DISCUSSION: CELLULAR DNA STRAND BREAKAGE

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Many techniques are now available for measuring DNA single- and double-strand breaks by ionizing radiation. As we are reminded by the results of Van Loon and co-workers, single-strand break measurements are far simpler to perform than double-strand break assays and are sensitive to damage by lower radiation doses. However, while the number of single-strand breaks is linearly dependent on dose, it is not predictive for cell killing by ionizing radiation. Conversely, the number of initial double-strand breaks has been shown to correlate with cell killing. Double-strand break damage, if unrepaired or inaccurately repaired, is believed to be the likely "lethal lesion", leading first to chromosome breakage and then to loss of reproductive capacity, mutation and perhaps transformation. While there is little doubt that initial DNA damage is responsible for the final outcome of cell death, time and multiple DNA repair events separate these two processes. It is therefore somewhat unexpected that such good predictions for cell killing across a variety of cell lines can be obtained with the filter elution assay based only on initial numbers of DNA double-strand breaks.

At various times during the Workshop, the concept of lesion subsets was addressed. All DNA double-strand breaks are not likely to be equal in terms of their chemical nature or biological effect; some double-strand breaks are more likely to lead to chromosome damage and cell killing perhaps because they are less able to be repaired. Methods for measuring double-strand breaks probably do not distinguish between these subsets. In addition, measurement of initial damage does not take into account differences in rates or accuracy of repair of lesions, factors which are known to play a major role in cell survival and mutation.

The relevance of DNA damage measurements in predicting cell response to radiation damage was discussed at length, especially with regard to the neutral filter.
elution method (or neutral "illusion" method, as one participant quipped) now in widespread use. Radford's excellent correlation between DNA damage and cell killing in fibroblasts (Radford et al., 1986) promises, within certain limitations, a method for predicting cell viability after radiation exposure. As shown by Prise and Michael, mammalian cell killing also correlates well with DNA double-strand break induction for high LET radiations, although the slope of the curve is increased due to the increased killing efficiency of the DNA damage. For low LET radiation, Radford showed that the correlation between initial DNA damage and cell killing is altered for ataxia telangectasia cells, similar to results reported by Iliakis (1988) for xrs-5 cells. It is not surprizing that the correlation breaks down for these cells which are deficient in repair. But perhaps this is telling us that the variety of radiation responses observed for different cell lines is not a result of relative repair deficiency or proficiency, but instead reflects some physical property of the cell which the neutral filter elution assay is able to detect.

The linear correlation between initial double-strand breaks and cell killing is of particular importance since conclusions about the mechanism of radiation killing have been drawn based on the presence of a shoulder on the dose-response curve for double-strand break induction (Radford et al., 1988). The mechanism for such a non-linear induction of double-strand breaks by low LET radiation remains an area of considerable controversy. Could the linear-quadratic dependency indicate one and two-track action as initially proposed by the Chadwick-Leenhouts model? Or perhaps the non-linearity reflects inherent differences in cell lines (e.g., levels of radioprotective molecules). On a statistical basis, the probability of two independent single-strand breaks interacting to produce a double-strand break is highly unlikely. The basis for a possible chemical saturation process needs to be defined. As shown by several investigators at this Workshop, other methods for measuring DNA double-strand breaks, such as gel electrophoresis, neutral DNA precipitation and neutral sucrose sedimentation generally demonstrate a linear dependency between DNA damage and radiation dose. Regardless of the reason for the non-linearity of double-strand break induction measured with the filter elution method, the lesion (or subset of lesions) measured by this method appears to correlate very well with cell killing. Understanding why non-linearity is detected in initial events could tell us much more about what factors influence radiation-induced cell killing.