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Molecular chaperones as regulatory elements of cellular networks

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Molecular chaperones help hundreds of signaling molecules to keep their activation-competent state, and regulate various signaling processes ranging from signaling at the plasma membrane to transcription. Besides these specific regulatory roles, recent studies have revealed that chaperones act as genetic buffers stabilizing the phenotypes of various cells and organisms. This may be related to their low affinity for the proteins they interact with, which means that they represent weak links in protein networks. Chaperones may uncouple protein, signaling, membrane, organelle and transcriptional networks during stress, which gives the cell additional protection. The same networks are preferentially remodeled in various diseases and aging, which may help us to design novel therapeutic and anti-aging strategies.

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Introduction

The term 'molecular chaperone' denotes a large family of abundant, ubiquitous proteins that form an ancient defense system in our cells. Chaperones promote cell survival by sequestering damaged proteins and preventing their aggregation. During stressful conditions, such as elevated temperature, they prevent protein aggregation by facilitating the refolding or elimination of misfolded proteins. The stress-induced response to damaged proteins is helped by a sophisticated regulatory system, which shuts down most cellular functions and, in parallel, induces the synthesis of several chaperones and other survival-promoting proteins. Therefore, many of the chaperones are also called stress or 'heat shock' proteins in reference to the archetype of cellular stress, heat shock. Besides their role during stress, chaperones have multiple roles under normal conditions. They promote the transport of macromolecules (e.g. proteins or RNA) and participate in almost every remodeling event involving larger protein complexes, including signaling, transcription, cell division, migration, differentiation, etc [1–3]. The multiple roles of chaperones have inflated the term, which is now used to describe almost any protein (or RNA) that transiently accompanies other molecules and promotes their transport or assembly to larger complexes. Thus chaperones for RNAs, copper and lipids have also been described. Certain chaperones are specialized to a single protein or to a small class of proteins, like the chaperones of catenin, collagen, the major histocompatibility complex, myosin and others. The term 'intramolecular chaperone' has been coined for protein segments (usually residing in the N terminus) that help the folding of the rest of the protein. Moreover, small compounds can be termed 'chemical chaperones', and are used in clinical practice to cure protein folding diseases. Space limitations restrict this review to the 'original' chaperones: those protein chaperones that have multiple protein substrates.

Chaperones mostly form low-affinity, dynamic, temporary interactions (weak links) in cellular networks. Given that chaperones generally have a large number of partners, they behave like hubs in protein–protein interaction networks. Moreover, many chaperone effects (e.g. cell survival or changes in the phenotype diversity) are typical integrative properties, which can rarely be understood by studying the individual chaperone–client interactions exclusively. Thus the network approach is a promising tool to explain some key aspects of chaperone function [3,4••,5•,6•]. We will highlight several potential connections between the individual chaperone–protein contacts and cellular networks, and will explain how some aspects of the network approach can be used to understand the integrative properties of chaperone-mediated regulation. Finally, we will show how the network approach is linked to chaperone-related therapeutic and anti-aging strategies.

Chaperones and cellular networks

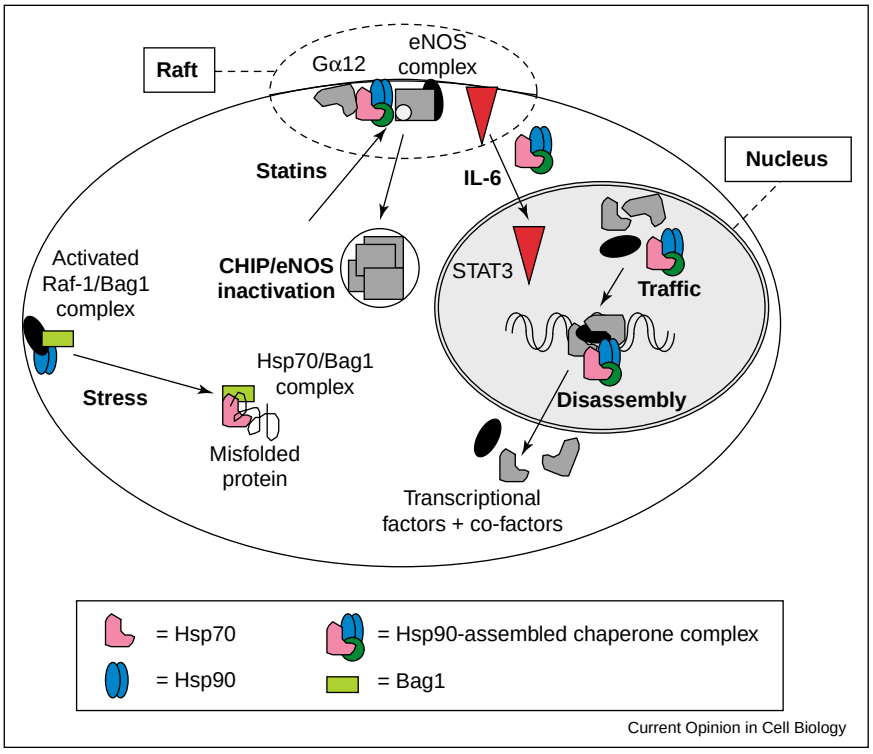
Chaperones form large complexes and have a large number of co-chaperones to regulate their activity, binding properties and function [1–3]. These chaperone complexes regulate local protein networks, such as the mitochondrial protein transport apparatus [7] and the assembly [8] and substrate specificity [9•] of the major cytoplasmic proteolytical system, the proteasome.

Chaperones and the signaling, membrane and organelle networks

The major chaperone-regulated cellular networks are related to signaling, membrane structure and transcription. Though the network approach has been worked out only for segments of the whole signaling network or 'signalome' [10], chaperones may be important elements in the promotion of cross-talk between various signaling processes. The Hsp90 chaperone complex promotes the maturation of >100 kinase substrates including several members of the Raf-1-related signaling pathway. The antiapoptotic protein Bag1 (Bcl-2-associated athanogene protein 1) activates this pathway. Under stress, Bag1 is associated with another chaperone, Hsp70 (70-kDa heat shock protein), which leads to the attenuation of Bag1-mediated Raf-1 activation (Figure 1). Thus, the Bag1/Raf-1 interaction may contribute to the mechanism underpinning how stress shuts down cell proliferation [11].

Another well-known chaperone-mediated signaling pathway, the activation of nitric oxide synthases, gives us an example of chaperone effects on various membranes. The endothelial nitric oxide synthase (eNOS) is activated if assembled to a raft-associated complex containing Hsp90 (90-kDa heat shock protein), the Akt kinase (protein kinase B) and calmodulin. The formation of this complex is helped by statins, the widely used anti-atherosclerotic drugs [12]. A co-chaperone of Hsp70 and Hsp90, CHIP (carboxyl terminus of Hsc70-interacting protein), redirects the maturating eNOS, which usually follows a Golgi-to-plasma-membrane route, into an insoluble cellular compartment, leading to its inactivation [13]. Both Hsp90 and Hsp70 are raft-associated chaperones [14]. Besides its role in eNOS trafficking, Hsp90 helps the GTP-binding protein $G_{\alpha 12}$ to associate with membrane rafts [15] and promotes the traffic of STAT3 (signal transducer and activator of transcription protein 3) from membrane rafts to the cell nucleus after interleukin-6 stimulation [16] (Figure 1). Rab3A, a key player in Ca-dependent exocytosis, is also regulated by the Hsp90/Hsp70/cysteine string protein chaperone complex in synaptic membranes [17]. Finally, studies of *Vígh et al.* showed that chaperones may have a general role in membrane stabilization [18]. All these examples link chaperones to the membrane network of the cell, which integrates the plasma membrane, the endoplasmic reticulum (ER), various vesicles, the nuclear membrane and

Figure 1



Molecular chaperones in the regulation of signaling: a few recent advances. Chaperones play an essential role in the maturation and activation of hundreds of protein kinases. Bag1, the co-chaperone of Hsp70, can activate the Hsp90-dependent Raf-1 kinase. Sequestration of Bag1 by Hsp70 during stress may provide a mechanism for how stress shuts down cell proliferation. Chaperones participate in raft-dependent signaling of eNOS, G-proteins, and STATs. Chaperones also help the subnuclear traffic and disassembly of transcriptional factors and related complexes. (Please note that members of the Hsp90-associated chaperone complex vary in the different pathways and are shown using the same symbol only for clarity.)

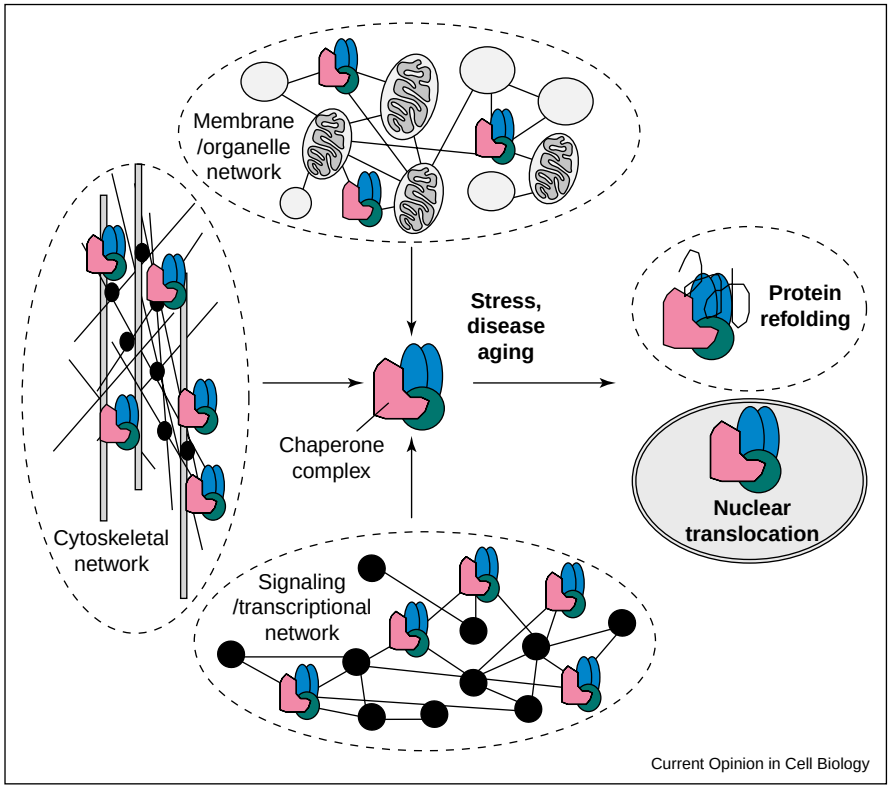
mitochondria [19–21]. Moreover, chaperones may facilitate cytoplasmic traffic [3,22–25]. Links between the ER, the mitochondria and the cytoplasm have already been shown to signal messages of cellular stress between these compartments [26–28]. Chaperones may emerge as stabilizers and regulators of the connectivity and traffic of these important networks.

The connections between elements of the mitochondrial network, the ER, nuclear membranes and the cytoplasmic meshwork may be key points of cellular integrity and information transfer, while de-coupling of these segments may be an efficient protection against any cellular damage. One might expect that chaperones are needed for an efficient coupling of these cellular networks. De-coupling of network elements and modules is a widely used method to stop the propagation of damage [4••,6•]. In case of stress, the increased occupancy of chaperones by damaged proteins together with the stress-induced translocation of chaperones to the nucleus [1–3,29] might lead to an 'automatic' de-coupling of network elements and modules, providing the cell periphery with an additional safety measure (Figure 2).

Chaperones and the transcriptional network

Chaperones are well known to protect the cell nucleus after stress. As a novel version of this role, Hsp70 was shown to drive damaged nuclear proteins to the nucleolus, clearing other nuclear components of misfolded proteins and decreasing the danger of their widespread aggregation [29]. In agreement with these findings, chaperones promote the transport of ribosomal subunits [30] and the mobility of steroid receptors inside the nucleus [31]. Molecular chaperones regulate both the activation [32–34] and the disassembly of numerous transcriptional complexes [35,36] (Figure 1). Thus, chaperones emerge

Figure 2



Chaperones as regulators of cellular networks. Chaperones emerge as integrative regulators of the signaling/transcriptional, cytoskeletal and membrane/organelle networks of the cell. Modification of chaperones as well as a change in the extent to which they are required in various networks may affect most of the other connected cellular functions. As an example, stress (disease or aging) may induce a chaperone-mediated de-coupling of cellular integrity, severing the connections between organelles (e.g. mitochondria, ER, the nuclear membrane and vesicles) as well as preventing cytoplasmic traffic. Signaling and transcriptional regulation are also likely to be impaired. Stress is accompanied by the translocation of chaperones to the nucleus, where they work to maintain the remodeling capacity in the nucleus while promoting the temporary fragmentation of all networks in the cell periphery. The residual or newly formed links between network members are typically weaker than the original connections were, which may decrease cellular noise and provide an additional level of system stabilization [6•]. Thick and thin lines denote strong and weak links, respectively. (Please note that link strengths change continuously in the cell; therefore, the clear discrimination between strong and weak links, as well as the identity of all chaperone complexes, are for clarity only.) Black circles denote protein elements of the cytoskeletal and signaling/transcriptional networks. Dotted lines demarcate various networks from each other. Obviously all these networks overlap in the cellular context.

as regulators of the transcriptional network [37]. Stress-induced nuclear translocation of chaperones may preserve nuclear remodeling capacity during environmental damage, and thus protect the integrity of DNA.

Emergent properties of the chaperone-regulated cellular networks

The previous examples showed that chaperones are involved in the regulation of signaling, organelle, membrane, cytoskeletal and transcriptional networks (Figure 2). However, relatively little is known about the chaperone-mediated, emergent properties of cellular functions. One of the most important advances in this area came from Susan Lindquist and her co-workers when they discovered that Hsp90 acts as a buffer of genetic changes in *Drosophila* [38] and in *Arabidopsis* [39]. A recent paper suggests that this effect might originate epigenetically from Hsp90-induced heritable changes in the chromatin structure [40•].

Chaperone overload

Chaperone-induced genetic buffering is diminished during stress, which causes the sudden appearance of the phenotype of previously hidden mutations, thereby promoting population survival by providing a possible molecular mechanism for fast evolutionary changes [38,39]. On the other hand, the stress-induced appearance of genetic variation at the level of the phenotype cleanses the genome of the population by allowing the disappearance of disadvantageous mutations by natural selection. Chaperones are highly conserved proteins [1–3], so similar mechanisms might operate in humans. Moreover, the tremendous advance of medicine in the last two hundred years has significantly reduced the effects of natural selection and potentially increased the accumulation of hidden mutations in the human genome. However, chaperones may become occupied by the damaged proteins in aged organisms (half of cellular proteins of 70–80 years old humans may be already oxidized), resulting in a chaperone overload. As a consequence the protein products harboring the 'hidden mutations' may be released and may contribute to the development of civilization diseases, such as cancer, atherosclerosis and diabetes [41–43]. This effect may be negligible today, although it will increase with each generation. Still, we probably have many hundreds of years to think about a possible solution.

Chaperones as weak links

Recent findings [44•,45•] raised the idea that not only chaperones but a large number of other proteins may also regulate the phenotypic diversity of the population. Though a relatively small number of other regulators have been uncovered so far, it seems unlikely that a common molecular mechanism, such as involvement in signaling or in modifications to the chromatin structure, can explain all the effects observed. If a general explanation is sought, it is more likely to be related to the network properties of the cell. In this context, chaperones are typical weak linkers, providing low-affinity, low-probability contacts with other proteins (Figure 2). Weak links are known to promote system stability in a large variety of networks from macromolecules to social networks and ecosystems, which suggests that this may be a general network-level phenomenon explaining many of the genetic buffering effects of chaperones [6•].

Chaperone therapies

Cellular networks are remodeled under stress [46] and in various diseases. Effective interventions to push the equilibrium towards the original state may not be limited to single-target drugs with a well-designed, high affinity interaction with one of the cellular proteins. In agreement with this general assumption, several examples show that multi-target therapy may be superior to the usual single-target approach [47]. The best known examples of multi-target drugs include Aspirin, Metformin or Gleevec as well as combinatorial therapy and natural remedies. Because of the multiple regulatory roles of chaperones, chaperone modulators provide additional examples of multi-target drugs. Indeed, chaperone substitution (in the form of chemical chaperones [48]), the pharmacological help of chaperone induction by stress, termed chaperone co-induction [49•], and chaperone inhibition [50•] are all promising therapeutic strategies. Both chaperone co-inducers and chaperone inhibitors, including geldanamycin analogues and other Hsp90 inhibitors, have recently completed successful clinical trials.

Conclusions

Chaperones regulate cellular functions at two levels. In several cases they interact with a specific target protein and help it to fold after synthesis, or re-fold after stress. These strong interactions make chaperones important parts of the central scaffold of cellular networks, such as the protein net, the signaling network, the membrane and organelle network and the transcriptional network. However, in most cases chaperones have only a low-affinity, temporary, weak interaction with most of their targets (Figure 2). Changes to these interactions do not affect the general behavior of the whole network, the cell. However, inhibition of these weak interactions might lead to a rise in cellular noise and the destabilization and disintegration of the whole network and by promoting an 'error catastrophe' help us to combat cancer [50•]. In contrast, chaperone activation might decrease cellular noise, and consequent cell-stabilization might give an additional, indirect help to prevent protein folding diseases including various forms of neurodegeneration, such as amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and Huntington's disease [48,49•]. Besides slowing or reversing the development these diseases, chaperone-based therapies may also generally benefit the aging organism by stabilizing its cells and functions. Thus properly working chaperones may be key

players to help us achieve improved life conditions at an advanced age. The assessment of the multiple roles of chaperones in the context of cellular networks is just beginning.

Update

Recently a promising model was developed to integrate various chaperone-dependent and other elements in the signaling network leading to the activation of heat shock factor-1 and the consequent synthesis of molecular chaperones [51]. Hsp90 was shown to act as a molecular switch of the Erb-B2 oncogenic tyrosine kinase signaling network by regulating the heterodimer formation between Erb-B2 and various other kinases [52]. This extends the membrane-dependent remodeling effects of Hsp90 to a novel field. As a theoretical contribution to chaperone therapies, the efficiency of multi-target drugs over single target drugs has been summarized, and a new drug-design paradigm was proposed in a recent publication [53].

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