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Summary

IN order to grow, divide, and to exert their specialized functions, our cells depend on a multitude of different proteins with specific roles in the cell. Proteins are characterized by the order of their building stones, the amino acids, the length of the amino acid chain, and the way this chain is folded into a three dimensional structure. This three-dimensional form determines the function of a protein. Proteins in their functional form are folded in such a way that the water repelling segments of the chains are not in contact with the surrounding aqueous environment in the cell. If these so-called hydrophobic domains would be exposed, they could, besides with hydrophobic domains within the same proteins, also non-functionally and irreversibly stick to hydrophobic domains of other proteins. These non-productive interactions could then lead to misfolding and inactivation of the proteins involved.

During a variety of processes in the cell, proteins need to be partially or completely unfolded, such as when the linear chain is transported from one compartment in the cell to the other. To prevent that the temporally unfolded proteins stick to each other, the cell contains chaperones. Chaperones are proteins that selectively bind to hydrophobic domains, prevent their aggregation, and enable refolding of the unfolded proteins. In case proteins escape the protection by chaperones and misfold, they will be removed by the degradation system of the cell, the proteases.

Some environmental factor or certain diseases cause an accumulation of unfolded or misfolded proteins in the cell. This happens for example when cells are exposed to high temperatures or in protein misfolding diseases such as Huntington's disease or Alzheimer's disease. In these cases, the balance between the amount of damaged proteins on one site and the chaperones and proteases on the other site is disturbed in such as way that there are not

enough chaperones for proteins refolding and not enough proteases for degradation of the damaged proteins. The unfolded or misfolded proteins are not functional anymore and accumulate in aggregates, which can have damaging effects on cells and even can lead to cell death. How aggregates lead to cell death is still unknown. Because it has been discovered only recently that protein aggregates are a hallmark of protein misfolding diseases, currently much research is done on the relation between protein aggregation and cell death.

The finding that heat can cause cell death is used in the treatment of cancer (Chapter 1). Heat treatments, also called hyperthermia, are successfully used in addition to radiotherapy or chemotherapy to increase the tumor cell killing effect of these therapies. However, there are some limitations to the use of hyperthermia. Frequent use of heat treatments within a too short time period can cause a temporal heat resistance of cancer cells. This phenomenon is called thermotolerance. It has been proposed that thermotolerance is, at least in part, caused by a rapid increase in the production of a specific group of chaperones: the heat shock proteins. After a heat treatment, these heat shock proteins are produced and remain present in the cell. The level of resistance of cells against heat strongly correlates with the expression levels of heat shock proteins. To prevent that tumors become heat resistant and to make optimal use of hyperthermia in the clinic, it is desirable to better understand the effect of heat on cells and the process of thermotolerance.

In thermotolerant cells, several heat shock proteins (Hsp's) are present at increased levels. One heat shock protein whose level strongly correlates with heat resistance is Hsp70, in which '70' stands for its molecular weight. This thesis concentrates on Hsp70. It describes how Hsp70 as a chaperone contributes to thermotolerance of cells (Chapter 2 and Chapter 4) and how the chaperone activity of Hsp70 can be influenced by another, just recently identified, non-heat shock protein Bag1, which binds to Hsp70 (Chapter 3 and Chapter 4).

So far, the function of Hsp70 has been studied mainly in test tubes (Chapter 1). These studies have revealed that Hsp70 functions as a chaperone: Hsp70 protects unfolded proteins from irreversible aggregation and helps them to refold to their native state. Although it is likely that this refolding process plays a role in thermoresistance of cells, it is not clear to what extent the chaperone activity of Hsp70 contributes to thermotolerance of living cells. This has been investigated in Chapter 2. Hamster cells, which under normal conditions do not produce Hsp70, were provided with a human gene that codes for Hsp70. The production of Hsp70 in these cells was controlled by the

antibiotic tetracycline, which enabled a precise control of the production of Hsp70, independent of stress or heat. Chapter 2 describes the contribution of Hsp70 as a chaperone to the heat resistance of living cells. The effect of the presence of Hsp70 alone is compared to the effect of all Hsp's produced after heat-stress. More production of Hsp70 increased the heat resistance of cells. This resistance, however, did not reach the level of resistance of cells that produced all Hsp's. In addition, Hsp70 contributed to the protection of proteins against the damaging effects of heat. This was investigated by measuring the extent of heat-denaturation of the model protein firefly luciferase. The gene coding for this protein was adapted for expression in the nucleus or in the cytoplasm. Cells with the nuclear or cytoplasmic luciferase were heated and the activity and aggregation of the protein were followed in time. Luciferase was protected by the presence of Hsp70, both in the cytoplasm and in the nucleus. Furthermore it was investigated whether expression of Hsp70 alone was sufficient for the protection of proteins similar to thermotolerant cells. Surprisingly, in the cytosol, Hsp70 could indeed protect luciferase to the same extent as all Hsp's together. This was not true for the nucleus. In the nucleus also other Hsp's appeared to be involved in protection.

Most cellular processes require the activities of complex networks of interacting and collaborating proteins. To understand the function of newly discovered proteins one therefore often searches for proteins with known functions that bind to the new protein. In this way it has been found that Hsp70 binds to a protein, Bag1 that has been proposed to play a role in the protection of cells against cell death after a variety of cellular stresses. Bag1 levels are elevated in certain tumor cells, but the precise function of Bag1 is still unclear. In cells, four different forms of Bag1 are expressed, which differ in the amino acid chain length and are identical at one site of the amino acid chain. The longest isoform of Bag1 is expressed only in the nucleus. Interestingly, all Bag1 isoforms bind to Hsp70. Experiments with purified Hsp70 and Bag1 have shown that the smallest isoform of Bag1 inhibits the chaperone activity of Hsp70. Because in cells, the isoforms of Bag1 bind to a variety of other, non-heat shock proteins, this study has investigated how these isoforms influence the Hsp70 chaperone activity in cells (Chapter 3 and Chapter 4). It appeared that when besides Hsp70 also the levels of individual Bag1 isoforms were increased, Hsp70 was inhibited by all Bag1 isoforms, both in the cytosol and in the nucleus. The location of the Bag1 isoforms appeared to correspond to their influence on Hsp70: the longest isoform of Bag1 selectively inhibited the Hsp70 chaperone activity in the nucleus. The inhibitory effect of Hsp70 there-

fore seems to be a general characteristic of Bag1 proteins. It is clear from these findings that the influence of Hsp70 is not only determined by the levels of Hsp70. In addition, the concentration of Hsp70 binding proteins, such as Bag1, appears to have an influence on the function of Hsp70.

Chapter 2 and Chapter 3 described the influence of Hsp70 on the activity of the model protein luciferase. It has remained unclear how and where the unfolded proteins are processed in the cell. In Chapter 4, a method has been developed to directly follow the fate of unfolded proteins in living cells. The nuclear from luciferase was fused to a green fluorescent protein (GFP). The coupling of GFP to luciferase enabled us to follow the changes in localization of luciferase in a living cell under the microscope. When cells with nuclear luciferase coupled to GFP were heated, the inactivated luciferase accumulated in small foci throughout the whole nucleus. The unfolded proteins in these small aggregates were not refolded if the cells were subsequently brought back to their normal growth temperature. If, however, the cellular levels of Hsp70 were increased, the insoluble and inactive proteins accumulated in a few large aggregates during heat shock. The localization of these aggregates appeared to overlap with the localization of a sub-compartment of the nucleus: the nucleolus. Surprisingly, from the nucleolus the proteins relocalized after return to normal growth conditions, which was associated with the reactivation of luciferase. How the unfolded proteins enter the nucleolus and what happens in the nucleolus to enable refolding of the proteins remains to be investigated.

Although experiments in test tubes have provided important information about the function of Hsp70, the situation in a living cell appears to be more complex. In addition to the prevention of protein aggregation and protein refolding, Hsp70 appears also to be involved in transport and temporal storage of the unfolded proteins. It has been described that misfolded cytoplasmic proteins are transported to the centrosome of the cell, which is associated with their degradation. Transport and temporal storage at specific sites within the cell might therefore be a common event in the processing of damaged proteins. A possible advantage of storage may be that is reduces damage to the cell, because it minimizes the chance that damaged proteins aggregate with other functional proteins or protein structures. A discussion about this possibility and about the putative role of Hsp's in transport and storage processes is given in Chapter 5. To understand more about the interplay between Hsp70 and other Hsp's in the protection of cells against protein damage, in the future, more studies in living cells will be required.