HYPERTHERMIA AND PROTEIN AGGREGATION
role of heat shock proteins

Hyperthermia (treatment of cells a few degrees above their growth temperature) can lead to cell kill (reproductive capacity), and can also enhance the sensitivity of cells for radiation and chemotherapeutics. Enhancement of the radiosensitivity by heat is of clinical importance. Combined treatments of heat and radiation are already practiced in a number of radiotherapeutic institutes. The mechanisms underlying the process of cell killing by heat and of heat radiosensitization are unclear as yet. Data from the literature indicate an important role for heat-induced protein denaturation and aggregation in hyperthermic cell killing. It has already been shown that thermostolerance (transient resistance induced by a pretreatment) at the survival level paralleled resistance at the level of protein denaturation/aggregation. So, protection against protein denaturation/aggregation may result in protection against heat killing. Cells exposed to heat (or other forms of stress) respond by synthesizing a specific set of proteins, the so called "heat shock proteins (hsp's)" or "stress proteins". These proteins are thought to be involved in protection against damage induced by heat (stress). The purpose of the experiments described in this thesis was to obtain more insight into the protective mechanism(s) of heat shock proteins in protein denaturation and aggregation.

Chapter 1 provides an extensive review of literature related to (1) protein denaturation and aggregation, (2) the effects of hyperthermia on cellular processes, (3) chaperone functions of heat shock proteins and their regulation, and (4) radiosensitization by hyperthermia.

Several investigators suggested that changes in the intracellular free calcium concentration ([Ca$^{2+}$]) might be a primary step in the mechanism of hyperthermic cell killing. In chapter 2 the role of [Ca$^{2+}$] in hyperthermic cell killing is described. [Ca$^{2+}$], was measured during hyperthermia using the fluorescent intracellular calcium probe
fura-2/AM. It was concluded from these studies that, although heat-induced alterations in $[\text{Ca}^{2+}]_i$ were observed in some cell lines, no relation with cell killing was present and therefore alterations of calcium homeostasis cannot be considered as a general cause for hyperthermic cell killing. Increases in $[\text{Ca}^{2+}]_i$ induced by different agents may lead to cell killing, but such a mode of cell killing is totally different from cell killing by heat. Calcium-toxicity is dependent on $[\text{Ca}^{2+}]_e$, whereas heat-toxicity usually is not. Heat, on the other hand, causes (nuclear) protein aggregation correlating with its killing potential, whereas $[\text{Ca}^{2+}]_i$ changes are not related to protein aggregation. Finally, the data in chapter 2 combined with those from the literature also indicated that neither the induction/synthesis of hsp's nor thermotolerance development seems to be regulated by changes in $[\text{Ca}^{2+}]_i$.

In the chapters 3-5 two hsp's were investigated regarding their involvement in the cell's ability to deal with heat-induced nuclear protein aggregation. Rat-1 cells transfected with human hsp70 showed less heat-induced nuclear protein aggregation when compared to non-transfected cells [chapter 3]. This indicates that hsp70 is able to protect cells against nuclear protein aggregation. The rate of disaggregation of these nuclear aggregates after the heat treatment was comparable for transfected and non-transfected cells, indicating that unlike as was suggested before, hsp70 is not involved in nuclear recovery processes. Overexpression of hsp70 missing its ATP-binding domain still resulted in some protection against heat-induced nuclear protein aggregation and tolerance at the survival level [chapter 4]. So, ATP-binding seems of minor importance for protection against protein aggregation and heat resistance. Overexpression of hsp70 missing its peptide binding/nucleolar localization domain resulted in wild-type heat sensitivity. Also, no protection was observed against nuclear protein aggregation. Thus, the peptide binding domain seems to be required for the protective effect of hsp70. Chinese hamster cells overexpressing human hsp27 did not show protection against initial heat-induced nuclear protein aggregation, although heat tolerance was observed [chapter 5]. These cells however, did show accelerated disaggregation of these protein aggregates. So, unlike hsp70, hsp27 might be involved in the recovery from nuclear heat damage.

In thermotolerant cells, several heat shock proteins are expressed at elevated levels. At maximum tolerance (survival level), thermotolerant human cells (HeLa S3) [chapter 6] showed, in contrast to thermotolerant rodent cells [chapters 3-5], no protection against heat-induced nuclear protein aggregation. However, further increases of the cellular hsp70 level during TT development and decay resulted in protection against nuclear protein aggregates, which is in agreement with previous observations [chapters 3-4]. At all stages during thermotolerance development and decay, thermotolerant cells showed a more rapid recovery (disaggregation) from the heat-induced nuclear protein aggregates than non-thermotolerant cells. The rates of disaggregation during development and decay of thermotolerance paralleled the cellular levels of hsp27 suggesting that hsp27 is somehow involved in this recovery
process from heat-induced nuclear protein aggregates supporting the observations made in chapter 5.

Comparable levels of thermotolerance could be induced by different agents (sodium-arsenite, A-TT; ethanol, E-TT; diamide, D-TT; heat, H-TT) [chapter 7]. Stress-induced expression of the major heat shock proteins (hsp27, hsc70, hsp70, and hsp90) was generally the highest in H-TT cells and the lowest in A-TT cells. Interestingly, the four types of TT cells showed distinct differences in resistant proteins. Thermal protein denaturation and aggregation determined in isolated cellular membrane fractions was found to be attenuated when they were isolated from H-TT and A-TT cells but not when isolated from E-TT and D-TT cells. The heat resistance in the proteins of the membrane fraction corresponded with elevated levels of hsp70 associated with the isolated membrane fractions. In the nuclear fraction, only marginal (not significant) attenuation of the formation of protein aggregates (as determined by TX-100 (in)solubility) was observed in all cases. The post-heat recovery from heat-induced protein aggregation in the nucleus was faster in H-TT, E-TT and D-TT cells, but not in A-TT cells correlating with total cellular levels of hsp27.

The data indicate that heat-induced loss of clonogenic ability may be a multitarget rather than single target event. A threshold of damage may exist in cells after exposure to heat; a set of critical proteins need to be damaged before this threshold is exceeded and the cell dies. As a consequence, stabilization of only one of these sets of proteins can already be sufficient to render cells thermotolerant at the clonogenic level.

Besides its relevance for heat toxicity, earlier data suggested that protein aggregation in the cell nucleus leads to a disturbance of the DNA organization. This disturbance has been implicated in the process of thermal inhibition of DNA repair leading to heat radiosensitization. As the expression of hsp70 and hsp27 has an impact on nuclear protein aggregation and disaggregation, it was tested whether this also had consequences for heat radiosensitization [chapter 8]. Cells transfected with hsp70, showing protection against heat-induced nuclear protein aggregation, indeed showed reduced thermal radiosensitization compared to non-transfected cells. Transfection with hsp27, resulting in an accelerated nuclear protein disaggregation, revealed an accelerated decline of thermal radiosensitization. So, hsp’s as regulators of protein denaturation, aggregation and disaggregation modulate the extent and duration of heat radiosensitization. This may explain why in many cases thermotolerant cells show reduced heat radiosensitization and/or a more rapid decay.

In the general discussion [chapter 9] attempts have been made to generate new concepts and working models in order to better understand the importance of protein denaturation/aggregation for cell killing and radiosensitization by heat. Special attention has been given to the role of hsp70 and hsp27 in these processes. A scheme is presented to explain the ATP-dependence in protection against protein aggregation by hsp70. Also suggestions for cooperations between hsp’s are given.
For hsp27 a scheme is presented regarding its availability in relation to actin. Also new approaches for future research are proposed.

An important feature in cellular heat sensitivity and tolerance is the "threshold" concept for protein damage. Heat-induced cell death takes place when a set of critical heat sensitive proteins are damaged (denatured, aggregated) beyond a threshold of total damage. In thermotolerant cells most critical proteins have become resistant because of hsp action. The degree of tolerance is related to the extend of resistance of the critical proteins. When the damage to the resistance proteins is below the threshold, these (thermotolerant) cells will not die at all.