6 Weak Links and Cellular Stability

This will be our second journey into Netland. We are going to visit cellular networks. I first introduce various cellular networks and continue with a wide variety of proteins which may all modulate the stability of our cells. In the last two sections, I also explain the role of stabilizing proteins in evolution, cancer and other diseases, and aging.

6.1 Cellular Networks

Cells are built from a wide variety of molecules. All these molecules build networks. Some of their interactions are largely unspecific and can be described in general terms. An example is the self-association of lipids to membranes. However, most of the interactions between cellular molecules are unique and specific, and require a network approach for a detailed description. One of the best examples for the description of unique cellular interactions between molecules is the protein–protein interaction network (see Fig. 6.1), where the elements of the network are proteins and the links between them are permanent or transient bonds. At a higher level of complexity, we have networks of protein complexes, where individual elements may be regarded as modules of the large protein–protein network. The cytoskeletal network and the membrane–organelle network are good examples of these larger networks. In the cytoskeletal network, the elements of the network are individual cytoskeletal filaments, like actin, tubulin filaments, or their junctions, and the links between them are the bonds. 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 protein complexes usually link them together.

Both the membranes and the organelles contain large protein–protein interaction networks (Sőti et al, 2005).[1]

"Something is not clear to me here. Are biochemists playing around when defining such overlapping networks? Why is a protein–protein interaction network not good enough to describe the whole cell?" The easy answer would be that we have other molecules than proteins in the cell. However, the real reason is a bit more complex. When we speak about protein–protein interaction networks, we never mean the interaction of this little green protein A on the right side of the cell with that larger pear-like protein B sitting beside it. The interaction of protein A and protein B in the network sense means that there is a good chance that we will find their complex in the given type of cell. In networks, the notions 'protein A' and 'protein B' refer to a population of these proteins, and not to individual proteins A or B. In contrast, many of the other networks which have larger protein complexes as elements are defined as networks of unique partners, where the interaction of mitochondrium A-245 with mitochondrium A-312 can be traced and monitored continuously by modern techniques of cell biology.

I am sorry to have to tell you, Spite, that the situation is even more complex with cellular networks than was suggested in our previous discussion. There are a rather large number of these networks in which the elements and links are functionally defined (see Fig. 6.1). As an example, in signaling 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. In the 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 another. Finally, the gene transcription network has two types of element, namely, 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 RNAs) and various parts of the gene sequences in the cellular DNA. As we begin to learn more about the molecular composition and regulation

[1] Here the examples (actin, tubulin, vesicles, rafts, lysosomes, etc.) are just given for those who are familiar with cell biology. It is not necessary to understand them here, since the basic concepts of this chapter can still be followed. I therefore ask the reader coming from a different background to skip these examples, or consult cell biology textbooks or publicly available glossaries for their explanation.

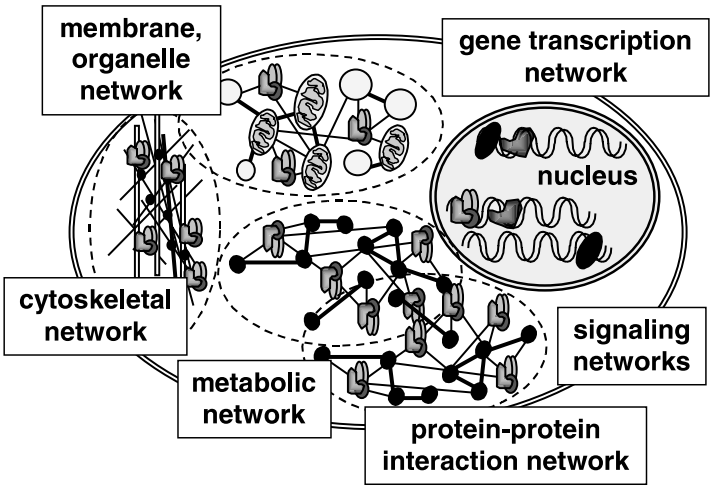

Fig. 6.1. Cellular networks. The figure illustrates the most important networks in our cells. Many of these networks, like the transcriptional or metabolic networks, are functionally defined. In the metabolic network, the various metabolites are the elements and the enzyme reactions are the links. In the gene transcription network, the transcription factors and genes are the elements and functional interactions between them are the connecting links. Other networks, like the protein–protein interaction network or the cytoskeletal network, have proteins as elements, and permanent or transient bonds between these proteins as links. All these networks overlap one another considerably, and some of them (like the membrane–organelle network) contain modules of other networks (e.g., the organelle specific modules of the protein–protein interaction networks)

of our cells, the definition of more and more networks becomes feasible. A sign of this is the fact that, recently, more and more specific transcriptional networks have been defined for various physiological events, such as development or aging, and for various human diseases.

Cellular networks have all the major network properties described in Chap. 2. They are often small worlds, with a scale-free degree distribution and a motif-rich, modular and hierarchical structure (Almaas et al., 2004; Bergmann et al., 2004; Bortoluzzi et al., 2003; Chen et al., 2003; Jeong et al., 2000; Park et al., 2005a; Ravasz et al., 2002; Stuart et al., 2003). However, when examining the above features in the context of cellular networks, it is important to scrutinize the validity of the dataset use a suitable sampling procedure and data analysis, as already described in Chap. 2 (Arita, 2004; Ma and Zeng, 2003; Tanaka, 2005).

Can we make an exact copy of ourselves? *"As you were listing all the interesting features of cellular networks, I kept asking myself: when we know the position of all the proteins in the cell, will we just be able to put them together and get another cell which is identical? Going even one step further, if it was a nerve cell and we copied this procedure with many of them, could we arrive at a cloned brain, which has the very same thoughts as the original?"* This is very good question, Spite. Congratulations! I seriously doubt whether we will ever be able to do this. The first objection is that we cannot yet isolate all the protein molecules, set their modification patterns and position them exactly as they were in the 'sample' cell. However, there is a much more serious objection than this. All these networks are self-organized networks. This means that they have a memory. A cellular protein may have several possible neighbors, but it will only bind to some of them. What determines which of the possible neighbors will be the real one? All the past events between these network elements determine their current interactions. Moreover, the events which changed the interactions of these elements with the rest of the network also influence complex formation between the proteins. Hence, for a correct copy we have to repeat many of the past events in the process of network self-organization, which makes the whole adventure quite impossible, not only in technical terms, but even theoretically.[2]

6.2 Stability of the Cellular Net

After a brief introduction to the various cellular networks, I will describe a landmark experiment which gave insight into the emergent properties of cellular networks. In 1998, Suzanne L. Rutherford and Susan Lindquist published ground-breaking results in Nature, showing that compromised chaperone function leads to the appearance of silent mutations in *Drosophila*. Molecular chaperones, or in other words, heat shock proteins or stress proteins are highly abundant proteins that have been conserved throughout evolution. Why are these proteins known by so many different names? The name 'chaperone' refers to their function. Chaperones are the physicians of the cell. They heal other proteins. As mentioned in the Preface and in the last chapter, chaperones form the most ancient defense system of our cells. They recognize both half-ready and damaged proteins, prevent their aggregation, and help them to complete their folding process or to re-fold. This is why chaperones are also called stress proteins. If our cells suffer any damage, the cellular proteins also become

[2] I am grateful to Gergely Hojdák for this question.

damaged. Therefore, we need more chaperones after stress. In agreement with this, chaperone synthesis is up-regulated in damaged cells. This happens in parallel with the inhibition of most other protein-synthetic events. The reason for the general no-go sign to protein synthesis is that it is very costly. If the cell is in danger, its energy reserves are low. A stressed cell is a very utilitarian hospital, where only doctors and nurses get their breakfast, lunch and dinner.[3] After stress, more or less only chaperones are synthesized in the cell. This is why they are called stress proteins. Heat shock is the archetypal stress, and heat shock was the damage under investigation when Ferruccio Ritossa discovered stress protein synthesis in 1962 (Hartl, 1996; Bukau and Horwich, 1998; Csermely et al., 1998; Ritossa, 1962).

What is stress? The word 'stress' was coined by Hans Selye (1955; 1956). In this book, I use a definition from the context of the cellular net. Stress is any unexpected, large and sudden perturbation of the cellular network, to which the network (1) does not have a prepared adaptive response or (2) does not have time to mobilize the adaptive response. In this book stress is used differently from stress in the usual sense in physics, where it is a force that produces strain in a physical body. I should note that this definition is very close to the relaxation problems mentioned in Sect. 3.2. If the perturbations are of unusual type, or if they are too big, or arrive one after the other in too great a number, the network may be in trouble. If the network has time, it will be able to remodel itself. This is called an adaptive response. However, if the perturbations come too fast, the network has to mobilize its general defense, the stress response.[4]

[3] In the hospital of the stressed cell, porters may also get some food, since the unequal ion balance must be maintained at the plasma membrane.

[4] Cells behave quite similarly to families, when they put grandma's silver, Tante Sissi's china and any other family treasures into a safe place in the hope of preserving a few resources for recovery after damage. Tante Sissi – Aunt Sissi – was our neighbor when I was a child. She was already unthinkably old when I was born, and as the widow of a high-ranking officer in the Austro-Hungarian monarchy, which had become obsolete half a century before, taught me German with a genuine Austrian accent every Wednesday. The highlight of these sessions was an original Meinl tea, which I had to welcome with the appropriate enthusiasm for such a rarity. Tante Sissi will appear on several occasions in later sections of the book to illustrate social behavior.

More on the name 'chaperone'. The word 'chaperone' comes from French and was coined by John Ellis (Hemmingsen et al., 1988) after Ronald Laskey used this expression for a histone chaperone, nucleoplasmin (Earnshaw et al., 1980). Originally, chaperones were old ladies who accompanied beautiful young girls to the grand ball many years ago. As mentioned above, cellular chaperones prevent the aggregation of proteins. Aggregation is an unplanned, rather tight interaction of two partners in which they stick together. Similarly to the old ladies who went to the ball to protect young girls from this kind of unplanned, tight interaction, their cellular counterparts do the same with the inexperienced and naïve proteins when they run into trouble.

Let me return to 1998 when Suzanne Rutherford bred her 10 400 fruit flies. All of them were sons and daughters of funny couples. One of the parents was normal. However, the other had a mutation in Hsp90 which compromised the function of this chaperone.[5] 10 226 flies looked normal. However, 174 of them were miniature Frankenstein monsters. Eyes were missing, distorted or repositioned, wings grew deformed, legs were transformed, bristles were duplicated and halteres were crippled. Altogether 23 types of malformation were catalogued with a minimum occurrence in 3 and a maximum in 48 flies (Rutherford and Lindquist, 1998).

What can be the reason for these malformations? Similarly to many other chaperones, Hsp90 is known to participate in embryogenesis (Csermely et al., 1998). The first explanation which comes to mind is rather straightforward: damaged Hsp90 derailed embryonic development. However, such an effect should derail the development of far more than just 174 fruit flies out of the 10 400. Moreover, many of these malformations were inheritable even after a transient inhibition of Hsp90. Exactly the same type of malformation was observed in the grandchildren of 9 monster types. This suggested a genetic background, which brings us to the next possible explanation: Hsp90 inhibition increases the mutation rate. The mutation rate was carefully checked and many other experiments were also performed. All suggested that the mutations causing the distortions were originally present in the *Drosophilas*. However, they were not visible.

These silent mutations affected the phenotype only if Hsp90 was inhibited. Once silent mutations got exposed, they destabilized the cells

[5] In the expression Hsp90, Hsp stands for heat shock protein and 90 refers to the molecular weight of this chaperone, which is 90 kDa.

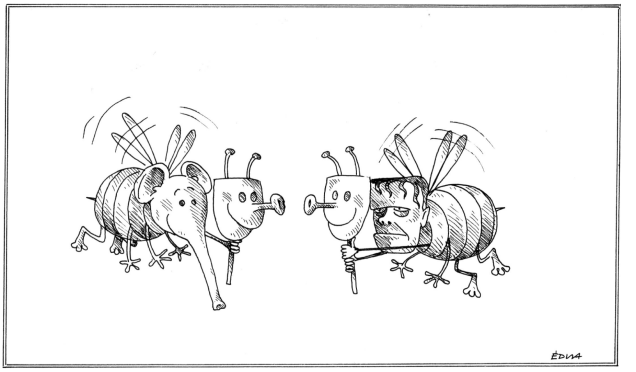

Fig. 6.2. Mutations causing the distortions were originally present in the *Drosophilas*. However, they were not visible

involved in development and led to their increased diversity, inducing a larger number of unexpected developmental phenotypes. A new conclusion was born: the Hsp90 chaperone buffers the effects of silent mutations to induce diversity in developmental morphology (Rutherford and Lindquist, 1998).

Mutations keep their silence in many different ways. In describing the findings of Rutherford and Lindquist (1998), I used the expression 'silent mutation'. Mutations can be silent in many ways. Here, silence means that, although the mutation causes a phenotype change at the level of the organism, this change is concealed by the buffering effect of chaperones. There are mutations which are more silent than those concealed here, since their presence does not lead to any change in the phenotype. This second type of silence is a permanent silence, as opposed to the conditional silence of chaperone-buffered mutations. Permanent silence is rather widespread due to gene duplications and degenerate pathways in various networks, which efficiently substitute the diminished or missing function.

"Now I understand the chaperones, and even the sudden outbreak of the monstrous Drosophilas. But you still owe me something. Where is the cellular net?" It is coming soon, Spite! We are almost there. First I would like to make a few extensions of the original statement:

1. Is *Drosophila* the only species where chaperones stabilize the effects of silent mutations? No, *Drosophila* is not unique. Chaperone-induced buffering has been found in other species, such as the plant

Arabidopsis thaliana or the bacterium *Escherichia coli* (Fares et al., 2002; Queitsch et al., 2002).

2. Is Hsp90 the only chaperone to stabilize the effects of silent mutations? Hsp90 is not alone in buffering developmental diversity. Over the past few years the findings of Rutherford and Lindquist (1998) have been extended to other chaperones like Hsp60 and Hsp70 (Fares et al., 2002; Roberts and Feder, 1999).
3. Are chaperones the only proteins to stabilize the effects of silent mutations? Chaperones are not unique in buffering developmental diversity. There are several other proteins which increase the morphological diversity of the *Drosophila* phenotype (de Visser et al., 2003; Gibson and van Helden, 1997; Gibson and Wagner, 2000; Scharloo, 1991). The generality of the effect has been addressed by Wilkins (1997). Moreover, Bergman and Siegal (2003) showed by modeling experiments that the deletion of many proteins both from a model network and from yeast cells may cause an increase in phenotypic diversity. These data warn us that there are many more proteins than just chaperones buffering phenotypic diversity.

Here we have to stop for a while. An exciting question arises: how do all these proteins buffer phenotypic diversity? While it was just a chaperone, we believed we knew the answer: the chaperone deals with the mutant proteins, preventing them from causing trouble. When stress comes, all types of mutants are released and they start to cause various unexpected effects and interactions leading to increased diversity in the next generation. However, this very same mechanism clearly cannot work with all the proteins uncovered. While some of the non-chaperone proteins like those participating in various cellular signaling steps may affect developmental diversity in a direct and rather specific way, there may also be a general effect here which should not be linked to the specific function of the individual proteins. Spite, listen! Here comes the cellular net. The network approach seems to be rather effective in keeping the specific features of the network elements (here the various proteins) in the background and concentrating on their position in the broader context, throughout the whole network, i.e., the cell.

Here is one more thought before really launching into the cellular network.[6] As mentioned in Sect. 3.1, the development of diversity is

[6] I know, this chapter is beginning to look like a dummy version of a Beethoven symphony: whenever you start to think: "Now he's really getting to the point of it!", a new development is inserted. But please bear with me. The coda will be quite good, I promise.

linked to noise. Higher noise brings greater diversity. Those mutations made the poor *Drosophilas* not only monstrous, but also noisier.[7]

Cellular noise and diversity. Kuznetsov et al. (2002) found that the messenger RNAs of at least 45% of yeast genes are present in less than 1 (less than one!) copy per yeast cell on average. Many genes are switched on sporadically, producing messenger RNAs in occasional pulses present in only a few cells. An individual cell may contain only a few molecules of several key proteins, which are distributed in various segments of the cell. Low reactant numbers can lead to significant statistical fluctuations in molecule numbers and reaction rates, producing a rather high level of noise and diversity (Rao et al., 2002). Moreover, in both prokaryotes (Elowitz et al., 2002; Ozbudak et al., 2002) and eukaryotes (Blake et al., 2003), it has been shown that an increase in the noise of gene transcription leads to an increased diversity of final transcripts, and hence to an increased diversity in phenotype (McAdams and Arkin, 1997; Levin, 2003).

We have finally arrived at the cellular net. We will use it to see if there might be a common mechanism behind the role of all those proteins inducing more noise and diversity. What do we know about the network properties of chaperones, which have the best established and most general effects on phenotypic diversity among the wide variety of proteins? Chaperones are typical weak hubs in the cellular protein net since they form a multitude of low affinity interactions with a large number of other proteins and with each other (Kovacs et al., 2005). Chaperones are forced to leave their weak links during stress. What happens if the system starts to lack weak links? Is there any connection between weak links and noise? Yes, there is: long-range or intermodular weak links make the system less noisy. Weak links help relaxation and increase system integrity. Let me sum up all of this: if we disturb the weak links of chaperones, the cellular integrity is decreased, the relaxation of perturbations also decreases, the cellular noise increases, and local tensions develop, which may all significantly contribute to the development of the observed diversity. This brings me to state the following hypothesis:

[7] The noise is cellular noise here, but one never knows: even their mutant buzz may be noisier, repelling the Beethoven-loving *Drosophila* members of the other sex.

Weak links of the cellular protein network buffer noise and diversity. Besides their specific effects, chaperones and other proteins may also buffer developmental diversity by forming a large number of weak links in the cellular protein network. The increase in diversity goes in parallel with an increase in the cellular noise level. As an additional support to these ideas, Tsigelny and Nigam (2004) recently demonstrated that chaperones decrease the noise of protein folding in a model system.

The effect of chaperones agrees with the functional definition of weak links. Let me reiterate here the functional definition of weak links from Sect. 4.2: a link is defined as weak when its addition or removal does not change the mean value of a target measure in a statistically discernible way. When he gave this definition, Berlow (1999) also stated that the removal of these weak links would increase the variation and noise in the system. We do have an agreement here. The incapacitation of the Hsp90 chaperone did not change the *Drosophila* population in a statistically discernible way, since 174 divergent monsters did not make any significant changes in the mean parameters of 10 400 *Drosophilas*. However, the variation did increase. Similarly, the increased diversity caused by the non-chaperone proteins listed above was usually also confined to a smaller segment of the population. Moreover, the above definition of weak links is also in agreement with the data of Bergman and Siegal (2003), who selected a gene set which had no significant change in its expression after knocking out 53 yeast genes (Hughes et al., 2000). Examining the variability of gene expression of the same selected gene set, Bergman and Siegal (2003) found that it was increased after the deletion of the 53 yeast genes, in agreement with the experiments with molecular chaperones.

Are there any other proteins which may be good candidates both for making a large number of weak links and showing a capacity to buffer cellular diversity?

- **The p53 tumor suppressor as a buffer of diversity.** One of the possible candidates to buffer cellular noise and diversity is p53, a transcription factor involved in the proper control of the cell cycle, which suffers various debilitating mutations in many types of cancer. It has long been regarded as a highly-connected node in the cellular network (Vogelstein et al., 2000) and as a buffer for developmental noise as well (Aranda-Anzaldo and Dent, 2003). The

potential role for p53 to buffer cellular noise is in agreement with the presumably increased noise during malignant transformation.

- **Prions as diversity generators.** Prions are peculiar proteins. They have two conformations, the normal and the infectious. The energy levels of the two prion conformations are separated by a high activation energy. This allows the conversion of normal prions to infectious prions only under specific conditions. One of these conditions can be the presence of an infectious prion, which catalyzes its own formation from normal prions. The conformational difference gives prions a molecular memory and serves as a source of epigenetic inheritance (Uptain and Lindquist, 2002).[8] In the case of yeast prions, the infectious [PSI$^+$] prion form makes ribosomes to skip the termination of protein synthesis at the stop codon of the messenger RNA in approximately 0.2 to 16 percent of cases. This will give rise to proteins extended at their C-terminus, which may bear a new function. The normal prion form is spontaneously converted to the infectious [PSI$^+$] form in approximately one of a million yeast cells. Therefore in a regular yeast population some of the members 'automatically' acquire a new phenotype. This phenotype might be eliminated from the population or fixed by mutations in the skipped stop codons (True and Lindquist, 2000; True et al., 2004; Wilson et al., 2005).

Protein aggregates as noise generators in neurodegenerative diseases. Infectious prions form protein aggregates (Uptain and Lindquist, 2002).[9] Prion aggregates display great diversity (DePace and Weissman, 2002), making them a good candidate for a noise generator. Moreover, aggregation itself increases noise and diversity, since chaperones and other proteins co-aggregate with prions and other aggregates. This may preferentially break the original weak links of the protein network of the cell, since proteins involved in strong links cannot easily be removed from their original position and so cannot easily be captured by the growing cellular aggregates. Besides infectious prions, there are numerous other forms of ex-

[8] We call inheritance epigenetic if the inheritable property is not transmitted via a DNA sequence, but is inherited with the help of other molecular mechanisms. Such a mechanism may use the modulation of DNA accessibility by DNA methylation or histone modification. Epigenetic molecular mechanisms also include RNA- and protein-based inheritance.

[9] The enhanced aggregation is what makes infectious prions dangerous for nerve cells in the sheep disease scrapie or in its human forms like the Creutzfeldt–Jakob disease. Aggregation may capture essential proteins and it disturbs cell life in many ways as described here.

tensive protein aggregation which are most characteristic of neural cells and cause severe neurodegenerative diseases, like Alzheimer's and Parkinson's diseases. Cellular noise may also be increased in these disease states, preventing the proper function of the affected nerve cells.

Ways of optimizing noise and diversity. As already mentioned in Sect. 3.1, we require an optimal level of noise to obtain stochastic resonance. In this chapter we have seen that we need an optimal level of diversity to survive. The optimization has to be finely tuned, since if buffering were too low or too high, silent mutations would never accumulate or get released, respectively. How are noise and diversity optimized at the cellular level? As discussed, chaperones help damaged proteins to refold, and hence promote cell survival. Consequently, a wise cell wants to be soaked in chaperones to obtain her bonus for life. But this would be a bad strategy. Noise and diversity cannot be dampened beyond a certain level. In other words, a lot of weak links are bad for your health! (Ask your physician or pharmacist!) In agreement with this, chaperone levels are tightly regulated, not only from the bottom, but also from the top. An increased chaperone capacity leads to a decrease in chaperone levels (Dressel et al., 2003; Feder et al., 1992; Gülow et al., 2002; Rubenstein and Zeitlin, 2000). In summary, it seems that if noise becomes suboptimal, then buffering gets reduced. We might have another solution to restore the balance between noise and buffering. If buffering cannot be decreased, increased noise generation might also help. As a possible example of this, yeast prions are much more frequent in laboratory strains than in natural or industrial isolates (Pal, 2001). Laboratory strains of the yeast fungi possibly enjoy stress levels several orders of magnitude smaller than those observed by wild-type yeast strains. Noise becomes suboptimal, and the noise-generating prions become over-expressed.

Aggregation as a friend: molecular crowding reduces noise. To show the complexity of life, let me describe another thought concerning aggregation. All the aggregation phenomena mentioned so far have been extensive, avalanche-type, irreversible processes, which produce strong links and capture various regular members of the cellular protein net, forcing them to leave their original weak links. These aggregation processes are most probably noise generators. However, we might have another type of unplanned protein association. If the association of proteins is a finely tuned, reversible process, than it results in numerous novel weak links which may actually decrease cellular diversity. If the cell can slightly modify its conditions, preferring the slow, self-organizing development of weak links, this would serve

as an ideal tool for noise regulation. This is a typical slow adaptive response, as opposed to the stress response which is always produced in a hurry. What are the cellular tools for the regulated development of weak links? In this chapter I have mentioned quite a few ways, such as chaperones, prions and other proteins, to fulfill this requirement. An additional mechanism is related to water. Water-mediated weak links are highly efficient at stabilizing protein structure (Kovacs et al., 2005). Water may act at the cellular level as well. However, its action here is completely different from that mentioned before. Cellular water content cannot be reduced to an extent that would seriously affect the weak links of protein structures. If cellular water is just slightly decreased, molecular crowding occurs (Hall and Minton, 2003). Here the 'free water' between proteins gets reduced, creating better conditions for low affinity protein association which develops weak links. It is quite likely that the cell learned to regulate its water content and, consequently, the level of molecular crowding as a finely tuned adaptation to varying conditions. [After writing this remark, I learned about the modeling results of Morishita and Aihara (2004), showing that molecular crowding may indeed reduce the noise of gene expression.]

Protein surface: a fractal for attachment optimization. *"In spite of your prediction of the Morishima and Aihara (2004) paper, something is not clear to me here. You said in Sect. 5.1 that proteins were like nano-LEGO. Their vastly different amino acids never fit together. And what happens now? You 'predict' that whenever they have a small chance of coming closer, they will just bind to each other. Do you not feel that there is a gap here?"* You are right, Spite. If proteins meet just by chance, they cannot make strong links. However their surface is just optimal to make low affinity interactions, i.e., weak links. Proteins have a self-similar, fractal surface. The fractal dimension which denotes the exponent of the scale-free distribution of self-similar elements of the protein surface is variable in individual protein regions. This means that protein surfaces are multifractal. This allows a good opportunity for the loose accommodation of a wide variety of partners (Lewis and Rees, 1985). Moreover, once two proteins get close to each other, water and chaperones help both their positioning and mutual conformational rearrangements (Kovacs et al., 2005; Liu et al., 2005).

Weak links can be fairly general in the protein net. In Sects. 2.4 and 4.2, I mentioned that most links are weak in a scale-free network. However, the link strength distribution of cellular protein–protein interactions has not yet been analyzed. Are there any data to support a large amount of weak links in the cellular protein net? Annotated protein–protein

interaction databases, e.g., that of von Mering et al. (2002), contain relatively few 'highly reliable' interactions and a vast number of 'unreliable' interactions. While many of these 'unreliable' interactions may indeed be artifacts, quite a number of them may represent low-affinity, weak links between proteins. Kinetic measurements of seemingly stable cellular complexes, such as membrane rafts, the cell nucleus, or clathrin skeleton, showed the surprising flexibility of these complexes. As an example, a core histone stays for only a few minutes in the tightly packed DNA nucleosome. This crazy flip-flopping may only occur if a large amount of the protein–protein links are in fact weak (Kenworthy et al., 2004; Misteli, 2001; Wakeham et al., 2003).

Are unstructured proteins noise buffers? Recently, a large number of proteins have been identified which contain shorter or longer sequences with no secondary structure. These sequences participate in many low affinity protein–protein interactions (Dunker et al., 2002; Tompa, 2002; Uversky, 2002; Wright and Dyson, 1999). Chaperones are unstructured proteins (Tompa and Csermely, 2004) which buffer noise and diversity. Whether unstructured proteins generally buffer cellular noise and diversity is an exciting question for future study.

Let me summarize what we have said so far. Cellular diversity is regulated by a complex array of noise buffers and noise generators. Chaperones and p53 help to decrease, while prions and other protein aggregates probably increase cellular noise and diversity. Both mechanisms might involve large changes in the configuration and the level of weak links in the cellular protein network. Are protein–protein interactions the only way for cells to stabilize themselves? Obviously not. There are many thousands of specific regulatory elements, like the negative feedbacks in genetic networks (Becskei and Serrano, 2000) described earlier. Besides these, modules of protein networks like cellular organelles may also play an important role in cellular stabilization.

Organelle diversity as a stabilizer of eukaryotic cells. Recent data indicate that mitochondria form a network which is able to show collective phenomena, such as synchronization, topological phase transition, etc. This significantly increases the speed with which a signal, like that of reactive oxygen species, can travel through a large cell, such as a cardiomyocyte spanning 0.1 mm (Aon et al., 2004a). The existence of a well-coordinated mitochondrial network and the established links between other

cellular organelles, such as mitochondria and the endoplasmic reticulum, the endoplasmic reticulum and the cell nucleus, etc., makes the presence of a cell organelle network possible inside eukaryotic cells. Subcellular organelles are not identical. They have qualitatively and quantitatively different components, different environment, different damage, different ages, etc. However, they do have a similar function, and they are therefore also linked to similar elements of the cellular net. This complex similarity and diversity pattern enables the emergence of several weak links between the individual organelle and other elements of the cellular net. Most probably, these weak links also help to stabilize cellular functions.

As we get close to the end of the first part of our trip amongst the cellular networks, I must make two things explicit. I always mentioned cellular stability, noise and diversity in general terms instead of saying, for example, that chaperones buffer developmental noise by acting on the protein network of the cell. This implies two generalizations:

- Chaperones and all the proteins and organelles mentioned above act on all cellular networks including protein, genetic, metabolic, cytoskeletal and organelle networks (Sőti et al, 2005).
- The second generalization means that chaperones and all the above mechanisms regulate not only developmental but presumably all types of diversity.

However, this leads us to the next section, since we need stress (Rao et al., 2002) to provoke the diversity of diversities.

6.3 Stress, Diversity and Jumps in Evolution

The story of our second trip into Netland is almost complete. We have got the following message: weak links may provide stability to the cellular net. It was also shown that a decrease in weak links brings greater noise and diversity to our cells. We have seen a number of potential molecular mechanisms. However, two elements are still missing:

- How and when are weak links decreased in natural conditions?
- What are the consequences of sudden increases in diversity?

Chaperone function may be compromised in several ways. Chaperones can be inhibited either pharmacologically or by introducing debilitating mutations into them. However, the most important inhibition is competitive and occurs during stress. Stress produces a large number

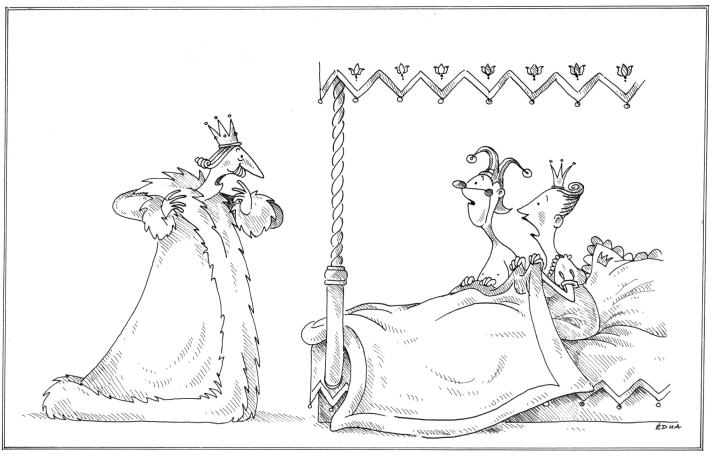

Fig. 6.3. Stress is any unexpected, large and sudden change in the life of the network, to which the network does not have a prepared adaptive response or does not have time to mobilize its adaptive response

of damaged proteins which all compete for the same set of chaperones. Chaperone levels do increase under stress, but damaged proteins may easily outnumber the available chaperones and cause a chaperone overload (Csermely, 2001b).

What happens in the cellular net if stress occurs? The cellular resources become exceedingly sparse and the cell has to concentrate all its energy to maintain the most important pathways. Everything which is not absolutely necessary is stopped. Weak links are released and do not reform again. However, the disappearance of weak links makes the system unstable. As a specific point, stress induces the reconfiguration of protein networks around chaperones by cutting their existing weak links. How is this done? Chaperones become overwhelmed with damaged proteins and release the silent mutations they protected before. As a consequence the phenotype will display profound changes.

Disintegration of the cellular net during stress. As mentioned above, stress deciphers the original weak links of the cellular protein network leading to cellular instability. The stress-induced reconfiguration of the cellular net resembles the topological phase transitions described in Sect. 3.4. From the random to scale-free topology of our protein network under normal conditions, stress may shift the net to a star phase and further to a disintegration to subgraphs. This latter phenomenon, which breaks the net-sistance (see Sect. 4.3) and implies the death of the cell, may actually occur

during apoptosis. Apoptosis often accompanies severe cellular stress (Sőti et al., 2003). Apoptosis is programmed cell death where, in the final executive phase, special proteases called caspases destroy several key proteins of the cell (Sreedhar and Csermely, 2004). It is an exciting question as to whether caspase substrates were 'selected' to destroy hubs of the cellular net in such a way as to ensure a fast and lethal disintegration of the cellular networks.

Stress increases population diversity, not only in development, but also in a surprisingly large number of cellular features, as summarized in the seminal review by Rao et al. (2002). A summary of the various forms of stress-induced diversity is given in Table 6.1.

How does stress induce all this diversity? The exposure of silent mutations described in detail in the last section is just one of the mechanisms which have been developed to increase diversity. Cell cycle progression, mitochondrial activity, oxidation and epigenetic regulation are all among the large number of events which will undergo variable changes during stress. Stress mobilizes several pathways to increase variability in the genotype, such as decreased fidelity in DNA replication, mutations, recombination, etc. (Radman et al., 2000). As an additional mechanism, an over-representation of repeats in stress-response genes has also been observed, which may induce phenotypic variability due to genetic recombination or slip-induced mispairing of bases (Rocha et al., 2002).

We have reached another point on our trip where we may take a rest. We have learned that stress induces a great diversity in an astonishingly large number of system properties. We have also learned that there are many mechanisms behind this. When we sit down for a while, we may start to think: what is missing? Just the essence is missing. Why is this good?

To give the answer let me return to the studies by Rutherford and Lindquist (1998) and repeat their conclusion here: Hsp90 buffers the phenotype of silent mutations. As I mentioned, chaperones (including Hsp90) get saturated by damaged proteins during stress, silent mutations became exposed, and diversity develops. This connects the results of Rutherford and Lindquist (1998) with evolution. If a larger stress comes, the *Drosophila* destiny has two ways to go:

- **Genome cleansing.** The normal *Drosophila* population survives. Silent mutations cause malformations and the crippled *Drosophilas* die out from the population, either directly or by natural selection due to their mating disadvantage. In both cases genome cleansing will occur and the *Drosophila* population may start to collect the

Table 6.1. Stress-induced diversity in various complex systems. As already mentioned in a general context in Sect. 4.1, due to the deductionist mainstream scientific approach, individual responses under stress have received little attention until recently. The list here is therefore far from complete. Most of the data, including those where no direct reference is given, are from the excellent reviews by Booth (2002), Himmelstein et al. (1990) and Rao et al. (2002)

Source of diversity	Reference
Prokaryotic cells	
Bacterial stress unpredictably activates lysis of bacteria by their pathogen, the lambda phage	Arkin et al., 1998; Vohradsky, 2001
Stress induces the death of individual bacteria at various times	Lewis, 2000
The doubling of different bacterial cultures varies under stress	Plank and Harvey, 1979
Stressed bacteria move towards their food with highly variable speeds and mechanisms	Alon et al., 1999; Levin, 2003; Spudich and Koshland, 1976
Stressed bacteria develop various numbers of small DNA fragments, called plasmids	
Pathogenic bacteria show a great variability in the different segments of their life cycle when stressed	
Stressed bacteria switch from one survival strategy like spore formation to quite another, rather unpredictably	
Eukaryotic cells	
Stress induces a large variation in the speed and selectivity of protein transport	Simon et al., 1992
The doubling of different cell cultures varies upon stress, together with the length of different parts of the cell cycle	Brooks, 1985
Stressed stem cells differentiate to a much more diverse set of differentiated cells and the speed of differentiation also varies greatly	Mayani et al., 1993
Heat shock induces the death of individual cells at various times	Yashin et al., 2002
The regularity and speed of the polymerization of actin, the major constituent of the cellular cytoskeleton, varies a lot in stressed cells	van Oudenaarden et al., 1999
Stress brings a great variability to coordinated gene transcription; both the onset and the extent of transcription varies	

Table 6.1. Cont. Stress-induced diversity in various complex systems

Source of diversity	Reference
Eukaryotic tissues, organisms	
Stress induces a great variability in the phenotype of the next generation	Rutherford and Lindquist, 1998; Queitsch et al., 2002
Stressed plants show a great variability in their regeneration from tissue culture, both in speed and in final shape	Finnegan, 2001
The flowering time of stressed plants shows a great variation	Finnegan, 2001
Certain tumors show a highly variable speed of development if the host becomes stressed, both at the primary tumor level and in metastasis development	Cook et al., 1998; Kemkemer et al., 2002
Artificially induced genes (transgenes) show a large variability in their expression and final effects in stressed mice	Elliott et al., 1995
Blood vessel formation shows a great variability both in speed and in the structure of the vessels in stressed animals	
The immune response of stressed animals becomes much less predictable than that of control animals	

new silent mutations again after the stressful event. This is the fate of the *Drosophila* gang in your orchard on an unusually hot summer's afternoon. However, on certain rare occasions, they may undergo a big enough stress for another outcome.

- **Evolutionary jump.** The *Drosophila* cannot survive the stress. If they had no diversity reserves in the form of silent mutations, all of them would die. This is THE END, folks. The last *Drosophila* may buzz a requiem, and after a decent decline, bring the whole species to the graveyard. However, if the effect of silent mutations can engender unprecedented diversity, one (some) of the stress-induced phenotypes may be able to survive under the drastically changed conditions. A charming *Drosophila* lady, e.g., with one eye on her hind leg and an excellent view of the hideously dangerous *Drosophila* back-catcher as it seeks its dinner for the day, will mate with a handsome guy, also with an eye on its hind leg, and become

> the Founding Mother of the new *Drosophila* population, to be remembered forever. I have to make the point explicit because you might not have got it yet: an evolutionary jump[10] has happened. (Actually, the eye jumped from the front to the hind leg, but this is not the important part of the story.)

Since the time of Charles Darwin (1859), the mechanism behind evolutionary jumps has remained a rather unexplained phenomenon. Wings do not develop gradually: first the forelimb is extended; then it turns a bit backwards; then it grows even longer. In the next step it begins to be covered by longer epidermal scales than usual. Then the scales are gradually transformed to feathers. After 150 years of debate we still do not know the exact chain of events explaining how wings actually developed (Dial, 2003). But we know something for sure: it was not as gradual as described above. Life simply does not work this way. Gould and Eldredge (1993) formulated the concept of punctuated equilibrium in 1972 to outline the theoretical background for evolutionary jumps.[11] However, the molecular mechanism was missing. Hsp90 gave the first clue to solve the puzzle as to how evolutionary jumps and punctuated equilibrium develop at the molecular level.

It is not buffering but the molecular mechanism itself that was new in the work by Rutherford and Lindquist (1998). The concept that developmental diversity can be buffered was introduced by the classic studies of Schmalhausen (1949) and Waddington (1942; 1953; 1959) who called it canalization. Hsp90 gave the first molecular explanation for the mechanisms behind canalization. Is the concept of cellular weak links novel? Sangster et al. (2004) already raised the idea that Hsp90 participates in the remodeling of protein networks. However, the assumption that Hsp90 may do this as part of the weakly linked elements of this network, and that other modulators may also behave as capacitors of evolution using this molecular mechanism was suggested later (Csermely, 2004; 2005).

Knowing the molecular mechanisms, it is not so surprising that the probability of evolutionary jumps can be regulated. Increased buffering causes fewer jumps. Conversely, less buffering leads to more jumps. Earl and Deem (2004) showed that the propensity for faster or slower evolution, which is called evolvability, can be selected. Their findings

[10] In strict terms, the expression 'evolutionary jump' is restricted to really big changes in evolution which obviously did not occur from one generation to the other.

[11] For speciation, however, the change requires a reproductive isolation in space (Gould and Eldredge, 1993).

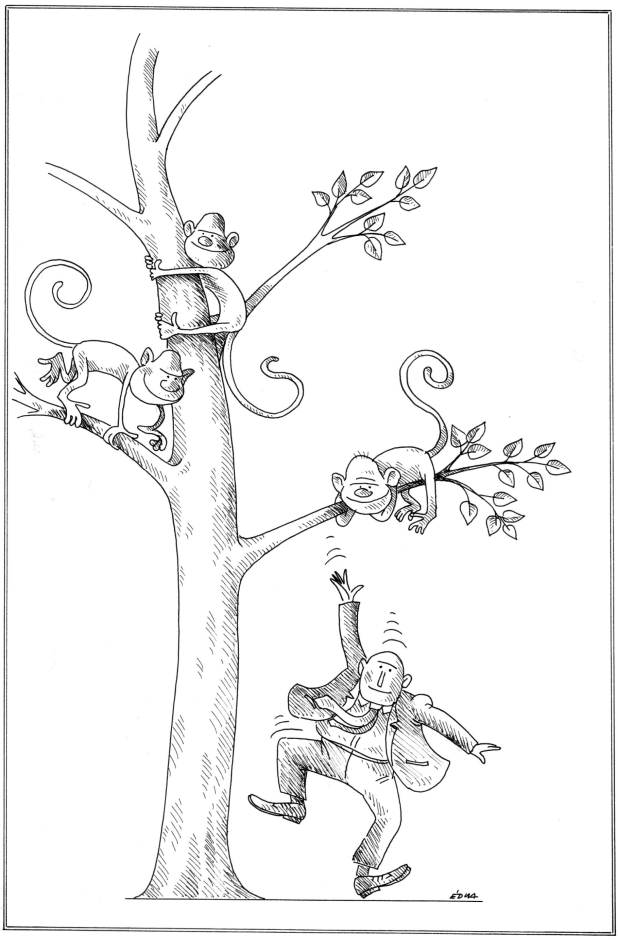

Fig. 6.4. An evolutionary jump had occurred

probably imply the regulation and selection of a number of molecular mechanisms during evolution.

1001 ways to adjust evolvability. Evolvability can be regulated by a number of mechanisms. Using the excellent summary by Molnar (2002), let me list some of the possible options here:

- **Weak-link-induced buffering.** As discussed above, weak links of the cellular network can adjust the level of buffering. A lower ratio of weak links may lead to a higher evolvability.
- **Redundancy and degeneracy.** As shown in Sect. 4.5, redundancy and degeneracy both stabilize networks and increase the number of weak links.

Decreased redundancy and degeneracy leads to higher evolvability (Willmore et al., 2005).

- **Noise generators.** On the other side of the coin, noise generation can also be achieved at the level of cellular networks. The higher the noise, the higher the evolvability of the system.
- **Error-prone replication.** When the DNA is copied, the evolvability can be regulated by regulating the fidelity of the process. More errors lead to a higher evolvability of the system.
- **Recombination.** An increased recombination rate leads to a more frequent repositioning of DNA segments in chromosomes. This also increases evolvability.
- **Level and fidelity of repair.** Repair of random damage is a key point for information stability. However, a carefully tuned regulation of the level and the fidelity of this repair can be a key mechanism for the regulation of evolvability. Lower and poorer repair leads to higher evolvability.
- **Replacement fidelity.** Repair can affect not only parts, but whole modules, or elements of the bottom network. This repair is better called replacement. A good example is the replacement of damaged cells by the proliferation of stem cells. If the fidelity of the replacement is lowered, evolvability may grow.
- **Redundancy.** Genetic information is often highly redundant. Several organisms contain duplicates of the whole genome, multiple copies of genes are even more prevalent. Decreased information redundancy or blocked release of spare copies may accelerate evolvability.
- **Storage.** Fluctuating resource density may be counteracted by efficient storage and proper mobilization. Although seemingly less efficient than the other mechanisms, inhibition of storage and release may also increase evolvability.
- **Multilevel selection.** The various sources of multilevel selection (Lewontin, 1970) may also regulate evolvability. As an example, a less tightly regulated spontaneous abortion during human pregnancy may increase evolvability.
- **Feedback.** Inhibition of regulating feedback mechanisms may significantly help the development of higher evolvability. Many other network motifs, such as balanced activation–inhibition circuits, may also regulate evolvability.
- **Network topology.** Besides weak links, cellular networks offer a number of other features which may all regulate evolvability. Jordan and Molnar (1999) described the bridge structure of cellular networks as a 'reliability factor', i.e., a factor reducing evolvability. Indeed, if we delete bridges which may act as stabilizing 'cross-links' of the network skeleton, the underlying fractal pattern of cellular networks (Song et al., 2005a), we may increase evolvability.

The 1002nd way: stress may induce evolvability via the topological phase transition of cellular networks. Liberman et al. (2005) explored various evolutionary scenarios as a function of the underlying graph structure. While random graphs suppressed evolutionary selection, scale-free and star structures were potent selection amplifiers. If we compare these findings with the topological phase transitions of network structure detailed in Sect. 3.4, we may summarize that under low levels of stress, a random network structure is preferred, and this has an inherently low evolvability. As resources diminish and stress grows, the network is transformed into a scale-free and a star topology, which increasingly speed up the evolvability of the system.

Laboratory strains evolve faster. *"A recent report by Gu et al. (2005) showed that the usual laboratory yeast strain which was isolated approximately 70 years ago from a rotten fig has elevated evolutionary rates, when compared to a wild strain isolated recently from the lungs of an AIDS patient. As far as I know, laboratory strains should enjoy a low-stress environment. They receive plenty of food, the nutrient composition is usually the same, the environment is more or less sterile, and in most cases there is plenty of room for daily life. If it is a high level of stress which speeds up evolution, how could the laboratory strain evolve more quickly?"* This is a nice reference, Spite. I looked at it, too. My first remark is that the difference was only 15%. However, the difference was significant and does indeed demand an explanation. The laboratory conditions provide an extreme example. Due to the lack of stress, the selection pressure here is usually low, and this allows 'experimentation' with a number of changes which would die out in normal, stressful conditions. Laboratory yeast strains have had time to grow a large number of silent mutations.[12] You will be surprised, Spite, but notwithstanding the long stress-free periods from time to time, the laboratory strains are under greater stress than the wild-type strains. When no experiments are performed, yeast strains are often set aside and the number of surviving yeast cells becomes extremely reduced. These periods result in extreme population bottlenecks which are known to accelerate evolution. The earlier data on the higher prion content of laboratory yeast strains (Pal, 2001) may provide an additional explanation. The lab strains may have made an 'overshoot', subsequently producing an overcompensating increase in their inherent noise which led to faster evolvability.

[12] It is worth noting that, in the original Rutherford and Lindquist (1998) experiment, the laboratory strains had more than twice as many silent mutations as the wild-type strains.

Evolutionary continuity at the molecular level. The requirement for continuity in evolution has also been formulated at the molecular level. Maynard-Smith (1970) emphasized the functional continuity of consecutive mutations in protein evolution by introducing the concept of protein space. Chaperones like Hsp90 serve perfectly to bring this concept into question as well, since they may smooth the transition between the original wild-type protein and a stable final mutant by helping the unstable mutant proteins to fold. At the protein level, the phenotype (a new enzyme activity, a novel site to get new binding partners, etc.) may also be revealed in stress, when chaperones are forced to leave the crippled protein mutants alone. Chaperones help jumps in protein evolution and actually make punctuated equilibrium (Gould and Eldredge, 1993) of the protein evolutionary landscape possible.[13]

For optimal evolvability, we require stress to expose the silent mutations. However, we also need peaceful periods to let the silent mutations develop. We need a certain level of noise to help the cellular network to reach the next equilibrium in the stability landscape. However, if noise grows too big, the system cannot dissipate the flow perturbations, and becomes continuously unbalanced.

Stress-management helps evolution. Major transitions in evolution (Maynard-Smith and Szathmary, 1995) may have required a finely tuned series of gross perturbations to induce these real evolutionary jumps, and peaceful periods to allow relative stability for the gradual development of network symbiosis and complexity.

Noise management helps the evolution of multicellular organisms. Noise management may have helped the development of the complex transcriptional control of multicellular organisms. Prokaryotes need a high translation rate for a high growth rate, which is a prerequisite for their fitness to compete efficiently for available food and outgrow other bacteria that might want to eat the same food. High translation invokes high noise. In contrast, for multicellular eukaryotes, a fast cellular growth rate is a ma-

[13] The dual role of chaperones here, i.e., their weak-link-induced stabilizing effect at the same network level, allowing unstable proteins to survive, and their weak-link-induced stabilizing effect at the cellular level, resembles the effects of weak links in society, which will be described in Chap. 8. We will see that weak links stabilize *both* the society and its members who participate in these weak links.

lignant suicide. Cellular association requires a low translation rate, which is less noisy, and provides a 'noise space' for the development of complex transcriptional control, which gives an advantage to form multicellular organisms (Levine and Tijan, 2003).

We have almost reached the end of our trip through the cellular net. Time to look again at our luggage and ask what we have learnt. Stress-induced development of diversity may help population survival and may be an important element of evolutionary jumps. Part of the mechanism behind this is a decrease in weak links in the cellular net, which leads in turn to increased noise and diversity. Both noise and diversity have to be kept at an optimal level. Noise and diversity management is an important element of long-term survival. As an introduction to our own involvement in the worlds of noise and diversity, let me end this section with a few examples illustrating the importance of optimal noise management.

Mona Lisa had an optimal level of weak links. We need an optimal amount of weak links. Too many of them would make us rather insensitive. Too few would mean a dangerously high level of potential instability. Developmental asymmetry shows the level of stabilization at sensitive and important points during embryonic development (Kowner, 2001; Willmore et al., 2005). A large asymmetry means a devastatingly high noise. On the other hand, perfect symmetry leads to over-stabilization and little room for evolutionary flexibility. A highly asymmetrical face is perceived as a monster. A fully symmetrical face will raise suspicions. Such a face is a baby face, which is not the best. If you doubt this, try to copy the left half of your beloved's face with your computer and see if you like the result. If asymmetry is present but not too dominant, we consider the face to be beautiful (Perrett et al., 1994; Swaddle and Cuthill, 1995). Eye development is another well-known example of developmental instability, behaving like litmus paper for the weak-link optimum. I have sobering news. When you are immersed in the beautiful eyes of your sweetheart, what is happening is no more and no less than an assessment of the weak-link optimum. We are not alone. Female birds select their mates by the beauty of their songs. Song-learning excellence shows the presence of the same stabilization (Nowicki et al., 2002) as the appearance of the most beautiful face.

Successful politicians may have an optimal level of weak links. Mate selection by birds and humans may not be

the only example where we use an assessment of weak-link content. The recent report by Todorov et al. (2005) shows that facial appearance may have an unexpectedly high impact on the final outcome of US elections. Congressional elections between 2000 and 2004 were mostly won by candidates who had a mature face as opposed to candidates with a baby face. In the light of the above findings, it would be very interesting to reassess the facial images and compare the level of symmetry. Personal and community preferences may have a common root.

6.4 Cancer, Disease and Aging

"This has indeed been quite an adventurous trip. I have seen crippled Drosophilas, networking mitochondria, a molecular crowd, even jumping evolution, but tell me, besides Mona Lisa and successful politicians, where does this leave us humans?" This section will be about us. As mentioned, chaperones are conserved throughout evolution. Chaperone-induced buffering is at work in you as you read this text (it may become somewhat compromised by the stress I have caused with some of my claims, but let me hope it is still functioning). I will review three situations when buffering becomes compromised and diversity develops in humans.

6.4.1 Cancer

Cancer cells live in constant stress. The tumor tissue in the suffering patient has gone through an evolution of months or years at best. Other organs are slightly better off, being products of evolutionary selection for times that are orders of magnitudes longer. Consequently, angiogenesis (blood vessel formation) is not part of the blueprint for most tumors. Hypoxia and nutrient deprivation of tumor cells are prevalent. These lead to increased glycolysis, a subsequent production of lactic acid, and the acidification of the cellular environment. Moreover, all these conditions vary abruptly due to the instability of microcirculation (Baish and Jain, 2000). In addition to all these factors, the immune system also attacks tumor cells and many types of cancer arise in a background of chronic inflammation, with permanently high immune activity (Loeb et al., 2003). Cancer cells do indeed live under constant stress. Besides the stepwise mutations of critical nodes in the protein network and the developing genetic instability (Hanahan and Weinberg, 2000), stress further enhances the instability, noise and diversity of malignant cells. Reish et al. (2003) have given a good example of the instability of cancer cells showing a decoupled replication of several

cancer genes in the two strands of DNA. These genes would normally be copied synchronously to the new DNA strands.

Weak-link-induced stabilization may play an important role in protecting us from malignant transformation and from the development of the more aggressive, metastatic forms of tumors (Csermely, 2001b). However, the instability of cancer cells gives us an additional tool to fight against them, namely, the error catastrophe (Eigen, 2002; Orgel, 1963). If we diminish the residual stabilizing weak links in tumor cells, the instability may go to such an extreme that the malignant cell is killed. Not surprisingly, inhibitors of the typical weak linkers, molecular chaperones, have been successfully introduced as anticancer agents (Neckers, 2003). Destruction of weak links may be one of the rich mechanisms triggered by these multitarget drugs.

Pink noise against cancer. Pink noise may be especially good at provoking the error catastrophe in tumor cells. As mentioned in Sect. 3.1, pink noise occasionally involves very large fluctuations. These fluctuations may push cancer cells beyond the instability threshold, without necessarily breaking the stability of healthy cells in an irreversible manner, as white noise of similar magnitude would do. Therefore in each of the chemotherapeutic, radiation and hyperthermic protocols, the use of a pink-noise-modulated flux of the noxious agent may prove to be highly beneficial.

6.4.2 Disease

Stress and cancer lead to destabilization of our cells. In modern medicine, Hans Selye (1955; 1956) was the first to note that, in spite of the various causes and final outcomes of diseases, most patients have many similar symptoms. In fact, they are simply sick. What if cellular instability in terms of disturbed relaxation and increased noise is a general phenomenon of the 'sick state' (West and Deering, 1994)? In agreement with these assumptions, heartbeats, circadian and sleep-phase variations all become noisier in sick patients (Goldberger et al., 2002; West and Deering, 1994).

Weak link therapy. Combination therapy is a success story in recent medicine. Combination therapy leads to smaller side effects. Moreover, with combined medicines, less resistance develops against the treatment. These benefits are usually regarded as an effect of the smaller doses of each

of the drugs one might use, compared with the dose of a single drug administered alone (Borisy et al., 2003; Huang, 2002; Sharom et al., 2004). This is obviously a great advantage. But what happens if we consider combination therapy, multitarget drugs (Morphy et al., 2004; Youdim and Buccafusco, 2005) and natural remedies in the context of the cellular net? These drugs all interact with their targets with low affinity. If they inhibit their target, they turn a strong link into a weak one. Weak activation also makes weak links (Agoston et al., 2005). Multitarget drugs stabilize the cell, besides their multiple effects. The smaller noise leads to fewer unexpected side effects and, since the whole system gets more stable, it is not forced to shift to a new equilibrium, the resistant state. Weak link therapy may be a winning strategy for future medicine (Csermely et al., 2005).

Hospitals dispensing Western-style medicine look more and more like automobile repair shops. We bring in our sick relative with the secret expectation that we will get back a repaired, shining, polished 'original'. In the hospital it is often only the 'sick part' of the person that is treated, whilst the process leaves the real disease of the complex living person untouched. In contrast, traditional Chinese, Indian (aryuvedic) medicine and alternative (complementary) medical treatment concentrate on the whole, and do not mobilize the vast knowledge mankind has collected about the molecular background of our body over the past two centuries. Hopefully, with the advent of the fast-propagating network and system biology approach, these two treasures will soon be reconciled.

6.4.3 Aging

We all have a sickness that cannot be cured: aging. There are three major theories of aging:

- **The mutation accumulation theory** says that the deleterious mutations that take effect 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.
- **The pleiotropy (or antagonistic pleiotropy)** theory suggests that pleiotropic genes with good early effects are favored by selection even if the same genes later show deleterious effects.
- **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 in these costly mechanisms

would help longevity but, in parallel, would decrease survival at a younger age (Kirkwood and Austad, 2000).

Aging is accompanied by a general increase in noise (Carney et al., 1991; Goldberger et al., 2002; Hayflick, 2000; Herndon et al., 2003), in parallel with a decrease in complexity (Goldberger et al., 2002). As an interesting example showing the similarities between stress and aging, fasting stress undergone by young individuals reproduces the irregularities of cortisol secretion in elderly people under normal, non-stressed conditions. The additive effects of stress and aging are shown by the fact that cortisol secretion in elderly people becomes even more irregular after fasting (Bergendahl et al., 2000).[14]

The age-induced destabilization of cellular nets is perceivable in everyday life, too. Sudden tears and running noses are both signs of the same phenomenon: the increased noise of aging. Synchronization goes downhill too. When we grow old, first jet-lags become more of a nuisance, and then suddenly you find yourself sleeping during a colleague's lecture and lying awake the whole night afterwards (Weinert, 2000). The seminal and unfortunately rather unnoticed paper by Himmelstein et al. (1990) gives a good summary of the erosion in homeostatic capacity during aging, which is more prevalent if elderly people have lived a life in poverty, under stress or under racist pressure. Not surprisingly, many of the longevity genes are hubs which control somatic maintenance and repair (Ferrarini et al., 2005; Kirkwood and Austad, 2000), most probably damping the increased disorder and noise of the aged organism.

The increased noise of aging and the wisdom of the youngest child. It is rather common in the fairy tales to find that the youngest child is the wisest. Elderly parents often have rather talented children. If our cells become noisier as we grow older, more cells will be unusual in an old woman or man than in a young person. Noisier maternal and epigenetic effects may give rise to a larger proportion of unusual gifts – and unusual problems.

Aging of cellular networks. 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

[14]The take-home message is eat well, if you grow old. But life is complicated! You should not eat too much, since it will oxidize your proteins, and accelerate your aging process.

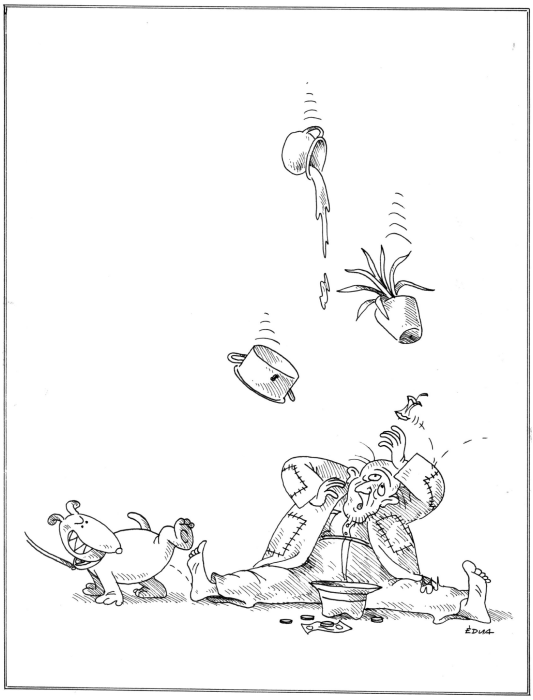

Fig. 6.5. The erosion in homeostatic capacity during aging becomes especially prevalent if elderly people have lived a life of poverty, stress or racist pressure

increased frequency of devastating falls by elderly people (Scheibel, 1985). The trabecular network of our bones and the network of bone-remodeling osteocytes have a scale-free and small-world pattern. The deterioration of these networks occurs if physical exercise is not sufficient, 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 (Benhamou et al., 2001; Bourrin et al., 1995; da Fontoura Costa and Palhares Viana, 2005; Gross et al., 2004; Hruza and Wachtlova, 1969; Mosekilde, 2000).

Do other complex systems also age? Do systems like the World Wide Web, the world economy, social networks, ecosystems, or Gaia get more unstable as they develop?[15]

[15] I am grateful to Bálint Pató for these questions.

It is difficult to establish a direct link between increased noise and loss of complexity. A loss of complexity (system integrity) will lead to increased noise and, conversely, increased noise with decreased repair and remodeling processes causes 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.

The weak-link theory of aging. Let me make an 'in neuro' experiment here. As we learned in Sect. 2.4, most of our cellular networks have a scale-free link strength distribution. Aging induces random damage in networks. At the molecular level, this means that mutations and free radicals affect various proteins in a random fashion. Most of this random damage will affect weak links. What is the result? The loss of weak links leads to a similar, but unbalanced system. What do we see in aging? I think you have the answer.[16]

Chaperone overload: a possible cause of civilization diseases. A special case of the loss of weak links is the chaperone overload in the cells of the aging body (Csermely, 2001b). Chaperones do not only buffer silent mutations in bacteria, fruit flies and plants. Obviously, the human being is no exception. We also harbor mutant genes in our cells, which remain silent because their damaged proteins are constantly repaired by chaperones. Due to the success of medicine and changes in our Western lifestyle, natural selection had been practically switched off by the 20th century. Moreover, we successfully avoid devastatingly large stresses in our life (life threatening diseases, etc.). These lead to the accumulation of silent mutations in the human genome. Even if there is any exposure of silent mutations, medical care saves us and no selection occurs. In principle this is not a problem. We have chaperones, and they will take care of our silent mutations. However, aging incapacitates and overloads chaperones. As one sign of this, 50% of our proteins become oxidized by the time we reach the age of 70 to 80. Chaperone overload leads to the exposure of silent mutations in aged people. This may contribute[17] to the development of age-related polygenic diseases, such as

[16] As I mentioned above, we see the same noisy, unbalanced system in aging as after the destruction of weak links. Actually, the loss of weak links may also account for the disintegration of responses (Goldberger et al., 2002), since in most cases the long-range, bridging links are also weak.

[17] The extent of contribution is not known. Currently, it may be negligible, but it will grow with each human generation. We may have a further 600 years until it becomes a serious threat. However, doctors should watch out today: with each

cancer, diabetes and others. As a rather surprising coincidence (or perhaps more than a coincidence), Azbel (1999) showed that human life-expectancy follows different rules before and after 75 years, which may signal that "very unusual latent mutations [...] are switched on [...] by some kind of evolvability" (Azbel, 1999). Chaperone overload may give an efficient and very general mechanism for this 'switch', which is applied to all silent mutations and not only the 'very unusual' ones. Thus chaperone overload may be a quite general reason behind age-induced disorganization of cellular responses.

We have come to the end of our trip into the cellular net. Before going a level higher, let me list the precious stones we have collected along the way:

- an optimal level of diversity and noise is important for survival;
- the cell uses a large number of noise buffers and noise generators to do this task well;
- weak links may provide an important element of noise and diversity control at the cellular level;
- noise management[18] may provide excellent opportunities for evolutionary jumps;
- diseases like cancer and aging are accompanied by an increased level of noise and diversity to the extent that the damage of weak links may be a cause of aging.

Finally and most importantly, all these mechanisms are astonishingly general and conserved.

coming generation the aging diseases will become more and more unpredictable due to the increasing exposure of random, silent mutations.

[18]The best recipe for a high evolution rate involves intermittent long periods of low noise, and short periods of high noise, or stress.