Mutation Research-Reviews in Mutation Research 794 (2024) 108507

Contents lists available at ScienceDirect

Mutation Research-Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/mutrev

Considerations on the scoring of telomere aberrations in vertebrate cells detected by telomere or telomere plus centromere PNA-FISH

Alejandro D. Bolzán [a,b]

[a] Laboratorio de Citogenética y Mutagénesis, Instituto Multidisciplinario de Biología Celular (IMBICE, CONICET-UNLP-CICPBA), calle 526 y Camino General Belgrano, La Plata, Buenos Aires B1906APO, Argentina
[b] Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, calle 60 y 122, La Plata, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Telomere
Incomplete chromosome elements
Telomere shortening
Telomere fragility
Telomere dysfunction
PNA-FISH

ABSTRACT

Given that telomeres play a fundamental role in maintaining genomic stability, the study of the chromosomal aberrations involving telomeric sequences is a topic of considerable research interest. In recent years, the scoring of these types of aberrations has been used in vertebrate cells, particularly human cells, to evaluate the effects of genotoxic agents on telomeres and the involvement of telomeric sequences on chromosomal aberrations. Currently, chromosomal aberrations involving telomeric sequences are evaluated in peripheral blood lymphocytes or immortalized cell lines, using telomere or telomere plus centromere fluorescence *in situ* hybridization (FISH) with Peptide Nucleic Acid (PNA) probes (PNA-FISH). The telomere PNA probe is more efficient in the detection of telomeric sequences than conventional FISH with a telomere DNA probe. In addition, the intensity of the telomeric PNA-FISH probe signal is directly correlated with the number of telomeric repeats. Therefore, use of this type of probe can identify chromosomal aberrations involving telomeres as well as determine the telomere length of the sample. There are several mistakes and inconsistencies in the literature regarding the identification of telomere aberrations, which prevent accurate scoring and data comparison between different publications concerning these types of aberrations. The aim of this review is to clarify these issues, and provide proper terminology and criteria for the identification, scoring, and analysis of telomere aberrations.

1. Introduction

Telomeres are specialized nucleoproteic complexes localized at the physical ends of linear eukaryotic chromosomes, which protect them from degradation, recombination or fusion, by preventing the chromosome ends from being recognized as DNA double-strand breaks (DSBs) by the DNA repair machinery, thus avoiding the activation of the DNA damage response and repair pathways [1–3]. Therefore, the proper functioning of telomeres is essential to maintain genome stability in the cells. When telomeres become unstable, different types of chromosomal aberrations arise [4,5]. These are known as "telomere aberrations" and may arise due to the loss of the chromosome end/s (due to a breakage event) or telomere dysfunction (Fig. 1). Telomere dysfunction may be due to alterations in the shelterin complex or other telomere-associated proteins [6–9], some DNA damage response (DDR) proteins required for proper telomere protection [3,10], the structure of telomeric DNA [11], the structure or activity of telomerase [12–16], the telomere RNA (TERRA) [17–20] or helicase enzymes [21–24].

Telomere aberrations are currently identified in metaphase spreads from vertebrate cells by FISH using a PNA (Peptide Nucleic Acid) pantelomeric probe (usually labeled with Cy3, FITC or FAM), which identifies all the telomeres present in a cell [5,25]. This is due to the fact that the commercially available human pantelomeric probes (several suppliers worldwide, including Panagene from Daejeon, Korea, DAKO, from Glostrup, Denmark, PNA Bio from Thousand Oaks, CA, USA, and Applied Biosystems, from Foster City, CA, USA, among others) share a common sequence with all vertebrate species, i.e., (TTAGGG)n/(AATCCC)n [26]. The telomere PNA probe is more efficient in the detection of telomeric sequences than conventional FISH with a telomere DNA probe [25,27,28]. In addition, the fluorescence intensity of the telomeric PNA-FISH

Abbreviations: Ace, acentric; AF/s, acentric fragment/s; Alt-NHEJ, alternative non-homologous end joining; BIR, break induced replication; CAF, compound acentric fragment; Cy3, Cyanine3; DAPI, 4′,6-diamidino-2-phenylindole; DDR, DNA damage response; DSBs, double-strand breaks; FAM, Carboxyfluorescein; FISH, fluorescence *in situ* hybridization; IAF, interstitial acentric fragment; IC, incomplete chromosome; ICE, incomplete chromosome elements; ITS, interstitial telomeric sequences; PNA, peptide nucleic acid; SSBs, single strand breaks; TAF, terminal acentric fragment; TDM, telomeric DNA-containing double minute chromosome.

E-mail addresses: adbolzan64@gmail.com, abolzan@imbice.gov.ar.

https://doi.org/10.1016/j.mrrev.2024.108507
Received 3 January 2024; Received in revised form 20 May 2024; Accepted 21 May 2024
Available online 25 May 2024

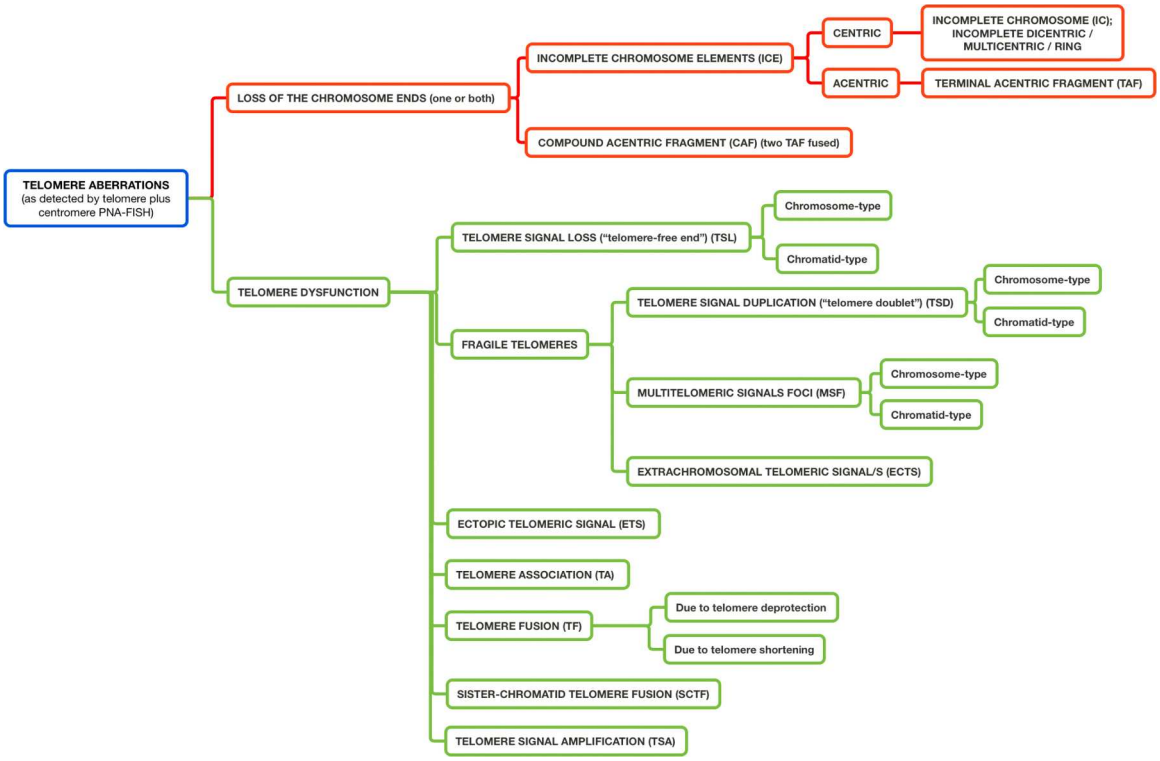


Fig. 1. Classification of telomere aberrations as detected by telomere or telomere plus centromere PNA-FISH. See text for details.

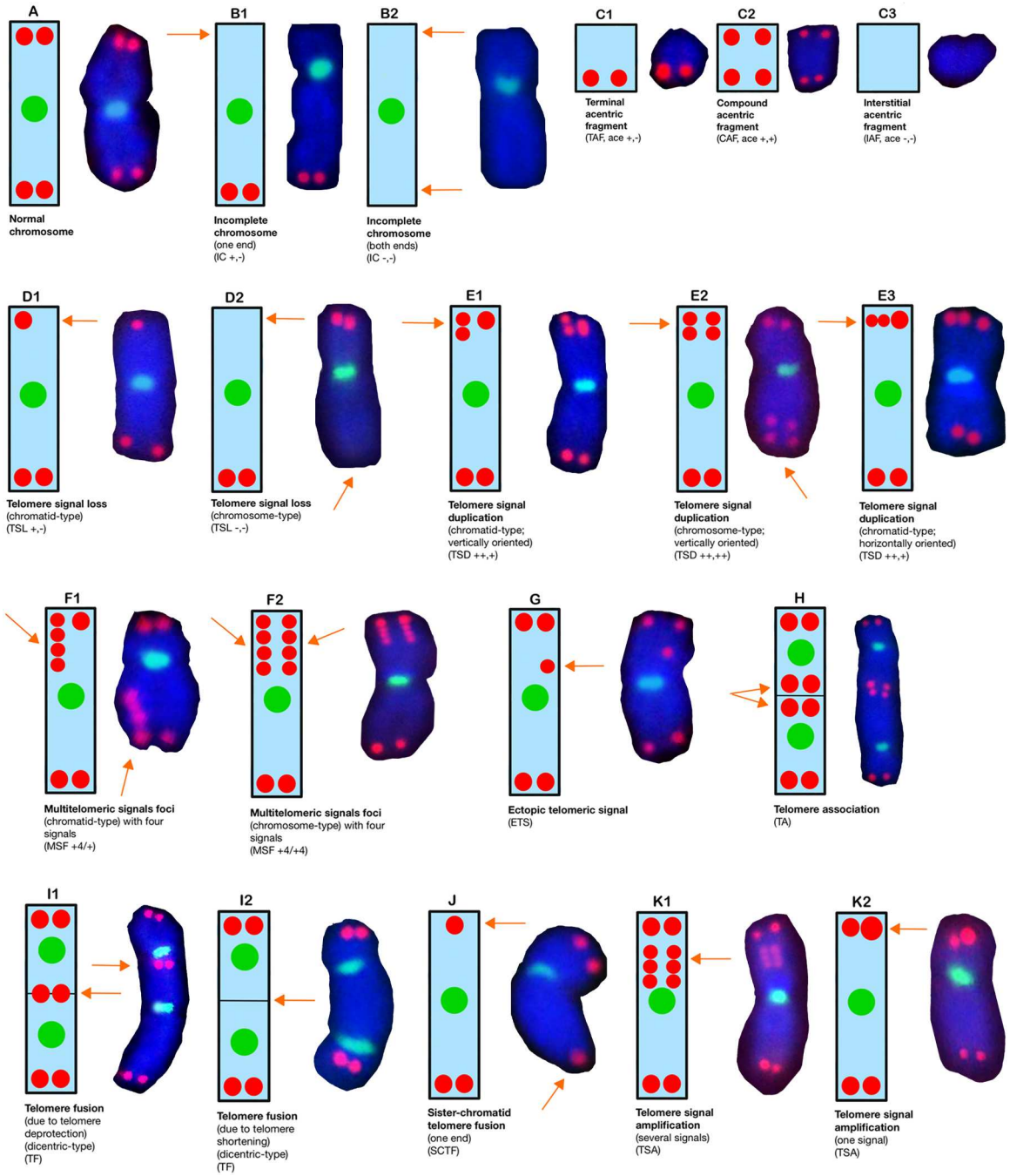
signals is directly correlated to the length of the telomeres [25,29–31]. Therefore, use of this type of probe can identify chromosomal aberrations involving telomeres as well as determine the telomere length of the sample. Telomere FISH signals correspond exclusively to clusters of telomeric DNA, constituted by a variable number of telomeric repeats, usually located at the ends of the chromosomes. To make the identification of chromosomes and telomere aberrations more accurate, when available, a PNA pancentromeric probe (usually labeled with FITC or FAM) is used simultaneously with the pantelomeric probe. The use of a pancentromeric probe is not always necessary, because in some cases 4′, 6-diamidino-2-phenylindole (DAPI) counterstain is sufficient to detect the centromeres of the chromosomes. Nevertheless, centromere detection with DAPI is not always 100% accurate, so, when available, the pancentromeric probe must be used to detect the centromeres. Therefore, depending on the sample type, telomere aberrations are detected by telomere or telomere plus centromere PNA-FISH [4,5]. For human and mouse, a pancentromeric PNA probe is commercially available from Panagene (Daejeon, Korea). For other species this probe can be synthesized on demand from several suppliers such as Panagene (Daejeon, Korea), PNA Bio (Thousand Oaks, CA, USA) or BOC Sciences (Shirley, NY, USA), if the relevant centromeric sequence is known. It is important to mention that several vertebrate species contain the so-called interstitial telomeric sequences (ITS) [26,32], which also hybridize with the telomeric probes. In these cases, the analysis of telomere aberrations should be performed with caution. This is the reason why, for a more accurate analysis of telomere aberrations, the better choice is a species or cell type with a telomere-only pattern of distribution of telomeric sequences in metaphase chromosomes after PNA-FISH (human lymphocytes, human fibroblasts, primary cells of rats, etc.).

Normally, after PNA-FISH, each chromosome at metaphase exhibits four telomeric signals, two at each end (each signal corresponding to a cluster of telomeric repeats), and one centromeric signal (Fig. 2A). In this way, by using telomere plus centromere PNA-FISH, a dicentric chromosome is detected at metaphase as a chromosome exhibiting two centromeric signals and four telomeric signals, and a centric ring as a ring chromosome with a centromeric signal and no telomeric signals. However, telomere and centromere staining allows not only the accurate and rapid identification of dicentric and ring chromosomes, but also the detection of the different types of acentric fragments (AFs) and the above mentioned telomeric aberrations [4,5]. Thus, PNA-FISH can be used for improved detection of unstable chromosomal aberrations in vertebrate cells, including those involving telomeric sequences [4,5,33–43]. In this way, short- and long-term chromosome damage involving telomeres induced by mutagenic agents like ionizing radiation and chemotherapeutic drugs can be determined in exposed cells, individuals, or populations. This is particularly valuable for cancer patients or individuals occupationally or accidentally exposed to radiation (for biodosimetry studies), to monitor the follow-up of exposed individuals and to predict the efficacy of chemo- and radiotherapies at the chromosome level [36–39,44,45].

For the above reasons, the use of PNA-FISH to detect chromosomal aberrations involving telomeres has become a powerful tool for detecting telomere aberrations and a common practice in several laboratories [33–43,46–48]. Despite increasing use of PNA-FISH, a review of the literature identified several inconsistencies and mistakes in the scoring and/or the nomenclature used for the detection of telomere aberrations. Therefore, in this review I will briefly discuss each type of chromosomal aberration involving telomeres (as identified by PNA-FISH in metaphase chromosomes of vertebrates). In addition, I will consider several issues regarding the scoring of these aberrations, point out common mistakes and inconsistencies to avoid in order to ensure a proper scoring, and provide proper terminology and criteria for the scoring, identification, and analysis of these aberrations. For a detailed review regarding the different types of telomere aberrations considered here, the reader is referred to works by Bolzán [4,5], Bolzán and Bianchi [49] and Cherdyntseva and Gagos [50].

2. Telomere aberrations

The two main types of telomere aberrations that can be distinguished by PNA-FISH are those that involve the loss of the chromosome ends (one or both), and those that involve dysfunctional telomeres (Fig. 1) [4,



(caption on next page)

Fig. 2. Schematic representation (left) and FISH images (right) of telomere aberrations as seen in a metaphase cell after telomere plus centromere PNA-FISH identified with appropriated nomenclature. FISH images correspond to metaphase chromosomes from a lymphoblastoid human cell line usually employed in the author's laboratory. Red signals correspond to telomere probe, green signals to centromere probe and blue color to DAPI counterstain). FISH images are for reference only and do not necessarily correspond exactly to the schematic representation of each aberration. In each scheme, red/pink dots represent the telomeres (telomeric FISH signals), green dots represent the centromere (centromeric FISH signal), and the light blue color represent the rest of the chromosome (DAPI counterstain). Orange arrows indicate the site of the aberration. The presence (and the number) or absence of telomeric signals at the aberration site is indicated by + or – sign, respectively. Please, note that only representative cases of each type of aberration are shown, and that one or more telomeres can be involved in the aberration. See Section 2.1 of the present review and [4,5] for further details. (A) **normal chromosome**, showing four telomeric signals and one centromeric signal; (B) **incomplete chromosomes (IC)**, formed after a breakage event which occurred at one (B1) or both (B2) ends of a chromosome at interphase (accompanying acentric fragments not shown); (C) **acentric fragments (ace)** (lacking centromeric signal): terminal fragment (**TAF**) (C1), derived from an incomplete chromosome lacking one end; compound fragment (**CAF**) (C2), formed by the fusion of two terminal fragments, and interstitial fragment (**IAF**) (C3), lacking telomeric signals (IAF are not considered telomere aberrations, but can be detected using telomere plus centromere FISH); (D) **telomere signal loss (TSL)** at one end (chromatid- and chromosome-type, i.e., one (D1) or both (D2) telomeric signals lost, respectively); please, note that the hybridization pattern depicted in Fig. D2 is the same as in Fig. B2, but no associated TAF or CAF is present in the cell; (E) **telomere signal duplication (TSD)/telomere doublet (TD)/doublet telomere signal (DTS)** at one end (chromatid- and chromosome-type, i.e., one (E1) or both (E2) telomeric signals duplicated, respectively), with telomere signals vertically oriented; a telomere signal duplication (chromatid-type) with fused signals is also shown (E3); (F) **multitelomeric signals foci (MSF)**, resulting from a telomere signal split into three or more signals (in this case, four signals: F1, chromatid-type multitelomeric signals foci; F2, chromosome-type multitelomeric signals foci; please, note that **MSF** usually involve only one telomere and, in some cases, the telomere is so fragmented that the exact number of telomeric signals cannot be determined and some signals may appear outside the chromosome end); (G) **ectopic telomeric signal (ETS)**, a single telomere signal located far from the chromosome end, which may arise as a *de novo* (additional) telomeric signal or as a consequence of the translocation of a telomere signal from a chromosome end to another chromosome (see text for details); (H) **telomere association (TA)** (four telomeric signals, two of each belonging to a different chromosome); (I1) **telomere fusion (TF)** due to telomere deprotection (this type of telomere fusion gives rise to a dicentric chromosome with interstitial telomeric signals at the site of fusion); (I2) **telomere fusion (TF)** due to telomere shortening (this type of telomere fusion gives rise to a dicentric chromosome without interstitial telomeric signals at the site of fusion); (J) **sister-chromatid telomere fusion (SCTF)**, which exhibits a single telomere signal at the fusion point; (K) **telomere signal amplification (TSA)**, manifested as an increase in the number of telomeric signals in the involved chromosome, resulting in several interstitial telomeric signals (K1), or an increase in the size of the telomeric signal (K2). Please, note that **extrachromosomal telomeric signals** (**ECTS**, located outside the metaphase chromosomes) were not included in this figure.

5]. In the first case, telomeric sequences are not directly involved in the aberration because it is the chromosome end itself that is lost due to chromosome breakage. In the second case, the aberration directly affects the telomeric sequences present in the chromosome/s exhibiting the aberration/s. Because telomere aberrations have been described in detail previously [4,5,49,50], here I will briefly refer to each type of aberration to give a proper background for the reader to understand the common mistakes and inconsistencies found in the literature regarding the identification, scoring, and analysis of telomere aberrations. I will also analyze the terminology used for the identification of telomere aberrations and propose a proper nomenclature system for the scoring of these aberrations.

2.1. Types of telomere aberrations and common mistakes regarding their identification, scoring, and analysis

2.1.1. Telomere aberrations involving the loss of the chromosome ends

Telomere aberrations involving the loss of the chromosome ends are known as incomplete chromosome elements (ICE) [27], since they arise from breakage at terminal regions of the chromosomes and thus exhibit "open" ends, and comprise those chromosomes lacking one or both ends (centric ICE, also referred to as "incomplete chromosomes" or IC) and their accompanying acentric fragments (also referred to as acentric ICE, which can be terminal or compound) [4,5,27,34,40,49]. ICE can give rise to the so-called breakage-fusion-bridge (BFB) cycles. This occurs when, after breakage and loss one chromosome end, chromosomes repeatedly fuse and break for many cell generations, thus generating chromosomal instability [4,5,11]. At metaphase, an IC exhibits one centromeric signal and two telomeric signals (if only one chromosome end is lost) or no telomeric signals (if both ends are lost) (Fig. 2B1 and 2B2, respectively). A terminal acentric fragment (TAF) possesses two telomeric signals, whereas a compound acentric fragment (CAF) possesses four telomeric signals, since it is the result of the fusion of two TAF (Fig. 2C1, and 2C2, respectively). Although a TAF could also be scored as a telomeric DNA-containing double minute chromosome (TDM) (extrachromosomal elements described by Zhu et al. using telomere PNA-FISH in metaphase spreads of immortalized ERCC1$^{-/-}$ mice embryonic fibroblasts [51]), the TAF would have to be very small to be misclassified as a TDM. A TAF cannot be considered an extrachromosomal element because it derives from an IC.

It is important to mention that ICE may also include those dicentric or multicentric chromosomes lacking one or both ends (i.e., accompanied by one or two TAF and the corresponding CAF resulting from the formation of the dicentric), and those ring chromosomes accompanied by two TAF (instead of a CAF, as in the case of a canonical ring chromosome) (figures not shown) (see [4,5,40,49,52] for details of the different types of incomplete dicentrics and rings). In addition, to be considered as an ICE, the chromosome element must have at least one open end (i.e., to be incomplete). Thus, only incomplete chromosomes (with one or more centromeres) and terminal fragments must be considered truly ICE. Accordingly, Pujol-Canadell et al. [40] consider CAFs as complete chromosome elements. However, CAFs (Fig. 2C2) must be included in the group of those aberrations derived from the loss of the chromosome ends (Fig. 1). For scoring purposes, the presence of telomeric signals in an ICE is recorded as (+), whereas the absence of telomeric signals is symbolized as (−) [4,5,40]. Thus, for example, an IC lacking one end is recorded as IC+/ - or IC+, −, whereas a TAF is recorded as ace (+,-), and a CAF as ace (+,+), "ace" means acentric fragment [4,5,40].

In metaphase cells containing all centromeres and telomeres, ICE are usually observed in pairs, namely, an IC plus a TAF, two IC plus a CAF, or two TAF accompanied by a conventional dicentric or centric ring [53]. Nevertheless, other combinations are possible: an incomplete dicentric or multicentric chromosome accompanied by one or two TAF or one CAF, an IC accompanied by one CAF or two TAF, etc. (see [4,5,49] for details). Those AFs not accompanying the formation of dicentrics or rings are termed "in excess" AFs, which might arise either from a complete exchange [an intra-arm intrachange, leading to an interstitial deletion or interstitial acentric fragment or IAF, represented as ace (-/- or -,-)] (Fig. 2C3) or an incomplete exchange or breakage (terminal deletions, leading to TAF or CAF), and their scoring provides additional information regarding chromosomal incompleteness [4,5,53]. It is important to highlight that IAFs are not considered as a form of ICE, because previous evidence strongly suggests that the majority of these fragments form a ring structure, without open ends (the so-called acentric rings or double minute chromosomes) [54].

Pujol-Canadell et al. [40], classify "in excess" AFs as "extra chromosome pieces", a terminology which could be misleading, because this expression does not necessarily refer to AFs (for example, extra chromosomal telomeric sequences [55,56] could also be considered as extra

chromosome pieces), and does not allow to distinguish *a priori* between the different types of AFs induced by a given mutagen. Also, the same authors describe incomplete and complete chromosome elements, including in the latter CAF and dicentrics without open ends (i.e., complete dicentrics). In fact, all the chromosomal aberrations without open ends must be considered as complete aberrations.

Another important issue regarding the scoring of ICE corresponds to AFs. An AF is, by definition, a chromosome fragment without centromere, derived from one or more breakage events occurring in one or two chromosomes at their ends, so the use of the expression "acentric chromosome" [38] when scoring AFs is inappropriate. In fact, a chromosome without a centromere cannot be considered as such. Therefore, the different types of acentric fragments showing no centromeric signals should be identified based on telomere or telomere plus centromere FISH, namely as CAF (with telomeric signals at both ends), TAF (with telomeric signals at one end) or IAF (without telomeric signals) (see Fig. 2C1–3). Moreover, chromatid-type AFs can also be formed if only one of the chromatids is affected by the breakage event (which occurs in the G2 phase of the cell cycle; figures not shown).

Finally, it is important to mention that a few authors refer to IC as a "deleted chromosome" [57]. This terminology is confusing, because it does not identify which part of the chromosome is missing (one end, both ends, an interstitial region or the entire chromosome?). In fact, in the case of an IC, it is not the chromosome itself which is lost or deleted, but one or two of its ends instead. Therefore, a chromosome without telomeric signals at one or both ends, accompanied by one or two TAF (or a CAF), respectively, must always be designated as "incomplete chromosome" or IC. We must bear in mind that the presence of the accompanying AFs is the only way to confirm that a suspected IC is truly an IC. Otherwise, the breakage event/s at terminal regions of the chromosome which originate/s the IC cannot be established. In the absence of a detectable AF, the observed aberration should be considered a telomere (signal) loss instead of an IC (see Section 2.1.2.1).

2.1.2. *Telomere dysfunction-related aberrations*

Telomere aberrations due to telomere dysfunction include telomere signal loss, telomere signal duplication, telomere association, telomere fusion, telomere recombination, and telomeric repeats translocation or amplification (see [4,5,49] for details). The common feature of these aberrations is that telomeric sequences are directly involved in the aberration. Telomere signal loss and duplications and fused telomeres have been scored as an indication of telomere fragility (i.e., telomere prone to breakage) [58]. However, fragile telomeres, as defined by Sfeir et al. [59], are those chromatid ends having multiple telomeric signals. In addition, these authors observed that, in some cases, the multiple signals were spatially separated from the chromatid end, suggesting that the telomeric DNA failed to condense or was broken. Therefore, only telomere signal duplication can be considered a telomere aberration implying telomere fragmentation (as shown in various images within figure 2A from ref. [59]). In this context, telomere signal loss could be due to the induction of single strand breaks at telomeres, leading to replication fork collapse and telomere loss [60] (see also figure 2 from ref. [61]). Alternatively, telomere signal duplication (also referred to as telomere doublets [38,50]) and other aberrations manifested as multi-telomeric foci at chromatid ends could be due to lesions that impede telomere replication and cause an accumulation of unrepaired single strand breaks, leading to fragile telomeres [59] (see also figure 2 from ref. [61]).

Telomere associations and fusions are usually considered indicators of telomere shortening [62,63]. However, telomere dysfunction is not always due to telomere shortening (see the Introduction section above) [3,6,9,10,12–24]. Therefore, the presence of a telomere association or fusion in a cell may be due to a phenomenon other than telomere shortening. Telomere fusions can give rise to the above mentioned BFB cycles, generating chromosomal instability [4,5,11]. Moreover, canonical dicentric chromosomes, resulting from the end-to-end fusion of two chromosomes with open ends, cannot be considered as aberrations indicating telomere fragility [58], because no fragile telomeres are involved in the aberration.

2.1.2.1. *Telomere (signal) loss.*

Telomere loss refers to the absence of the telomeric FISH signal at one or both ends in one or both chromatids (Fig. 2D) [4,5]. Therefore, after PNA-FISH, telomere loss can be defined as a chromosome end lacking telomeric signals or a chromosome end with undetectable telomeric signals (one or both). If one telomeric signal is lost or absent, the aberration is considered a chromatid-type telomere loss (Fig. 2D1). If both telomeric signal are lost or absent, the aberration is considered a chromosome-type telomere loss (Fig. 2D2). In addition, because is not the telomere itself that is lost in a telomere loss event, but a significant amount of telomeric repeats needed to be detected by PNA-FISH instead (i.e., telomere length less than 1 Kb; see ref. [31]), the appropriate terminology for a telomere loss should be "telomere signal loss" (this also applies to telomere duplications; see Section 2.1.2.2) [50].

A few authors refer to telomere "loss" when only one signal is absent at the chromosome end, and telomere "deletion" when both telomeric signals are lacking (for example see ref. [38, figure 3c]). This is confusing, because in both cases telomeric repeats (in the form of a telomere FISH signal) are lost. Telomere loss has also been named "telomere-free end" (see [64] figure 2c) and "telomeric-signal-free chromosome end" (see [41,50] figure 1A-c), but I believe that the latter expression is more appropriate than the former, because, as pointed out before, a telomere loss event implies that the affected chromosome end lacks telomeric DNA repeats in an amount sufficient to become undetectable by PNA-FISH, not the loss of the telomere itself. In addition, the expression "telomere or telomeric-signal-free chromosome end/s" [41,50] does not distinguish between chromosome- and chromatid-type telomere signal loss. Therefore, to avoid confusion, I propose that those aberrations implying the loss of telomeric repeats to an extent that cannot be detected by PNA-FISH, should be named chromatid- or chromosome-type telomere signal loss, as previously reported [4,5]. In addition, the distinction between chromatid- and chromosome-type telomere signal loss clarifies whether one or two telomere signals are lost in the chromosome ends involved in the aberration, and whether telomere attrition (visualized as telomere signal loss) occurred during the G1(chromosome-type) or the G2 (chromatid-type) phases of the cell cycle.

On the other hand, for a proper scoring of telomere signal loss, all the chromosomes in the metaphase should be analyzed, to exclude the presence of AFs (TAF or CAF), indicative of ICE instead of telomere signal loss. As indicated before, telomeres undetectable by FISH could be due to the loss of the chromosome end (with a TAF accompanying the abnormal chromosome) or the loss of telomeric repeats (no signals and no accompanying acentric fragment). It is worth mentioning that when scoring telomere PNA-FISH signal loss even the weakest telomeric signals must be scored, because every signal, no matter its size, is indicative of the presence of telomeric repeats. These signals could be visualized after amplifying the fluorescence intensity in the captured images. This is the reason why the presence of telomere signals of different intensity at one end should not be scored as a telomere signal loss (or even worse, "sister telomere loss", as reported in ref. [65]). Loss of intensity in a telomere signal is not the same as (complete) loss of the signal. For scoring purposes, it makes no sense to distinguish between a strong and a pale telomeric signal, because signals with different degrees of intensity are usually found in each sample/metaphase. This depends on several factors, such as the image capture system employed, the FISH protocol applied, the software used for image analysis, the heterogeneity in telomere length between different chromosomes or even between sister chromatids, etc. Although telomere signals of different intensity at the same chromosome end could be an indication of telomere length differences between chromatids, for a proper evaluation of telomere

shortening by using PNA-FISH telomere probes, the telomere length of the samples being analyzed must be determined using the Q-FISH technique [4,5,31].

2.1.2.2. Fragile telomeres. Telomere signal duplication. Extrachromosomal telomeric sequences. Ectopic telomeric signals.. Fragile telomeres are detected by telomere PNA-FISH as a telomere showing two or more signals at one or both chromatids. This results in telomere signal duplication, triplication, quadruplication, etc. (Figs. 2E and 2F). Accordingly, when scoring fragile telomeres, the number of telomere signals in the affected chromosome end must be reported (two, three, four, etc.). Particularly, when the telomeric signal is split into two signals, this aberration is referred to as a telomere signal duplication, which can be of chromatid- or chromosome type, depending on whether the signal duplication affects one (Fig. 2E1) or both (Fig. 2E2) chromatids, respectively [4,5,49,52]. In addition, duplicated telomeric signals can be horizontally (Fig. 2E3) or vertically (Fig. 2E1 and 2E2) oriented in the chromosome end (affecting one or both chromatids). Telomere signal duplication has been also termed "telomere doublet" [38,50,61] (for example see ref. [36, figure 3c]) or "multitelomeric foci" [61] or "multitelomeric signals" [66], because this type of aberration implies the presence of multiple telomeric signals, due to telomere fragility [59]. Although all the above-mentioned aberrations correspond to a fragile telomere, the nomenclature "telomere signal duplication" or "telomere doublet" should be restricted to those chromosomal aberrations where only two telomere signals are present, one close to the other, whereas the term "multitelomeric foci" or, more precisely "multitelomeric signals foci" must be restricted to those aberrations where the telomere signal is comprised of three or more signals (usually three). In this case, the number and distribution of signals must be recorded, because it gives a proper idea of how fragile the telomere is and whether the signals are distributed in one (chromatid-type multitelomeric signals foci, Fig. 2F1) or both chromatids (chromosome-type multitelomeric signals foci, Fig. 2F2). Therefore, telomere signal duplications, being usually more frequently observed than telomeric signals split in three or more signals, should not be scored as "multitelomeric foci" or "multitelomeric signals", as reported elsewhere (see [66] figure 2C, which includes a telomere signal duplication as a "multitelomeric signal" aberration). Fragile telomeres can sometimes give rise to telomeric FISH signals located outside chromosome(s) (i.e., extrachromosomal telomeric signals), likely due to excessive telomere fragmentation of telomeric DNA [55,56,59]. Extrachromosomal telomeric DNA has been observed in cells from *Atm*$^{-/-}$ mice and patients with ataxia telangiectasia [55,67], in *Atm*$^{-/-}$ mouse embryo cells after X-rays exposure (a phenomenon observed up to many cell divisions post-irradiation) [56], and in mouse embryonic fibroblasts lacking TRF1 [59].

On the other hand, fragile telomeres, no matter the number of telomeric signals involved, should not be termed "extra telomere signals" nor categorized as "abnormal telomere signals" [56,68], because an ectopic telomeric signal (see below) is also an "extra telomere signal" and it is not the signal itself, but its number that is affected in a fragile telomere. In addition, telomere signal duplications must not be confused with ectopic telomeric signals, as showed in Lacoste et al. [64] (figure 2e; in fact, this figure shows some examples of true ectopic telomeric signals mixed with telomere signal duplications). If a chromosome end exhibits two telomeric signals with one of them close to the other in one or both chromatids, the aberration corresponds to a telomere signal duplication, not an ectopic telomeric signal. An ectopic telomeric signal is one located between the telomeric and the centromeric region of the chromosome and far from the chromosome end, which is the proper location of a true telomeric signal (Fig. 2G). Usually, ectopic signals (one or more) appear in one chromatid (Fig. 2G), but sometimes they can be present in both chromatids (figure not shown). Ectopic telomeric signals may arise as *de novo* telomeric signals (giving rise to additional intrachromosomal telomeric signals in the metaphase) or as a consequence of the translocation of a telomere signal from a chromosome end to an interstitial location of another chromosome of the same metaphase. In the latter case, the metaphase does not exhibit additional telomeric signals, and the telomere signal translocation event is accompanied by a chromosome lacking a telomeric signal present in the same metaphase (the original location of the translocated telomeric signal). Clearly, fragile telomeres, including telomere signal duplications (two signals) and multitelomeric signals foci (three or more signals), must be scored separately from ectopic telomeric signals.

Although the molecular nature of fragile telomeres is not yet well understood, several authors proposed that they may result from inadequate telomere DNA replication (due to the formation of single strand breaks or other lesions that stall replication fork progression, causing an accumulation of unreplicated single stranded DNA), induced by oxidative damage [61], X-rays [68], aphidicolin (an inhibitor of DNA replication) [59,68], ATM deficiency [56], or the loss of shelterin proteins like TRF1 [59] or helicases like BLM and RTEL1 [59]. Alternatively, it was recently proposed that telomere fragility arises from break induced replication (BIR) and alt-NHEJ of the cleaved end after BIR [69]. Because the appearance of the multitelomeric signals depends on the quality of the FISH image obtained and sometimes the telomeric signals appear overlapped or fused, the analysis of the different telomere aberrations involving fragile telomeres should be made with caution and using good quality images.

2.1.2.3. Telomere association. After telomere or telomere plus centromere PNA-FISH, a telomere association is detected by the presence of four telomeric signals, each pair belonging to one end of two different chromosomes, which are very close to each other but do not fuse [4,5,49,52] (Fig. 2H). Telomere associations are assumed to be the result of telomere shortening or attrition [4,5,62,63]. Telomere associations (four telomere FISH signals) should not be confused with telomere fusions (two telomere FISH signals, see below), as reported elsewhere [58]. Alternatively, a telomere association can be present in the affected cell as a ring-like chromosome with four telomeric signals, since the telomeres of both chromosome arms are not fused but appear very close one to each other (figure not shown) [4,5].

2.1.2.4. Telomere fusion. Telomere dysfunction is usually expressed as telomere fusion [4,5,70,71], a type of aberration consisting of a dicentric chromosome with a double interstitial telomeric signal located at the fusion point (Fig. 2I1; see also [64] (figure 2a), and [72] (figure 3)). This is because the telomeres of two different chromosomes have fused into two telomeric signals, one per chromatid [4,5,49,52]. Alternatively, a telomere fusion can be present in the form of a true ring chromosome with interstitial telomeric signals (figure not shown) [4,5]. In addition, telomere fusions can be of chromatid-type (forming a "chromatid dicentric"), if just one chromatid of each chromosome is involved in the fusion event (figure not shown), although this is a rare event ([4,73] figure 1).

In a few cases, dicentric chromosomes resulting from telomeric fusions do not show telomeric FISH signals at the fusion site because the fused telomeres contain a very low number of telomeric sequences to be detectable by PNA-FISH (i.e., they arise from telomere shortening, giving rise to critically short telomeres) (Fig. 2I2). However, even in this case, a telomeric fusion can be easily distinguished from a canonical dicentric because no associated acentric fragment (i.e., one CAF or, less frequently, two TAF) is present in the metaphase with the aberration, as is the case of canonical dicentric chromosomes. This is because a true telomere fusion results from a dysfunctional telomere, not from a breakage event [4,5,49,52]. Therefore, a canonical dicentric (a chromosome with end-to-end fusion and no telomeric signals at the fusion site) is not a telomeric fusion and should not be scored as a type of telomere aberration, as reported elsewhere [58]. Thus, when scoring dicentrics, the presence or absence of the corresponding CAF or TAF

should be confirmed; otherwise, the dicentric chromosome corresponds to a true telomere fusion (with or without telomeric signals at the fusion site).

Telomere fusions are usually the result of telomere shortening, but this is not always the case, as dysfunctional telomeres may arise from causes other than telomere shortening (as discussed in the Introduction section of this review). Furthermore, for a proper discrimination of the different types of telomere fusions (i.e., telomere-telomere or telomere-DSB fusion), the Chromosome Orientation FISH (CO-FISH) technique must be applied (see [4,5,70] for details).

Beyond the classic telomere fusions, a type of telomere aberration named "sister-chromatid telomere fusion" or "sister-telomere chromatid fusion" has been described in some cells. This type of aberration involves a single chromosome and is manifested as a single telomere FISH signal located at the center of the chromosome ends (one or, less frequently, both), resulting from the fusion of the sister chromatids and their corresponding telomeric signals ([50], figure 1A-e; [64], figure 2b) (Fig. 2J). However, at least in a few cases, this type of aberration should be considered a chromatid-type telomere signal loss instead of a true sister-chromatid telomere fusion. This is because sometimes the fusion of the chromatids is not clearly visible (even under DAPI counterstain), the telomeric FISH signal size does not correspond to the size of two merged signals, or the telomeric signal is not precisely located at the center of the chromosome end [50,64]. To be considered as a true sister-chromatid telomere fusion, the chromatids of the affected chromosome end must be fused and the telomere signal (resulting from the fusion of both telomeric signals) must be at the center of the chromosome end. Therefore, the scoring of this type of aberration should be made with caution. In addition, the usage of the term "sister chromatid fusion" to describe this type of aberration as reported elsewhere [50,64] is inaccurate, because it does not include the term "telomere", although it is classified as a telomere aberration. Interestingly, the question as to whether this type of aberration should be considered a telomere aberration or not remains because it is not possible to determine if the chromatids are fused as a result of a true telomere fusion or if the telomere signal fusion is simply a consequence of the fusion of the chromatids.

2.1.2.5. Telomere signal amplification. Telomere signal amplification occurs when telomeric FISH signals increase in size (Fig. 2K1), number (more than two signals, appearing as interstitial telomeric sequences, vertically oriented in the chromosome) (Fig. 2K2) or intensity in relation to the typical hybridization pattern of the cell type being analyzed. Sometimes, the amplification of telomeric repeats extends to an entire chromosome arm or along the entire chromosome. These features can distinguish between multitelomeric foci (fragile telomeres) and telomere signal amplification events, since multitelomeric foci are constituted by telomeric signals of similar size and intensity and/or are not dispersed along an entire chromosome or chromosome arm, as is the case of a telomere signal amplification. Telomere signal amplification arises by unequal sister-chromatid exchange, breakage-fusion-bridge cycles, or excision and reintegration events (the so-called "rolling circle" mechanism) [4,5,11]. Therefore, the amplification of telomeric sequences must not be confused with telomere fragility. Also, telomere signal amplification events should be scored with caution, using good quality images, because otherwise they could be confused with artifacts due to image capturing.

2.1.2.6. Telomere sister-chromatid exchanges (T-SCEs). T-SCEs are recombination events at telomeres and imply an exchange of telomeric DNA between sister chromatids [4,5,49,50,52]. Because this type of telomere aberration cannot be identified using conventional telomere or telomere plus centromere PNA-FISH (rather is detected only using CO-FISH), it will not be considered here (see [4,5,50,70] for details).

3. Number of cells to be analyzed and other considerations for the scoring of telomere aberrations

Even though at least a hundred metaphases are usually scored for chromosomal aberrations induced by a given mutagen, in our recent experience, fifty metaphase cells are enough to determine the frequency of telomere aberrations in a sample (Bolzán, unpublished data), as also reported by others [42,51,68]. In fact, a few recently published papers report the analysis of just or about twenty metaphase spreads per slide for the scoring of telomere aberrations, but in these cases the scoring is limited to telomere-dysfunction related aberrations, and ICE are not included in the scoring [41,58]. Despite the above considerations, when analyzing samples from cells or individuals chronically or acutely exposed to very low doses of ionizing radiation or very low concentrations of a chemical mutagen, it could be necessary to score several hundreds of metaphases to detect statistically significant differences in the frequency of telomere aberrations between control and treated samples, as is usually done when scoring unstable chromosomal aberrations by Giemsa staining or chromosomal aberrations by whole chromosome painting. Despite the above observations, the number of metaphases to be analyzed should be based on power calculation, taking into account the basal frequency of telomere aberrations (in control cells or individuals, without treatment), and the frequency of these aberrations in the exposed or treated cells.

Telomere aberrations are usually scored as frequency per cell [41,42,46–48,58]. Alternatively, when cells exhibit different numbers of chromosomes, telomere aberrations can be expressed as aberrations per chromosome. In the particular case of telomere signal loss, some authors prefer to report the loss of telomeric signals through the measurement of the "mean telomere signal count" instead of the frequency of (chromatid- and chromosome-type) telomere signal loss per cell [43]. Furthermore, when scoring telomere signal loss or duplications (and other forms of telomere fragility), each chromosome end must be scored separately, because one or both ends of the chromosome can be affected. In other words, the same chromosome can have one or two chromosome- and/or chromatid-type telomere signal loss or duplication/multitelomeric signals or a combination of both types of aberrations.

On the other hand, whenever possible, when scoring telomere aberrations, the absence of a telomeric signal should be indicated by the – symbol, whereas the presence of a telomeric signal should be indicated by the + symbol (see Fig. 2).

Finally, good quality FISH images (neither too bright, nor too dark, and with low background) should be used when analyzing telomere aberrations because, for a proper scoring, all telomeric signals should be clearly seen. In addition, because Cy3 can bind to tiny dust particles on the slide, when using a Cy3-labeled telomeric probe, PNA-FISH must be performed on carefully pre-cleaned slides to avoid artifacts and a high background, which seriously impairs the hybridization and analysis of the aberrations.

4. Scoring of telomere aberrations by PNA-FISH: concluding remarks

The analysis of telomere instability produced by genotoxic agents in vertebrate cells contributes to a better understanding of genomic instability, specifically that observed in immortalized cell lines and in individuals chronically or acutely exposed to ionizing radiation or chemical mutagens [4,5,37–39]. As previously reported, physical (ionizing radiation), chemical (mainly anticancer drugs), and biological (viruses) mutagens can induce telomere aberrations in vertebrate cells (see [4,5,74] for review). The increasing use of the PNA-FISH technique to analyze telomere aberrations, requires an appropriate identification and scoring system established for these aberrations. The scoring, identification and analysis issues reviewed here regarding telomere aberrations are of paramount importance for achieving proper

Table 1
Probable causes and potential consequences of the formation of each type of telomere aberrations. The ultimate consequences of telomere aberrations can be cellular senescence, cell death or cellular transformation (the latter leading to cancer development), depending on the frequency and the severity of aberrations. See text and reference list for more details.

Aberration	Causes	Consequences
Incomplete chromosome elements	Chromosome breakage at terminal regions due to exposure to physical, chemical or biological mutagens which induce DSBs.	Chromosomal/genomic instability due to the loss of the chromosome end/s and the subsequent formation of BFB cycles.
Telomere signal loss	DNA lesions that block replication fork progression (replication stress). Mutagens which cause oxidative stress and telomere shortening, etc., can cause telomere signal loss.	Telomere shortening and subsequent telomere and chromosomal instability.
Telomere signal duplication / Multitelomeric signals foci	Lesions that impede telomere replication and cause accumulation of unreplicated single stranded DNA due to replication fork stalling (replication stress). DSBs due to radiation exposure, loss of telomeric proteins (TRF1 and others), etc., can lead to these types of aberrations.	Telomere fragility and subsequent chromosomal instability.
Extrachromosomal telomeric signals	ATM/Atm deficiency, TRF1 loss, etc.	Telomere DNA fragmentation and subsequent chromosomal instability.
Ectopic telomeric signals	Telomere fragility (due to several causes).	Chromosomal instability due to telomeres located outside chromosome ends.
Telomere association	Telomere shortening (caused by oxidative stress or other factors).	Chromosomal instability.
Telomere fusion	Telomere shortening or deprotection (due to loss or alteration of telomeric proteins, DDR proteins, telomeric RNA, etc.).	Chromosomal/genomic instability (formation of BFB cycles).
Sister-chromatid telomere fusion	Telomere shortening or deprotection (not clear at present).	Chromosomal instability due to the fusion of sister chromatids or telomeres.
Telomere signal amplification	Unequal sister-chromatid exchange, BFB cycles, “rolling circle” mechanism, etc.	Chromosomal instability due to the presence of altered telomeric sequences (in size or number).

cytogenetic analysis of telomere damage in vertebrate cells, particularly human cells. Recent studies using telomere plus centromere PNA-FISH demonstrate the importance of using this technique to analyze the chromosomal instability in human cells [37–43]. In addition, telomere plus centromere PNA-FISH can be complemented with multicolor or multiplex FISH (M-FISH) to study chromosome damage in cancer patients, thereby improving diagnosis for these patients [38,39]. Undoubtedly, the improvement of the FISH techniques and the combination of different molecular cytogenetic techniques will allow a better understanding of telomere aberrations in the near future. Of course, to fully understand the role of telomeres in overall effect on the genome, the scoring by PNA-FISH of telomere aberrations should be accompanied by other methodologies that characterize other parameters related to telomere dysfunction (absolute telomere length, telomerase activity, expression levels of telomeric proteins, etc.). I hope that the concepts expressed in the present review are useful to researchers and clinicians interested in performing analysis of telomere aberrations and will serve as a methodology guideline for future experimental work on these types of aberrations, and for accurately and consistently scoring of them. To help readers to design future experiments concerning telomere damage and the analysis of telomere aberrations, a summary of the probable causes and potential consequences of each type of telomere aberrations is presented in Table 1. Obviously, the direct consequence or biological impact of telomere aberrations is chromosomal/genomic instability, which can ultimately lead to cellular senescence, cell death or cellular transformation (the latter giving rise to cancer development), both depending on the frequency and the severity of the telomere aberrations [11,45]. In particular, severe telomere shortening or attrition (manifested as telomere signal loss) is related to cellular senescence and aging [6,8]. According to Boccardi et al. [65], fragile telomeres and sister telomere chromatid fusion (or sister-chromatid telomere fusion) could also be related to aging. Moreover, the role of telomere aberrations in the so-called “telomere syndromes” or “telomere biology disorders” [75] remains to be determined. It is important to highlight that present data indicate that there is no specific telomeric aberration type associated with a given mutagen, i.e., all types of physical, biological and chemical mutagens tested so far are capable of inducing the different types of telomere aberrations [4,5,74]. Future studies using telomere/telomere plus centromere PNA-FISH will be needed to clarify the causes and consequences of telomere aberrations formation, and to determine whether or not specific telomere aberrations can be used as biomarkers of genotoxic exposures, as previously suggested by Zeegers et al. [36] for chromosome ends without telomeres (i.e., chromosome-type telomere signal loss) induced by ionizing radiation in human peripheral lymphocytes.

Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by fundings from CONICET, CICPBA, and UNLP of Argentina. The author thanks MSc. Andrea Gabriela Cardozo (fellowship from CONICET, working in the author’s lab) for providing the FISH images used in Fig. 2.

This review is dedicated to the memory of Dr. Néstor Oscar Bianchi (1931–2023), one of the most eminent geneticists of Latin America, who passed away on February 4, 2023.

References

[1] E.H. Blackburn, Switching and signaling at the telomere, Cell 106 (2001) 661–673.
[2] J.W. Shay, W.E. Wright, Telomeres and telomerase: three decades of progress, Nat. Rev. Genet. 20 (2019) 299–309.
[3] Y. Doksani, The response to DNA damage at telomeric repeats and its consequences for telomere function, Genes 10 (2019) 318.
[4] A.D. Bolzán, Chromosomal aberrations involving telomeres and interstitial telomeric sequences, Mutagenesis 27 (2012) 1–15.
[5] A.D. Bolzán, Using telomeric chromosomal aberrations to evaluate clastogen-induced genomic instability in mammalian cells, Chromosome Res 28 (2020) 259–276.
[6] R.J. O’Sullivan, J. Karlseder, Telomeres: protecting chromosomes against genome instability, Nat. Rev. Mol. Cell Biol. 11 (2010) 171–181.
[7] I. Schmutz, T. de Lange, Shelterin, Curr. Biol. 26 (2016) R397–R399.
[8] J.W. Shay, Telomeres and aging, Curr. Op. Cell Biol. 52 (2018) 1–7.
[9] A. Cicconi, S. Chang, Shelterin and the replisome: at the intersection of telomere repair and replication, Curr. Opin. Genet. Dev. 60 (2020) 77–84.

[10] N. Arnoult, J. Karlseder, Complex interactions between the DNA-damage response and mammalian telomeres, Nat. Struct. Mol. Biol. 22 (2015) 859–866.
[11] J. Maciejowski, T. de Lange, Telomeres in cancer: tumour suppression and genome instability, Nat. Rev. Mol. Cell. Biol. 18 (2017) 175–186.
[12] S.R. Chan, E.H. Blackburn, Telomeres and telomerase, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 359 (2004) 109–121.
[13] E.H. Blackburn, Telomeres and telomerase: their mechanisms of action and the effects of altering their functions, FEBS Lett. 579 (2005) 859–862.
[14] S.E. Artandi, R.A. DePinho, Telomeres and telomerase in cancer, Carcinogenesis 31 (2010) 9–18.
[15] I. Chiodi, C. Belgiovine, C. Mondello, Telomerase and telomeric proteins: A life beyond telomeres, in: A.N. Gagnon (Ed.), Telomerase: Composition, Functions and Clinical Implications, Nova Science, New York, 2010, pp. 35-58.
[16] A. Romaniuk, A. Paszel-Jaworska, E. Totoń, N. Lisiak, H. Holysz, A. Królak, S. Grodecka-Gazdecka, B. Rubis, The non-canonical functions of telomerase: to turn off or not to turn off, Mol. Biol. Rep. 46 (2019) 1401–1411.
[17] C.M. Azzalin, J. Lingner, Telomeres: the silence is broken, Cell Cycle 7 (2008) 1161–1165.
[18] B. Luke, J. Lingner, TERRA: telomeric repeat-containing RNA, EMBO J. 28 (2009) 2503–2510.
[19] E. Cusanelli, P. Chartrand, Telomeric repeat-containing RNA TERRA: a noncoding RNA connecting telomere biology to genome integrity, Front. Genet. 6 (2015) 1–9.
[20] N. Bettin, C.O. Pegorar, E. Cusanelli, The emerging roles of TERRA in telomere maintenance and genome instability, Cells 8 (2019) 246.
[21] A. Chavez, A.M. Tsou, F.B. Johnson, Telomeres do the (un)twist: helicase actions at chromosome termini, Biochim. Biophys. Acta 1792 (2009) 329–340.
[22] K. Paeschke, K.R. McDonald, V.A. Zakian, Telomeres: structures in need of unwinding, FEBS Lett. 584 (2010) 3760–3772.
[23] E.J. Uringa, K. Lisaingo, H.A. Pickett, J. Brind'Armour, J.H. Rohde, A. Zelensky, J. Essers, P.M. Lansdorp, RTEL1 contributes to DNA replication and repair and telomere maintenance, Mol. Biol. Cell 23 (2012) 2782–2792.
[24] V. Popuri, J. Hsu, P. Khadka, K. Horvath, Y. Liu, D.L. Croteau, V.A. Bohr, Human RECQL1 participates in telomere maintenance, Nucleic Acid. Res 42 (2014) 5671–5688.
[25] P.M. Lansdorp, N.P. Verwoerd, F.M. van den Rijke, V. Dra- Gowska, M.T. Little, R. W. Dirls, A.K. Raap, H.J. Tanke, Heterogeneity in telomere length of human chromosomes, Hum. Mol. Genet. 5 (1996) 685–691.
[26] J. Meyne, R.L. Ratliff, R.K. Moyzis, Conservation of the human telomere sequence (TTAGGG)n among vertebrates, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 7049–7053.
[27] J.J.W.A. Boei, S. Vermeulen, J. Fomina, A.T. Natarajan, Detection of incomplete exchanges and interstitial fragments in X-irradiated human lymphocytes using a telomeric PNA probe, Int. J. Radiat. Biol. 73 (1998) 599–603.
[28] W. Deng, J.N. Lucas, Combined FISH with pan-telomeric PNA and whole chromosome-specific DNA probes to detect complete and incomplete chromosomal exchanges in human lymphocytes, Int. J. Radiat. Biol. 75 (1999) 1107–1112.
[29] U.M. Martens, J.M. Zijlmans, S.S. Poon, W. Dragowska, J. Yui, E.A. Chavez, R. K. Ward, P.M. Lansdorp, Short telomeres on human chromosome 17p, Nat. Genet. 18 (1998) 76–80.
[30] N. Serakinci, J. Koch, Detection and sizing of telomeric repeat DNA in situ. A performance comparison of PRINS and PNA assays, Nat. Biotechnol. 17 (1999) 200–201.
[31] S.S. Poon, U.M. Martens, R.K. Ward, P.M. Lansdorp, Telomere length measurements using digital fluorescence microscopy, Cytometry 36 (1999) 267–278.
[32] A.D. Bolzán, Interstitial telomeric sequences in vertebrate chromosomes: origin, function, instability and evolution, Mutat. Res. 773 (2017) 51–65.
[33] L. Benkhaled, L. Barrios, M. Mestres, M.R. Caballin, M. Ribas, J.F. Barquinero, Analysis of gamma-rays induced chromosome aberrations: a fingerprint evaluation with a combination of pan-centromeric and pan-telomeric probes, Int. J. Radiat. Biol. 82 (2006) 869–875.
[34] L. Benkhaled, M. Xunclá, M.R. Caballín, L. Barrios, J.F. Barquinero, Induction of complete and incomplete chromosome aberrations by bleomycin in human lymphocytes, Mutat. Res. 637 (2008) 134–141.
[35] P. Rodríguez, J.F. Barquinero, A. Duran, M.R. Caballín, M. Ribas, L. Barrios, Cells bearing chromosome aberrations lacking one telomere are selectively blocked at the G2/M checkpoint, Mutat. Res. 670 (2009) 53–58.
[36] D. Zeegers, S. Venkatesan, S.W. Koh, G.K. Low, P. Srivastava, N. Sundaram, S. Sethu, B. Banerjee, M. Jayapal, O. Belyakov, R. Baskar, A.S. Balajee, M.P. Hande, Biomarkers of ionizing radiation exposure: a multiparametric approach, Genome Integr. 8 (2017) 6.
[37] R. M'Kacher, E.E. Maalouf, M. Ricoul, L. Heidingsfelder, E. Laplagne, C. Cuceu, W. M. Hempel, B. Colicchio, A. Dieterlen, L. Sabatier, New tool for biological dosimetry: reevaluation and automation of the gold standard method following telomere and centromere staining, Mutat. Res. 770 (2014) 45–53.
[38] R. M'Kacher, B. Colicchio, C. Borie, S. Junker, V. Marquet, L. Heidingsfelder, K. Soehnlen, W. Najar, W.M. Hempel, N. Oudrhiri, et al., Telomere and centromere staining followed by M-FISH improves diagnosis of chromosomal instability and its clinical utility, Genes 11 (2020) 475.
[39] R. M'Kacher, B. Colicchio, S. Junker, E. El Maalouf, L. Heidingsfelder, A. Plesch, A. Dieterlen, E. Jeandidier, P. Carde, P. Voisin, High resolution and automatable cytogenetic biodosimetry using *in situ* telomere and centromere hybridization for the accurate detection of DNA damage: an overview, Int. J. Mol. Sci. 24 (2023) 5699.
[40] M. Pujol-Canadell, R. Puig, G. Armengol, L. Barrios, J.F. Barquinero, Chromosomal aberration dynamics through the cell cycle, DNA Repair 89 (2020) 102838.
[41] A. George, S. Venkatesan, N. Ashok, R. Saraswathy, M.P. Hande, Assessment of genomic instability and proliferation index in cultured lymphocytes of patients with Down syndrome, congenital anomalies and aplastic anaemia, Mutat. Res. 836 (2018) 98–103.
[42] V. Hande, K. Teo, P. Srikanth, J.S.M. Wong, S. Sethu, W. Martinez-Lopez, M. P. Hande, Investigations on the new mechanism of action for acetaldehyde-induced clastogenic effects in human lung fibroblasts, Mutat. Res. 861 (862) (2021) 503303.
[43] A. Chakraborty, S. Roy, M.P. Hande, B. Banerjee, Telomere attrition and genomic instability in unexplained recurrent pregnancy loss in humans: a preliminary study, Mutat. Res. 886 (2023) 503580.
[44] A. Ayouaz, C. Raynaud, C. Heride, D. Revaud, L. Sabatier, Telomeres: hallmarks of radiosensitivity, Biochimie 90 (2008) 60–72.
[45] S. Venkatesan, A.T. Natarajan, M.P. Hande, Chromosomal instability-mechanisms and consequences, Mutat. Res. 793 (2015) 176–184.
[46] N.S. Paviolo, I.Y. Quiroga, D.C. Castrogiovanni, M.S. Bianchi, A.D. Bolzán, Telomere instability is present in the progeny of mammalian cells exposed to bleomycin, Mutat. Res 734 (2012) 5–11.
[47] N.S. Paviolo, D.C. Castrogiovanni, A.D. Bolzán, The radiomimetic compound streptonigrin induces persistent telomere dysfunction in mammalian cells, Mutat. Res. 760 (2014) 16–23.
[48] N.S. Paviolo, F.F. Santiñaque, D.C. Castrogiovanni, G.A. Folle, A.D. Bolzán, The methylating agent streptozotocin induces persistent telomere dysfunction in mammalian cells, Mutat. Res 794 (2015) 17–24.
[49] A.D. Bolzán, M.S. Bianchi, Telomeres, interstitial telomeric repeat sequences, and chromosomal aberrations, Mutat. Res. 612 (2006) 189–214.
[50] V. Cherdyntseva, S. Gagos, Chromosome extremities under the microscopy lens: molecular cytogenetics in telomere research, Curr. Opin. Genet. Dev. 60 (2020) 69–76.
[51] X.D. Zhu, L. Niedernhofer, B. Kuster, M. Mann, J.H. Hoeijmakers, T. de Lange, ERCC1/XPF removes the 3' overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes, Mol. Cell 12 (2003) 1489–1498.
[52] A.D. Bolzán, Cytogenetic evaluation of telomere dysfunction: Chromosomal aberrations involving telomeres and interstitial telomeric sequences, in: L. Mancini (Ed.), Telomeres: Function, Shortening and Lengthening, Nova Science Publishers Inc., New York, 2009. pp. 133-185.
[53] J.J.W.A. Boei, S. Vermeulen, A.T. Natarajan, Analysis of radiation-induced chromosomal aberrations using telomeric and centromeric PNA probes, Int. J. Radiat. Biol. 76 (2000) 163–167.
[54] J.J.W.A. Boei, A.T. Natarajan, Combined use of chromosome painting and telomere detection to analyse radiation induced chromosomal aberrations in mouse splenocytes, Int. J. Radiat. Biol. 73 (1998) 125–133.
[55] M.P. Hande, A.S. Balajee, A. Tchirkov, A. Wynshaw-Boris, P.M. Lansdorp, Extra-chromosomal telomeric DNA in cells from Atm(-/-) mice and patients with ataxia-telangiectasia, Hum. Mol. Genet. 10 (2001) 519–528.
[56] B. Undarmaa, S. Kodama, K. Suzuki, O. Niwa, M. Watanabe, X-ray-induced telomeric instability in Atm-deficient mouse cells (M), Biochem. Biophys. Res. Commun. 315 (2004) 51–58.
[57] C. Herate, L. Sabatier, Retrospective biodosimetry techniques: Focus on cytogenetics assays for individuals exposed to ionizing radiation, Mutat. Res. 783 (2020) 108287.
[58] J.F. Tričković, A.V. Šobot, I. Joksić, G. Joksić, Telomere fragility in radiology workers occupationally exposed to low doses of ionising radiation, Arh. Hig. Rada. Toksikol. 73 (2022) 23–30.
[59] A. Sfeir, S.T. Kosiyatrakul, D. Hockemeyer, S.L. MacRae, J. Karlseder, C. L. Schildkraut, T. de Lange, Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication, Cell 138 (2009) 90–103.
[60] T. von Zglinicki, Oxidative stress shortens telomeres, Trends Biochem. Sci. 27 (2002) 339–344.
[61] R.P. Barnes, E. Fouquerel, P.L. Opresko, The impact of oxidative DNA damage and stress on telomere homeostasis, Mech. Ageing Dev. 177 (2019) 37–45.
[62] C.M. Counter, F.M. Botelho, P. Wang, C.B. Harley, S. Bacchetti, Stabilization of short telomeres and telomerase activity accompany immortalization of EpsteinBarr virus-transformed human B lymphocytes, J. Virol. 68 (1994) 3410–3414.
[63] M. Volleth, M. Zenker, I. Joksic, T. Liehr, Long-term culture of EBV-induced human lymphoblastoid cell lines reveals chromosomal instability, J. Histochem. Cytochem. 68 (2020) 239–251.
[64] S. Lacoste, E. Wiechec, A.G. dos Santos Silva, A. Guffei, G. Williams, M. Lowbeer, K. Benedek, M. Henriksson, G. Klein, S. Mai, Chromosomal rearrangements after *ex vivo* Epstein-Barr virus (EBV) infection of human B cells, Oncogene 29 (2010) 503–515.
[65] V. Boccardi, L. Cari, G. Nocentini, C. Riccardi, R. Cecchetti, C. Ruggiero, B. Arosio, G. Paolisso, U. Herbig, P. Mecocci, Telomeres increasingly develop aberrant structures in aging humans, J. Gerontol. A Biol. Sci. Med. Sci. 75 (2020) 230–235.
[66] L. Bejarano, G. Bosso, J. Louzame, R. Serrano, E. Gómez-Casero, J. Martínez-Torrecuadrada, S. Martínez, C. Blanco-Aparicio, J. Pastor, M.A. Blasco, Multiple cancer pathways regulate telomere protection, EMBO Mol. Med. 11 (2019) e10292.
[67] P. Srikanth, A.R. Chowdhury, G.K.M. Low, R. Saraswathy, A. Fujimori, B. Banerjee, W. Martinez-Lopez, M.P. Hande, Oxidative damage induced telomere mediated genomic instability in cells from ataxia telangiectasia patients, Genome Integr. 13 (2022) 1, 0003.

[68] Y. Sakamoto, K. Shiraishi, S. Kodama, Enhanced induction of abnormal telomere FISH signals in response to oxidative DNA damage, J. Radiat. Res. (2024) 1–7.
[69] Z. Yang, K.K. Takai, C.A. Lovejoy, T. de Lange, Break-induced replication promotes fragile telomere formation, Genes Dev. 34 (2020) 1392–1405.
[70] S.M. Bailey, M.N. Cornforth, R.L. Ullrich, E.H. Goodwin, Dysfunctional mammalian telomeres join with DNA double-strand breaks, DNA Repair 3 (2004) 349–357.
[71] S. Stroik, E.A. Hendrickson, Telomere fusions and translocations: a bridge too far? Curr. Opin. Genet. Dev. 60 (2020) 85–91.
[72] P. Slijepcevic, S. Al-Wahiby, Telomere biology: integrating chromosomal end protection with DNA damage response, Chromosoma 114 (2005) 275–285.
[73] S.M. Bailey, J. Meyne, D.J. Chen, A. Kurimasa, G.C. Li, et al., DNA double-strand break repair proteins are required to cap the ends of mammalian chromosomes, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 14899–14904.
[74] A.D. Bolzán, Mutagen-induced telomere instability in human cells, Mutat. Res. (2021) 868–869, 503387.
[75] L.F.Z. Batista, I. Dokal, R. Parker, Telomere biology disorders: time for moving towards the clinic? Trends Mol. Med. 28 (2022) 882–891.