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REVIEW ARTICLE

Cellular networks and the aging process

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Abstract
The most important interactions between cellular molecules have a high affinity, are unique and specific, and require a network approach for a detailed description. After a brief introduction to cellular networks (protein – protein interaction networks, metabolic networks, gene regulatory networks, signalling networks and membrane – organelle networks) an overview is given on the network aspects of the theories on aging. The most important part of the review summarizes our knowledge on the aging of networks. The effects of aging on the general network models are described, as well as the initial findings on the effects of aging on the cellular networks. Finally we suggest a 'weak link theory of aging' linking the random damage of the network constituents to the overwhelming majority of the low affinity, transient interactions (weak links) in the cellular networks. We show that random damage of weak links may lead to an increase of noise and an increased vulnerability of cellular networks, and make a comparison between these predictions and the observed behaviour of the emergent properties of cellular networks in aged organisms.

Key words: *Aging, network integrity, protein – protein interaction network, signalling network, transcriptional regulatory network.*

Cellular networks

The tremendous increase of our knowledge on the cross-reacting pathways of our cells requires the integration of the analytical description of the individual interactions. This integration is conveniently served by the network description of the cellular organization. Networks can be defined, where specific entities (the elements of the network) and their interactions (the links of the network) can be described. From the point of network description usually we are not interested in the structure or detailed properties of the individual elements, but only the topology and weight of their contacts. This simplified description allows the comparison of various networks operating at different levels and in different environments (Barabasi & Oltvai, 2004; Albert, 2005; Csermely, 2006). In the protein – protein interaction network the elements of the network are proteins and the links between them are permanent or transient bonds (Rual *et al.*, 2005; Stelzl *et al.*, 2005; von Mering *et al.*, 2002). In the cytoskeletal network, we have individual cytoskeletal filaments, like actin, tubulin filaments, or their junctions as the elements of the network, and the bonds between them are the links. In the membrane – organelle network various membrane segments (membrane vesicles, domains, rafts of cellular membranes) and cellular organelles (mitochondria, lysosomes, segments of the endoplasmic reticulum, etc.) are the elements and usually protein complexes link them together. Both the membranes and the organelles contain large protein – protein interaction networks. In signalling networks the elements are proteins or protein complexes and the links are highly specific interactions between them, which undergo a profound change (either activation or inhibition), when a specific signal reaches the cell (White & Anderson, 2005). Gene transcription networks have two types of elements, transcriptional factor complexes and the DNA gene sequences, which they regulate. Here the transcriptional factor complexes may initiate or block the transcription of the gene's messenger RNA. The links between these elements are the functional (and physical) interactions between the proteins (sometimes RNA-s) and various parts of the gene sequences in the cellular DNA (Blais & Dynlacht, 2005). Finally, in metabolic networks the network elements are metabolites, such as glucose, or adenine, and the links between them are the enzyme reactions, which make one metabolite from the other (Borodina & Nielsen, 2005).

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Cellular networks are often small worlds, where from a given element any other elements of the network can be reached via only a few other elements. Networks of our cells usually have a scale-free degree distribution, which means that these networks have hubs, i.e. elements, which have a large number of neighbours. These networks are rich in motifs, which are regularly appearing combinations of a few adjacent network elements, and contain hierarchical modules, or in other words: are forming hierarchical communities (Albert, 2005; Barabasi & Oltvai, 2004). The complex architecture of cellular networks is needed to solve four major tasks (Figure 1): the first task is the local dissipation of the perturbations/noise coming from outside the cell, and from the stochastic elements of intracellular reactions; the second task is the efficient and reliable global transmission of signals from one distant element of the cell to another; the third task is the discrimination between signals and noise via the continuous remodelling of these networks during the evolutionary learning process of the cell; and the fourth task is the protection against the continuous random damage of free radicals and other harmful effects during stress and aging (Csermely, 2006). The general features of cellular networks mentioned above all help to solve these tasks. As a relatively simplified view, hubs help to confine most of the perturbations to a local environment, while the small world character allows the global propagation of signals. Motifs and hierarchical modules help both the discrimination between the two, and provide stability at the network level (which is helped by a number of repair functions at the molecular level). However, at the end of our brief summary of the major features of cellular networks, we have to warn that the above features of the cellular networks are only general characters, which need to be scrutinized for the validity of the dataset, correct sampling procedure and method of data analysis (Ma & Zheng, 2003; Arita, 2004; Tanaka *et al.*, 2005).

Network models of aging

Aging is accompanied by a general increase in noise parallel with a decrease in complexity (Hayflick, 2000; Goldberger *et al.*, 2002; Herndon *et al.*, 2003). The seminal and unfortunately rather unnoticed paper of Himmelstein *et al.* (1990) gives a good summary of the erosion in homeostatic capacity during aging. It is difficult to establish a direct link between the increased noise and the loss of complexity. Deterioration of complex structures is seen all around our body during aging. Loss of the fractal structure of the dendritic arbor from neural cells of the motor cortex may lead to the increased frequency of devastating falls of elderly people (Scheibel, 1985). The trabecular network of our bones and the network of bone-remodelling osteocytes have a scale-free and small-world pattern. The deterioration of these networks occurs, if there is not enough physical exercise, during osteoporosis and other age-related diseases. The analysis of bone network integrity can be used for the prediction of bone fractures often having fatal consequences in advanced age (Hruza & Wachtlova, 1969; da Fontoura Costa & Palhares Viana, 2005). The loss of complexity (system integrity) will lead to an increased noise and

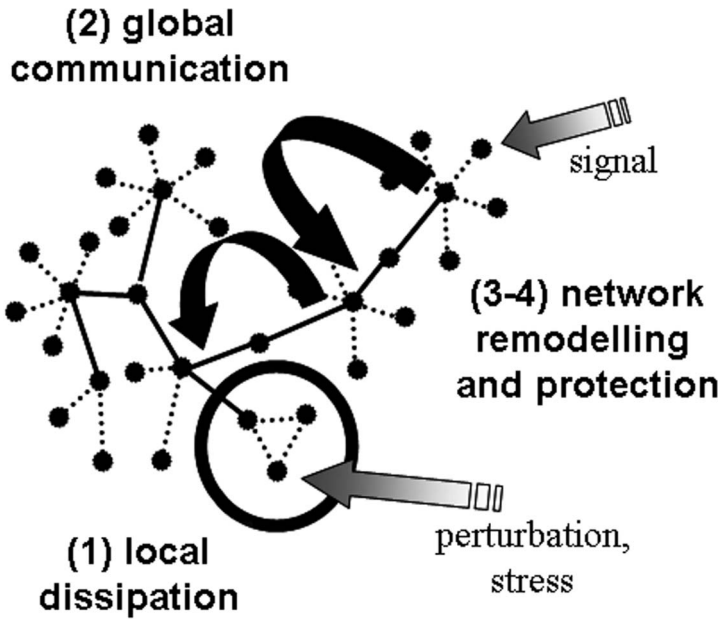

Figure 1. Major tasks of cellular networks. (1) Local dissipation of the perturbations/noise coming from outside the cell, and from the stochastic elements of intracellular reactions. (2) Efficient and reliable global transmission of signals from one element of the cell to another. (3) Discrimination between signals and noise via the continuous remodelling of these networks during the evolutionary learning process of the cell. (4) Protection against the continuous random damage of free radicals and other harmful effects during stress and aging.

vice versa, an increased noise with decreased repair and remodelling processes is causing a loss of complexity. These two phenomena are different sides of the same coin and may form a vicious circle aggravating the status of the aging body.

There are three major theories of aging. (1) The mutation accumulation theory says that the deleterious mutations having their effects at an advanced age are not cleansed by evolutionary selection, since aging occurs after the peak of reproduction. Therefore, these mutations can accumulate through generations. (2) The pleiotropy (or antagonistic pleiotropy) theory suggests that pleiotropic genes with good early effects are favoured by selection even if later the same genes will show deleterious effects. (3) The disposable soma theory states that the prevention of late defects (like better scavenging of free radicals) would require a lot of intrinsic resources. Large investments to these costly mechanisms would help longevity but, in parallel, would decrease survival at a younger age (Kirkwood & Austad, 2000).

Aging is probably the most complex phenotype and is accompanied by the life-long accumulation of random damage in somatic cells and tissues. Due to the random nature of the defects, if we would like to give a general description of the molecular events during the aging process we run into trouble. Aging is accompanied by an increase in damaged proteins and an increased load of free radicals (Hayflick, 2000; Soti & Csermely, 2000; Nardai *et al.*, 2002). The parallel and gradual overload and loss of the protective mechanisms led Kirkwood and Kowald (1997) describe the 'network theory of aging', which takes a large number of these events into account.

All these events become especially pronounced in aged mitochondria, which is a primary site of damage by reactive oxygen species (ROS). In agreement with a central role of oxidative events and mitochondria in aging, caloric restriction is the most effective way to delay the aging process (Sohal & Weindruch, 1996). In contrast, raising extracellular pyruvate levels induces cellular senescence of human diploid fibroblasts, which is a cellular model of aging (Xu & Finkel, 2002). Mitochondrial mass is increasing (Lee *et al.*, 2002), and the expression of the ADP/ATP translocase is decreasing during cellular senescence (Fan *et al.*, 1998), which may be a part of a vicious circle producing more ROS leading to a further loss in energy production, inducing more mitochondria, which will produce even more ROS.

However, mitochondrial aging is not exclusively linked to enhanced ROS production and the consequent random damage. When the mutation rate of mitochondrial DNA was increased by decreasing the proofreading capacity of the mitochondrial DNA polymerase, a complex aged phenotype was developed in transgenic mice (Trifunovic *et al.*, 2004). However, later studies showed no increase in oxidative damage in the same animals (Trifunovic *et al.*, 2005). Additionally, decreased accuracy of mitochondrial ribosomes might be another key mechanism of aging at the cellular level (Hipkiss, 2003).

Mitochondrial mutations clonally expand in the affected cells, which leads to a cellular mosaic with a large heterogeneity of mitochondria and their hosting cells in aged organisms (Hagen *et al.*, 1997; Kraytsberg *et al.*, 2003). Mitochondria form a network in the cell (Aon *et al.*, 2004), where the network architecture may group more and less efficient mitochondria together. Network clustering of 'viable', high-flux and 'damaged', low-flux mitochondria may contribute to the sequestration of damaged mitochondria and oxidized proteins to the mother yeast cells, which are important mechanisms to prevent yeast aging (Lai *et al.*, 2002; Aguilaniu *et al.*, 2003). The damage of mitochondrial cluster formation may generally accompany the aging process.

Aging of cellular networks

In the network theory aging is usually described as a loss of the attractiveness of the initial elements of the network. In this sense, aging contributes to the development of a hierarchical network structure, where hubs are connected to a large number of single elements and hub–hub contacts are only sporadic. A more important network model of aging introduces a permanent random damage. Here, deletion of links or deletion of network elements gives a different outcome. However, both are leading to a fast deteriorating of emergent network properties (Dorogovtsev & Mendes, 2000; Zhu *et al.*, 2003; Agoston *et al.*, 2005). The two types of network aging, decreasing attractiveness and permanent random damage are related, since a model of the former quite efficiently describes the effects of the latter, when the extent of random damage is intermediate (Middendorf *et al.*, 2005). The interesting study of Chan *et al.* (2004) showed that a rapidly aging network makes the older nodes rather isolated and has only a local spread of information instead of global coupling. This property of aging networks may contribute to the loss of integration in aged organisms as discussed above. After generalized damage the function of the network can be significantly restored by a limited intervention on its hubs (Ferrarini *et al.*, 2005).

As it can be expected from the network models above, elements of the cellular networks are not equally important in the aging process. Many of the longevity genes were found to be hubs, which control somatic maintenance and repair (Promislow, 2004; Ferrarini *et al.*, 2005) and probably dampen the increased disorder and noise of the aged organism. As another example, oxidative damage in a transgenic model of Alzheimer's disease preferentially affected signalling networks related to the iNOS-integrin

pathway, CRE/CRB transcription regulation and Rab-Lyst vesicular trafficking (Soreghan *et al.*, 2005).

Aging induces random damage in networks. It has been already noted by Promislow (2004) that most of this random damage will affect links between weakly connected proteins due to the simple fact that these links and these proteins are the most common. However, an important question arises here. What is the result, if a large number of weak links is deleted from a network? Weak links stabilize a vast variety of networks including all those listed as cellular networks at the beginning of this review. It is a general phenomenon that the loss of weak links does not affect the major responses of these networks. However, the numerous examples prove that deletion of weak links leads to an unbalanced system, with a larger noise and instability (Figure 2, Csermely, 2004, 2006). These are exactly those major properties of aging networks, which have been mentioned before. Thus, a 'weak link theory of aging' can be formed, where the preferential loss of low affinity, transient, low probability interactions of cellular networks emerges as an important mechanism explaining the increase in noise parallel with a decrease in complexity in aging organisms.

An additional mechanism for the 'weak link theory of aging' may be that the aging cell loses one by one new segments of its original function due to the permanent random damage and insufficient repair. Consequently, the shrinking resources of the aging cell withdraw the pathways to the most important, vital routes (the so-called 'skeleton' of the network; Garlaschelli *et al.*, 2003; Song *et al.*, 2005) and preferentially lose the secondary, weak pathways, which were back-ups, or gave an extra stability. This again induces a preferential loss of weak links in cellular networks leading to the instability and noise mentioned above.

The proof of the 'weak link theory of aging' requires further studies on the position of aging genes in cellular network, on the distribution of the strength of their contacts, and on the effects of permanent random damage as well as network rearrangements during this process. However, even in its current, preliminary form the theory shows the strength of the connection of network topology and dynamics with the emergent network properties to explain the complex aging phenotype.

Conclusions

Aging has the most complicated phenotype and is accompanied by a general increase in noise parallel with a decrease in complexity. The analysis of cellular networks already helped to explain several aspects of aging and longevity including:

- the importance of mitochondrial networks in aging (Aon *et al.*, 2004; Trifunovic *et al.*, 2004);
- the de-coupling of global communication in aging cellular networks (Chan *et al.*, 2004);
- the importance of hubs (e.g. proteins with a large number of partners) in the aging process (Promislow, 2004; Ferrarini *et al.*, 2005); and
- the effects of the preferential random damage and rearrangement-induced loss of weak links (the 'weak link theory of aging') in the explanation of the increased noise of aging cells and organisms (Csermely, 2006).

Further progress elucidating the details of the topology and dynamics of cellular networks, advances in the functional genomic, transcriptomic and proteomic analysis as well as extended databases and bioinformatics (Raghothama *et al.*, 2005) will all help us to identify the key members of cellular networks and the emergent network properties in aging and longevity.

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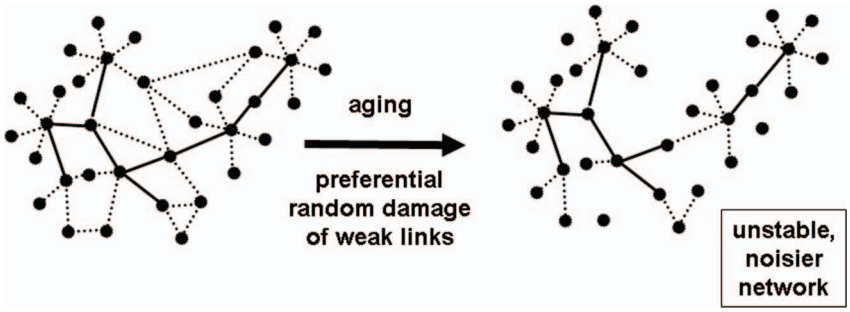

Figure 2. Weak link theory of aging. Continuous random damage affects those elements of cellular networks more often, which bind to each other with low affinity, low probability and transient interactions, since these interactions are much more common than strong interactions in these networks. Consequently, the networks will be more unstable and the emergent properties of the cellular networks will be noisier. These effects may emerge as an important mechanism explaining the increase in noise parallel with a decrease in complexity in the aging process.

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