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Molecular chaperones, stress proteins and redox homeostasis[1]

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Abstract. Protection against oxidative stress is highly interrelated with the function of the most ancient cellular defense system, the network of molecular chaperones, heat shock, or stress-proteins. These ubiquitous, conserved proteins help other proteins and macromolecules to fold or re-fold and reach their final, native conformation. Redox regulation of protein folding becomes especially important during the preparation of extracellular proteins to the outside oxidative milieu, which should take place in a gradual and step-by-step controlled manner in the endoplasmic reticulum or in the periplasm. Several chaperones, such as members of the Hsp33 family in yeast and the plethora of small heat shock proteins as well as one of the major chaperones, Hsp70 are able to act against cytoplasmic oxidative damage. Abrupt changes of cellular redox status lead to chaperone induction. The function of several chaperones is tightly regulated by the surrounding redox conditions. Moreover, our recent data suggest that chaperones may act as a central switchboard for the transmission of redox changes in the life of the cell.

1. Introduction: Protein folding, heat shock proteins, molecular chaperones

Chaperones are ubiquitous, highly conserved proteins, which utilize a cycle of ATP-driven conformational changes to fold or refold their targets, and which probably played a major role in the molecular evolution of modern enzymes [1,2]. Environmental stress (a sudden change in the cellular environment, to which the cell is not prepared to respond, such as heat shock) leads to the expression of most chaperones, which therefore are called heat-shock, or stress proteins. Lacking a settled view about their action in the molecular level [3], chaperones are still best classified by their molecular weights (Table 1).

Higher levels of cellular organization also need a constant remodeling. About twenty years ago Keith A. Porter and co-workers suggested the existence of a cellular meshwork, called as "microtrabecular lattice" to organize cytoplasmic proteins and RNA-s [4]. As time passes more and more data provide indirect evidence for a high-order organization of the cytoplasm [5]. Chaperones are ideal candidates for being a major constituent of this cytoplasmic meshwork: they are highly abundant, form a loose and dynamic complex with all the elements of the cytoskeleton and each other, and also attach to a plethora of other proteins. Several lines of initial evidence shows that chaperones are necessary for the cellular trafficking of several proteins such as the steroid receptors and that disruption of chaperone/protein complexes accelerates cell lysis [6–8].

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Table 1
Major classes of molecular chaperones

Most important eukaryotic representatives[a]	Reviews
small heat shock proteins (e.g. Hsp27[b])	26
Hsp60	1, 62
Hsp70 (Hsc70[c], Grp78)	1, 62
Hsp90	6, 63
Hsp104	64
Peptidyl-prolyl *cis/trans*-isomerases	65
Protein disulfide isomerases	17,18

[a]Co-chaperones (chaperones which help the function of other chaperones listed) were not included in this table, albeit almost all of these proteins also possess a "traditional" chaperone activity in their own right. Several chaperones of the endoplasmic reticulum (e.g. calreticulin, calnexin, etc.), which do not belong to any of the major chaperone families, as well as some heat shock proteins (e.g. ubiquitin), which do not possess chaperone activity were also not mentioned.

[b]The abbreviation "Hsp" and "Grp" refer to heat shock proteins, and glucose regulated proteins, chaperones induced by heat shock or glucose deprivation, respectively. Numbers refer to their molecular weight in kDa.

[c]Hsc70 denotes the cognate (constitutively expressed) 70 kDa heat shock protein homologue in the cytoplasm.

2. Redox chaperones in the endoplasmic reticulum and in the periplasm: quality control of secreted proteins

Secreted proteins have to be prepared for the oxidative milieu of the extracellular space. A rapid oxidation would result in the formation of numerous incorrect disulfide bridges, which would lock the protein in a distorted conformation. Therefore folding of secreted/plasma membrane proteins is most probably accompanied by their gradual oxidation in the endoplasmic reticulum (ER). This would imply the existence of a redox gradient along the secretory pathway. The first tools, such as the redox sensitive green fluorescent protein (GFP, which, in fact, has a yellow color in this case, 9) to measure this putative gradient have already been established.

Another mechanism for the control of gradual oxidation is the reorganization of rapidly formed, incorrect disulfide bonds. This is performed by the numerous protein disulfide isomerases (PDI-s). These proteins have been discovered independently by the group of Christian Anfinsen [10], and by Pal Venetianer and Bruno Straub [11] in Hungary almost forty years ago. In the meantime the existence of a large number of the family has been uncovered, such as Erp29, Erp57, Erp59, Erp72 and others. The exact substrate specificity of these enzymes is not known. However, Erp59 seems to be the most abundant member of the family and Erp72 acts mainly on glycosylated proteins. PDI-s have two types of activity: beside the disulfide isomerase function they also act as conventional chaperones [12]. PDIs cooperate with other chaperones, such as calnexin, calreticulin and Grp78 in the ER [13,14]. The correct chaperone/target ratio is very important in the action of PDI-s. In case of a large excess of targets PDI acts as an "anti-chaperone" promoting inter- and not intramolecular disulfide bridge formation [15]. Therefore, in case of ER-overload or after the poisoning by reducing agents PDI-s may promote the formation of covalently linked aggregates instead of their usual role as redox-chaperones. However, this aggregates are not always useless. In certain cases this protein aggregates containing of PDI and

misfolded proteins are limited in size, and remain degradable by the mechanism of ER-associated protein degradation (ERAD) instead of forming large, hopelessly insoluble deposits [16].

Besides the reorganization of incorrect disulfide bridges protein disulfide isomerases also participate in the direct oxidation of secreted proteins [17,18]. In these cases oxidized protein disulfide isomerases are reduced by the ER transmembrane proteins Ero1-L-alpha and Ero1-L-beta requiring flavin adenine dinucleotide as a cofactor. The two Ero-s seem to act on different PDI-s, and the more active form, Ero1-L-beta is over-regulated, if the ER experiences an excess of unfolded proteins [19]. In contrast of yeast PDI, which is present predominantly in the oxidized state, most mammalian PDI-s are partially reduced. The small pool of oxidized PDI suggests that in mammalian cells oxidative equivalents are rapidly transferred to cargo proteins [19] or the redox gradient along the secretory pathway is more expressed than in yeast.

Protein disulfide isomerases are not always ER-resident proteins. They are also secreted to the extracellular medium, where they continue their folding assistance, and prevent the formation of extracellular protein aggregates [20,21]. In the *E. coli* periplasm a slightly different mechanism controls the oxidation of proteins to that of the ER: oxidation is achieved by a separate arm of proteins involving DsbA, which is reoxidized by the inner membrane protein DsbB. The *E. coli* protein disulfide isomerases are DsbC and DsbG, which are reoxidized by the membrane protein DsbD [17].

3. Redox chaperones in the cytoplasm: Another defense against oxidative damage

In all aerobic organisms active oxygen species are produced even under physiological conditions. A variety of antioxidant systems exists in the cytoplasm to diminish the oxidative damage. This important task is performed by enzymes like superoxide dismutase, peroxidase or catalase, or by antioxidants, such as ascorbic acid, coenzyme Q and glutathione (GSH) which can be easily oxidized and provide a large redox buffer capacity for the cell. Gluthationylation, i.e. thiol-disulfide exchange between a cysteine residue in a protein, and the oxidized form of glutathione (GSSG) occurs in oxidative stress. This process serves as a redox-dependent regulator of various protein functions [22], like the inactivation of the AP-1 or p53 transcription factors, the PTP-1B phosphotyrosine phosphatase [23] or the cAMP-dependent protein kinase [24].

Other systems specifically protect the sensitive molecules, mostly proteins from the damage. In this process traditional chaperones (Table 1), e.g. the small heat shock proteins and Hsp70 can also serve as cytoplasmic “antioxidants” (Table 2). They protect their target proteins by covering their sensitive sites. Sometimes all this mechanisms are not effective enough, and the oxidative damage is prevails. In this case Hsp-s capture denatured proteins and hold them until their refolding or degradation.

These mechanisms are connected to each other in many ways. Small heat shock proteins elevate reduced glutathione levels by promoting an increase in glucose-6-phosphate dehydrogenase activity and by a somewhat smaller activation of glutathione reductase and glutathione transferase [25,26]. Heme oxygenase is a heat shock protein responsible for the production of the antioxidants biliverdin and bilirubin [27].

Sulfur containing amino acids (cysteine and methionine) are susceptible to oxidation. SH-groups of cysteine residues of some specific proteins, (such as the glucocorticoid receptor) are maintained in the reduced state or just inversely: oxidized by the thioredoxins in the cytoplasm [28].

Oxidized methionines can be reduced by special enzymes, the methionine sulfoxide reductases, MSRs [29–31]. An increasing number of evidence indicate that this system may serve as an additional antioxidant mechanism scavenging oxidative agents [32,33]. The prokaryotic homologue of

Table 2
Cytoplasmic redox chaperones

Chaperone	Function	References
small heat shock proteins (Hsp25, Hsp27)	increases reduced glutathione levels by increased glucose-6-phosphate dehydrogenase, glutathione reductase and glutathione transferase activities	25,26
Hsp32a	heme oxygenase-1, an important component of oxidative stress-mediated cell injury	27
Hsp33	oxidation-activated chaperone in yeast	49
Hsp70	has (probably indirect) anti-oxidant properties	38
cytochrome c	released from mitochondria in apoptosis, chaperone function has been detected	35
thioredoxin[a]	promotes cytoplasmic oxidation of selected proteins	28
ERV1/ALR[a]	promotes the cytoplasmic formation of disulfide bridges	29
MsrA and MsrB[a]	methionine sulfoxide reductase: regenerates functional methionine	30

[a]No direct chaperone activity has been demonstrated yet.

MSRs can be integrated into the outer membrane helping the survival of the pathogen in the presence of reactive oxygen species secreted by the host [34].

An interesting member of redox cytoplasmic chaperones is cytochrome c, which is only a “guest” in the cytoplasm during its apoptotic release from mitochondria and has been established as a chaperone a long time ago [35].

4. Redox control of chaperone induction

Oxidative stress leads to a massive induction of heat shock proteins. This is partly mediated by the oxidatively damaged proteins [36], which occupy chaperone-binding sites, and liberate heat shock factor 1 (HSF1), the transcription factor responsible for Hsp induction [37]. However, the redox state of the cell also influences HSF1 activity directly. Thus a decrease in reduced glutathione level [38] may lead to a direct activation of HSF1 [39]. Strong oxidative agents inhibit the trimerization of HSF1 blocking its DNA-binding ability [40]. A more reduced cellular environment also helps chaperone induction [41] though millimolar concentration of a reducing agent impairs the activation of HSF1 [42]. As a summary of these results, mild changes of redox homeostasis lead to the activation of HSF1. However, large changes in redox homeostasis cause HSF1 inhibition. HSF1 also regulates the redox state of the cell. HSF1 activation leads to a rapid elevation of reduced glutathione [43]. Morever, HSF1 seems to play a key role in maintaining the intracellular redox balance even at physiological conditions [44].

5. Redox regulation of chaperone function

Cytoplasmic chaperones, such as small heat shock proteins, or Hsp90 usually loose their activity after the oxidation of their cysteine or methionine residues [45,46]. Both in Hsp70 and Hsp90 the oxidation-prone cysteine is in the close vicinity of an ATP binding site [46,47], which may explain the rapid loss of their chaperone activity after oxidation. Small heat shock proteins were recently established as ATP-binding chaperones, therefore the above explanation may have a more general implication than we previously thought. Chaperone-inactivation may also occur by S-nitrosylation after NO, or peroxonitrite addition.

On the contrary to the above general trend, Hsp70 was shown to acquire both enhanced peptide binding ability and peptide complex stability under oxidative conditions [48]. Similarly, the yeast cytoplasmic chaperone, Hsp33 is activated after oxidative stress [49]. Though its homologues have not been found in higher eukaryotes, a similar mechanism would be very logical to operate in other cells. Rapoport and co-workers [50] raised the interesting possibility that oxidation of PDI may trigger a release of its substrate, which would then travel further in the secretory chain or, as in the case of cholera toxin, would be a subject of a retrograde transport back to the cytoplasm.

The activity of thioredoxins is also under redox control, i.e. thioredoxins can undergo glutathionylation during oxidative stress [51]. The picture becomes even more composed considering the fact that these proteins have a variety of other functions in cell life: they act as a cytokine, chemokine as well as a growth factor extracellularly, and modify the effect of several regulatory factors intracellularly [52,53]. The question arises, whether these functions are modified under glutathionylation and participating in the signalisation of the oxidative stress.

6. Changes of chaperones and redox function in disease and aging

In several diseases, such as in endothelial dysfunction, in diabetes, in Alzheimer and Parkinson diseases the redox homeostasis becomes severely damaged [54,55]. The amount of oxidized proteins increases, which requires a larger amount of chaperones to cope with the conformational damage and leads to chaperone induction. However, chronic stress exhausts the chaperone-induction signalling mechanisms, and damaged proteins begin to accumulate. Moreover, oxidized proteins are much poorer substrates, but highly effective inhibitors of the proteasome [56]. All these changes also occur during aging with the concomitant decrease in the chaperone-induction capacity of the aging organism [57]. In contrast, enhanced antioxidant systems (such as the overexpression of Cu-Zn-superoxide dismutase) as well as an increased amount of heat shock proteins leads to longevity [58].

7. Perspectives: Chaperones as central players in the transmission of redox changes to the life of the cell

Oxidative damage, together with other proteotoxic insults during the propagation of various diseases and aging results in a change between the ratio between damaged proteins and available chaperone capacity. The chaperone-overload, which is a consequence of these events leads to rather unexpected changes. Recently one of the major cytoplasmic chaperones, Hsp90, was shown to act as posttranslational "silencer" of several genetical changes by assisting in an efficient repair of folding defects [59]. After a large stress transient chaperone-overload prevents the conformational repair of misfolded mutants. Therefore many, previously hidden genotypical changes appear in the phenotype resulting in a "boom" of genetical variations in the whole population. This may help the selection of a beneficial change, which, in turn, may help the adaptation of the population to the changing environmental conditions.

Under stressful conditions most of the exposed mutations are disadvantageous, and tend to disappear from the population by natural selection. According to a recent hypothesis [60] the development of modern medical practice depressed natural selection by its groundbreaking achievements to reduce prenatal and infant mortality leading to a rise of phenotypically silent mutations in the genome. As a consequence we carry more and more chaperone-buffered, silent mutations from generation to generation. The chance of the phenotypic manifestation of these mutations becomes especially large in aged subjects, where

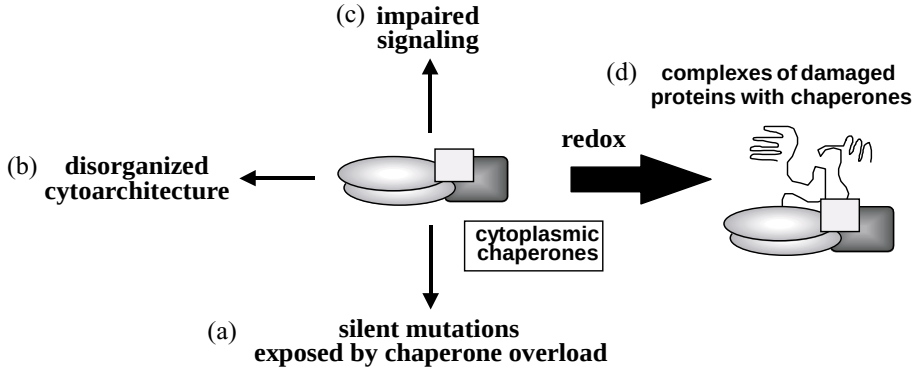

Fig. 1. Chaperones as major transmitters of changes in redox homeostasis to the life of the whole cell. Clockwise from bottom: (a) phenotypically buffered, silent mutations require the assistance of chaperones to rescue them from folding traps [59,60]. (b) Chaperones form low affinity and highly dynamic extensions of the cytoskeleton participating in cellular traffic and in the organization of the cytoarchitecture [6–8]. (c) Cytoplasmic chaperones of eukaryotic cells participate in the maintenance of the conformation of some, selected protein substrates. Most of these unstable proteins are parts of various signalling cascades [6, 63]. (d) After changes in the redox homeostasis, chaperones become more and more occupied by damaged proteins. As a consequence of this: (a) silent mutations escape and contribute to the onset of polygenic diseases; (b) cell architecture becomes disorganized; (c) signalling is impaired. The verification of these presently largely hypothetical changes requires further experimentation.

protein damage is abundant, and chaperone induction is impaired [61]. The background of misfolded proteins increases and by competition prevents the chaperone-mediated buffering of silent mutations. Phenotypically exposed mutations contribute to a more abundant manifestation of multigene-diseases, such as atherosclerosis, autoimmune-type diseases, cancer, diabetes, hypertensive cardiovascular disease and several psychiatric illnesses (Alzheimer disease, schizophrenia, etc.). The “chaperone overload” hypothesis emphasizes the need for efficient ways to enhance chaperone-capacity in aging subjects, and calls for the identification and future “repair” of silent mutations [60].

After oxidative damage in the cytoplasm or reductive damage in the endoplasmic reticulum, the resulting chaperone overload changes the whole life of the cell. Besides the exposure of the previously hidden mutations signaling becomes impaired and the cellular architecture is disorganized (Fig. 1). Chaperones may act as central players of the transmission of redox changes in the life of the cell.

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