Induction of DNA Damage by Light Ions Relative to $^{60}$Co $\gamma$-rays

Robert D. Stewart
Department of Radiation Oncology, University of Washington School of Medicine, Seattle, WA, USA

Abstract

The specific types and numbers of clusters of DNA lesions, including both DNA double-strand breaks (DSBs) and non-DSB clusters, are widely considered 1 of the most important initiating events underlying the relative biological effectiveness (RBE) of the light ions of interest in the treatment of cancer related to megavoltage x-rays and $^{60}$Co $\gamma$-rays. This review summarizes the categorization of DNA damage, reviews the underlying mechanisms of action by ionizing radiation, and quantifies the general trends in DSB and non-DSB cluster formation by light ions under normoxic and anoxic conditions, as predicted by Monte Carlo simulations that reflect the accumulated evidence from decades of research on radiation damage to DNA. The significance of the absolute and relative numbers of clusters and the local complexity of DSB and non-DSB clusters are discussed in relation to the formation of chromosome aberrations and the loss of cell reproductive capacity. Clinical implications of the dependence of DSB induction on ionization density is reviewed with an eye towards increasing the therapeutic ratio of proton and carbon ion therapy through the explicit optimization of RBE-weighted dose.

Keywords: DSB; SSB; base damage; MCDS; particle RBE; light ions

Introduction

This review summarizes key mechanisms of action and the overall quantitative trends in the numbers and types of DNA damage created by charged particles relative to $^{60}$Co $\gamma$-rays and megavoltage (MV) x-rays. The review is presented through the lens of the Monte Carlo damage simulation (MCDS) developed by Stewart and colleagues [1–4]. The MCDS software is freely available from the author or as an Internet download (see Appendix A). At present, the MCDS is the only available Monte Carlo model, to our knowledge, capable of generating nucleotide-level maps of the DNA damaged when cells are irradiated under aerobic or anoxic (or reduced) oxygen conditions [4]. The MCDS has been extensively benchmarked against measurements and the results of detailed track-structure simulations for light ions ($Z = 1–26$) of varying linear energy transfers (LETs) [1, 2, 4, 5]. The MCDS has also been tested against measurements of DNA double-strand break (DSB) induction for low-energy electrons and photons [6–8].

Monte Carlo simulations of radiation-induced DNA damage are, in effect, the modeled embodiment of what we know—or believe we know—from the analysis of a large number of published studies on the induction of DNA damage by ionizing radiation. Monte Carlo simulations are also useful for the generation and testing of hypothesized mechanisms of action [9–13], for developing quantitative algorithms for the classification of clusters of DNA lesions as DSB and non-DSB clusters [1, 14–17], and for the analysis of measured fragment-size distributions [15, 18, 19]. The fundamental radiobiologic and clinical significance of particle relative biological effectiveness (RBE) for the end point of DNA...
damage is reviewed in relation to the RBE for mutagenesis, chromosome aberrations, and reproductive cell survival. Areas in need of additional research are highlighted by comparing the relative and absolute numbers of different types of DNA damage from the MCDS to data from published track-structure simulations [5, 18, 20–25]. Differences in the yields and types of radiation damage to DNA are an indication of significant differences in either the modeled mechanisms of action or uncertainties associated with determinations of model inputs (parameters). The potential clinical significance of using the RBE for DNA DSB induction is briefly discussed.

### The 3 Mutually Exclusive Categories of Initial DNA Damage

Exposure to ionizing radiation produces many different types of DNA lesion. As explained in Appendix B, a DNA lesion is a single nucleotide that has sustained an abnormal chemical alteration, such as a damaged or missing base or a strand break [26]. Strand breaks may arise from direct or indirect damage to the deoxyribose sugar or as a break in the phosphodiester bond connecting adjacent nucleotides on the same side of the DNA helix. Groups of several DNA lesions within 1 or 2 helical turns of the DNA, which have been referred to as multiply damaged sites [26], clustered damages [27], and clusters of DNA lesions or simply clusters [28, 29], are considered one of the distinguishing hallmarks of ionizing radiation [30–32]. Theoretical considerations suggest that, in addition to isolated DNA lesions, low linear energy transfer (LET) radiation (< 2 keV/μm) can create clusters with ≥ 10 individual DNA lesions [1], although the average number of lesions per cluster is usually closer to ~ 1.4 to 2 [29]. High-LET radiation (> 100 keV/μm) is capable of producing damage of even greater complexity, that is, an average of 4 to 5 lesions per cluster [29] and ≥ 25 lesions in some clusters [1].

All configurations of initial DNA damage can be classified into 1 of 3 mutually exclusive category of damage: (1) DSBs, (2) single-strand breaks (SSBs), and (3) base damage (BD). To provide additional information about the nature of the initial damage formed by radiation, SSBs and DSBs are sometimes subdivided into various types of simple and complex damage by the number of lesions forming the cluster [1, 2]. Idealized schematics with several types of damage are shown in Figure 1. Other damage-classification schemes that emphasize varying aspects of cluster complexity, such as the small-scale (less than a few 10s of base pairs [bp]) spatial arrangement of strand breaks on the same or different strands of the DNA helix, have also been proposed [14, 17]. Terms such as regionally multiply damaged sites (RMDS), DSB clusters, and DSB sites have also been proposed to capture the larger-scale clustering of DNA damage in kilobase pair to megabase pair segments of DNA [5, 15]. One pragmatic measure of cluster complexity is the dimensionless ratio of the number SSBs (or BDs) per DSB. That ratio is a measure of local cluster complexity because a DSB can only be formed in clusters with 2 or more individual DNA lesions, whereas the other 2 major categories of mutually exclusive DNA damage (SSB and BD) are composed of ≥ 1 lesions. The range of the ratio of SSB per DSB is from ≥ 20 for low LET radiation down to < 3 for high LET radiation [29]. A large value for...
the ratio of SSB per DSB indicates a low level of cluster complexity (average of ~ 1.4 lesions/cluster) and a small value for that ratio indicates a high level of cluster complexity (average of < 4-5 lesions/cluster).

**Mechanisms of DNA Damage Induction by Ionizing Radiation**

Radiation damages the DNA through direct and indirect mechanisms [26]. As a direct effect, radiation transfers energy to the DNA molecule, and indirect effects arise when the deposition of energy near the DNA creates reactive species that diffuse and interact with the DNA. The primary chemical species believed responsible for indirect effects is the hydroxyl radical (-OH).

First, radiation ionizes a water molecule:

\[
\text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^+ + e^-. \tag{1}
\]

The \(\text{H}_2\text{O}^+\) is a positively charged ion that interacts with a nearby water molecule to form an -OH radical through the following reaction:

\[
\text{H}_2\text{O}^+ + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{OH}. \tag{2}
\]

Hydroxyl radicals have 9 electrons, and 1 of them is unpaired and highly reactive. The interactions of -OH radicals with DNA can produce BD and strand breaks, that is, the same types of lesions direct mechanism. The average diffusion distance of an -OH radical in a cellular milieu is about 6 nm [33] or about 3 times the diameter of the DNA double helix, which implies that the chance an -OH radical will damage the DNA decreases rapidly with distance beyond about 6 nm.

On the spatial scales in which individual DNA lesions and clusters of \(\geq 2\) lesions are created within a turn or two of the DNA. The direct ionization of the DNA, as well as the diffusion and attacks of -OH radicals, must happen within subnuclear volumes of \(\sim 5\) to 10 nm. Otherwise, individual lesions formed by the passage of an ion near a specific stretch of the DNA would be separated by 10s, 100s, or even 1000s of base pairs of DNA and considered (counted) as part of a different, rather than the same, cluster. For example, track-structure simulations suggest that DSB and other types of non-DSB cluster are formed by \(\geq 2\) to 5 ionizations localized in targets with diameters \(\sim 1\) to 4 nm [34]. Are those ionizations formed by a single (primary) charged ion passing within or near the nanometer-sized target or by \(\geq 2\) statistically independent charged particle passing through or near target? A 1-event (hit) mechanism implies that the initial yield of DNA damage (ie, the yield of damage before any chemical or enzymatic repair) is proportional to the absorbed dose, whereas a 2-event mechanism implies that the initial yield of DSB and non-DSB clusters will be a linear-quadratic function of the absorbed dose. Experiments show that the induction of many types of DNA damage is proportional to an absorbed dose of \(\leq 100\) s or even 1000s of Gy [35]. For human cells with a DNA content of \(\sim 6\) gigabase pairs (Gbp)/cell, the initial number of DSBs/cell increases linearly with increasing dose from a few milligray up to \(\geq 100\) Gy [36]. In yeast cells, the initial DSB yield/cell increases linearly with a dose \(\geq 2400\) Gy [37]. The production of BD is also linear for absorbed doses up to at least a few 100s of Gy [32]. These observations all imply that the 1-hit mechanism is responsible for the induction of DNA damage by radiation.

The observation that DSB induction is a linear function of absorbed dose up to several 100s or 1000s of Gy implies that strand breaks formed by individual (separate and statistically independent) charged particle tracks are rarely created in close enough spatial proximity to form a DSB. That is, all DSBs are created by individual (primary) particle tracks,\(^1\) rather than through the combined action of \(\geq 2\) independent particle tracks, which produce individual lesions within a specific 10- to 20-bp segment of DNA. If DSBs were created through the action of \(\geq 2\) independent particle tracks, DSB induction would be a linear-quadratic function of absorbed dose, rather than a linear function of absorbed dose, as experimentally observed. Similar experimental and theoretical considerations imply that non-DSB clusters (simple or complex SSBs and clusters of \(\geq 1\) BD) are also created through the action of individual, rather than multiple, particle tracks. A 1-hit mechanism of action for the creation of DSB and non-DSB clusters also implies that local cluster complexity is the same for small and large doses of the same type of ionizing radiation. That is, the number of clusters per gigabase pair of DNA increases with increasing dose, but the small-scale number of lesions per cluster (cluster complexity) is independent of dose up to a few 100s or 1000s of Gy of absorbed dose.

**Effects of Particle Type, Kinetic Energy, and O₂ Concentration on DSB Induction**

Figure 2 shows the RBE for DSB induction (\(\text{RBE}_{\text{DSB}}\)) for electrons and light ions as a function of the square of the effective charge on the ion divided by the speed of the particle, \((Z_{\text{eff}}/b)^2\). In general, particle LET increases with increasing \((Z_{\text{eff}}/b)^2\) and

\(^1\)The “primary particle track” includes spatially and temporally correlated \(\delta\)-rays and other secondary ions.
with decreasing particle kinetic energy (Appendix C, Figure C.1). The main advantage of using \( \frac{Z_{eff}}{b} \)\(^2\), rather than LET for the presentation of RBE data is that particles with the same \( \frac{Z_{eff}}{b} \)\(^2\) have approximately the same collisional stopping power (ionization density), regardless of particle type or kinetic energy, whereas particles with the same LET can have very different kinetic energies and biological effects. For example, a 4 keV and 900 keV \(^1\)H\(^+\) ions both have an LET of ~28 keV/\( l_{\mu m} \), but a \( \frac{(Z_{eff})^2}{b} \) of 10 970 and 518, respectively. Based on LET, those ions would be expected to be equally damaging regarding molecular and cellular end points; however, as shown in Figure 2, the low-energy 4 keV \(^1\)H\(^+\) ion is ~70% more effective at creating DSBs, per unit of absorbed dose, than the higher-energy 900 keV \(^1\)H\(^+\) ion.

Except for a few of the results from track-structure simulations, which appear to be outliers, estimates of \( RBE_{DSB} \) from MCDS and track-structure simulations are in excellent agreement for values of \( \frac{(Z_{eff})^2}{b} \) up to ~500 for normoxic conditions (Figure 2, left). This implies good agreement in the \( RBE_{DSB} \) estimates for protons with an LET \( \leq 27 \) keV/\( l_{\mu m} \) (\( \geq 1 \) MeV kinetic energy), 39.4 keV/\( l_{\mu m} \) (\( \geq 15 \) MeV kinetic energy) for \(^4\)He\(^{2+}\) ions, and ~60 keV/\( l_{\mu m} \) (\( \geq 400 \) MeV) for \(^{12}\)C\(^{6+}\) ions. For intermediate values of \( \frac{(Z_{eff})^2}{b} \) (\( \sim 500 \) to \( \leq 1000 \)), the MCDS estimates of the \( RBE_{DSB} \) are slightly larger than the values from track-structure simulations. In general, estimates of \( RBE_{DSB} \) from the MCDS increase in monotonic fashion with increasing \( \frac{(Z_{eff})^2}{b} \) and approach an asymptotic RBE of ~3.4 for values of \( \frac{(Z_{eff})^2}{b} > 10000 \). Track-structure simulations predict the same general trends in \( RBE_{DSB} \) with increasing \( \frac{(Z_{eff})^2}{b} \), but approach values \( \sim 2 \) to 2.5, that is, a factor of 1.4 to 1.7 less than the asymptotic value predicted by the MCDS. Under fully anoxic conditions (Figure 2, right), the overall trend in \( RBE_{DSB} \) is about the same as for normoxic conditions (pO\(_2\) \( > 5\% - 7\% \)). However, \( RBE_{DSB} \) approaches an asymptotic value of ~9.9 for large \( \frac{(Z_{eff})^2}{b} \), which corresponds to an oxygen enhancement ratio for very high LET radiations of ~2.91, that is, \( \text{Asymptotic } RBE_{DSB(\text{anoxic})} = \text{Asymptotic } RBE_{DSB(\text{normoxic})} \times \text{Oxygen enhancement ratio (OER)}. \)

Figure 3 shows \( RBE_{DSB} \) as a function of the continuous slowing-down approximation (CSDA) range of \(^1\)H\(^+\), \(^4\)He\(^{2+}\), and \(^{12}\)C\(^{6+}\) ions in water. Small values in the CSDA range correspond to scenarios in which a high-energy (low-LET) ions, such as a clinically relevant 200 MeV proton beam incident on a patient, has lost most of its kinetic energy and is nearing the so-called track end. For \(^1\)H\(^+\) and \(^4\)He\(^{2+}\) ions, estimates of \( RBE_{DSB} \) from the MCDS are in excellent agreement with track-structure simulations [5] when the particle range is large compared with the dimensions of a typical human cell (~10 \( \mu m \)). For \(^{12}\)C\(^{6+}\)
ions, estimates of $RBE_{DSB}$ from the MCDS are in good agreement with the track-structure simulations [5] for ions with a range of about > 5 mm, which corresponds to a $^{12}C^{6+}$ ion capable of passing through a few 100 cells. For shorter range $^{12}C^{6+}$ ions, estimates of $RBE_{DSB}$ from the MCDS are ≤ 30% larger than the value for the track-structure simulations [5].

The differences in track structure and MCDS estimates of $RBE_{DSB}$ for short-range particles are most likely due to differences in the modeled irradiation geometry. The MCDS simulations are premised on an irradiation scenario in which the entire cell nucleus is uniformly irradiated by charged particles, regardless of the range of the particle. In contrast, Friedland et al [5] modeled DNA damage arising from ions sampled from a circular disk located outside the cell nucleus but inside the cytoplasm. Monoenergetic, monodirectional, charged particles are directed from that disk toward the cell nucleus. For high-energy, long-range particles, the differences in the irradiation geometry have a negligible effect on $RBE_{DSB}$. However, for short-range particles, the fine details of the irradiation geometry can become quite significant because of the effects of energy and path-length straggling, changes in charged particle stopping power while passing through the cytoplasm and cell nucleus. The effects of stoppers (ions with a range < diameter of the cell nucleus) will become very significant in track-structure simulations [5] because only part of the nuclear DNA will be irradiated, whereas the entire volume of nuclear DNA will still be irradiated in the MCDS. In addition to differences in the irradiation geometry at the cellular level (~ 5-10 μm), the algorithms used to group DNA lesions into DSB and non-DSB clusters (16), small-scale (< 10 nm) modeling of the DNA and chromatin structure, and saturation effects (eg, radical-radical -OH recombination or multiple ionizations in the same nucleotide) may have a significant effect on Monte Carlo simulations of very high LET radiation (eg, data shown in Figure 3 for $^{12}C^{6+}$ ions with a range of < 2-4 mm).

**Effects of Particle Type, Kinetic Energy, and O2 Concentration on non-DSB Clusters**

Figure 4 illustrates the predicted trends in the induction of non-DSB clusters (SSBs and BDs) as a function of $(Z_{eff}/b)^2$ under normoxic (left panel) and anoxic (right panel) conditions. As Figure 4 (left) shows, the relative numbers of non-DSB clusters decreases in monotonic fashion with increasing LET under normoxic conditions. Except near the track ends of very high LET particles ($(Z_{eff}/b)^2 > 10^4$), MCDS estimates of $RBE_{SSB}$ (Figure 4, solid line) are within ± 5% to 10% (Figure 4, filled symbols) of the track-structure simulations of Friedland et al [5]. The MCDS predicts that the same general trend in the yield of non-DSB clusters as a function of $(Z_{eff}/b)^2$ holds for cells irradiated under anoxic conditions, although there is a small peak in $RBE_{SSB}$ in the $(Z_{eff}/b)^2$ range from about 400 to 2000. The peak value of $RBE_{SSB}$ at $(Z_{eff}/b)^2 \sim 10^3$ ultimately arises because of the complex interaction between particle-ionization density, shifts in the relative importance of the indirect and direct mechanisms of DNA damage (ie, high-LET radiation favors direct ionization of the DNA rather than indirect action -OH radical attack), changes in DNA radical scavenging and chemical repair, and the categorization of clusters of DNA lesions into 3 mutually
exclusive categories of damage (SSB, BD, and DSB). As illustrated in Figure 2 (right), there is an almost a 3-fold increase in RBE_{DSB} as (Z_{eff}/b)^2 goes from 10^{3} to 10^{4}. Throughout that same range, there is about a 3-fold decrease in the relative number of BD clusters (RBE goes from 0.38 to 0.12), whereas the relative number of SSBs only decreases by a factor of 1.9 (RBE goes from 0.72 to 0.38). It is that difference in the rates of change in the RBE_{DSB} and RBE_{SSB} with (Z_{eff}/b)^2 that gives rise to the peak RBE_{SSB} at (Z_{eff}/b)^2 \sim 10^3. A similar peak does not occur for BD because the rates of change in the RBE_{DSB} and RBE_{BD} with (Z_{eff}/b)^2 are about the same.

Figure 5 shows the ratio of the number non-DSB clusters to the number of DSBs as a function of (Z_{eff}/b)^2. The ratio of non-DSB to DSB clusters is a pragmatic measure of small-scale (< 10-20 bp of DNA) cluster complexity because all DSBs must contain \geq 2 individual DNA lesions (ie, 2 opposed strand breaks plus other possible DNA lesions), whereas non-DSB clusters\footnote{For the sake of notational and conceptual convenience, SSBs and BDs separated by \geq 10 bp from any other DNA lesions are, except where otherwise explicitly noted, added together with non-DSB clusters composed of \geq 2 individual DNA lesions.} may be composed of \geq 1 individual lesions. Under normoxic conditions (Figure 5, left), low-LET radiation [(Z_{eff}/b)^2 < \sim 10)] creates 20-25 SSBs/DSB and \sim 50 BDs/DSB. The larger number of BD clusters/DSB arises because of the approximately 3:1 ratio of SSBs/nucleotide with BDs [1, 2]. For cells irradiated under anoxic conditions, the ratio of SSB to DSB is \sim 40 compared with \sim 105 for the ratio of BD to DSB. The number of non-DSB (SSB and BD) clusters per DSB decreases with increasing (Z_{eff}/b)^2 and approaches an asymptotic value \sim 1 to 2 for cells irradiated under normoxic (Figure 5, left) and anoxic (Figure 5, right) conditions. The ratio of SSB to DSB from the MCDS (Figure 5, solid line) are in excellent agreement with data from track-structure simulations (Figure 5, left, filled symbols) for light ions with (Z_{eff}/b)^2 up to \sim 5 \times 10^4.

Discussion and Conclusions

The DSB has long been considered one of the most critical, if not the most critical, forms of initial molecular damage caused by ionizing radiation [4, 27, 38–42]. Individual DNA lesions (strand breaks and base damage) are rapidly and effectively repaired through an excision repair process [43] and are many orders of magnitude less likely to initiate reproductive cell death than...
clusters of DNA lesions [25, 27, 32, 44, 45]. Non-DSB clusters are mutagenic and potentially lethal in part because an abortive excision-repair process sometimes converts non-DSB clusters into DSBs [32, 46–50] that require an additional (separate) homologous or nonhomologous enzymatic repair step [51]. Experiments and Monte Carlo simulations of non-DSB clusters suggest that the ratio of delayed DSBs (DSBs formed through enzymatic processing of non-DSB clusters) to prompt DSBs (DSBs formed directly by ionizing radiation) is \(~1.3\) to \(2\) [47, 48].

A putative link between DSB induction and cell survival is plausibly motivated by the hypothesis that a small subset (\(<1\%\)–\(2\%\))3 of the break-ends associated with 2 or more DSB are incorrectly rejoined to form a variety of lethal and nonlethal exchange-type chromosome aberrations [52–59]. The incomplete processing of initial DSB into a chromosomal exchange may also produce DNA fragments that manifest at later times as micronuclei, acentric fragments or as residual chromosome breaks. Pairs of DSB formed along a chromosome by high LET radiations also produce DNA fragments with lengths characteristic of the organization and structure of the chromatin [15, 60]. In a study of density-inhibited (nondividing) normal human fibroblasts (AG1522 cells), Cornforth and Bedford [61] report a 1-to-1 relationship between the mean number of lethal events (\(\alpha\)) and the average number of chromosome-type aberrations, primarily deletions and asymmetric exchanges. In mutants of yeast exhibiting no DSB repair, the mean number of lethal events produced by low LET (30 MeV) electrons and high LET (3.5 MeV) \(\alpha\) particles equals the number of initial DSB per cell [62].

As illustrated in Figures 2 and 4, DSB are the only major category of initial DNA damage in which the relative number of DSB increases with increasing LET [increasing \((Z_{\text{eff}}/\beta)^2\)] under both normoxic and anoxic conditions. The trend in DSB induction with particle LET and oxygen concentration are consistent with experimental observations for reproductive cell survival (4). In contrast to DSB, the relative numbers of non-DSB clusters (SSBs and BDs) decrease with increasing LET [increasing \((Z_{\text{eff}}/\beta)^2\)] under both normoxic and anoxic conditions. The accumulated evidence from decades of research and

\[3\text{In repair-proficient human cells, MV x-rays and low-LET (}\geq 50\text{ MeV)) protons, create about 8 to 8.5 DSBs/Gy/Gbp (Stewart et al [3, 4] and references therein). A diploid human cell containing 6 Gbp of DNA, therefore, sustains } \geq 50 \text{ DSB/Gy/cell. For comparison, } \alpha \text{ (per gray) represents the average number of lethal lesions per gray per cell formed under low-dose rates and/or for low doses of radiation. Therefore, the fraction of the initial DSB that becomes lethal must be } \leq \alpha, \text{ divided by the number of DSBs/Gy/cell, } < (0.1-1/ \text{ Gy})/(50 \text{ DSBs/Gy/cell}) \sim 0.2\% \text{ to } 2\%.\]
many lines of evidence provide compelling evidence that (1) DSBs are the most critical form of initial DNA damage, and (2) it is the processing of a subset of the initial DSBs into chromosome aberrations (fragments and exchanges) that are the primary cause of reproductive cell death, rather than the initial DSBs themselves. That is, the initial DSBs formed by low- and high-LET radiation are the dominant form of sublethal and potentially lethal damage [54, 61]. Non-DSB clusters tend to be less lethal than DSBs but may remain an important source of small-scale mutations as well as a secondary source of (time-delayed) DSBs.

The usefulness of measures of local DSB complexity, such as the average number of lesions per cluster or other measures (eg, the DSB, DSB, or DSBcb of Charlton and Humm [17] and Nikjoo et al [14]) in models for cell killing and the formation of chromosome aberrations is open to debate. Mechanistic studies have shown that specific enzymatic steps involved in DSB repair are sensitive to the types and spatial arrangement of the individual lesions forming a DSB (63, 64). Ottolenghi et al [65] have also suggested that pairs of DSBs formed within a few base pair of DNA (eg, a DSB++) might have an especially important role in cell killing. On the other hand, Carlson et al [11]) found that the number of lesions per DSB has a small (second-order) effect on DSB reparability and cell killing; they instead found that cell killing was primarily determined by the relative (total) number of DSBs/cell (ie, REBDSB), regardless of the local DSB complexity. Additional research is needed to unravel the significance and the most-appropriate metrics of local DSB complexity relating to DSB repair, chromosome aberrations, and cell killing.

Prise et al [66] compiled large amounts of data on DSB induction by ionizing radiation in various eukaryotic cells. The DSB yields measured using up-to-date techniques, such as pulsed-field gel electrophoresis, and expressed per unit of genome length are similar among yeast and mammalian cells (all estimates fall in the range of 4.2-6.9 DSBs/Gy/Gbp for low-LET radiation), despite orders-of-magnitude differences in genome sizes. That observation suggests that the induction of DSBs and other forms of DNA damage are, as a first approximation, proportional to genome size. To produce equal numbers of DNA clusters/cell, yeast cells need to be exposed to ~250 times as much dose as a human cell, that is, diploid humans cells have ~6 Gbp of DNA compared with ~24.2 Mbp of DNA for Saccharomyces cerevisiae (bakers’ yeast). Because damage anywhere within the DNA has the potential to kill or cause mutations, cells with a small genome are much more resistant to ionizing radiation than are cells with large genomes [35, 37, 62, 67].

The approximate linear scaling of DNA damage with cell DNA content implies that cells in the G2 and M phases of the cell cycle sustain up to 2-fold more initial DSB and non-DSB clusters than cells in nondoning G0 and G1 phase cells. In addition, in rapidly dividing tumor cells and in tissues with rapid cell turnover (eg, epithelial cells in skin or the lining of the gastrointestinal tract) will, on average, be challenged to repair greater numbers of DSB and non-DSB clusters than cells in slowly dividing or nondividing tissues. For comparison to measured values in the range from 4.2 to 6.9 DSB/Gy/Gbp, the MCDS predicts 8.3 DSBs/Gy/Gbp for 60Co γ-ray irradiation of mammalian cells under normoxic conditions (Stewart et al [3]) compared with the 22% lower value of 6.8 DSB/Gy/Gbp reported by Friedland et al [5]. The comparison of measured and Monte Carlo simulations of DNA damage in mammalian cells suggests an absolute accuracy ~20% to 30% for the absolute numbers of DSBs/Gy/Gbp compared with an accuracy that may be as low as ±5% for the relative numbers of SSBs and DSBs for low and intermediate LET radiation [(Zeff/β)2 < ~103], as illustrated in the left panels of Figures 2, 4, and 5. Uncertainties in the absolute and relative numbers of DSB and non-DSB clusters are most significant for very low energy (high LET, large (Zeff/β)2) particles (eg, Figure 3), which includes end-of-range particles in the tip of the Bragg peak formed by therapeutic proton and carbon ion beams. Some of the differences shown in Figure 3, especially for short-range, very high LET 12C6+ ions may be due to the algorithms used to group individual DNA lesions along a short segment of DNA into DSB and non-DSB clusters. For example, Pater et al [16] reported that differences in the algorithms used to group lesions into DSBs can alter the apparent DSB yield by ~30%.

The use of light ions, especially protons and carbon ions, in the treatment of cancer is of increasing interest in the United States and elsewhere [68–70]. In addition to the physical (dosimetric) advantages associated with the finite range in tissue of charged particles (ie, little or no “exit dose”), there is compelling experimental and theoretic evidence that initial molecular damage, especially including DSB induction, increases with increasing ionization density. The subsequent processing of potentially lethal DSBs into chromosome aberrations is a major mechanisms of action underlying the reproductive cell death of tumor cells as well as cells in at-risk organs and tissues in radiation therapy. Corrections for LET-dependent spatial variations in particle RBE, especially near the distal edge of a spread-out Bragg peak, are essential in carbon ion radiation therapy [71–73]. A peak RBE value of 2.6 to 3.4 for high-LET carbon ions, as illustrated in Figure 3, is generally consistent with the RBE for fast neutron [74] and carbon ion [71] therapy clinical end points. Detailed Monte Carlo modeling of the University of
Washington’s clinical neutron therapy system [75, 76], which is based on 50.5 MeV protons incident on a beryllium target, gives, for example, an $RBE_{DSB}$ of 2.8 ± 0.1 (Stewart et al [3]).

Although a constant (spatially invariant, independent of LET) proton RBE = 1.1 provides reasonable equivalence to MV x-ray treatments [77], a constant proton RBE is at odds with many decades of research on the molecular and cellular mechanisms of action underlying particle RBE [78]. Near the tip of a pristine Bragg peak and a few millimeters beyond, Monte Carlo simulations of proton $RBE_{DSB}$ can easily approach 1.2 to 1.4, whereas at the entrance (plateau) region, the $RBE_{DSB}$ is ~ 1 to 1.05 [3, 79]. Preliminary studies [80, 81] of the $RBE_{DSB}$ in a series of patients treated with proton therapy found a tumor RBE that ranged from 1.02 to 1.10 and organ at-risk RBE values that ranged from 1.0 to 1.4. Biological optimization of the $RBE_{DSB}$ by dose has the potential to improve the therapeutic ratio of proton therapy by ≥ 10% to 20% of the total treatment dose, and, at the same time, reduce healthy-tissue toxicity [80, 81]. As summarized and reviewed in this article, $RBE_{DSB}$ is a well-characterized and -understood end point that is useful for quantifying the effects of particle LET and oxygen concentration on the related molecular and cellular end points of chromosome aberrations and reproductive cell survival.

### ADDITIONAL INFORMATION AND DECLARATIONS

**Conflicts of Interest:** The author has no conflicts of interest to disclose.

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**Appendix A. About the MCDS**

The details of the MCDS model and software have been described in detail elsewhere [1–4]. Version 3.10A of the MCDS software is freely available by contacting the author at trawets@uw.edu and from [http://faculty.washington.edu/trawets/mcds/](http://faculty.washington.edu/trawets/mcds/).

**Appendix B. Key Concepts and Terminology Related to the Classification of DNA Damage within the MCDS**

The terminology and concepts used to categorize DNA damage are sometimes used in conflicting, incompatible, and confusing ways in the literature and even among the various groups that have developed Monte Carlo simulations of DNA damage. As reported by Pater et al. [16], differences in algorithms used to group individual lesions into DSBs can, for example, alter the DSB yield by ~ 30%. This Appendix briefly summarizes and defines some of the key concepts and terminology used in the MCDS for the classification of groups of DNA lesions into DSB and non-DSB clusters.

**DNA Lesion**

As illustrated in Figure B.1, a DNA lesion is a single nucleotide with abnormal chemical alternations in the (1) deoxyribose sugar, (2) the phosphodiester bond connecting adjacent nucleotides on the same side of the DNA helix, or (3) the organic base (A, G, T, and C). Chemical alterations to the deoxyribose sugar or phosphodiester bond usually manifest as a strand break shortly (within a few milliseconds) after passage of the primary particle, whereas a BD manifests as either an abasic site (loss of the base) or a multitude of specific types chemical alterations in the A, G, T, or C base [82]. The fine details of the chemical alterations forming a strand break or BD have a significant effect on the repair of both DSB and non-DSB clusters [43, 63, 64, 83].

**Cluster of DNA Lesions**

In the MCDS, ≥ 2 individual DNA lesions in a short, but variable, length of DNA are included in the same cluster when no 2 lesions on the same or opposed DNA strands are separated by more than 10 bp. For convenience, individual DNA lesions separated by > 10 bp from all other DNA lesions are counted as a cluster of DNA lesions. The length (in base pairs) of a cluster may be > 10 bp and varies with particle LET because the cluster-identification algorithm used in the MCDS ends 1 clusters and starts a new cluster only when a there is a segment of undamaged DNA > 10 bp in length. For example, consider Figure 1B, if the 2
adjacent nucleotides with BD (filled red squares) were separated by 9 bp instead 1 or 2 bp from the strand break, that cluster would have a length of \(~18\) to 20 bp. Additional lesions on the same or opposed strand might further increase the length of the cluster to \(\geq 30\) bp. Cluster length (in base pairs) tends to increase with increasing particle LET because of the trend toward an increase in ionization density (and hence the number of lesions/base pair) with increasing particle LET.

**Double-Stranded Break**

A cluster of DNA lesions that contains \(\geq 2\) strand breaks on the opposed DNA strand within 10 bp. A cluster of DNA lesions categorized as a DSB may contain additional BD, abasic site (As), or strand break (Sb) within 10 bp of another lesion within the cluster (ie, a “complex DSB”).

**Single-Stranded Break**

A cluster of DNA lesions that contains \(\geq 1\) strand break that does not also have an additional strand break within 10 bp on the opposed DNA strand. A cluster categorized as an SSB may contain (1) additional BD or abasic site (As) on the same or opposed DNA strands within 10 bp, (2) an additional Sb within the same strand of DNA, and (3) a Sb on the opposed strand of DNA that is \(\geq 10\) bp away.

**BD (or Cluster of Damaged Bases)**

A cluster of DNA lesions that contains \(\geq 1\) BDs or As within 10 bp of each. A BD cluster does not contain any strand breaks.

**Non-DSB Cluster**

Any type of DNA lesion cluster that is not categorized as a DSB. SSB and BD clusters are the 2 main types of subcategories of non-DSB clusters. Intrastrand and interstrand cross-links (**Figure B.1**, right) are other subcategories of non-DSB cluster of special interest in DNA repair.

**Cluster Complexity**

There are many potential measures of cluster complexity, including the average number of lesions per cluster and the linear density of lesions per cluster (eg, lesions per base pair of DNA). The ratio of non-DSB (SSB and BD clusters) to DSB clusters is also an indirect metric of cluster complexity because non-DSB clusters contain, on average, a smaller number of lesions per cluster than DSBs do.
Appendix C. Relationship between \( (Z_{\text{eff}}/\beta)^2 \), Kinetic Energy, and Particle LET

Large values of \( (Z_{\text{eff}}/\beta)^2 \) correspond to low energy, short-range, high LET particles. For very high energy ions, the speed \( \beta \) of the ion relative to the speed of light approaches unity, and the effective charge \( Z_{\text{eff}} \) is computed as follows [3]:

\[
Z_{\text{eff}} = Z \left[ 1 - \exp(-125 \times \beta \times Z^{-2/3}) \right]
\]

and

\[
\beta = \sqrt{\frac{1}{1 + \frac{T}{m_0c^2}}}
\]

where \( T \) is the kinetic energy of the charged particle (in MeV), and \( m_0c^2 \) is the rest mass energy of the charged particle (in MeV). Figure C.1 shows the relationship between the kinetic energy, \( (Z_{\text{eff}}/\beta)^2 \) and the unrestricted LET (total stopping power) in water of \(^1\text{H}^+\), \(^4\text{He}^2+\), and \(^{12}\text{C}^6+\) ions, as reported by the MCDS [4].

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