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Review

Interstitial telomeric sequences in vertebrate chromosomes: Origin, function, instability and evolution

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ABSTRACT

By definition, telomeric sequences are located at the very ends or terminal regions of chromosomes. However, several vertebrate species show blocks of (TTAGGG)n repeats present in non-terminal regions of chromosomes, the so-called interstitial telomeric sequences (ITSs), interstitial telomeric repeats or interstitial telomeric bands, which include those intrachromosomal telomeric-like repeats located near (pericentromeric ITSs) or within the centromere (centromeric ITSs) and those telomeric repeats located between the centromere and the telomere (i.e., truly interstitial telomeric sequences) of eukaryotic chromosomes. According with their sequence organization, localization and flanking sequences, ITSs can be classified into four types: 1) short ITSs, 2) subtelomeric ITSs, 3) fusion ITSs, and 4) heterochromatic ITSs. The first three types have been described mainly in the human genome, whereas heterochromatic ITSs have been found in several vertebrate species but not in humans.

Several lines of evidence suggest that ITSs play a significant role in genome instability and evolution. This review aims to summarize our current knowledge about the origin, function, instability and evolution of these telomeric-like repeats in vertebrate chromosomes.

Contents

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1. Introduction. What are interstitial telomeric sequences?

1.1. Telomeres

Telomeres are specialized nucleoproteic complexes localized at the physical ends of linear eukaryotic chromosomes that maintain their stability and integrity [1]. They provide a protective "cap" for chromosomal DNA against illegitimate recombination, exonucleolytic attack and degradation, and oxidative damage [1,2]. In all vertebrates, the DNA component of telomeres consists of extended arrays of the TTAGGG hexamer [3,4]. Interestingly, this "vertebrate" telomere motif was also found in most Metazoa (except nematodes and arthropods) and in the unicellular metazoan sister group Choanozoa (see [5] for review). Telomeric DNA is bound by a specialized multiprotein complex known as shelterin, constituted by six proteins (POT1, TPP1. TIN2, TRF1, TRF2 and RAP1) and their variants (for example, mice have two forms of POT1, POT1a and POT1b) [1,6]. Besides telomeric repeats and shelterin, telomeres also comprise (UUAGGG)n-containing RNA molecules (telomeric repeat containing RNA or TERRA), a novel class of RNA transcribed from the subtelomere towards the telomere which plays critical roles in telomere biology, such as heterochromatin formation at chromosome ends and regulation of telomerase activity [7–11]. Spontaneous or induced telomere shortening is usually prevented by telomerase, a reverse transcriptase which adds telomeric repeats to the chromosome ends, thus elongating telomeres [1,12–14]. Telomerase activity is usually inactive in somatic cells, so telomere shortens with each cell division, but it is active in germline cells, stem cells, immortalized cell lines, activated lymphocytes, and most of the tumor cells analyzed so far [14]. Alternatively, telomere elongation can occur in the absence of telomerase through the so-called ALT (for 'Alternative Lengthening of Telomeres') mechanism, which involves homologous recombination between telomeres and has been described in several tumor cells and immortalized cell lines [14–17]. Interestingly, telomerase and ALT mechanisms of telomere elongation coexist in some human tumor cells [14].

1.2. Interstitial telomeric sequences

By definition, telomeric sequences are located at the very ends or terminal regions of chromosomes. However, several vertebrate species show blocks of (TTAGGG)n repeats present in non-terminal regions of chromosomes, the so-called interstitial telomeric sequences (ITSs), interstitial telomeric repeats or interstitial telomeric bands, which include those intrachromosomal telomeric-like repeats located near (pericentromeric ITSs) or within the centromere (centromeric ITSs) and those telomeric repeats located between the centromere and the telomere (i.e., truly interstitial telomeric sequences) of eukaryotic chromosomes [18–20] (Fig. 1). The presence of ITSs has been assumed to be the result of tandem chromosome fusions (telomere–telomere fusions) during evolution or the insertion of telomeric DNA within unstable sites during the repair of DNA double-strand breaks (DSBs) [4,18,19,21]. We will consider the origin and evolution of ITSs in detail in sections 3 and 4 of this review.

1.3. Relationship between ITSs and true telomeres

It has been shown that ITSs do not represent a functional telomere [4]. The only exception reported so far is represented by an Indian Muntjac cell line, where in a small percentage of cells ITSs get amplified and chromosomes fall apart into many small fragments with functional telomeres on most chromosome ends [22]. Moreover, unlike terminal telomeric sequences (i.e., true telomeres), ITSs seem not to be directly associated with the nuclear matrix [23]. Nevertheless, ITSs can interact with telomeres, as demonstrated by the recent discovery of structures named "interstitial telomere loops" or ITLs. These ITLs are chromosome-end structures which result from the interaction of telomeres and ITSs, and are dependent on the telomere-repeat binding factor 2 (TRF2, from the shelterin complex) and lamin A/C (a canonical component of the nucleoskeleton) [24,25]. This structure has important implications in organismal aging, telomere and genome stability, regulation of gene expression and chromosome condensation [25].

1.4. ITSs detection

ITSs are usually detected at the chromosome level by using Fluorescence *in situ* hibridization (FISH) with a DNA or PNA (Peptide Nucleic Acid) telomeric probe or the primed *in situ* labeling (PRINS) technique, but for most short ITSs (<100 bp) molecular methods such as Southern blot or pulsed field gel electrophoresis (PFGE) are necessary to detect these sequences and to determine the exact co-localization of ITSs and the associated breakage or recombination sites. Only a few short ITSs can be detected by FISH or PRINS, due to the relatively low sensitivity of these techniques (about 1 kb, being PRINS more sensitive than FISH) [26,27]. It is important to note that when we refer to ITSs, we

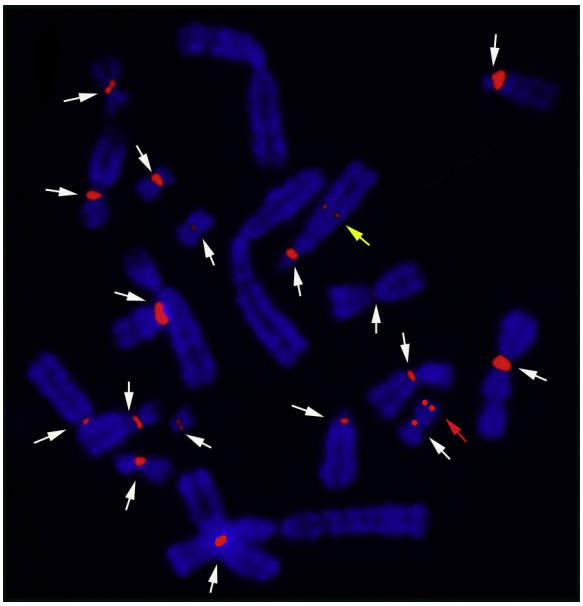

Fig. 1. Examples of ITSs. Patterns of hybridization of (TTAGGG)n repeats in a metaphase spread from a Chinese hamster ovary (CHO) cell after FISH with a telomeric probe. Note the presence of telomeric signals at the centromeric regions of all chromosomes (heterochromatic centromeric ITSs) (white arrows) except the largest pairs, one interstitial signal in the long arm of chromosome 4 (i.e., truly interstitial ITSs) (yellow arrow), and terminal signals (true telomere) (red arrow) at one of the ends of a small metacentric chromosome.
(modified from [132])

refer to a block of telomeric repeats, not a single TTAGGG repeat, since ITSs sites contain several TTAGGG repeats (even in the case of short ITSs). Thus, when using FISH or PRINS for chromosomal detection of ITSs, each fluorescent signal means a block of ITSs, not a single ITS or TTAGGG repeat. Moreover, specific chromosome regions containing ITSs are named "ITSs sites". Each ITSs site contains several TTAGGG repeats. Since methods for detection and quantification of ITSs have been described and discussed in detail elsewhere [18,20,28], they will not be considered in the present review.

1.5. ITSs could represent a significant part of telomeric DNA in vertebrates

Even if the extent to which ITSs vary between individuals and among different species of vertebrates is almost completely unknown, a recent study in passerine birds showed that ITSs constitute 15–45% of total telomeric DNA [29]. This finding suggests that ITSs could represent a significant part of the telomeric DNA in several vertebrate species. Given that the presence of ITSs in vertebrate chromosomes –particularly in their centromeric or pericentromeric regions- is a rather widespread phenomenon (see next sections for details), the study of these sequences is of great interest. Therefore, in this review we will summarize our current knowledge about the origin, function, instability and evolution of ITSs. It is important for the reader to bear in mind that this review will focus on ITSs only in vertebrate cells. We will consider in detail several aspects related to ITSs, including types, structure, organization, instability, function, and evolutionary origin of these telomeric-like repeats.

2. Types, structure and organizatian of ITSs

ITSs can be classified into four different types or classes, according to their sequence organization, localization and flanking sequences: short, subtelomeric, fusion, and heterochromatic ITSs [18,30] (Table 1). The first three have been identified in the human genome.

2.1. Classes of ITSs found mainly in the human genome

2.1.1. Short ITSs

These ITSs comprise few exact telomeric TTAGGG repeats, usually up to 20 hexamers (i.e., 120 bp, although most human short ITSs are less than 100 bp long) tandemly oriented [21,30,31]. Short ITSs are present at over 50 loci in human chromosomes (for example, 21q22, 2q31 and 7q36) [27,30,32] and on mitotic chromosomes of the Chinese hamster [33]. They have also been found in other primates and rodents like mouse, chimpanzee, gorilla and rat [19,27,30–32,34,35]. Short ITSs can be divided into five subclasses based upon their flanking sequences [30] (Fig. 2): in class A, the telomeric array is flanked by the same repetitive element on both sides, such as short interspersed nuclear element (SINE), long terminal repeat (LTR) or long interspersed element (LINE); in class B, the telomeric array is flanked by the same direct repeat on both sides; in class C, the telomeric array is flanked by unique sequences; in class D, the telomeric array is flanked by transposable elements on one side (i.e., interrupts a transposable element) and unique sequences on the other side, and in class E, the telomeric array is inserted at the junction between two different repetitive elements.

2.1.2. Subtelomeric ITSs

These ITSs are present on all human chromosome ends and are composed of head-to-tail tandem arrays containing several hundreds of base pairs of exact and degenerate tandem repeats (which differ from the canonical TTAGGG sequence by one or more base substitutions or small indels) inserted into subtelomeric domains. Subtelomeric ITSs are polymorphic and unstable sequences, and have been observed to be associated with telomeric proteins and can even contain genes. ITSs were isolated from the subtelomeric region of human chromosomes 4p, 6p, 16p, 20p, 22q, and Xq [21,30,31].

2.1.3. Fusion ITSs

These ITSs are composed of head-to-head blocks of TTAGGG repeats flanked by subtelomeric sequences, that originated from end fusions of ancestral chromosomes [30,32]. They are very rare in the human genome: the telomeric-like sequences in 2q13 and 1q41 represent the only fusion ITSs so far characterized in the

Table 1
Types of ITSs found in vertebrate cells (see Section 2 of this review for details).

Type of ITSs	Characteristics
Short	Few exact telomeric repeats (usually less than 100 bp long) tandemly oriented. Comprise five subclases, depending on their flanking sequences (see Fig. 2 of this review for details).
Subtelomeric	Head-to-tail tandem arrays containing several hundreds of bp of exact and degenerative tandem repeats inserted into subtelomeric regions of chromosomes.
Fusion	Head-to-head blocks of telomeric repeats flanked by subtelomeric low copy repeats, originated from end fusions of ancestral chromosomes.
Heterochromatic	Large blocks of telomeric-like sequences, spanning several hundreds of kb, usually found at the centromeric regions of chromosomes.

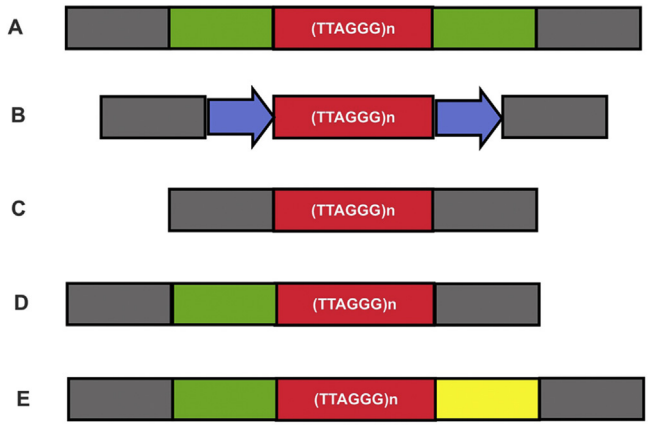

Fig. 2. Schematic representation of the different types of short ITSs found in vertebrate cells (see Section 2.1 of this review for details). Short ITSs can be divided into five subclasses based upon their flanking sequences: in class A, the telomeric array is flanked by the same repetitive element on both sides (green boxes); in class B, the telomeric array is flanked by the same direct repeat (blue arrows) on both sides; in class C, the telomeric array is flanked by unique sequences (grey boxes); in class D, the telomeric array is flanked by transposable elements on one side (green box) and unique sequences from the other side (grey box), and in class E, the telomeric array is inserted at the junction between two different repetitive elements (green and yellow boxes). Red boxes represent blocks of telomeric repeats, comprising each one several units of the hexamer TTAGGG (usually up to 20 hexamers).

human genome [18,30,32]. Fusion ITSs were also found in the Indian muntjac [36], but in this case, the fusion sites contain telomeric repeats immediately adjacent to satellite I (a class of Cervidae-specific centromeric satellite sequence), suggesting that ITSs played a role in the fusion event [36,37].

2.2. Heterocromatic ITSs

Besides the above types of ITSs, a fourth class of ITSs, named heterochromatic ITSs, which comprise large blocks of telomeric-like sequences (spanning several hundreds of kb) usually located within or at the margin of constitutive heterochromatin, has been found in several species of vertebrates other than human [4,18,19]. Due to its predominant location in the chromosomes, this type of ITSs has also been named "pericentromeric" or "centromeric" ITSs [18]. However, the term "heterochromatic" is more appropriated, since this type of ITSs can be also located in non-centromeric or truly interstitial regions of vertebrate chromosomes [4,19,34,35,38–42] or distributed along an entire chromosome arm (as is the case of several chromosomes of the hamsters *Phodopus* sp. [40]), or even an entire (micro)chromosome, like in some birds [43]. The existence of heterochromatic ITSs at the pericentromeric regions of vertebrate chromosomes has been recently demonstrated by FISH in the Mongolian gerbil (*Meriones unguiculatus*) [44], where FISH signals corresponding to ITSs included a wider area than the centromere (detected by anticentromere antibodies). In Chinese hamster (*Cricetulus griseus*) chromosomes, heterochromatic ITSs is organized as extended uninterrupted arrays of telomeric repeats, without restriction enzyme sites, being the telomeric sequences in the middle of two blocks of satellite DNA [45]. These ITSs localize at pericentromeric regions and associate with nucleosomes that have a shorter repeat length than bulk chromatin [46]. Therefore, a sequence-driven telomeric chromatin organization has been proposed [46]. Gámez-Arjona et al. [47] proposed that the short nucleosomal organization of higher eukaryotes telomeres and hamster ITSs could be explained by the presence of specific proteins or chromatin features that could lead to compaction of nucleosomes, which might require the presence of perfect telomeric repeat arrays. However, this is not always the case. In arvicoline rodents, a co-distribution of heterochromatic ITSs and the satellite DNA Msat-160 (another repeat located in the pericentromeric regions of chromosomes) has been found, in which both repeats occupe adjacent regions in some chromosomes, with a variable overlapping region in some of them [34]. In addition, it has been shown that some telomeric proteins, such as TRF1, TRF2 and RAP1 can also locate to ITSs [48–52], suggesting a role of the shelterin complex in the organization and functioning of heterochromatic ITSs (see Section 5.3 of the present review for details). One of the best studied ITSs are those ones from the Chinese hamster (*Cricetulus griseus*). In this species, heterochromatic ITSs comprise 250–500 kb of DNA on each chromosome, constitutes the major component of satellite DNA, and represents about 5% of the genome [53]. Accordingly, Chinese hamster cell lines (like CHO or CHE) usually contain a high proportion of ITSs, easily visualized using FISH with a telomeric probe [54,55]

2.3. ITSs identifiable by cytogenetic methods in vertebrate chromosomes

Despite the above classification, using a cytogenetic approach (i.e., telomere FISH or PRINS), two kinds of ITSs can be usually identified in vertebrate chromosomes: heterochromatic ITSs (seen as strong signals after telomere FISH or PRINS), and short ITSs (of about 1 Kb of size and seen as faint signals after telomere FISH or PRINS, depending on the sequence copy number of the ITSs) [19,30,32,34,35,40–42]. Large ITSs blocks, either pericentromeric or non-centromeric ones, are very likely the result of repeated events of telomeric repeats amplification [34,40]. We will consider this and other issues concerning the evolutionary origin of ITSs in the next section of this review.

3. Evolutionary origin of ITSs

3.1. Evolutionary origin of heterochromatic ITSs

End-to-end joining of two telomeres can lead to the formation of a block of ITSs (heterochromatic ITSs), like the one observed in several chromosomes of many vertebrate species [4]. In some cases, these ITSs may undergo amplification, leading to large blocks of heterochromatic ITSs within the pericentromeric or centromeric regions of chromosomes [4]. Moreover, deletion or translocation of ITSs is also possible, leading to the absence of ITSs in one of several branches derived from the same ancestor [56] or the transposition of ITSs into euchromatic locations (i.e., truly ITSs) [38,39–42], respectively. Fusion ITSs, like those found at 1q41 and 2q13 in the human genome, are supposed to be also originated by telomere–telomere fusion of ancestral chromosomes [30,32].

Concerning the evolutionary origin of heterochromatic ITSs, Ruiz-Herrera et al. [19] proposed several years ago a four-step mechanism to explain the presence of this kind of ITSs in vertebrate chromosomes, which is in line with the "centromere-from-telomere" hypothesis by Villasante et al. [57] and the notion of "chromosome plasticity" (which refers to the transforming potential of the chromosome material in which its functional elements, i.e., centromeres and telomeres, are the key players, giving rise to species-specific karyotypes; see [58] for an update): (1) *initial fusion events* telomere–telomere fusions of ancestral chromosomes during evolution, giving rise to ITSs, located mainly at the pericentromeric regions of chromosomes derived from Robertsonian (Rb) fusions, (a chromosome rearrangement involving centric fusion of two acro- or telocentric chromosomes to form a single metacentric), (2) *amplification of (TTAGGG)n repeats*

present in the ancestral karyotypes (through mechanisms such as unequal crossing-over or DNA polymerase slippage and gene conversion; see Section 6.1 of this review for details), (3) *subsequent reorganization of chromosomes* (inversions, translocations and tandem fusions) and further redistribution of ITSs to internal parts of the genome (alternatively, small-scale reorganizations and point mutations would cause progressive degeneration of the original telomeric array) and (4) *breakage/fission* (chromosome rupture in which the presence of telomeric repeats at the breakpoints would provide the substrate for a new stable telomere, or a chromosome fission event leading to the generation of new acrocentric chromosomes). This last step in the mechanism of origin of heterochromatic ITSs proposed by Ruiz-Herrera et al. [19], is supported by several studies showing that ITSs are naturally prone to breakage [52,58–61] and is in line with the notion of chromosome plasticity, particularly centric fission [58]. In this context, the presence of heterochromatic ITSs in vertebrate chromosomes is explained by assuming that ITSs represent remnants of structural chromosome rearrangements that occurred during karyotypic evolution, such as Robertsonian (Rb)-like fusions, tandem chromosome fusions or pericentric inversions [34,40,42,62–65]. Published data suggest that tandem chromosome fusions mostly involve telocentric chromosomes [36,43,66–68].

3.2. Sometimes heterochromatic ITSs are just a component of centromeric satellite DNA

Despite the above considerations, several studies have shown that, in some cases, the presence of centromeric ITSs in vertebrate chromosomes simply reflects the fact that these telomeric sequences are a component of the centromeric satellite DNA [34,35,42,69–74]. In fact, ITSs represent a primordial component of the repetitive DNA in cetaceans, fishes and rodents [34,45,75–78] and constitutes a major motif of repetitive DNA in some species of other vertebrate groups, like amphibians (frogs) [72] and marsupials (kangaroos and wallabies) [69,70]. In these cases, ITSs are assumed to be the result of amplifications of telomeric repeats that occurred independently during the chromosomal evolution of species. Moreover, studies in cotton rats (*Sigmodon* sp.) and in several species of fishes (from the Orders Anguilliformes, Mugiliformes, Salmoniformes and Syngnathiformes) strongly suggest that ITSs may also be the result of movement of heterochromatin during chromosomal rearrangements or that nucleolus organizer regions (NORs) played some role in the formation of ITSs, since these telomeric sequences have been found in the vicinity or within NORs sites of some chromosomes [35,78,79].

3.3. Evolutionary origin of short and subtelomeric ITSs

Although it is widely accepted that the evolutionary origin of both heterochromatic and fusion ITSs can be explained by telomere–telomere fusions between ancestral chromosomes during the evolution of organisms (with or without further amplification, translocation or deletion of these telomeric sequences), the evolutionary origin of other ITSs, especially short ITSs is far from being solved. Several years ago, Ruiz-Herrera et al. [19] proposed a model to explain the presence of short ITSs in the genome of vertebrates which implies that these sequences are originated by the insertion of telomeric repeats during the repair of DSBs during evolution, with or without the intervention of telomerase. Thus, telomerase-mediated repair of DSBs ("chromosome healing") could lead to the appearance of ITSs, at least in the chromosomes of rodents and primates [19,80]. In this view, the short ITSs found in the chromosomes of vertebrates are considered relics of ancient breakage within fragile sites rather than fragile sites themselves [19,21,30]. Short ITSs may be inserted with (i.e., deletion, insertion or duplication) or without modification of the flanking sequences [18,19,21,31] (we refer the reader to Fig. 4 in [19] and Fig. 2 in [18] for details). As suggested by previous studies, deletion of flanking sequences seems to be the most frequent target site modification occurring during the insertion of short ITSs in primates and rodents [21,27]. Moreover, the comparison of the loci of 10 human ITSs with their genomic orthologs in 12 primate species by Nergadze et al. [21] showed that short ITSs appeared suddenly during primate evolution, not from the expansion of pre-existing telomeric repeats, as is the case for other microsatellites. These telomeric-like sequences were inserted in a pre-existing and well conserved unrelated sequence [21].

Despite the above findings concerning the short ITSs insertion events in primates and rodents, at present there is not sufficient evidence that telomerase *per se* is capable to seed telomeric repeats into genomic regions. In fact, Hanish et al. [81], examining the requirements for telomere formation in human HeLa cells, found that the size distribution and the total yield of telomerase products did not correlate with the capacity of the telomeric sequences to seed new telomeres in these cells. In other words, neo-telomere formation did not correlate with the ability of human telomerase to elongate telomeric sequences *in vitro*. Thus, short ITSs in vertebrate chromosomes could have arisen by the repair of DSBs but without the intervention of telomerase. Recent evidence provided by studies about the Breakage-Induced-Replication (BIR) [82–87] mechanism of DNA repair, and the Targeted Telomere Insertion (TTI) mechanism of telomere maintenance [17], support this view.

The BIR mechanism, also known as recombination-dependent DNA replication, has been extensively investigated in *Escherichia coli* and its bacteriophages and in budding yeasts (see [87] for review). BIR is one of the three major homology-dependent repair pathways of DNA DSBs in yeast (the other ones are gene conversion and single-strand annealing), and occurs when only one DSB end shares homology with a donor sequence. Thus, BIR is responsible for the repair of one-ended DSBs [87]. This mechanism is believed to be responsible for restarting DNA replication at broken replication forks and allows yeast telomeres to be maintained in the absence of telomerase, resembling the ALT mechanism of telomere maintenance that occurs in human cancer cells [87,88]. Moreover, studies in yeast demonstrated that both post-replication repair (PRR) and homologous recombination (HR) pathways (like BIR) are involved in the expansion of ITSs, thus contributing to ITSs instability [84]. The rate of repeats expansion is determined by the interplay between PRR and HR. Thus, BIR could explain length polymorphism characteristic of ITSs in human cells [83]. In fact, BIR, which originates non-reciprocal translocations or insertions, was recently demonstrated in mammals [83]. It has been shown that BIR is often interrupted soon after initiation, giving rise to properly repaired DNA [85,86]. However, under some circumstances –such as the collapse of replication fork, changes in the regulation of DNA replication or in the timing of DNA synthesis initiation- BIR can be triggered, giving rise to genetic instability in the form of trinucleotide repeats expansion and other mutations such as translocations or copy-number variations [85,86] (Fig. 3A).

We know now that microhomology-mediated BIR or MM-BIR, a BIR-like mechanism involving template switching at positions of microhomology [90,91]] can elongate human telomeres, and that BIR repair of damaged forks induces genomic duplications of up to 200 Kb in human cells [83], and can even be involved in disease associated trinucleotide expansions [82,83,85,86]. Moreover, the MMBIR model has been used to explain telomere healing in human cells [92,93]. MMBIR-like events have been described in various model systems, including mouse embryonic stem cells [94] and

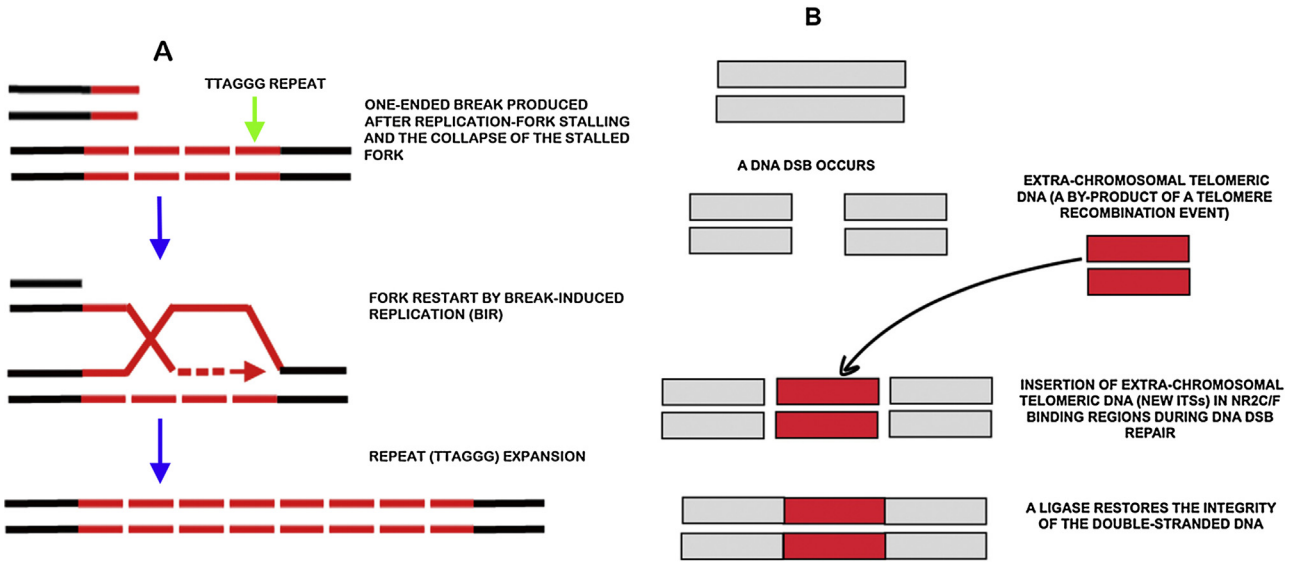

Fig. 3. Possible origin of short and subtelomeric ITSs by Break-Induced-Replication (BIR) and Targeted Telomere Insertion (TTI) mechanisms (see Section 3.3 of this review for further details). BIR is often interrupted after initiation, giving rise to a properly repaired DNA. However, under some circumstances, after a replication-fork stalling event, the stalled fork may collapse, leading to the formation of a one-ended DSB, which initiates the BIR process, which involves strand invasion of the single-stranded 3′ end (red line with arrowhead). After BIR, repeat expansion occurs (A). Dashed lines represent new DNA synthesis (in this case, new interstitial telomeric repeats). Please note that this Figure is a simplified representation of the BIR process. In TTI (B), extra-chromosomal telomeric DNA (resulting from a previous telomere recombination event, not shown in the Figure) is inserted in a NR2C/F binding site during the repair of a DNA DSB. After the insertion, a new potential fragile site containing ITSs appears in the repaired chromosome. Upon breakage of ITSs sites, intra- or inter-chromosomal rearrangements could appear. In B, a double-stranded DNA (belonging to an unreplicated chromosome) is represented; red boxes represent blocks of TTAGGG repeats and grey boxes represent each one of the strands of chromosomal (non-telomeric) DNA.

human cells [95,96]. According to current models [90,91], MMBIR is initiated by DNA breakage generating a single DNA end, and proceeds with multiple template switches at positions of microhomologies that could be as short as 1–3 bp, leading to varying levels of amplification and rearrangements (see [97] for review). Interestingly, studies in yeast showed that BIR can switch to MMBIR, i.e., the collapse of classical BIR can lead to MMBIR [97].

It has been proposed more than a decade ago, that DNA polymerase slippage can give rise to ITSs [89]. Thus, the misalignment between template and primer strand during DNA replication can lead to insertion or deletion of repeated units, which results in the expansion or shortening of a block of ITSs, like it happens with microsatellites. This process could explain the formation of few (short) ITSs, like those located at 21q22, 2q31 and 7q36, and the subtelomeric ITSs found at 6pter in the human genome [18,89]. However, the recent evidence reviewed above favors BIR-like mechanisms (like MMBIR) instead of a DNA polymerase slippage process to explain the insertion and expansion of short ITSs in the vertebrate genome [82,87,89].

Besides BIR-like mechanisms, the abovementioned TTI mechanism, recently described in ALT human cancer cells, could also explain the evolutionary origin of ITSs in vertebrate cells [17] (Fig. 3B). Previously, Londoño-Vallejo et al. [15] found that some chromosomes of human cancer cells using the ALT mechanism of telomere elongation exhibit interstitial telomeric signals after Chromosome Orientation FISH (CO-FISH) with a telomeric probe. The authors suggested that the presence of interstitial telomeric repeats in ALT cells were probably the mark of past non-homologous end-joining (NHEJ) events between two chromosome ends still carrying telomeric repeats [15]. However, Marzec et al. [17] showed recently that these ITSs (supposedly expanding several kb pairs, since they could be detected by telomere FISH) originate from the insertion of extra-chromosomal telomeric DNA (a by-product of telomere recombination or telomere sister-chromatid exchanges, characteristic of ALT cells) in broken chromosomal sites. Since this mechanism of telomere-driven genomic instability is different from the common breakage-fusion-bridge (BFB) cycles mechanism, and is due to the activation of a telomere maintenance mechanism, these authors named it "targeted telomere insertion" (TTI) [17]. The chromosome regions where telomeric repeats are inserted correspond to regions regulated by orphan nuclear receptors of the NR2C/F classes, which belong to the nuclear hormone receptor family of transcription factors [17]. ITSs originated by this mechanism generate potential common fragile sites, thus contributing to the elevated genomic/chromosome instability found in ALT cells [17]. Upon breakage of ITSs sites, intra- (due to a chromosome break) or inter-chromosomal (translocation or recombination between chromosomes) rearrangements may arise [17]. Although TTI was found in ALT human cells, we cannot ruled out the possibility that this mechanism also occurs in another cell types in vertebrates.

In conclusion, even if the hypothesis of Ruiz-Herrera et al. that the presence of short ITSs in the chromosomes of vertebrates could be the result of the repair of DSBs during evolution could be valid, present evidence suggests that short ITSs could be inserted in the genome of vertebrates via DNA repair mechanisms (such as MMBIR or TTI) but without involving the intervention of telomerase. These mechanisms could explain the insertion of a few bp up to several Kb pairs of telomeric repeats and the origin of both subtelomeric and short ITSs. Nevertheless, further studies will be needed to determine the precise mechanism of origin of these ITSs in vertebrates.

4. ITSs and karyotypic evolution in vertebrates

4.1. ITSs in vertebrate chromosomes: the study by Meyne et al.

In 1990, Meyne et al. [4] published a seminal work about the chromosomal distribution of telomeric sequences in 100 vertebrate species using FISH with a telomeric DNA probe. All of the species studied showed telomeric FISH signals at the termini of all chromosomes, but 55 of these species also showed one or more non-telomeric sites of hybridization, meaning that telomeric sequences were also located at interstitial regions of

chromosomes, mostly near or within the centromere [4]. Thus, Meyne et al. [4] demonstrated that the chromosomes of many vertebrate species contain centromeric, pericentromeric or even truly ITSs. Given that the probe used in this study allowed to detect only ITSs longer than 1 kb, the figure of 55/100 cases is very likely an underestimate, so small or very small ITSs blocks could be also present in the chromosomes of the species analyzed. Several studies performed following the one by Meyne et al. [4], confirm that the chromosomal distribution of telomeric sequences in vertebrates (i.e., telomere-only or telomere plus ITSs pattern) depends on the species considered (see for example [33,34,40,41,43,69,70,78,98–105]). Both terminal telomeric and ITSs can be present in the autosomes (even B chromosomes and microchromosomes) [4,43,98] and the sex chromosomes [41,98,106,107], depending on the species. Moreover, the number, localization and degree of amplification of ITSs varies with the karyotype and the morphology of the chromosomes [34,40]. In addition, the evolution of ITSs, specially pericentromeric ITSs, seems to be associated with the one from satellite DNA repeats [40].

4.2. Are ITSs and the evolutionary status of species related?

From their study, Meyne et al. [4] proposed that a relationship exists between the presence of non-telomeric sequences in the chromosomes and the evolutionary status of vertebrate species. Thus, primitive species (having "primitive" karyotypes) show a telomere-only pattern of telomeric sequences (TTAGGG)n distribution, intermediate or evolving species possess chromosomes with ITSs (acquired by amplification or translocation of telomeric sequences or by chromosome end-to-end fusion events), and highly evolved species present chromosomes with no or a few ITSs, since the amplified regions may be lost after extensive chromosome rearrangements occurring during evolution [4]. Although some studies in rodents [34,35] or bats [73] support this view (at least for most of the species studied), other studies in birds (where numerous ITSs were found in the chromosomes of primitive species) [43], reptils (where ITSs are common in both basal and derived lineages within the suborder Serpentes [42] and where species from the Order Squamata present a high frequency of ITSs despite their generally conserved karyotypes [98]), and mammals (where several ITSs were found in recent species) [40,70] do not fully support it, and suggest that the presence, absence or amplification of ITSs in vertebrate chromosomes is related to the evolutionary status of single chromosomes, rather than that of entire karyotypes or species. In this view, "primitive" chromosomes are considered those ones containing only a few or none ITSs, "evolving" chromosomes are the ones derived from fusions and other rearrangements which occurred in "primitive" chromosomes, and "highly evolved" chromosomes are the ones containing several ITSs [70].

4.3. ITSs and the role of Rb fusions in the karyotypic evolution of vertebrates

Since Rb fusions are one of the most frequent type of events occurring during the karyotypic evolution in vertebrates (specially in mammals and birds), it is important to consider that there are three different types of Rb fusions, based on the relationship between telomeric sequences and the fusion event [58,62] (Fig. 4): In the first case (Fig. 4A), the acrocentric chromosomes fuse and a dicentric chromosome results, without the involvement of telomeric sequences (because they were lost due to extensive telomere shortening), as observed *in vitro* by Blasco et al. in mTR-/- mice (where telomere shortening was induced by the deletion of the telomerase RNA gene in the mouse germ-line) [108], and recently by Sánchez-Guillén et al. in wild house mice populations [109]; in the second case (Fig. 4B), a chromosome breakage event occurring within minor satellite sequences results in the loss of the telomeres and part of the centromeres involved in the fusion, as seen in feral mouse populations [110,111] (in the newly formed centromeric region of every Rb chromosome about 20–60 kb of minor satellite DNA are retained, flanked by two blocks of about 6 megabases each of major satellite DNA) [110]; finally, there is another mechanism by which a Rb fusion may arise (Fig. 4C), in which the telomeres of the chromosomes involved in the fusion event are retained but presumably inactivated (since the presence of functional telomeres prevents chromosome fusion [62]), forming a block of telomeric sequences at the centromeric region of the rearranged chromosome, which appears like an ITSs signal after telomere FISH or PRINS, as seen in many vertebrate species [4,40,73,99]. Inactivation or the loss of function of telomeres could be due the loss of telomere-associated proteins, changes in telomere chromatin or telomerase inactivation [62]. Thus, end-to-end joining of two telomeres can lead to the formation of interstitial telomeric sites (heterochromatic pericentromeric or centromeric ITSs). This latter type of chromosome fusion is termed "Robertsonian-like" fusion, since telomere loss or inactivation is a prerequisite for the formation of true Rb fusions (otherwise, telomeres stabilize the chromosomes so they cannot be fused). It is important to note that all Rb fusions give rise to dicentric chromosomes [62], although one of the centromeres is usually inactivated and the resulting chromosome visualized as a monocentric one. The process of telomere/centromere inactivation or telomere transformation into centromeres involves epigenetic mechanisms which are still not fully understood [58,112]. In summary, Rb fusions may arise by telomere shortening, chromosomal breakage within centromeric satellite sequences, or telomere inactivation.

5. ITSs and genome instability

5.1. The instability of ITSs in vertebrate cells

Many studies demonstrated that ITSs may act as hotspots for breakage, recombination, rearrangement and amplification sites, conferring fragility to the region where they are inserted [25,52,59,113–116], and that these sequences may participate in DNA repair and regulation of gene expression [54,113,117]. According with Revaud et al. [113], ITSs instability is related to the cell cycle phases, the occurrence of breaks within ITSs, the functions of DNA-PKcs, and the DNA DSBs repair pathways recruited in accordance with the chromatin state of the exposed cells (see next section for details). Moreover, a co-localization of ITSs and fragile sites has been found at the cytogenetic level in rodents and primates, and previous cytogenetic studies in non-human primates have indicated that there is a relationship among evolutionary breakpoints, fragile sites and the existence of ITSs [14,48]. Interestingly, Barros et al. [118] found that in the fish *Ancistrus* sp. (Loricariidae) 5S rDNA regions contain fragile sites which co-localize with ITSs sites. These sites provide chromosomal instability, resulting in telomeric recombinations via TRF2 and BFB cycles. In the human genome, al least 19 ITSs (involving chromosomes 1, 2, 3, 6, 7, 8, 10, 11, 12, 14 and 18) were found to co-localize with fragile sites in the same chromosome [18]. Moreover, several unstable ITSs loci were found to be associated with human sporadic gastric tumors [89].

Data from Bosco and de Lange [52] showed that telomeric DNA is inherently fragile, regardless of its genomic localization. A recent study in the human genome demonstrates that very small ITSs (defined as two TTAGGG repeats separated by less than 100 bp) are recombination hot spots, being the average recombination rate at

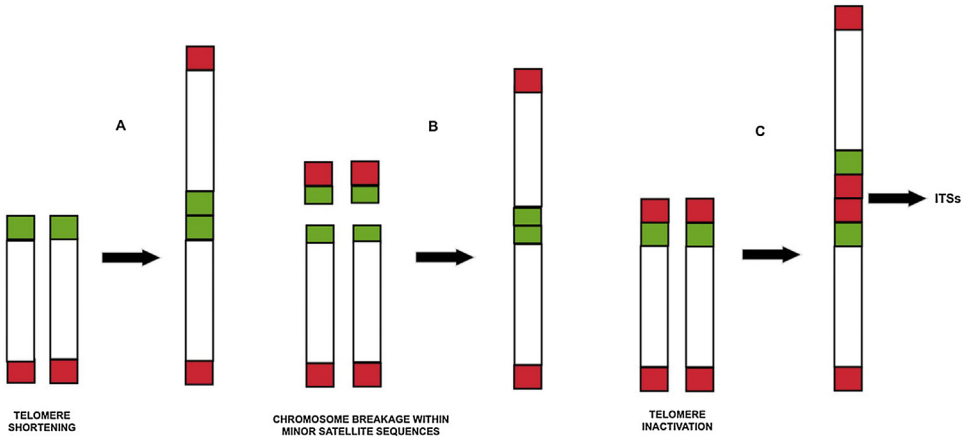

Fig. 4. Types of Robertsonian (Rb) fusions based on the relationship between telomeric sequences and the fusion event (see Section 4.3 of the present review for details). In the first case (A), the acrocentric chromosomes fuse and a dicentric chromosome results, without the involvement of telomeric sequences (because they were lost due to extensive telomere shortening); in the second case (B), a chromosome breakage event occurring within minor satellite sequences results in the loss of the telomeres and part of the centromeres involved in the fusion; finally, in the third case, (C), the telomeres of the chromosomes involved in the fusion event are retained but inactivated (due to inactivation or loss of telomere-associated proteins, changes in telomere chromatin, telomerase inactivation, etc.), forming a block of telomeric sequences at the centromeric region of the rearranged chromosome, which appears like an ITSs signal. Red boxes represent the telomeres of the chromosomes involved in the fusion and green boxes represent their centromeres.
(based on [62])

these ITSs higher than expected compared to sites placed randomly troughout the genome [25]. Since no significant correlation was observed between the number of ITSs in a 10 kb genomic window and the frequency of recombination of these sequences, it was suggested that isolated ITSs recombine as frequently as more dense ITSs regions, and that it is not necessarily the occurrence of a given number of repetitive DNA that influences recombination, but the presence of a certain type of ITSs [25]. Therefore, ITSs represent a favorable substrate for chromosomal breakage, thus promoting genome instability.

Several studies have shown that ITSs (interstitial telomeres) are present in some constitutional autosomal structural rearrangements in humans [114,119–123], in which telomeres lose their ability to prevent chromosome fusion, leading to the presence of ITSs at the fusion point of the derivative chromosome/s either in stable or jumping telomeric translocations (unbalanced translocations involving a donor chromosome arm or chromosome segment that has fused to two or more different recipient chromosomes) [124,125], telomeric associations, ring chromosomes and duplications. Some of these rearrangements were in mosaic form [114,123]. The instability observed in these structural rearrangements involving ITSs indicates that these telomeric-like sequences are prone to breakage and may be involved in generating mosaicism in humans [114,120,121].

5.2. Some ITSs are not hotspots for rearrangement or recombination

All of the abovementioned studies showed that ITSs are naturally prone to breakage, a view in line with the notion of chromosome plasticity, in particular centric fission [58]. However, not all ITSs are hotspots for rearrangement or recombination [126–128]. Azzalin et al. [30] proposed that short ITSs may not be fragile sites but simply mark sites of DSBs that occurred within unstable chromosome regions. In this way, short ITSs may be envisaged as relics of ancient breakage within fragile sites, rather than fragile sites themselves [30]. This hypothesis does not rule out the possibility that very extended blocks of ITS, such as those present in Chinese hamster cells could be prone to breakage and recombination. In fact, several studies have shown that heterochromatic ITSs can be involved in spontaneous and clastogen-induced chromosomal aberrations [55,59,116,129–133], being the frequency of aberrations involving these sequences higher than expected based on the percentage of the genome covered by ITSs.

5.3. Factors affecting ITSs stability

Several external and internal factors decide the fate of ITSs either as unstable hotspots or stable sequences [18] (Table 2):

Table 2
Factors affecting ITSs stability (see Section 5.3 of this review for details and references).

Factor	Condition	Effect
Nature of the sequence	Conserved	Promotes ITSs instability
	Degenerative	Favors ITSs stability
Length of ITSs	High copy number	Promotes ITSs instability
	Low copy number	Favors ITSs stability
Chromatin status	Condensed or relaxed/Hetero- or euchromatin	Variable effect (can increase or decrease ITSs stability)
Epigenetic status of the telomeric sequence	Methylated or hypermethylated	Favors ITSs stability
	Demethylated or hypomethylated	Promotes ITSs instability
Telomere-associated proteins	Present	Favors ITSs stability
	Absent	Promotes ITSs instability
Clastogen	Radiaton/chemical mutagens	Variable effect (can increase or decrease ITSs stability, depending on the clastogen

5.3.1. Nature of the sequence

The primary sequence of ITSs consists of an AT-rich sequence comprised of 50–60% of guanine. According to *in vitro* studies, this composition favors the formation of guanine tetrad pairing and other secondary higher-order DNA structures [52,134,135], which could promote ITSs instability, since these structures are preferential sites for chromosomal recombination and exchange. On the contrary, if the primary sequence of ITSs is degenerative (i.e., contains degenerative sequences which disrupts the guanine tetrad pairing structure), this could favor the stability of ITSs, as supported by the finding that an increased number of degenerative sequences confers stability to microsatellites [89].

5.3.2. Length of the ITSs

Being ITSs considered as a kind of microsatellite DNA [18], a high copy number of telomeric repeats can promote genomic instability of ITSs (like the one observed in heterochromatic ITSs), whereas a low copy number (short ITSs) could favor the stability of ITSs. For example, in the human genome, short ITSs at 2q31 and 7q36, were found to be more stable than the one at 21q22 (which contains a higher copy number of telomeric repeats than the ITSs at 2q31 and 7q36) [89].

5.3.3. Chromatin status

Chromatin conformation (i.e., condensed or relaxed) may have a variable effect on ITSs stability. Those ITSs usually involved in chromosomal aberrations are the ones located at the centromeric regions of chromosomes (heterochromatic ITSs) [28,59]. Balajee et al. [54] and Fernandez et al. [130] suggested that the increased frequency of chromosomal aberrations at ITSs loci might be related to their propensity to form secondary structures, thus promoting recombination events. Some studies demonstrated that hamster ITSs possess a particular chromatin structure, enriched in short unpaired DNA segments, which confers alkali-sensitivity and affects the DNA repair process in ITSs-rich chromosome regions and thus influences their involvement in the formation of chromosomal aberrations [46,117]. The highly compacted chromatin present in heterocromatic ITSs may result in a greater torsional stress that will produce a high density of short unpaired DNA segments within, which turns these regions sensitive to damage [117]. Moreover, the induction and repair kinetics of DNA SSBs and DSBs induced by ionizing radiation in hamster cells showed that the initial rejoining rate of DSBs within ITSs is slower than that in the whole genome, demonstrating an intragenomic heterogeneity in DSBs repair. In the absence of DNA-PKcs or Rad51C, the rejoining rate of DSBs within ITSs was not modified, unlike in the whole genome [117]. Moreover, interstitial telomeric chromatin in hamster cells shares common structural features with truly telomeric chromatin, and a higher nucleosome density than bulk chromatin, which correlates with a highly regular chromatin structure [46]. The size and location of ITSs appear to be very important in their instability, since small ITSs located in the euchromatin of human chromosomes do not display increased radiation-induced instability compared with non-telomeric sequences [128].

5.3.4. Epigenetic status of the telomeric sequence

Based on data on the epigenetic regulation of repetitive sequences in mammalian cells, Lin and Yan [18] suggested several years ago that histone modifications and DNA methylation likely occurs in subtelomeric ITSs, which could protect ITSs-rich chromosome regions from breakage and play important roles in gene expression regulation. These authors proposed that hypermethylation favors ITSs stability, whereas demethylated or hypomethylated ITSs tend to be unstable. Interestingly, in cells that lack DNA methyltransferase, demethylation of subtelomeric ITSs are associated with increased homologous recombination between telomeric regions [136]. Despite the above observations, data on the epigenetic control of ITSs in vertebrate cells are still very scarce, mainly due to the fact that methods used for analysis of telomeric chromatin usually cannot distinguish between ITSs and telomeres.

5.3.5. Telomere-associated proteins (i.e., those belonging to the shelterin complex)

The binding of these proteins (like TRF1, TRF2 and RAP1) to ITSs [48–51,60,137,138], favors their stability by reducing unequal homologous recombination events between telomeric sequences, whereas in the absence of one or more of these proteins ITSs instability arises [18,139,140]. Interestingly, it has been found that human chromosome 2q14 presents a TRF1-controlled common fragile site (induced by aphidicolin) containing ITSs [52]. TRF1 binds to and stabilizes this fragile site, but does not affect other common fragile sites, so 2q14 is the first common fragile site controlled by a sequence-specific DNA binding protein.

5.3.6. Clastogen

Clastogens such as radiation or chemical mutagens may have a variable effect, since they can increase or decrease ITSs instability. We will consider this issue in detail in the next section of this review.

On the other hand, even if some studies suggest that telomerase could play a role in the insertion of short ITSs in mammalian genomes [19,21,59,81,141] thus affecting ITSs stability, there is not sufficient evidence that telomerase *per se* is capable to insert telomeric repeats into genomic regions (see Section 3 of the present review for details). Therefore, the role of telomerase as a factor affecting ITSs stability (as suggested by Lin and Yan [18]) remains to be determined.

6. Induced ITSs instability: the effect of clastogens on ITSs

6.1. Chromosomal aberrations directly involving ITSs

As previously mentioned, ITSs instability depends on several factors, and clastogens may have a variable effect on these telomeric-like sequences. Despite the involvement of ITSs in chromosomal rearrangements such as fusions, fissions and inversions, there are four different types of spontaneous or clastogen-induced chromosomal rearrangements in which ITSs may be directly involved: interstitial fragments, amplification, deletion and transposition or translocation of ITSs (see [20,28,142] for review) (Fig. 5). These aberrations can be identified using telomere FISH (either with a DNA or a PNA probe) or PRINS [28].

Breaks occurring at the centromeric region of chromosomes having pericentromeric or centromeric ITSs may give rise to acentric interstitial fragments or microchromosomes, which appear labeled along their entire length after FISH or PRINS, since they contain blocks of ITSs (Fig. 5A) [20,28,142]. Centromeric breaks may also occur within the ITSs heterochromatic block but without apparent chromosome break (visualized as a split signal after telomere FISH or PRINS) or at the centromeric region of a chromosome containing heterochromatic ITSs but not directly involving ITSs themselves (the telomeric signal remains as a single signal) [28].

Amplification of ITSs is visualized as an increase in the number and/or the size and intensity of the ITSs hybridization signals after telomere FISH or PRINS compared with the normal telomeric hybridization pattern of the cell type or species being studied [55,59,116,129,131,132,143] (Fig. 5B). Spontaneous amplification of interstitial telomeric bands on specific marker chromosomes has been observed in different sub-clones of the CHO cell line by

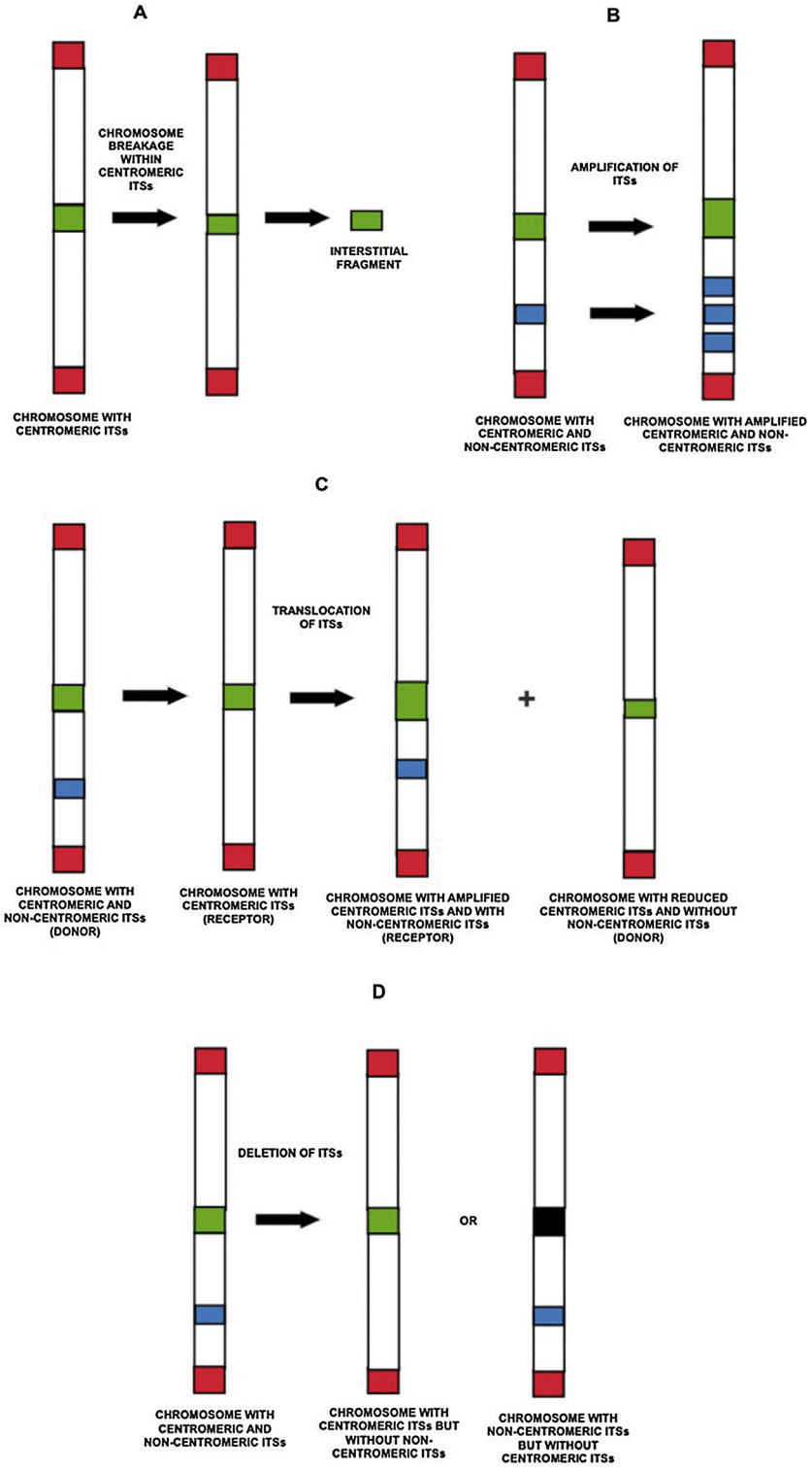

Fig. 5. Chromosomal aberrations directly involving ITSs (see Section 6.1 of the present review for more details). A) interstitial fragment (due to breakage at centromeric ITSs); B) amplification of centromeric and non-centromeric ITSs; C) translocation or transposition of centromeric and non-centormeric ITSs and D) deletion or loss of centromeric and non-centromeric ITSs. Red boxes represent the telomeres, black boxes represent the centromere, green boxes represent blocks of centromeric heterochromatic ITSs, and blue boxes represent non-centromeric ITSs present in the chromosomes involved in the aberration. Please, note that only representative cases of each type of aberration are shown.

several authors [59,116,128,129,143]. Different mechanisms have been proposed to explain amplification of ITSs, including unequal crossing over between repeats of sister chromatids, breakage-fusion-bridge cycles, hyper-recombinogenicity of ITSs, replication slippage, gene conversion and excision and reintegration events, i.e. the 'rolling circle' mechanism [18,20,28,142].

Translocation or transposition of ITSs means that a relocation of one or more pairs of ITSs signals (i.e., blocks of ITSs, one per

chromatid) compared with the normal telomeric hybridization pattern of the species or cell type being studied has taken place (Fig. 5C) [20,28,142].

Deletion or loss of ITSs can occur at the centromeric or interstitial regions of chromosomes containing ITSs, and can be total or partial. In the first case, the chromosome exhibits no telomeric signal at ITSs sites (even using a PNA probe) (Fig. 5D), whereas in the second case, the chromosome shows reduced hybridization signals after telomere FISH (either with a DNA or a PNA probe) or PRINS at ITSs sites [20,28,142]. Obviously, to detect loss or deletion of ITSs in clastogen-exposed cells, the normal pattern of distribution of these sequences in the cells under study must be known. Additionally, loss of ITSs during the karyotypic evolution of a given group of species can be detected by comparing the distribution pattern of ITSs between ancestral and descendant species.

6.2. Effects of clastogens on ITSs

Despite the spontaneous instability of ITSs, several studies demonstrated that clastogenic agents can induce ITSs instability, and provided further insights into the instability of ITSs sites in vertebrate chromosomes [28].

It has been shown that chromosome regions rich in heterochromatic ITSs are prone to breakage, fragility and recombination, both spontaneous and induced by ionizing radiation [23,59,129,130,143–149], restriction endonucleases [54], mitomycin C and teniposide (VM-26) [130], and the radiomimetic compounds bleomycin and streptonigrin [55,131,132]. The percentage of chromosomal aberrations involving ITSs was found to be higher than expected based on the percentage of the genome composed by telomeric sequences [55,129–132]. Moreover, telomeric FISH signals have been observed at the site of breakage in chromatid exchanges like tri- and quadriradials [130–132], which suggests that ITSs are directly involved in the breakage event. Despite the above observations, Desmaze et al. [128] studied the radiation sensitivity of a short ITSs localized in the human chromosome 2q31 and several human cell lines, and found that the presence of ITSs did not enhance the formation of radiation-induced chromosomal aberrations, indicating that ITSs are not preferentially involved in these aberrations. More recently, Revaud et al. [113] found that the exposure to 1 Gy of γ-radiation irradiation to hamster cells significantly increased the frequency of aberrations involving pericentromeric ITSs but had no effect on those ITSs located in the long arm of chromosome 9 (i.e., non-centromeric ITSs). They also found that DNA-PKcs prevent the formation of radiation-induced chromosomal aberrations in pericentromeric ITSs [113], indicating that these enzymes are involved in the repair of radiation-induced DSBs in these loci. In addition, these authors [113] proposed that alternative non-homologous end joining (A-NHEJ) mechanisms of repair are preferentially recruited at pericentromeric ITSs for the repair of radiation-induced DSBs.

Pandita and DeRubeis [150] exposed CHO cells to different DNA-damaging agents (including bleomycin at the same dose and time of exposure that those used in another study with Chinese hamster cell lines [55]) and DNA synthesis inhibitors, applied FISH with a telomeric DNA probe, and found that none of these treatments induced the acquisition of interstitial telomeric bands on marker chromosomes, suggesting that DNA damage itself do not induce amplification of ITSs. However, some of the abovementioned studies showed amplification of telomeric sequences at breakpoints and fragile sites [54,55,130] and more recently, Sánchez et al. [131,132], by using FISH with a telomeric PNA probe (a more efficient probe than the one used by Pandita and DeRubeis) and Quantititative FISH ("Q-FISH"), showed that the antibiotics bleomycin and streptonigrin can induce amplification of telomeric repeat sequences in CHO cells, expressed both as an increased in the number of ITSs FISH signals and in the size of ITSs. The amplification of telomeric sequences in CHO cells by bleomycin and streptonigrin seems to occur mainly in G1 or S phases of the cell cycle [131,132]. The underlying mechanism involved in the amplification of telomeric repeats by bleomycin and streptonigrin in Chinese hamster cells remains to be established, but one possible cause of the induced amplification could be hyper-recombinogenicity of ITSs following treatment with these compounds [146]. The amplification of ITSs induced by bleomycin and streptonigrin in Chinese hamster cells (CHE and CHO cell lines) is not accompanied by an increase in the activity of telomerase [55,131,132], at least in the short-term. These data strongly suggest that telomerase is not involved in the amplification of ITSs induced by these compounds.

On the other hand, it was also found that bleomycin and streptonigrin induce terminal as well as interstitial translocation of telomeric sequences, and chromosome breaks at centromeric regions rich in heterochromatic ITSs, although these regions are not the preferential target of the clastogenic action of these compounds [131,132]. Moreover, it has been observed that the involvement of heterochromatic ITSs in the aberrations induced by radiomimetic clastogens is not random. In effect, heterochromatic ITSs were found to be preferentially involved in the chromosome- and chromatid-type breaks and chromatid exchanges induced by bleomycin and streptonigrin compared with other types of unstable aberrations induced by these compounds [131,132]. In addition, most of the chromosome breaks involving ITSs in CHO cells induced by bleomycin occur at the centromeric region of chromosomes, whereas in streptonigrin-exposed cells these breaks occur outside the centromere [131,132]. More recently, it was found that the methylating compound streptozotocin induces the formation of acentric fragments, additional telomeric FISH signals (which implies amplification and/or translocation of ITSs) and centromeric breaks involving ITSs in CHO cells [151]. However, these telomeric-like sequences are not preferentially involved in the chromosome damage induced by streptozotocin, since the percentage of aberrations involving ITSs did not differ between control and exposed cells [151]. Moreover, no effect of streptozotocin on telomerase activity in CHO cells was found 18 h after treatment, indicating that this enzyme is not involved in the ITSs instability induced by this compound [151].

Overall, the studies performed so far concerning the effects of clastogens on ITSs show that the involvement of these telomeric-like sequences in the induced chromosomal aberrations and the sensitivity of ITSs to clastogens depend on the size, location and chromatin structure of the ITSs loci involved, as well as the clastogen and the cell type exposed to it. Moreover, at least in the short-term, telomerase activity seems to be unrelated to the ITSs instability caused by chemical mutagens, since none of the clastogens tested so far produced alterations of the enzyme activity [55,131,132,151].

Two studies carried out a few years ago in our laboratory provided the first evidence of long-term instability of ITSs caused by clastogens, showing that bleomycin and streptonigrin induce delayed instability of ITSs in CHO cells [152,153]. This instability was cytogenetically detectable as additional (new) telomere FISH signals (bleomycin and streptonigrin) or centromeric breaks involving dissociation of the telomeric signal (streptonigrin) 6 days after treatment [152,153]. These effects probably resulted from breakage of heterochromatic ITSs blocks and further insertion of these sequences at the sites of mono- or isochromatid breaks occurring at G2 or G1-S phases of the cell cycle respectively, since most of the additional FISH signals were present as single (bleomycin and streptonigrin) or double (streptonigrin) dots and

located at interstitial sites of the involved chromosomes [152,153]. It is noteworthy to mention that the observed instability was a temporary effect, since it was no longer present in CHO cells 15 days after treatment. Further studies will be needed to established the causes and the significance of the delayed instability of ITSs.

7. Conclusions: importance and biological functions of ITSs

Despite ITSs do not work as functional telomeres and their biological functions have not yet been clearly elucidated, the studies reviewed here show that these telomeric-like sequences play a significant role in genome instability and chromosomal evolution. In effect, ITSs have been associated with chromosomal rearrangements, fragile sites and recombination hot spots [18–20,28,118,142]. Particularly, heterochromatic ITSs are involved in chromosome fusions, fissions and inversions, promoting the formation of new telomeres, and can undergo several types of rearrangements, including breakage, amplification, translocation or deletion, which can lead to the development of new karyotypes and new species, thus favoring karyotypic evolution, in line with the recent "centromere-from-telomere hypothesis" and the notion of "chromosome plasticity" [57,58]. A recent work in three hamster species of the genus *Phodopus* clearly demonstrates that ITSs play a role in the reshaping of karyotypes [40]. Thus, heterochromatic ITSs seem to have played a significant role in karyotypic evolution in vertebrates. However, heterochromatic ITSs are not always correlated with the rearrangements which occurred during karyotypic evolution in vertebrate species, and in several cases their presence in the chromosomes simply reflects the fact that these sequences are a component of the centromeric satellite DNA [34,35,42,69–74]. Moreover, the recent findings of the coexistence of vertebrate (TTAGGG) and *Arabidopsis*-like (TTTAGGG) telomeric sequences at the pericentromeric ITSs of pig chromosome 6 and in the ITSs region of several different chromosomes in plants from the Hyacinthaceae and Amaryllidaceae families [154,155], suggest that plant and vertebrate telomeres share certain characteristics and open new horizons in the study of telomere evolution. In addition, short ITSs can be used as markers for phylogenetic studies, since they behave as genetic mobile elements and can be considered as rare genomic changes (i.e., they are unlikely to arise multiple times independently in evolution), exhibiting low levels of homoplasy (character state similarity not due to shared descent, i.e., produced by convergent evolution or evolutionary reversal), thus being a good tool to determine common ancestry [19]. Moreover, there is a direct relationship between the percentage of short ITSs conserved in different vertebrate species and the time of divergence between them: the closer the species are (like, for example, humans and chimps), the higher the percentage of ITSs conserved (human and chimps share 90%, whereas mouse and rat only share 24% of short ITSs) [19].

According with the recent "centromere-from-telomere" hypothesis, telomeres evolved as the first functional element of the eukaryotic chromosome, whereas the centromere emerged from the telomere [57,58]. The emergence and subsequent rearrangements of ITSs in the chromosomes of vertebrates perfectly fit with this scenario. Also, the fact that ITSs are naturally prone to breakage is in line with the notion of chromosome plasticity, in which centric fissions play a major role [58].

On the other hand, ITSs exhibit polymorphism in terms of both sequence and copy number (reflected by the different numbers of alleles determined for each ITSs locus) [18], are associated to fragile sites, are involved in DNA DSBs repair, and influence the clastogenic effect of mutagens. These properties of ITSs makes them a good endpoint for medical or clinical research, including the study of hereditary diseases (chromosome instability syndromes), the study of cancer (detection of genetic instability in tumors) and the development of markers for linkage studies and forensic genetics. For example, the ITSs at 22q11.2 identified in the human genome by Yan et al. [27] using PRINS, is associated with hotspots for disease-related chromosome breaks for multiple disorders, such as DiGeorge syndrome and chronic myeloid leukemia [156,157]. Moreover, deletion and translocation of ITSs were frequently found in gastric carcinoma cells [158] and ITSs have been implicated in several cases of mosaicism for autosomal structural rearrangements in humans (see [114] and references therein). The recent finding of ITSs in human BRCA2 deficient breast tumors and cell lines suggests that these telomeric-like sequences play a role in chromosomal instability related to carcinogenesis [159].

Regarding the possible biological function of ITSs, some studies suggest that heterochromatic ITSs may undergo epigenetic modifications (like a higher nucleosome density compared with non-telomeric chromatin, the association of ITSs with telomeric proteins, formation of interstitial telomere loops, etc.) affecting gene expression in particular cell lineages [19,25,46]. It has been proposed that telomeric proteins possibly regulate gene expression through looping mechanisms or by modifying the chromatin landscape [51]. Cellular levels of TRF1 and TRF2 proteins could influence their binding to ITSs and thus the expression of neighboring genes [51]. Further epigenetic and functional analyses in vertebrate cells are required to better clarify the constitution and functional significance of pericentromeric ITSs chromatin.

For short ITSs no direct indication on any particular function has been provided so far. Current knowledge supports the view that short ITSs could simply represent "relics" of ancient breakage within fragile sites that occurred in the germ line during evolution [19].

Thus, future research should focus on the possible biological significance of ITSs by investigating the role of these telomeric-like sequences in the regulation of gene expression (especially those genes close to the ITSs sites), in meiotic and mitotic recombination and in DNA repair occurring in ITSs-rich chromosome regions. Further understanding of ITSs biology and functions is fundamental to get a full picture of the genomic instability and DNA repair phenomena, and karyotypic evolution as well. Finally, despite the recent evidence provided by studies about the BIR and TTI mechanisms, further studies are needed to determine the precise mechanism underlying the evolutionary origin if ITSs, especially short and subtelomeric ITSs, and if telomerase played some role in the insertion of ITSs in the chromosomes of vertebrates during their karyotypic evolution.

Conflict of interest statement

The author declares that there are no conflicts of interest.

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References

[1] R.J. O'Sullivan, J. Karlseder, Telomeres: protecting chromosomes against genome instability, Nat. Rev. Mol. Cell Biol. 11 (2010) 171–181.
[2] E.H. Blackburn, Switching and signaling at the telomere, Cell 106 (2001) 661–673.
[3] 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.
[4] J. Meyne, R.J. Baker, H.H. Hobart, T.C. Hsu, O.A. Ryder, O.G. Ward, J.E. Wiley, D. H. Wurster-Hill, T.L. Yates, R.K. Moyzis, Distribution of nontelomeric sites of

(TTAGGG)n telomeric sequences in vertebrate chromosomes, Chromosoma 99 (1990) 3–10.
[5] N.M. Gomes, J.W. Shay, W.E. Wright, Telomere biology in metazoa, FEBS Lett. 584 (2010) 3741–3751.
[6] I. Schmutz, T. de Lange, Shelterin, Curr. Biol. 26 (2016) R397–R399.
[7] C.M. Azzalin, J. Lingner, Telomeres: the silence is broken, ABBV Cell Cycle 7 (2008) 1161–1165.
[8] B. Luke, J. Lingner, TERRA: telomeric repeat-containing RNA, EMBO J. 28 (2009) 2503–2510.
[9] S. Feuerhahn, N. Iglesias, A. Panza, A. Porro, J. Lingner, TERRA biogenesis, turnover and implications for function, FEBS Lett. 584 (2010) 3812–3818.
[10] E. Cusanelli, P. Chartrand, Telomeric repeat-containing RNA TERRA: a noncoding RNA connecting telomere biology to genome integrity, Front. Genet. 6 (2015) 1–9.
[11] J.J. Montero, I. López de Silanes, O. Graña, M.A. Blasco, Telomeric RNAs are essential to maintain telomeres, Nat. Commun. 7 (2016) 12534.
[12] 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, 1st ed., New York, Nova Science, 2010, pp. 35–58.
[13] M.A. Jafri, S.A. Ansari, M.H. Alqahtani, J.W. Shay, Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies, Genome Med. 8 (2016) 69.
[14] Z. Zhao, X. Pan, L. Liu, N. Liu, Telomere length maintenance shortening, and lengthening, J. Cell. Physiol. 229 (2014) 1323–1329.
[15] J.A. Londoño-Vallejo, H. Der-Sarkissian, L. Cazes, S. Bacchetti, R. Reddel, Alternative lengthening of telomeres is characterized by high rates of telomeric exchange, Cancer Res. 64 (2004) 2324–2327.
[16] D. Conomos, H.A. Pickett, R.R. Reddel, Alternative lengthening of telomeres: remodeling the telomere architecture, Front. Oncol. 3 (2013) 27.
[17] P. Marzec, C. Armenise, G. Pérot, F.-M. Roumelioti, E. Basyuk, S. Gagos, F. Chibon, J. Déjardin, Nuclear-receptor-mediated telomere insertion leads to genome instability in ALT cancers, Cell 160 (2015) 913–927.
[18] K.W. Lin, J. Yan, Endings in the middle: current knowledge of interstitial telomeric sequences, Mutat. Res. 658 (2008) 95–110.
[19] A. Ruíz-Herrera, S.G. Nergadze, M. Santagostino, E. Giulotto, Telomeric repeats far from the ends: mechanisms of origin and role in evolution, Cytogenet. Genome Res. 122 (2008) 219–228.
[20] A.D. Bolzán, M.S. Bianchi, Telomeres interstitial telomeric repeat sequences, and chromosomal aberrations, Mutat. Res. 612 (2006) 189–214.
[21] S.G. Nergadze, M.A. Santagostino, A. Salzano, C. Mondello, E. Giulotto, Contribution of telomerase RNA retrotranscription to DNA double-strand break repair during mammalian genome evolution, Genome Biol. 8 (2007) R260.
[22] Y. Zou, X. Yi, W.E. Wright, J.W. Shay, Human telomerase can immortalize Indian muntjac cells, Exp. Cell Res. 281 (2002) 63–76.
[23] A.S. Balajee, I. Dominguez, V.A. Bohr, A.T. Natarajan, Immunofluorescent analysis of the organization of telomeric DNA sequences and their involvement in chromosomal aberrations in hamster cells, Mutat. Res. 372 (1996) 163–172.
[24] A.M. Wood, J.M. Rendtlew Danielsen, C.A. Lucas, E.L. Rice, D. Scalzo, T. Shimi, R.D. Goldman, E.D. Smith, M.M. Le Beau, S.T. Kosak, TRF2 and lamin A/C interact to facilitate the functional organization of chromosome ends, Nat. Commun. 5 (2014) 5467.
[25] M.A. Wood, K. Laster, E.L. Rice, S.T. Kosak, A beginning of the end: new insights into the functional organization of telomeres, Nucleus 6 (2015) 172–178.
[26] S.S. Poon, U.M. Martens, R.K. Ward, P.M. Lansdorp, Telomere length measurements using digital fluorescence microscopy, Cytometry 36 (1999) 267–278.
[27] J. Yan, E.F. Bouchard, O. Samassekou, B.Z. Chen, Identification of a human chromosome-specific interstitial telomere-like sequence (ITS) at 22q11.2 using double-strand PRINS, Cytogenet. Genome Res. 116 (2007) 29–37.
[28] A.D. Bolzán, Chromosomal aberrations involving telomeres and interstitial telomeric sequences, Mutagenesis 27 (2012) 1–15.
[29] C.G. Foote, D. Vleck, C.M. Vleck, Extent and variability of interstitial telomeric sequences and their effects on estimates of telomere length, Mol. Ecol. Resour. 13 (2013) 417–428.
[30] C.M. Azzalin, S.G. Nergadze, E. Giulotto, Human intrachromosomal telomeric-like repeats: sequence organization and mechanisms of origin, Chromosoma 110 (2001) 75–82.
[31] S.G. Nergadze, M. Rocchi, C.M. Azzalin, C. Mondello, E. Giulotto, Insertion of telomeric repeats at intrachromosomal break sites during primate evolution, Genome Res. 14 (2004) 1704–1710.
[32] C.M. Azzalin, E. Mucciolo, L. Bertoni, E. Giulotto, Fluorescence in situ hybridization with a synthetic (T2AG3)n polynucleotide detects several intrachromosomal telomere-like repeats on human chromosomes, Cytogenet. Cell Genet. 78 (1997) 112–115.
[33] L. Bertoni, C. Attolini, M. Faravelli, S. Simi, E. Giulotto, Intrachromosomal telomere-like DNA sequences in Chinese hamster, Mamm. Genome 7 (1996) 853–855.
[34] M.T. Rovatsos, J.A. Marchal, I. Romero-Fernández, F.J. Fernández, E.B. Giagia-Athanosopoulou, A. Sánchez, Rapid, independent, and extensive amplification of telomeric repeats in pericentromeric regions in karyotypes of arvicoline rodents, Chromosome Res. 19 (2011) 869–882.
[35] V.J. Swier, F.A. Anwarali Khan, R.J. Baker, Do time, heterochromatin NORs, or chromosomal rearrangements correlate with distribution of interstitial telomeric repeats in Sigmodon (cotton rats)? J. Hered. 103 (2012) 493–502.
[36] M.G. Tsipouri, S. Hu, NISC Comparative Sequencing Program, A. Dutra, E. Pak, H. Riethman, E.D. Green, Comparative sequence analyses reveal sites of ancestral chromosomal fusions in the Indian muntjac genome, Genome Biol. 9 (2008) R155.
[37] N. Hartmann, S. Scherthan, Characterization of ancestral chromosome fusion points in the Indian muntjac deer, Chromosoma 112 (2004) 213–220.
[38] C.J. Metcalfe, M.D. Eldridge, L.R. McQuade, P.G. Johnston, Mapping the distribution of the telomeric sequence (T2AG3)n in rock-wallabies, Petrogale (Marsupialia: macropodidae) by fluorescence in situ hybridization. I. The penicillata complex, Cytogenet. Cell Genet. 78 (1997) 74–80.
[39] C.J. Metcalfe, M.D. Eldridge, R. Toder, P.G. Johnston, Mapping the distribution of the telomeric sequence (T2AG3)n in the Macropodoidea (Marsupialia), by fluorescence in situ hybridization. I. The swamp wallaby Wallabia bicolour, Chromosome Res. 6 (1998) 603–610.
[40] A. Paço, R. Chaves, A. Vieira-da-Silva, F. Adega, The involvement of repetitive sequences in the remodelling of karyotypes: the *Phodopus* genomes (Rodentia Cricetidae), Micron 46 (2013) 27–34.
[41] C. Lanzone, C. Labaroni, N. Suárez, D. Rodríguez, M.L. Herrera, A.D. Bolzán, Distribution of telomeric sequences (TTAGGG)n in rearranged chromosomes of phyllotine rodents (Cricetidae, sigmodontinae), Cytogenet. Genome Res. 147 (2015) 247–252.
[42] P.F. Viana, L.B. Ribeiro, G.M. Souza, M. Chalkidis Hde, M.C. Gross, E. Feldberg, Is the karyotype of neotropical boid snakes really conserved? Cytotaxonomy, chromosomal rearrangements and karyotype organization in the boidae family, PLoS One 11 (2016) e0160274.
[43] I. Nanda, D. Schrama, W. Feichtinger, T. Haaf, M. Schartl, M. Schmid, Distribution of telomeric (TTAGGG)(n) sequences in avian chromosomes, Chromosoma 111 (2002) 215–227.
[44] R. de la Fuente, M. Manterola, A. Viera, M.T. Parra, M. Alsheimer, J.S. Rufas, J. Page, Chromatin organization and remodeling of interstitial telomeric sites during meiosis in the Mongolian gerbil (*Meriones unguiculatus*), Genetics 197 (2014) 1137–1151.
[45] M. Faravelli, C.M. Azzalin, L. Bertoni, O. Chernova, C. Attolini, C. Mondello, E. Giulotto, Molecular organization of internal telomeric sequences in Chinese hamster chromosomes, Gene 283 (2002) 11–16.
[46] D. Revaud, J. Mozziconacci, L. Sabatier, C. Desmaze, C. Lavelle, Sequence-driven telomeric chromatin structure, Cell Cycle 8 (2009) 1099–1100.
[47] F.M. Gámez-Arjona, C. López-López, M.I. Vaquero-Sedas, M.A. Vega-Palas, On the organization of the nucleosomes associated with telomeric sequences, Biochim. Biophys. Acta 1803 (2010) 1058–1061.
[48] V.A. Zakian, Telomeres: beginning to understand the end, Science 270 (1995) 1601–1607.
[49] C. Mignon-Ravix, D. Depetris, B. Delobel, M.F. Croquette, M.G. Mattei, A human interstitial telomere associates in vivo with specific TRF2 and TIN2 proteins, Eur. J. Hum. Genet. 10 (2002) 107–112.
[50] R.I. Krutilina, A.N. Smirnova, O.S. Mudrak, N.M. Pleskach, M.P. Svetlova, S.L. Oei, P.M. Yau, E.M. Bradbury, A.O. Zalensky, N.V. Tomilin, Protection of internal (TTAGGG)n repeats in Chinese hamster cells by telomeric protein TRF1, Oncogene 22 (2003) 6690–6698.
[51] T. Simonet, L.E. Zaragosi, C. Philippe, K. Lebrigand, C. Schouteden, A. Augereau, S. Bauwens, J. Ye, M. Santagostino, E. Giulotto, F. Magdinier, B. Horard, P. Barbry, R. Waldmann, E. Gilson, The human TTAGGG repeat factors 1 and 2 bind to a subset of interstitial telomeric sequences and satellite repeats, Cell Res. 21 (2011) 1028–1038.
[52] N. Bosco, T.A. de Lange, A TRF1-controlled common fragile site containing interstitial telomeric sequences, Chromosoma 121 (2012) 465–474.
[53] M. Faravelli, D. Moralli, L. Bertoni, C. Attolini, O. Chernova, E. Raimondi, E. Giulotto, Two extended arrays of a satellite DNA sequence at the centromere and at the short-arm telomere of Chinese hamster chromosome 5, Cytogenet. Cell Genet. 83 (1998) 281–286.
[54] A.S. Balajee, H.J. Oh, A.T. Natarajan, Analysis of restriction enzyme-induced chromosome aberrations in the interstitial telomeric repeat sequences of CHO and CHE cells by FISH, Mutat. Res. 307 (1994) 307–313.
[55] A.D. Bolzán, G.L. Páez, M.S. Bianchi, FISH analysis of telomeric repeat sequences and their involvement in chromosomal aberrations induced by radiomimetic compounds in hamster cells, Mutat. Res. 479 (2001) 187–196.
[56] J.E. Wiley, J. Meyne, M.L. Little, J.C. Stout, Interstitial hybridization sites of the (TTAGGG)n telomeric sequence on the chromosomes of some North American hylid frogs, Cytogenet. Cell Genet. 61 (1992) 55–57.
[57] A. Villasante, J.P. Abad, M. Méndez-Lago, Centromeres were derived from telomeres during the evolution of the eukaryotic chromosome, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 10542–10547.
[58] P. Slijepcevic, Mechanisms of the evolutionary chromosome plasticity: integrating the centromere-from-telomere hypothesis with telomere length regulation, Cytogenet. Genome Res. 148 (2016) 268–278.
[59] P. Slijepcevic, Y. Xiao, I. Dominguez, A.T. Natarajan, Spontaneous and radiation-induced chromosomal breakage at interstitial telomeric sites, Chromosoma 104 (1996) 596–604.
[60] S.T. Sfeir, D. Kosiyatrakul, S.L. Hockemeyer, J. MacRae, C.L. Schildkraut, T. de Lange, Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication, Cell 138 (2009) 90–103.
[61] M. Fumagalli, F. Rossiello, M. Clerici, S. Barozzi, D. Cittaro, J.M. Kaplunov, G. Bucci, M. Dobreva, V. Matti, C.M. Beausejour, U. Herbig, M.P. Longhese, F.

d'Adda di Fagagna, Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation, Nat. Cell Biol. 14 (2012) 355–365.
[62] P. Slijepcevic, Telomeres and mechanisms of Robertsonian fusion, Chromosoma 107 (1998) 136–140.
[63] Y.C. Li, C. Lee, D. Sanoudou, T.H. Hsu, S.Y. Li, C.C. Lin, Interstitial colocalization of two cervid satellite DNAs involved in the genesis of the Indian muntjac karyotype, Chromosome Res. 8 (2000) 363–373.
[64] R. Matoso Silva, F. Adega, H.J. Kjöllerström, K. Labuschagne, A. Kotze, C. Fernandes, R. Chaves, M. do Mar Oom, Classical and molecular cytogenetics of the panther genet genetta maculata (Mammalia carnivora, viverridae), Cytogenet. Genome Res. 149 (2016) 274–281.
[65] M. Rovatsos, M. Johnson Pokorná, M. Altmanová, L. Kratochvíl, Mixed-Up sex chromosomes: identification of sex chromosomes in the X1 × 1 × 2 × 2/X1 × 2Y system of the legless lizards of the genus lialis (Squamata: gekkota: pygopodidae), Cytogenet. Genome Res. 149 (2016) 282–289.
[66] N. Hartmann, H. Scherthan, Characterization of ancestral chromosome fusion points in the Indian muntjac deer, Chromosoma 112 (2004) 213–220.
[67] J.X. Chi, L. Huang, W. Nie, J. Wang, B. Su, F. Yang, Defining the orientation of the tandem fusions that occurred during the evolution of Indian muntjac chromosomes by BAC mapping, Chromosoma 114 (2005) 167–172.
[68] A. Ropiquet, A. Hassanin, E. Pagacova, M. Gerbault-Seureau, H. Cernohorska, S. Kubickova, C. Bonillo, J. Rubes, T.J. Robinson, A paradox revealed: karyotype evolution in the four-horned antelope occurs by tandem fusion (Mammalia Bovidae, Tetracerus quadricornis), Chromosome Res. 18 (2010) 277–286.
[69] C.J. Metcalfe, M.D.B. Eldridge, P.G. Johnston, Mapping the distribution of the telomeric sequence (T2AG3)n in the 2n = 14 ancestral marsupial complement and in the macropodines (Marsupialia: macropodidae) by fluorescence in situ hybridization, Chromosome Res. 12 (2004) 405–414.
[70] C.J. Metcalfe, M.D. Eldridge, P.G. Johnston, Mapping the distribution of the telomeric sequence (T2AG3)n in the Macropodoidea (Marsupialia) by fluorescence in situ hybridization. II. The ancestral 2n = 22 macropodid karyotype, Cytogenet. Genome Res. 116 (2007) 212–217.
[71] I. Nanda, M. Fugate, C. Steinlein, M. Schmid, Distribution of (TTAGGG)n telomeric sequences in karyotypes of the Xenopus species complex, Cytogenet. Genome Res. 122 (2008) 396–400.
[72] D.P. Bruschi, M. Rivera, A.P. Lima, A.B. Zúñiga, S.M. Recco-Pimentel, Interstitial Telomeric Sequences (ITS) and major rDNA mapping reveal insights into the karyotypical evolution of Neotropical leaf frogs species (Phyllomedusa Hylidae, Anura), Mol. Cytogenet. 7 (2014) 22.
[73] S. Calixto Mda, I.S. de Andrade, D.C. Cabral-de-Mello, N. Santos, C. Martins, V. Loreto, M.J. de Souza, Patterns of rDNA and telomeric sequences diversification: contribution to repetitive DNA organization in Phyllostomidae bats, Genetica 142 (2014) 49–58.
[74] E.Y. Suárez-Villota, R.E. Haro, R.A. Vargas, M.H. Gallardo, The ancestral chromosomes of Dromiciops gliroides (Microbiotheridae), and its bearings on the karyotypic evolution of American marsupials, Mol. Cytogenet. 9 (2016) 59.
[75] U. Arnason, B. Widegren, Composition and chromosomal localization of cetacean highly repetitive DNA with special reference to the blue whale, Balaenoptera musculus, Chromosoma 98 (1989) 323–329.
[76] J.A. Adegoke, U. Arnason, B. Widegren, Sequence organization and evolution in all extant whalebone whales, of a satellite DNA with terminal chromosome localization, Chromosoma 102 (1993) 382–388.
[77] M.A. Garrido-Ramos, R. Herrán, R. Rejón, M.R. Rejón, A satellite DNA of the Sparidae family (Pisces, Perciformes) associated with telomeric sequences, Cytogenet. Cell Genet. 83 (1998) 3–9.
[78] K. Ocalewicz, Telomeres in fishes, Cytogenet. Genome Res. 141 (2013) 114–125.
[79] A. Śliwińska-Jewsiewicka, M. Kuciński, L. Kirtiklis, S. Dobosz, K. Ocalewicz, M. Jankun, Chromosomal characteristics and distribution of rDNA sequences in the brook trout Salvelinus fontinalis (Mitchill, 1814), Genetica 143 (2015) 425–432.
[80] S. Venkatesan, A.T. Natarajan, M.P. Hande, Chromosomal instability-mechanisms and consequences, Mutat. Res. 793 (2015) 176–184.
[81] J.P. Hanish, J.L. Yanowitz, T. de Lange, Stringent sequence requirements for the formation of human telomeres, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 8861–8865.
[82] A.Y. Aksenova, P.W. Greenwell, M. Dominska, A.A. Shishkin, J.C. Kim, T.D. Petes, S.M. Mirkin, Genome rearrangements caused by interstitial telomeric sequences in yeast, PNAS 49 (2013) 19866–19871.
[83] L. Costantino, S.K. Sotiriou, J.K. Rantala, S. Magin, E. Mladenov, T. Helleday, J.E. Haber, G. Iliakis, O. Kallioniemi, T.D. Halazonetis, Break-induced replication repair of damaged forks induces genomic duplications in human cells, Science 343 (2014) 88–91.
[84] A.Y. Aksenova, G. Han, A.A. Shishkin, K.V. Volkov, S.M. Mirkin, Expansion of interstitial telomeric sequences in yeast, Cell Rep. 13 (2015) 1545–1551.
[85] J.C. Kim, S.T. Harris, T. Dinter, K.A. Shah, S.M. Mirkin, The role of break-induced replication in large-scale expansions of (CAG)n/(CTG)n repeats, Nat. Struct. Mol. Biol. 23 (2017) 55–60.
[86] J. Kramara, B. Osia, A. Malkova, Break-induced replication: an unhealthy choice for stress relief? Nat. Struct. Mol. Biol. 24 (2017) 11–12.
[87] R.P. Anand, S.T. Lovett, J.E. Haber, Break-induced DNA replication, Cold Spring Harb. Perspect. Biol. 5 (2013) a010397.
[88] F.M. Roumelioti, S.K. Sotiriou, V. Katsini, M. Chiourea, T.D. Halazonetis, S. Gagos, Alternative lengthening of human telomeres is a conservative DNA replication process with features of break-induced replication, EMBO Rep. 17 (2016) 1731–1737.
[89] C. Mondello, L. Pirzio, C.M. Azzalin, E. Giulotto, Instability of interstitial telomeric sequences in the human genome, Genomics 68 (2000) 111–117.
[90] P.J. Hastings, G. Ira, J.R. Lupski, A microhomology-mediated break-induced replication model for the origin of human copy number variation, PLoS Genet. 5 (2009) e1000327.
[91] P.J. Hastings, J.R. Lupski, S.M. Rosenberg, G. Ira, Mechanisms of change in gene copy number, Nat. Rev. Genet. 10 (2009) 551–564.
[92] M.R. Lowden, S. Flibotte, D.G. Moerman, S. Ahmed, DNA synthesis generates terminal duplications that seal end-to-end chromosome fusions, Science 332 (2011) 468–471.
[93] S.A. Yatsenko, P. Hixson, E.K. Roney, D.A. Scott, C.P. Schaaf, Y.T. Ng, R. Palmer, R. B. Fisher, A. Patel, S.W. Cheung, J.R. Lupski, Human subtelomeric copy number gains suggest a DNA replication mechanism for formation: beyond breakage-fusion-bridge for telomere stabilization, Hum. Genet. 131 (2012) 1895–1910.
[94] M.F. Arlt, S. Rajendran, S.R. Birkeland, T.E. Wilson, T.W. Glover, *De novo* CNV formation in mouse embryonic stem cells occurs in the absence of Xrcc4-dependent nonhomologous end joining, PLoS Genet. 8 (2012) e1002981.
[95] D.F. Conrad, C. Bird, B. Blackburne, S. Lindsay, L. Mamanova, C. Lee, D.J. Turner, M.E. Hurles, Mutation spectrum revealed by breakpoint sequencing of human germline CNVs, Nat. Genet. 42 (2010) 385–391.
[96] Y. Wang, P. Su, B. Hu, W. Zhu, Q. Li, P. Yuan, J. Li, X. Guan, F. Li, X. Jing, R. Li, Y. Zhang, C. Férec, D.N. Cooper, J. Wang, D. Wang, J.M. Chen, Y. Wang, Characterization of 26 deletion CNVs reveals the frequent occurrence of micro-mutations within the breakpoint-flanking regions and frequent repair of double-strand breaks by templated insertions derived from remote genomic regions, Hum. Genet. 134 (2015) 589–603.
[97] C.J. Sakofsky, S. Ayyar, A.K. Deem, W.-H. Chung, G. Ira, A. Malkova, Translesion polymerases drive microhomology-mediated break-induced replication leading to complex chromosomal rearrangements, Mol. Cell. 60 (2015) 860–872.
[98] M. Rovatsos, L. Kratochvíl, M. Altmanová, M. Johnson Pokorná, Interstitial telomeric motifs in squamate reptiles: when the exceptions outnumber the rule, PLoS One 10 (2015) e0134985.
[99] J.R. Vermeesch, W. De Meurichy, H. Van Den Berghe, P. Marynen, P. Petit, Differences in the distribution and nature of the interstitial telomeric (TTAGGG)n sequences in the chromosomes of the Giraffidae, okapai (Okapia johnstoni), and giraffe (Giraffa camelopardalis): evidence for ancestral telomeres at the okapi polymorphic rob(5;26) fusion site, Cytogenet. Cell Genet. 72 (1996) 310–315.
[100] M. Lizarralde, A.D. Bolzán, M.S. Bianchi, Karyotypic evolution in south american subterranean rodents *Ctenomys magellanicus* (Rodentia Octodontidae): chromosome rearrangements and (TTAGGG)n telomeric sequence localization in 2n = 34 and 2n = 36 chromosomal forms, Hereditas 139 (2003) 13–17.
[101] M.S. Lizarralde, A.D. Bolzán, S. Poljak, M.I. Pigozzi, J. Bustos, M.S. Merani, Chromosomal localization of the telomeric (TTAGGG)n sequence in four species of Armadillo (Dasypodidae) from Argentina: an approach to explaining karyotype evolution in the Xenarthra, Chromosome Res. 13 (2005) 777–784.
[102] M.D. Mudry, M. Nieves, A.D. Bolzán, Chromosomal localization of the telomeric (TTAGGG)n sequence in eight species of New World Primates (Neotropical Primates, Platyrrhini), Cytogenet. Genome Res. 119 (2007) 221–224.
[103] E.R. Steinberg, L. Cortés-Ortiz, M. Nieves, A.D. Bolzán, F. García-Orduña, J. Hermida-Lagunes, D. Canales-Espinosa, M.D. Mudry, The karyotype of *Alouatta pigra* (Primates: platyrrhini): mitotic and meiotic analyses, Cytogenet. Genome Res. 122 (2008) 103–109.
[104] P.A. Martinez, J.M. Boeris, J. Sánchez, M.C. Pastori, A.D. Bolzán, M.A. Ledesma, Karyotypic characterization of *Trachemys dorbigni* (Testudines: emydidae) and *Chelonoidis (Geochelone) donosobarrosi* (Testudines: testudinidae) two species of cryptodiran turtles from Argentina, Genetica 137 (2009) 277–283.
[105] J. Sánchez, L. Alcalde, A.D. Bolzán, First evidence of chromosomal variation within *Chelonoidis chilensis* (Testudines: testudinidae), Herpetol. J. 25 (2015) 83–89.
[106] E. Gornung, A.M. Bezerra, R. Castiglia, Comparative chromosome mapping of the rRNA genes and telomeric repeats in three Italian pine voles of the Microtus savii s.l. complex (Rodentia, Cricetidae), Comp. Cytogenet. 5 (2011) 247–257.
[107] K. Matsubara, Y. Uno, K. Srikulnath, Y. Matsuda, E. Miller, M. Olsson, No interstitial telomeres on autosomes but remarkable amplification of telomeric repeats on the W sex chromosome in the sand lizard (Lacerta agilis), J. Hered. 106 (2015) 753–757.
[108] M.A. Blasco, H.W. Lee, M.P. Hande, E. Samper, P.M. Lansdorp, R.A. DePinho, C. W. Greider, Telomere shortening and tumor formation by mouse cells lacking telomerase RNA, Cell 91 (1997) 25–34.
[109] R.A. Sánchez-Guillén, L. Capilla, R. Reig-Viader, M. Martínez-Plana, C. Pardo-Camacho, M. Andrés-Nieto, J. Ventura, A. Ruiz-Herrera, On the origin of Robertsonian fusions in nature: evidence of telomere shortening in wild house mice, J. Evol. Biol. 28 (2015) 241–249.
[110] S. Garagna, D. Broccoli, C.A. Redi, J.B. Searle, H.J. Cooke, E. Capanna, Robertsonian metacentrics of the house mouse lose telomeric sequences but retain some minor satellite DNA in the pericentromeric area, Chromosoma 103 (1995) 685–692.

[111] I. Nanda, S. Schneider-Rasp, H. Winking, M. Schmid, Loss of telomeric sites in the chromosomes of Mus musculus domesticus (Rodentia: muridae) during Robertsonian rearrangements, Chromosome Res. 3 (1995) 399–409.
[112] D.J. Amor, K.H. Choo, Neocentromeres: role in human disease, evolution, and centromere study, Am. J. Hum. Genet. 71 (2002) 695–714.
[113] D. Revaud, L.M. Martins, F.D. Boussin, L. Sabatier, C. Desmaze, Different DNA-PKcs functions in the repair of radiation-induced and spontaneous DSBs within interstitial telomeric sequences, Chromosoma 120 (2011) 309–319.
[114] J. Lévy, A. Receveur, G. Jedraszak, S. Chantot-Bastaraud, F. Renaldo, J. Gondry, J. Andrieux, H. Copin, J.P. Siffroi, M.F. Portnoï, Involvement of interstitial telomeric sequences in two new cases of mosaicism for autosomal structural rearrangements, Am. J. Med. Genet. A 67A (2015) 428–433.
[115] A.E. Kilburn, M.J. Shea, R.G. Sargent, J.H. Wilson, Insertion of a telomere repeat sequence into a mammalian gene causes chromosome instability, Mol. Cell. Biol. 21 (2001) 126–135.
[116] L. Bertoni, C. Attolini, L. Tessera, E. Mucciolo, E. Giulotto, Telomeric and nontelomeric (TTAGGG)n sequences in gene amplification and chromosome stability, Genomics 24 (1994) 53–62.
[117] M.T. Rivero, A. Mosquera, V. Goyanes, P. Slijepcevic, J.L. Fernandez, Differences in repair profiles of interstitial telomeric sites between normal and DNA double-strand break repair deficient Chinese hamster cells, Exp. Cell Res. 295 (2004) 161–172.
[118] A.V. Barros, M.A. Vier Wolski, V. Nogaroto, M.C. Almeida, O. Moreira-Filho, M. R. Vicari, Fragile sites, dysfunctional Telomere and chromosome fusions: what is 5S rDNA role? Gene 608 (2017) 20–27.
[119] V.M. Park, K.M. Gustashaw, T.M. Wathen, The presence of interstitial telomeric sequences in constitutional chromosome abnormalities, Am. J. Hum. Genet. 50 (1992) 914–923.
[120] E. Rossi, G. Floridia, M. Casali, C. Danesino, G. Chiumello, F. Bernardi, I. Magnani, L. Papi, M. Mura, O. Zuffardi, Types stability, and phenotypic consequences of chromosome rearrangements leading to interstitial telomeric sequences, J. Med. Genet. 30 (1993) 926–931.
[121] J.R. Vermeesch, P. Petit, F. Speleman, K. Devriendt, J.P. Fryns, P. Marynen, Interstitial telomeric sequences at the junction site of a jumping translocation, Hum. Genet. 99 (1997) 735–737.
[122] D. Sanlaville, C. Baumann, J.M. Lapierre, S. Romana, N. Collot, V. Cacheux, C. Turleau, G. Tachdjian, De novo inverted duplication 9p21pter involving telomeric repeated sequences, Am. J. Med. Genet. 83 (1999) 125–131.
[123] F. Fortin, M. Beaulieu, M. Bergeron, R. Fetni, N. Lemieux, Frequency of chromosome healing and interstitial telomeres in 40 cases of constitutional abnormalities, Cytogenet. Genome Res. 125 (2009) 176–185.
[124] R. Berger, O.A. Bernard, Jumping translocations, Genes. Chromosomes Cancer 46 (2007) 717e23.
[125] K.S. Reddy, The conundrum of a jumping translocation (JT) in CVS from twins and review of JTs, Am. J. Med. Genet. A 152A (2010) 2924–2936.
[126] S.D. Bouffler, Involvement of telomeric sequences in chromosomal aberrations, Mutat. Res. 404 (1998) 199–204.
[127] C. Desmaze, C. Alberti, L. Martins, G. Pottier, C.N. Sprung, J.P. Murnane, L. Sabatier, The influence of interstitial telomeric sequences on chromosome instability in human cells, Cytogenet. Cell Genet. 86 (1999) 288–295.
[128] C. Desmaze, L.M. Pirzio, R. Blaise, C. Mondello, E. Giulotto, J.P. Murname, L. Sabatier, Interstitial telomeric repeats are not preferentially involved in radiation-induced chromosome aberrations in human cells, Cytogenet. Genome Res. 104 (2004) 123–130.
[129] L. Alvarez, J.W. Evans, R. Wilks, J.N. Lucas, M. Brown, A.J. Giacia, Chromosomal radiosensitivity at intrachromosomal telomeric sites, Genes. Chromosomes Cancer 8 (1993) 8–14.
[130] J.L. Fernández, J. Gosálvez, V. Goyanes, High frequency of mutagen-induced chromatid exchanges at interstitial telomere-like DNA sequence blocks of Chinese hamster cells, Chromosome Res. 3 (1995) 281–284.
[131] J. Sánchez, M.S. Bianchi, A.D. Bolzán, Effect of bleomycin on interstitial telomeric sequences of immortalized Chinese hamster cells, Mutat. Res. 669 (2009) 139–146.
[132] J. Sánchez, M.S. Bianchi, A.D. Bolzán, Relationship between heterochromatic interstitial telomeric sequences and chromosome damage induced by the radiomimetic compound streptonigrin in Chinese hamster ovary cells, Mutat. Res. 684 (2010) 90–97.
[133] N. Camats, A. Ruiz-Herrera, J.J. Parrilla, M. Acien, P. Payá, E. Giulotto, J. Egozcue, F. García, M. García, Genomic instability in rat: breakpoints induced by ionizing radiation and interstitial telomeric-like sequences, Mutat. Res. 595 (2006) 156–166.
[134] E. Salvati, M. Scarsella, M. Porru, A. Rizzo, S. Iachettini, L. Tentori, G. Graziani, M. D'Incalci, M.F. Stevens, A. Orlandi, D. Passeri, E. Gilson, G. Zupi, C. Leonetti, A. Biroccio, PARP1 is activated at telomeres upon G4 stabilization: possible target for telomere-based therapy, Oncogene 29 (2010) 6280–6293.
[135] J.B. Vannier, V. Pavicic-Kaltenbrunner, M.I. Petalcorin, H. Ding, S.J. Boulton, RTEL1 dismantles T loops and counteracts telomeric G4-DNA to maintain telomere integrity, Cell 149 (2012) 795–806.
[136] S. Gonzalo, I. Jaco, M.F. Fraga, T. Chen, E. Li, M. Esteller, A. Blasco, DNA methyltransferases control telomere length and telomere recombination in mammalian cells, Nat. Cell Biol. 8 (2006) 416–424.
[137] D. Yang, Y. Xiong, H. Kim, Q. He, Y. Li, R. Chen, Z. Son-gyang, Human telomeric proteins occupy selective interstitial sites, Cell Res. 21 (2011) 1013–1027.
[138] A. Sfeir, T. de Lange, Removal of shelterin reveals the telomere end-protection problem, Science 336 (2012) 593–597.
[139] P. Slijepcevic, The role of DNA damage response proteins at telomeres: an integrative model, DNA Repair (Amst.) 5 (2006) 1299–1306.
[140] S. Misri, S. Pandita, R. Kumar, T.K. Pandita, Telomeres histone code, and DNA damage response, Cytogenet. Genome Res. 122 (2008) 297–307.
[141] M.A. Barnett, V.J. Buckle, E.P. Evans, A.C. Porter, D. Rout, A.G. Smith, W.R. Brown, Telomere directed fragmentation of mammalian chromosomes, Nucleic Acids Res. 21 (1993) 27–36.
[142] 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.
[143] P. Slijepcevic, P.E. Bryant, Chromosome healing, telomere capture and mechanisms of radiation-induced chromosome breakage, Int. J. Radiat. Biol. 73 (1998) 1–13.
[144] P. Slijepcevic, A.T. Natarajan, P.E. Bryant, Telomeres and radiation-induced chromosome breakage, Mutagenesis 13 (1998) 45–49.
[145] P. Slijepcevic, Y. Xiao, A.T. Natarajan, P.E. Bryant, Instability of CHO chromosomes containing interstitial telomeric sequences originating from Chinese hamster chromosome 10, Cytogenet. Cell Genet. 76 (1997) 58–60.
[146] P. Day, C.L. Limoli, W.F. Morgan, Recombination involving interstitial telomere repeat-like sequences promotes chromosomal instability in Chinese hamster cells, Carcinogenesis 19 (1998) 259–266.
[147] S. Bouffler, A. Silver, J. Coates, D. Papworth, R. Cox, Murine radiation myeloid leukaemogenesis: the relationship between interstitial telomere-like sequences and chromosome 2 fragile sites, Genes. Chromosomes Cancer 6 (1993) 98–106.
[148] R. Jordan, A.A. Oroskar, B.A. Sedita, J. Schwartz, Characterisation of a potential radiation-sensitive fragile site, Environ. Mol. Mutagen. 25 (Suppl) (1995) 25.
[149] K. Ocalewicz, S. Dobosz, H. Kuzminski, Distribution of telomeric DNA sequences on the X-radiation-induced chromosome fragments observed in the genome of androgenetic brook trout (Salvelinus fontinalis, Mitchill 1814), Cytogenet. Genome Res. 137 (2012) 1–6.
[150] T.K. Pandita, D. DeRubeis, Spontaneous amplification of interstitial telomeric bands in Chinese hamster ovary cells, Cytogenet. Cell Genet. 68 (1995) 95–101.
[151] I.Y. Quiroga, N.S. Paviolo, A.D. Bolzán, Interstitial telomeric sequences are not preferentially involved in the chromosome damage induced by the methylating compound streptozotocin in Chinese hamster cells, Environ. Mol. Mutagen. 54 (2013) 147–152.
[152] M. Vidal Bravo, M.S. Bianchi, A.D. Bolzán, Bleomycin induces delayed instability of interstitial telomeric sequences in Chinese hamster ovary cells, Mutat. Res. 731 (2012) 133–139.
[153] M.V. Mencucci, M. Vidal Bravo, M.S. Bianchi, A.D. Bolzán, Streptonigrin induces delayed chromosomal instability involving interstitial telomeric sequences in Chinese hamster ovary cells, Mutat. Res. 747 (2012) 46–52.
[154] G. Ji, K. Liu, C. Chen, W. Ruan, C. Glytsou, Y. Yang, M. Okuka, W. Song, S. Gagos, N. Li, L. Liu, Conservation and characterization of unique porcine interstitial telomeric sequences, Sci. China Life Sci. 55 (2012) 1029–1037.
[155] G. Souza, A.L. Vanzela, O. Crosa, M. Guerra, Interstitial telomeric sites and Robertsonian translocations in species of Ipheion and Nothoscordum (Amaryllidaceae), Genetica 144 (2016) 157–166.
[156] O. Samassekou, J. Yan, Polymorphism in a human chromosome-specific interstitial telomere-like sequence at 22q11.2, Cytogenet. Genome Res. 134 (2011) 174–181.
[157] K. Shimojima, N. Okamoto, T. Inazu, T. Yamamoto, Tandem configurations of variably duplicated segments of 22q11.2 confirmed by fiber-FISH analysis, J. Hum. Genet. 56 (2011) 810–812.
[158] K. Kashima, A. Nanashima, T. Yasutake, T. Sawai, T. Tsuji, S. Hidaka, F. Akama, K. Miyashita, Y. Tagawa, T. Nagayasu, Decrease of telomeres and increase of interstitial telomeric sites in chromosomes of short-term cultured gastric carcinoma cells detected by fluorescence in situ hybridization, Anticancer Res. 26 (2006) 2849–2855.
[159] S.K. Bodvarsdottir, M. Steinarsdottir, H. Bjarnason, J.E. Eyfjord, Dysfunctional telomeres in human BRCA2 mutated breast tumors and cell lines, Mutat. Res. 729 (2012) 90–99.