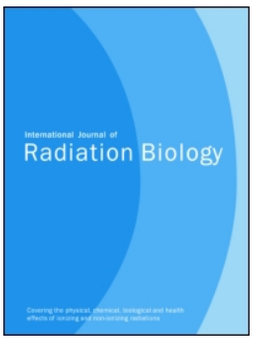

International Journal of Radiation Biology

ISSN: 0955-3002 (Print) 1362-3095 (Online) Journal homepage: http://www.tandfonline.com/loi/irab20

Influence of nuclear structure on the formation of radiation-induced lethal lesions

Daniel A. Friedman, Lauren Tait & Andrew T. M. Vaughan

To cite this article: Daniel A. Friedman, Lauren Tait & Andrew T. M. Vaughan (2016): Influence of nuclear structure on the formation of radiation-induced lethal lesions, International Journal of Radiation Biology, DOI: 10.3109/09553002.2016.1144941

To link to this article: http://dx.doi.org/10.3109/09553002.2016.1144941

Published online: 26 Feb 2016.

INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, 2016
http://dx.doi.org/10.3109/09553002.2016.1144941

Taylor & Francis
Taylor & Francis Group

REVIEW

Influence of nuclear structure on the formation of radiation-induced lethal lesions

Daniel A. Friedman[a], Lauren Tait[b] and Andrew T. M. Vaughan[b]

[a]Department of Biology, Gilbert Hall, Stanford University, California; [b]Department of Radiation Oncology, University of California, Davis, Sacramento, California, USA

ABSTRACT

Purpose The rejoining of fragmented nuclear DNA caused by ionizing radiation may lead to lethal chromosome rearrangements, such as rings or dicentrics. The clinically useful linear quadratic relationship between dose and cell survival has been interpreted as the generation of lethal lesions secondary to damage occurring in two separate chromosomes simultaneously (α component), or as potentially repairable separate events (β component). Here, the generation of such lesions is discussed, synthesizing existing knowledge with new insights gleaned from spatial proximity data made possible by high-throughput sequencing of chromosome conformation capture experiments. Over a range of several Mbp, the linear DNA strand is organized as a fractal globule generating multiple sites of contact that may facilitate deletions or inversions if the points of contact are damaged. On a larger scale, transcriptionally active euchromatin occupies a physically identifiable space separate from inactive areas and is preferentially susceptible to free radical attack after irradiation. Specific transcriptional programs link genomic locations within that space, potentially enhancing their interaction if subject to simultaneous fragmentation by a single radiation event.

Conclusions High throughput spatial analysis of the factors that control chromosome proximity has the potential to better describe the formation of the lethal chromosome aberrations that kill irradiated cells.

ARTICLE HISTORY
Received 4 February 2015
Revised 9 December 2015
Accepted 13 January 2016

KEYWORDS
Cellular radiobiology; chromosome abberations; models of cell killing; double-strand breaks; molecular radiobiology; microdosimetry

Introduction

Over the past five decades, developments in treatment delivery, computerization and imaging have enabled the practice of radiation oncology to make substantial advances. Megavoltage accelerators have provided improved depth dose profiles while subsequent onboard imaging technology has helped spare more normal tissue from damage (Jaffray 2012). Throughout the substantial technological advances that have significantly improved treatment outcomes, a comprehensive description of the process whereby radiation generates lethal lesions is lacking. The biological focus has rightly been the response of the cell to radiation-induced DNA double-strand breaks, the key lethal radiation event, their signaling to regulatory pathways and the mechanism(s) of their repair (Jackson and Bartek 2009, Thompson 2012, Price and D'Andrea 2013, Kavanagh et al. 2013). However technical limitations in detecting the location of such breaks, have limited studies on their potential to form a lethal lesion that restricts mitosis, such as a dicentric, ring structure or anaphase bridge (Costes et al. 2007). At least part of the reason for this discrepancy is the architectural complexity of the genome. At its simplest, two DNA double-strand breaks generated by irradiation within the same or separate chromosomes need to be ligated together producing an aberration rather than be individually repaired. The ability to generate such lesions is therefore determined by the spatial distribution of radiation energy within the complex structure of the genome itself. It is clear the latter aspect has lagged the development of sophisticated models of radiation deposition that describe radiation interactions (Goodhead 2006). However recent advances in the field(s) of genomic analysis have provided a more detailed spatial description of the genome that provide a more robust platform to map the generation of radiation induced rearrangements (Lieberman-Aiden et al. 2009).

In addition to the spatial orientation of radiation-induced lesions within the genome, their generation is also constrained by time dependent factors that exert their effects over a very large range (Figure 1). Thus the earliest detectable events within the genome, the physical photon or particle interaction with biological material, will occur in the range of femtoseconds. Subsequently, activated chemical species, primarily free radicals, are generated and react over a range of nano to microseconds with all cellular constituents, including DNA. Actual lesion processing, through either repair or aberrant ligation, will be resolved over a period of hours.

Therefore the introduction of lethal lesions by irradiation is impacted both by the complex spatial orientation of its genomic target and the sequential development of events that operate over an extremely broad time scale.

Radiation interactions in human cells

There is a long and rich history modelling the generation of DNA breaks and lethal lesions following irradiation of human

CONTACT Dr Andrew Vaughan, PhD atvaughan@ucdavis.edu University of California, Davis, Radiation Oncology, 4501 X Street, Sacramento, CA 95817, USA

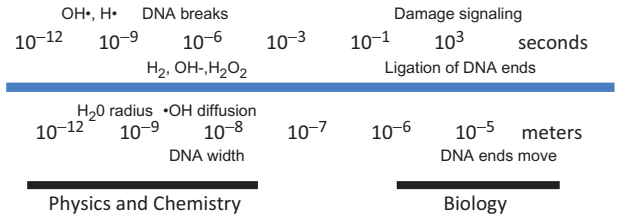

Figure 1. Scale of radiation-induced events in both time and space. Direct DNA damage and free radical mediated events such as those produced by the hydroxyl and hydrogen radicals ($OH^{\bullet}$, $H^{\bullet}$) occur over a time scale that is orders of magnitude faster and within a dimension that is also much smaller than the recognizable lesions they produce.

cells. Intrinsic to all of these is the discrete distribution of ionization events throughout the cell. Of such models, the 'Target Theory' of Lea was one of the first to propose the presence of multiple nuclear 'targets' that when 'hit' or inactivated lead to the death of the cell (Lea 1955). In its 'single hit – multiple target' formulation of Equation (1), S is the fraction surviving dose D, D_0 describes the slope of the exponential portion of the survival curve and the extrapolation number, n, the width of the shoulder region.

$$S(D) = 1 - (1 - e^{D/D0})^n \quad (1)$$

In this form the probability of inactivating any one target is the same, cell death will follow when a specific number of targets are inactivated, the number predicted by the size of the extrapolation number, n. Other variants such as the 'multiple hits – multiple target' model have been described that increase the overall model complexity but still use the same general approach (Nomiya 2013). The overall strategy was however criticized by many in that no clear biological entity corresponded with the proposed targets; however, the key emphasis on sub-nuclear volumes as mediators of nuclear change was, and still is, a valid approach in consideration of the discontinuous distribution of energy in irradiated systems.

Subsequently, two additional models, the 'Molecular theory' of Chadwick and Leenhouts and the 'Theory of Dual Radiation Action' described by Kellerer and Rossi have been described that, though starting from different sets of assumptions, each arrives at a similar description of cell kill, that of a linear quadratic relationship between survival and dose (Chadwick and Leenhouts 1973, Kellerer and Rossi 2012). Here, both *in vitro* cell killing and *in vivo* responses can be described as an effect 'E', dependent on the dose 'd' delivered over 'n' fractions, at least up to moderate doses (Equation 2).

$$E = n(\alpha d + \beta d^2) \quad (2)$$

In the 'Molecular theory' model of Chadwick and Leenhouts, the significance of the DNA double-strand break and its repair were used to derive a linear quadratic (LQ) association with survival after radiation (Chadwick and Leenhouts 1973). However this model incorporated a role for the recombination of proximal DNA single-strand breaks as a source for generating DNA double-strand breaks, an idea that has not gained wide acceptance due to the likely rarity of two such independent events occurring in close proximity. Perhaps the most significant treatment in terms of its long-term impact to the field is the 'Theory of Dual Radiation Action' described by Kellerer and Rossi (2012). In this approach they incorporated the relatively new technology of microdosimetry, utilizing large detectors filled with a gas that simulated the density of very small sub-nuclear volumes. They proposed that irradiation generated a population of sub-lesions that had the potential to interact to cause a lethal lesion, if they were in sufficiently close proximity. Such lethal lesions clearly include the generation of chromosome rearrangements, such as dicentrics and more complex aberrations, which interfere with cell division (Ballarini 2010, Hall and Giaccia 2012). The interaction distances calculated were of the order of a micrometer, a finding that quickly ran counter to experimental observation following the experiments of Goodhead et al. (1979). In these later series of experiments, using very low energy irradiation from ultrasoft aluminum or carbon K edge X-rays, it was shown that the interaction distances of such sub-lesions was much closer than the micrometer proposed in the model of Kellerer and Rossi (Goodhead et al. 1979). Nevertheless the LQ approach has proved to be a robust model of X- or gamma-ray-induced cell killing that has provided a useful and biologically grounded framework to view experimental data. For the purpose of this review the role of such modelling strategies is clear, they are clearly relatively simple, and therefore flexible tools to predict the likely survival of cells post irradiation. Viewed in this light they have provided useful service, especially in the clinic, where the linear quadratic formalism has provided key information on the relative potency of various fractionation strategies for treatment (Fowler 2010). However, none are likely to offer the final word in describing the effects of ionizing irradiation in complex biological systems. As an example, the recent utilization of a few large fractions to treat a range of cancers has been modelled using a combination of both the linear quadratic and target theory approaches to better describe anomalies of the LQ formulae in high dose regions (Park et al. 2008).

Microdosimetry at the biological interface

The models discussed above emphasize a single outcome of irradiation, cell survival vs. death, and include relatively simple assumptions. In contrast, the study of microdosimetry, the physical description of ionization energy deposition at the level of discrete photon/electron interactions, has benefited greatly from both the development of tissue equivalent proportional counters and Monte Carlo-based analyses of track structure (Goodhead 2006, Wiklund et al. 2011). These tools have provided detailed and three-dimensional descriptions of radiation interactions with targets the size, composition and density of cells. The energy from incident photons may be dissipated as localized clusters of ionizations and generation of free radicals that have the potential to initiate both chemical changes in the cell or physical disruptions, such as DNA strand breaks. The radiation chemistry of such interactions has been extensively reviewed and will not be repeated in detail here (O'Neill and Wardman 2009). Energetic photons, such as those used in radiation therapy, produce DNA damage directly and indirectly. Directly, high-energy photons can break the phosphodiester backbone of DNA.

Indirectly, high-energy photons can produce free radicals through the homolytic cleavage of covalent bonds in nuclear molecules, such as water. Free radicals then may react with molecules in spatial proximity, which can include the DNA phosphodiester backbone. Either process may generate breaks in one or both strands of DNA (Figure 1). The transfer of photon energy to the target molecules is complete within picoseconds and the free radicals execute chemical change within microseconds. Thus the resolution of the initial physical and chemical events following irradiation is complete within a time frame where the DNA organization may be considered a static entity, a 'snapshot' of fixed geometry. Subsequently, the resolution of damaged DNA proceeds at a many magnitudes slower pace, over a time frame of hours, where DNA fragments will be able to move in respect to both each other and the rest of the genome.

Substantial amounts of data, generated over many decades, have highlighted the DNA double-strand break as the lesion most closely linked to cell death (Iliakis 1991, Olive 1998, Xu and Price 2011). For a double-strand break, each end may physically separate prior to attempts at repair by either the Non Homologous End Joining (NHEJ), Microhomology Mediated End Joining (MMEJ) or Homologous recombination (HR) pathways (McVey and Lee 2008, Chapman et al. 2012, Kavanagh et al. 2013, Schipler and Iliakis 2013). Such a separation of free DNA ends may complicate repair and lead to the generation of a lethal lesion, such as a dicentric, as the free ends encounter alternate breaks. Thus both the local environment of the lesion(s) and activity of appropriate repair pathways may either promote or suppress such errors in DNA rejoining (Pfeiffer et al. 2004). Perhaps the most convincing support for the key role of such breaks in mediating lethality is the documented increase in radiation sensitivity for cells that lack key components of the DNA double-strand break repair pathways (Adachi et al. 2001, Woodbine et al. 2014).

Most microdosimetry-based studies on radiation-induced lethality have been limited by the lack of a comparably detailed three-dimensional description of DNA organization, its presumed target. Despite this drawback, studies have been undertaken using such data that is available, including a Monte Carlo simulation of radiation events matched with the generation of DNA double-strand breaks and subsequent dicentric formation (Edwards et al. 1996). The model successfully recapitulated the LQ relationship between dose and the generation of dicentric lesions discussed above, though utilizing only a random distribution of DNA breaks within a regular nuclear volume as its biological input. Others have included the observation that nuclear DNA is packed into constrained, supercoiled DNA loops and have modelled this level of organization as the targets for single or complex distributions of lesions (Cook and Brazell 1976, Yokota et al. 1995, Khodarev et al. 1997, Herr et al. 2014). Such studies clearly represent an advance over the assumption of a random distribution of DNA breaks within the genome, and they may readily recapitulate the admittedly simple LQ relationship between dose and cell survival. However the low-complexity assumptions of biological distributions that are employed prohibit any prospective assessments of the role of nuclear architecture on survival. Perhaps the most comprehensive series of studies to date are represented by those incorporating the PARTRAC (PARticle TRACk) code, combining Monte Carlo analysis of ionizing radiation track structure with multi-level modeling of DNA organization (Friedland et al. 2011, Friedland and Kundrat 2013). In this application, individual structures such as nucleosomes, looped DNA, hetero and euchromatin are modeled as defined structural entities, in addition to the action of repair processes targeting the key DNA double-strand break. Such a system has potential to help in the study of radiation-induced lesions in the context of classically organized nuclear DNA, though in its current version it does not accurately predict the number of cytotoxic dicentrics that are observed experimentally.

In this review we will emphasize the two extremes of radiation-induced lethality, the nature and location of the initial breaks, and the impact of genomic organization on the formation and subsequent biological effect of chromosome aberrations that are generated.

The lethal lesion

All the models discussed above are compatible with the lethal lesion introduced by irradiation being the result of the inappropriate fusion of two DNA double-strand breaks, generating toxic rearrangements such as a dicentric. Ionizing radiation cleaves DNA by physical and/or chemical attack, leaving DNA ends that contain a range of chemical moieties that need to be processed to 'clean' 5′ phosphates and 3′ hydroxyl termini to facilitate ligation (Obe et al. 2010, Schipler and Iliakis 2013, Averbeck et al. 2014). It is clear this process is highly efficient in normal cells where the majority of double-strand breaks are successfully repaired. The subsequent inappropriate ligation of two such breaks on different chromosomes can however generate an aberration with two centromeres (a dicentric chromosome) that will stop cell division by physically restricting the partition of daughter cells at mitosis as each linked centromere is pulled to opposite poles of the cell. Specifically, the linear and quadratic terms within the LQ equation are commonly assumed to represent DNA fragmentation events that are generated either simultaneously, such as within a specific particle track (α), or from discrete, separate, ionization events separated in space and time (β) (Brown and Attardi 2005, Vakifahmetoglu et al. 2008, Ballarini 2010, Hall and Giaccia 2012). Such an understanding is consistent with all the modeling procedures discussed above. In addition, the relevance of the LQ model in particular has been confirmed by decades of its practical application in a clinical setting, where it has proven capable of predictive power in illustrating the potency of a wide range of treatment strategies. Here, the biologically effective dose (BED), of treatment schedules of varying fraction size (d) can be determined in relation to variations in the dose response curve as represented by the ratio of α and β (Equation 2) (Fowler 2010).

The models themselves provide no information on the influence of DNA organization on the generation of such lesions. However, from simple considerations of biophysics,

larger chromosomes are more likely to participate in lesion formation as they are larger targets for the creation of the initial DNA break (Cornforth et al. 2002). Nevertheless, both historic and more recent data utilizing high throughput genome analysis suggest that such simple assumptions may not fully describe the earliest events in radiation-induced lethality. In particular, as discussed further below, the organization of DNA within the nucleus is not random, its organization into separate functional domains, each consisting of looped DNA, place a high degree of order on its packing within the nuclear boundary (Lieberman-Aiden et al. 2009, Rao et al. 2014). Thus the effects of irradiation at the sub-nuclear level may be illustrated using the tools and techniques of microdosimetry placed in the context of the increasingly well-defined biological organization of the intact eukaryote nucleus.

Radiation induced lethal lesions are not generated at random

To a first approximation, the generation of DNA breaks following ionizing irradiation would appear to be randomly distributed, notwithstanding the greater access to open chromatin by free radicals (Chiu et al. 1986, Savage 1993, Takata et al. 2013). After radiation exposure a variety of species are produced from the radiolysis of water, the hydroxyl radical (•OH) being the most frequent. Such radicals have extremely short lifetimes, of the order of a nanosecond, corresponding to a diffusion distance of ~5 nm, and are capable of damaging DNA if contact is made (Natarajan et al. 2010). The generation of radiation-induced free radicals is responsible for approximately two thirds of all DNA lesions and their effects are more frequently observed within open regions of the genome that are actively being transcribed, or are prepared to do so (Chiu et al. 1986, Magnander et al. 2010, Takata et al. 2013). This imposes a degree of selectivity on the distribution of DNA damage following low Linear Energy Transfer (LET) radiation exposure that will exhibit a bias to those locations that are actively engaged in transcription, and which are readily accessible to free radicals. Even if DNA breaks themselves were assumed to be induced at random, multiple lines of evidence suggest that their inappropriate rejoining, such as translocations and other rearrangements, are influenced by local genome architecture and post-break processing (Engreitz et al. 2012, Hakim et al. 2012). Thus there are inherent patterns to subsequent lesion formation. The generation of rearrangements has historically been described with reference to either the 'contact first' or 'breakage first' mechanisms (Aten et al. 2004, Meaburn et al. 2007). In the contact first mechanism, the two chromosome elements are in physical proximity prior to joint fragmentation – equivalent in principle to 'α type' damage described in Equation (1), where a cluster of ionization events may fragment more than one DNA strand (Sutherland et al. 2000, Sankaranarayanan et al. 2013). Alternatively, in the breakage first mechanism, the breaks are generated as separate elements that may require some mobility to meet and interact, broadly compatible with 'β type' damage. Recent data has suggested that the contact first mechanism may predominate in translocation induction, though not to completely exclude break mobility and subsequent interaction (Roukos et al. 2013). Here, to simplify the discussion, the role of initial chromosome proximity will be the focus in the genesis of aberrations.

Break proximity: Genome structure

Early studies on radiation-induced rearrangements identified two common types of lethal rearrangements; centric rings, formed by double-strand breaks on opposite arms of the same chromosome, and dicentrics, from breaks on different chromosomes (Figure 2A and B). The lesion may be expressed subsequent to an attempt at cell division where such rearrangements restrict proper partitioning of genetic material (Vakifahmetoglu et al. 2008). Though each type of rearrangement requires two DNA double-strand breaks, careful microscopic analysis of irradiated cells showed a smaller ratio of dicentric to ring formation than would be predicted by chance if all breaks interacted randomly (Sachs et al. 1997, Sankaranarayanan et al. 2013). The explanation for such a finding was assumed to be the physical localization of individual chromosomes in their own 'territories', which favored intra-chromosomal events (generating rings) over those that required the interaction of two different chromosomes that might be widely separated within the nucleus (Figure 2C). This conclusion, reached from analysis of chromosome rearrangements in plant cells, was among the earliest observations suggesting the existence of chromosome territories in the interphase nucleus and has been experimentally verified many times since (Sax 1940, Cremer and Cremer 2001).

Break proximity: Low energy photoelectrons

The energy of photoelectrons generated within irradiated tissue reach a maximum equal to that of the incident photon minus its binding energy. Using this principle, very low energy carbon K edge X-rays (0.28 keV), were employed as discrete tools to initiate localized DNA damage due to the very short path length (~7 nm) of the photoelectrons generated. Using equipment that allowed the irradiation of single cells, a three-fold enhancement in dicentric production was observed, compared to 250 kVp X rays over a range of 1–4 Gy (Figure 2D) (Thacker et al. 1986, Sachs et al. 1997, Griffin et al. 1998). In addition, the generation of aberrations by such short-range electrons was found to be linearly related to dose, D rather than D^2, suggesting the two breaks required were introduced as a single event (Sachs et al. 1997). This implies an event (α type using the LQ formalism), occurring in a small volume described by the range of the photoelectron, was responsible for both breaks leading to the rearrangements observed. Thus the proximity of individual chromosomes (or chromosome arms) were impacted by the same induced photoelectron and/or associated free radical production or, less likely, a single DNA double-strand break was able to initiate a rearrangement involving a proximal, undamaged, chromosome (Thacker et al. 1986, Cornforth 1990, Sachs et al. 1997, Griffin et al. 1998,

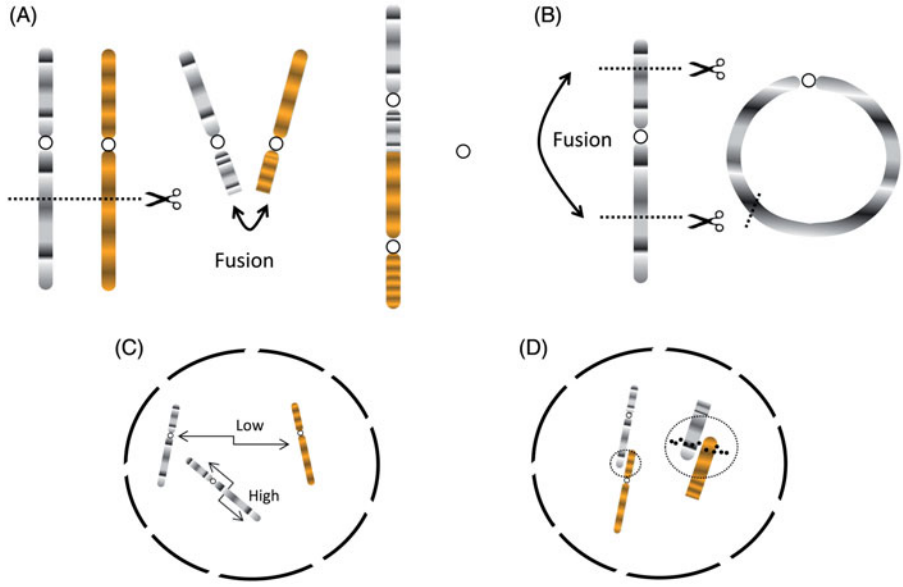

Figure 2. Dicentric and ring aberrations produced by irradiation lethal chromosome rearrangements produced by fusion two DNA double-strand breaks (dotted lines) that are sub subsequently fused (arrows) either (A) between different chromosomes (dicentric), or (B) within the same chromosome (ring). (C) Chromosome breaks (arrows) within the same chromosome have a higher probability of interacting, forming a ring structure, than breaks between chromosomes generating dicentrics or a translocation. (D) Two chromosome elements that are physically adjacent are more likely to be fragmented by a single ionization cluster, and subsequently interact, a process that is linearly related to dose, not dose squared.

Natarajan et al. 2010). In either case, however, both chromosome partners would likely need to be located close together within the nucleus.

Break proximity: Ion pair and high LET irradiation

Similar proximity effects were found using cellular irradiation with associated ion pairs (Geard 1985). In this approach, molecular ion pairs are produced by stripping electrons from accelerated diatomic species using Mylar films. When the source diatomic material is deuterium (D_2) the accelerated particles are deuterons – one proton and one neutron. Thicker Mylar films provide greater particle separation and those particle pairs that were separated by 50 nm or less were most effective at generating rearrangements (Geard 1985). Similarly to the data gathered using short-range photoelectrons, this suggests that simultaneous fragmentation of two DNA locations, generating 'α' type damage as defined above (Equation 1), occurs efficiently in chromosome locations that are spatially related. Substantial data also exists on the generation of chromosome rearrangements in relation to LET. In particular, high LET radiations, such as alpha particles or heavy ions, are up to 15-fold more efficient at generating chromosome rearrangements than the same dose of low LET radiation (Hada et al. 2011, Franken et al. 2012). These data show that the number of DNA double-strand breaks induced by such high LET irradiation, as measured by γH2AX (phosphorylated form of Histone H2A family member 'X') foci, are similar to that seen after the same dose of low LET radiation, suggesting it is the spatial distribution of the breaks, in addition to their number, that influence aberration formation and hence toxicity (Franken et al. 2012). Simplistically, a broadly linear track across a cell nucleus from a high LET particle places the majority of DNA breaks within the track core itself – a cylinder of approximately 10 nm diameter (Cucinotta et al. 1998). Here, due to spatial proximity and increased concentration, these breaks are more likely to interact than those scattered throughout the genome induced by sparsely ionizing low LET radiation. Further, particles that deliver ~100 keV/μm energy, alpha particles and some neutron energies, provide maximal cytotoxicity. This energy-dependent cytotoxicity has been attributed to the inter-ionization distance of such particles that matches the width of the DNA alpha helix, optimizing the probability of a DNA double-strand break (Hall and Giaccia 2012).

Direct measurement of lesions

The discussion above is derived from observations of lethal rearrangements obtained primarily with discrete and non-traditional radiation sources and shows that chromosome proximity is a key element in rearrangement formation. To specifically address the question of chromosome orientation on the generation of rearrangements after conventional high energy radiation exposures, a comprehensive study was initiated, in a single human cell line, to screen for the frequency of such rearrangements across all autosomes (Cornforth et al. 2002). In this analysis, chromosomes were uniquely labeled using multiplex FISH (mFISH – Fluorescence In-Situ Hybridization), such that rearrangement partners could be assigned to specific chromosomes (Cornforth et al. 2002). As expected, larger chromosomes, being larger radiation targets, were damaged more frequently. Somewhat surprisingly, and with few exceptions that included a group of centrally located gene rich chromosomes (Chrs. 1, 16, 17, 19 and 22) that showed increased mutual interactions, each chromosome showed the potential to fuse with any other. This finding appears to run counter to any role of chromosome proximity in mediating rearrangement frequency. However, though a comprehensive survey, such experiments carried out using

chromosome specific FISH probes can only offer analytical resolution at the level of whole chromosomes within a population of cells. Thus individual chromosome territories, if they vary in terms of their immediate contact partners from cell to cell, will generate a random distribution of rearrangements even if initial chromosome contact is the key element in rearrangement formation. To dissect this aspect chromosome organization on rearrangement probability, further technical refinements were required. These advances are provided by 'next generation' or high throughput chromatin technologies, which, as discussed below, offer base-pair-level sequencing accuracy assayed at a resolution of a single cell.

Hierarchical organization of eukaryote DNA

The folding of 3×10^9 bp of linear DNA molecule into the cell nucleus, approximating a sphere of diameter 8–15 μm, is classically described by the regular interaction of DNA with a histone octamer, in the 'beads on a string' model. Through increasing levels of compaction that involves a 30 nm fiber containing 6–11 nucleosome elements per radial turn, chromatin eventually compacts into microscopically identifiable chromosomes during mitosis (Hubner et al. 2013). Both the 'beads on a string' and chromosome models are likely accurate descriptions of the two extremes of nuclear organization. However, increasing doubt has been placed on the intermediate organization of DNA, mostly due to the as yet unresolved degree to which *in vitro* artifacts perturb analysis (Fussner et al. 2012, Nishino et al. 2012). Despite this important caveat, a substantial body of work has addressed the question of nuclear structure, using a range of different tools that provides some consensus. Using FISH labelling of sequential tracts of DNA on the same chromosome it was shown that the DNA backbone behaves as a flexible polymer, loosely constrained within its own domain (Yokota et al. 1995). In addition, by examination of the orientation of individual FISH targets (loci closer than predicted by simple linear sequence) it was deduced that the DNA was also organized in a series of loops. Such a finding is supported by prior work that inferred the presence of such constrained loops by their ability to relax and rewind their native supercoiling under the influence of intercalating agents such as ethidium bromide, a process completely inhibited by a break within the constrained loop (Cook and Brazell 1976, Khodarev et al. 1997). Estimates of the loop size vary considerably, from < 0.1 Mbp at the low end, with most however clustering in the 1–2.5 Mbp range, such differences likely linked to the difficulty in working with such delicate structures and the specific technique used (Jackson et al. 1990, Khodarev et al. 1997, Johnston et al. 1998, Ostashevsky et al. 1999). Nevertheless, the not unexpected detection of a sub-chromosomal fine structure within the nucleus has stimulated the generation of biologic modeling strategies, this time focused on the impact of such organization on lesion generation.

Rearrangements and genome proximity mapping

Next-generation sequencing (NGS) refers to the biochemical and bioinformatic techniques that enable whole genomes to be sequenced from experimentally fragmented DNA. A combination of new NGS technology and experimental ingenuity produced a method called chromosome conformation capture (3C), which has since been elaborated into many variants, such as Hi-C (Engreitz et al. 2012, Dekker et al. 2013, Koboldt et al. 2013). These techniques assay the spatial structure of the nuclear chromatin at a whole-genome scale, a tool of particular relevance to radiation-induced aberrations. In 3C, the three-dimensional architecture of the genome is determined by chemically cross-linking genomic DNA, which binds together segments of DNA that are physically adjacent to each other at the moment of analysis. Cross-linked loci may be on the same or different chromosomes. The genome is then digested with a frequently cutting restriction endonuclease, to fragment the genome and, in the case of Hi-C, tagged with a biotin molecule at the fragmentation site (Figure 3). In Hi-C, the biotin tag is then used to isolate the individual chimeric molecules produced. Finally, using a dilute concentration of DNA to enrich for intramolecular religation, the circular ligation products formed are again fragmented, and sequenced. With this system, the proximity of any specific genomic sequence to any other may be assessed by the sequence composition of the chimeric fusions, generating a

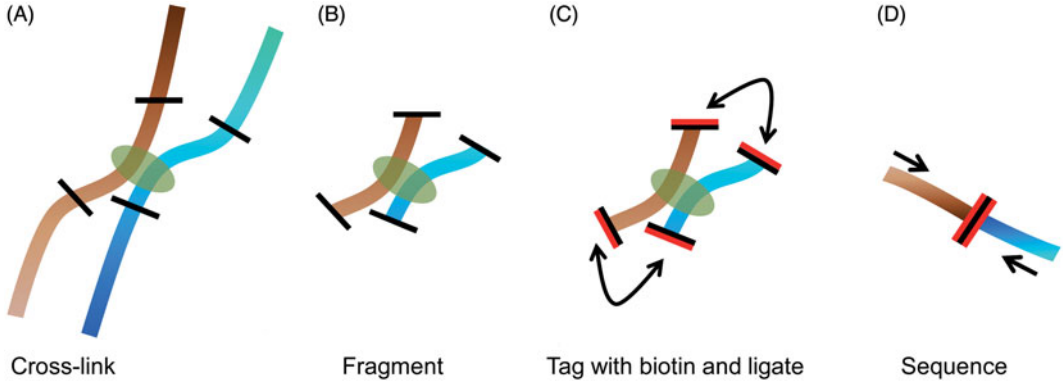

Figure 3. Hi-C variant of Chromosome Conformation Capture. (A) Genome is cross-linked with formaldehyde, locations in close proximity are physically tethered to each other. (B) Digesting the genome with a frequent (4 bp) restriction endonuclease generates free ends held together by the crosslink. (C) Each breaksite may be tagged with biotin that is then ligated to its partner fragment (arrows), the entire construct cleaved into smaller fragments and the junctions themselves isolated by pulling the biotin fragments out of solution. (D) DNA fragments containing biotin at the sequence junction now contain non-contiguous sequence elements that may be identified when screened with next generation paired-end sequencing (arrows) technologies.

spatial proximity map for the entire genome (Lieberman-Aiden et al. 2009, Rao et al. 2014).

Physical chromosome territories and chromosome fine structure

Using Hi-C to interrogate the nuclear organization of human lymphoblastoid cells, it was found that the most frequent sites of contact for any location are sequences that are on the same linear strand of DNA. The enhanced contact observed decreases monotonically over ~90 Mbp, interactions within the same chromosome exceeding that observed between different chromosomes, by 1–2 orders of magnitude (Lieberman-Aiden et al. 2009). This therefore provides comprehensive support for the presence of chromosome territories – locations on the same chromosome that are spatially close together as discussed above. Experiments such as these provide the genomic documentation for why the ratio of intra-chromosomal rearrangements (rings) to inter-chromosomal associations (dicentrics) varies from a simple random distribution; the former are, on average, formed by elements that are physically closer together prior to damage. Though the study quoted only addresses a physical association of DNA, others have shown genes that are coordinately expressed are often situated close to together on the same chromosome (Caron et al. 2001, Cohen et al. 2000). The detailed analysis of intra-chromosomal associations available through Hi-C provides information on the organization of the DNA strand that is most satisfactorily explained as a fractal globule, at least over the range of approximately 0.5–90 Mbp (Mirny 2011, Hahn and Kim 2013). The term 'fractal' used here refers to a pattern that displays similarity independent of scale, this type of fractal packing of linear DNA into 3-dimensional space is known as a Hilbert curve (Lieberman-Aiden et al. 2009). Such a structure, which does not contain knots, would permit the rapid access to specific regions of the genome, by simple unfolding, looping out and subsequent refolding, a level of packaging that would be appropriate for rapid gene access (Figure 4). The presence of a fractal globule polymer that persists over substantial (Mbp) distances indicates that over short segments of DNA there must be substantial flexibility and repetitive contact between DNA sequences; with such contacts decreasing in a mathematically predictable fashion as the distances between specific points on the linear DNA strand increase. This behavior may underpin the documented potency of radiation to generate large scale chromosomal deletions, a process likely to be facilitated if two adjacent points on the same chromosome are in physical contact when both are impacted by an ionization event (Sankaranarayanan et al. 2013).

A practical example of this level of organization may already have been observed in papillary thyroid cancer where fusion between RET (REarranged during Transfection) and either of its two frequent fusion partners, NCOA4 (Nuclear receptor COActivator 4) and CCDC6 (Coiled-Coil Domain Containing 6), is strongly linked to the disease. These three genes are all located within 18 Mbp on the long arm of chromosome 10 (Nikiforov et al. 1999, Nikiforova et al. 2000). Fusion of RET to either of these partners was commonly observed in those children developing thyroid tumors after the release of radioactivity, notably I-131, from the Chernobyl reactor explosion (Williams 2008). Careful matching of individual breakpoints in RET and NCOA4, separated by 8 Mbp, from patient tumors suggested that both genes were located in a fixed position prior to their joint fragmentation and subsequent fusion (Figure 5) (Nikiforov et al. 1999). It is possible (though unknown) that a fractal globule level of organization would reach a common solution in terms of unpacking and repacking a single region of DNA in a reproducible orientation, such that RET and NCOA4 are repetitively placed in juxtaposition, at least in the thyroid. This may specifically be the case however if both genes were transcribing at the same time, acting in response to similar signals or looped together via regulatory interactions (Cohen et al. 2000, Fullwood et al. 2009, Rao et al. 2014). This would make them statistically favored candidates for joint fragmentation and rearrangement subsequent to radiation induced fragmentation. The link to transcription was made more intriguing by an examination of RET and CCDC6 locations with FISH. Here, in an *in vitro* setting, RET and CCDC6 were found to be in close proximity in thyroid cells, but less so in cells of other types, suggesting a cell specific

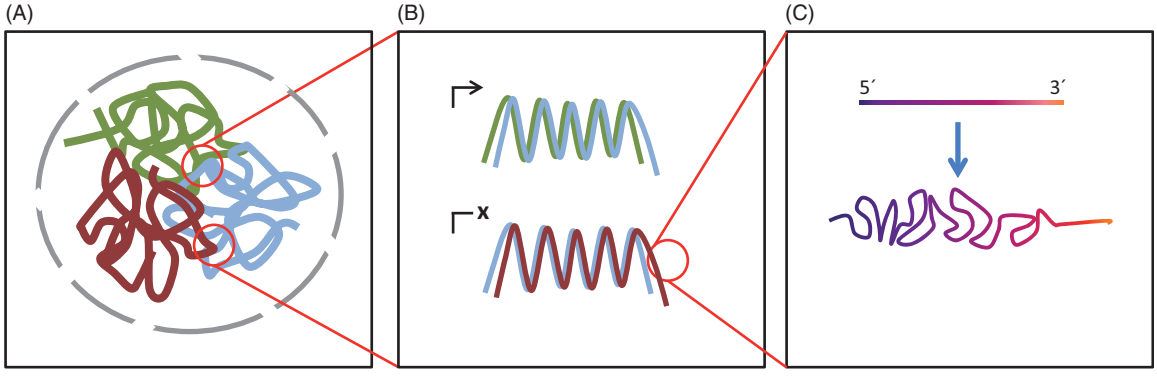

Figure 4. Chromatin organization interpreted from Hi-C experiments. (A) Chromosomes are localized in territories (three shown) however with substantial interdigitation or physical overlap into other chromosome territories. (B) The locations of inter-chromosome (and intra-chromosome) contact is linked through sharing transcriptional status; either on (arrow) or off (cross). (C) At the level of a discrete linear DNA strand the DNA is organized and efficiently packed within the genome as a fractal globule where short-range self-associations predominate. DNA self-association is thus greatest over short distances (bp to Kbp) and reduces monotonically as the distance (~90 Mbp) increases. Diagrams adapted from concepts developed by Lieberman-Aiden et al. (2009).

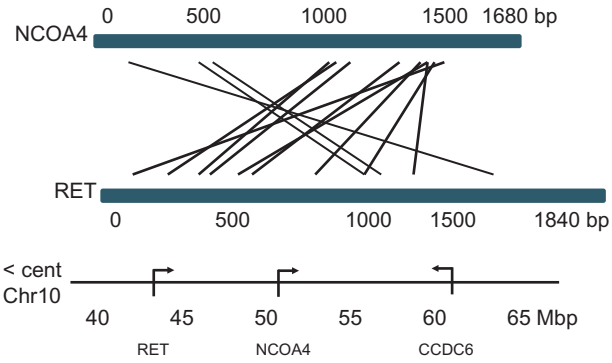

Figure 5. Proximity mapping of breakpoints in papillary thyroid cancer, redrawn using data from Nikiforov et al. (1999). (Top) Shown are the breakpoints mapped in specific segments of intron 11 and intron 5 of the RET-NCOA4 (REarranged during Transfection – Nuclear receptor COActivator 4) gene fusion respectively, and shown to scale. Here 9/12 breakpoints share a broadly parallel relationship with each other (bold lines), as do the 3/12 remaining breakpoints (faint lines). Such arrangements would be generated if both genes are located in a reproducible relationship with each other, such that a single ionization event affects both genes simultaneously. (Bottom) Location and transcriptional direction of each gene on chromosome 10. Despite the linear separation the data are consistent with reproducible gene contacts during radiation-associated cleavage that arise from a single radiation event.

and potentially transcription specific localization (Nikiforova et al. 2000).

Functional chromosome territories

Using the same data set generated by Hi-C as discussed above, Lieberman-Aiden also showed that the genome can be compartmentalized into two broadly identifiable regions that are self-associated, likely equivalent to euchromatin and heterochromatin (Lieberman-Aiden et al. 2009). The link to actively transcribing DNA was made by cross referencing Hi-C proximity data to both transcriptional activity, DNAse I sensitivity and levels of DNA methylation within Histone H3 lysine 36 (H3K36) that are associated with gene activity (Mikkelsen et al. 2007). Such associations transcend the linear organization of DNA, placing regions of gene activity into one physically interacting compartment, and heterochromatic DNA in another. This may seem a trivial reiteration of the well-known composition of chromatin. However, these data extend the description of these regions as viewed by microscopy to show, on a genome wide scale, that actively transcribing regions from the same and different chromosomes are in close physical contact; these are the same regions that are also more prone to attack by radiation-induced free radicals (Chiu et al. 1986, Cowell et al. 2007, Magnander et al. 2010, Vasireddy et al. 2010, Takata et al. 2013). The compartmentalized proximity of such damage may facilitate the subsequent rearrangement within compartments rather than between them (Cornforth et al. 2002, Lieberman-Aiden et al. 2009).

Functional compartmentalization as described was analyzed in more detail with a particular emphasis on intra-chromosomal contacts (Kalhor et al. 2012). Here, Kalhor and colleagues, using a variant of the Hi-C procedure executed on a solid matrix rather than in solution, found that for the largest chromosomes (Chrs. 1–6, 8 and 10), intra-chromosomal interactions across the centromere were restricted for those genomic regions that were composed of transcriptionally inactive chromatin. Actively transcribing regions were not so restricted and exhibited a higher contact frequency over a greater range of linear DNA sequence than non-transcribing elements. These data therefore suggest that, for these chromosomes, contact across the centromere, and any subsequent rearrangement such as a ring structure if fragmented, would likely be restricted to regions containing actively transcribing genes. These data parallel a similar whole genome survey examining translocations where experimental breaks inserted by the meganuclease, I-SceI into unique genes were commonly fused to partner locations that were actively transcribing (Chiarle et al. 2011).

Population-based analysis of the genome

Though a very useful approach, the 3C technique as discussed can only provide an average spatial mapping of specified locations across an entire population of cells. It is unclear whether such organization is uniformly maintained within defined cell lines, or potentially oligoclonal human tumor systems, or even between neighboring cells of the same genotype. To address this question, Kalhor subjected multiple 3C datasets to population based analysis. Here the genome was allocated into 428 chromatin blocks that contained similar contact profiles with the rest of the genome (Kalhor et al. 2012). These were then tested through 10,000 genome simulations to optimize fitting of the 'block' data with its interaction with the rest of the genome. This presentation of the data suggested that multiple genomic conformations provided the best fit for the 3C data obtained within an otherwise identical cell line. To address the question of variability directly, Nagano and colleagues subjected a series of otherwise identical single cells to Hi-C analysis (Nagano et al. 2013). Using mouse splenic CD4 (Cluster of Differentiation 4 or T helper) cells, they found substantial variability between inter-chromosomal contacts between the different individual cells that were examined. Individual cells were found to have relatively few chromosome contacts. These data suggest that previous maps showing a high frequency of genomic interaction, and multiple points of contact, represent an averaging of discrete data generated from uniquely organized single cells. This implies that even within a single cell type, the genome adopts multiple unique orientations, perhaps linked to cell cycle stage, tissue type or transcriptional profile such as that controlling stem cell potential (Charafe-Jauffret et al. 2009, 2013, de Wit et al. 2013, Aranda-Anzaldo et al. 2014). Extrapolating these findings to the mechanism whereby individual chromosomes fuse suggests that any specific chromosome contacts between unique chromosomes may be lost in the noise of averaging multiple cell interactions.

These data are significant in terms of the generation of radiation-induced rearrangements in that it questions their broadly random generation, as shown by mFISH studies (Cornforth et al. 2002). Here, the argument for randomness was made by examining an entire population of irradiated cells, showing that each chromosome has a broadly equal opportunity to interact with any other. The data discussed above brings this interpretation into question, suggesting

that within a single cell type, multiple genomic states may be generated, each of which brings together specific genomic components to execute a required function (Fullwood et al. 2009, Sandhu et al. 2012). This will provide differing probabilities, among single cells, for simultaneous DNA fragmentation and subsequent interaction of the regions that are uniquely in contact. For cell killing by irradiation it is the impact on single cells that is the key outcome, reflected in the adoption of the clonogenic (single cell) assay as the 'gold standard' for toxicity testing. There may be multiple factors that drive the specific organization of genomic components; and the role of transcription has already been implicated in such structural reorganization as a source of more 'open' and free radical accessible chromatin. Clearly for tumor eradication it is the response of individual cells, particularly those with an infinite reproductive future, which is of most concern.

Transcription linked genome organization

The advent of NGS and 3C technology has uncovered a complex interacting web of both short and long range (interchromosomal) interactions that sustain the cells transcriptional profile and execute tissue specific functions (Sandhu et al. 2012). These networks involve the physical association of widely separated (in terms of assigned chromosome) genome locations that may preferentially interact if jointly fragmented by irradiation. Such an association has been demonstrated using the I-SceI meganuclease as a surrogate for radiation, a widely used system for fragmenting defined genomic locations (Richardson and Jasin 2000, Chiarle et al. 2011, Schipler and Iliakis 2013). With this system targeting either c-myc (cellular myelocytomatosis oncogene) or IgH (Immunoglobulin H) genes, it was found that the experimentally fragmented genes preferentially underwent rearrangement with actively transcribing genes, particularly the histone depleted transcriptional start sites (Chiarle et al. 2011). These specific rearrangements are likely to occur, at least in part, due to the proximity of the fragmented gene and its subsequent partner, perhaps localized by sharing a common function, such as transcription. As an example, the TMPRSS2/ERG (TransMembrane PRoteaSe, Serine 2/Erythroblast transformation-specific Related Gene) gene fusion, observed in prostate tumors of ~50% of men with this disease, may be generated by androgen mediated co-localization of the two genes, in this case located on the same chromosome (Clark and Cooper 2009, Lin et al. 2009). The subsequent addition of a small radiation dose facilitates their fragmentation and fusion (Lin et al. 2009). The androgen mediated co-localization of these genes provides support for sub-nuclear functional compartments described as transcription factories, where genes with linked function aggregate in response to discrete transcriptional stimuli (Osborne et al. 2007, Fullwood et al. 2009). The presence of such factories was also inferred from the co-localization of the murine c-myc and IgH genes, on chromosomes 15 and 12 respectively, which are common translocation partners in plasmacytomas and Burkitts lymphoma (Osborne et al. 2007). Thus it is apparent that transcriptional programs are involved in defining the higher order structure of the genome and such functions may influence the contact proximity of both transcribing genes and their promoter/enhancer interactions, if appropriate stimuli are applied (Fullwood et al. 2009). In support of the general role of genome proximity in generating translocations, chromosome/chromosome proximity has been directly linked to the potential to undergo translocations following irradiation, at least in a murine system (Zhang et al. 2012). In addition, the Hi-C contact database generated by Lieberman-Aiden was cross-referenced with a large set of clinically relevant translocations, including the Mittelman database of rearrangements (Engreitz et al. 2012). Here, a strong correlation was found showing that for multiple clinically relevant translocations the partner genes are preferentially closely associated, prior to any fusion. It is a logical extension that genomic regions in contact may be targets for lethal fusion formation if both are damaged by irradiation, or other genotoxic agents. Though only translocations are discussed in this section, as they may be observed in surviving clones of cells, rearrangements that have the potential to be lethal, such as a dicentric, differ only in the inclusion of a second centromere (Meaburn et al. 2007). The process that drives the physical association of potential partners and the subsequent formation of the lesion may be very similar, thus supporting the 'contact first' hypothesis for radiation induced rearrangements.

Transcriptional upregulation may therefore have two, potentially synergistic, effects on radiation-induced lesion formation. First, regions that are transcriptionally active are more likely to undergo radiation-induced free radical damage due to their relaxed conformation facilitating free radical access. Second, transcriptionally active genes and their promoter/enhancer targets may preferentially stay in contact, making them susceptible to simultaneous fragmentation from either free radicals or direct ionization. As an example, the ERG transcription factor that is commonly over expressed in a range of tumors, was subject of an in-depth analysis of its effect on genomic organization, using 3C techniques. Here, over expression of ERG showed reproducible alterations in the spatial organization of the genome, a process that was consistent with the activation of genes linked to its role in oncogenic development (Rickman et al. 2012). Perhaps of greater interest however is the study of pluripotent stem cells, a cell type implicated as the key driver cell in the maintenance of a viable tumor mass and a logical target for therapeutic attack (Al-Hajj et al. 2003, Singh et al. 2004). It was found that the binding sites of those factors linked to the stem cell state, such as Nanog and Oct4, were preferentially associated, generating a stem cell specific set of chromosome contacts (de Wit et al. 2013, Ay et al. 2014). Both examples were generated from population based analyses, indicating the commonality of the associations found.

Relevance to radiation biology and treatment

The discussion above identifies transcriptionally active regions of the genome as more likely to be fragmented by ionizing radiation, specifically through free radical access, and also more likely to fuse when broken due to their

compartmentalized proximity. Placed in the language of radiation biology and the LQ formalism, such events may correspond to 'α' type damage where fragmentation occurs in two chromosomes simultaneously and is linearly proportional to dose, rather than separately identifiable events proportional to dose squared, as events induced by separate ionizations interact (Fowler 2010). This interpretation may however be too simplistic as experimental data shows that most survival curves tend to adhere to a simple exponential at higher doses, not the continuous curve as implied by Equation (2) (Park et al. 2008). It follows therefore that at high doses, perhaps >10 Gy, lethal lesions are generated by single events, such as the simultaneous fragmentation of two chromosomes, rather than the interaction of separate sub-lesions that provides the curvature to the LQ response. If this is correct then spatial contacts within the genome will have an influence on what specific rearrangements will be most likely to occur during the transition from low to very high single doses. Specifically, as the dose and number of breaks increase, the probability of two breaks interacting within the same nuclear compartment as defined by transcriptional status, also increases. This is particularly relevant as cell-type specific transcriptional programs activate their complement of genes, a feature that is a signature corruption within the transformed phenotype (Lee and Young 2013).

In terms of practical utility, a key question is whether knowledge of DNA organization at the level discussed here provides any useful information to the radiobiologist or radiation oncologist. Transcriptional programs that enhance specific chromosome contact likely suppress others, the potential net effect being a 'zero sum' of site-restricted lethal aberrations with no change in absolute lethality. Alternatively, cell-specific transcriptional programs, such as those characteristic of individual tissues and/or tumors, may generate a spatial proximity interactome that offers a common physical target for the insertion of DNA breaks and their subsequent processing. Though the focus in this review is the spatial organization of chromatin, the formation of any such lethal lesions will depend on the recruitment and effective action of DNA damage repair programs, such as NHEJ and HR, recently the subject of a comprehensive review in the context of radiation damage (Thompson 2012). By definition, if such programs operate efficiently no lethal lesions will occur as the chromosome integrity and orientation is unchanged. It is the inappropriate ligation of two disparate chromosome fragments that are in close physical contact that generates lesions. It follows that the contribution of either corrupt or intact DNA repair programs present in human cancers to radiation lethality may be impacted by the spatial organization of the DNA breaks they act upon, not just their ability to rejoin naked DNA templates.

To demonstrate the clinical utility of a more nuanced view of nuclear organization, it is important to identify any mechanistic relationship between the spatial arrangement of the genome pre-irradiation and the rearrangements produced by DNA repair programs post-irradiation. Does the chromatin state of the cell pre-irradiation bias towards certain structural rejoining events? If such a role is observed, then an unexplored avenue of radiation treatment sensitization may be the judicious administration of transcriptome-modulating systems (such as, RNA interference or microRNA) to manipulate specific genetic programs within the cancerous tissue before treatment.

The following questions need to be addressed:

1. Does chromosome contact in individual cells vary between cell types (different tissues, as well as normal vs. transformed)?
2. What controls the association of chromosome contacts in individual cells? How do these factors interact and influence each other?
3. If, as suggested from current Hi-C data in single cells, chromosome contact is restricted; do radiation-induced aberrations occur predominately within this class of physically associated chromosome contacts?
4. How does the spatial location of individual DNA breaks impact the efficiency of their restitution by the known repair pathways?
5. What types of therapeutic approaches could alter the nuclear architecture of cancerous tissue in a way that would increase the efficacy of radiation treatment?

Resolving the above questions will likely require the use of single cell Hi-C technology merged with aberration analysis in irradiated material, a challenging application, which is now technically feasible. The effects on single cells is the preferred platform for experiment as the variability of lesions within irradiated populations are difficult to tease out using current NGS systems. Such questions will determine whether the generation of lethal aberrations may be manipulated, for eventual therapeutic gain, through targeted alterations of chromosome contacts in normal tissues, tumors, or both.

Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

References

Adachi N, Ishino T, Ishii Y, Takeda S, Koyama H. 2001. DNA ligase IV-deficient cells are more resistant to ionizing radiation in the absence of Ku70: Implications for DNA double-strand break repair. Proc Natl Acad Sci USA. 98:12109–12113.

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. 2003. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA. 100:3983–3988.

Aranda-Anzaldo A, Dent MA, Martinez-Gomez A. 2014. The higher-order structure in the cells nucleus as the structural basis of the post-mitotic state. Prog Biophys Mol Biol. 114:137-145.

Aten JA, Stap J, Krawczyk PM, Van Oven CH, Hoebe RA, Essers J, Kanaar R. 2004. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. Science. 303:92–95.

Averbeck NB, Ringel O, Herrlitz M, Jakob B, Durante M, Taucher-Scholz G. 2014. DNA end resection is needed for the repair of complex lesions in G1-phase human cells. Cell Cycle. 13:2509–2516.

Ay F, Bailey TL, Noble WS. 2014. Statistical confidence estimation for Hi-C data reveals regulatory chromatin contacts. Genome Res. 24:999-101.

Ballarini F. 2010. From DNA radiation damage to cell death: Theoretical approaches. J Nucleic Acids. 2010:350608.

Brown JM, Attardi LD. 2005. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer. 5:231–237.

Caron H, Van Schaik B, Van Der Mee M, Baas F, Riggins G, Van Sluis P, Hermus MC, Van Asperen R, Boon K, Voute PA, et al. 2001. The human transcriptome map: Clustering of highly expressed genes in chromosomal domains. Science. 291:1289–1292.

Chadwick KH, Leenhouts HP. 1973. A molecular theory of cell survival. Phys Med Biol. 18:78–87.

Chapman JR, Taylor MR, Boulton SJ. 2012. Playing the end game: DNA double-strand break repair pathway choice. Mol Cell. 47:497–510.

Charafe-Jauffret E, Ginestier C, Bertucci F, Cabaud O, Wicinski J, Finetti P, Josselin E, Adelaide J, Nguyen TT, Monville F, et al. 2013. ALDH1-positive cancer stem cells predict engraftment of primary breast tumors and are governed by a common stem cell program. Cancer Res. 73:7290–7300.

Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur MH, Diebel ME, Monville F, Dutcher J, et al. 2009. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res. 69:1302–1313.

Chiarle R, Zhang Y, Frock RL, Lewis SM, Molinie B, Ho YJ, Myers DR, Choi VW, Compagno M, Malkin DJ, et al. 2011. Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. Cell. 147:107–119.

Chiu SM, Friedman LR, Xue LY, Oleinick NL. 1986. Modification of DNA damage in transcriptionally active vs. bulk chromatin. Int J Radiat Oncol Biol Phys. 12:1529–1532.

Clark JP, Cooper CS. 2009. ETS gene fusions in prostate cancer. Nat Rev Urol. 6:429–439.

Cohen BA, Mitra RD, Hughes JD, Church GM. 2000. A computational analysis of whole-genome expression data reveals chromosomal domains of gene expression. Nat Genet. 26:183–186.

Cook PR, Brazell IA. 1976. Conformational constraints in nuclear DNA. J Cell Sci. 22:287–302.

Cornforth MN. 1990. Testing the notion of the one-hit exchange. Radiat Res. 121:21–27.

Cornforth MN, Greulich-Bode KM, Loucas BD, Arsuaga J, Vazquez M, Sachs RK, Bruckner M, Molls M, Hahnfeldt P, Hlatky L, et al. 2002. Chromosomes are predominantly located randomly with respect to each other in interphase human cells. J Cell Biol. 159:237–244.

Costes SV, Ponomarev A, Chen JL, Nguyen D, Cucinotta FA, Barcellos-Hoff MH. 2007. Image-based modeling reveals dynamic redistribution of DNA damage into nuclear sub-domains. PLoS Comput Biol. 3:e155.

Cowell IG, Sunter NJ, Singh PB, Austin CA, Durkacz BW, Tilby MJ. 2007. gammaH2AX foci form preferentially in euchromatin after ionising-radiation. PLoS One. 2:e1057.

Cremer T, Cremer C. 2001. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet. 2:292–301.

Cucinotta FA, Nikjoo H, Goodhead DT. 1998. The effects of delta rays on the number of particle-track traversals per cell in laboratory and space exposures. Radiat Res. 150:115–119.

De Wit E, Bouwman BA, Zhu Y, Klous P, Splinter E, Verstegen MJ, Krijger PH, Festuccia N, Nora EP, Welling M, et al. 2013. The pluripotent genome in three dimensions is shaped around pluripotency factors. Nature. 501:227–231.

Dekker J, Marti-Renom MA, Mirny LA. 2013. Exploring the three-dimensional organization of genomes: Interpreting chromatin interaction data. Nat Rev Genet. 14:390–403.

Edwards AA, Moiseenko VV, Nikjoo H. 1996. On the mechanism of the formation of chromosomal aberrations by ionising radiation. Radiat Environ Biophys. 35:25–30.

Engreitz JM, Agarwala V, Mirny LA. 2012. Three-dimensional genome architecture influences partner selection for chromosomal translocations in human disease. PLoS One. 7:e44196.

Fowler JF. 2010. 21 years of biologically effective dose. Br J Radiol. 83:554–568.

Franken NA, Hovingh S, Ten Cate R, Krawczyk P, Stap J, Hoebe R, Aten J, Barendsen GW. 2012. Relative biological effectiveness of high linear energy transfer alpha-particles for the induction of DNA-double-strand breaks, chromosome aberrations and reproductive cell death in SW-1573 lung tumour cells. Oncol Rep. 27:769–774.

Friedland W, Dingfelder M, Kundrat P, Jacob P. 2011. Track structures, DNA targets and radiation effects in the biophysical Monte Carlo simulation code PARTRAC. Mutat Res. 711:28–40.

Friedland W, Kundrat P. 2013. Track structure based modelling of chromosome aberrations after photon and alpha-particle irradiation. Mutat Res. 756:213–223.

Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, et al. 2009. An oestrogen-receptor-alpha-bound human chromatin interactome. Nature. 462:58–64.

Fussner E, Strauss M, Djuric U, Li R, Ahmed K, Hart M, Ellis J, Bazett-Jones DP. 2012. Open and closed domains in the mouse genome are configured as 10-nm chromatin fibres. EMBO Rep. 13:992–996.

Geard CR. 1985. Charged particle cytogenetics: Effects of LET, fluence, and particle separation on chromosome aberrations. Radiat Res (Suppl. 8):S112–121.

Goodhead DT. 2006. Energy deposition stochastics and track structure: What about the target? Radiat Prot Dosimetry. 122:3–15.

Goodhead DT, Thacker J, Cox R. 1979. Effectiveness of 0.3 keV carbon ultrasoft X-rays for the inactivation and mutation of cultured mammalian cells. Int J Radiat Biol Relat Stud Phys Chem Med. 36:101–114.

Griffin CS, Hill MA, Papworth DG, Townsend KM, Savage JR, Goodhead DT. 1998. Effectiveness of 0.28 keV carbon K ultrasoft X-rays at producing simple and complex chromosome exchanges in human fibroblasts *in vitro* detected using FISH. Int J Radiat Biol. 73:591–598.

Hada M, Wu H, Cucinotta FA. 2011. mBAND analysis for high- and low-LET radiation-induced chromosome aberrations: A review. Mutat Res. 711:187–192.

Hahn S, Kim D. 2013. Physical origin of the contact frequency in chromosome conformation capture data. Biophys J. 105:1786–1795.

Hakim O, Resch W, Yamane A, Klein I, Kieffer-Kwon KR, Jankovic M, Oliveira T, Bothmer A, Voss TC, Ansarah-Sobrinho C, et al. 2012. DNA damage defines sites of recurrent chromosomal translocations in B lymphocytes. Nature. 484:69–74.

Hall EJ, Giaccia AJ. 2012. Radiobiology for the radiologist. Philadelphia: Wolters Kluwer Health.

Herr L, Friedrich T, Durante M, Scholz M. 2014. A model of photon cell killing based on the spatio-temporal clustering of DNA damage in higher order chromatin structures. PLoS One. 9:e83923.

Hubner MR, Eckersley-Maslin MA, Spector DL. 2013. Chromatin organization and transcriptional regulation. Curr Opin Genet Dev. 23:89–95.

Iliakis G. 1991. The role of DNA double strand breaks in ionizing radiation-induced killing of eukaryotic cells. Bioessays. 13:641–648.

Jackson DA, Dickinson P, Cook PR. 1990. The size of chromatin loops in HeLa cells. EMBO J. 9:567–571.

Jackson SP, Bartek J. 2009. The DNA-damage response in human biology and disease. Nature. 461:1071–1078.

Jaffray DA. 2012. Image-guided radiotherapy: From current concept to future perspectives. Nat Rev Clin Oncol. 9:688–699.

Johnston PJ, MacPhail SH, Banath JP, Olive PL. 1998. Higher-order chromatin structure-dependent repair of DNA double-strand breaks: Factors affecting elution of DNA from nucleoids. Radiat Res. 149:533–542.

Kalhor R, Tjong H, Jayathilaka N, Alber F, Chen L. 2012. Genome architectures revealed by tethered chromosome conformation capture and population-based modeling. Nat Biotechnol. 30:90–98.

Kavanagh JN, Redmond KM, Schettino G, Prise KM. 2013. DNA double strand break repair: A radiation perspective. Antioxid Redox Signal. 18:2458–2472.

Kellerer AM, Rossi HH. 2012. A generalized formulation of dual radiation action. Radiat Res. 178:Av204–213.

Khodarev NN, Narayana A, Constantinou A, Vaughan AT. 1997. Topologically constrained domains of supercoiled DNA in eukaryotic cells. DNA Cell Biol. 16:1051–1058.

Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. 2013. The next-generation sequencing revolution and its impact on genomics. Cell. 155:27–38.

Lea DE. 1955. Actions of radiation on living cells. New York: Cambridge University Press.

Lee TI, Young RA. 2013. Transcriptional regulation and its misregulation in disease. Cell. 152:1237–1251.

Lieberman-Aiden E, Van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science. 326:289–293.

Lin C, Yang L, Tanasa B, Hutt K, Ju BG, Ohgi K, Zhang J, Rose DW, Fu XD, Glass CK, et al. 2009. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell. 139:1069–1083.

Magnander K, Hultborn R, Claesson K, Elmroth K. 2010. Clustered DNA damage in irradiated human diploid fibroblasts: Influence of chromatin organization. Radiat Res. 173:272–282.

McVey M, Lee SE. 2008. MMEJ repair of double-strand breaks (director's cut): Deleted sequences and alternative endings. Trends Genet. 24:529–538.

Meaburn KJ, Misteli T, Soutoglou E. 2007. Spatial genome organization in the formation of chromosomal translocations. Semin Cancer Biol. 17:80–90.

Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP,et al. 2007. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature. 448:553–560.

Mirny LA. 2011. The fractal globule as a model of chromatin architecture in the cell. Chromosome Res. 19:37–51.

Nagano T, Lubling Y, Stevens TJ, Schoenfelder S, Yaffe E, Dean W, Laue ED, Tanay A, Fraser P. 2013. Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature. 502:59–64.

Natarajan AT, Palitti F, Hill MA, Stevens DL, Ahnstrom G. 2010. Influence of DMSO on Carbon K ultrasoft X-rays induced chromosome aberrations in V79 Chinese hamster cells. Mutat Res. 691:23–26.

Nikiforov YE, Koshoffer A, Nikiforova M, Stringer J, Fagin JA. 1999. Chromosomal breakpoint positions suggest a direct role for radiation in inducing illegitimate recombination between the ELE1 and RET genes in radiation-induced thyroid carcinomas. Oncogene. 18:6330–6334.

Nikiforova MN, Stringer JR, Blough R, Medvedovic M, Fagin JA, Nikiforov YE. 2000. Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. Science. 290:138–141.

Nishino Y, Eltsov M, Joti Y, Ito K, Takata H, Takahashi Y, Hihara S, Frangakis AS, Imamoto N, Ishikawa T, et al. 2012. Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure. EMBO J. 31:1644–1653.

Nomiya T. 2013. Discussions on target theory: Past and present. J Radiat Res. 54:1161–1163.

O'Neill P, Wardman P. 2009. Radiation chemistry comes before radiation biology. Int J Radiat Biol. 85:9–25.

Obe G, Johannes C, Ritter S. 2010. The number and not the molecular structure of DNA double-strand breaks is more important for the formation of chromosomal aberrations: A hypothesis. Mutat Res. 701:3–11.

Olive PL. 1998. The role of DNA single- and double-strand breaks in cell killing by ionizing radiation. Radiat Res. 150:S42–51.

Osborne CS, Chakalova L, Mitchell JA, Horton A, Wood AL, Bolland DJ, Corcoran AE, Fraser P. 2007. Myc dynamically and preferentially relocates to a transcription factory occupied by Igh. PLoS Biol. 5:e192.

Ostashevsky JY, Reichman B, Lange CS. 1999. Higher-order structure of mammalian chromatin deduced from viscoelastometry data. J Biomol Struct Dyn. 17:567–580.

Park C, Papiez L, Zhang S, Story M, Timmerman RD. 2008. Universal survival curve and single fraction equivalent dose: Useful tools in understanding potency of ablative radiotherapy. Int J Radiat Oncol Biol Phys. 70:847–852.

Pfeiffer P, Goedecke W, Kuhfittig-Kulle S, Obe G. 2004. Pathways of DNA double-strand break repair and their impact on the prevention and formation of chromosomal aberrations. Cytogenet Genome Res. 104:7–13.

Price BD, D'Andrea AD. 2013. Chromatin remodeling at DNA double-strand breaks. Cell. 152:1344–1354.

Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell. 159:1665–1680.

Richardson C, Jasin M. 2000. Frequent chromosomal translocations induced by DNA double-strand breaks. Nature. 405:697–700.

Rickman DS, Soong TD, Moss B, Mosquera JM, Dlabal J, Terry S, MacDonald TY, Tripodi J, Bunting K, Najfeld V, et al. 2012. Oncogene-mediated alterations in chromatin conformation. Proc Natl Acad Sci USA. 109:9083–9088.

Roukos V, Voss TC, Schmidt CK, Lee S, Wangsa D, Misteli T. 2013. Spatial dynamics of chromosome translocations in living cells. Science. 341:660–664.

Sachs RK, Chen AM, Brenner DJ. 1997. Review: Proximity effects in the production of chromosome aberrations by ionizing radiation. Int J Radiat Biol. 71:1–19.

Sandhu KS, Li G, Poh HM, Quek YL, Sia YY, Peh SQ, Mulawadi FH, Lim J, Sikic M, Menghi F, et al. 2012. Large-scale functional organization of long-range chromatin interaction networks. Cell Rep. 2:1207–1219.

Sankaranarayanan K, Taleei R, Rahmanian S, Nikjoo H. 2013. Ionizing radiation and genetic risks. XVII. Formation mechanisms underlying naturally occurring DNA deletions in the human genome and their potential relevance for bridging the gap between induced DNA double-strand breaks and deletions in irradiated germ cells. Mutat Res. 753:114–130.

Savage JR. 1993. Interchange and intra-nuclear architecture. Environ Mol Mutagen. 22:234–244.

Sax K. 1940. An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. Genetics. 25:41–68.

Schipler A, Iliakis G. 2013. DNA double-strand-break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice. Nucleic Acids Res. 41:7589–7605.

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. 2004. Identification of human brain tumour initiating cells. Nature. 432:396–401.

Sutherland BM, Bennett PV, Sidorkina O, Laval J. 2000. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. Proc Natl Acad Sci USA. 97:103–108.

Takata H, Hanafusa T, Mori T, Shimura M, Iida Y, Ishikawa K, Yoshikawa K, Yoshikawa Y, Maeshima K. 2013. Chromatin compaction protects genomic DNA from radiation damage. PLoS One. 8:e75622.

Thacker J, Wilkinson RE, Goodhead DT. 1986. The induction of chromosome exchange aberrations by carbon ultrasoft X-rays in V79 hamster cells. Int J Radiat Biol Relat Stud Phys Chem Med. 49:645–656.

Thompson LH. 2012. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. Mutat Res. 751:158–246.

Vakifahmetoglu H, Olsson M, Zhivotovsky B. 2008. Death through a tragedy: Mitotic catastrophe. Cell Death Differ. 15:1153–1162.

Vasireddy RS, Karagiannis TC, El-Osta A. 2010. gamma-radiation-induced gammaH2AX formation occurs preferentially in actively transcribing euchromatic loci. Cell Mol Life Sci. 67:291–294.

Wiklund K, Fernandez-Varea JM, Lind BK. 2011. A Monte Carlo program for the analysis of low-energy electron tracks in liquid water. Phys Med Biol. 56:1985–2003.

Williams D. 2008. Radiation carcinogenesis: Lessons from Chernobyl. Oncogene. 27(Suppl. 2):S9–18.

Woodbine L, Gennery AR, Jeggo PA. 2014. The clinical impact of deficiency in DNA non-homologous end-joining. DNA Repair (Amst). 16:84–96.

Xu Y, Price BD. 2011. Chromatin dynamics and the repair of DNA double strand breaks. Cell Cycle. 10:261–267.

Yokota H, Van Den Engh G, Hearst JE, Sachs RK, Trask BJ. 1995. Evidence for the organization of chromatin in megabase pair-sized loops arranged along a random walk path in the human G0/G1 interphase nucleus. J Cell Biol. 130:1239–1249.

Zhang Y, Mccord RP, Ho YJ, Lajoie BR, Hildebrand DG, Simon AC, Becker MS, Alt FW, Dekker J. 2012. Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. Cell. 148:908–921.