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Review

Telomeres and DNA double-strand breaks: ever the twain shall meet?

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Dedication. In appreciation of his heart-felt commitment to research and education, and the life-long influence he has had on the lives of students and colleagues, the authors wish to dedicate this paper to Professor Joel S. Bedford.

Abstract. Telomeres were first recognized as a *bona fide* constituent of the chromosome based on their inability to rejoin with broken chromosome ends produced by radiation. Today, we recognize two essential and interrelated properties of telomeres. They circumvent the so-called end-replication problem faced by genomes composed of linear chromosomes, which erode from their termini with each successive cell division. Equally vital is the end-capping function that telomeres provide, which is necessary to deter chromosome ends from illicit recombination. This latter property is critical in facilitating the distinction between the naturally occurring DNA double-strand breaks (DSBs) found at chromosome ends (*i.e.*, telomeres) and DSBs produced by exogenous agents. Here we discuss, in a brief historical narrative, key discoveries that led investigators to appreciate the unique properties of telomeres in protecting chromosome ends, and the consequences of telomere dysfunction, particularly as related to recombination involving radiation-induced DSBs.

Keywords. Telomeres, double-strand breaks, ionizing radiation, DNA repair.

Scope of discussion

As a general topic, telomeres and their relationship to DNA double-strand breaks (DSBs) is too broad to be contained within the pages of a brief review. We have, therefore, chosen to limit our discussion to DSBs that are produced exogenously, principally by ionizing radiation (IR). In addition, while occasional mention is made of studies involving lower eukaryotes, our intended emphasis is on the telomeres of higher eukaryotes, particularly mammals. It is instructive to address this topic from a brief historical perspective.

In the beginning…

The turn of the 20th century witnessed the fields of genetics and cytology merge, as researchers sought to understand the relationship between genes and chromosomes. During that time, there was lively, even bitter, debate as to the rudimentary mechanics of homologous chromosome pairing during meiosis. Many subscribed to the theory of 'telosynapsis',

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whereby homologues in certain plant species were thought to pair end-to-end during prophase [1]. Others argued for the universality of 'parasynapsis' instead, whereby homologues were said to pair with each other side-by-side along their lengths, a notion that would ultimately be proven correct [2, 3]. By then it was recognized that terminalization of chiasmata eventually led to the pairing of chromosomal termini in chromatids of the tetrad just prior to reductional division. But otherwise, and in contrast to the centromere, the ends of chromosomes were not given any special status or notice.

In many ways our understanding of the relationship between telomeres and DSBs parallels advances in (what would later become) the field of radiation biology. The first real appreciation of telomere function derived from early studies utilizing IR to induce structural changes in eukaryotic genomes. Muller [4] discovered various rearrangements in the chromosomes of *Drosophila* following exposure to X-rays, including inversions, which he noted never involved the rejoining of a natural chromosome end with a radiation-induced chromosome break. Muller recognized that the lack of such "terminal inversions" conveyed a special protective property to chromosome ends, which he later termed *telomeres*. Meanwhile, Stadler [5], who had been using X-rays to produce heritable chromosome aberrations in maize (*Zea*) sent McClintock several irradiated strains, including some that she discovered contained ring chromosomes. She reasoned that these ring chromosomes did not contain telomeres, but were formed instead by the rejoining of two proximal broken ends on either side of the centromere. This, together with her work leading to the discovery of breakage-fusion-bridge cycles [6, 7], cemented the notion that telomeres represented specialized structures, which behaved very differently from exogenously induced chromosome breaks. The idea that a principle function of telomeres was to promote and maintain chromosomal stability began to take hold.

Dealing with DNA ends

It would be decades before the structure of DNA was elucidated [8] and the implications that this discovery entailed were realized, a time during which the fledgling telomere field seemed to languish. By the 1970 s much progress had been made toward understanding the deposition of energy in matter by IR, and its effect on cells and molecules. By the 1980's it had become dogma that the principle target for chromosome aberration formation was the DNA DSB [9]. From a biophysical standpoint, however, controversy existed as to how IR interacted with DNA to form DSBs. In hindsight, the controversy was due, in no small part, to the fact that methods to quantify DSBs in mammalian cells were either insensitive, fraught with potential artifact, or both. In early studies, radiation chemists observed that the yield of DSBs in DNA irradiated with X-rays in aqueous solution showed an upward quadratic curvature with dose [10, 11]. This suggested to Chadwick and Leenhouts [12] that a principle component of DSB formation required cooperative damage of two closely spaced charged particle tracks (i.e., fast electrons set in motion by separate photo absorptive events). More specifically, each of the two independent tracks was envisioned to produce a DNA 'single-strand break' (SSB) situated within a few base pairs of one another, the net result being a DSB. This conclusion and its ramifications have since been vigorously challenged on the basis of both biophysical considerations and by direct experimental measurements [13–15]. In the mean time, adopters of this theory were faced with a dilemma whose proposed solution, as discussed below, involved telomere-DSB interactions.

Classical cytogenetic theory dictates that an exchange aberration occurring between two different chromosomes (*e.g.*, a dicentric or reciprocal translocation) requires the illegitimate rejoining of broken ends produced by a pair of breaks, one on each of the participating chromosomes [16]. Modernized versions of this theory merely substitute "DSB" for "chromosome break" in this context. If a DSB does, in fact, require the coincident passage of two independent particle tracks, then DSBs formed by this mechanism should be produced with dose-squared kinetics. It follows that if two such DSBs are needed for an exchange, then dicentrics should increase with the fourth power of dose. Of course, they do not, being formed instead with kinetics described by the venerable linear-quadratic equation $\alpha D + \beta D^2$, where D is the dose, α and β being proportionality constants [9, 16–18]. In attempting to provide an explanation for this discrepancy, Chadwick and Leenhouts proposed an altogether different mechanism for the origin of radiation-induced chromosome aberrations. In discordance with the notions set forth by Muller and McClintock, they proposed that most IR-induced interchanges involved a DSB from a broken chromosome rejoining with a telomere of another chromosome [19]. Limited experimental evidence, in the flowering plant *Haplopappus*, was offered in support of this mechanism [20].

With the revelation of the anxiously sought-after sequence of the human telomere [21], came molecular probes that enabled a direct re-examination of the above assertion in mammalian cells. Fluorescence *in*

situ hybridization (FISH) was used to examine, in human cells exposed to gamma rays, the breakpoint interfaces between chromosomes involved in dicentric formation. Using a fluorescently tagged oligomer complementary to the newly identified canonical $(T_2AG_3)_n$ human telomere sequence, over 200 radiation-induced dicentrics and 30 ring chromosomes were examined. Virtually never was a telomeric signal observed between the centromeres of a dicentric, and none of the rings contained a telomere either [22]. The obvious conclusion from this study was that IR-induced exchange formation in normal human cells *via* a telomere-DSB rejoining mechanism is extraordinarily rare, if it occurs at all. Muller and McClintock had been essentially correct, and the issue had been settled – or so it seemed.

Not long after the discovery of telomerase – a specialized reverse transcriptase responsible for addition of species-specific telomere repeats *de novo* – in the ciliated protozoan *Tetrahymena* [23, 24], there was a flurry of interest and research into the structure and function of telomeres of numerous organisms. The collective result of such work was that telomeres were no longer viewed as static structures composed of "junk" repetitive DNA, nobly, but dumbly, protecting the ends of chromosomes. The more modern view of the telomere is one of a complex dynamic structure, composed of many intricately interacting components [25], the identity of some coming as both a surprise and a challenge to existing paradigms of chromosome structure.

Dysfunctional telomeres *via* loss of terminal sequences/shortening

The turn of the 21st century saw powerful new molecular techniques applied to telomere research, including the use of genetically modified organisms. It gradually became apparent that telomeres can become dysfunctional in their endcapping function by one of two mechanisms, the first of which involves erosion or loss of terminal $(T_2AG_3)_n$ sequence. Once again, response to IR proved informative. For example, when bred over several generations, mice deficient in the RNA component of telomerase ($Terc^{-/-}$) experienced critically shortened telomeres and exhibited symptoms reminiscent of radiation-sensitivity syndromes. On the cellular level, their gastrointestinal crypt stem cells and primary thymocytes showed increased rates of apoptosis, and mouse embryonic fibroblasts derived from such mice showed increased radiosensitivity as measured by clonogenic survival. This cellular radiosensitivity correlated with delayed repair of DNA DSBs, persistent chromosomal breaks, and cytogenetic anomalies characterized by complex chromosomal rearrangements and widespread chromosome fragmentation [26].

Speaking more directly to the issue of telomere-DSB interactions, it was also observed in telomerase knockout mice that the incorrect repair of IR-induced DSBs resulted in rearrangements involving chromosomes with the shortest telomeres rejoining with radiation-induced breaks, a type of fusion responsible for the increased chromosome instability observed in this experimental model after exposure to IR [27, 28]. These observations are consistent with the demonstration that the fusion of critically shortened telomeres with DNA DSBs represents a repair pathway that competes with the formation of oncogenic translocations by the more common DSB-DSB rejoining reaction, thereby reducing tumorigenesis [29]. Additionally, *in vivo* evidence has recently been obtained in a mouse cancer model, demonstrating that short telomeres limit tumor progression by inducing senescence [30]. Because most somatic tissues do not express active telomerase, while the majority of tumors do [31], inhibition of telomerase has become an active area of research as regards its therapeutic potential. In human breast cancer cell lines, it was recently shown that the telomerase inhibitor GRN163L leads to shortening of telomeres and increased cell killing [32]. This was confirmed *in vivo* by decreased tumor growth in mice.

Chinese Hamster cells contain chromosomes with large, naturally occurring interstitial blocks of telomeric sequences, and these are reported to be hotspots for X-ray induced exchange aberrations [33], presumably through their interaction with radiation-induced DSBs. Terminal telomeric FISH signals have been reported to associate with gamma ray-induced chromosome breaks in rodent cells, although the mechanism for this is thought to involve cryptic translocations rather than true DSB-telomere rejoining [34]. Truncated chromosomes lacking telomere signals have been reported in human lymphocytes for up to three cell divisions following exposure to energetic ^{56}Fe ions [35], an observation for which there is a straightforward explanation. For sparsely ionizing radiations, such as X- and gamma rays, the vast majority of chromosome damage involves complete exchanges (*e.g.*, dicentrics and translocations) whereby all broken chromosome ends find partners with which to rejoin. But for certain densely ionizing radiations, such as accelerated charged particles of high atomic number, a substantial proportion of chromosome breaks remain "open", either in the form of terminal deletions or incomplete exchanges. It is, therefore, not surprising that a substantial proportion of telomeres would be missing from the cell after

multiple cell divisions, since the telomere-containing acentric fragments initially produced by radiation (*e.g.*, terminal deletions) would be lost. We find this explanation infinitely preferable to the alternative viewpoint that high-energy ^{56}Fe ions produce missing telomeric signals on chromosomes because this type of radiation somehow preferentially targets and destroys telomeres.

Dysfunctional telomeres *via* loss of protective protein function

A second, equally important, manner by which telomeres can become dysfunctional is now recognized that does not rely on gradual erosion or stochastic loss of terminal $(T_2AG_3)_n$ sequences. Endcapping function can also be compromised *via* abrogation of function of various protein components that are now known to comprise the functional telomere. Curiously, these proteins include those usually associated with the repair of IR-induced DSBs in mammalian cells, the majority of which are rejoined by nonhomologous end-joining (NHEJ) [36]. A key component of the NHEJ pathway is the DNA-dependent protein kinase (DNA-PK), a holoenzyme composed of the Ku70/Ku80 heterodimer and the catalytic subunit DNA-PKcs [37]. Deficient function of DNA-PKcs in mice leads to the well-known radiosensitive severe combined immunodeficiency (SCID) phenotype, and has been implicated in radiosensitivity and cancer susceptibility in BALB/c mouse strains [38]. It is also of interest to note that decreased levels of DNA-PKcs have been found in various human cancers [39–41].

DNA-PK was the first DSB repair protein shown to be required for capping the ends of mammalian chromosomes [42]. As observed using FISH, the chromosomes of mice with deficiencies in Ku70/Ku80 or DNA-PKcs were involved in end-to-end fusions involving telomeres of normal length. Further insight was provided by the demonstrations of a strand-specific postreplicative requirement for DNA-PKcs in processing mammalian telomeres produced *via* leading-strand synthesis [43], and a requirement for the kinase activity of DNA-PK to protect telomeres following replication [44]. The strand-specific technique of Chromosome-Orientation FISH (CO-FISH) was employed [45] to demonstrate that deficiency of DNA-PKcs resulted not only in telomeres inappropriately fusing to one another, but also with IR-induced DSBs [46] (Figs 1 and 2).

By presenting additional reactive chromosome ends in the context of slowed kinetics for DSB repair, uncapped telomeres in repair-deficient backgrounds create additional opportunity for misrepair. The relatively high frequency of these events in DNA-PKcs-deficient backgrounds indicates telomere dysfunction makes a significant, previously unrecognized, contribution to the radiosensitivity seen with repair deficiency. The discovery of a DSB-telomere rejoining pathway simultaneously vindicates and refutes aspects of the previously discussed model of aberration formation proposed by Leenhouts and Chadwick [19]. Although it is important to point out that their formation appears to be limited to repair-deficient backgrounds, interchanges of the type envisioned by these investigators can (and do) occur. On the other hand, the dose response for DSB-telomere misrejoining, as shown in Figure 3, is decidedly linear, consistent with them being formed in response to a single IR-induced DSB from a single charged particle track [14, 15]. Such linearity would most certainly not be expected for DSB formation that required cooperative damage in the form of two nearby SSBs produced by independent radiation tracks. Thus, from a biophysical standpoint these exchanges are produced from the interaction of a radiation-induced DSB with DNA not damaged by radiation. From a molecular standpoint they are essentially the result of DSB-DSB misrejoining, the second DSB being provided by an uncapped (dysfunctional) telomere.

The consequences of interstitial $(T_2AG_3)_n$ sequences that result from DSB-telomere fusions are not well understood, but they may destabilize chromosomes, as suggested by cytogenetic studies in mammalian cells that correlate interstitial telomere sequence with sites of spontaneous and radiation-induced chromosome rearrangements [47]. Studies in budding yeast suggest another possible source of instability: internal tracts of telomeric sequence inhibit DNA damage checkpoint signaling from nearby DNA DSBs [48]. From Figure 1, it should also be apparent that any rejoining reaction involving a DSB and a telomere will result in an "orphaned" chromosomal fragment containing a "raw" (reactive) terminal DSB, a situation analogous to that known to promote ongoing genomic instability [49].

Other contemporary issues

Telomeres may be viewed as being vulnerable to illicit acts of recombination immediately following their replication, before the necessary protein components required for endcapping have reassembled [43]. When Replicative Detargeting FISH (ReD-FISH) was used to study replication timing of mammalian telomeres, it was discovered that – in stark contrast to yeast, whose telomeres coordinately replicate at the end of S-phase

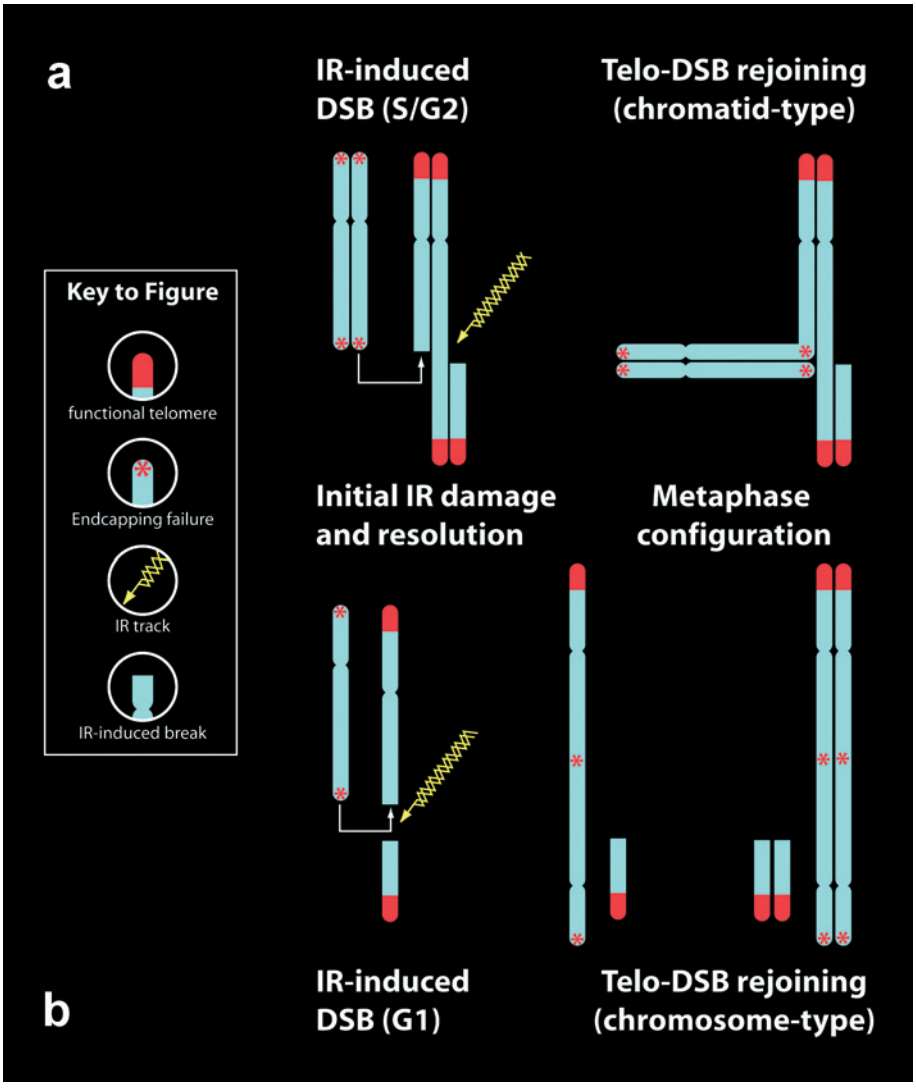

Figure 1. Rejoining of dysfunctional telomeres with radiation-induced double-strand breaks (DSBs): an overview. The term dysfunctional is meant to convey loss of endcapping function, which causes chromosomal termini to behave like DSBs. As explained in the text, there are two different mechanisms by which telomeres can loose endcapping function. Endcapping failure occurs when telomeres are lost or become critically shortened as, for example, when cells approach replicative senescence. The other mechanism, unrelated to telomere length maintenance, is *via* loss-of-function of various proteins required for proper telomere protection. These include TRF2, and a variety of proteins normally associated with the repair of DNA damage, such as DNA-PK. The figure makes no distinction between the two mechanisms of generating telomere dysfunction, since the net result is similar as regards the formation of chromosomal rearrangements. (*a*) In S and G2 phases of the cell cycle, an ionizing radiation (IR)-induced DSB takes the form of a chromatid break on one sister chromatid of a duplicated region of the chromosome. This DSB has the potential to rejoin with a dysfunctional telomere from another chromosome, producing a chromatid-type exchange at the following mitosis. (*b*) In G1 phase, IR produces a DSB in an unreplicated chromosome that can potentially rejoin with a dysfunctional telomere of another chromosome; this appears, following replication, as a chromosome-type exchange at mitosis. In both scenarios, either dicentric chromosomes or translocations (not shown) may be formed. Irrespective of telomeric dysfunction, DSBs induced by IR frequently interact with one another (not shown) to form exchange-type aberrations. In addition, even in the absence of exogenous IR-induced DSBs, chromosomes with dysfunctional telomeres tend to fuse with one another, producing dicentrics. To distinguish telomere-telomere from telomere-DSB rejoinings, special techniques are required (see Fig. 2).

– the telomeres of Indian Muntjac [50] and human cells (Cornforth, unpublished results) replicate throughout S phase. More specifically, in the Muntjac, there is a strong tendency for homologous chromosomes to replicate synchronously, and telomeres on opposite arms of the same chromosome to replicate asynchronously. It is tempting to speculate that this may reflect an evolutionary adaptation that serves to minimize the number of newly replicated telomeres at any given time, thereby reducing the number of vulnerable ends available for misrejoining – with each other or with exogenously induced DSBs.

Verdun and colleagues [51, 52] have advanced the intriguing proposal that it is actually necessary for

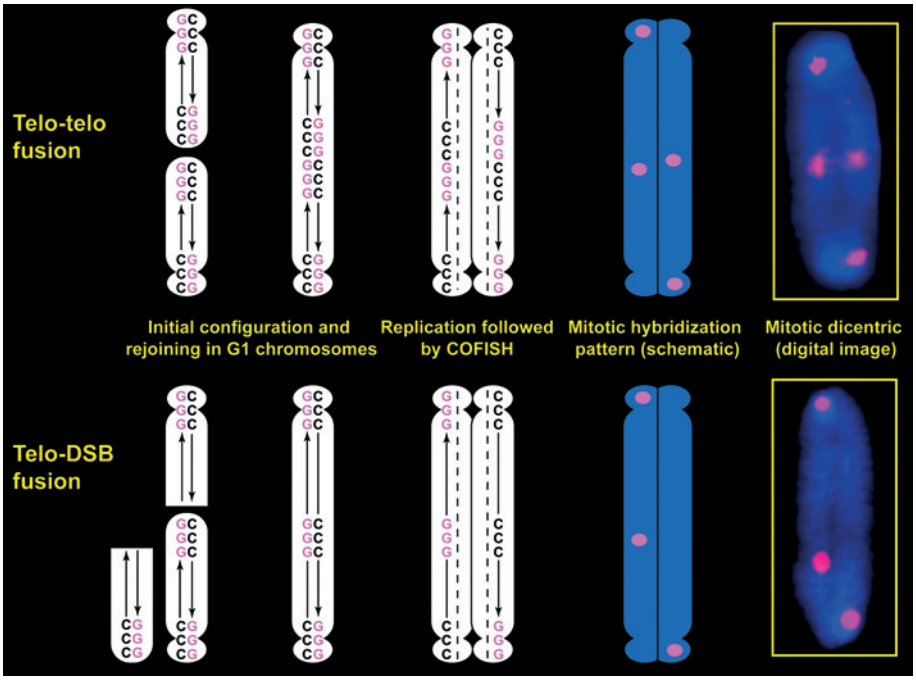

Figure 2. Schematic of chromosome-orientation fluorescence *in situ* hybridization (CO-FISH) and the telomeric hybridization patterns used to distinguish fusions between two telomeres (upper row) from fusions between a telomere and a DSB (bottom row). The chromosomes depicted are of mouse, for which p-arms are very short. The G-rich telomeric sequences are shown in red. Following replication and selective degradation of the newly synthesized DNA strands by CO-FISH (vertical dashed lines), hybridization to the complementary C-rich probe will produce the indicated staining patterns. Owing to the requisite conservation of DNA polarity, fusions between two telomeres will always produce two signals (one on each sister chromatid) at the point of fusion, whereas a telomere-DSB fusion can only produce one (single-sided) signal. The same is true for reciprocal translocations. Identical conclusions and staining patterns result if one considers the fusion event to have occurred in S/G_2 instead. In this case, the resulting chromatid-type exchanges would require conversion to chromosome-type exchanges in the following cell division (not shown). Illustration inspired by Fig. 1 of [46].

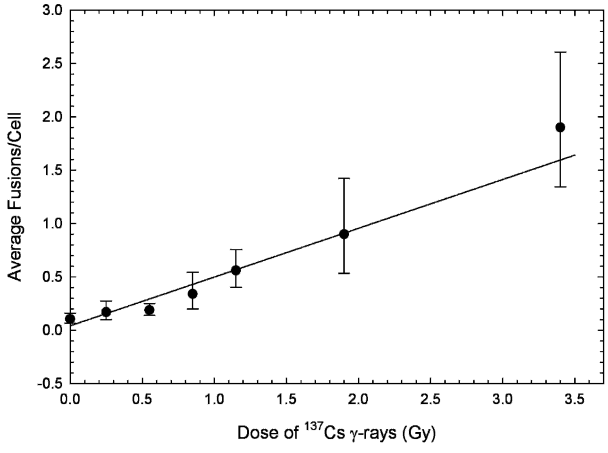

Figure 3. Dose response for telomere-DSB fusions in mouse $p53^{-/-}$ SCID cells. Owing to decreased DNA-PKcs function, the telomeres of these cells become uncapped, frequently becoming involved in fusions involving exogenous DSBs produced by gamma rays. Error bars represent 95% confidence limits about a Poisson-distributed random variable. The fit to the data is a weighted least-squares linear regression. Re-plotted from data presented in Table 3 of [46].

normal functional telomeres to be recognized as DSBs (immediately after replication and into G_2) to generate a localized damage response, recruit the necessary processing machinery, and acquire a protective end-capping structure. Earlier results utilizing so-called ChIP-(on)-chip analysis, which couples chromatin immuno-precipitation with DNA microarrays, demonstrated that phosphorylation of H2AX (γH2AX) occurs preferentially in telomeric regions that become critically shortened as they approach replicative senescence [53]. A pertinent finding of a more recent, comprehensive ChIP-chip study was that this type of damage response was observed, at reduced levels, even in relatively early-passage, actively growing, pre-senescent primary human cultures, but it was not found in cells whose telomere lengths were kept long through the activation of hTERT [54]. Taken together, this seems to suggest that at least some of the damage response observed in non-immortalized cell cultures is due to a subpopulation of early-senescing cells containing chromosomes with critically shortened telomeres, possibly arising *via* stochastic loss, as opposed to a transient uncapping of telomeres in S/G_2. With the exception of Artemis [55], no individuals null for NHEJ proteins have been identified in the population. This, of course, does not discount the possibility that partial deficiencies may be relevant to issues of human health. Utilizing an RNA interference

(RNAi) strategy with small interfering RNAs (siRNA), we have shown that frequencies of IR-induced telomere-DSB fusion are increased when protein levels of DNA-PKcs are reduced to roughly 50% that of controls [56]. No telomere-telomere fusions were observed under these conditions, but uncapping of telomeres did occur, as evidenced by numerous telomere-DSB fusions, proving these events to be sensitive markers of telomere dysfunction, reflective of underlying repair deficiency. It is noteworthy that no further increase in the frequency of telomere-DSB fusions was seen when the dose was raised from 1.5 to 5 Gy. Such a plateau in the dose-response relationship supports the hypothesis that uncapped telomeres are rate limiting. This study demonstrated that the NHEJ protein DNA-PKcs serves to suppress telomere dysfunction in a manner dependent on protein abundance. Partial deficiencies in DNA-PK relevant to the human condition, if they exist, are more likely to involve haploinsufficiencies, rather than complete loss of function, and would probably involve low penetrance genes that are fixed in the human population at relatively high frequencies. If this notion is correct, the potential impact of inter-individual susceptibility regarding the incidence of telomere-DSB interactions requires examination in the context of human cancer risk.

The concept is now firmly ensconced that DSB repair proteins are intimately associated with proper telomere function. But what of the *vice versa* scenario; do telomere proteins interact with exogenous DSBs? There are several studies generally supportive of this idea, one most recently that focused on the misrepair of enzymatically induced I-Sce1 DSBs [57]. Here it was concluded that DSB repair by the homologous recombination (HR) pathway was inhibited when TRF2 – a critical telomere-specific binding protein required for mammalian end-capping [43, 58] – was depleted, whereas such depletion had no effect on DSB repair by the NHEJ pathway. Interestingly, overexpression of TRF2 actually inhibited NHEJ, while simultaneously stimulating HR. Based on TRF2's ability to facilitate strand invasion during t-loop formation, the authors suggest that perhaps it plays a similar role at HR-mediated recombination intermediates. The first study suggestive of TRF2 involvement in an early DNA damage response (presumptively to DSBs) was based on its recruitment to sites damaged by high-intensity ultra-violet (UV) laser beams, as evidenced by co-localization of TRF2 with γH2AX and other damage markers [59]. This prompted models of telomere function that assumed a more-or-less reciprocal relationship between DNA repair proteins and telomeres [60].

Arguing against the above viewpoint, however, is the observation that recruitment of TRF2 to IR-induced damage foci does not occur following exposure to gamma rays [61, 62]. More recent experiments have utilized focused beams of alpha particles to produce copious quantities of DSBs within defined subnuclear target dimensions of $\leq 5\ \mu m^2$. TRF2 recruitment to these locally damaged sites, quantitatively visualized as co-localization of fluorescent foci, was not observed [63]. Although these results do not altogether preclude a possible functional role for telomeric proteins in DNA repair, they strongly suggest that TRF2 does not bind to exogenous DSBs as an early damage response. This conclusion was promptly questioned on the basis that the ability to form foci at sites of IR-induced damage may not be a universal property of DSB response proteins, and that such proteins, even if recruited to sites of DSB damage, may not exist in sufficient local concentration to yield visible foci following IR. In addition, the dense local deposition of energy following the traversal of an alpha particle may produce DSBs that are qualitatively different from those produced by X- and gamma rays, perhaps eliciting a qualitatively different damage response [64]. If there is a point of harmony regarding these otherwise contradictory viewpoints [63, 64], it is that localized damage from high-intensity lasers is not well characterized in terms of the cellular lesions that are produced.

Without discussing the relative merit of the aforementioned arguments, suffice it say that disagreement exists as to whether telomere proteins (*e.g.*, TRF2) interact directly with exogenous DSBs. We, nevertheless, feel compelled to opine that we find it difficult to envision a useful purpose served by having key components of the telomere promptly assemble at sites of IR-induced DSBs. Our difficulty with this scenario is made more acute in light of evidence suggesting that TRF2 actually represses ATM-mediated damage response [61], and the recent demonstration that the RAP1/TRF2 complex inhibits NHEJ at human telomeres [65]. This latter study further suggests a distinct polarity-dependent nature to the end-joining inhibition, so as to not interfere with repair of DSBs upstream of telomeric repeats. We imagine, perhaps naively, that the purported recruitment of telomeric proteins to DSBs would tempt cellular repair machinery towards all sorts of recombinational mischief. In this context, the overexpression of TRF2 [57], to the extent that it is biologically relevant, may be viewed as a situation leading to the inappropriate up-regulation of DSB repair by HR, at the expense of the more common and robust NHEJ pathway. In any case, whereas the issues highlighted above may not yet be resolved to the point of universal

consensus, for the time being, we tend to view the overlapping relationships between telomeres and DSB repair proteins as a one-way street. In other words – at least as regards an early damage response – DSB repair proteins play at telomeres, but telomere proteins do not play at DSBs (perhaps they know better).

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1 Gates, R. R. (1911) The mode of chromosome reduction. Bot. Gaz. 51, 321 – 344.
2 Darlington, C. D. (1931) The cytological theory of inheritance in *Oenothera.* J. Genet. 24, 405 – 474.
3 Darlington, C. D. (1932) Recent Advances In Cytology. Churchill, London.
4 Muller, H. J. (1938) The remaking of chromosomes. Collecting Net 13, 181 – 198.
5 Stadler, L. J. (1931) The experimental modification of heredity in crop plants. 1. Induced chromosomal irregularities. Sci. Agric. 11, 557 – 572.
6 McClintock, B. (1938) The production of homozygous deficient tissues with mutant characteristics by means of the aberrant behavior of ring-shaped chromosomes. Genetics 23, 215 – 376.
7 McClintock, B. (1940) The stability of broken ends of chromosomes in *Zea mays.* Genetics 26, 234 – 282.
8 Watson, J. D. and Crick, F. H. (1953) Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. Nature 171, 737 – 738.
9 Cornforth, M. N. and Bedford, J. S. (1993) Ionizing radiation damage and its early development in chromosomes. In: Advances in Radiation Biology, vol. 17, pp. 423 – 496, Lett, J. T. and Sinclair, W. K. (eds.), Academic Press, San Diego.
10 Hagen, U. (1967) Determination of single and double breaks in irradiated DNA from the distribution of molecular weight. Biochim. Biophys. Acta 134, 45 – 58.
11 Freifelder, D. and Trumbo, B. (1969) Matching of single-strand breaks to form double-strand breaks in DNA. Biopolymers 7, 681 – 693.
12 Chadwick, K. H. and Leenhouts, H. P. (1973) A molecular theory of cell survival. Phys. Med. Biol. 18, 78 – 87.
13 Savage, J. R. K. (1975) Radiation-induced chromosomal aberrations in the plant Tradescantia. Radiat. Bot. 15, 87 – 140.
14 Ward, J. F. (1990) The yield of DNA double-strand breaks produced intracellularly by ionizing radiation: a review. Int. J. Radiat. Biol. 57, 1141 – 1150.
15 Boudaiffa, B., Cloutier, P., Hunting, D., Huels, M. A. and Sanche, L. (1658) Resonant formation of DNA strand breaks by low-energy (3 to 20 eV) electrons. Science 287, 1658 – 1660.
16 Lea, D. E. (1946) Actions of Radiations on Living Cells. Cambridge University Press, London.
17 Sax, K. (1938) Chromosome aberrations induced by x-rays. Genetics 23, 494 – 516.
18 Lloyd, D. C., Purrott, R. J., Dolphin, G. W., Bolton, D., Edwards, A. A. and Corp, M. J. (1975) The relationship between chromosome aberrations and low LET radiation dose to human lymphocytes. Int. J. Radiat. Biol. 28, 75 – 90.
19 Leenhouts, H. P. and Chadwick, K. H. (1974) Radiation induced DNA double strand breaks and chromosome aberrations. Theor. Appl. Genet. 44, 167 – 172.
20 Spanjers, W. A., Engles, F. M., Werry, P. A. T. J., Chadwick, K. H. and Leenhouts, H. P. (1975) New evidence on chromosome joining after radiation. In: Fifth Symposium on Microdosimetry, vol. 2, pp. 545, Booz, J., Ebert, H. G. and Smith, G. B. R. (eds.) Commission of the European Communities, Luxembourg.
21 Moyzis, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., Meyne, J., Ratliff, R. L. and Wu, J. R. (1988) A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc. Natl. Acad. Sci. USA 85, 6622 – 6626.
22 Cornforth, M. N., Meyne, J., Littlefield, L. G., Bailey, S. M. and Moyzis, R. K. (1989) Telomere staining of human chromosomes and the mechanism of radiation-induced dicentric formation. Radiat. Res. 120, 205 – 212.
23 Greider, C. W. and Blackburn, E. H. (1985) Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 43, 405 – 413.
24 Greider, C. W. and Blackburn, E. H. (1987) The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 51, 887 – 898.
25 de Lange, T. (2005) Shelterin: The protein complex that shapes and safeguards human telomeres. Genes Dev. 19, 2100 – 2110.
26 Wong, K. K., Chang, S., Weiler, S. R., Ganesan, S., Chaudhuri, J., Zhu, C., Artandi, S. E., Rudolph, K. L., Gottlieb, G. J., Chin, L., Alt, F. W. and DePinho, R. A. (2000) Telomere dysfunction impairs DNA repair and enhances sensitivity to ionizing radiation. Nat. Genet. 26, 85 – 88.
27 Latre, L., Tusell, L., Martin, M., Miro, R., Egozcue, J., Blasco, M. A. and Genesca, A. (2003) Shortened telomeres join to DNA breaks interfering with their correct repair. Exp. Cell Res. 287, 282 – 288.
28 Genesca, A., Martin, M., Latre, L., Soler, D., Pampalona, J. and Tusell, L. (2006) Telomere dysfunction: a new player in radiation sensitivity. Bioessays 28, 1172 – 1180.
29 Qi, L., Strong, M. A., Karim, B. O., Huso, D. L. and Greider, C. W. (2005) Telomere fusion to chromosome breaks reduces oncogenic translocations and tumour formation. Nat. Cell Biol. 7, 706 – 711.
30 Feldser, D. M. and Greider, C. W. (2007) Short telomeres limit tumor progression *in vivo* by inducing senescence. Cancer Cell 11, 461 – 469.
31 Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L. and Shay, J. W. (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266, 2011 – 2015.
32 Gomez-Millan, J., Goldblatt, E. M., Gryaznov, S. M., Mendonca, M. S. and Herbert, B. S. (2007) Specific telomere dysfunction induced by GRN163L increases radiation sensitivity in breast cancer cells. Int. J. Radiat. Oncol. Biol. Phys. 67, 897 – 905.
33 Alvarez, L., Evans, J. W., Wilks, R., Lucas, J. N., Brown, J. M. and Giaccia, A. J. (1993) Chromosomal radiosensitivity at intrachromosomal telomeric sites. Genes Chromosomes Cancer 8, 8 – 14.
34 Slijepcevic, P., Natarajan, A. T. and Bryant, P. E. (1998) Telomeres and radiation-induced chromosome breakage. Mutagenesis 13, 45 – 49.
35 Durante, M., George, K. and Cucinotta, F. A. (2006) Chromosomes lacking telomeres are present in the progeny of human lymphocytes exposed to heavy ions. Radiat. Res. 165, 51 – 58.
36 Iliakis, G., Wang, H., Perrault, A. R., Boecker, W., Rosidi, B., Windhofer, F., Wu, W., Guan, J., Terzoudi, G. and Pantelias, G. (2004) Mechanisms of DNA double strand break repair and chromosome aberration formation. Cytogenet. Genome Res. 104, 14 – 20.
37 Jeggo, P. A. (1998) Identification of genes involved in repair of DNA double-strand breaks in mammalian cells. Radiat. Res. 150, S80 – 91.
38 Ponnaiya, B., Cornforth, M. N. and Ullrich, R. L. (1997) Radiation-induced chromosomal instability in BALB/c and

C57BL/6 mice: The difference is as clear as black and white. Radiat. Res. 147, 121 – 125.
39 Moll, U., Lau, R., Sypes, M. A., Gupta, M. M. and Anderson, C. W. (1999) DNA-PK, the DNA-activated protein kinase, is differentially expressed in normal and malignant human tissues. Oncogene 18, 3114 – 3126.
40 Someya, M., Sakata, K., Matsumoto, Y., Yamamoto, H., Monobe, M., Ikeda, H., Ando, K., Hosoi, Y., Suzuki, N. and Hareyama, M. (2006) The association of DNA-dependent protein kinase activity with chromosomal instability and risk of cancer. Carcinogenesis 27, 117 – 122.
41 Auckley, D. H., Crowell, R. E., Heaphy, E. R., Stidley, C. A., Lechner, J. F., Gilliland, F. D. and Belinsky, S. A. (2001) Reduced DNA-dependent protein kinase activity is associated with lung cancer. Carcinogenesis 22, 723 – 727.
42 Bailey, S. M., Meyne, J., Chen, D. J., Kurimasa, A., Li, G. C., Lehnert, B. E. and Goodwin, E. H. (1999) DNA double-strand break repair proteins are required to cap the ends of mammalian chromosomes. Proc. Natl. Acad. Sci. USA 96, 14899 – 14904.
43 Bailey, S. M., Cornforth, M. N., Kurimasa, A., Chen, D. J. and Goodwin, E. H. (2001) Strand-specific postreplicative processing of mammalian telomeres. Science 293, 2462 – 2465.
44 Bailey, S. M., Brenneman, M. A., Halbrook, J., Nickoloff, J. A., Ullrich, R. L. and Goodwin, E. H. (2004) The kinase activity of DNA-PK is required to protect mammalian telomeres. DNA Repair 3, 225 – 233.
45 Bailey, S. M., Goodwin, E. H. and Cornforth, M. N. (2004) Strand-specific fluorescence *in situ* hybridization: the CO-FISH family. Cytogenet. Genome Res. 107, 14 – 17.
46 Bailey, S. M., Cornforth, M. N., Ullrich, R. L. and Goodwin, E. H. (2004) Dysfunctional mammalian telomeres join with DNA double-strand breaks. DNA Repair 3, 349 – 357.
47 Kilburn, A. E., Shea, M. J., Sargent, R. G. and Wilson, J. H. (2001) Insertion of a telomere repeat sequence into a mammalian gene causes chromosome instability. Mol. Cell. Biol. 21, 126 – 135.
48 Michelson, R. J., Rosenstein, S. and Weinert, T. (2005) A telomeric repeat sequence adjacent to a DNA double-stranded break produces an anticheckpoint. Genes Dev. 19, 2546 – 2559.
49 Sabatier, L., Ricoul, M., Pottier, G. and Murnane, J. P. (2005) The loss of a single telomere can result in instability of multiple chromosomes in a human tumor cell line. Mol. Cancer Res. 3, 139 – 150.
50 Zou, Y., Gryaznov, S. M., Shay, J. W., Wright, W. E. and Cornforth, M. N. (2004) Asynchronous replication timing of telomeres at opposite arms of mammalian chromosomes. Proc. Natl. Acad. Sci. USA 101, 12928 – 12933.
51 Verdun, R. E. and Karlseder, J. (2006) The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. Cell 127, 709 – 720.
52 Verdun, R. E., Crabbe, L., Haggblom, C. and Karlseder, J. (2005) Functional human telomeres are recognized as DNA damage in G2 of the cell cycle. Mol. Cell 20, 551 – 561.
53 d'Adda di Fagagna, F., Reaper, P. M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki, G., Carter, N. P. and Jackson, S. P. (2003) A DNA damage checkpoint response in telomere-initiated senescence. Nature 426, 194 – 198.
54 Meier, A., Fiegler, H., Munoz, P., Ellis, P., Rigler, D., Langford, C., Blasco, M. A., Carter, N. and Jackson, S. P. (2007) Spreading of mammalian DNA-damage response factors studied by ChIP-chip at damaged telomeres. EMBO Journal 26, 2707 – 2718.
55 Li, L., Moshous, D., Zhou, Y., Wang, J., Xie, G., Salido, E., Hu, D., de Villartay, J. P. and Cowan, M. J. (2002) A founder mutation in Artemis, an SNM1-like protein, causes SCID in Athabascan-speaking Native Americans. J. Immunol. 168, 6323 – 6329.
56 Zhang, Y., Zhou, J., Cao, X., Zhang, Q., Lim, C. U., Ullrich, R. L., Bailey, S. M. and Liber, H. L. (2007) Partial deficiency of DNA-PKcs increases ionizing radiation-induced mutagenesis and telomere instability in human cells. Cancer Lett. 250, 63 – 73.
57 Mao, Z., Seluanov, A., Jiang, Y. and Gorbunova, V. (2007) TRF2 is required for repair of nontelomeric DNA double-strand breaks by homologous recombination. Proc. Natl. Acad. Sci. USA 104, 13068 – 13073.
58 van Steensel, B., Smogorzewska, A. and de Lange, T. (1998) TRF2 protects human telomeres from end-to-end fusions. Cell 92, 401 – 413.
59 Bradshaw, P. S., Stavropoulos, D. J. and Meyn, M. S. (2005) Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. Nat. Genet. 37, 193 – 197.
60 Slijepcevic, P. (2006) The role of DNA damage response proteins at telomeres – An "integrative" model. DNA Repair 5, 1299 – 1306.
61 Karlseder, J., Hoke, K., Mirzoeva, O. K., Bakkenist, C., Kastan, M. B., Petrini, J. H. and de Lange, T. (2004) The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. Plos Biol. 2, E240.
62 Zhu, X. D., Kuster, B., Mann, M., Petrini, J. H. and de Lange, T. (2000) Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat. Genet. 25, 347 – 352.
63 Williams, E. S., Stap, J., Essers, J., Ponnaiya, B., Luijsterburg, M. S., Krawczyk, P. M., Ullrich, R. L., Aten, J. A. and Bailey, S. M. (2007) DNA double-strand breaks are not sufficient to initiate recruitment of TRF2. Nat. Genet. 39, 696 – 698.
64 Bradshaw, P. S. and Meyn, M. S. (2007) Reply to "DNA double-strand breaks are not sufficient to initiate recruitment of TRF2". Nat. Genet. 39, 698 – 699.
65 Bae, N. S. and Baumann, P. (2007) A RAP1/TRF2 complex inhibits nonhomologous end-joining at human telomeric DNA ends. Mol. Cell 26, 323 – 334.