MICROBIAL PROTEINS: FROM STRUCTURE TO PATHOGENICITY – GROEL AS AN EXAMPLE

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Introduction

Genomic and proteomic analysis bring to biomedical labs qualitatively new technologies, which enable the complex look to genes and gene-related products – proteins. In contrary to genes, which number is definitive for given living system, the number of protein species varied substantially according the immediate living conditions of the system studied. Moreover, most of the proteins have the function associated with the tertiary structure of their molecule, e.g. with the space orientation so called folding and intracellular compartment where are they located. In *in vitro* systems the process of folding is inefficient and only minority of synthesized molecules have correct shape. *In vivo* most molecules must rapidly fold to proper tertiary conformation, otherwise unfolded and misfolded proteins would be degraded. The promotion of protein folding is most probably the chaperones and chaperonins role. These molecules create a family of conserved proteins found in all compartments of prokaryotic and eukaryotic cells. Moreover, in *in vivo* systems synthesized and folded proteins must occupy the proper intracellular niche to express their function. Very frequently the intracellular location, in connection with posttranslational modification of proteins produced, dictated the spectrum of their biological functions. This assumption is generally accepted and is valid for chaperonins as well. Recent report accentuate the evaluation of proteomic results obtained from extensive bacterial analysis on the example of GroEL protein with respect to structure-function relationship and to intracellular localization of individual protein species what might have significant impact for the understanding of pathogenicity of bacteria.

Molecular chaperones and chaperonins

According their function and space orientation, chaperones can be divided into two chaperone classes. Chaperones that bind and stabilize a wide range of unfolded or partially folded proteins, prevented them from being degraded, are called molecular chaperones. The function of molecular chaperones is to bind to all nascent polypeptide chains as they are synthesized on ribosomes. Chaperones exerted the protective function in cytosol, mitochondrial matrix, chloroplasts, and nucleus of the cell. Molecular chaperones are represented by the heat shock protein 70 family of proteins, which includes Hsp70 in the cytosol and mitochondria, Bip protein in endoplasmic reticulum, and DnaK chaperone in bacterial cells. The second group of chaperones represents chaperonins. Chaperonins directly facilitate the folding of proteins. The typical representative of chaperonins is bacterial GroEL molecule that creates multimeric barrel-shaped complexes composed from 14 identical subunits (Fig. 1). The partially folded or misfolded proteins enter the cavity of GroEL complex, where they bind to inner wall and undergo the process of folding. The exit of folded protein from GroEL cavity is an ATP-dependent process assisted by co-chaperonin GroES. The identical function has the TCiP molecule in eukaryotic cells. TCiP is thus the eukaryotic homologue of bacterial GroEL molecule with one substantial difference – TCiP lacks the analogue of GroES co-chaperonin. The parameters of GroEL/GroES cavity were estimated to be 18.4 nm long and 3.3 nm in diameter, what enables the folding of small- and medium-sized proteins [for reviews see publications 1 or 2].

Folding of proteins and something more

Generally, the protection and folding of proteins is only a basic but not exclusive function of chaperones. Another function, which is related to the common property of chaperones to interact with proteins, is a selective transport of proteins among individual cellular compartments. The chaperones of the Hsp70 family within eukaryotic cells excel in this respect. The transportation of pre-proteins through the mitochondrial membrane is facilitated by their binding to mitochondrial Hsp70, which

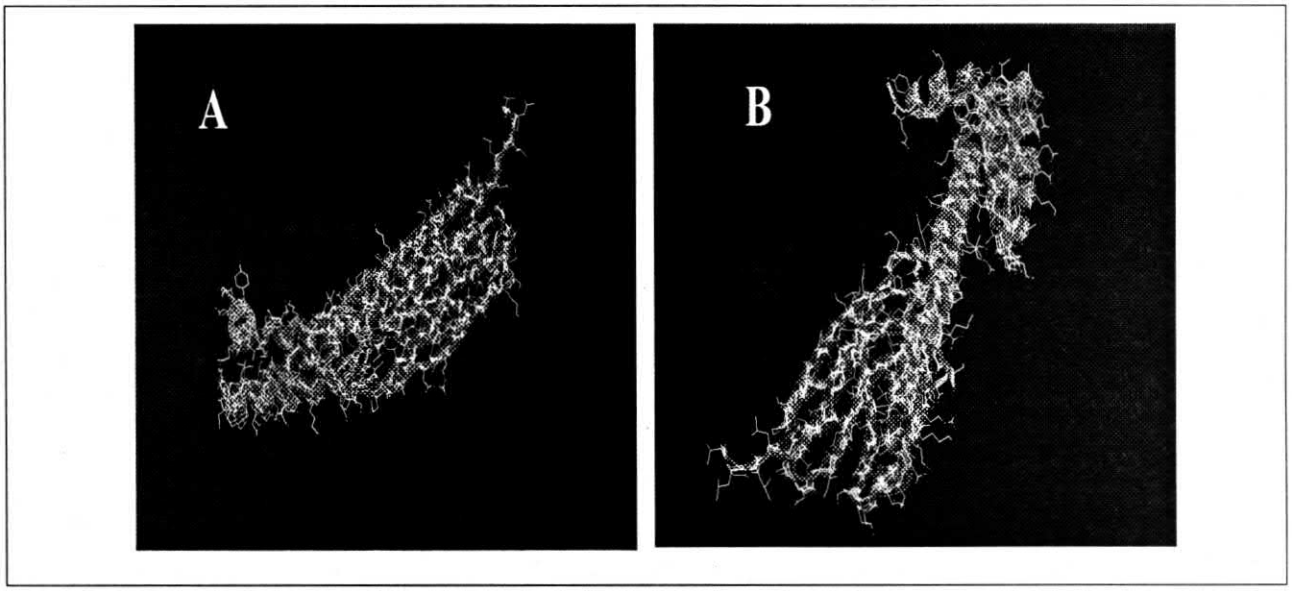

Fig. 1: Horizontal (A) and vertical (B) view on the model of Francisella tularensis strain SCHU GroEL molecule. Model was created using the Deep View Swiss-Pdb Viewer – Swiss-Model program version 36.0002.

limit the dependency of the transport on the membrane potential of mitochondria [3]. The chaperone--mediated autophagy is another example of selective transport associated with the function of chaperones. In the frame of this process, the cytosolic proteins are transported to lysosomes to be degraded. The chaperones of Hsp70 type are associated with the lysosomal membrane from the cytosolic site and together with other molecular chaperones (Hsp90, Hsp40, Hop, Hip, and BAG-1) create a molecular complex that facilitate the transport across the lysosomal membrane [4].

Molecules of Hsp60 family have apparently broader spectrum of functions, which originated from several forms of Hsp60 molecule and their subcellular and extracellular locations recognized until now. Based on original studies of GroEL in bacteria and yeast, Hsp60s were thought to reside in cytoplasm and/or stroma of organelles. This notion was further supported by the fact that no member of GroEL family possesses the leader sequence or other motifs, which predict the molecule to be located in the cell membrane or to be secreted. However, a number of recent studies documented an extracytoplasmic location for chaperonins in bacteria [5, 6]. Membrane-associated localization of bacterial chaperonins were reported in *Salmonella typhimurium* [7], *Mycobacterium leprae* [8], and several other microbes [9, 10]. Moreover, under the *in vitro* conditions *L. pneumophila* Hsp60 molecules are associated predominantly with the bacterial cell wall mainly and transported across the bacterial membrane and accumulated inside the vesicles of eukaryotic host cells where the *Legionella pneumophila* resides [11]. Proteome analyses of *Francisella tularensis* LVS whole cell lysates revealed the dominant expression of several Hsp60 molecule species known as CH60FRATU under the accession number P94798 (see Fig. 2). Western blot analyses of outer membrane proteins extracted from *Francisella tularensis* LVS microbes with subsequent immunodetection with tularemia patient sera and combined with identification of proteins using mass spectrometry revealed the CH60FRATU molecules among the integral membrane proteins (Fig. 3).

As the existence of multiple protein species of Hsp60 molecule differing in pI and molecular mass, and according the localization inside and outside the bacterial cell seems to be proved from proteome studies, the functional profile of individual protein species should be determined. The membrane-associated molecular form will have probably different structure and different biological function than cytosolic or secreted ones. The main biological function accomplished by cytosolic form of chaperonins was mentioned above. The recognized functional profile of membrane-associated stress proteins covers the adherence of bacteria to structural components of eukaryotic cell membranes and the promotion of bacterial invasiveness, which accompanies the process of adhered bacteria internalization. This can be demonstrated by the fact that various stresses augmented the adherence of bacteria to cultured cells. The implication of GroEL molecules at the process of cell adherence was demonstrated for microbes *Haemophilus ducreyi* [12, 13], *Helicobacter pylori* [14], and *Clostridium difficile* [15].

Among the ligands for surface-exposed Hsp(s) recognized till now are sulfoglycolipids for 70-kDa--related heat shock protein of *Haemophilus influenzae* [16], membrane sulfated glycolipids for Hsp70

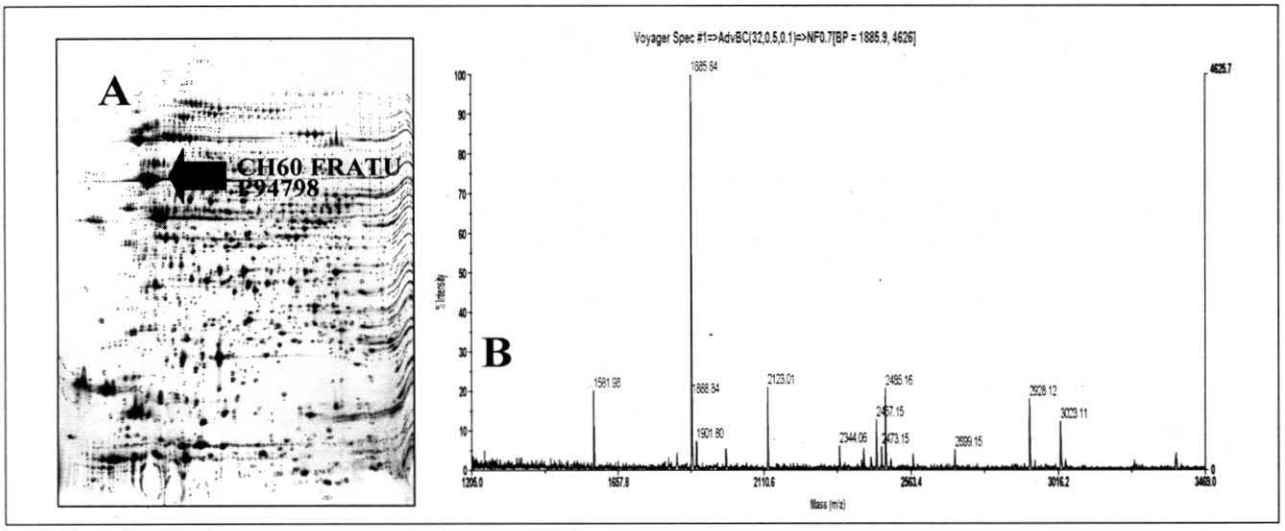

Fig. 2: Identification of Francisella tularensis Cpn60 molecule. (A) Two-dimensional gel electrophoretogram with identified spot containing Cpn60 molecules.(B) Mass spectrum obtained by MALDI TOF analysis of spot marked in (A)

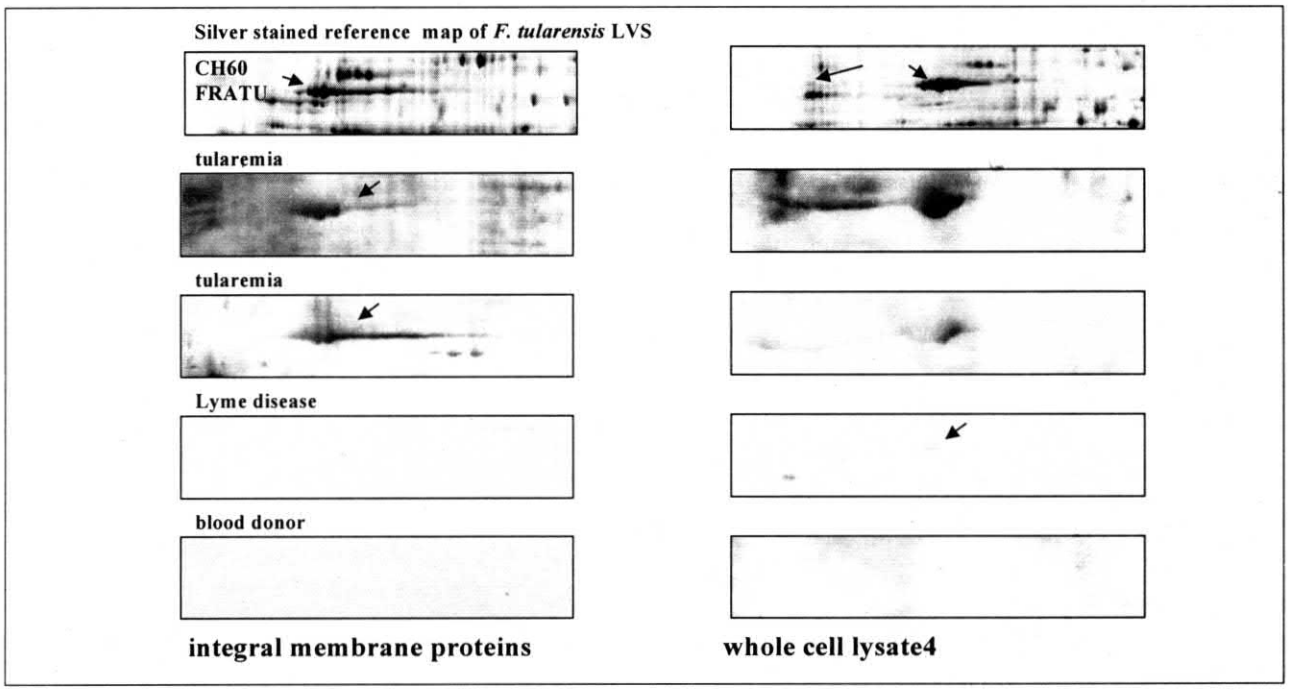

Fig. 3: The sections of Western blots with immunostained Francisella tularensis Cpn60 molecules (arrows)

of *Helicobacter pylori* [17], galactosylceramide for 62 kDa Hsp of *Borrelia burgdorferi* [18], and components of intestinal mucus for membrane associated 66-kDa stress proteins of *Salmonella typhimurium* [19]. However, none of them was explicitly proved to be the ligand for Hsp60 family members. The protein receptors with signaling function, as are Toll-like receptors or CD14, engaged in the induction of innate immune response rather than structural components of surface membranes or extracellular matrix seems to have the capacity to bind effectively 60 kDa family stress proteins of bacterial as well as eukaryotic origin [20, 21].

In addition to the function in bacterial adherence to eukaryotic cells, the surface-associated Hsp60 chaperonin of *Legionella pneumophila* was demonstrated to be a bacterial invasin, which promotes invasiveness of two virulent *Legionella* strains and in ***in vitro*** artificial system predetermine the shape of endosomes in which the particles expressed GroEL resided [22].

Protein structure, pathogenicity of infection and autoimmune diseases

The consequence of bacterial stress protein interaction with cell surface receptors after subsequent signal transduction is a functional activation of cell. The proliferation cell response [20], as well as pro-inflammatory cytokine production [23], enzyme secretion (metalloproteinase) [24] and phenotype transformation (expression of adhesion molecules) [25] was reported as a cell reaction to the bacterial heat shock 60 kDa proteins. All activities mentioned above are prerequisite for the proper functioning of innate immune mechanism that creates first barrier against invaded microbes. Macrophages, once infected, produce pro-inflammatory cytokines, attract the lymphocyte subpopulation to the site of infection, and present peptides to corresponding T lymphocyte subsets. The epitopes of bacterial Hsp60 can be presented by the MHC class I molecules or by non-polymorphic class Ib molecule Qa-1. The activated $CD8^+$ cytotoxic T lymphocytes recognized the bacterial peptide derived from Hsp60 in the context of self-MHC molecule and cross-recognize also a homologous peptide derived from mammalian Hsp60 [26, 27]. It seems likely that the surface expression or secretion of heat shock proteins is common immediate response of both interacting organisms and can be considered as a "danger" signal for defence systems. Due to the homology of this phylogenetically conserved proteins there are the identical epitopes on peptides derived from prokaryotic and eukaryotic Hsps [28]. Cross-recognition of self-Hsps peptides expressed on stressed cells, based on the stress induced by infection and activation of relevant lymphocyte clones by prokaryotic molecules, is an unwanted tribute of effective anti-bacterial immune response of organism, which might constitute the link between infection and autoimmunity. The complex effect of both chlamydial and human Hsp60 during the atherogenesis was recognized recently what can serve as an example and can help to understand the association of infections with pathophysiology of sever human illnesses [29].

Moreover, when we consider the role of Hsp60 molecules for the pathogenesis of infections and their consequences, we should still take in mind the chaperonin function. The example of chaperone's substantial role in the pathogenicity of infection is their involvement in the formation of beta-sheet aggregate of prion protein (PrPSc), which is the infectious form derived by conformational conversion from PrPC, largely alpha-helical isoform of prion protein. The efficacy to bind and to convert the PrPC into protease-resistant form PrPSc was demonstrated for eukaryotic chaperonin and bacterial GroEL molecules as well [30, 31]. In spite of the artificial system, this data documented the broad range of functional activities of bacterial GroEL molecules.

Conclusion

The members of Hsp60 chaperonin family are multifunctional molecules, which are over-expressed in prokaryote as well as eukaryote cells in response to stress conditions. Bacterial GroEL molecules have probably the function associated with the precisely defined structural forms (protein species) and their localization inside or outside the bacterial body. Until now, we have a limited knowledge about the number of protein species, which are coded by *groESL* gene and we rather assess the spectrum of activities associated with this gene products. On the other hand we anticipate the deep impact of bacterial chaperonine molecules to the pathogenesis of human illnesses. From these reasons, the care should be paid to detailed structure-function relationship studies of individual candidate protein species. In this respect, the proteome study of individual subcellular compartments, which can contain the microbial components, can bring decisive knowledge to the understanding of pathogenicity of infections.

Literature

1. Molecular cell biology, 4th Edition, Eds.: Freeman, W.H., and comp., NY, 2000.
2. Molecular biology of the cell, 3rd Edition, Eds.: Alberts, B., and comp., Garland Publ., NY, 1994.
3. GEISSLER, A., et al. Mitochondrial import driving forces: anhanced trapping by matrix Hsp70 stimulates translocation and reduces the membrane potential dependence of loosely folded preproteins. *Mol. Cell. Biol.*, 2001, vol. 21, no. 20, p. 7097–7104.
4. AGARRABERES, FA. – DICE, JF. A molecular chaperone complex at the lysosomal membrane is required for protein translocation. *J. Cell. Sci.*, 114, 2001, 13, p. 2491–2499
5. KOVÁCS, E., et al. Heat stress induces association of the GroEL analog chaperonin with thylakoid membranes in cyanobacterium, Synechocystis PCC 6803. *Plant Physiol. Biochem.*, 1994, vol. 32, p. 285–293.
6. SCORPIO, A., et al. Subcellular localization and chaperone activities of Borrelia burgdorferi Hsp60 and Hsp70. J. Bacteriol. 176, 1994, p. 6449–6456
7. ENSGRABER, M. LOOS, M. A 66-kilodalton heat shock protein of *Salmonella tyohimurium* is responsible for binding of the bacterium to the intestinal mucus. *Infect. Immun.*, 1992, vol. 60, p. 3072–3078.
8. GILLIS, TP., et al. Immunochemical characterization of a protein associated with Mycobacterium leprae cell wall.

Infect. *Immun.*, 1985, vol. 49, p. 371–377.
9. YAMAGUCHI, H., et al. Induction and epitope analysis of Helicobacter pylori heat shock protein. *J. Gastroenterol.*, 1996, vol. 31, p. 12–15.
10. PLIKAYTIS, BB., et al. Purified 60-kilodalton Legionella protein antigen with Legionella-specific and nonspecific epitopes. *J. Clin. Microbiol.*, 1987, vol. 25, p. 2080–2084.
11. GARDUŇO, RA., et al. Immunolocalization of Hsp60 in Legionella pneumophila. *J. Bacteriology*, 1998, vol. 180, no. 3, p. 505–513.
12. PARSONS, LM. – LIMBERGER, RJ. – SHAYEGANI, M. Alterations in levels of DnaK and GroEL result in diminished survival and adherence of stressed Haemophilus ducreyi. *Infect. Immunol.*, 1997, vol. 65, p. 2413–2419.
13. FRISK, A. – ISON, CA. – LAGERGARD, T. GroEL heat shock protein of Haemophilus ducreyi: association with cell surface and capacity to bind to eukaryotic cells. *Infect. Immunol.*, 1998, vol. 66, p. 1252–1257.
14. YAMAGUCHI, H., et al. Heat-shock protein 60 homologue of Helicobacter pylori is associated with adhesion of H. pylori to human gastric epithelial cells. *J. Med. Microbiol.*, 1997, vol. 46, p. 825–831.
15. HENNEQUIN, C., et al. GroEL (Hsp60) of Clostridium difficile is involved in cell adherence. *Microbiology*, 2001, vol. 147, p. 87–96.
16. HARTMANN, E. – LINGWOOD, C. Brief heat shock treatment induces a long-lasting alteration in the glycolipid receptor binding specificity and growth rate of Haemophilus influenzae. *Infect. Immun.*, 1997, vol. 65, p. 1729–1733.
17. HUESCA, M., et al. Characterization of an acidic-pH-inducible stress protein (hsp70), a putative sulfatide binding adhesin, from Helocobacter pylori. *Infect. Immun.*, 1998, vol. 66, p. 4061–4067.
18. KANEDA, K., et al. Glycosphingolipids-binding protein of Borrelia burgdorferi sensu lato. *Infect. Immun.*, 1997, vol. 65, p. 3180–3185.
19. ENSGRABER, M. – LOOS, M. A 66-kilodalton heat shock protein of Salmonella typhimurium is responsible for the binding of the bacterium to intestinal mucus. *Infect. Immun.*, 1992, vol. 60, p. 3072–3078.
20. SASU, S., et al. Chlamydia pneumoniae and chlamydial heat shock protein 60 stimulate proliferation of human vascular smooth muscle cells via toll-like receptor 4 and p44/p42 mitogen-activated protein kinase activation. *Circ. Res.*, 2001, vol. 89, p. 244–250.
21. UEKI, K., et al. Self-heat shock protein 60 induces tumour necrosis factor-alpha in monocyte-derived macrophage: possible role in chronic inflammatory periodontal disease. *Clin. Exp. Immunol.*, 2002, vol. 127, p. 1–3.
22. GARDUNO, RA. – GARDUNO, E. – HOFFMAN, PS. Surface-associated hsp60 chaperonin of Legionella pneumophila mediates invasion in a HeLa cell model. *Infect. Immun.*, 1998, vol. 66, p. 4602–4610.
23. TABONA, P., et al. Homogeneous Escherichia coli chaperonin 60 induces IL-1 beta and IL-6 gene expression in human monocytes by a mechanism independent of protein conformation. *J. Immunol.*, 1998, vol. 161, p. 1414–1421.
24. VEHMAAN-KREULA, P., et al. Chlamydia pneumoniae proteins induce secretion of the 92-kDa gelatinase by human monocyte-derived macrophages. Arterioscler. Thromb. *Vasc. Biol.*, 2001, vol. 21, p. E1-E8.
25. KOL, A., et al. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J. Clin Invest.*, 1999, vol. 103, p. 571–577.
26. ZUGEL, U., et al. Crossrecognition by CD8 T cell receptor alpha beta cytotoxic T lymphocytes of peptides in the self and the mycobacterial hsp60 which share intermediate sequence homology. *Eur. J. Immunol.*, 1995, vol. 25, p. 451–458.
27. SOLOSKI, MJ. – METCALF, ES. The involvement of class Ib molecules in the host response to infection with Salmonella and its relevance to autoimmunity. *Microbes Infect.*, 2001, vol. 3, no. 1, p. 1249–1259.
28. KIMURA, Y., et al. Evidence for the early recruitment of T-cell receptor gamma delta+ T cells during rat listeriosis. *Immunology*, 1996, vol. 87, p. 21–28
29. KOL, A., et al. Cutting edge: heat shock protein (Hsp) 60 activates the innate immune response: CD14 is an essential receptor for Hsp60 activation of mononuclear cells. *J. Immunol.*, 2000, vol. 164, p. 13–17.
30. DeBURMAN, SK., et al. Chaperone-supervised conversion of prion protein to its protease-resistant form. *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, p. 13938–13943.
31. STOCKEL, J. – HARTL, FU. Chaperonin-mediated de novo generation of prion protein aggregates. *J. Mol. Biol.*, 2001, vol. 313, p. 861–872.

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