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Gregory L. Blatch

Networking of Chaperones by Co-Chaperones

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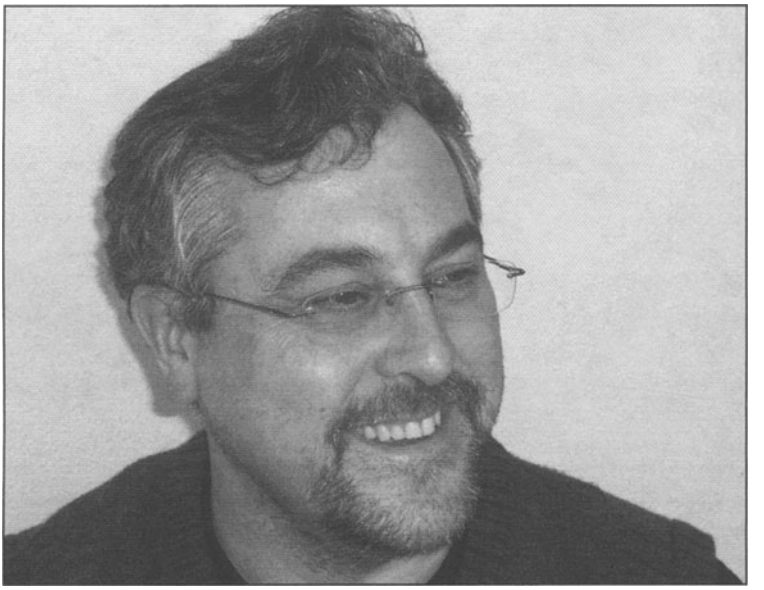

About the Editor...

GREGORY L. BLATCH is Professor of Biochemistry at Rhodes University, Grahamstown, South Africa. His research interests fall within the broad field of cell stress and chaperones, with a focus on the role of co-chaperones in the regulation and networking of the major molecular chaperones, Hsp70 and Hsp90. He was recently awarded a Wellcome Trust International Senior Research Fellowship for his research on the biomedical aspects of chaperones and co-chaperones. He received his Ph.D. from the University of Cape Town (UCT), South Africa, and did his Postdoctoral at Harvard University Medical School, U.S.A.

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PREFACE

There are a number of books dedicated to the cellular and molecular biology of chaperones and their important role in facilitating protein folding; however, this is the first book dedicated to the co-chaperones that regulate them. This book is perhaps long overdue, as the concept of co-chaperones has been in place for more than a decade. The chapters reflect many of the emerging themes in the field of co-chaperone-chaperone biology, with a particular emphasis on the co-chaperones of the major molecular chaperones, Hsp70 and Hsp90.

What constitutes a co-chaperone? In formal terms, a co-chaperone may be defined as any non-substrate protein that interacts specifically with a molecular chaperone and is important for efficient chaperone function. Many co-chaperones are dedicated to a specific chaperone and play a regulatory role (e.g., Hsp40 regulates the nucleotide-bound state of Hsp70). This regulatory role is highly substrate-sensitive, with some co-chaperones having the ability to directly interact with the substrate protein and target it to the chaperone. Indeed, some co-chaperones have the capacity to carry out some of the functions of a chaperone, such as the prevention of protein aggregation (e.g., some Hsp40s, UNC-45 and Cdc37). However, co-chaperones do not always have the ability to interact with substrate or to act as true chaperones in their own right. Nevertheless, whether they directly bind the substrate or indirectly "sense" its presence, in many cases co-chaperones provide specificity to their somewhat promiscuous chaperone partner.

The structure of co-chaperones suggests that they have evolved through domain recruitment, manifesting as highly sophisticated protein scaffolds for the efficient spatial orientation of protein-protein interaction domains (e.g., J domain) and motifs (e.g., tetratricopeptide repeat [TPR] motif). A number of the chapters document the rapidly emerging structural data on domains and motifs, giving us insight into the elegant manner in which these structural features are the functional engines driving the optimal docking and regulation of chaperones by co-chaperones. Interestingly, evidence has also emerged for "fractured" co-chaperones (e.g., Zim17 in yeast), which represent the evolution of physically uncoupled, yet functionally linked, partner domains, allowing for the flexibility of multiple roles.

Contrary to the perception that co-chaperones are merely auxiliary components of the cell's molecular chaperone machinery, a number of chapters suggest that co-chaperones are core components of, and can sometimes transcend, the chaperone machinery (e.g., the role of GrpE as a thermosensor; and Hop may not be dedicated to Hsp70 and Hsp90). Furthermore, co-chaperones not only play an important role in the regulation of Hsp70 and Hsp90 protein folding pathways, but also integrate these folding pathways with protein degradation pathways so as to maintain

cellular homeostasis. Therefore, co-chaperones can be broadly viewed as quality control factors enabling the major molecular chaperones to integrate diverse cellular signals and make the correct decision on whether to hold, fold, or degrade; the global safety of the cell being paramount. Finally, the dogma that chaperones interact only with misfolded or denatured substrate proteins is being challenged by mounting evidence to indicate that co-chaperones are able to target chaperones to act with near native proteins to facilitate conformational change (e.g., targeting of clathrin to Hsp70 by auxilin). The name co-chaperone is perhaps limiting, and as more details on the global cellular roles of co-chaperones are revealed, we will no doubt have to re-evaluate the co-chaperone paradigm.

Gregory L. Blatch, Ph.D.

Acknowledgments

I have been very privileged to have had the opportunity to edit the first book dedicated to co-chaperones. Privileged, firstly because it has given me many new and exciting insights into this fascinating field of research, and secondly because it has allowed me to enter into a thoroughly enriching process of interacting with a highly professional network of biologists. Like any typical network, there were many weak links (the email conversations) and a few strong links (the book chapters) in the network of interactions between editor and authors! And so it was that this book on the "Networking of Chaperones by Co-chaperones" was born. I hope that each of the contributors to this book enjoyed the process as much as I have; thank you for your immense creative input. I am also very grateful to the Rhodes University Chaperone Research Group for so eagerly assisting me at the whole book proofing stage: Dr. Aileen Boshoff, Melissa Botha, Sheril Daniel, Dr. Linda Stephens, Dr. Victoria Longshaw, Michael Ludewig, Dr. Eva-Rachele Pesce, Mokgadi Setati and Addmore Shonhai. I went to many people for advice; thanks to all of you for your valuable time, but especially Dr. Graeme Bradley (Rhodes University), Dr. Peter Lund (Birmingham University, U.K.) and my wife Heather Yule.

CHAPTER 1

Nucleotide Exchange Factors for Hsp70 Molecular Chaperones

Jeffrey L. Brodsky* and Andreas Bracher

Abstract

Hsp70 molecular chaperones hydrolyze and re-bind ATP concomitant with the binding and release of aggregation-prone protein substrates. As a result, Hsp70s can enhance protein folding and degradation, the assembly of multi-protein complexes, and the catalytic activity of select enzymes. The ability of Hsp70 to perform these diverse functions is regulated by two other classes of proteins: Hsp40s (also known as J-domain-containing proteins) and Hsp70-specific nucleotide exchange factors (NEFs). Although a NEF for a prokaryotic Hsp70, DnaK has been known and studied for some time, eukaryotic Hsp70s NEFs were discovered more recently. Like their Hsp70 partners, the eukaryotic NEFs also play diverse roles in cellular processes, and recent structural studies have elucidated their mechanism of action.

Introduction

To cope with environmental stresses, such as heat shock, oxidative injury, or glucose-depletion, the expression of a large number of heat shock proteins (Hsps) is induced in all cell types examined. Early work defined these Hsps (some of which are identical to the glucose-responsive proteins, or Grps) by their apparent molecular masses; thus, Hsps with a mass of ~70 kDa became known as Hsp70s, and ~40 kDa Hsps are Hsp40s.[1] Subsequent studies indicated that many Hsps also function as molecular chaperones, factors that aid in the maturation, processing, or sub-cellular targeting of other proteins.

Perhaps the best-studied group of molecular chaperones is the Hsp70s.[2] Hsp70s are found in every organism (with the exception of some *archaea*[3]) and in eukaryotes reside in or are associated with each sub-cellular compartment. Hsp70s are highly homologous to one another and are comprised of three domains: A ~44 kDa amino-terminal ATPase domain, a central ~18 kDa peptide-binding domain (PBD), and a carboxy-terminal "lid" that closes onto the PBD to capture peptide substrates.[4] In some Hsp70s, a short carboxy-terminal amino acid motif also mediates the interaction between Hsp70s and co-chaperones containing tetratrico peptide repeat (TPR) domains (see Chapters by Cox and Smith, and Daniel et al). By virtue of their preferential binding to hydrophobic peptides, Hsp70s retain these aggregation-prone substrates in solution, which in turn permits Hsp70s to enhance: (1) the folding of nascent or temporarily unfolded proteins; (2) the degradation of mis-folded polypeptides; (3) the assembly of multi-protein complexes; and (4) the catalytic activity of enzyme complexes that might require quaternary assembly. It should come as no surprise, then, that Hsp70 over-expression permits the cell to

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withstand cellular stresses, and that Hsp70s and constitutively expressed Hsp70 homologues, or Hsp70 "cognates" (also known as Hsc70s) play vital roles in cellular physiology.

Hsp70s bind loosely to their peptide substrates when the ATPase domain is occupied by ATP, and tightly when the enzyme is in the ADP-bound state;[5-8] therefore, ADP-ATP exchange is critical for peptide release, and both ATP hydrolysis and nucleotide exchange are accelerated by Hsp70s co-chaperones. Specifically, Hsp40s—which are defined by the presence of an ~70 amino acid "J" domain—enhance ATP hydrolysis (see Chapter by Rosser and Cyr), whereas ADP release is catalyzed by another group of proteins, known as nucleotide exchange factors (NEFs). In fact, these factors do not "exchange" one nucleotide for another, but because ATP is present at much higher concentrations than ADP in the cell, ATP binding most commonly follows ADP release.

The physiological consequences of eukaryotic Hsp70-Hsp40 interaction are well-characterized.[9-11] In contrast, the contributions of Hsp70 NEFs in eukaryotic cell homeostasis are only now becoming apparent. Therefore, the purpose of this review is to summarize briefly what is known about the best-characterized Hsp70 NEF, the bacterial GrpE protein, and then to discuss in greater detail the more recent discovery of eukaryotic NEFs in the cytoplasm and in the endoplasmic reticulum (ER). Particular emphasis will be placed on the molecular underpinnings by which these NEFs function, and on important but unanswered questions in this field of research.

GrpE: The Bacterial Nucleotide Exchange Factor for Hsp70

The replication of the λ bacteriophage genome in *E. coli* requires DNA helicase activity at the origin of replication (*ori*). The helicase is initially inhibited by the λP protein, but the protein is displaced by host-encoded Hsp70 and Hsp40 chaperones, which were first named DnaK and DnaJ, respectively, based on the inability of *dnaK* and *dnaJ* mutants to support λ replication.[12] Another mutant that prevented λ replication is encoded by the *grpE* locus.[13] DnaK-DnaJ-dependent liberation of λP from the *ori* and replication of the phage genome can be recapitulated in vitro, and it was discovered that decreased amounts of DnaK are required in these assays if GrpE is also present.[14,15] This phenomenon results from the fact that GrpE strips ADP from DnaK, and the combination of DnaJ and GrpE synergistically enhances DnaK's ATPase activity in single-turnover measurements by 50-fold, or even up to 5000-fold, depending on whether GrpE is saturating.[8,16] The DnaK-DnaJ-GrpE "machine" not only regulates multi-protein complex assembly—as observed during phage λ replication—but assists in the folding of newly synthesized and unfolded polypeptides, and homologues of each of these proteins reside in the mitochondria and help drive the import or "translocation" and maturation of nascent polypeptides in this organelle (see Chapter by Bursać and Lithgow).[17,18]

The Discovery of Hsp70 Nucleotide Exchange Factors in Eukaryotes: Fishing Pays Off

The cytoplasm and ER lumen in eukaryotes contain several Hsp70 and Hsp40 homologues, and it was assumed that GrpE homologues would also reside in these compartments. After many years, the failure to identify them was ascribed either to the fact that GrpE homologues are highly divergent and/or that the Hsp70s in the ER and eukaryotic cytoplasm might have evolved such that GrpE-assisted ADP release is dispensable.[19] Thus, it came as a complete surprise when BAG-1—which was first identified as a cellular partner for Bcl-2, a negative regulator of apoptosis[20]—was found to catalyze ADP release from mammalian Hsp70.[21] The binding between BAG-1 and the ATPase domain of Hsp70 is mediated by a ~50 amino acid "BAG" domain,[22-24] which is present in each of the many isoforms and splice variants of BAG-1 that have been identified. However, it is clear that BAG domain-containing NEFs do not function identically to GrpE, at least in part because their structures are distinct (also see below). For example, GrpE catalyzes the release of both ADP and ATP from DnaK, whereas BAG-1 triggers only ADP release.[25] In addition, GrpE augments DnaK-DnaJ-mediated pro-

tein folding and assembly, whereas BAG-1 has been found to exert either positive or negative effects on Hsp70-Hsp40-directed protein folding and chaperone activity. These contradictory results stem primarily from the concentrations of BAG-1 employed and the presence or absence of specific co-chaperones.[26,27] Thus, future work is needed to define how BAG domain-containing proteins impact known chaperone activities and how each of the various isoforms function under normal, cellular conditions and at their native concentrations.

For some time it was thought that yeast lacked a BAG domain-containing protein, but the available structure of an Hsp70 ATPase domain in complex with a BAG domain fragment[28] brought about the discovery of a highly divergent BAG-1 homologue in the yeast database, Snl1.[29] *SNL1* was originally identified as a high-copy suppressor of the toxicity produced by the C-terminal fragment of a nuclear pore protein, and one consequence of this fragment is the generation of nuclear membrane "herniations".[30] Therefore, it was proposed that Snl1 modulates nuclear pore complex (NPC) integrity, and consistent with this hypothesis, Snl1 is an integral membrane protein that resides in the nuclear envelope/ER membrane. Proof that Snl1 is a bona fide BAG homologue derived from the fact that Snl1 associates with Hsp70s from yeast and mammals, and that a purified soluble fragment of Snl1 stimulates Hsp40-enhanced ATP hydrolysis by Hsp70 to the same extent as a mammalian BAG domain-containing protein.[29]

Because the lumen of the ER houses a high concentration of Hsp70 and because of its prominent role in catalyzing the folding of nascent proteins, it was also assumed that a NEF would reside in this compartment. Almost all secreted proteins associate with BiP, the ER lumenal Hsp70, during translocation and folding.[31] During translocation, BiP is anchored to an integral membrane J-domain-containing protein, but if the subsequent folding of the nascent secreted protein is compromised, BiP interacts instead with soluble Hsp40s to facilitate the "retro-translocation" of the aberrant protein from the ER and into the cytoplasm where it is degraded by the proteasome.[32] This process was termed ER associated degradation (ERAD[33]) and is conserved amongst all eukaryotes.

To identify BiP partners that might include NEFs and that might facilitate protein translocation, folding, and/or ERAD, genetic selections were performed in different yeasts. First, the *SLS1* gene was identified in a synthetic lethal screen in *Y. lipolytica* strains that lacked a component of the signal recognition particle, which is essential in this organism for protein translocation.[34] Later studies established that the Sls1 homologue in *S. cerevisiae* interacts preferentially with the ADP-bound form of BiP, that Sls1 enhances the Hsp40-mediated stimulation of BiP's ATPase activity, and that Sls1 accelerates the release of ADP and ATP from BiP.[35] Second, Stirling and colleagues isolated a gene that at high-copy number suppressed a growth defect in *S. cerevisiae* lacking an Hsp70-related protein, known as Lhs1, and that were unable to mount an ER stress response.[36] The gene, *SIL1*, is identical to *SLS1*, and the Sil1 protein was shown to bind selectively to BiP's ATPase domain. Together, these data suggested strongly that Sls1/Sil1 is a BiP NEF. Further support for this hypothesis was provided by the discovery that Sls1/Sil1 is the yeast homologue of BAP, a resident of the mammalian ER that strips nucleotide from BiP and synergistically enhances the J-domain-mediated activation of BiP's ATPase activity.[37]

Surprisingly, Lhs1, mentioned above as an Hsp70-related protein, also appears to function as a NEF. Lhs1 is a member of the Hsp110/Grp170 family of mammalian molecular chaperones that possess N-terminal ATP binding domains with some homology to the Hsp70 ATPase domain; however, the C-terminal halves are comprised of extended, nonconserved polypeptide binding domains.[38] Recent studies from the Stirling laboratory indicate that Lhs1 interacts with BiP in the yeast ER and can strip ADP/ATP from BiP as efficiently as Sls1/Sil1, thus activating BiP's steady-state ATPase activity when combined with a J-domain-containing protein.[39] In turn, BiP activates the ATPase activity of Lhs1, and in both cases the ATP-binding properties of the chaperones are essential for activity. These results indicate that BiP and Lhs1 reciprocally enhance one another's activities, perhaps to coordinate the transfer of polypeptide substrates. Although it is not yet clear whether all