HEAT-INDUCED ALTERATIONS IN THE CELL NUCLEUS
Relation to hyperthermic cell killing and radiosensitization

Hyperthermia (exposure of cells to temperatures above their normal growth temperature) may kill eukaryotic cells and may also enhance the radiosensitivity of those cells that survived the heat treatment. Studies on the action of hyperthermia are of biological as well as of clinical importance. Biologically, hyperthermia serves as a tool to investigate cellular responses to environmental stress. Knowledge of regulation and function(s) of stress induced proteins ("heat shock proteins": HSPs) are helpful in elucidating the mechanisms of gene control and, cell proliferation, and understanding adaptation or protection of cells to an altered environment. Clinically, the possible use of hyperthermia as an adjuvant in the radiotherapeutic treatment of cancer needs the understanding of mechanisms that underlay heat-induced cell death and radiosensitization. By in vitro heating of established human (HeLa S3) and rodent (Ehrlich Ascites Tumor and LM fibroblast) cell lines, both heat killing and radiosensitization were investigated.

Eukaryotic cells are progressively killed by hyperthermia (40-46°C) with increasing time and temperature. The effects of such heat treatments were investigated at the level of the cell nucleus. Upon exposure of cells to hyperthermic temperatures, changes in the tightness of protein binding to nuclear structures were observed (Chapters 2 and 7). The amount and duration of such binding was found to be related to ensuing the extent of heat killing; this correlation held under conditions that both enhanced (using heat sensitizers like procaine or ethanol) and reduced (using the heat protector glycerol or via the induction of thermotolerance) thermal killing (Chapters 2 and 3).

The enhanced binding of proteins appeared to occur specifically at the nuclear matrix (Chapters 4,5 and 11) and at least in part to regulatory (topoisomerase II) sites present at the basis of DNA loops attached to the nuclear matrix (Chapter 6), thereby affecting the nature (Chapter 5) but not the number (Chapter 4) of DNA-matrix attachment sites. Since the nuclear matrix is a highly dynamic structure, involved in the regulation of various DNA-associated processes (see 1.3), it is suggested that the enhanced binding of proteins to this structure may affect these functions and result in thermal cytotoxicity. A less malleable matrix, an inhibition of DNA supercoiling ability, the restriction of matrix-attached regulatory sequences, and "late" DNA damage, may cause this enhanced nuclear protein binding may to become cytotoxic (Chapter 12). The proteins involved in the enhanced binding to the nuclear structure appear to be non-histone proteins and are not of cytoskeletal origin. More DNA polymerase α and β activity was found to be retained in nuclei isolated from heated cells (Chapter 7). Polyacryl-
amide gel electrophoretic analysis (SDS-PAGE) revealed an abundance of polypeptides that remained bound to nuclear structures after heating of cells while they were only present to a minor extent or even absent in similar structures from unheated cells (Chapters 5 and 11). One of these proteins was characterized by immunoblotting as belonging to the group of HSP70s. The aberrant protein-protein binding in the nucleus directly after heating may serve as a trigger for HSP synthesis. Combined with data from the literature about the properties of the HSP70s, our results point to a role of HSP70 in the restoration of the heat-induced alterations in nuclear protein binding. Hence, the presence of an increased amount of HSP70s in the nucleus at the time of heating of cells (e.g., in thermotolerant cells) will lead to an enhanced rate of restoration of normal nuclear architecture after the heat treatment, leading to protection against thermal cell death (Chapter 2 and 3).

Radiation-induced cell killing is probably caused by non- or misrepaired damage to the DNA (see 1.3; 12.2). The observed increased radiation sensitivity upon exposure of cells to heat was investigated with respect to DNA damage induction and repair. Hyperthermia mainly affects the latter. Repair of radiation-induced damage as measured using the alkaline unwinding technique (Chapter 9) and the fluorescent halo-assay (Chapter 11) was inhibited. The effect of hyperthermia on DNA repair rates as such is not sufficient to fully explain radiosensitization (Chapter 12). The efficiency of DNA repair is dependent on several, yet unknown factors which may contribute to the extent of thermal radiosensitization.

Initially a good correlation was found between the loss of cellular activity of the repair enzymes DNA polymerase α and β and the extent of heat radiosensitization (Chapters 8 and 12). The effect of thermotolerance on heat-induced loss of polymerase activities resembled the extent of radiosensitization when heat treatment was immediately followed by radiation (Chapter 10). However, as the time interval between heat and radiation was increased, this correlation did not hold. Heat radiosensitization disappeared more rapidly in tolerant than in nontolerant cells. The recovery of cellular polymerase activities, however, occurred with similar kinetics in both tolerant and nontolerant cells (chapter 10). Combined application of heat and aphidicolin (a DNA polymerase α inhibitor) on DNA repair also revealed that heat-inactivation of cellular DNA polymerase α activity cannot be a (major) determinant in hyperthermic inhibition of repair (Chapter 9). Analysis of data from the literature (see 12.2) also sheds doubt on a functional relation between heat-induced loss of cellular DNA polymerase activity and radiosensitization. Changes in availability of DNA polymerases for the damaged DNA in the cells, due to enhanced binding of these enzymes to the nuclear matrix and to intracellular reallocation after heating, may be important factors and should be taken into consideration.

Data were obtained showing that the overall observed increase in binding of proteins to the nuclear matrix at the moment of radiation was related to the inhibition of DNA repair (using the halo-assay: Chapter 11) and the extent
of radiosensitization (survival: Chapter 10) after hyperthermia. This correlation held under conditions of thermotolerance; also the more rapid recovery from radiosensitization in tolerant as compared to nontolerant cells was reflected at the level of nuclear protein binding (Chapter 10). Enhanced nuclear protein binding at the time of irradiation may lead to radiosensitization through:

1. a change in distribution (functional activity) of repair enzymes as suggested for the DNA polymerases (Chapter 7).
2. a reduction in the accessibility of the damaged DNA for the repair enzymes as suggested by the reduced detectability of damage using the halo-assay (Chapter 11), the reduced accessibility of the topoisomerase II sites in the DNA (Chapter 6), and data from the literature.

In conclusion, the results show that hyperthermia causes changes at the level of the cell nucleus that may be important for both thermal cell death as well as for thermal radiosensitization. The parameter "enhanced nuclear protein binding" correlates with hyperthermic killing when both the extent and duration of this binding are taken into account. For the enhanced radiosensitivity, the heat-induced alterations in intranuclear protein binding may play a determining role, altering the normal interactions between damaged DNA and repair enzymes, leading to less adequate repair.