. Proteostasis is regulated by a diverse family of proteins known as molecular chaperones. Molecular chaperones act as catalysts for protein homeostasis by preventing protein aggregation, promoting protein folding and mediating appropriate protein degradation under both physiological and stressful conditions. These chaperones rely on a network of accessory proteins, termed co-chaperones, to fine-tune their function. As a consequence, co-chaperones are important mediators of the outcome of chaperone assisted protein homeostasis. Indeed, Hsp70 molecular chaperones cannot participate in productive protein folding without an Hsp40 co-chaperone. Equally, the co-chaperones Hop and CHIP interact with the Hsp70/Hsp90 chaperones to control triage of protein clients towards folding or degradation pathways. A co-chaperone can be defined as a non-client protein that interacts with a protein chaperone and/or its client protein to regulate chaperone function. Co-chaperones are evolutionarily conserved together with their chaperone counterparts (even being identified in the recently sequenced genome and transcriptome of the Coelacanth). Co-chaperones often outnumber their respective chaperones and are hence a way to induce specialisation of a relatively small number of chaperone isoforms. Co-chaperones may fulfil this function in a number of ways; by inducing conformational changes, delivering client proteins or regulating inherent enzymatic activities of chaperones. Many co-chaperones are modular proteins that combine the ability to bind client proteins with the capacity to interact with or modulate the activity of chaperones. Therefore, whilst co-chaperones are structurally diverse, there are conserved structural features within some families (such as the J domain of Hsp40 and the tetratricopepetide repeat (TPR) domain of some Hsp90/Hsp70 co-chaperones). Some co-chaperones (e.g. many Hsp40 isoforms) have chaperone-like activity in that they can bind and prevent aggregation of client proteins. However, most co-chaperones lack the inherent ATPase activity of chaperones and hence cannot actively refold proteins in the absence of chaperones. This second edition is timely since research in recent years has substantially expanded our understanding of co-chaperone function. For some co-chaperones, a number of new isoforms have been discovered, including FKBP immunophilin isoforms, virally encoded GroES

and the first putative co-chaperone for the organelle Hsp90, Gp96. However, the role of many of the numerous Hsp40 co-chaperones remains undefined. Our understanding and integration of the roles of known co-chaperones into cytosolic chaperone pathways has expanded. In particular, the roles of the structurally diverse Hsp90 co-chaperones during the ATP-dependent Hsp90 folding cycle have begun to emerge. We are beginning to appreciate that certain co-chaperones also function independently of chaperones and have features that are not normally associated with co-chaperone function. In particular, the established Hsp90/Hsp70 co-chaperone, Hop, is the first of this group to be shown to have independent ATPase activity; a characteristic not associated with co-chaperones. Does this suggest that it is time to reclassify Hop as a chaperone? Or will future analyses discover similar features of other co-chaperones, necessitating us to redefine the features of a co-chaperone? We have a new understanding of the role played by co-chaperones in human disease. Cell biological studies have demonstrated that some co-chaperones, like Hop and Cdc37, are expressed at higher levels in cancer, where they may contribute to maintenance of the malignant state and as such are now being considered as drug targets. We are starting to recognise that some co-chaperones are collaborative whilst others are mutually exclusive, although we perhaps don't fully appreciate the functional redundancy between co-chaperones yet. However, we still do not have a complete understanding of the spatial and temporal control of co-chaperone function. The mechanisms that control co-chaperone expression and subcellular localisation are poorly understood. Furthermore, the global control of co-chaperone and chaperone function through fluctuations of ATP levels ("energy" levels) in the cell, has not been studied in any detail. This represents a logical area to investigate towards understanding how the co-chaperone-chaperone network is tuned for different cellular states from normal through to stress and disease states. How do chaperones select their co-chaperones, particularly in cases of potential functional redundancy between certain isoforms? Likewise, while many co-chaperone isoforms (e.g. Hop) have been detected in the extracellular environment, we do not know whether these proteins function as co-chaperones outside of the cell. Indeed, many chaperones are now known to have extracellular functions and therefore it is likely that co-chaperones may too. Are there any co-chaperokines waiting to be identified? Are extracellular co-chaperones analogous to their intracellular counterparts? Our recent advances in analysis of co-chaperone function has demonstrated that there is still much to learn, and led to new questions that will ensure that research into our understanding of this important family of proteins continues.

Acknowledgements

We would like to thank all of those who have assisted us with the preparation of this book. In particular, without the commitment of new authors and re-commitment of previous authors, this book would not have been possible. We are very grateful for the essential contributions of the reviewers to the completion of the book. A special mention of thanks is also due to Mike Cheetham (UCL) for his many contributions to this process.

Contents

Contributors

Swati Baindur-Hudson College of Health and Biomedicine, Victoria University, Melbourne, Australia

Centre for Chronic Disease Prevention and Management, Victoria University, Melbourne, Australia

Institute of Sport, Exercise and Active Living, Victoria University, Melbourne, Australia

Gregory L. Blatch College of Health and Biomedicine, Victoria University, Melbourne, Australia

Centre for Chronic Disease Prevention and Management, Victoria University, Melbourne, Australia

Institute of Sport, Exercise and Active Living, Victoria University, Melbourne, Australia

Department of Biochemistry and Microbiology, Biomedical Biotechnology Research Unit (BioBRU), Rhodes University, Grahamstown, South Africa

Aileen Boshoff Biomedical Biotechnology Research Unit (BioBRU), Biotechnology Innovation Centre, Rhodes University, Grahamstown, South Africa

Andreas Bracher Dept. of Cellular Biochemistry, Max-Planck-Institute of Biochemistry, Martinsried, Germany

Johannes Buchner Department Chemie, Technische Universität München, Munich, Germany

Dejan Bursać School of Medicine, Deakin University, Geelong, Australia

Stuart K. Calderwood Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Center for Life Sciences, Boston, MA, USA

J. Paul Chapple Molecular Endocrinology Centre, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Michael E. Cheetham Department of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, London, UK

Marc B. Cox Department of Biological Sciences, Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, USA

Douglas M. Cyr Department of Cell Biology and Physiology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA

Emma J. Duncan Molecular Endocrinology Centre, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Adrienne L. Edkins Department of Biochemistry and Microbiology, Biomedical Biotechnology Research Unit (BioBRU), Rhodes University, Grahamstown, South Africa

Kip Gabriel Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Melbourne, Australia

Mario D. Galigniana Departamento de Química Biológica/IQUIBICEN, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Instituto de Biología y Medicina Experimental/CONICET, Buenos Aires, Argentina

Yenni A. Garcia Department of Biological Sciences, Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, USA

Naihsuan C. Guy Department of Biological Sciences, Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, USA

Armin Melnyk Medical Biochemistry & Molecular Biology, Saarland University, Homburg, Germany

Weiming Ni Department of Genetics, Howard Hughes Medical Institute, Yale School of Medicine, New Haven, CT, USA

Odutayo O. Odunuga Department of Chemistry and Biochemistry, Stephen F. Austin State University, Nacogdoches, TX, USA

Carlos H. Ramos Department of Organic Chemistry, Institute of Chemistry, University of Campinas-UNICAMP, Campinas—SP, Brazil

Neta Regev-Rudzki Department of Biological Chemistry, The Weizmann Institute of Science, Rehovolt, Israel

Alexandra Beatrice Rehn Department Chemie, Technische Universität München, Munich, Germany

Heiko Rieger Statistical Physics, Saarland University, Saabrücken, Germany

Jeffrey C. Sivils Department of Biological Sciences, Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, USA

Jacqueline van der Spuy Department of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, London, UK

Jacob Verghese Dept. of Cellular Biochemistry, Max-Planck-Institute of Biochemistry, Martinsried, Germany

Richard Zimmermann Medical Biochemistry & Molecular Biology, Saarland University, Homburg, Germany

About the Editors

Gregory Lloyd Blatch is Professor and Dean of the College of Health and Biomedicine, Victoria University (Australia). His personal research interests fall within the broad field of stress biology, and he is internationally recognized for his work on the role of molecular chaperones and co-chaperones in disease (e.g. cancer) and infection (e.g. malaria). He was awarded a Wellcome Trust International Senior Research Fellowship (UK) for biomedical aspects of his research (2002 -2007). He received his PhD from the University of Cape Town (South Africa; 1990) and did his postdoctoral at Harvard University Medical School (USA). He started his university career at the University of the Witwatersrand (South Africa), before moving to Rhodes University (South Africa). While he is now based at Victoria University, he is also a Rhodes University Visiting Professor.

Adrienne L. Edkins is Senior Lecturer in Biochemistry at Rhodes University, Grahamstown, South Africa and Acting Director of the Biomedical Biotechnology Research Unit (BioBRU). Her research interests are in the area of Hsp90 complex in cancer and stem cells, with emphasis on the analysis of extracellular Hsp90 and the TPR-containing co-chaperone Hop. Her group is funded by the NRF, MRC and CANSA and she holds a Y1 rating from the NRF. She received her PhD from the University of Glasgow (Scotland, UK; 2009) and was the recipient of a Wellcome Trust 4 Year PhD Fellowship and Beit International Postgraduate Fellowship for Zimbabwe.

Chapter 1
GrpE, Hsp110/Grp170, HspBP1/Sil1 and BAG Domain Proteins: Nucleotide Exchange Factors for Hsp70 Molecular Chaperones

Andreas Bracher and Jacob Verghese

Abstract Molecular chaperones of the Hsp70 family are key components of the cellular protein folding machinery. Substrate folding is accomplished by iterative cycles of ATP binding, hydrolysis and release. The ATPase activity of Hsp70 is regulated by two main classes of cochaperones: J-domain proteins stimulate ATPase hydrolysis by Hsp70, while nucleotide exchange factors (NEF) facilitate its conversion from the ADP-bound to the ATP-bound state, thus closing the chaperone folding cycle. Beginning with the discovery of the prototypical bacterial NEF GrpE, a large diversity of Hsp70 nucleotide exchange factors has been identified, connecting Hsp70 to a multitude of cellular processes in the eukaryotic cell. Here we review recent advances towards structure and function of nucleotide exchange factors from the Hsp110/Grp170, HspBP1/Sil1 and BAG domain protein families and discuss how these cochaperones connect protein folding with quality control and degradation pathways.

Keywords Disaggregase activity · Proteostasis · Protein structure · Protein quality control

Introduction

Cells are confronted with a variety of adverse environmental conditions such as heat shock, oxidative injury, heavy metals and glucose-depletion and pathologic states such as inflammation, tissue damage, infection, ischemia and reperfusion. To cope with this plethora of stresses, cells induce the expression of cytoprotective genes including heat shock proteins (Hsps). Many Hsps function as molecular chaperones that aid the folding, assembly and targeting of their substrate proteins. Under stress conditions, chaperones shield denatured proteins from aggregation, disassemble protein aggregates and assist protein refolding or targeting to the degradation

A. Bracher (✉) · J. Verghese
Dept. of Cellular Biochemistry, Max-Planck-Institute of Biochemistry,
82152 Martinsried, Germany
e-mail: bracher@biochem.mpg.de

G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_1

machinery in order to maintain protein homeostasis (proteostasis) in the cell (Hartl et al. 2011; Balch et al. 2008). Hsps can be classified into families based on their molecular mass: Hsp60, Hsp70, Hsp90, Hsp100 and small heat shock proteins. Importantly, these general molecular chaperones do not work by themselves, but are dependent on a plethora of cochaperones, which control their function. As a whole, these factors form an elaborate network that orchestrates protein folding in the cell (Kim et al. 2013; Bukau et al. 2006). Within this proteostasis network, the Hsp70 system forms a central hub at the crossroads between the translation apparatus, specialized downstream chaperones and the cellular degradation machinery. Hsp70 function is regulated by cochaperones which control its ATP hydrolysis activity. In this review we will focus on a specific group of Hsp70 cochaperones, the nucleotide exchange factors (NEF). We will present the structures and molecular function of NEFs, and discuss their role in the cellular protein folding and degradation machinery.

Hsp70 Architecture and Functional Cycle

Hsp70 was initially identified in the bacterium *Escherichia coli*, where it is named DnaK. Later Hsp70 proteins were found to be conserved in eukaryotes as well (Gupta 1998). In eukaryotes, compartment-specific isoforms were identified in cytosol/nucleus, endoplasmic reticulum (ER) lumen, and mitochondria. Human cytosol contains multiple Hsp70 paralogs, including constitutively expressed (Hsc70/HSPA8) and stress-inducible isoforms (Hsp72/HSPA1A/B). The ER-lumenal and mitochondrial forms are named BiP/Grp78/HSPA5 and mortalin/Grp75/HSPA9, respectively.

Hsp70 proteins share a conserved domain architecture containing two major domains (Fig. 1.1): an amino-terminal nucleotide binding domain (NBD) and a carboxy-terminal substrate-binding domain (SBD) (Mayer and Bukau 2005). The NBD is approximately 44 kDa in size and forms a bilobular structure that encloses a cleft with the nucleotide binding pocket at the bottom (Fig. 1.1c) (Flaherty et al. 1990). The structurally homologous lobes (I and II) of the NBD are subdivided into regions A and B. The SBD comprises of a β-sandwich subdomain with a groove that binds hydrophobic polypeptides and a carboxy-terminal α-helical "lid" that folds over the peptide binding site and facilitates high affinity substrate interaction (Zhu et al. 1996). The conserved hydrophobic NBD-SBD inter-domain linker plays an important role in conveying conformational information between the domains (Vogel et al. 2006; Swain et al. 2007).

Studies on DnaK from *E. coli* showed that Hsp70 functions through an ATP-dependent cycle (Fig. 1.1a). When ATP is bound to the NBD, the Hsp70 SBD rearranges to a conformation with low affinity for the substrate (Fig. 1.1b) (Kityk et al. 2012; Qi et al. 2013). ATP hydrolysis induces a conformational rearrangement in the NBD that detaches the SBD to assume a conformation with high affinity for segments with five consecutive hydrophobic amino acid residues in client proteins (Fig. 1.1c) (Rüdiger et al. 1997; Zhuravleva et al. 2012). Substrate binding increases

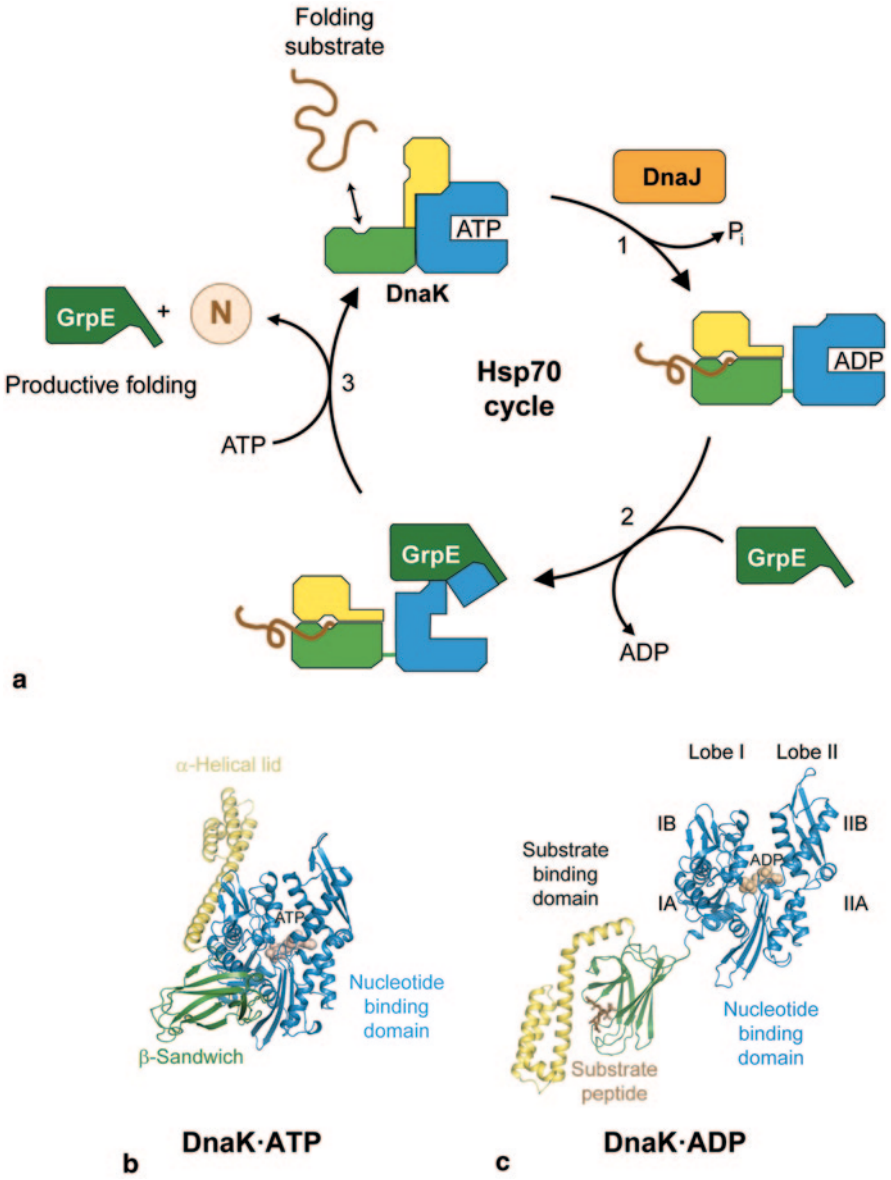


Fig. 1.1 DnaK structure and folding cycle. **a** Model for the Hsp70 folding cycle. The DnaK·ATP complex has weak substrate affinity. ATP binding to the NBD (*blue*) stabilizes a compact domain arrangement, which leaves the SBD (*yellow* and *green*) in an open conformation. This conformation exhibits dynamic interactions with the substrate (indicated in *brown*). ATP hydrolysis stimulated by DnaJ (*1*) causes a conformational change in the NBD that triggers formation of the closed SBD conformation, which has higher affinity for the substrate, resulting in a stable substrate complex. The binding of the NEF GrpE (*2*) promotes a slight opening of the NBD, which results in the release of ADP from DnaK. The cycle is reset (*3*) when a new ATP molecule binds to the NBD, triggering the release of NEF and substrate. **b** Crystal structure of the DnaK·ATP complex. The

the ATP hydrolysis rate of DnaK substantially. The spontaneous transition between the two states is slow as Hsp70 has intrinsically only weak ATPase activity. This prevents substrate-free cycling. The cycle is reset with the release of ADP and replacement with ATP, which releases the client protein for a new folding attempt.

DnaK, DnaJ and GrpE: The Eubacterial Hsp70 System

For its proper functioning in protein folding, DnaK is dependent on the ATPase-stimulating cochaperone DnaJ and the nucleotide exchange function of GrpE (Fig. 1.1a). Although interactions with substrate protein trigger ATP hydrolysis in DnaK, meaningful folding rates with model proteins are only achieved in presence of DnaJ, the prototypical Hsp40 protein (Laufen et al. 1999). Hsp40 and other J-domain proteins are reviewed in Chapter 4. Because of DnaK's slow off-rate for ADP, additional presence of GrpE is essential for *E. coli* cells to reset the Hsp70-folding cycle (Ang and Georgopoulos 1989). The combined action of the two cofactors is thought to drive the folding cycle of the molecular chaperone, resulting in repetitive rounds of substrate binding and release.

GrpE functions as the nucleotide exchange factor for DnaK by stabilizing a NBD conformation with an open nucleotide binding cleft (Harrison et al. 1997) (Fig. 1.1a). The crystal structure revealed that subdomain IIB of DnaK is rotated outwards in the complex, which weakens the contacts to ADP (Fig. 1.2).

The *E. coli* cytosol comprises of two additional isoforms of Hsp70, HscA and HscC, and five more proteins containing a J-domain. These isoforms and their associated J-protein cofactors have more specialized functions than DnaK, such as incorporation of Fe–S clusters into substrates using the IscU scaffold protein. In contrast, DnaK appears to be the more general-purpose protein-folding machine. Interestingly, functioning of HscA does not require the NEF GrpE (Brehmer et al. 2001).

The Evolution of Eukaryotic Hsp70 Systems

In eukaryotes, close sequence homologs to GrpE are only found in mitochondria and chloroplast, i.e. organelles of eubacterial origin, whereas orthologs to DnaK and DnaJ are found in the cytosol/nucleus and the ER lumen. These endosymbiont-derived organelles have thus preserved an eubacterial protein folding machinery (homologs to GroEL, GroES, HtpG and ClpA are further evidence for this), al-

peptide backbone is shown in ribbon representation, and the bound nucleotide as space-filling model (PDB code 4B9Q (Kityk et al. 2012)). The nucleotide binding, β-sandwich and α-helical domains are indicated in *blue*, *green* and *yellow*, respectively. **c** NMR model for the DnaK·ADP complex. In this state, the NBD and SBD are loosely associated (PDB code 2KHO (Bertelsen et al. 2009)). The representation mode is the same as in **b**. The peptide NRLLLTG from the complex structure with the SDB alone (PDB code 1DKZ (Zhu et al. 1996)) is superposed

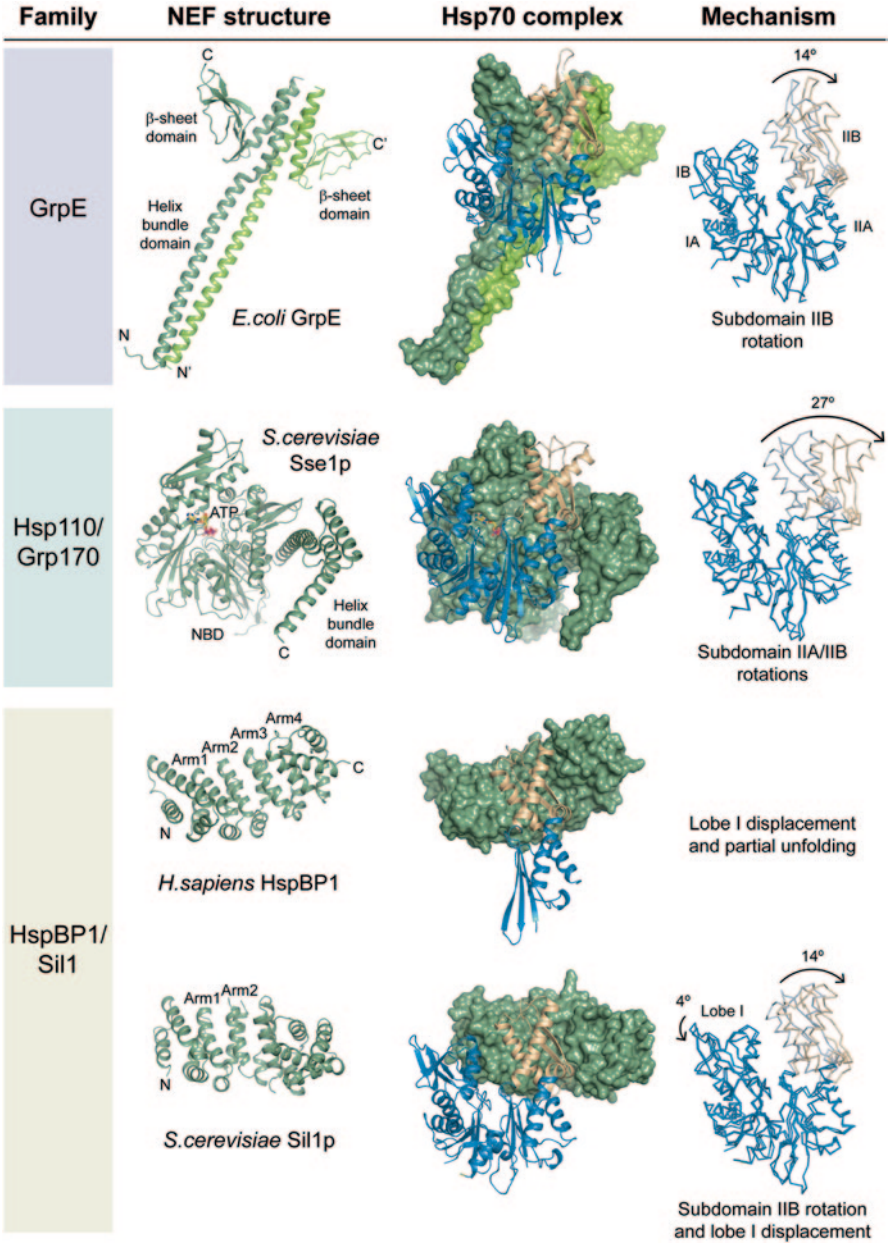
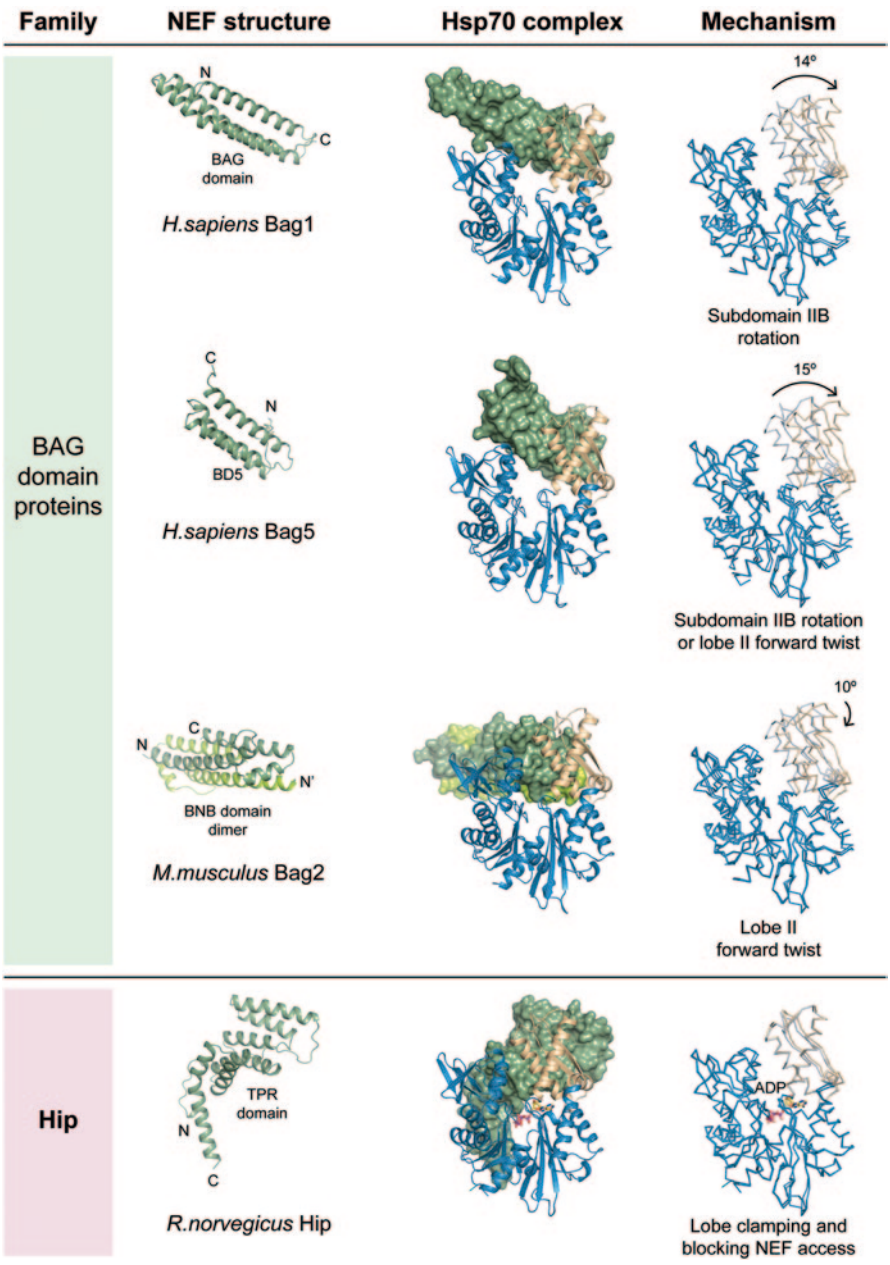


Fig. 1.2 Structure and mechanism of nucleotide exchange factors Structures for the four NEF families are shown together the respective Hsp70 complexes. The NEF is always shown in *green*; the Hsp70 NBD in *blue* with subdomain IIB highlighted in *beige*. On the *right* the structure of the NBD in the complex is superposed with the ADP-bound conformation, and the putative nucleotide exchange mechanism indicated. For comparison, the structure of the NEF-antagonist Hip is shown. The drawings are based on the PDB coordinate sets 1DKG (GrpE·DnaK (Harrison



et al. 1997)), 2V7Y (DnaK·ADP (Chang et al. 2008)), 3D2F (Sse1p·Hsp70 (Polier et al. 2008)), 1HPM (Hsc70·ADP (Wilbanks and McKay 1995)), 1XQS (HspBP1·Hsp70-lobeII (Shomura et al. 2005)), 3QML (Sil1p·Kar2p (Yan et al. 2011)), 1HX1 (Bag1·Hsc70 (Sondermann et al. 2001)), 3A8Y (Bag5·Hsp70 (Arakawa et al. 2010)), 3CQX (Bag2·Hsp70 (Xu et al. 2008)) and 4J8F (Hip·Hsp70 (Li et al. 2013))

though the respective genes were eventually transferred to the host nuclear genome. The "paralogs" of DnaK, Hsp70, Hsc70 and Bip, have somewhat different properties and are only found in eukaryotes. These proteins might thus have derived from an independent genetic transfer to the archaeal progenitor of eukaryotes, perhaps of a more specialized isoform or without the NEF. Note that the genes of DnaJ and DnaK are often part of an operon in bacteria, whereas GrpE is independently transcribed. Consistently, archaea in general do not harbor components of the Hsp70 system, unless presence of other typical bacterial genes suggests a relatively recent fusion event with an eubacterium. These archaeal Hsp70 are clearly more closely related to their eubacterial counterparts than to the Hsp70 proteins of the eukaryotic cytosol and ER lumen.

For a long time the eukaryotic Hsp70 proteins were assumed to require no NEF assistance. The measured ADP off-rates were at least one order of magnitude higher than for *E.coli* DnaK. Hence it came as quite a surprise when the first cytosolic NEF was discovered, Bag1, which belongs to a large family of BAG proteins (Höhfeld and Jentsch 1997; Takayama et al. 1999). Soon after, Sil1p and Fes1p of *Saccharomyces cerevisiae* were recognized as members of a second family of NEF proteins, the HspBP1/Sil1 proteins (Kabani et al. 2000; Kabani et al. 2002b). Finally, the Grp170/Hsp110 family of Hsp70 homologs was identified as potent NEFs to ER-lumenal and cytosolic Hsp70, respectively (Dragovic et al. 2006a; Raviol et al. 2006b; Steel et al. 2004). The fascinating details of this discovery process were reviewed earlier (Brodsky and Bracher 2007).

Now it is clear that under cellular conditions, the function of eukaryotic Hsp70 proteins is strongly dependent on nucleotide exchange factors. The combined deletion of the yeast Hsp110 homologs, Sse1p and Sse2p, is lethal (Raviol et al. 2006b; Shaner et al. 2004); the deletion of Fes1p results in a temperature-sensitive phenotype, suggesting severe problems in protein folding (Shomura et al. 2005; Kabani et al. 2000). The probable reason for the early misconception of NEF expendability is the presence of considerable amounts of inorganic phosphate (P_i) in cellular fluids (17–27 mM in *S. cerevisiae* according to ^{31}P-NMR measurements (Gonzalez et al. 2000)). Additional binding of P_i lowers the spontaneous off-rate of ADP from eukaryotic Hsp70 by approximately one order of magnitude, apparently through reduced nucleotide binding domain (NBD) dynamics (Arakawa et al. 2011; Gässler et al. 2001). Thus the spontaneous off-rate of eukaryotic Hsp70 under physiological conditions is actually close to that of DnaK.

The Grp170/Hsp110 family of Hsp70 NEFs appears to be the most ancient and universal type of eukaryotic Hsp70 NEFs (Table 1.1). Coding sequences for probable homologs were identified in virtually every eukaryotic genome so far. Humans have three genes for cytosolic isoforms (Hsp105/Hsp110, Apg-1 and Apg-2) and one ER-lumenal form (Grp170); *S. cerevisiae* has two cytosolic (Sse1p and Sse2p) and one ER-resident form (Lhs1p). Grp170/Hsp110 family proteins are distantly related to eukaryotic Hsp70. Apparently they have emerged from functional specialization of Hsp70 paralogs. The other NEF families, BAG domain proteins and Sil1/HspBP1 homologs, have rather generic structures frequently found in the eukaryotic proteome, specifically helix bundles and successions of Armadillo repeats,

Table 1.1 Eukaryotic NEFs for Hsp70 proteins

		Species	Name	Proposed involvement in	References
GrpE	Mitochondria	*S. cerevisiae*	Mge1p	Protein import and maturation	
		A. thaliana	*At*Mge1	Possible UV-B stress tolerance	(Hu et al. 2012)
			*At*Mge2	Chronic heat stress tolerance	
		H. sapiens	Mge1	Possible thermosensor function	
	Chloroplast	*Chlamydomonas reinhardtii*	Cge1	Protein import, temperature tolerance, VIPP1 oligomer assembly	(Liu et al. 2010; Willmund et al. 2007)
		Physcomitrella patens	*Pp*Cge1, *Pp*Cge2	Protein import and maturation	(Shi and Theg 2010)
Hsp110/Grp170	Cytosol/nucleus	*Plasmodium falciparum*	*Pf*Hsp110c	Proteome stabilization in malarial fevers	(Muralidharan et al. 2012)
		S. cerevisiae	Sse1p	Substrate binding, constitutive expression	(Mukai et al. 1993)
			Sse2p	Stress-inducible isoform	
		S. pombe	Pss1	Ras1 GTPase signaling	(Chung et al. 1998)
		N. crassa	Hsp88	Direct binding to small Hsp Hsp30	(Plesofsky-Vig and Brambl 1998)
		A. thaliana	Hsp91/Hsp70-14	Possible role in thermotolerance	(Jungkunz et al. 2011; Storozhenko et al. 1996)
		D. melanogaster	HSC70cb	Suppresses aggregation-induced toxicity along with DnaJ-1	(Kuo et al. 2013)
		H. sapiens	Hsp105α,β/Hsp110/HspH1	Dissaggregase activity, substrate binding	
			Apg-1/Osp94/HspA4L	Spermatogenesis	
			Apg-2/HspA4	Spermatogenesis, dissaggregase activity	
	ER	*S. cerevisiae*	Lhs1p	Protein translocation, lumenal folding, UPR, ERAD	
		H. sapiens	Grp170/Hyou1/Orp150	Response to oxygen deprivation	

Table 1.1 (continued)

		Species	Name	Proposed involvement in	References
HspBP1/Sil1	Cytosol/nucleus	*S. cerevisiae*	Fes1p	Co-translational folding, degradation of misfolded proteins	
		A. thaliana	*At*Fes1A	Thermotolerance, response to salt stress	(Zhang et al. 2010)
		H. sapiens	HspBP1	Hsp70 inhibition?	
	ER	*S. cerevisiae*	Sls1p/Sil1p	Protein translocation, lumenal folding, UPR, ERAD	
		H. sapiens	Sil1/BAP	Neuronal morphology, migration and axon growth	
BAG-domain proteins	Cytosol/nucleus	*S. cerevisiae*	Snl1p	Ribosomal editing, co-translational folding	
		S. pombe	Bag101/Bag-1A	Protein QC and degradation	
			Bag102/Bag-1B	Kinetochore integrity	
		A. thaliana	*At*Bag1–*At*Bag4	Plant programmed cell death	(Kabbage and Dickman 2008; Doukhanina et al. 2006)
			*At*Bag5	Calmodulin signaling?	
			*At*Bag6	Calmodulin signaling?	
		C. elegans	Bag-1	Protein QC and degradation?	
			Bag-2/unc-23	Muscle maintenance?	
		D. melanogaster	Starvin	Muscle maintenance, recovery from cold stress	(Coulson et al. 2005)
		H. sapiens	Bag1/Rap46/HAP	Apoptosis, protein QC and degradation	
			Bag2	CHIP Ub-ligase inhibition	
			Bag3/CAIR-1	Autophagy, cell adhesion and migration	
			Bag4/SODD	Apoptosis	
			Bag5	Parkin Ub-ligase inhibition	
			Bag6/Scythe/BAT-3	GET pathway, protein degradation	
	ER	*A. thaliana*[a]	*At*Bag7	UPR	(Williams et al. 2010)

[a] *A. thaliana* has homologs to Sil1 (F4F15.90) and Grp170 (AtHsp70-17). These were however not characterized at the protein level yet

respectively. Such scaffolds can rapidly (on an evolutionary timescale) adapt to a new function after a gene duplication event, and have been employed over and over again in eukaryotic protein evolution. Helix bundles are for example also found in syntaxin SNARE proteins; Armadillo and HEAT repeat proteins in nuclear transport factors and β-catenin (Tewari et al. 2010). It is conceivable that BAG proteins have emerged multiple times, having short and long 3-helix bundle structures (Bag1 and Bag4/Bag5), insertions or 4-helix bundle dimer structures (Bag2). Their few common signature residues are forced by the evolutionary constraints on the binding partner, the NBD of Hsp70, which exhibits high surface conservation (for details see below). It moreover appears that the ER-lumenal NEF Sil1 from yeast and animals have evolved independently: Although yeast Sil1p resembles the mammalian HspBP1 at the secondary and tertiary structure level (Shomura et al. 2005; Yan et al. 2011), it appears to employ a binding mode and mechanism of action distinct from mammalian Sil1, which acts more similar to HspBP1 (Hale et al. 2010; Howes et al. 2012). Consequently, the ancestry and exact functional role of BAG and Sil1/HspBP1 protein homologs in different species is difficult to rationalize on sequence data alone. Humans and *Arabidopsis thaliana* have six and seven known cytosolic BAG isoforms, respectively (Table 1.1); yeast has one ER-membrane-bound homolog, Snl1p, but exact functional homologs to Snl1p have not been identified in humans and *Arabidopsis* either (Sondermann et al. 2002; Takayama et al. 1999).

In addition to the emergence of three Hsp70 NEF families in multiple isoforms in eukaryotes, an even more dramatic expansion in J-domain protein diversity has occurred, resulting in approximately 40 isoforms in humans (see review in (Kampinga and Craig 2010)).

Molecular Structure and Function of Eukaryotic NEFs

Eukaryotic GrpE Homologs

Structural data for eukaryotic GrpE homologs are not yet available. Judging from sequence alignments, their structures are likely fairly similar to bacterial GrpE proteins, which have been solved for the *E. coli* (Harrison et al. 1997), *Thermus thermophilus* (Nakamura et al. 2010) and *Geobacillus kaustophilus* (Wu et al. 2012) homologs. All these proteins have dimeric two-domain structures composed of a coiled-coil helix bundle and a wing-like β-domain (Fig. 1.2). One β-domain engages in contacts with subdomains IB and IIB, assisted by additional contacts from the helix bundle, stabilizing a NBD conformation with an open nucleotide binding cleft. Opening is enabled by an outwards rotation of subdomain IIB.

Simulations suggest a highly dynamic structure for the NBD of Hsp70 proteins, allowing shearing motions between the lobes and an outwards rotation of subdomain IIB around an inbuilt hinge, which likely influence the nucleotide exchange rate (Ung et al. 2013). GrpE and the other Hsp70 NEFs appear to capture and

stabilize open states in which a subset of the interactions between NBD and ADP is disabled, thereby lowering ADP affinity. Substantial parts of the NBD contact area with GrpE become buried near the lobe interface in the ADP-bound conformation of DnaK, suggesting that GrpE captures open conformations, but cannot 'force' the NBD to open. ATP binding induces a conformational change in the NBD of DnaK, displacing the binding sites on lobes I and II by inter-lobe shearing, resulting in strongly decreased affinity to GrpE. So both ADP and ATP compete with GrpE for binding to DnaK.

The Hsp110 Family of Nucleotide Exchange Factors

The Hsp110/Grp170 proteins belong to the Hsp70 protein family (Easton et al. 2000). Crystal structures of the yeast Hsp110 protein Sse1p revealed a shared domain composition comprising a N-terminal actin-type nucleotide binding domain, followed by a β-domain and a α-helix bundle (Liu and Hendrickson 2007; Polier et al. 2008; Schuermann et al. 2008). Hsp110 family protein sequences are however much less conserved than canonical Hsp70, with the greatest divergence found in the C-terminal domains. Backbone extensions compared to canonical Hsp70 proteins are found at the C-terminus and within the β-domain (Fig. 1.3). The Grp170 homologs have even larger extensions than cytosolic homologs and always bear N-terminal import and C-terminal ER-retention signal sequences (Table 1.1).

In the crystal structures of Sse1p, the α-helix bundle is associated with the flank of the NBD, resulting in a compact conformation (Fig. 1.2). The β-domain undergoes extensive interactions with the bottom of the NBD, but not with the α-helix bundle domain, which extends in the opposite direction. Sse1p exhibits a pronounced twist of the NBD lobes, revealing a bound ATP molecule in the center. Structures of an ATPase-inactive DnaK mutant later demonstrated that the binding of ATP induces a very similar conformation in canonical Hsp70 proteins (Kityk et al. 2012; Qi et al. 2013).

In the crystal structures of the complex, the NBDs of Sse1p and mammalian Hsp70 face each other in a pseudo-symmetrical fashion (Polier et al. 2008; Schuermann et al. 2008). The NBD of Hsp70 is captured in an open conformation by additional interactions of subdomain IIB with the α-helix bundle domain of Sse1p. In this conformation, ADP cannot simultaneously engage in direct interactions with all four subdomains and is thus more likely to dissociate, explaining the nucleotide exchange activity of Sse1p. The residues mediating key contacts to Hsp70 are conserved in all Hsp110/Grp170 proteins (Andreasson et al. 2010; Hale et al. 2010). Only the compact, ATP-bound conformation of Hsp110/Grp170 proteins provides the necessary geometry required for simultaneous interactions between NBD·NBD and α-helix bundle·subdomain IIB of Hsp110/Grp170 and Hsp70, respectively (Raviol et al. 2006b; Shaner et al. 2004; Andreasson et al. 2008).

Besides serving as essential NEFs for Hsp70, Hsp110/Grp170 proteins potently stabilize denatured proteins against aggregation (Goeckeler et al. 2002;

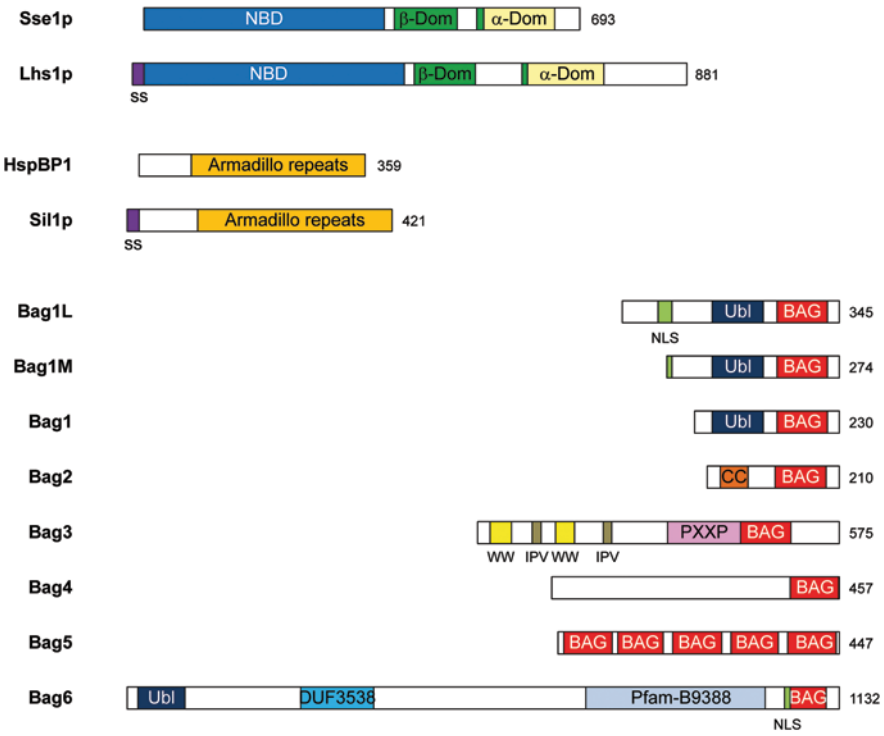


Fig. 1.3 Domain architectures of different NEF families As examples for Hsp110 and Grp170 proteins the yeast homologs Sse1p and Lhs1p are shown, respectively. Both consist of an N-terminal nucleotide binding domain (NBD, *blue*), a β-sandwich (β-Dom, *green*) and a α-helix bundle domain (α-Dom, *pale yellow*). SS indicates a signal sequence for ER import. The HspBP1/Sil family proteins have characteristic Armadillo repeat folds (*orange*). All members of the BAG family in humans, Bag1–6, contain C-terminal Hsp70-binding BAG domains (*red*), but have otherwise divergent domain architecture. Bag5 has four additional BAG domains of unknown function. Bag1 isoforms and the large Bag6 contain Ubiquitin-like domains (Ubl, *dark blue*), which might associate with the regulatory particle of the 26S proteasome. Bag6 has furthermore two probable domains, which have not yet been characterized further. Bag2 contains a coiled-coil dimerization domain (CC, *orange*). Bag3 comprises multiple N-terminal sequence motifs, WW domains (WW, *yellow*), IPV sequence motifs (*brown*) and PXXP repeats (*pink*). Bag1 L and Bag6 have NLS sequences (*light green*) for nuclear targeting

Oh et al. 1997; Oh et al. 1999). The molecular basis for this holdase activity is still controversial. Canonical Hsp70 proteins stably interact with substrate proteins only in the ADP state, enclosing hydrophobic peptide segments between β-domain and α-helix bundle. While Sse1p appears to have no intrinsic ATPase activity—bound ATP survived in the crystallization experiments for weeks—ATPase stimulation by J-domain proteins has been observed (Mattoo et al. 2013; Raviol et al. 2006a). Consistently, binding of Sse1p and human Hsp105 to hydrophobic peptides has been reported, although with a preference towards aromatic residues in contrast to canonical

Hsp70s, which prefer aliphatic sidechains and prolines (Goeckeler et al. 2008; Xu et al. 2012; Rüdiger et al. 1997; Zahn et al. 2013). Because of their low sequence conservation in the β-sheet domain, Hsp110 orthologs may differ considerably in their substrate binding properties. For example, Sse1p potently stabilizes the model protein firefly luciferase (FLuc) at 42 °C for subsequent refolding, while its close homolog Sse2p is inactive (Polier et al. 2010). The reason for this surprising difference seems to be that Sse1p unfolds partially at 37 °C with a concomitant increase in aggregation prevention capacity, while the paralogous Sse2p is stable until 46 °C, similar to human Apg-2, which unfolds at 51 °C (Polier et al. 2010; Raviol et al. 2006a).

While the Hsp110 holdase activity appears to be important, its NEF function is critical (Raviol et al. 2006b; Shaner et al. 2004). Only mutant forms of Sse1p that abolish interactions with Hsp70 and nucleotide exchange were lethal in the *SSE1*/*SSE2* deletion background (Polier et al. 2008). Similar requirements were found for the 'mammalian disaggregase' function of Hsp110, Hsp70 and Hsp40 (see below).

Sil1/HspBP1 homologs

HspBP1 (Hsp70 binding protein 1) is the mammalian homolog of the cytosolic Fes1p protein in *S. cerevisiae* (Kabani et al. 2002a; Kabani et al. 2002b; Raynes and Guerriero 1998). The ER-lumenal paralogs are named Sls1p/Sil1p or Sil1/BAP (Bip associated protein) in yeast and mammals, respectively (Kabani et al. 2000; Chung et al. 2002). Sil1 homologs occur almost ubiquitously in eukaryotes. Homologs to HspBP1 are found in most animal, plant, algal and fungal genomes. Sil1/HspBP1 proteins are composed of a divergent N-terminal part of ~85 residues and a conserved C-terminal core domain, which alone is sufficient to mediate nucleotide exchange (Fig. 1.3). Crystal structures showed that the core domains of human HspBP1 and yeast Sil1p consist of Armadillo repeats flanked by capping helix pairs (Shomura et al. 2005; Yan et al. 2011) (Fig. 1.2). Surprisingly, the complex structures with the respective Hsp70 binding partner revealed distinct binding modes for the paralogs. The curved-shaped HspBP1 associates so extensively with subdomain IIB of the Hsp70 NBD that the bulk of the NEF clashes severely with lobe I, thereby destabilizing its fold as judged from tryptophan fluorescence quenching and increased sensitivity against protease degradation (Shomura et al. 2005). Yeast Sil1p also embraces subdomain IIB, however using different molecular contacts, resulting in a distinct region covered by the NEF (Yan et al. 2011). This binding mode just induces an outward rotation of subdomain IIB and a slight sideways displacement of lobe I, more similar to the complexes with GrpE (Harrison et al. 1997) and the Hsp110 protein Sse1p (Polier et al. 2008; Schuermann et al. 2008). The binding mode of animal and plant Sil1 appears to resemble HspBP1 closer than yeast Sil1, as judged from mutational analysis (Hale et al. 2010; Howes et al. 2012).

BAG domain-containing NEFs

BAG (Bcl-2 associated athanogene) family proteins have a modular domain architecture comprising a conserved region of ~100 amino acids at the C-terminus, called the BAG domain (Takayama et al. 1999). In the N-terminal part diverse domains and sequence motifs were found for BAG domain proteins (Fig. 1.3). The human genome comprises six BAG family protein sequences, which were numbered Bag1–6 (Takayama and Reed 2001) (Table 1.1). As pointed out above, these proteins are structurally and functionally quite heterogeneous, and will be discussed here one after the other. Only Bag1 and Bag3 appear to be conserved in most metazoans. Homologs have been described in the fruit fly *Drosophila melanogaster* (Arndt et al. 2010), the nematode worm *Caenorhabditis elegans* (Nikolaidis and Nei 2004) and the tunicate *Ciona intenstinalis* (Wada et al. 2006).

The first structures to be solved were the BAG domain of Bag1 in isolation and in complex with the NBD of Hsc70, revealing a bundle structure with three long α-helices for the BAG domain (Sondermann et al. 2001; Briknarova et al. 2001) (Fig. 1.2). Interactions with α-helices 2 and 3 of Bag1 stabilize a conformational change in the Hsc70 NBD similar to the GrpE·DnaK complex (Harrison et al. 1997; Sondermann et al. 2001). Three different isoforms of Bag1 exist in cells, which are generated by alternative translation initiation from a single mRNA (Fig. 1.3). All Bag1 isoforms contain an ubiquitin-like (Ubl) domain that serves as a sorting signal to facilitate interaction with the 26S proteasome (Alberti et al. 2003). The Bag1 L isoform contains an additional nuclear localization signal (NLS) at the extreme N-terminus, whereas the other two isoforms are present in the cytosol (Takayama et al. 1998). Interestingly, the BAG domain shares binding sites with Hsc70 and Raf1, a stress-signaling anti-apoptotic kinase, and the two proteins bind Bag1 in a mutually exclusive manner (Song et al. 2001). The structure of the Ubl domain from mouse Bag1 has been solved by NMR, revealing a characteristic ubiquitin-like fold (Huang and Yu 2013). In mice, this domain of Bag1 mediates interaction with the cytoplasmic tail of the heparin-binding EGF-like growth factor (HB-EGF) precursor, thereby altering cell adhesion and secretion of the mitogen HB-EGF (Lin et al. 2001).

Bag3 is expressed prominently in striated muscle tissue, but is also necessary for development and blood cell formation. Bag3 deletion in mice resulted in severe myopathy (Homma et al. 2006) and loss of hematopoietic stem cells (Kwon et al. 2010). Interestingly, Bag3 is the only heat stress-inducible BAG-domain protein (Franceschelli et al. 2008; Jacobs and Marnett 2009). Bag3 contains various sequence motifs and domains, such as WW domains and proline-rich repeats (PXXP), which mediate interactions with numerous partner proteins other than Hsp70. For example, the first WW domain was shown to interact with PXXP motifs at the C-terminus of PDZGEF2, a regulatory protein involved in cell adhesion (Iwasaki et al. 2010); binding to the small heat shock proteins HspB8 and HspB6 is mediated by two IPV motifs (Fuchs et al. 2010). The PXXP repeats of Bag3 likely interact with SH3 domains found in regulatory proteins of cell adhesion and migration (Doong et al. 2000). These interactions link Bag3 to processes such as development, autophagy and cytoskeletal organization (reviewed in (Rosati et al. 2011)). The

complex of Bag3, Hsc70 and HspB8 was strongly implicated in macroautophagy (Arndt et al. 2010; Lamark and Johansen 2012), a process in which portions of the cytosol are engulfed by a membrane and digested. The complex appears to be involved in targeting aggregated proteins to aggresomes for degradation. Aggresomes are microtubule-dependent collection points for such terminally misfolded proteins in the cell (Kopito 2000). Details are unclear, but Bag3 interacts and co-localizes with p62/SQSTM1, a key regulator of the macroautophagy pathway (Gamerdinger et al. 2009). An association of Bag3 with the adaptor protein 14-3-3γ is dependent on phosphorylation at Ser136 and Ser173, and may serve to attach aggregates to the motor protein Dynein that travels along microtubules (Xu et al. 2013). Macroautophagy appears to be vitally important for muscle maintenance. In *D. melanogaster* muscles, the Bag3 ortholog Starvin is required for Z-disk maintenance through a process named 'chaperone-assisted selective autophagy' (CASA) (Arndt et al. 2010). A complex of Bag3, Hsc70 and HspB8 is needed for autophagy of the large muscle protein filamin after mechanical tension-induced unfolding (Ulbricht et al. 2013). Autophagosome formation is dependent on the interaction of the Bag3 WW domain with the filamin-interacting protein synaptopodin-2.

Bag4 is alternatively named "silencer of death domains" (SODD) as it binds to the cytoplasmic regions of receptors that signal cell death, namely TNFR1 and DR3, and prevents ligand-independent receptor signaling and apoptosis (Jiang et al. 1999). Surprisingly, NMR structures showed that the three-helix bundle in Bag4 is about 25 amino acids shorter than in Bag1, although it comprises the signature residues needed for interaction with Hsp70 proteins, suggesting that it might have evolved independently (Brockmann et al. 2004; Briknarova et al. 2002). Bag1, Bag3 and Bag4 have been shown to bind the anti-apoptotic protein Bcl-2 (Antoku et al. 2001). Together with their ability to interact with Hsp70, which also has an anti-apoptotic function, this suggests linked mechanisms for apoptosis inhibition (Antoku et al. 2001). It is not known whether these BAG domain proteins can simultaneously bind Hsp70 and Bcl-2, but it has been hypothesized that these two proteins compete for binding as they both can interact with the BAG domain (Doong et al. 2002).

Among the Bag proteins, Bag5 is unique in containing five consecutive short BAG domains similar in structure to the BAG domains of Bag3 and Bag4 (Arakawa et al. 2010). Of these, only the fifth BAG domain is active in Hsp70 NBD binding and assisting Hsp70-mediated substrate refolding. The crystal structure of this domain with the Hsp70 NBD revealed two distinct conformations of the complex; one where the NBD is in an open state similar to the Bag1 complex and the other with a NBD exhibiting a binding pocket distorted by inter-lobe shearing (Arakawa et al. 2010; Fig. 1.2). Both conformational states likely have reduced affinity for ADP. The functional consequences that could result from the shorter BAG domain structures in Bag3, Bag4 and Bag5 are currently unknown. Interestingly, Bag5 was shown to associate with the E3-ubiquitin ligase Parkin, modulating substrate protein ubiquitylation (Kalia et al. 2004).

Bag2 is the most distantly related member of the BAG family. In the crystal structures, what was supposed to be the BAG domain adopted an unanticipated dimeric