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Molecular chaperones, evolution and medicine

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Abstract

Protein folding has numerous steps, which need assistance in vivo. Molecular chaperones are required for many proteins to fold, or re-fold into native structures forming an ancient, primary system for 'intracellular self-defense'. Molecular chaperones participate in the organization of the cytoarchitecture, were necessary for the development of modern enzymes and—by stabilizing the genome—for the development of the first stable cells. They have a profound importance in medical practice. Chaperone induction provides cytoprotection in various pathological conditions, while chaperone inhibition can be an efficient tool to fight against cancer. Chaperones are inefficient enzymes and have low-affinity interactions, therefore their assays require unusual methods, which will be summarized in the concluding part of the paper.

Keywords: Heat shock proteins; Low affinity; Molecular chaperones; Protein folding; Stress proteins

1. Chaperones and protein folding

Protein folding is characterized by three major steps in vitro (Fig. 1) [1–6]. Under in vitro conditions in the *first few milliseconds* most of the secondary structure is already formed. In most cases folding starts with the formation of alpha-helices, since here the participation of adjacent amino acids is required. Beta-sheet formation establishes H-bonds between amino acids, which are far from each other in the primary sequence, therefore a greater decrease of entropy occurs than in the formation of alpha-helices. In the end of this first step, the hydrophobic segments are segregated by the surrounding water and they form a hydrophobic core of an intermedier, which is often called as the 'molten globule'. If the protein is larger than 30 kDa, this intermedier can be fairly stable.

The partially folded state of *molten globule*s can be characterized by a developed secondary structure, which is mostly un-organized showing almost no tertiary structure [3–5]. Molten globules still have large unburied hydrophobic surfaces, therefore are subjects of extensive aggregation. The volume of molten globules, however, is almost as small as that of the final, folded protein.

The *last steps* of protein folding are the slow, rate-limiting steps [1,2]. Here the inner, hydrophobic core of the protein is re-organized [6]. Parallel with this, unique, high-energy bonds are formed, such as disulfide bridges, ion-pairs, and the isomerization of proline *cis*/*trans* peptide bonds occurs. The free energy gain of these processes enables the formation of local, thermodynamically unstable, 'high-energy' protein structures, which are stabilized by

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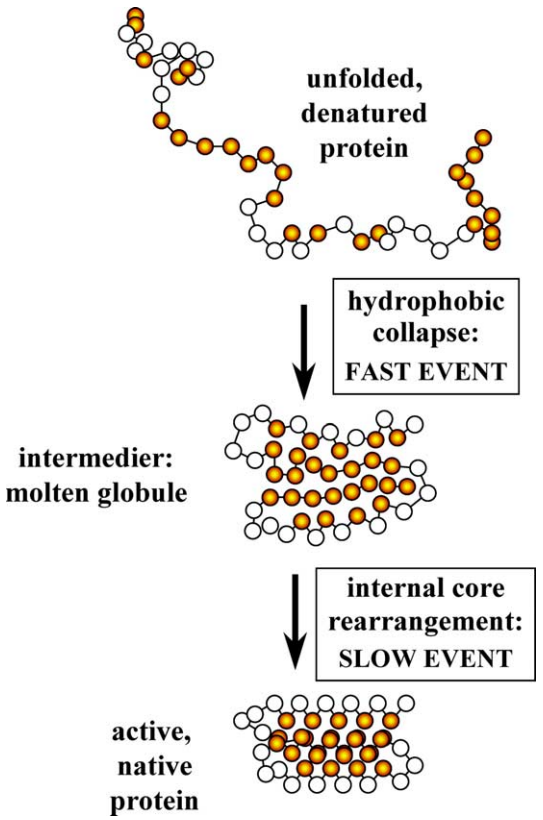

Fig. 1. Major steps of protein folding in vitro. Adapted from Csermely [6].

thermodynamically favorable conformation of the rest (bulk) of the protein. These high-energy segments of proteins can stabilize themselves by forming complexes with another molecule, thus they often serve as active centers of enzymes or as contact surfaces between various proteins involved, e.g. in signal transduction.

Protein folding is not a straightforward process. Dead-end streets, reverse reactions, futile cycles are all characteristic to it. A minor amount of fully folded, native protein always co-exists with various forms of molten globules and with traces of remaining unfolded specii. This unordered flow of events needs a lot of help. Aggregation of unfolded proteins and of molten globules is a great danger, which would drive the majority of folding intermediates to a nonproductive side-reaction, much before reaching their fully folded, competent state. Molecular chaperones serve to prevent this. They recognize and cover hydrophobic surfaces successfully competing with the aggregation process. Moreover, molecular chaperones can leave their complex with misfolded proteins, utilizing the energy of ATP-hydrolysis-driven conformational changes.

Unaided protein folding often leads to folding traps. Steric hindrance (and most probably the lack of water in the inner, hydrophobic core of the protein) many times prevent the rearrangement of the hydrophobic core. Unfolding of the core is aided by periodic pulling and water-percolation [6] and the 'traditional' molecular chaperones may also provide better circumstances for ion-pair formation and for the establishment of high-energy protein segments. Disulfide bridge formation and proline *cis/trans* isomerization are promoted by protein disulfide isomerases and by peptidyl-prolyl-*cis/trans* isomerases, respectively [7,8].

Nascent proteins have the unique situation that they have to fold, when they are not even ready yet. The first protein segment, which leaves the ribosome surely has a different energy minimum, than the whole protein. In many cases, in vivo protein folding has to be delayed. Molecular chaperones are attached to the ribosomes 'waiting' for the nascent protein chain. When it appears, the chaperones 'sit on it' preventing premature protein folding before the rest of the protein is synthesized [9,10].

Chaperones also direct proteins inside the cell. Pores of the mitochondria or of the endoplasmic reticulum are too small to accommodate fully folded, globular proteins. Proteins have to unfold to get through, and to re-fold in the lumen of the organelle [11].

Molecular chaperones not only help, but also destroy. Some incorrectly folded proteins—maybe those which have lost not only their tertiary, but also secondary structure leaving their peptide bonds accessible—are presented to the lysosomal protein degradation [12] or to the extralysosomal proteasome [13]. In case of massive protein damage, when the amount of degradable proteins exceeds the capacity of the intracellular proteolytic systems, chaperones help to form inclusion bodies to segregate damaged proteins [14].

2. Nonconventional roles for chaperones: organization of the cytoarchitecture

Heat shock proteins are regarded as molecular chaperones, thus their major cellular function is considered to be established. However, most of the protein folding experiments are conducted in an in vitro environment. When protein folding is studied in vitro, the experimenter has to use rather diluted conditions to prevent unwanted aggregation. Dilution also helps to make the kinetical analysis easier, and spares precious research materials. On the contrary to these usual experimental conditions, the cellular environment is crowded [15]. Molecular crowding promotes protein aggregation thus calls for an enhanced need of chaperone action. On the other hand, bona fide chaperones are not the only cellular solutions for aggregation-protection. Several 'innocent bystanders', such as tubulin [16] or even small molecules (lipids, other amphiphyles, sugars, a class of compounds called as chemical chaperones [17]) may assist folding and prevent aggregation albeit at much higher concentrations than the efficient concentration of heat shock, or other stress-induced proteins. Though we have several important lines of evidence, which undoubtedly show the necessity of chaperones in folding of numerous protein kinases, receptors, actin, tubulin, etc. [18] we do not really know, how big is the segment of the life of an ordinary chaperone, when it 'chaperones' unfolded or misfolded proteins in eukaryotic cells.

To make it clear, with the argumentation above we do not want to question the importance of chaperones in folding-assistance. Nevertheless, we would like to stress, that there is enough room to think about other important functions of chaperones related to, but not equal with their participation in protein folding. One of these possibilities is, that peptide-binding chaperones are the 'dustmen' of the cells. The proteasomal apparatus is most probably linked with oligo- and dipeptidases and therefore the 'leaking' peptide-endproducts of proteasomal degradation [19] are usually cleaved further into single amino acids. However, the coupled protein/peptide degradation can leak especially under stressed conditions, like in oxidative stress. Released peptide segments may often contain elements of important binding sites and thus may efficiently interfere with signaling and, metabolic processes. If this happened at a massive scale, this would be a disaster for the cell. Peptides need to be eliminated, and safeguarding mechanisms must exist to correct the occasional 'sloppiness' of degradative processes. Chaperones are excellent candidates for this purpose and their role in collection of 'peptide-rubbish' must be considered besides their well-established function in peptide presentation for the immune system [20].

As yet another important, and nonconventional aspect of chaperone action (from the many more possible) lies in their incredible stickiness. Chaperones often form dimers, and tend to associate to tetra-, hexa-, octamers and to even higher oligomers [21–23]. Oligomerization usually affects only a few percent of the total protein; but addition of divalent cations, certain nucleotides, heat treatment enhances oligomer formation. It is important to note that oligomerization studies were usually performed under 'normal', in vitro experimental conditions, using a few μg/ml of purified chaperone. The in vivo concentration of chaperones is estimated to be around a hundred-, or thousand-fold higher. This may significantly enhance the in vivo oligomerization tendencies of these proteins. Oligomer formation of chaperones might be further promoted by the large excluded volume effect of the 'molecularly crowded' cytoplasm [15].

Different chaperones also associate with each other. The Hsp90-organized foldosome may contain almost a dozen independent chaperones, or co-chaperones. The stoichiometry and affinity of these associations dynamically varies, and the variations are affected by the folding state of the actual target (or targets) which associate with these extensive folding machinery [23].

Besides binding to themselves, to their sibling-chaperones, and to their targets, many chaperones bind to actin filaments, tubulin, and other cellular filamentous structures, such as intermediate filaments. There is a chaperone complex associated with the centrosome [24] and several chaperones, especially Hsp90 were considered to be involved in the direction of cytoplasmic traffic [25].

The above model describing chaperones as a highly dynamic 'appendix' of various, and often quite poorly identifiable, cytoplasmic filamentous structures is reminiscent of the early view [26,27]

about the microtrabecular lattice of the cytoplasm. Although later studies efficiently questioned the validity of the original electronmicroscopical evidence of the microtrabeculae, pointing out many possibilities for artifact formation during sample preparation, several indirect evidence, such as diffusion anomalies support the existence of a cytoplasmic mesh-like structure [28–31]. The major cytoplasmic chaperones (Hsp90, TCP1/Hsp60 and their associated proteins) may well form a part of this network in cells [32].

Our experiments showing the acceleration of the efflux of cytoplasmic constituents after the inhibition of the major cytoplasmic chaperone, Hsp90, both in case of Jurkat cells [33] and erythrocytes [34] (Fig. 2) suggest the involvement of the 90 kDa molecular chaperone, Hsp90 in the maintenance of the cytoarchitecture. Interestingly, we did not see an acceleration of cytoplasmic release in *E. coli* [33], which is in agreement with the lower level of cytoplasmic organization of prokaryotes compared to eukaryotes. We cannot ascertain at the moment that the faster release of cytoplasmic proteins after the disruption of Hsp90 complexes by Hsp90 inhibitors [33,34] or anti-Hsp90 ribozyme treatment [33] is a consequence of a disrupted cytoplasmic meshwork or shows the involvement of Hsp90 in the stabilization of the traditional cytoskeleton. However, our ongoing experiments may show the reorganization of Hsp90 in the cytoplasm after these treatments as well as changes in the intracellular diffusion rates.

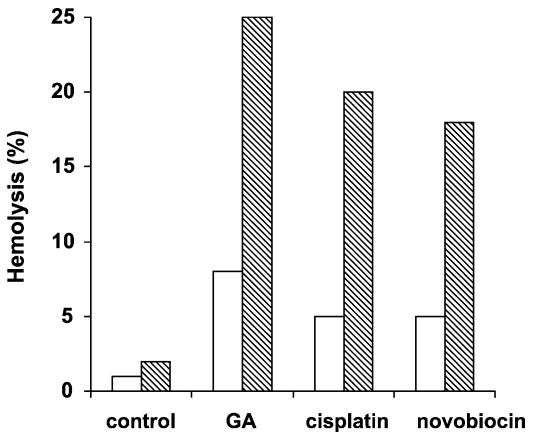

Fig. 2. Hsp90 inhibition induces an accelerated hemolysis. Hemolysis was induced by a 15 min (open bars) or 30 min (filled bars) treatment of 0.002 % of the nonionic detergent, Brij58 at room temperature. Mouse red blood cells were pre-incubated with 1 μM geldanamycin, 100 μM cisplatin or 1 mM novobiocin, inhibitors of Hsp90 at its N-terminus, C-terminus or both termini, respectively [60]. Hemolysis was measured by monitoring the amount of released hemoglobin after centrifugation of lysed cells at 528 nm. Data are representatives of three independent experiments. (The results were published in an abstract form in Ref. [34].)

3. Chaperones and evolution

Chaperones are ancient protein structures, which were highly conserved throughout all the known parts of evolution, and are repeatedly emerging as parts of the minimal genomes of various organisms suggesting their presence in the hypothetical Last Universal Common Ancestor (LUCA) [35]. The increasing size of constituent proteins (a necessity for modern enzyme action, where conformational changes make induced-fit, and allosteric regulation possible) caused more and more folding traps. To prevent this, and to help de novo protein folding an increase of chaperone capacity was probably needed [36,37]. According to the above assumptions, chaperone capacity was likely to grow in parallel with cellular complexity of primordial cells.

As we described in Section 2, chaperones help the organization of the cytoarchitecture [32–34]. Chaperones also stabilize lipid membranes [38]. Both effects may have helped the occurrence of the first stable ancestors of modern cells by worsening the chances for ‘membrane-leaks’, which would help the exchange of various cell constituents (including genetically coding material) between neighboring organisms.

In the last few years, several experiments were published, which suggested that chaperones behave as ‘buffers of evolutionary changes’. Chaperones may correct the conformational changes caused by various mutations, and make the genetical changes phenotypically silent in various organisms studied [39–42]. Thus chaperones were probably not only contributors to the emerging cellular organization of primordial cells, but in parallel, they also increased genetical stability by buffering the phenotypical consequences of mutational events.

After a large stress, the suddenly increased amount of damaged proteins may cause a ‘chaper-

one overload', and may prevent the conformational repair of misfolded mutants. Therefore, many previously hidden genotypical changes may appear in the phenotype resulting in a 'boom' of genetical variations in the whole population. This may help the selection of a beneficial change, which, in turn, may help the adaptation of the population to changed environmental conditions. However, most of the exposed mutations are disadvantageous, and tend to disappear from the population by natural selection.

Changes in living conditions and the significantly better medical care throughout life in the last 150 years have significantly reduced the occurrence of large physiological stresses that would normally result in significant intracellular proteotoxicity. There is little chaperone overload during reproductive years in the present times. Even major stressful events, such as critical infections and extreme and unexpected changes in the environment, etc. that do cause a massive chaperone overload, can be mitigated by improved medical care, thus saving lives that would otherwise have been lost. Thus, a larger proportion of people harboring deleterious mutations probably survive today and transmit their genes to later generations. Thus improved medical care may have led to a rise in phenotypically silent mutations in the human genome. As a consequence we may be carrying more and more chaperone-buffered, silent mutations from generation to generation [43].

The chance of the phenotypic manifestation of these mutations becomes especially large in aged subjects, where protein damage is abundant, and both chaperone induction and chaperone function are impaired [44]. Here the background of misfolded proteins increases and by competition prevents the chaperone-mediated buffering of silent mutations. Phenotypically exposed mutations may contribute to a more abundant manifestation of multigene-diseases, such as atherosclerosis, autoimmune-type diseases, cancer, diabetes, hypertensive cardiovascular disease and several psychiatric illnesses (Alzheimer disease, schizophrenia, etc.). The chaperone overload hypothesis emphasizes the need for efficient ways to enhance chaperone-capacity in aging subjects [43].

4. Beneficial effects of chaperone induction

Molecular chaperones are responsible for the 'conformational homeostasis' of cellular proteins. When the homeostasis of the host organism is perturbed, an increased capacity of the 'chaperone machines' is highly advantageous. Many of the perturbations (such as alcohol, other poisons, sunburn, anxiety, etc.) may induce the synthesis of these chaperone proteins per se, but in case of bacterial and viral infections the developing fever also helps this process. Ischemia and the consecutive oxidative damage of reperfusion are also common environmental perturbations in higher organisms. Since Currie et al. [45] have shown that the induction of molecular chaperones, most notably Hsp70, may prevent the cardiac muscle from the damage of both ischemia and reperfusion, molecular chaperones are actively investigated as possible tools in the treatment of heart attack or stroke. Their protective role is also used in organ transplantation, where a prior heat treatment induces a more efficient organ-survival and diminishes the occurrence of rejection by the host organism. Several common drugs, such as aspirin [46] promote the induction of the chaperone defense system, however, recently a specific chaperone co-inducer drug family [47,48] interacting with the heat shock factor [49] has been also described.

5. Advantages of chaperone inhibition

The above examples show the advantages of chaperone induction. Chaperones protect our cells—chaperones are good. Not always. When chaperones protect our malignant cells—they are not really beneficial. Still, chaperone inhibition as a pharmacological tool to prevent cancer development seems to be a wild idea. However, if we consider that chaperones are necessary for the folding of numerous cyclin-dependent kinases, which promote the cell cycle [50], and some of the chaperone inhibitors are selectively enriched in tumor cells [51], we begin to believe that chaperone inhibition might be a valid pharmacological intervention against tumors. Indeed, many chaperone inhibitors are currently in clinical trials against various forms of cancer [52].

Since the 90 kDa molecular chaperone (Hsp90) has the most specific and most cell-permeable inhibitors, and since this chaperone is the center of the kinase-related chaperone machinery, in most cases chaperone-based inhibition is achieved by using Hsp90 inhibitors. The first Hsp90 inhibitor drug was geldanamycin, a natural product isolated from *Streptomyces hygroscopicus*. Though the antitumor effects of geldanamycin were initially thought to be due to specific tyrosine kinase inhibition, later studies revealed that the antitumor potential relies on depletion of oncogenic protein kinases via the proteasome [53]. The major regulatory signaling proteins, which are affected by geldanamycin, include the proto-oncogene kinases ErbB2, EGF, v-Src, Raf-1 and Cdk4 [50].

Radicicol, another Hsp90 inhibitor [54], is a macrocyclic antibiotic isolated from *Monosporium bonorden*. However, radicicol lacks antitumor activity in vivo in experimental models because of its instability. The oxime derivatives of radicicol [55] exhibit antitumor activity in vivo as well as in vitro, hence serve as good anticancer drug candidates. Radicicol binds to the N-terminal domain of Hsp90 with much higher affinity than the structurally different drug, geldanamycin [56]. Moreover, radicicol reduces hypoxia-induced VEGF expression, which is an efficient way to decrease hypoxia-induced angiogenesis [57]. As a recent development, PU3, a purine-based Hsp90 inhibitor was designed using X-ray crystallographic data [58]. PU3 behaves like geldanamycin in inhibiting Hsp90 client protein degradation, and possesses a robust antitumor potential [58].

Recently it was shown that Hsp90 contains a second nucleotide binding site at the C-terminal domain [59–61]. Nucleotide binding to this site can be inhibited by the commonly used chemotherapeutic agent, cisplatin [60], which displays a rather selective binding to Hsp90 among proteins [62]. Inhibition of the C-terminal site of Hsp90 by cisplatin results in the differential inhibition of Hsp90-assisted protein folding: that of Raf kinase remains unaffected, however, folding of luciferase is strongly inhibited under these conditions [60]. The C-terminal nucleotide binding site displays a markedly different nucleotide specificity if compared to that of the N-terminal site [63], where all the known inhibitors (geldanamycin, radicicol and PU3) bind. It is the task of the future to find truly selective and high affinity inhibitors of the C-terminal Hsp90 nucleotide binding site.

6. Assays for chaperone action

Chaperone induction can be monitored by assessing the mRNA or protein levels. However, the paramount importance of molecular chaperones in medicine necessitates the measurement of their activity in patients. It is rather likely that the 'free chaperone capacity' will be a common marker of health in the near future. Unfortunately, until recently we did not have easy methods to determine chaperone-related activities in whole cellular homogenates. However, recent progress in the biochemistry of molecular chaperones enables us to construct and try such methods.

Chaperone activity can be assessed by measuring the chaperone-induced prevention of protein aggregation [64]. This is a measure of passive chaperone function which does not require ATP. The active, ATP-dependent assistance in the refolding of misfolded proteins may be assessed using several test systems such as the luciferase-renaturation assay of whole cell homogenates [64]. To check the chaperone activity of purified chaperones and chaperone-complexes (immune precipitates) several other assays, such as the citrate synthase-assay, are also available [64].

Autophosphorylation in the presence of Ca-ATP is a common feature of almost all molecular chaperones [23]. Since most of protein kinases cannot utilize Ca-ATP, phosphorylation of cellular proteins in the presence of Ca-ATP gives a surprisingly clear pattern. As an example, we have shown earlier, that in streptozotocin-diabetic rats the phosphorylation of the 94 kDa glucose-regulated protein, Grp94, is diminished. Insulin-treatment reversed the effect [65].

Unfortunately, the *ATPase* reaction is not so specific, than the autophosphorylation. Therefore, specific measurement of chaperone/ATPases may only be accomplished by using specific inhibitors of ATPase activity, such as geldanamycin for Hsp90 [23]. Moreover, in many cases the chaperone ATPase activity is too small for efficient measurements.

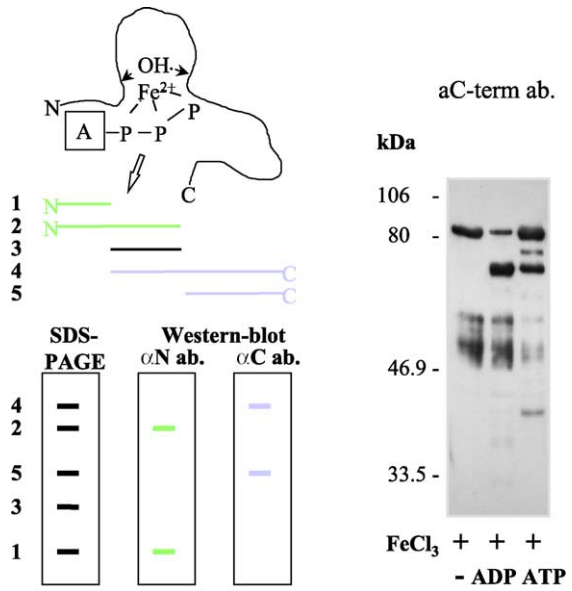

Fig. 3. Nucleotide affinity cleavage: an efficient method to analyze nucleotide binding of molecular chaperones. The Fe-ATP (Fe-nucleotide) complex at the nucleotide-binding site induces a local Fenton reaction producing hydroxyl radicals. These reactive compounds cleave the protein (in this case the 90 kDa heat shock protein, Hsp90) just in the vicinity of the binding site producing a remarkably clean cleavage pattern, which can be detected by antibodies against the N- or C-terminus of the protein (see more details in Soti et al. [60]).

Another efficient method to test the integrity of molecular chaperones is the nucleotide affinity cleavage assay, which has been recently applied to this class of nucleotide binding proteins [60]. This assay is good to detect the ability of the nucleotide binding site to bind the nucleotide, thus to be able to assess the ability of the given chaperone to perform the ATP-dependent, active refolding of various proteins (Fig. 3).

7. Summary and perspectives

In recent years we have learned a lot about the molecular mechanism of protein folding in vitro, and some important features were also revealed of the in vivo formation and repair of protein structure. Many aspects of the molecular mechanism of chaperone action were cleared, and we also recognized the importance of these proteins in the clinical practice. Chaperones participate in the maintenance of the cytoarchitecture and played a prominent role during various important steps of evolution. Both their induction and inhibition have grown to important pharmacological targets and several methods have been established to assess their function both in vitro and in vivo. We hope that with this short review we may increase the courage of some fellow scientists to enter this difficult, but very promising path of multidisciplinary research.

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References

[1] P.S. Kim, R.L. Baldwin, Annu. Rev. Biochem. 59 (1990) 631.
[2] C.R. Matthews, Annu. Rev. Biochem. 62 (1993) 653.
[3] C.M. Dobson, P.A. Evans, S.E. Radford, Trends Biochem. Sci. 19 (1994) 31.
[4] K. Kuwajima, Proteins 6 (1989) 87.
[5] O.B. Ptitsyn, FEBS Lett. 285 (1991) 176.
[6] P. Csermely, BioEssays 21 (1999) 959.
[7] S. Raina, D. Missiakas, Annu. Rev. Microbiol. 51 (1997) 179.
[8] G.S. Hamilton, J.P. Steiner, J. Med. Chem. 41 (1998) 5119.
[9] R.P. Beckmann, L.A. Mizzen, W.J. Welch, Science 248 (1990) 850.
[10] J. Frydman, E. Nimmesgern, K. Ohtsuka, F.U. Hartl, Nature 370 (1994) 111.
[11] W.J. Chirico, M.G. Waters, G. Blobel, Nature 332 (1988) 805.
[12] H.-L. Chiang, S.R. Terlecky, C.P. Plant, J.F. Dice, Science 246 (1989) 382.
[13] D. Voges, P. Zwickl, W. Baumeister, Annu. Rev. Biochem. 68 (1999) 1015.
[14] R.J. Mayer, J. Arnold, L. Laszlo, M. Landon, J. Lowe, Biochim. Biophys. Acta 1089 (1991) 141.
[15] S.B. Zimmerman, A.P. Minton, Annu. Rev. Biophys. Biomol. Struct. 22 (1993) 27.

[16] S. Guha, T.K. Manna, K.P. Das, B. Bhattacharya, J. Biol. Chem. 273 (1998) 30077.
[17] W.J. Welch, C.R. Brown, Cell Stress Chaperones 1 (1996) 109.
[18] F.-U. Hartl, Nature 381 (1996) 571.
[19] A.F. Kisselev, T.N. Akopian, A.L. Goldberg, J. Biol. Chem. 273 (1998) 1982.
[20] P.K. Srivastava, A. Menoret, S. Basu, R.J. Binder, K.L. McQuade, Immunity 8 (1998) 657.
[21] N. Benaroudj, F. Triniolles, M.M. Ladjimi, J. Biol. Chem. 271 (1996) 18471.
[22] J.D. Trent, H.K. Kagawa, T. Yaoi, E. Olle, N.J. Zaluzec, Proc. Natl Acad. Sci. USA 94 (1997) 5383.
[23] P. Csermely, T. Schnaider, Cs. Soti, Z. Prohászka, G. Nardai, Pharmacol. Ther. 79 (1998) 129.
[24] W.C. Wigley, R.P. Fabunmi, M.G. Lee, C.R. Marino, S. Muallem, G.N. DeMartino, P.J. Thomas, J. Cell Biol. 145 (1999) 481.
[25] W.B. Pratt, A.M. Silverstein, M.D. Galigniana, Cell. Signal. 11 (1999) 839.
[26] J.J. Wolosewick, K.R. Porter, J. Cell Biol. 82 (1979) 114.
[27] M. Schliwa, J. van Blerkom, K.R. Porter, Proc. Natl Acad. Sci. USA 78 (1981) 4329.
[28] J.S. Clegg, Am. J. Physiol. 246 (1984) R133.
[29] K. Jacobson, J. Wojcieszyn, Proc. Natl Acad. Sci. USA 81 (1984) 6747.
[30] K. Luby-Phelps, F. Lanni, D.L. Taylor, Annu. Rev. Biophys. Biophys. Chem. 17 (1988) 369.
[31] A.S. Verkman, Trends Biochem. Sci. 27 (2002) 27.
[32] P. Csermely, News Physiol. Sci. 15 (2001) 123.
[33] A.S. Sreedhar, K. Mihály, B. Pató, T. Schnaider, A. Steták, K. Kis-Petik, J. Fidy, T. Simonics, A. Maráz, P. Csermely, J. Biol. Chem. 278 (2003).
[34] B. Pato, K. Mihaly, P. Csermely, Eur. J. Biochem. 268 (2001) S107.
[35] E.V. Koonin, M.Y. Galperin, Sequence, evolution, function, Computational Approaches in Comparative Genomics, Kluwer, Dordrecht, 2003.
[36] P. Csermely, Trends Biochem. Sci. 22 (1997) 147.
[37] S. Walter, J. Buchner, Angew. Chem. 41 (2002) 1098.
[38] Z. Torok, I. Horvath, P. Goloubinoff, E. Kovacs, A. Glatz, G. Balogh, L. Vigh, Proc. Natl Acad. Sci. USA 94 (1997) 2192.
[39] S.L. Rutherford, S. Lindquist, Nature 396 (1998) 336.
[40] S.P. Roberts, M. Feder, Oecologia 121 (1999) 323.
[41] M.A. Fares, M.X. Ruiz-Gonzalez, A. Moya, S.F. Elena, E. Barrio, Nature 417 (2002) 398.
[42] C. Queitsch, T.A. Sangster, S. Lindquist, Nature 417 (2002) 618.
[43] P. Csermely, Trends Genet. 17 (2001) 701.
[44] G. Nardai, P. Csermely, Cs. Soti, Exp. Gerontol. 37 (2002) 1255.
[45] R.W. Currie, M. Karmazyn, M. Kolc, K. Mailer, Circulation Res. 63 (1988) 543.
[46] D.A. Jurivich, L. Sistonen, R.A. Kroes, R.I. Morimoto, Science 255 (1992) 1243.
[47] L. Vigh, P. Literati Nagy, I. Horvath, Zs. Torok, G. Balogh, A. Glatz, E. Kovacs, I. Boros, P. Ferdinandy, B. Farkas, L. Jaszlits, A. Jednakovits, L. Koranyi, B. Maresca, Nat. Med. 3 (1997) 1150.
[48] Zs. Török, N.M. Tsvetkova, G. Balogh, I. Horváth, E. Nagy, Z. Pénzes, J. Hargitai, O. Bensaude, P. Csermely, J.H. Crowe, B. Maresca, L. Vígh, Proc. Natl Acad. Sci. USA 100 (2003) 3131.
[49] J. Hargitai, H. Lewis, I. Boros, T. Rácz, A. Fiser, I. Kurucz, I. Benjamin, Z. Pénzes, L. Vígh, P. Csermely, D.S. Latchman, Biochem. Biophys. Res. Commun. 307 (2003) 689.
[50] W.B. Pratt, D.O. Toft, Exp. Biol. Med. 228 (2003) 111.
[51] G. Chiosis, H. Huezo, N. Rosen, E. Mimnaugh, L. Whitesell, L. Neckers, Mol. Cancer Ther. 2 (2003) 123.
[52] L. Neckers, Trends Mol. Med. 8 (2002) S55.
[53] L. Whitesell, E.G. Mimnaugh, B. De Costa, C.E. Myers, L.M. Neckers, Proc. Natl Acad. Sci. USA 91 (1994) 8324.
[54] S. Soga, T. Kozawa, H. Narumi, S. Akinaga, K. Irie, K. Matsumoto, S.V. Sharma, H. Nakano, T. Mizukami, M. Hara, J. Biol. Chem. 273 (1998) 822.
[55] T. Agatsuma, H. Ogawa, K. Akasaka, A. Asai, Y. Yamashita, T. Mizukami, S. Akinaga, Y. Saitoh, Bioorg. Med. Chem. 10 (2002) 3445.
[56] S.M. Roe, C. Prodromou, R. O'Brien, J.E. Ladbury, P.W. Piper, L.H. Pearl, J. Med. Chem. 42 (1999) 260.
[57] E. Hur, H.H. Kim, S.M. Choi, J.H. Kim, S. Yim, H.J. Kwon, Y. Choi, D.K. Kim, M.O. Lee, H. Park, Mol. Pharmacol. 62 (2002) 975.
[58] G. Chiosis, M.N. Timaul, B. Lucas, P.N. Munster, F.F. Zheng, L. Sepp-Lorenzino, N. Rosen, Chem. Biol. 8 (2001) 289.
[59] M.G. Marcu, A. Chadli, I. Bouhouche, M.G. Catelli, L.M. Neckers, J. Biol. Chem. 275 (2000) 37181.
[60] Cs. Soti, A. Racz, P. Csermely, J. Biol. Chem. 277 (2002) 7066.
[61] C. Garnier, D. Lafitte, P.O. Tsvetkov, P. Barbier, J. Leclerc-Devin, J.M. Millot, C. Briand, A.A. Makarov, M.G. Catelli, V. Peyrot, J. Biol. Chem. 277 (2002) 12208.
[62] H. Itoh, M. Ogura, A. Komatsuda, H. Wakui, A.B. Miura, Y. Tashima, J. Biochem. 343 (1999) 697.
[63] Cs. Sõti, A. Vermes, T.A. Haystead, P. Csermely, Eur. J. Biochem. 270 (2003) 2421.
[64] B.C. Freeman, A. Michels, J. Song, H.H. Kampinga, R.I. Morimoto, Meth. Mol. Biol. 88 (2001) 393.
[65] P. Csermely, Cell Biol. Int. 18 (1994) 566.