Tracking DNA double-strand breaks

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Citation for published version (APA):

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CHAPTER 8

SUMMARY AND GENERAL DISCUSSION
DSBs are the most hazardous of all DNA lesions. DSBs may lead to carcinogenesis. On the other hand, they are the critical lesions leading to cell death in various cancer therapies. For these reasons, induction and cellular responses to DSBs are studied in great detail.

In experimental settings, induction of DSBs is a critical step. In the present thesis, we present two methods to induce DSBs in small areas of the cell nucleus. Both methods have, in contrast to many existing techniques for local DSB induction, a well-characterized damage spectrum. This is an important advantage, because different proteins are recruited in the various cellular responses to different DNA lesions. DSBs are analyzed using fluorescence microscopy and both methods for damage induction are suitable for studying live cells as well as fixed cells.

In Chapter 2, a method is presented to induce DSBs by irradiating cells with $\alpha$-particles. DSBs are induced in easily recognizable linear tracks. It is possible to apply a low dose by inducing one track of DSBs per cell, each track corresponding with a dose of only 0.5 Gy.

In Chapter 3, we describe a soft X-ray microbeam system for local DSB induction. In contrast to the $\alpha$-particles method, large numbers of DSBs are induced simultaneously in a large number of cells. The method is very convenient to detect proteins that accumulate at DSBs in low copy numbers, because the system can be operated at a high dose rate.

Next, supporting techniques are described, that facilitate application of the DSB induction methods. This concerns the attachment of cells to the mylar membrane of culture dishes used in both irradiation set-ups (Chapter 4). In Chapter 5, procedures are provided for the analysis of movement of DSBs in nuclei.

In Chapter 6, we present a study in which the $\alpha$-particle method was applied to analyse the dynamic behaviour of DSBs. It was found for the first time that chromatin domains that contain DSBs are mobile and can fuse. This observation is relevant for cancer therapy, because fusion of two or more chromatin domains that contain unrepaired DSBs may increase the probability of chromatin exchanges. Chromatin exchanges, induced by radiotherapy in normal tissue around a tumor, may contribute to the risk to develop a new type of cancer. We estimate that DSB-containing chromatin domains can travel a distance of 1-2 $\mu$m. This seems a limited distance, but we have preliminary results indicating that chromatin domains that contain undamaged DNA are less mobile. Furthermore, we observed that movement of DSB-containing domains is non-directional. The obvious question that remains to be discussed is: is movement of DSB-containing domains functional or accidental? It has been suggested that DSBs move to repair centers. Our observation that
movement of DSB-containing domains is non-directional, argues against this concept. Bringing together two or more domains containing open DNA ends increases the probability of chromosome exchanges, leading to cell death or carcinogenesis. In future experiments, we will study the relation between distances between DSBs and the probability of chromosome exchanges. The soft X-ray system described in Chapter 3 is the ideal tool to address this question, because it allows comparison of cell populations irradiated with the same dose but with different distribution patterns of DSBs.

Telomeres are nucleoprotein structures at the end of chromosomes that disguise and protect chromosome ends so that they are not recognized as DSBs. Many DNA repair-related proteins are associated with telomeres and recent studies suggest that the reverse might be true as well, i.e., that telomeric proteins such as TRF2 are recruited to DSBs. In Chapter 7, we demonstrate that at this crossroad of DSBs and telomeres, characterization of the damage spectrum is essential. We found that accumulation of the telomeric protein TRF2 at DSBs can only be detected at DNA damage sites induced by multiphoton laser irradiation and not at DSBs induced by induction techniques with a better characterized damage spectrum. This result is not surprising, considering that a role of TRF2 at DSBs is unlikely, the more so because it has been suggested that TRF2 inhibits ATM to avoid a DNA damage response to proper chromosome ends. Inhibition of ATM at DSBs would not be a good strategy for cells, because ATM plays a central role in the cellular response to DSBs.

In conclusion, it can be stated that the DSB-inducing techniques described here are useful tools to unravel processes induced by DSBs.