analysis of Cl^- fluxes in the lens shows that the peripheral fiber cells have a membrane voltage that favors Cl^- efflux. Thus these cells are able to undergo a regulatory volume decrease and appear normal (Fig. 4*C*). In deeper regions of the lens, the membrane voltage favors an influx rather than an efflux of Cl^-. In these cells, an opening of volume-regulated Cl^- channels will therefore cause an influx of Cl^- and further increase the rate of cell swelling. Thus the knowledge now accumulated on the lens circulation system for the first time provides a satisfactory explanation for the early localized tissue damage observed in the diabetic rat lens.

Future outlook

Assuming that the circulation is essential for homeostasis of the central fiber cells, any reduction in the circulatory system may eventually lead to cataract formation. It is possible that some reduction does occur with age, and this could lead to some forms of the senile cataract. However, it is also possible that the system can be upregulated to offset deleterious effects and avoid cataract formation. Indeed, the work on the Na^+-K^+ pumps (4) suggests that the circulation may be physiologically regulated. If so, then it should be possible to pharmacologically manipulate it. In this sense it is intriguing that a number of receptor isoforms (3) as well as isoforms of the Na^+-K^+ pumps (9) have been recently identified in the lens. Although the functional role of these receptors has yet to be determined, it is interesting to speculate that they could be used as the targets of novel therapies to prevent cataract formation by modulating the activity of the lens internal circulation.

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A Nonconventional Role of Molecular Chaperones: Involvement in the Cytoarchitecture

Peter Csermely

A hallmark of chaperone action is assistance in protein folding. Indeed, folding of nascent prokaryotic proteins proceeds mostly as a chaperone-assisted, posttranslational event. On the contrary, in nonstressed eukaryotic cells folding-related tasks of eukaryotic chaperones are restricted to a subset of proteins, and "jobless" chaperones may form an extension of the cytoarchitecture, facilitating intracellular traffic of proteins and other macromolecules.

Protein folding is characterized by two major steps in vitro (Fig. 1; Ref. 4). In the first steps, most of the secondary structure is already formed. Folding usually starts with the formation of α-helices, since β-sheet formation requires hydrogen bonds between amino acids, which are far from each other in the primary sequence. In this step, the unfolded protein is collapsed and a (more or less) stable intermediary, the molten globule, is formed. The partially folded state of molten globules can be characterized by a developed secondary structure that is mostly unorganized, showing almost no tertiary structure. Molten globules still have large hydrophobic surfaces and

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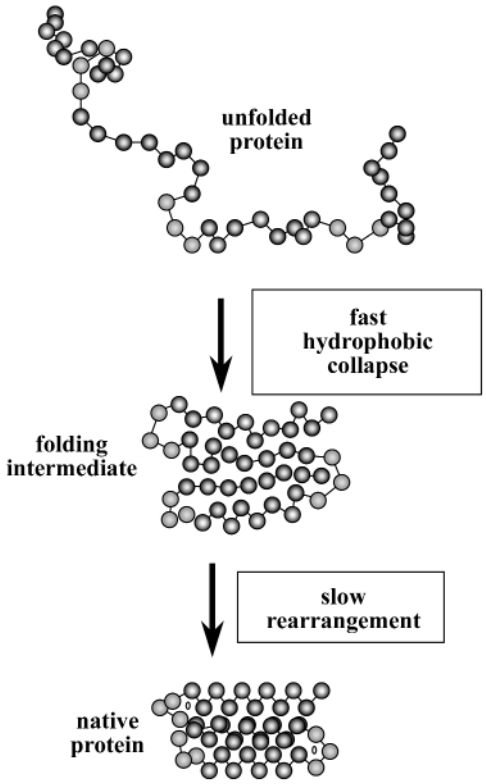

FIGURE 1. Major steps of protein folding in vitro. The unfolded protein first undergoes fast hydrophobic collapse, during which most of its hydrophobic surfaces become buried. The folding intermediate develops its final, native structure in a slower process. Although folding of small proteins (in the range of 10–30 kDa) may be a rather straightforward process, larger proteins are often trapped in various misfolded states. These trapped intermediates usually have hydrophobic surfaces and are prone to aggregation. Figure is adapted from Ref. 2.

therefore are subjects of extensive aggregation. The volume of molten globules, however, is almost as small as that of the native, folded protein.

The last steps of protein folding are the slow, rate-limiting steps. In these steps the inner, hydrophobic core of the protein becomes tightly packed (2) and unique, high-energy bonds are formed, such as disulfide bridges or ion pairs. The free energy gain of these latter processes enables the formation of local, unstable protein structures, which are stabilized by the favorable conformation of the rest of the protein. These unstable protein segments 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.

For larger proteins, folding is not a straightforward process. Their unordered, hesitating, zigzag pathways need a lot of help. Besides this, aggregation of partially unfolded or misfolded proteins is a great danger. Molecular chaperones serve to prevent aggregation and to rescue misfolded proteins from their folding traps (1, 5). In the case of chaperone machines that surround their targets, rearrangement of the hydrophobic core of the target protein is aided by periodic pulling and water percolation (Fig. 2; Ref. 2), whereas other molecular chaperones grab a hydrophobic peptide segment of their client proteins.

Protein folding in crowded cells: conventional roles of chaperones

In vivo protein folding first occurs when a protein is born. Prokaryotic proteins are synthesized quickly. Most of their folding occurs after translation and needs the help of chaperones. In eukaryotes, protein synthesis is a slower process: proteins fold during their emergence from the ribosomes, i.e., cotranslationally (8). Folding of these proteins may occur sequentially. Different domains of the protein fold one after the other, and the process is helped by the ribosomal machinery itself. After synthesis, chaperones help the translocation of proteins through membranes. Pores of most cellular membranes (with the notable exception of the nuclear pores) are too small to accommodate fully folded, globular proteins. Proteins have to unfold to get through and refold in the lumen of the organelle. These processes are facilitated by molecular chaperones.

The cellular environment is much more crowded than usual in vitro experimental conditions in protein folding studies. Estimated protein concentrations reach 200–300 mg/ml (20–30% wt/vol), which is close to the theoretical "overlap" concentration for a typical 50-kDa protein (7). Molecular crowding promotes aggregation, which makes the chaperone-mediated protection of folding proteins even more desirable. Crowding also stabilizes chaperone-target complexes, which increases the efficiency and fidelity of chaperone action.

Chaperones also help refolding of damaged proteins. After environmental stress, protein damage becomes abundant; therefore, an increased capacity of the "chaperone machines" is highly advantageous. Indeed, many stressors (such as alcohol, other poisons, sunburn, anxiety, etc.) induce the synthesis

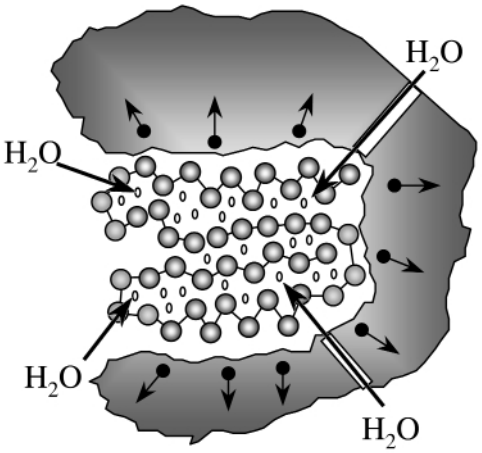

FIGURE 2. A possible mechanism of chaperone action. Anfinsen-cage type molecular chaperones, which surround their target proteins, exert a periodic multidirectional pulling of the target by using their periodic conformational changes governed by the hydrolysis of ATP. In the pulling process, the hydrophilic exterior of the target protein becomes immobilized, whereas its hydrophobic core becomes mobilized and gets another chance to rearrange itself. During the chaperone-mediated expansion of the target, water molecules enter its hydrophobic core and facilitate its rearrangement further. This (partially hypothetical) view is supported by fluorescence anisotropy, electron spin resonance measurements, and hydrogen/deuterium exchange studies. Figure is adapted from Ref. 2.

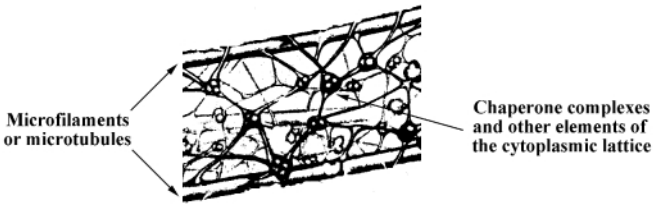

FIGURE 3. Chaperones as putative constituents of the cytoarchitecture. The figure summarizes the current view about the complex cytoplasmic meshwork attached to the microfilamental, microtubular, and intermediate filamental structures. Recent data indicate that molecular chaperone complexes may be an important parts of this meshwork. Figure is adapted from Ref. 14.

of these chaperones (called heat-shock or stress proteins), and in case of bacterial and viral infections the developing fever also helps this process (13). In stressed cells, chaperones not only help proteins to survive but also help their destruction by presenting ultimately damaged proteins to the lysosomal protein degradation or to the proteasome. Chaperones may also play an important role in helping RNA folding and association of RNA-protein complexes.

Are eukaryotic chaperones jobless?

Eukaryotes accommodated mitochondria, which enabled them to produce a vast amount of chemical energy in the form of ATP. This energy richness may be one reason why eukaryotes discard rather than repair a lot of misfolded proteins (10, 12). Similarly, as much as 97% of RNAs never leave the nucleus but become almost instantly degraded (6). In resting eukaryotic cells, chaperones have a smaller role in folding than in prokaryotes. Most cytoplasmic chaperone machineries are specialized to help the folding of a small subset of proteins, such as nuclear hormone receptors, protein kinases, actin, or tubulin. On the other hand, many of the eukaryotic chaperones, such as the 90-kDa heat shock protein (Hsp90) are expressed constitutively and form 1–5% of cellular proteins. Moreover, Hsp90 and other chaperones are mandatory for the life of eukaryotic cells; their deletion is lethal (3, 9). Why do we need so much of these chaperones, if their specific targets are at least a hundred times less abundant than the chaperones themselves? Are they just waiting for a stress to occur? Recent data from Pratt et al. (9), and from our own lab (unpublished observations) indicate that eukaryotic chaperones may also participate in the cytoplasmic organization and traffic. This eukaryote-specific role fits the increased need for compartmentalization and organization in eukaryotic cells.

A nonconventional role: chaperones and the cytoarchitecture

In the late 1970s, Porter and co-workers (14) found a cytoplasmic meshwork of various filaments called the "microtrabecular lattice." Although a rather energetic debate has developed about the validity of the electronmicroscopical evidence for the microtrabeculae, several independent findings support the existence of a cytoplasmic mesh-like structure (7). However, the identity of the constituents of this lattice, besides the regular microtubular, microfilamental, and intermediate filamental network, remained rather elusive. The extensions of the regular cytoarchitecture obviously must bind to the microtubular, microfilamental, and intermediate filamental network, must be abundant proteins by themselves, and their association must be a low affinity, highly dynamic association, making them difficult to isolate and study by conventional biochemical techniques.

Besides other proteins, such as members of the glycolytic pathway (7), molecular chaperones are excellent candidates for this purpose (Fig. 3; Ref. 3). They are abundant and all bind to filamentous actin, to tubulin, and most probably to the intermediate filaments. They form low affinity and highly dynamic complexes with each other, with the filaments, and with their target proteins. Besides their structural and perhaps protective role, chaperones also participate in the direction of cytoplasmic protein (9) and maybe RNA traffic. Disruption of Hsp90-organized chaperone complexes (often called the foldosome) leads to a slower translocation of various signaling molecules, including steroid receptors and several protein kinases. Fast translocation of these signaling components is linked to the cytoskeleton and directed by the foldosome-component immunophilins or $p50^{cdc37}$ chaperone (9).

Summary and perspectives

Besides the well established role of molecular chaperones in aiding protein folding, recent data raised the possibility of their participation in the eukaryotic cytoarchitecture, facilitating intracellular traffic of proteins and other macromolecules. Further studies are needed to explore whether these roles may be performed in parallel or whether they, at least in part, compete with each other. The early observations that cellular stress provokes an increased Brownian motion of endogenous intracellular particles (7) as well as the reduced chaperone efficiency of filamentous archebacterial 60-kDa heat shock proteins (11) indicate that chaperone-assisted folding and participation in

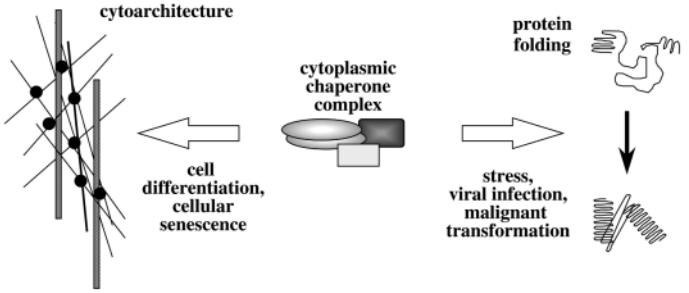

FIGURE 4. Possible interplay between chaperone-assisted protein folding and participation in the cytoarchitecture. Indirect evidence suggests that chaperone-assisted protein folding and chaperone participation in the cytoarchitecture may be competitive processes. This raises the possibility that cellular conditions requiring a more intensive assistance in protein folding (environmental stress, malignant transformation, viral infection, etc.) may disrupt the cytoplasmic meshwork and vice versa; a more developed cytoplasmic meshwork (e.g., in differentiated or senescent cells) may impair chaperone-assisted protein folding.

the cytoarchitecture may be competing processes. Cellular stress induces the buildup of massive amounts of misfolded proteins; chaperones help to rescue and refold them. This may impair their anchorage to the cytoarchitecture, and an increased diffusion may develop. Similarly, an accelerated protein synthesis, such as that of malignant or virally infected cells, may also impair cellular rigidity by shifting the chaperone aid toward protein folding instead of the regular support of the cytoarchitecture (Fig. 4).

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Molecular Structure of Tight Junctions and Their Role in Epithelial Transport

James Melvin Anderson

Tight junctions create a paracellular barrier with physiological properties that differ among epithelia. Among these differences are electrical resistance and discrimination for solute size and charge. Emerging evidence suggests that a large family of transmembrane proteins called the claudins create these variable properties.

Tight junctions create the major barrier regulating paracellular movement of water and solutes across vertebrate epithelia. This barrier is variable and physiologically regulated, and its disruption contributes to human diseases (13, 15). This review will first briefly define the basic physiological properties and morphological features of the tight junction. These have been known for decades but lacked a molecular explanation. The evidence will be presented that a large family of transmembrane proteins called the claudins, and to a lesser extent a single gene product called occludin, are responsible for these properties.

Morphology of tight junctions and their role in epithelial transport

Epithelial transport occurs through both transcellular and paracellular pathways (Fig. 1). Transcellular transport is directional, energy dependent, and governed by the cell-specific profile of transporters and channels positioned on the apical and basolateral cell membranes. In contrast, paracellular transport is passive and results from diffusion, electrodiffusion, or osmosis down the gradients created by transcellular mechanisms. The paracellular route does not show directional discrimination; however, it varies enormously among epithelia in terms of electrical resistance and shows small differences in ionic selectivities (Table 1). Thus the paracellular pathway complements transcellular mechanisms by defining the degree and selectivity of back leak for ions and solutes, making an important tissue-specific contribution to overall transport (Table 1) (2, 15). In some cases, the lateral intercellular space can influence paracellular electrical resistance; however, the tight junction is the major physical structure defining the specific properties of the paracellular barrier.

In transmission electron micrographs the tight junction appears as a series of close cell-cell contacts, and in freeze-fracture electron micrographs the contacts correspond to continuous rows of transmembrane protein particles (Figs. 2 and 3). The barrier is created where the particles meet in the para-

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