|  |
| --- |
| **Prof. Abdussalam Azem’s Research** |
| **Protein transport and folding**  Proteins are formed as long chains of amino acids that fold into very specific three-dimensional conformations, which are essential for their activity. In the crowded cellular environment, proteins are assisted by chaperone proteins to fold into their final, functional conformation [1, 2]. Nascent proteins take advantage of chaperone proteins to reach their functional structure, as do stress-denatured proteins. Additionally, most proteins that are transported across biological membranes traverse the membrane in an unfolded conformation [3-8]. The processes of unfolding for transport and refolding on the other side of the membrane both make use of chaperone proteins. Our laboratory focuses on two types of protein systems: the 60 kDa protein-folding system and the mitochondrial inner-membrane protein import system.  **Protein folding by Cpn60**  The 60 kDa family of chaperone proteins, also known as "chaperonins", is composed of two types. We are studying type I chaperonins, tetradecameric oligomeric proteins composed of 60 kDa subunits (cpn60) that work together with small heptameric ring-shaped helper proteins composed of 10 kDa (cpn10) subunits [9, 10]. This system is over-expressed in response to heat-shock, prevents protein aggregation and helps stress-denatured proteins refold. Thus, the proteins are also called the 60 and 10 kDa heat-shock proteins (hsp's). Due to their tremendous stability, the best studied of these proteins are the GroEL (60 kDa subunits) and GroES (10 kDa subunits) of *E. coli*. Homologous proteins have been identified in mammalian, yeast and plant mitochondria as well as in plant chloroplasts [11, 12]. In our laboratory, we are studying the structure and function of mammalian mitochondrial and plant chloroplast chaperonins, both of which exhibit different structural characteristics than GroEL.  The mammalian mitochondrial cpn60 is involved in folding proteins that have been transported into the mitochondrial matrix, as well as in refolding stress-denatured mitochondrial protein [13]. The presence of extra-mitochondrial chaperonins has been associated with a number of diseases. In contrast to the highly stable GroEL tetradecamer, the mitochondrial cpn60 oligomer has very weak contacts between the two heptamers that make up the protein [14]. We have extensively described the equilibrium between the oligomeric forms of this protein under various conditions [15]. Another outstanding feature of the mammalian mitochondrial cpn60 is its specificity for the mammalian mitochondrial cpn10 [16]. Both the bacterial GroEL and the plant chloroplast cpn60 can function equally well with cpn10 from any source.  Plant chloroplast cpn60 was originally discovered as a protein that was bound to Rubisco large subunits [17]. Early research implicated this protein in Rubisco assembly. However, tobacco plants expressing antisense to the hsp60 b subunit accumulated equal or higher levels of Rubisco than control plants [18]. Other roles for chloroplast cpn60's have been suggested, including: general chloroplast protein import [19], chromoplast differentiation (Bonk et al., 1996), fruit ripening [20] and import of carotenogenic enzymes [21]. Mutants in a number of the plant chaperonins were shown to result in phenotypes ranging from stunted growth to lethality [22, 23]. The significance of these proteins is very wide-ranging.  In contrast to the GroEL oligomer, which is composed of 14 identical subunits, the chloroplast cpn60 oligomer is composed of two subunit types, α and β. The organization of these subunits within the tetradecamer has yet to be shown, however, various lines of evidence suggest that one heptamer consists solely of a subunits and one ring of β subunits. Analysis of the Arabidopsis thaliana genome shows that chloroplasts of this plant contain 6 putative homologs of cpn60, 4 of the β gene and 2 of the α gene [11].  Another intriguing phenomenon in the chloroplast system is its "double" cpn10. In addition to 2 "normal"-sized cpn10 homologs, the chloroplast contains a cpn10 gene (cpn20) composed of two cpn10's joined head-to-tail. The final structure of this "double" protein has yet to be determined, as does its role in vivo [9]. Until now, only limited studies have been carried out on the plant chaperonins and the proteins studied have been from widely varying sources.  **Mitochondrial protein import**  In yeast, mitochondrial DNA encodes only 12 proteins, most of them integral membrane proteins. The majority of the over 1000 proteins found in the mitochondria are encoded by nuclear genes, synthesized in the cytoplasm and then imported into one of the four mitochondrial compartments: the outer membrane, the inter-membrane space, the inner membrane and the matrix. In our laboratory, we are studying the mechanism of protein import into the mitochondrial matrix with a focus on the role of the mitochondrial protein translocation motor. The importance of this process is demonstrated by the fact that most mitochondrial proteins essential for yeast viability are involved in the import of proteins into the mitochondria [24, 25].  Proteins destined for the mitochondrial matrix are usually synthesized as precursors with an N-terminal extension that is recognized and bound by cytosolic chaperones to prevent misfolding and aggregation. Once the mitochondrial precursor protein reaches the mitochondrial surface, it is transferred to the import receptor, and then inserted into the mitochondrial import channel, which consists of two separate complexes, one in the outer membrane (Translocase Outer Membrane, or TOM) and one in the inner membrane (Translocase Inner Membrane, or TIM). Mitochondrial import is mediated not only by membrane proteins but also by two matrix-localized soluble proteins, the mitochondrial hsp70 chaperone (70 kDa heat shock protein- mhsp70) and its cofactor, mitochondrial GrpE (mGrpE or Mge1). These two proteins are recruited to the import channel by forming a complex with an essential protein, Tim44. The Tim44-mhsp70-mGrpE complex mediates the final step of import into the matrix, in which the precursor protein bound to the import channel is pulled into the matrix. Recently it was shown that additional proteins [Tim14 (PAM18) and Tim16 (PAM16), probably participate in regulating of the function of the translocation motor. Our work is aimed at analyzing the structure-function relationship of the translocation motor, mainly using purified proteins and mutagenesis analysis.  After their import, mitochondrial proteins are folded with the help of mitochondrial chaperones localized in the mitochondrial matrix. In addition to its role in protein import, mhsp70 is also involved in folding the newly-imported proteins. In order to carry out its folding function, mhsp70 forms a second matrix-localized complex with two proteins, Mdj1 and mGrpE (Mge1). Subsequently, some of the imported proteins are passed on to the hsp60 folding machinery. Both the hsp70 and hsp60 protein folding systems are essential and conserved throughout evolution.  1. Ellis, R. J. (1997) Molecular chaperones: avoiding the crowd, *Curr Biol. 7*, R531-3. 2. Ellis, R. J. & Hartl, F. U. (1999) Principles of protein folding in the cellular environment, *Curr Opin Struct Biol. 9*, 102-10. 3. Neupert, W. (1997) Protein import into mitochondria, *Annu Rev Biochem. 66*, 863-917. 4. Schatz, G. & Dobberstein, B. (1996) Common principles of protein translocation across membranes, *Science. 271*, 1519-26. 5. Matouschek, A. (2003) Protein unfolding--an important process in vivo?, *Curr Opin Struct Biol. 13*, 98-109. 6. Ma, Y. & Hendershot, L. M. (2001) The unfolding tale of the unfolded protein response, *Cell. 107*, 827-30. 7. Schmidt, S., Strub, A. & Voos, W. (2001) Protein translocation into mitochondria, *Biol Signals Recept. 10*, 14-25. 8. Strub, A., Lim, J. H., Pfanner, N. & Voos, W. (2000) The mitochondrial protein import motor, *Biol Chem. 381*, 943-9. 9. Levy-Rimler, G., Bell, R. E., Ben-Tal, N. & Azem, A. (2002) Type I chaperonins: not all are created equal, FEBS Lett. 529, 1-5. 10. Bukau, B. & Horwich, A. L. (1998) The Hsp70 and Hsp60 chaperone machines, *Cell. 92*, 351-66. 11. Hill, J. E. & Hemmingsen, S. M. (2001) Arabidopsis thaliana type I and II chaperonins, *Cell Stress Chaperones. 6*, 190-200. 12. Hartl, F. U. (1996) Molecular chaperones in cellular protein folding, *Nature. 381*, 571-9. 13. Rospert, S., Looser, R., Dubaquie, Y., Matouschek, A., Glick, B. S. & Schatz, G. (1996) Hsp60-independent protein folding in the matrix of yeast mitochondria, *Embo J. 15*, 764-74. 14. Nielsen, K. L. & Cowan, N. J. (1998) A single ring is sufficient for productive chaperonin-mediated folding in vivo, *Mol Cell. 2*, 93-9. 15. Levy-Rimler, G., Viitanen, P., Weiss, C., Sharkia, R., Greenberg, A., Niv, A., Lustig, A., Delarea, Y. & Azem, A. (2001) The effect of nucleotides and mitochondrial chaperonin 10 on the structure and chaperone activity of mitochondrial chaperonin 60, *Eur J Biochem. 268*, 3465-72. 16. Richardson, A., Schwager, F., Landry, S. J. & Georgopoulos, C. (2001) The importance of a mobile loop in regulating chaperonin/ co-chaperonin interaction: humans versus Escherichia coli, *J Biol Chem. 276*, 4981-7. 17. Ellis, R. J., Robinson, C., van der Vies, S. M. & Kirwin, P. M. (1988) Processing and assembly of chloroplast proteins,*Biochem Soc Trans. 16*, 703-4. 18. Zabaleta, E., Assad, N., Oropeza, A., Salerno, G. & Herrera-Estrella, L. (1994) Expression of one of the members of the Arabidopsis chaperonin 60 beta gene family is developmentally regulated and wound-repressible, *Plant Mol Biol. 24*, 195-202. 19. Lubben, T. H., Donaldson, G. K., Viitanen, P. V. & Gatenby, A. A. (1989) Several proteins imported into chloroplasts form stable complexes with the GroEL-related chloroplast molecular chaperone, *Plant Cell. 1*, 1223-30. 20. Schmitz, G., Schmidt, M. & Feierabend, J. (1996) Comparison of the expression of a plastidic chaperonin 60 in different plant tissues and under photosynthetic and non-photosynthetic conditions, *Planta. 200*, 326-36. 21. Bonk, M., Hoffmann, B., Von Lintig, J., Schledz, M., Al-Babili, S., Hobeika, E., Kleinig, H. & Beyer, P. (1997) Chloroplast import of four carotenoid biosynthetic enzymes in vitro reveals differential fates prior to membrane binding and oligomeric assembly, *Eur J Biochem. 247*, 942-50. 22. Ishikawa, A., Tanaka, H., Nakai, M. & Asahi, T. (2003) Deletion of a chaperonin 60 beta gene leads to cell death in the Arabidopsis lesion initiation 1 mutant, *Plant Cell Physiol. 44*, 255-61. 23. Apuya, N. R., Yadegari, R., Fischer, R. L., Harada, J. J., Zimmerman, J. L. & Goldberg, R. B. (2001) The Arabidopsis embryo mutant schlepperless has a defect in the chaperonin-60alpha gene, *Plant Physiol. 126*, 717-30. 24. Wiedemann, N., Frazier, A. E. & Pfanner, N. (2004) The protein import machinery of mitochondria, *J Biol Chem. 279*, 14473-6. 25. Herrmann, J. M. & Neupert, W. (2003) Protein insertion into the inner membrane of mitochondria, *IUBMB Life. 55*, 219-25. |