

## A Universal DNA Lesion Yield in Irradiated Cells: 0.005 DSB/Gy/Haploid Genome

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It is generally assumed that X-rays and  $\gamma$ -rays indiscriminately damage cellular macromolecules, principally by indirect effects mediated by reactive oxygen species (ROS). The premise is simple: water is the most abundant chemical found in living cells and hydroxyl radicals ( $\text{HO}^\bullet$ ) are the primary species derived from the ionization of water. As  $\text{HO}^\bullet$  are extremely reactive with organic compounds, radiation damage should be distributed uniformly across cell targets. This, however, is not the case. **For phylogenetically distinct bacterial and archaeal species, radioresistance consistently coincides with a greatly diminished susceptibility to ROS-mediated protein oxidation, but with similar DNA lesion-yields as sensitive cells.**

Conflict between theory and experiment first developed as inferences from *in vitro* studies on radiation effects on DNA were broadly applied to *in vivo* conditions. Eighty percent of radiation damage to purified DNA in aqueous solution is caused by  $\text{HO}^\bullet$ , and these non-specific reactions are readily abolished by the addition of small organic compounds or DNA binding proteins; *in vitro*, 20% of DNA damage which is not preventable by ROS-scavengers is assigned to direct effects. Accordingly, DNA damage in irradiated cells was attributed mainly to indirect effects. Yet, in diverse cell-types with vastly different antioxidant statuses and radiation resistances, lesion-yields for DNA double-strand breaks (DSBs) were near parity, and similar to DSB levels in irradiated samples of dried DNA where damage is limited to direct effects. DNA packaged in cells and viruses is highly resistant to ROS during irradiation, at least 20 times less susceptible to damage than when the same DNA is purified and irradiated in water. Remarkably, for organisms spanning the limits of radiation resistance and sensitivity, including bacteria, human cells, yeast, animals and viruses, the DSB lesion-yields for ionizing radiation fall within a narrow range (0.003-0.006 DSB/Gy/Mbp per haploid genome). Indeed, the most severe form of DNA damage in irradiated cells – the DSB – is caused mainly by processes which are not overtly affected by their capacity to scavenge ROS. In contrast, the amount of protein damage in irradiated cells is strongly influenced by their

antioxidant status. **The primacy of DSBs in contemporary radiation toxicity models is not consistent with these findings.**

In marked contrast to DNA damage in cells, inter-species comparisons of bacteria showed that the levels of protein damage in irradiated cells are highly variable, quantitatively related to survival, and mechanistically linked to the accumulation of  $Mn^{2+}$ . Proteins in irradiated *D. radiodurans* are protected from ROS but lose their resistance when purified from the cells. Recent studies showed that protein-free cell extracts of *D. radiodurans* are armed with ROS-scavenging orthophosphate and metabolite complexes of  $Mn^{2+}$  which specifically protect proteins (Daly *et al.*, 2010). When reconstituted *in vitro* at physiologically relevant concentrations, the ligands displayed multifactoral antioxidant synergism when combined with  $Mn^{2+}$  and preserved the activity of multimeric enzymes exposed to 50,000 Gy, conditions which obliterated DNA. When applied *in vivo*, they protected bacteria and human cells from extreme cellular insults caused by  $\gamma$ -rays. **The key difference between naturally sensitive and resistant bacteria is that the latter appear to have developed chemical mechanisms for protecting their proteins, which include those needed for DNA repair.**