Systems biology of molecular chaperone networks

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Abstract. Molecular chaperones are not only fascinating molecular machines, which help the folding, refolding, activation or assembly of other proteins, but also have a number of functions, which can be understood only by considering the emergent properties of cellular networks—and that of chaperones as special network constituents. As a notable example for the network-related roles of chaperones they may act as genetic buffers stabilizing the phenotype of various cells and organisms, and may serve as potential regulators of evolvability. Why are chaperones special in the context of cellular networks? Chaperones: (1) have weak links, i.e. low affinity, transient interactions with most of their partners; (2) connect hubs, i.e. act as 'masterminds' of the cell being close to several centre proteins with a lot of neighbours; and (3) are in the overlaps of network modules, which confers them a special regulatory role. Importantly, chaperones may uncouple or even quarantine modules of protein–protein interaction networks, signalling networks, genetic regulatory networks and membrane organelle networks during stress, which gives an additional chaperone-mediated protection for the cell at the network-level. Moreover, chaperones are essential to rebuild inter-modular contacts after stress by their low affinity, 'quasi-random' sampling of the potential interaction partners in different cellular modules. This opens the way to the chaperone-regulated modular evolution of cellular networks, and helps us to design novel therapeutic and anti-ageing strategies.

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System biology and the utility of the network approach

The complexity of cells can be described reasonably well if we use the network approach and catalogue the interactions between cellular molecules, processes or organelles. Here the interacting macromolecules are treated as network elements, and their interactions form the weighted and sometimes directed links of the respective network. We may also assemble a network-set of directed links as representations of signalling, genetic regulatory or metabolic processes of these functional networks in the cell (Barabasi & Oltvai 2004, Boccaletti et al 2006, Csermely 2006).

Cellular networks often form small worlds, where any other element of the network can be reached from a starting element via only a few links. Networks of our cells contain hubs, i.e. elements, which have a large number of neighbours. These networks can be dissected to overlapping modules, which form hierarchical communities. Small world-ness, the importance of hubs and modules in network organization, is a characteristic of most cellular networks, which makes the network approach a highly useful conceptual framework to understand the complexity of the cell. Moreover, the hierarchical modules of cellular networks enables us to treat larger segments of these networks as single entities (elements), which greatly simplifies the multitude of the hundreds of thousands of interactions, and gives us a chance for understanding and visualization. Last but not least, the above features of network organization are much more general than the realm of cellular networks, and can be extended to the networks of cells (such as the nervous system), to social networks, to ecosystems and to cultural networks, such as power grids, the worldwide web or the internet. The general network organization principles pinpoint those features of the special cellular network, which have a key importance in our understanding of the specific cellular functions. This holistic view of network properties greatly helps the interdisciplinary approach of systems biology in the cellular domain (Boccaletti et al 2006, Csermely 2006, Newman 2003, Palla et al 2005).

Special roles of chaperones in cellular networks

Molecular chaperones are not only fascinating molecular machines, which help the folding, refolding, activation or assembly of other proteins, but also have a number of functions, which can be understood only by considering the emergent properties of cellular networks—and that of chaperones as special network constituents. What are the special roles of chaperones in the context of cellular networks?

- *Chaperones have weak links, i.e. low affinity, transient interactions with most of their partners.* This feature is actually a consequence of the assistance of chaperones in folding and re-folding of cellular proteins. Chaperones bind and release a large number of newly born or damaged proteins, which requires a large promiscuity and low affinity of their interactions (Csermely 2004, Tsigelny & Nigam 2004).
- *Chaperones preferentially connect hubs.* In other words, chaperones act as 'masterminds' of the cell being neighbours of several centre proteins with a lot of secondary neighbours. This may help the chaperone-mediated cross-talk between signalling and gene regulatory pathways enabling chaperones to act as a central switchboard of the cell re-programming cellular functions during and after stress (Korcsmaros et al 2007a).
- *Chaperones are in the overlaps of network modules.* This key position gives them a special regulatory role, since they can easily couple, uncouple or even quarantine

network communities, i.e. protein complexes, cellular organelles, such as damaged mitochondria, signalling pathways, metabolic routes or genetic regulatory circuits (Korcsmaros et al 2007a, Soti et al 2005, Szabadkai et al 2006).

Many chaperones are also known as 'stress' or 'heatshock' proteins, since they are synthesized when the cell experiences stress. During stress, chaperones become increasingly occupied by damaged proteins, and a so-called 'chaperone overload' may easily occur (Csermely 2001, Nardai et al 2002). This 'competitive inhibition' of molecular chaperones might lead to a de-coupling of all the chaperone-mediated contacts between network modules mentioned above. De-coupling may even be so extensive that the damaged module becomes quarantined, and practically all of its contacts are efficiently severed isolating it from the rest of the cell. Since de-coupling of modules stops the propagation of network damage at the modular boundaries, chaperone-induced module de-coupling provides an additional safety measure for the cell (Soti et al 2005, Szabadkai et al 2006, Szalay et al 2007).

When the stress is over, and cellular resources slowly start to get back to normal again, cellular networks start to re-establish those links, which were ceased to operate during stress. Bridges, local hubs are re-built, modules are re-coupled. As a gross summary of these processes, the cell re-establishes its lost repertoire of weak links, which enable its networks to a large number of dynamic and flexible changes. In this way the re-gaining of the links shed during stress can be envisioned as a purchase of a general 'insurance', which enables the stressed cell to recover from its former, rigid state highly specialized to the given form of stress, and to attain a more flexible structure, which will be able cope with a large number of unexpected changes in the 'peaceful' period. (NB, this 'network-based' conceptualization of stress defines stress as an environmental change strong and/or repeated enough to exhaust cellular resources requiring an efficient, cheap and simple defence.)

Cellular remodelling steps after stress may be greatly helped by the newly synthesized molecular chaperones, since their low affinity interactions effectively sample a large number of proteins, and allow the re-arrangement of hubs, re-formation of bridges and binding of de-coupled modules each other in a very flexible, partially stochastic manner. Thus, chaperones give the cell a refined and flexible way for the gradual build-up of the complex modular structure and function, when the stress is already over (Szalay et al 2007).

One of the best examples of chaperone-mediated emergent network properties was shown by Susannah Rutherford and Susan Lindquist, when they discovered that Hsp90 acts as a buffer to conceal the phenotype of the genetic changes in *Drosophila melanogaster* (Rutherford & Lindquist 1998). Chaperone-induced genetic buffering is released upon stress, which causes the sudden appearance of the phenotype of previously hidden mutations, helps population survival and gives a

possible molecular mechanism for fast evolutionary changes. On the other hand, stress-induced appearance of genetic variation at the level of the phenotype cleanses the genome of the population by allowing the exposure and gradual disappearance of disadvantageous mutations by natural selection. After the initial report of Rutherford and Lindquist (1998), the effect was extended to other chaperones and to *Escherichia coli*, *Arabidopsis thaliana* and the evolution of resistance in fungi (Cowen & Lindquist 2005, Fares et al 2002, Queitsch et al 2002). In recent years the scientific community has became increasingly aware of the idea that not only chaperones but a large number of other proteins may also regulate the diversity of the phenotype (Bergman & Siegal, 2003, Csermely 2004, 2006). If a general explanation is sought, it is more likely to be related to the network properties of the cell than conferred to a simple bi-molecular interaction. In this context, the weak links of chaperones, their central position linking hubs to each other and their inter-modular links may all help their regulatory role in the evolvability of complex systems. The remodelling of the inter-modular contacts is an especially intriguing idea for the explanation of chaperone-mediated sudden changes in the emergent properties of cellular networks (such as the phenotype of the hosting organism). Different assembly of slightly changed cellular modules may cause profound and abrupt changes of the functional repertoire without major changes of the underlying structure of protein–protein interactions. This gives an exploratory but stable mechanism for the evolution of cellular networks (Korcsmaros et al 2007a).

Chaperone networks

Chaperones never act alone. They form highly dynamic complexes with each other (forming sometimes truly extensive homo- or hetero-oligomers), with their co-chaperones (regulating and modifying their function) and with the plethora of their client proteins (Soti et al 2005, Zhao et al 2005). This large set of primary, secondary and more distant chaperone-neighbours can be easily perceived as a network. Indeed, in yeast, two interrelated, but separated chaperone networks have been reported (Fig. 1). One of them, the CLIPS chaperones (chaperones linked to protein synthesis exemplified by the SSB Hsp70 proteins and by the TriC/CCT complex) operates to help the folding of *de novo* synthesized proteins, while the other, the HSP chaperone group (containing the SSA Hsp70 and Hsp90) assists in the re-folding of damaged proteins after stress. While the synthesis of CLIPS chaperones becomes repressed during stress, the synthesis of HSP chaperones is grossly activated (Albanese et al 2006). A closer look to these two 'separate' chaperone networks uncovers a large set of connecting, overlapping chaperones, such as the SSE1 (Hsp104) chaperone, which acts as a nucleotide exchanger for both key Hsp70 proteins in the different groups (Raviol et al 2006). Additionally,

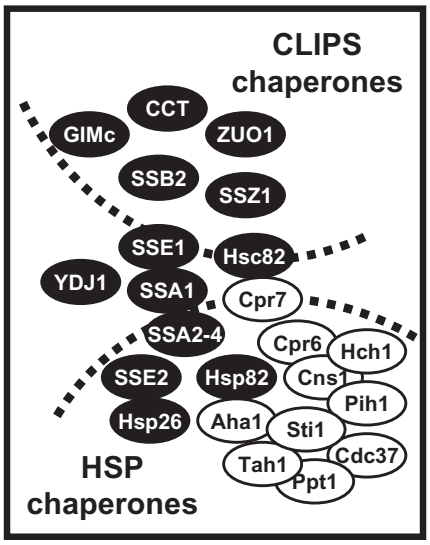

FIG. 1. The two interrelated yeast chaperone networks. CLIPS chaperones (chaperones linked to protein synthesis) operate to help the folding of *de novo* synthesized proteins, while the HSP chaperone group mostly assists in the refolding of damaged proteins after stress. A large set of chaperones connects the two chaperone networks, such as the SSE1 (Hsp104) chaperone, which acts as a nucleotide exchanger for both key Hsp70 proteins in the different groups (Albanese et al 2006, Raviol et al 2006, Zhao et al 2005).

members of the yeast Hsp90-related chaperone co-factor complex also have extensive contacts with both Hsp70 complexes (Zhao et al 2005).

The utility of chaperone-networks is further supported by the bacterial chaperones from the Mycoplasma genus, if compared to those of *E. coli*. Mycoplasmas evolve 50% faster than related organisms. This high mutation rate allows them an easy escape from the detection mechanisms of the host organism. A likely consequence of this high mutation rate is an increase in the frequency of misfolded Mycoplasma proteins. Indeed, recent estimates using comparative structural genomics resulted in generally lower protein stability of 11 protein families in Mycoplasmas compared to other bacteria. The interesting fact, that most Mycoplasmas have lost either the gene or the activity of their central chaperone, GroEL strongly suggests that protein quality control is mostly mediated by protein degradation in these bacteria. This is even more likely, since most major bacterial proteases are more intimately linked to the GroE system than to the more distant DnaK system (Fig. 2), which makes such a rearrangement plausible (Wong & Houry 2004).

As it has been already mentioned above, chaperones couple other cellular networks, besides those of their own. The mitochondrial matrix chaperone, Hsp78

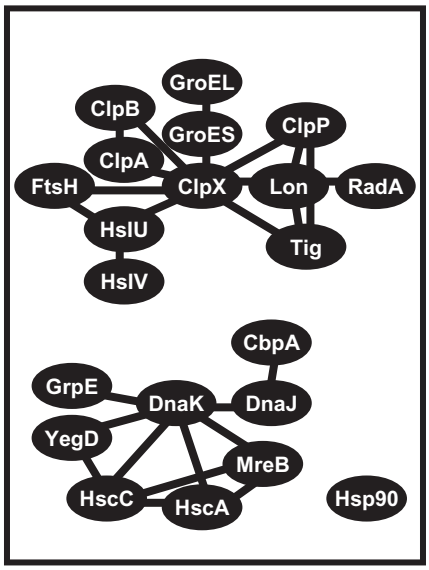

FIG. 2. The bacterial chaperone network of *Escherichia coli*, and its possible rearrangement in Mycoplasmas. The figure shows a sketch of the chaperone network of *E. coli* after Wong and Houry (2004). In most Mycoplasmas the key chaperone, GroEL is either missing or became inactivated. This may trigger a shift towards protein degradation in the quality control of damaged proteins (Wong & Houry 2004), which is in agreement with the structure of the chaperone network.

has been shown to mediate the restoration of mitochondrial network after stress in yeast (Lewandowska et al 2006). The involvement of chaperones in the coupling of mitochondria and the endoplasmic reticulum was also observed in higher eukaryotes (Fig. 3, Szabadkai et al 2006). A member of the small heat shock protein family, αB-crystallin was shown to regulate the dynamics of actin filament networks protecting the remaining network integrity after stress (Launay et al 2006). The role of chaperone networks at the emergent properties of the whole-cell level was further supported by a genetic screen searching for synthetically damaging mutations with an inactive Hsp70/SSB system in yeast. Surprisingly, the uncovered synthetically damaging mutants could not be associated with protein damage needing a direct assistance of the damaged molecular chaperones. This showed that chaperones may stabilize by the damage of many mutant proteins indirectly, by opening alternative routes in various cellular networks (Bobula et al 2006, Csermely 2004, 2006).

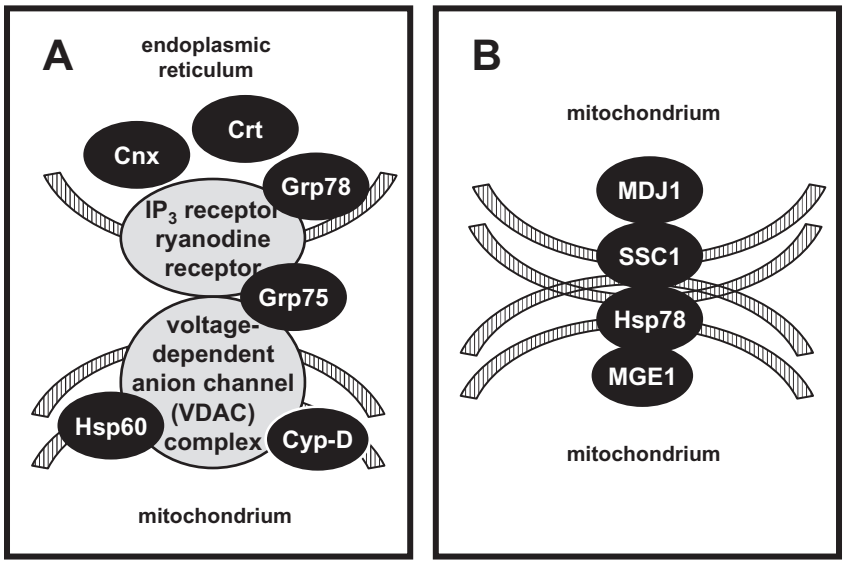

FIG. 3. Chaperone complexes help the coupling of mitochondria to the endoplasmic reticulum (A) and the re-assembly of the mitochondrial network after stress (B). The chaperone complex including calnexin (Cnx), calreticulin (Crt), Grp78, Grp75, Hsp60 and Cyclophylin D (Cyp-D) is involved in the coupling of mitochondria to the endoplasmic reticulum (Panel A, Szabadkai et al 2006). The mitochondrial chaperone complex, Hsp70(SSC1)/MDJ1 plays a key role in the maintenance of the yeast mitochondrial network, while the cooperating mitochondrial chaperone complex, Hsp78/MGE1 plays a key role in the re-assembly of the mitochondrial network after stress (Panel B, Lewandowska et al 2006).

Extracellular chaperones and networks

Though recent studies uncovered a number of highly important roles for extracellular chaperones (see Asea 2003, Eustace et al 2004, Schmitt et al 2007, Yerbury et al 2005 and other chapters in this volume), our understanding of extracellular chaperone networks is still lagging behind. Components of the Hsp90 chaperone complex, such as Hop or p23, have already been identified as extracellular chaperones (Eustace & Jay 2004), and the concerted tyrosine phosphorylation of both Grp94 (endoplasmin) and Hsp60 were shown to mediate sperm-zona recognition (Asquith et al 2004). The extraordinarily dynamic nature of the plasma membrane and its special permeability at the boundaries of its microdomains (Marguet et al 2006) suggests that we will discover many more elements of intracellular chaperone networks at the extracellular space. During this build-up of the databases for extracellular chaperone networks we will have to consider special, extracellular chaperones as well, such as the SPARC (secreted protein, acidic and rich in

cysteine), a matricelluar glycoprotein modulating cell proliferation, adhesion, migration and extracellular matrix production (Emerson et al 2006).

Conclusions and open questions

Recent progress in network science and, especially, our emerging knowledge of network dynamics provides a unique chance to understand chaperone function at a novel level. However, the lack of adequate and verified information on the several hundred thousands of interactions in cellular networks, the incomplete accuracy and sensitivity of currently available analytical methods as well as inadequate methods for network analysis give much more open questions than satisfying answers at the moment. We list a few of these questions in the following:

- What can be the *in vivo* distribution of chaperone function between the folding and refolding of single proteins, *versus* the assistance in the assembly of protein–protein, protein–RNA and protein–DNA complexes? How are these two functions related to the role of chaperones in regulating protein degradation? What mechanisms regulate the shift between these functions during and after stress, in the onset and propagation of disease and during aging?
- Do we have extracellular chaperone networks? How are these chaperone networks regulated by the local fluctuations of extracellular ATP levels? What are the chaperones assisting in the assembly of the extracellular matrix? Do we have polysaccharide chaperones?

We are quite certain that chaperone networks will give a lot of excitement and pleasure for systems biologists, who would like to understand and modify the function of our cells in health, stress, disease and aging. As a result of these studies a renaissance of network-based and chaperone-based therapies is expected, where target sets of multi-target drugs will be identified using our knowledge on the vulnerable points (hot spots) of cellular networks, including molecular chaperones and their sub-networks (Csermely et al 2005, Korcsmáros et al 2007b).

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DISCUSSION

Asea: How would you design a network for the extracellular molecular chaperones?

Csermely: We know very little about the chaperone interactions outside the cell. On one hand, the extracellular space is much less crowded than the intracellular space and, therefore, we would expect fewer interactions, especially in the low-affinity range that is typical for chaperones. However, the extracellular matrix adds a lot of possibilities for a highly dynamic organization. This may put the networking features back to the stage. There is an additional trick here. The intracellular chaperone network might simply be continued in the extracellular space, due to the high dynamism of the plasma membrane. I am sorry for not being able to give an exact answer to the question, but this field is in its infancy.

Macario: At a much simpler level, now we know that chaperones interact with other chaperones to form a team, such as the Hsp70(DnaK) team formed by it and Hsp40(DnaJ) and the nucleotide-exchange factor (e.g. GrpE in bacteria) and that some of these teams interact with other teams, for example the Hsp70(DnaK) team interacts with the GroEL/S team (Macario & Conway de Macario 2007), how do you integrate this simple knowledge with the more complex picture provided by systems biology?

Csermely: This approach is relatively young. We have data sets for six years good enough for analysis, and the scope of these data sets is relatively limited even today. Regarding the protein–protein interaction networks, so far the bacterial system has not been adequately addressed. The example you mentioned, GroEL and DnaK, cannot be put into a bacterial network because of the paucity of the data. However, in the yeast system, we can work with good reliability and efficiency. I hope in two or three years it will change because the other systems will have enough data. These molecular chaperone networks can be put in as integrators of the other complexes in the cell. That's our current view.

Hightower: One thing I thought was missing was work on integrative cellular functions. I think more of this is now happening, but more needs to come. Network theory will be helpful there. Recently, Juliet Lee and I have studied cell movement as an integrated cellular activity. Juliet has studied cell movement in fish keratocytes for many years. Recently, we have been studying human colonocytes. We can paint actin microfilaments red and Hsp27 green. As we watch the cells move around, when they make their projections from their surface they flash yellow indicating colocalization of actin and Hsp27. This really made me think of your network theory. Hsp27 may be linking signalling domains with cytoskeletal domains.

Csermely: When people measure these integrative cellular responses, what is usually neglected is measuring the scatter. If the scatter is large, it is not always the problem of the graduate student: it can tell you that there is an imbalance in the system, or the system is not so well buffered by molecular chaperones or other proteins. Let me stress, that we have an important take-home message here: the scatter does have a meaning.

Nixon: It is becoming obvious that some extracellular chaperones are spatially constrained in lipid raft structures of cells. What role do you think lipid rafts play in the emergence of these networks within the extracellular domain of the cell?

Csermely: In the future I think the lipid raft structures will assume much more importance, along with microheterogeneities of the membrane. Hsp90, Hsp70 and other molecular chaperones are already part of lipid rafts (Triantafilou et al 2004), but at the moment we don't know much about how rafts and microheterogeneities change in response to various stresses, and disease and ageing. It is an unexplored field.

Calderwood: I was interested in what you said about comparing the chaperones with signalling molecules. You said they were enriched in unstructured disordered regions. When we think of signalling molecules we think of molecules that are in a precisely defined place in the pathway. If you were to look at the same pathway with Hsp90, it interacts with numerous enzymes along the pathway.

Csermely: The reason for this enrichment in segments may not help a functional role at the level of the whole cell, but may participate in a functional role at the

level of the protein–protein interactions. Unstructured regions expand the capture radius of a particular protein, because this segment can point well out of the main bulk of the protein, and can capture proteins that are outside the average radius of the original protein. What often happens, both in signalling and in molecular chaperones, is that these proteins expand the space where they can interact with other proteins by these features. In signalling, this helps the assembly of the pathway under conditions where it hasn't been assembled before. In terms of molecular chaperones, they have to connect those proteins which need assistance to assemble. Therefore unstructured regions in both protein classes are probably more important in the 'local' functional sense than in the general functional sense meaning their function in the whole cellular network.

Henderson: For obvious reasons you have had to present these networks as static structures. Clearly, networks are dynamic. Are you utilizing mathematical modelling to try to capture network dynamics?

Csermely: Yes. At the moment the topology of the networks can be elucidated by relatively simple mathematics. We are just at the beginning. At the moment people working on these structural networks are at the point, where we start to build in the weights of the links (whether they are weak or strong), their direction and also the colouring of the links (whether they are activating or inhibiting). Mathematically, the theory isn't good enough that we could accommodate all these link/edge properties at the moment. The perturbations are currently used in a simplistic form: we see how a single perturbation to a small degree is propagating through the network. We haven't reached the point where we can do a gross differential equation treatment. This would be a major advance, indeed, but if you have a network with 100 000 elements, then this many differential equations will be obviously too much.

Henderson: You don't know how many interacting links you have. Perhaps there are hundreds of proteins in the network.

Csermely: If it is the yeast, you have 6000, and in the human perhaps 30 000, but take into account post-translational modifications and you have 100 000.

Henderson: You have to curtail the modelling to a subnetwork of a subnetwork.

Csermely: Yes. These are two different approaches, and both are fruitful. The usual modelling approach is to confine the network to a small subset, say of 10 proteins, and find out all you can from this. This was fruitful in the elucidation of the role of molecular clocks and circadian rhythms (Elowitz & Leibler 2000, Ueda et al 2005), for example. Currently, the sort of network theory I introduced applies to the whole cell, and gives up a little of the precision of the interactions, because you can't have both.

Henderson: The network theory allows you to posit a hypothesis, but the big problem is testing it biologically. For years I have been trying to model cytokine

networks, and our big problem is that we can model them in a couple of days, but it takes the biologist a couple of years to test the hypothesis. Can you see yourself testing hypotheses generated by your network modelling systems?

Csermely: Sometimes testing is not that difficult. One example of what we are doing in the lab is that we are elucidating signalling networks, and we assembled a general signalling network compiled from three different species. Along this process we found a lot of cross-talk candidates between various signalling pathways. In *C. elegans* it is relatively easy to test whether a protein is a cross-talk protein or not.

Multhoff: Returning to the topic of lipid rafts, you were talking about protein–protein interactions, but for cell signalling protein–lipid interactions might be important, or protein–carbohydrate interactions, also.

Csermely: Absolutely. The problem is twofold. First, we don't have such a big database on protein–lipid interactions: the lipidome is just emerging (Lu et al 2005). Secondly, many of these interactions are not that specific. I agree this is important, but there are conceptual problems at the moment.

Gupta: You showed the largest number of interactions for Hsp70. There are many different forms of this protein. Are you distinguishing them in your analyses? Once you do that, do you still see a large number of interactions?

Csermely: We do have data individually on all the isoforms of the HSPs. Interestingly, most of the interactions are coming from the cytosolic Hsp70. This doesn't mean that this is disproportionally having a large number of interactions. It is probably the bias of the experimental methods, which provided the databases for the interactions (Korcsmaros et al 2007).

Gupta: The chaperone protein is supposed to interact with a large number of proteins. So how can you distinguish between a network-type interaction and a normal functional interaction?

Csermely: One of the problems with the current data on chaperones is that we don't have a good discrimination between highly transient chaperone-client interactions and network-type, regularly occurring chaperone-protein interactions. There might be later on a basis of discrimination by means of the affinity constants, but we only have a few of these now. But a warning is appropriate here: I'm sure we will have a continuum of binding affinities, and it will be difficult to dissect from which affinity value on a client-chaperone interaction is 'just a normal functional interaction' and not a regularly occurring network-type interaction.

Lund: I was intrigued by the idea that when a stress occurs, chaperones are wrapped up with dealing with this problem, and this causes a separate module to become disconnected. When the stress is finished these modules become rewired, but may be rewired in subtly different ways. This is a mechanism for epigenetic change, because you are not changing the protein, but the way in which the network is put together. Is there any evidence for this that you are aware of?

Csermely: Not direct evidence, just a bit of indirect evidence (Korcsmaros et al 2007, Bobula et al 2006). The basic assumption does not seem to be completely valid that if you inhibit molecular chaperones and the system is beginning to behave differently in this epigenetic way, then the reason is that molecular chaperones cannot fold a certain number of proteins, and therefore those proteins will be in a different form in the cell. This may still be valid, but it is not the complete picture.

Hightower: There are interesting observations about the properties of the cytoprotected state of cells in tissues being different from normal cell and tissue functions. Cells are less responsive to extracellular cues, i.e. they are non-mitotic and non-apoptotic, when they are in the cytoprotected state, suggesting the kinds of changes you might expect a different network reassembly to be able to accomplish.

Lund: This is a mechanism of cellular learning or cellular memory, then.

Csermely: Learning in the sense of cells or other unconscious assemblies can be rationalized as a topological change in the network system. This may be actually the underlying mechanism of learning in our brains, where neural cells change their network assembly (both in the form of changing the topology of their anatomical contacts in the long-term, as well as changing the emerging oscillation networks in the short-term).

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