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Minireview

Type I chaperonins: not all are created equal

Galit Levy-Rimler, Rachel E. Bell, Nir Ben-Tal, Abdussalam Azem*

Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69778, Israel

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Abstract **Type I chaperonins play an essential role in the folding of newly translated and stress-denatured proteins in eubacteria, mitochondria and chloroplasts. Since their discovery, the bacterial chaperonins have provided an excellent model system for investigating the mechanism by which chaperonins mediate protein folding. Due to the high conservation of the primary sequence among Type I chaperonins, it is generally accepted that organellar chaperonins function similar to the bacterial ones. However, recent studies indicate that the chloroplast and mitochondrial chaperonins possess unique structural and functional properties that distinguish them from their bacterial homologs. This review focuses on the unique properties of organellar chaperonins.** **© 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Chaperonin; cpn60; cpn10; GroEL; GroES; Type I chaperonin

1. Introduction

Chaperonin proteins are indispensable for the proper functioning of all prokaryotic and eukaryotic organisms. Their primary role is to mediate the folding of newly translated, imported and stress-denatured proteins [1]. Although, a number of studies have also implicated chaperonins in processes that occur in membranes, their precise involvement is not well understood [2,3]. The chaperonins are divided into two groups. Type I chaperonins are present in eubacteria, mitochondria and chloroplasts. The protein-folding activity of Type I chaperonins is executed by the concerted action of two proteins, chaperonin 60 (cpn60) and chaperonin 10 (cpn10). The central cavity of the cylindrical cpn60 tetradecamer provides an isolated environment for protein folding, while the co-chaperone, cpn10, binds to cpn60 and synchronizes the release of the folded protein from cpn60 in an ATP-dependent manner [4,5]. Type II chaperonins comprise only a cpn60 member. This family is found in the eukaryotic cytosol and in *Archaebacteria*.

As proposed by Anfinsen more than three decades ago, the primary sequence contains all the information needed for a protein to fold correctly [6]. Under the proper conditions, most soluble proteins will fold, in vitro, spontaneously. However, in the crowded and continuously stressed cellular environment, most proteins will not fold without the assistance of molecular chaperones. The vital role played by chaperonins in cellular function is demonstrated by the fact that deletion of the mitochondrial yeast chaperonins (also called hsp60 and hsp10) and the bacterial chaperonins (GroEL and GroES) is lethal in both organisms [7–9]. Moreover, deletion of one of the chloroplast cpn60 subunits is lethal at the embryonic stage of development [10]. In humans, a recent study showed that hereditary spastic paraplegia spg13 is associated with a mutation in the gene encoding the human mitochondrial cpn60 [11].

Due to the profound stability of the bacterial chaperonin system and the relative ease with which GroEL can be purified, most of our understanding of chaperonin structure and function comes from investigations of the *Escherichia coli* GroE chaperonin proteins. The mitochondrial and chloroplast chaperonins are highly unstable proteins that dissociate to monomers, in some instances, under conditions close to those of the physiological environment [12,13]. Additionally, due to their evolutionary conservation, it has been assumed that the chloroplast and mitochondrial chaperonins function in a manner similar to their bacterial homologs. Consequently, very little has been done to study these chaperonin systems. However, an increasing number of studies are showing that there are variations in both the structure and function of organellar chaperonins. Possibly, these differences reflect a special adaptation of specific homologs to their endogenous substrates.

2. The bacterial chaperonins

The bacterial chaperonin system comprises two partner proteins, GroEL and the co-chaperonin GroES, that mediate protein folding in an ATP-dependent manner. High-resolution structures of the individual GroE subunits, individual oligomers and their mutual complexes have been determined (for reviews, see [4,5]) [14]. X-ray studies showed that each GroEL subunit contains three structurally distinct domains: an apical, an intermediate and an equatorial domain (Fig. 1). The apical domain contains the binding sites for both GroES and the unfolded protein substrate. The equatorial domain contains the ATP-binding site and most of the oligomeric contacts. The intermediate domain links the apical and equatorial domains and transfers allosteric information between them. The GroEL oligomer is a tetradecamer, cylindrically shaped, that is organized in two heptameric rings stacked back to back. Each GroEL ring contains a central cavity, known as the 'Anfinsen cage' [15], that provides an isolated environment for protein folding. The identical 10 kDa subunits of GroES

*Corresponding author. Fax: (972)-3-640 6834.
E-mail address: azem@post.tau.ac.il (A. Azem).

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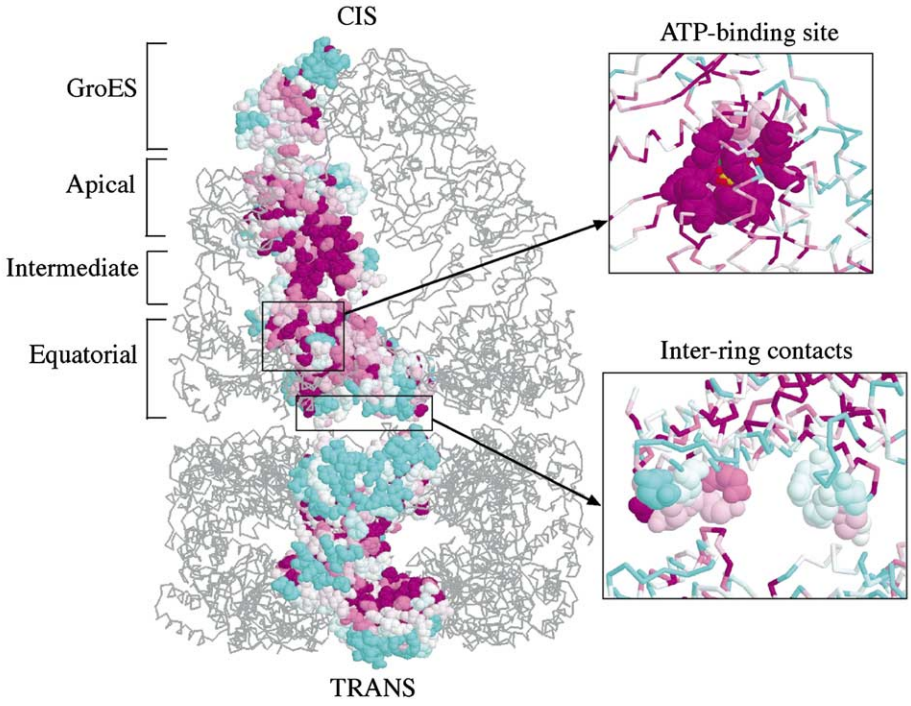

Fig. 1. Evolutionary conservation pattern in chaperonins. The evolutionary rate at each amino acid site was calculated using the maximum likelihood based-algorithm: Rate4Site [37]. These rates were then color-coded onto one GroES heptamer and two GroEL monomers, shown in Van der Waals spheres mode. Bordeaux, white and turquoise correspond to slowly, medium and rapidly evolving amino acids, respectively. The GroEL monomer in the CIS ring is viewed from the inside, while that in the trans ring is viewed from the outside. The rest of the subunits in the complex were presented in gray colored backbone mode. Several chains were removed for easier presentation. Insets: conservation pattern of the ATP-binding site and the inter-ring contacts.

form a dome-like heptameric oligomer ($GroES_7$) in solution [16]. The binding of GroES to GroEL occurs only in the presence of adenine nucleotides and is mediated by the 'mobile loop' ($\sim$20 residues of the GroES structure). The latter is an unstructured loop that becomes ordered upon formation of the GroEL–GroES complex [17]. The symmetrical nature of the free GroEL oligomer suggests that each ring can harbor one binding site each for GroES and unfolded protein. Indeed, two types of GroEL–GroES hetero-oligomers are formed, in vitro, in the presence of nucleotides. One is an asymmetric, bullet-shaped complex that contains one GroES heptamer bound to the opening of the central cavity of one GroEL ring [18]. The second is a symmetric, football-shaped complex in which two $GroES_7$ molecules cap the cavities of both $GroEL_{14}$ rings [19–22]. The GroEL/GroES chaperonins provide the best example that a high-resolution structure is essential, but not sufficient, to elucidate the mechanism of protein function. A variety of biochemical and structural solution methods have led to essential insights into the mechanism of chaperonin function [23]. The folding reaction is initiated by the binding of an unfolded substrate to one of two possible acceptor states: the $GroEL_{14}$ oligomer or the asymmetric complex (Fig. 2A, forms I and VII). The in vivo environment may allow for the formation of only the asymmetric complex as an acceptor form. However, this suggestion has yet to be demonstrated directly [24].

The current model of chaperonin-mediated protein folding suggests that regardless of the identity of the initial substrate acceptor form, the CIS complex (Fig. 2A, form III), containing the unfolded substrate together with GroES bound to the same GroEL ring, is the chaperonin intermediate that is folding-competent [25]. Following the formation of the CIS complex, a sequence of steps, controlled by ATP hydrolysis, results in the formation of a new type of CIS complex. The latter accommodates in its central cavity the refolded protein, which is release-competent (Fig. 2A, form IV). ATP binding to the trans ring triggers the release of the refolded protein [26]. Thus, release of ligands from the CIS ring is controlled by the allosteric information transferred from the trans ring [27]. The molecular steps that occur during the release of the refolded substrate are largely unclear. Single turnover experiments have shown that the discharge of ligands from the CIS ring is accelerated following binding of ATP and unfolded substrate, but not GroES, to the trans ring. This suggests that the release step proceeds directly through bullet-to-bullet (Fig. 2A, form V to VII) and not bullet-to-football transition (Fig. 2A, form V to VI) [26]. In contrast, other studies showed that the efficiency of protein folding is increased when the symmetric complexes constitute the majority of GroEL/GroES hetero-oligomers present in the refolding solution, suggesting that the symmetric complexes may have a functional role in vivo [28]. The fact that central intermediates in the chaperonin reaction cycle may exist only transiently explains the difficulty in determining the character of the obligatory forms in the cycle. Until recently it was generally accepted that an unfolded substrate bound to the trans ring of an asymmetric complex will fold efficiently only after adopting the CIS orientation (Fig. 2A, the transition from form VIII to III). However, more recently it was shown that proteins too large to be encapsulated in the CIS cavity could also be refolded efficiently by the bacterial chaperonins when they are bound by the trans ring [29].

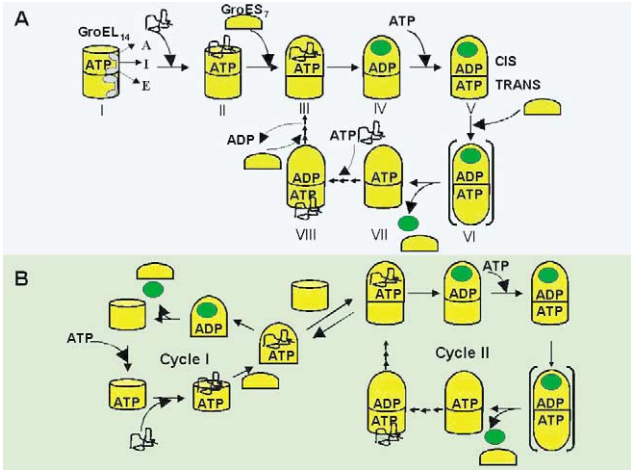

Fig. 2. Models for chaperonin-mediated protein folding. A: A scheme for the reaction cycle of bacterial chaperonins. B: A scheme for the reaction cycle of mitochondrial chaperonins. The model depicted in the scheme suggests that mt-cpn60 can function as a single ring (cycle I). However, at high protein concentration mt-cpn60 can also function as a tetradecamer (cycle II).

3. The mitochondrial chaperonins

Mitochondrial matrix proteins can be translocated from the cytosol only in an unfolded conformation. Therefore, in addition to protecting stress-denatured proteins, mitochondrial chaperonins mediate the folding of newly imported matrix proteins. Despite high sequence homology to the bacterial chaperonins, the mammalian mitochondrial cpn60 is unique among Type I chaperonins even when compared with its yeast mitochondrial homolog. The first difference is related to the oligomeric state of mammalian mt-cpn60. While the bacterial, yeast mitochondrial and chloroplast chaperonins exist and function as tetradecamers composed of two heptameric rings, the mammalian mitochondrial chaperonin was initially isolated as a heptameric protein and was suggested to function as such [13,30]. The latter observation is especially interesting considering the importance of the inter-ring communication for GroEL function. Further comprehensive analysis of the oligomeric state of mammalian mt-cpn60 showed that it exists in solution in a dynamic equilibrium between monomers, heptamers and tetradecamers. At very low protein concentrations, the mt-cpn60 dissociates to monomers, while in the presence of ATP and mt-cpn10, the equilibrium is shifted toward the formation of tetradecamers [31]. The ability of the mammalian mitochondrial chaperonins to undergo concentration-dependent self-assembly is not unique among the chaperonins. It is well known that at low protein concentrations the chloroplast chaperonins dissociate to monomers, while at high protein concentrations they assemble into tetradecamers [32]. Additionally, the chaperonin from *Thermoanaerobacter brockii* has been isolated as a single-ring heptameric protein that assembles into tetradecamers in the presence of ATP and cpn10 [33]. However, the fact that the mammalian mt-cpn60 forms tetradecamers in vitro does not exclude the possibility that the double-ring structure is not an obligatory intermediate in its reaction cycle [30]. Subsequent studies showed that even GroEL single-ring mutants could be functional in mediating protein folding [34,35]. Thus, it is possible that each heptamer represents an independent folding unit and that dimerization of the rings acts to enhance chaperonin-folding activity (Fig. 2B) [24,34,35]. Support for this suggestion is obtained from sequence alignment of a large number of chaperonins (Fig. 1). While the ATP-binding site is almost completely conserved among chaperonins, the inter-ring contacts are not entirely conserved (Fig. 1) [36]. Thus, the variations in the stability of chaperonins that are observed in vitro are not surprising.

Another property that is unique to all mitochondrial chaperonins is related to the interaction of mt-cpn10 with mt-cpn60. In contrast to the bacterial and chloroplast cpn60s, which are able to refold denatured proteins with the help of cpn10 from any source, the mitochondrial cpn60s are able to refold denatured proteins only with the help of mitochondrial cpn10s [13,38]. A structural explanation for this phenomenon was provided by elegant experiments carried out by Richardson and coworkers [39]. Swapping the mobile loop of GroES with that of mt-cpn10, or substitution of three amino acids within the GroES mobile loop, renders the bacterial co-chaperonin able to bind mt-cpn60. It was suggested that a higher affinity for the mobile loop of mt-cpn10 compensates for the lower affinity of mt-cpn60 for the co-chaperonins. The modulation of the mt-cpn60/mt-cpn10 interaction by nucleotides is also different than the nucleotide dependence observed for other chaperonins. The bacterial and chloroplast cpn60s bind strongly to their respective co-chaperonins in the presence of ADP, while the mt-cpn60 does not bind mt-cpn10 under similar conditions [30]. In contrast to the mt-cpn10 mobile loop, the nucleotide-binding domain of mt-cpn60 is highly identical to that of the bacterial cpn60 (Fig. 1). Thus, structural features that dictate the unique nucleotide-binding properties of mt-cpn60 remain to be elucidated.

4. The chloroplast chaperonins

By the time the concept of molecular chaperones had been established in the 1980's, the spotlight was focused on the extremely stable GroEL chaperonin [1]. The chloroplast chaperonin was very unstable, and research on this protein took a back seat (for a review, see [40]). Due to its high sequence homology to GroEL, it was easy to assume that its structure and function were also very similar. However, accumulating evidence points to many significant differences between the chloroplast chaperonins and their bacterial homologs. First of all, in contrast to the bacterial and mitochondrial chaperonins that are composed of 14 identical subunits, the chloroplast chaperonins are composed of two distinct subunit types, α and β. The primary sequences of α and β subunits are only ~50% identical, similar to their respective homologies to GroEL. Additionally, α and β subunits were shown to be present in roughly equal amounts in chaperonin oligomers purified from chloroplast [40]. Two types of oligomers were obtained when the chloroplast chaperonins were reconstituted, from purified monomers, in vitro [32]. One is composed of 14 identical β monomers (β_{14}) and the other composed of seven α and seven β subunits ($\alpha_7\beta_7$). Attempts to reconstitute oligomers from purified α monomers alone were unsuccessful. Interestingly, the β_{14} oligomers promote the folding of denatured proteins with the help of the mitochondrial cpn10, but not with the help of any authentic chloroplast co-chaperonins. Thus we conclude that the chloroplasts contain only $\alpha_7\beta_7$ oligomers and that the β_{14} oligomers are formed only in vitro [41]. The observation that the α and β subunits form mixed

oligomers raises the question: how are these subunits organized within the tetradecamer? The fact that the oligomers exhibit sevenfold symmetry, and that α subunits do not form oligomers on their own, suggests that perhaps heptamers of α are formed on templates of β_7, forming a tetradecamer composed of two homogeneous rings [32]. To add to the complexity of the ch-cpn60 system, a recent examination of the complete sequences of *Arabidopsis thaliana* showed that the genome of this plant encodes four isoforms of β and two of α subunits [42].

Another difference that characterizes the chloroplast chaperonins concerns the structure of their co-chaperonin. The latter was first purified from crude pea extract by taking advantage of its ability to form a complex with GroEL [43]. The purified co-chaperonin was able to assist both GroEL and ch-cpn60 in the refolding of denatured proteins. However, complete sequencing of its gene led to a surprising finding. The chloroplast co-chaperonin polypeptide chain is composed of two GroES-like domains that are held together by a short linker [43]. Thus the molecular mass of the chloroplast co-chaperonin monomer is approximately double the size (20 kDa) of the bacterial and chloroplast cpn10s. The oligomeric structure of the ch-cpn20 is still a mystery. When examined by electron microscopy, ch-cpn20 forms ring structures that are similar to GroES oligomers [43,44]. Other studies have reported that the *Arabidopsis thaliana* ch-cpn20 forms tetramers, in vitro, that interact with GroEL as such [45]. Although little is known about the structure of the ch-cpn20–ch-cpn60 hetero-oligomers, it is possible that each domain of the co-chaperonins has adapted to interact with a different subunit of ch-cpn60.

Until recently, it has been thought that chloroplasts contain only the double domain co-chaperonins. However, two independent works have provided evidence that *Arabidopsis thaliana* chloroplasts may contain at least one normal size cpn10 in addition to the ch-cpn20 [42,46]. The in vivo roles of each of the chloroplast co-chaperonins are not yet known.

5. Concluding remarks

Since their discovery, chaperonins have been studied extensively in order to understand the mechanism by which they function. In the case of the bacterial chaperonins, comprehensive research over the past two decades has unraveled essential steps in the reaction cycle at the molecular level. While it has been generally assumed that the mechanism used by the bacterial chaperonins is also utilized by the eukaryotic chaperonins, in this review we have cited several lines of evidence demonstrating that this is not the case. The great complexity of the eukaryotic chaperonins can best be seen in the genome of *Arabidopsis thaliana* which contains nine cpn60 genes: two types of α, four of β and three in the mitochondria [42]. Additionally, it contains one ch-cpn20 and four cpn10's, the latter of which are divided equally between mitochondria and chloroplasts [42]. What is the functional significance of the greater complexity of the eukaryotic chaperonins? The answer to this question will be the subject of future extensive research.

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References

[1] Hemmingsen, S.M., Woolford, C., van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W. and Ellis, R.J. (1988) Nature 333, 330–334.
[2] Bochkareva, E.S., Solovieva, M.E. and Girshovich, A.S. (1998) Proc. Natl. Acad. Sci. USA 95, 478–483.
[3] Torok, Z., Horvath, I., Goloubinoff, P., Kovacs, E., Glatz, A., Balogh, G. and Vigh, L. (1997) Proc. Natl. Acad. Sci. USA 94, 2192–2197.
[4] Sigler, P.B., Xu, Z., Rye, H.S., Burston, S.G., Fenton, W.A. and Horwich, A.L. (1998) Annu. Rev. Biochem. 67, 581–608.
[5] Hartl, F.U. (1996) Nature 381, 571–579.
[6] Anfinsen, C.B. (1973) Science 181, 223–230.
[7] Fayet, O., Ziegelhoffer, T. and Georgopoulos, C. (1989) J. Bacteriol. 171, 1379–1385.
[8] Rospert, S., Junne, T., Glick, B.S. and Schatz, G. (1993) FEBS Lett. 335, 358–360.
[9] Reading, D.S., Hallberg, R.L. and Myers, A.M. (1989) Nature 337, 655–659.
[10] Apuya, N.R., Yadegari, R., Fischer, R.L., Harada, J.J., Zimmerman, J.L. and Goldberg, R.B. (2001) Plant Physiol. 126, 717–730.
[11] Hansen, J.J., Dür, A., Cournu-Rebeix, I., Georgopoulos, C., Ang, D., Nielsen, M.N., Davoine, C.-S., Brice, A., Fontaine, B., Gregersen, N. and Bross, P. (2002) Am. J. Hum. Genet. 70, 1328–1332.
[12] Musgrove, J.E., Johnson, R.A. and Ellis, R.J. (1987) Eur. J. Biochem. 163, 529–534.
[13] Viitanen, P.V., Lorimer, G.H., Seetharam, R., Gupta, R.S., Oppenheim, J., Thomas, J.O. and Cowan, N.J. (1992) J. Biol. Chem. 267, 695–698.
[14] Chen, L. and Sigler, P.B. (1999) Cell 99, 757–768.
[15] Ellis, R.J. (1994) Curr. Biol. 4, 633–635.
[16] Hunt, J.F., Weaver, A.J., Landry, S.J., Gierasch, L. and Deisenhofer, J. (1996) Nature 379, 37–45.
[17] Landry, S.J., Zeilstra-Ryalls, J., Fayet, O., Georgopoulos, C. and Gierasch, L.M. (1993) Nature 364, 255–258.
[18] Martin, J., Mayhew, M., Langer, T. and Hartl, F.U. (1993) Nature 366, 228–233.
[19] Azem, A., Kessel, M. and Goloubinoff, P. (1994) Science 265, 653–656.
[20] Harris, J.R., Pluncktun, A. and Zahn, R. (1994) J. Struc. Biol. 112, 216–230.
[21] Llorca, O., Marco, S., Carrascosa, J.L. and Valpuesta, J.M. (1994) FEBS Lett. 345, 181–186.
[22] Schmidt, M., Rutkat, K., Rachel, R., Pfeifer, G., Jaenicke, R., Viitanen, P., Lorimer, G. and Buchner, J. (1994) Science 265, 656–659.
[23] Ranson, N.A., Farr, G.W., Roseman, A.M., Gowen, B., Fenton, W.A., Horwich, A.L. and Saibil, H.R. (2001) Cell 107, 869–879.
[24] Thirumalai, D. and Lorimer, G.H. (2001) Annu. Rev. Biophys. Biomol. Struct. 30, 245–269.
[25] Weissman, J.S., Hohl, C.M., Kovalenko, O., Kashi, Y., Chen, S., Braig, K., Saibil, H.R., Fenton, W.A. and Horwich, A.L. (1995) Cell 83, 577–587.
[26] Rye, H.S., Roseman, A.M., Chen, S., Furtak, K., Fenton, W.A., Saibil, H.R. and Horwich, A.L. (1999) Cell 97, 325–338.
[27] Horovitz, A., Fridmann, Y., Kafri, G. and Yifrach, O. (2001) J. Struct. Biol. 135, 104–114.
[28] Grallert, H. and Buchner, J. (2001) J. Struct. Biol. 135, 95–103.
[29] Chaudhuri, T.K., Farr, G.W., Fenton, W.A., Rospert, S. and Horwich, A.L. (2001) Cell 107, 235–246.
[30] Neilsen, K.L. and Cowan, N.J. (1999) Mol. Cell. 2, 93–99.
[31] Levy-Rimler, G., Viitanen, P., Weiss, C., Sharkia, R., Greenberg, A., Niv, A., Lustig, A., Delarea, Y. and Azem, A. (2001) Eur. J. Biochem. 268, 3465–3472.
[32] Dickson, R., Weiss, C., Howard, R.J., Alldrich, S.P., Ellis, R.J., Lorimer, G.H., Azem, A. and Viitanen, P.V. (2000) J. Biol. Chem. 275, 11829–11835.
[33] Todd, M.J., Walke, S., Lorimer, G., Truscott, K. and Scobes, R.K. (1995) Biochemistry 34, 14932–14941.

[34] Erbset, A., Yifrach, O., Jones, S. and Lund, P.A. (1999) J. Biol. Chem. 274, 20351–20357.
[35] Chatellier, J., Hill, F., Foster, N.W., Goloubinoff, P. and Fersht, A.R. (2000) J. Mol. Biol. 304, 897–910.
[36] Brocchieri, L. and Karlin, S. (2000) Protein Sci. 9, 476–486.
[37] Pupko, T., Bell, R.E., Mayrose, I., Glaser, F. and Ben-Tal, N. (2002) Bioinformatics 18, S71–S77.
[38] Rospert, S., Glick, B.S., Jeno, P., Schatz, G., Todd, M.J., Lorimer, G.H. and Viitanen, P.V. (1993) Proc. Natl. Acad. Sci. 90, 10967–10971.
[39] Richardson, A., Schwager, F., Landry, S.J. and Georgopoulos, C. (2001) J. Biol. Chem. 276, 4981–4987.
[40] Boston, R.S., Viitanen, P.V. and Vierling, E. (1996) Plant Mol. Biol. 32, 191–222.
[41] Nishio, K., Hirohashi, T. and Nakai, M. (1999) Biochem. Biophys. Res. Commun. 266, 584–587.
[42] Hill, J.E. and Hemmingsen, S.M. (2001) Cell Stress Chaperones 3, 190–200.
[43] Bertsch, U., Soll, J., Seetharam, R. and Viitanen, P.V. (1992) Proc. Natl. Acad. Sci. 89, 8696–8700.
[44] Viitanen, P.V., Schmidt, M., Buchner, J., Suzuki, T., Vierling, E., Dickson, R., Lorimer, G.H., Gatenby, A. and Soll, J. (1995) J. Biol. Chem. 270, 18158–18164.
[45] Koumoto, Y., Shimada, T., Kondo, M., Takao, T., Shimonishi, Y., Hara-Nishimura, I. and Nishimura, M. (1999) Plant J. 17, 467–477.
[46] Koumoto, Y., Shimada, T., Kondo, M., Hara-Nishimura, I. and Nishimura, M. (2001) J. Biol. Chem. 276, 29688–29694.