# Chaperones as possible elements of eukaryotic cytoarchitecture

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After the discovery of the need for extensive assistance in protein folding in the case of many nascent or damaged proteins, heat shock proteins and other stress-induced proteins have come to be regarded as molecular chaperones; thus their major cellular function is considered to be established. 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 conserves precious research materials. Contrary to these usual experimental conditions, the cellular environment is crowded (Zimmerman and Minton, 1993). Molecular crowding promotes protein aggregation and thus enhances the need for chaperone action. On the other hand, bona fide chaperones are not the only cellular solutions for aggregation-protection. Several "innocent bystanders," such as tubulin (Guha *et. al.,* 1998) or even small molecules (lipids, other amphiphyles, sugars, a class of compounds called as chemical chaperones, Welch and Brown, 1996) may assist folding and prevent aggregation albeit at 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. (Hartl, 1996), we do not really know how big is the segment of the life of an ordinary chaperone during which it “chaperones" unfolded or misfolded proteins in eukaryotic cells.

I should make it clear that with the above argumentation, I do not want to question the importance of chaperones in folding-assistance. Nevertheless, I would like to stress that there is enough room to think about other important functions of chaperones related, but not equal to 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- end products of proteasomal degradation (Kisselev *et. al.,* 1998) are presumably cleaved further into single amino acids. However, direct evidence for this efficient degradation-completion is missing.

Released peptide segments may often contain elements of important binding sites and thus might efficiently interfere with signaling and, metabolic processes. If this happened, 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 (Srivastava *et. al.,* 1998).

As yet another important, and non-conventional, 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 (Csermely *et. al.*, 1998, Trent *et. al.,* 1998, Benaroudj *et. al.,* 1996). Oligomerization usually affects only a few percent of the total protein; but addition of divalent cations, certain nucleotides, heat

treatment, etc enhances oligomer formation. It is important to note that oligomerization studies were usually performed under "normal," *in vitro* experimental conditions, using a few mg/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 (Zimmerman and Minton, 1993).

Different chaperones 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 (Csermely *et. al.,* 1998).

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 (Wigley *et. al.,* 1999), and several chaperones, especially Hsp90 were considered to be involved in the direction of cytoplasmic traffic (Pratt, 1997).

The above model, which describes chaperones as a highly dynamic "appendix" of various, and often quite poorly identifiable, cytoplasmic filamentous structures, is reminiscent of the early view (Wolosewick and Porter, 1979; Schliwa *et al*., 1981) about the microtrabecular network of the cytoplasm. Although a rather energetic debate has developed about the validity of the electron microscopical evidence of the microtrabeculae, several independent findings support the existence of a cytoplasmic and nuclear mesh-like structure (Clegg, 1984; Jacobson and Wojcieszyn, 1984; Luby-Phelps *etal*., 1988; Penman and Penman, 1997; Hendzel *et al.,* 1999). The major cytoplasmic chaperones (TCP1/Hsp60 and Hsp90 and their associated proteins) may well form a part of this network in cells.

One of the major advances of the eukaryotic cell is probably centered around its superior compartmentalization and organization compared with that of the prokaryotic organisms. However, cellular order must be maintained and repaired. Chaperones may be important elements of this job in eukaryotes. Further studies to explore the details of this putative function may easily lead to exciting, novel aspects of chaperone action.

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