Preventing illicit liaisons in Poland

Workshop on the Biology of Molecular Chaperones: Heat Shock Proteins in Molecular Medicine, Misfolding Diseases and Cancer

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This EMBO-FEBS Workshop on the Biology of Molecular Chaperones: Heat Shock Proteins in Molecular Medicine, Misfolding Diseases and Cancer took place from 28 May to 2 June 2005 in Zakopane, Poland, and was organized by U. Hartl and M. Zylicz.

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misfolded proteins by colluding with the ubiquitin–proteasome system (Fig 1).

Such quality control is vital for the survival of unicellular and multicellular organisms alike and therefore it is not surprising that there is a high degree of evolutionary conservation of chaperone structure and function. At this recent EMBO-FEBS-sponsored meeting, 130 researchers from around the globe met to consider the roles of molecular chaperones and, in particular, their contribution to the survival of an organism.

In this selective review of some of the meeting's highlights, we have separated the presentations according to the main chap-
erone system to which speakers referred—an artificial distinction,
as a recurrent theme of many talks (and an explicit point made by
P. Csermely (Budapest, Hungary)) was that chaperones are
involved in complex networks of interactions with each other and
with their numerous cochaperones and client proteins.

Many of the talks impressively illustrated the range of advanced biophysical and imaging techniques that are now being applied to studies on protein folding. However, in his opening keynote lecture, C. Georgopoulos (Geneva, Switzerland) showed how simple genetic experiments with bacteria and their viruses not only led to the discovery of many of the chaperones discussed at this meeting, but also continue to provide important insights into the key players in protein folding pathways in these and other (such as yeast and worm) systems.

The heat shock protein 70 chaperones

The heat shock protein 70 (Hsp70) chaperones have long interested researchers, largely because they participate in several cellular processes and are found in many diverse locations. A complete structure for any Hsp70 is still elusive, but they all contain a peptide-binding and a nucleotide-binding domain, with the strength of peptide binding modulated by nucleotide binding. Hsp70 proteins interact with cochaperones, known as Hsp40 proteins, and with a host of nucleotide exchange factors (NEFs). Some of the details of these interactions and how they modulate Hsp70 function are now being unravelled.

Two NEFs for Hsp70, Bcl2-associated athanogene 1 (BAG1, found in eukaryotic cytosol) and GrpE (found in prokaryotes and

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Introduction

Although amino-acid composition and the cellular environment have crucial roles in determining the fold of a nascent polypeptide chain, the polypeptide is often tempted by many 'illicit liaisons' before it attains its final, native fold. This is where the chaperones step in to help. Although originally thought simply to prevent these liaisons, it has become increasingly evident that chaperones can also rescue polypeptides that have strayed from the normal folding pathway, sequester misfolded proteins to reduce potentially toxic effects and facilitate the degradation of

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Fig 1 | Molecular chaperones are involved in several cellular processes in addition to facilitating folding of the nascent polypeptide chain. Under normal physiological conditions, the polypeptide chain with the help of ribosome-associated and cytosolic chaperones (C) follows a folding pathway with defined partially folded intermediates (I) that eventually lead to the native folded structure. However, if this folding pathway is in some way perturbed, for example by mutation or cellular stresses such as heat shock, then 'off-pathway' misfolded intermediates may form (I^*), which can be prone to form largely unordered aggregates. Such non-functional aggregates can be dealt with in various ways by the chaperone network, resulting in either restoration of the native structure or sequestration/degradation of the aggregated form. All these aspects of chaperone function were discussed at the meeting.

their organellar descendants) have been studied in great detail, but a more recently discovered factor, Hsp-binding protein 1 (HspBP1), is providing some surprises. Y. Shomura (Martinsried, Germany) showed that this cofactor, which is required for efficient protein folding in yeast at 37 °C, acts through a different mechanism to BAG1 and GrpE. Structural studies on HspBP1 crystallized with part of the ATPase domain of Hsp70 suggest that it acts sterically to distort two lobes of the ATPase domain, thus weakening the affinity of the chaperone for ADP. Not only does HspBP1 have a distinct mode of action, but its biological role also seems to be profoundly different from the other known NEFs (Shomura *et al.*, 2005). J. Höfheld (Bonn, Germany) showed that HspBP1 inhibits the action of the carboxyl terminus of Hsc-interacting protein (CHIP), which is a ubiquitin ligase that associates with both Hsp70 and Hsp90 and directs their client proteins to the proteasome (Alberti *et al.*, 2004). This inhibition can decrease the degradation of proteins such as the cystic fibrosis transmembrane conductance regulator (CFTR) precursor. By contrast, BAG1 stimulates CHIP action. This is supported by work from D. Cyr (Chapel Hill, NC, USA) who showed that overexpression of CHIP reduces the maturation of CFTR. He showed that

inactivation of a multisubunit E3 ubiquitin ligase that contains CHIP combined with other proteins, including Hsc70, can improve the cell-surface expression of CFTR Δ 508, the most common mutated form of CFTR in cystic fibrosis (Younger *et al.*, 2004). Höfheld also showed that another cochaperone, Hsj1, is important in directing Hsp70 clients to the proteasome, again by interaction with CHIP (Westhoff *et al.*, 2005). Hsj1 interacts with misfolded proteins—experimentally demonstrated with a huntingtin variant fused to green fluorescent protein (GFP)—and loads them onto ATP-bound Hsc70 before CHIP binding. This process may be important in attempts by the cell to defend itself against the toxic effects of misfolded or aggregated proteins.

The role of Hsp70 proteins in cancer is receiving increasing attention. G. Multhoff (Regensburg, Germany) has shown that some tumour cells have Hsp70 on their surface and a 14-mer peptide from Hsp70 can stimulate natural killer cells through the CD94 receptor. These natural killer cells can subsequently lyse Hsp90-presenting cells. The ability of natural killer cells to find such expressing cells *in vivo* may be attributable to the production of lipid vesicles with Hsp70 on their surface by the tumour cells (Gastpar *et al.*, 2005). A model in severe combined immunodeficiency (SCID) mice has been established, in which injection of CD94⁺ natural killer cells leads to a significant reduction of Hsp70-expressing tumours. A successful Phase I trial of a similar process in humans has recently been published (Krause *et al.*, 2004). R. Issels (Munich, Germany) has been studying the observed beneficial effects of hyperthermia in tumour treatment. He presented data from a melanoma model suggesting that this may be due to the stimulation of an anti-tumour response to peptides cross-presented to immunocompetent T cells by heat-induced Hsp70 proteins released from the tumour cells. M. Jäätelä (Copenhagen, Denmark) described a detailed study in which four different cytosolic Hsp70s were individually depleted using RNA interference (RNAi) in a variety of tumour- and non-tumour-derived human cell lines. Two Hsp70 proteins, Hsp70 and Hsp70-2, were shown to be required for the growth of tumorigenic cells, owing to the induction of cell-cycle arrest when these proteins were depleted. Furthermore, the effects of depleting these two proteins were different, which implies that they have distinct roles in tumorigenesis. Gene-chip analysis showed that the expression of several genes was altered in the Hsp70- and Hsp70-2-depleted cells, including many involved in the cell cycle and apoptosis. Two important genes that were upregulated by Hsp70-2 depletion in tumour, but not non-tumour, cells were macrophage inhibitory cytokine 1 (MIC1), which has several roles in the cell cycle, and p53. The expression of Hsp70-2 is also elevated in one-third of breast cancers studied (Rhode *et al.*, 2005).

Hsp90 and its cochaperones

Chaperones do not carry out their functions in protein folding unaided. Increasingly the role of the so-called cochaperones has come into focus primarily—but not solely—through genetic and biochemical studies on the molecular chaperone Hsp90 (Terasawa *et al.*, 2005; see also www.hsp90.com). As discussed by J. Buchner (Munich, Germany), there are at least eight Hsp90 cochaperones, and most of these have homologues in yeast. Hsp90 forms homodimers and has a specific set of client proteins, many of which are involved in cell-signalling pathways that are activated through the Hsp90 chaperone cycle. Cochaperones modulate this cycle by binding to the amino- or C-terminus of

Hsp90, thus affecting its intrinsic ATPase activity, which is necessary for the binding and release of its client proteins. A recent addition to the Hsp90 cochaperone family in yeast is Ppt1, which is a homologue of the human protein phosphatase 5 (PP5) protein. Buchner described recent studies from his group showing that Ppt1 is a serine/threonine protein phosphatase that acts on Hsp90. Yeast mutants carrying a deletion of *ppt1* have hyperphosphorylated Hsp90, which in turn is defective in protein activation of substrates such as the v-Src protein kinase. Although the phosphorylation site on Hsp90 is not yet known, these findings clearly show the importance of Hsp90 phosphorylation in the chaperone cycle.

D. Picard (Geneva, Switzerland) described his work on a second Hsp90 cochaperone, p23 (known in yeast as Sba1; Richter *et al*, 2004). This cochaperone modulates the stability of the complex formed between Hsp90 and its substrate. Like the Ppt1 cochaperone, Sba1 is not essential for viability in yeast. By studying the effects of p23 depletion in homozygous and heterozygous mice, however, Picard's group was able to demonstrate an important role for p23 in perinatal survival. It remains to be established whether this is a direct consequence of an effect on the Hsp90 chaperone cycle or is due to prostaglandin E2 synthase activity associated with p23.

One of the themes that linked many of the talks was the importance of chaperones in human health and disease, particularly Hsp90. In cancer cells, Hsp90 is mainly in an activated, high-affinity conformation because many of its clients are deregulated in such cells. This conformation is more sensitive to Hsp90-specific inhibitors such as geldanamycin and radicicol. Consequently, Hsp90 represents a therapeutic target for cancer and S. Pacey (Potters Bar, UK) reported on the current state of clinical trials with a geldanamycin derivative, 17-AAG (17-allylamino-17-demethoxygeldanamycin; Banerji *et al*, 2005). Now in phase II clinical trials, the studies have shown that geldanamycin derivatives could be used to treat melanoma and prostate cancers with manageable toxicity, but whether these compounds (or newer derivatives) will reach the clinic remains to be seen.

The chaperonins

The subset of chaperone proteins known as chaperonins continue to fascinate. They have an intriguing double-ring structure with each ring comprising seven or eight sub-units. The chaperonins bind to and then sequester protein-folding intermediates in the cavity in the centre of one of the rings, where folding can take place without interference from other unfolded proteins. GroEL is the archetypal bacterial chaperonin and its substrates in *Escherichia coli* have been defined by U. Hartl (Martinsried, Germany) and his colleagues by pull-down experiments and mass spectrometry analysis. Among the substrates that absolutely require GroEL for folding are several essential proteins, which explains why *E. coli* cannot tolerate deletion of *groEL*. Proteins with TIM- β/α barrel structures are particularly enriched among the substrates. The group of H. Saibil (London, UK) is using cryo-electron microscopy to analyse the structure of complexes of GroEL and its substrates. They have observed close contact between one of the helices (helix I) in the apical domain of GroEL and two different substrates, mitochondrial malate dehydrogenase and the bacteriophage coat protein gp23. The folding pathways of two other GroEL substrates (β -trypsinogen and dihydrofolate reductase) are being studied in the group of A. Horwich (New Haven, CT,

USA) using either the progressive formation of disulphides, or the progressive protection of amide nitrogens, as a probe for structure. In both cases, the expected progressive gain of structure is seen as the folding reaction proceeds—this requires the cochaperone GroES and ATP. This work brings us closer to the goal of comparing folding pathways of client proteins on and off chaperonins, which will enable us to determine whether the mechanism of chaperonins is purely one of sequestration or whether they can actually alter the pathways taken to the folded state.

Chaperonin proteins fall into two distinct phylogenetic groups: type I proteins are found in bacteria, chloroplasts and mitochondria whereas the type II proteins are found in archaea and the eukaryotic cytosol. This latter group is now receiving increasing attention. A combination of studies of protein folding in reticulocyte lysates and pull-down experiments with a glutathione-S-transferase (GST)-fusion library in yeast has led J. Freedman (Stanford, CA, USA) to propose that the number of proteins that interact with the eukaryotic chaperonin (variously known as CCT, TRiC and TCP-1) is higher than previously thought. The figure is between 5% and 8% of newly synthesized proteins and this group is enriched in essential proteins. Proteins with long stretches of β -sheet seem to be particularly likely to interact with CCT. An intriguing result was presented by P. Stirling (Burnaby, Canada), who has identified a protein that strongly interacts with CCT in its native, rather than unfolded, state. Referred to as PhLP3 (for phosducin-like protein), this protein can block the CCT-mediated folding of actin and tubulin (which is stimulated by the CCT cochaperone prefoldin). Moreover, deletion of the yeast homologue of PhLP3 can suppress effects arising from the mutation of a yeast *prefoldin* gene, implying that these two proteins may have opposite effects on the action of CCT.

Chaperones and protein (dis)aggregation: Hsp104/ClpB

When any cell is subjected to a heat or chemical stress, one of the immediate responses is the production of a range of molecular chaperones. The main role of the stress-induced chaperones is to keep proteins on their folding pathway to prevent the formation of aggregates of the misfolded and non-functional protein. Should this fail, a second class of chaperones moves in to disassemble these aggregates and reactivates the proteins by refolding them into their soluble, native conformation (Fig 1). The conserved ClpB/Hsp104 chaperone has such disaggregation activity. Studies in *E. coli* using a GFP-based reporter protein (K. Liberek, Gdansk, Poland) have shown that the reactivation of the aggregated GFP substrate requires cooperation between ClpB/Hsp104 and Hsp70 (DnaK) and its cochaperones DnaJ and GrpE (Zietkiewicz *et al*, 2004). DnaK/J/GrpE initiate the reactivation step, probably by reducing the size of the aggregates through remodelling. Reactivation of these smaller aggregates to generate soluble refolded proteins requires ClpB/Hsp104 for completion, but as shown by Liberek, the small heat shock proteins IbpA and IbpB also have an important role by stabilizing the aggregates in a refolding-competent state. This pathway of disaggregation of thermally denatured proteins is also found in eukaryotes, in which it operates in the cytosol and in mitochondria.

Inappropriate protein aggregation is also associated with several neurodegenerative diseases, the so-called 'protein-misfolding diseases' typified by Huntington's disease (aggregation of the polyglutamine-rich huntingtin protein) and Alzheimer's disease (aggregation of the A β protein; Muchowski &

Wacker, 2005). These aggregates may be intra- or, more usually, extra-cellular and seem to have evaded the cytoplasmic chaperone machinery and its associated quality control. Two groups at the meeting (R. Morimoto, Evanston, IL, USA and E. Nollen, Utrecht, The Netherlands) reported how genome-wide screens—using RNAi and chemical mutagenesis—in *Caenorhabditis elegans* led to the identification of genes that either enhanced or suppressed the aggregation of proteins associated with human ‘protein-misfolding diseases’ (Nollen *et al.*, 2004). The RNAi screens revealed not only genes that encode molecular chaperones, particularly components of the CCT complex, but also those that encode proteins involved in protein degradation, RNA and protein synthesis and vesicular transport. The chemical mutagenesis screen also revealed some surprises; for example, Morimoto reported that a mutation in a neuronal-specific transcription factor resulted in increased aggregation of polyglutamine in the muscle. These screens also identified the importance of proteins that negatively regulate the heat shock response, some in a tissue-specific manner. Nollen reported that the mutation of some of the genes identified by her group resulted in a 90% reduction in aggregation; however, the genes identified in a genome-wide RNAi screen that resulted in increased protein aggregation were also essential for viability. The identity of these genes is now under investigation.

Another interesting finding reported by Morimoto was that over-expression of the *hsf-1* gene, which encodes the transcription factor heat shock-factor protein 1 that regulates the expression of many heat shock-inducible chaperones and other proteins involved in quality control, suppressed polyglutamine aggregation. This study led his group to discover a link between the stress response and cell longevity, as reduced levels of HSF-1 in neurons and muscle cells suppressed the longevity of *C. elegans*.

A different approach to determining the cellular factors that control protein aggregation in cultured mammalian cells was reported by A. Bertolotti (Paris, France). She found that when a polyglutamine-rich protein that aggregates in the nucleus or cytoplasm was targeted to a different cellular compartment—in this case either the endoplasmic reticulum or mitochondria—the protein no longer aggregated (Rousseau *et al.*, 2004). When the protein was transported back to the cytoplasm, it again formed aggregates. This indicates that either the endoplasmic reticulum (and most probably mitochondria) contain one or more anti-aggregation factors that presumably are compartment-specific molecular chaperones—or conversely, pro-aggregation factors exist in the cytoplasm and nucleus. By using yeast to follow up these findings, it should be possible to define the nature of these factors using a genetic screen similar to the studies reported at this meeting with *C. elegans*.

Prions represent an unusual class of transmissible protein aggregates and are usually thought to be amyloid in nature. Two speakers (R. Melki, Paris, France, and M. Tuite, Canterbury, UK) described recent work on prions in yeast and the roles of molecular chaperones in the formation and propagation of two of these prions, Ure2/[URE3] and Sup35/[PSI⁺]. Melki described the work of his group on the assembly of full-length Ure2 and challenged the idea that it forms amyloid fibrils under ‘physiological conditions’ *in vitro* as they seem to lack the cross- β core typical of an amyloid. Tuite described *in vivo* studies showing that the disaggregation of Sup35 prion aggregates by the molecular chaperone Hsp104 is essential for the propagation of the [PSI⁺] prion.

Modifying the levels of Hsp104 by gene knockout or over-expression of the *HSP104* gene blocks propagation but by apparently different mechanisms. The findings he described correlate in part with recently described *in vitro* studies (Shorter & Lindquist, 2004) but also raise the possibility that Hsp104 may control the transmission of prions to daughter cells.

Chaperones and protein degradation

Links between Hsp70 and proteasomal degradation have been described above, and several speakers discussed interactions between different chaperones and degradation by the proteasome. C. Garrido (Dijon, France) showed how the degradation of the cyclin-dependent kinase inhibitor p27Kip1 is enhanced by the small heat shock protein Hsp27, which binds to ubiquitylated proteins and stimulates their further ubiquitylation. For the cell to progress through the cell cycle, p27Kip1 needs to be degraded, so the action of Hsp27 on this protein may aid quiescent cells to re-enter the cell cycle under stressful conditions. A further link between the proteasomal pathway and molecular chaperones was described by M. Zyllicz (Warsaw, Poland), who also chaired the meeting. He showed that the product of the *mdm2* oncogene, which has E3 ubiquitin ligase activity, seems to have chaperone activity. It can reactivate heat-denatured luciferase and block the aggregation of citrate synthase, which are both classic diagnostic assays for chaperone proteins. Among other properties, Mdm2 also seems to act synergistically with Hsp90 in maintaining the activity of p53 and this activity is independent of its ubiquitin ligase activity. This implies that Mdm2 can either assist the degradation of p53 through the proteasome, or activate p53 together with Hsp90.

Concluding remarks

When the field of molecular chaperone biology began to emerge as a separate discipline about 15 years ago, the suggestion that understanding chaperone activity might lead to advances in therapeutic strategies against cancer or neurodegenerative diseases would have been dismissed as fanciful. However, research into the most basic properties of these ubiquitous proteins is now informing clinical and pharmaceutical developments in these areas. Meetings such as these, that bring together researchers both in pure chaperone biology and in the study of their numerous applications, are likely to cause this trend to accelerate.

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