Redox Regulation in Protein Folding and Chaperone Function

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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 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). Besides to promote the formation of the correct conformation of nascent or damaged proteins chaperones also assist in the formation of correct disulfide bridges offering the help protein disulfide isomeras (PDI-s) [4,5].

Higher levels of cellular organization also need a constant remodeling. Chaperones are obvious candidates to provide help in these processes. About twenty years ago based on high-voltage electron microscopy Keith A. Porter and co-workers suggested the existence of a cellular meshwork, called as "microtubular lattice" to organize cytoplasmic proteins and RNA-s [6]. Almost instantly a fierce debate arose considering the lattice as an artifact of the techniques used. However, as time passes more and more data provide indirect evidence for a high-order organization of the cytoplasm [7]. Chaperones are ideal candidates for being a major constituent of a 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 disruption of chaperone/protein complexes disturbs the organization of cytoplasmic traffic of several proteins, such as the steroid receptors, and accelerates cell lysis [8-10].

2. Redox Chaperones in the Endoplasmic Reticulum and in the Periplasm: Quality Control of Secreted Proteins

Secreted proteins have to 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 colour in this case, [11]) to measure this putative gradient have already been established.

Table 1. Major classes of molecular chaperones

<table>
<thead>
<tr>
<th>Most important eukaryotic representatives</th>
<th>Reviews</th>
</tr>
</thead>
<tbody>
<tr>
<td>small heat shock proteins (e.g. Hsp27)</td>
<td>21</td>
</tr>
<tr>
<td>Hsp60</td>
<td>1,46</td>
</tr>
<tr>
<td>Hsp70 (Hsc70, Grp78)</td>
<td>1,46</td>
</tr>
<tr>
<td>Hsp90</td>
<td>8,47</td>
</tr>
<tr>
<td>Hsp104</td>
<td>18</td>
</tr>
<tr>
<td>Proline- and prolyl cis/trans-isomerases</td>
<td>45</td>
</tr>
<tr>
<td>Protein disulfide isomeraseases</td>
<td>45</td>
</tr>
</tbody>
</table>

All chaperones whose expression was not included in this review; among all of these proteins also possess a "traditional" chaperone activity in their own right.

The chaperones of the endoplasmic reticulum (i.e. a calreticulin, calnexin, etc.) which do not belong to one 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.

For abbreviations: Hsp and Grp refer to heat shock proteins, and glucose regulated proteins, chaperones targeted by heat shock or glucose deprivation, respectively. Numbers refer to their molecular weight in kDa.

As to denote: the prokaryotically constitutively expressed 23 kDa heat shock protein homologue in the prokaryon.

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 isomerasers (PDIs). These proteins have been discovered independently by the group of Christian Anfinsen [12], and by Pal Venetianer and Bruno Straub [13] in Hungary almost forty years ago. In the meantime the existence of a large number of the family has been discovered, 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. The formation of disulfide bridges requires the accessibility and correct positioning of SH groups. This is achieved by the "conventional" chaperone activity of protein disulfide isomerasers [14] as well as by their cooperation with other chaperones, such as Grp78 in the ER [15]. The correct chaperone/target ratio is very important in the action of PDIs. In case of a large excess of targets PDI acts as an "anti-chaperone" promoting inter- and not intramolecular disulfide bridge formation [16]. Therefore, in case of ER-overload or after the poisoning by reducing agents PDIs may promote the formation of covalently linked aggregates instead of their usual role as redox-chaperones.

Besides the reorganization of incorrect disulfide bridges protein disulfide isomerasers also participate in the direct oxidation of secreted proteins. In these cases oxidized protein disulfide isomerasers are reduced by the ER transmembrane proteins Erg1-L-alpha and Erg1-L-beta requiring flavin adenine dinucleotide as a cofactor. The two Erg-s seem to act on
different PDI-s, and the more active form, Ero1-L-beta is overregulated if the ER experiences an excess of unfolded proteins [17]. 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 [17] 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 [18,19]. 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 [4,5].

### Table 2. Cytoplasmic redox chaperones

<table>
<thead>
<tr>
<th>Chaperone</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>small heat shock proteins (Hsp25, Hsp27)</td>
<td>increases reduced glutathione levels by increased glucose-6-phosphate dehydrogenase, glutathione reductase and glutathione transferase activities</td>
<td>20,21</td>
</tr>
<tr>
<td>Hsp32</td>
<td>heme oxygenase-1, an important component of oxidative stress-mediated cell injury</td>
<td>22</td>
</tr>
<tr>
<td>Hsp33</td>
<td>oxidation-activated chaperone in yeast</td>
<td>23</td>
</tr>
<tr>
<td>Hsp70</td>
<td>has (probably indirect) anti-oxidant properties</td>
<td>50</td>
</tr>
<tr>
<td>cytochrome c</td>
<td>released from mitochondria in apoptosis, chaperone function has been detected</td>
<td>27</td>
</tr>
<tr>
<td>thioredoxin</td>
<td>promotes cytoplasmic oxidation of selected proteins</td>
<td>24</td>
</tr>
<tr>
<td>ERV1/ALR*</td>
<td>promotes the cytoplasmic formation of disulfide bridges</td>
<td>25</td>
</tr>
<tr>
<td>MsrA and MsrB*</td>
<td>methionine sulfoxide reductase: regenerates functional methionine</td>
<td>26</td>
</tr>
</tbody>
</table>

*No direct chaperone activity has been demonstrated yet.

### 3. Redox Chaperones in the Cytoplasm: Another Defense against Oxidative Damage

From the traditional chaperones (Table 1) the small heat shock proteins and Hsp70 act as cytoplasmic "antioxidants" (Table 2). 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 [20,21]. Heme oxygenase a heat shock protein responsible for the production of the antioxidants...
bilevelurin and bilirubin is an important component of cellular defense mechanisms against oxidative stress [22]. Yeast cells contain a rather unique chaperone, Hsp33, which is activated after oxidative stress [23]. SH-groups of some specific proteins (such as the glucocorticoid receptor) are maintained in the reduced state or just inversely: oxidized by thioredoxins in the cytoplasm [24]. In turn, these proteins are most probably oxidized by members of the ERV1/ALR protein family [25]. Oxidized methionines are reduced by special enzymes, the methionine sulfoxide reductases [26]. An interesting member of the oxidase cytoplasmic chaperones is cytochrome c, which is only a 'guest' in the cytoplasm due to its apoprotein release from mitochondria and has been established as a chaperone a long time ago [27].

4. Redox Control of Chaperone Induction

Oxidative stress leads to a massive induction of heat shock proteins. This is partly mediated by the oxidation-induced formation of damaged proteins [28], which occupy chaperone-binding sites and liberate heat shock factor 1 (HSF1), the transcription factor responsible for Hsp induction [29]. However, a decrease in reduced glutathione levels (by oxidation or by the formation of S-nitroso-glutathione by NO) [30] may also lead to a direct activation of HSF1 [31]. Interestingly, a more reduced cellular environment also helps chaperone induction [32]. In agreement with this, heat shock itself leads to a rapid elevation of reduced glutathione [33]. On the other hand, millimolar concentration of a reducing agent impairs the activation of HSF1 [34].

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 [35,36]. Both in Hsp70 and Hsp90 the oxidation-prone cysteine is in the close vicinity of an ATP binding site [36,37], 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 peroxynitrite addition. On the contrary to this general trend, the yeast cytoplasmic chaperone, Hsp33 is activated after oxidative stress [23]. 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 [38] 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.

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 [39,40]. 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 [41]. All these changes also occur during ageing with the
concomitant decrease in the chaperone-induction capacity of the aging organism [42]. 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 [43].

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 [44,45]. (b) Chaperones form low affinity and highly dynamic extensions of the cytoskeleton participating in cellular traffic and in the organisation of the cytoarchitecture [8-10]. (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 [8,47]. (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 disorganised; (c) signalling is impaired. The verification of these - presently largely hypothetical - changes requires further experimentation.

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 ageing 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 genetic changes by assisting in an efficient repair of folding defects [44]. 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 [45] 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 phenotype manifestation of these mutations becomes especially large in aged subjects, where protein damage is abundant, and chaperone induction is impaired. The background of unfolded 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 ageing subjects, and calls for the identification and future “repair” of silent mutations [45].

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 (Figure 1). Chaperones may act as central players of the transmission of redox changes in the life of the cell.

References


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