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Telomeres and mechanisms of Robertsonian fusion

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Abstract. The Robertsonian (Rb) fusion, a chromosome rearrangement involving centric fusion of two acro-(telo)centric chromosomes to form a single metacentric, is one of the most frequent events in mammalian karyotype evolution. Since one of the functions of telomeres is to preserve chromosome integrity, a prerequisite for the formation of Rb fusions should be either telomere loss or telomere inactivation. Possible mechanisms underlying the formation of various types of Rb fusion are discussed here. For example, Rb fusion in wild mice involves complete loss of p-arm telomeres by chromosome breakage within minor satellite sequences. By contrast, interstitial telomeric sites are found in the pericentromeric regions of chromosomes originating from a number of vertebrate species, suggesting the occurrence of Rb-like fusion without loss of telomeres, a possibility consistent with some form of telomere inactivation. Finally, a recent study suggests that telomere shortening induced by the deletion of the telomerase RNA gene in the mouse germ-line leads to telomere loss and high frequencies of Rb fusion in mouse somatic cells. Thus, at least three mechanisms in mammalian cells lead to the formation of Rb fusions.

Introduction

Chromosome rearrangements involving centric fusion of two telocentric or acrocentric chromosomes in which a single metacentric chromosome is formed are termed Robertsonian (Rb) fusions (Robertson 1916). This type of chromosome rearrangement is of major importance in mammalian karyotype evolution (Holmquist and Dancis 1979). Since telomeres, specialized structures at chromosome ends, are required for maintaining chromosome stability and integrity (Zakian 1997), a prerequisite for the formation of Rb fusions should be either elimination or inactivation of telomeres. In feral mouse populations p-arm telomeres are eliminated by chromosome breakage prior to the formation of Rb fusions (Garagna et al. 1995; Nanda et al. 1995). In addition, a recent report suggests that telomere shortening induced by the deletion of the telomerase RNA gene in the mouse germ-line leads to telomere loss and high frequencies of Rb fusion in mouse somatic cells (Blasco et al. 1997). By contrast, interstitial telomeric sites (ITS) are found in the pericentromeric regions of chromosomes originating from a number of vertebrate species (Meyne et al. 1990), suggesting the occurrence of Rb-like fusion without loss of telomeres, a possibility consistent with telomere inactivation. Possible mechanisms underlying the formation of various types of Rb fusion are discussed here in the light of recent advances in telomere research.

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Robertsonian fusion involving loss of telomeric sequences

Loss of telomeric sequences by telomere shortening

Telomeres are specialized structures at chromosome ends that are thought to function as buffers against end-to-end chromosome fusion (Zakian 1997). Conventional DNA replication mechanisms cannot replicate telomeres com-

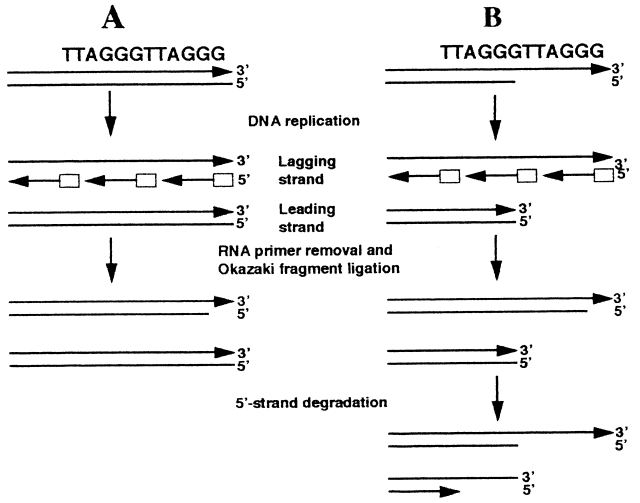

Fig. 1A, B. Telomere replication [vertebrate DNA sequence (TTAGGG)n shown]. **A** Conventional model (according to Lingner et al. 1995). **B** Revised model (according to Wellinger et al. 1996; Makarov et al. 1997). Lagging strand synthesis produces a series of Okazaki fragments attached to short RNA primers (*boxes*) in both models. According to the conventional model, degradation of the primers, gap repair and ligation replace all but the last primer, resulting in a gap at half of 5' termini, an effect known as the end replication problem. According to the revised model, DNA is lost from both ends of the chromosomes, owing to degradation of the 5' strand by an S-phase-specific exonuclease activity, resulting in long 3' overhangs at both ends

pletely because of the so-called end replication problem (Fig. 1A). Conventional mechanisms are expected to yield one blunt DNA end, the other end having a 3' overhang (Fig. 1A). However, at the end of S-phase all chromosome ends in yeast (Wellinger et al. 1996) and 80%–100% of chromosome ends in human cells (Makarov et al. 1997) have 3' overhangs (Fig. 1B). It has been proposed that a C-strand specific exonuclease activity converts blunt DNA ends into ends with G-rich 3' overhangs in a cell cycle-dependent manner (Wellinger et al. 1996; Makarov et al. 1997). Thus, telomeric DNA in eukaryotic cells is being lost as a result of both incomplete replication and strand-specific exonuclease activity (Fig. 1B). The loss of telomeric DNA is counterbalanced by a specialized enzyme termed telomerase (reviewed in Greider 1995). Telomerase is a reverse transcriptase that synthesizes telomeric repeats using its own RNA component as a template (Greider 1995; Lingner et al. 1997). Telomerase is constitutively expressed in lower eukaryotes, plants, rodents and human germ-line cells, but human somatic cells generally lack detectable telomerase activity (Greider 1995). In some cases telomeres may be elongated by telomerase-independent mechanisms as described in the yeast *Kluyveromyces lactis*, human cells and in cells of other species (reviewed in Biessman and Mason 1997). Thus, telomere maintenance is a dynamic process consisting of competitive degradation (end-replication problem and strand-specific exonuclease activity) and elongation (telomerase and telomerase-independent mechanisms) of telomeric DNA. In single cell organisms and in human germ-line cells the elongation and degradation mechanisms are well balanced so that the overall telomere length remains constant. By contrast, telomeres shorten with cellular ageing in human somatic cells, suggesting that telomere degradation mechanisms dominate in these cells. Telomere shortening in turn leads to increased frequencies of end-to-end chromosome fusion in ageing human somatic cells (Counter et al. 1992), suggesting that intact telomeres normally prevent end-to-end chromosome fusion.

This view is reinforced by recent experiments using mice lacking functional telomerase. The telomerase RNA gene was deleted in the mouse germ-line and viable mice lacking detectable telomerase activity ($mTR^{-/-}$) were obtained (Blasco et al. 1997). Although $mTR^{-/-}$ mice were apparently normal and viable for at least six generations they lost telomeric DNA at a rate of 4.8±2.4 kb per generation (Blasco et al. 1997). As a result, mice from the fourth generation onwards possessed chromosome ends lacking detectable telomere repeats. This was evident in primary cell cultures obtained from $mTR^{-/-}$ mice (Blasco et al. 1997). Mouse chromosomes (with the exception of the Y chromosome) are classified as acrocentrics by cytological criteria. However, mouse chromosomes are probably truly telocentric because as yet no gene-coding DNA sequences are found between centromeres and mouse p-arm telomeres (Kipling et al. 1991). Some of the chromosomes lacking detectable telomere repeats formed Rb fusions in primary $mTR^{-/-}$ cells (Blasco et al. 1997) (Fig. 2, panel 1). In mouse telocentric chromosomes p-arm telomeres are significantly shorter than their q-arm counterparts as revealed by a novel quantitative fluorescence in situ hybridization technique (Zijlmans et al. 1997). Also, this technique revealed that mouse telomeres are on average shorter than previously anticipated, i.e. 10–60 kb rather than 50–100 kb (Zijlmans et al. 1997). Since the rate of telomere shortening is expected to be similar for all chromosome ends and given the rate of telomere shortening of 4.8 kb per generation, p-arms of mouse chromosomes are more sensitive to telomere loss in the absence of telomerase activity than q-arm telomeres. Thus, telomere shortening as a result of telomerase inactivation leads to increased frequencies of Rb fusion in mouse cells (Blasco et al. 1997) (Fig. 2, panel 1).

It would be of interest to examine further generations (if viable) of $mTR^{-/-}$ mice. At least three possible outcomes are predicted. First, if telomere shortening continues this could cause high levels of Rb fusion (i.e. dicentric chromosomes) leading to chromosome instability (breakage-fusion-bridge cycles, aneuploidy etc.). Chromosome instability in germ-line cells may cause a decrease in fertility and/or increased levels of malformations due to the high levels of karyotypically abnormal spermatozoa and eggs. This in turn could lead to lower survival rates of $mTR^{-/-}$ mice and their eventual extinction. If this possibility is correct then telomerase inactivation may be tolerated only temporarily. Second, telomerase-independent mechanisms of telomere elongation (Biessman and Mason 1997) may become more active in further generations of $mTR^{-/-}$ mice, thus preventing telomere shortening. As a result $mTR^{-/-}$ mice may be viable for longer than six genera-

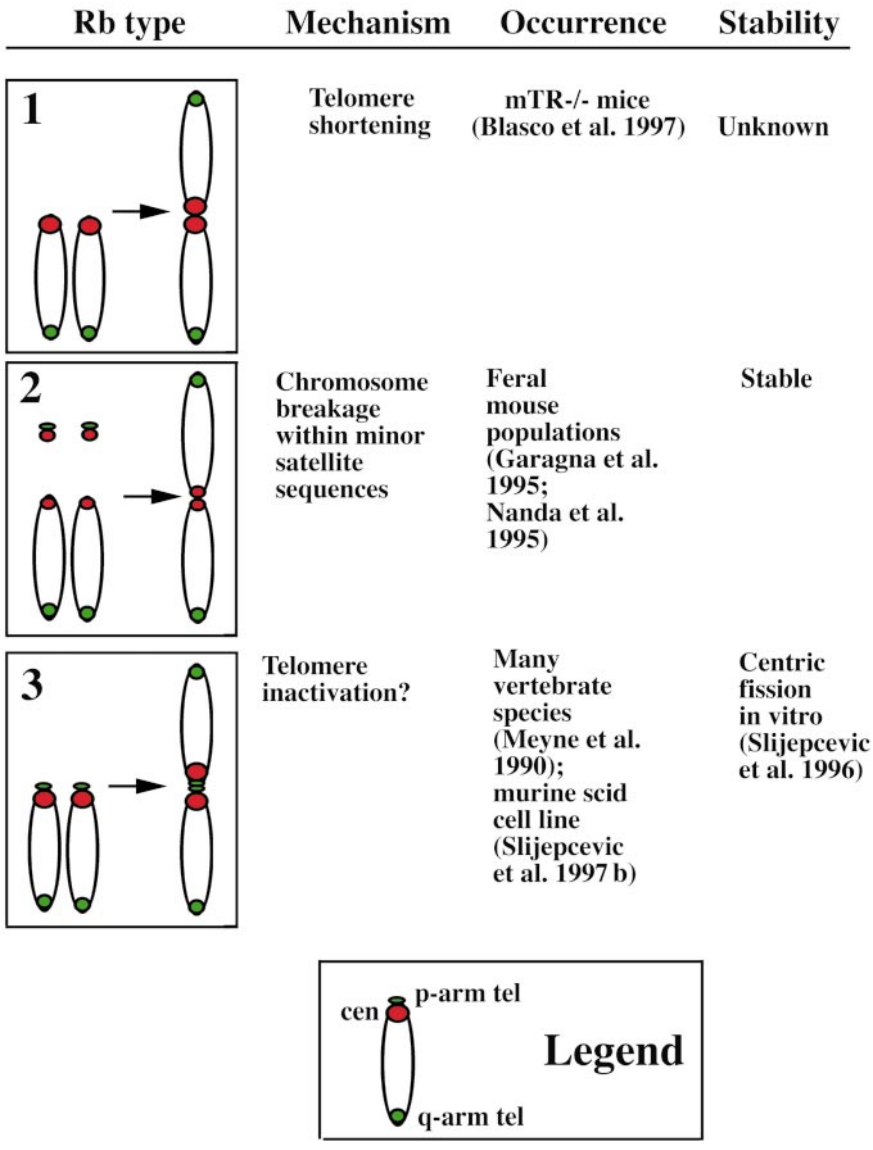

Fig. 2. Types of Robertsonian (*Rb*) fusion and their mechanisms

tions. Third, formation of Rb fusions is observed exclusively in vitro, i.e. in tissue cultures established from $mTR^{-/-}$ mice (Blasco et al. 1997). However, telomere shortening may not have the same effects in vivo. For example, normal *Drosophila* chromosomes lacking telomeric sequences and *Drosophila* chromosomes exhibiting terminal deletions that lose DNA from the break-sites are quite stable (Biessman et al. 1990).

Loss of telomeric sequences by chromosome breakage

Since telomerase is expressed in normal germ-line cells (Kim et al. 1994), telomere shortening is likely to be prevented in germ-line cells of normal mice. As a result, formation of constitutive Rb fusions in karyotypes of normal mice (which must occur in germ-line cells) is likely to be the result of mechanisms other than telomere shortening. In line with this possibility, Rb fusions in wild mice are the result of chromosome rearrangements that involve chromosome breakage within minor satellite sequences (which constitute part of centromeric heterochromatin), resulting in the complete loss of p-arm telomeres (Garagna et al. 1995; Nanda et al. 1995) (Fig. 2, panel 2). The exact mechanism leading to chromosome breakage within minor satellite sequences is not known at present. It is tempting to speculate that one scenario may involve a phenomenon known as TEL+CEN antagonism. Circular plasmids containing either telomere or centromere sequences are stably propagated in yeast, in contrast to plasmids containing both telomere and centromere sequences, which as a result become unstable (Enomoto et al. 1994). This effect is termed TEL+CEN antagonism. TEL+CEN antagonism is also observed in short yeast artificial chromosomes (YACs) (Enomoto et al. 1994). It has been proposed that TEL+CEN plasmids, or short YACs, are unstable as a result of being pulled to two different regions of the nucleus, i.e. telomeres and centromeres usually display a polarized, Rabl configuration (Enomoto et al. 1994). TEL+CEN antago-

nism in yeast is regulated by gene products that regulate telomere length (i.e. Rap1 protein) and telomere position effect (i.e. Sir proteins) (Enomoto et al. 1994). Since mouse p-arm telomeres are very close to centromeres they must occupy the same nuclear pole as centromeres, while q-arm telomeres occupy the opposite pole. A possible reason why mouse p-arm telomeres are shorter than q-arm telomeres may be to reduce TEL+CEN antagonism to a tolerable level, which will facilitate a stable polarization of telomeres and centromeres in interphase nuclei. In other words, short p-arm telomeres in mouse telocentric chromosomes are expected to behave like centromeres for the sake of the proper nuclear organization, rather than like telomeres (Lima-de-Faria and Bose 1962). If the balance in TEL+CEN antagonism is altered by changes in p-arm telomere length (elongation), cellular mechanisms may not be able to distinguish between p- and q-arm telomeres, leading to a tension within the chromosome as a result of forces that pull p-arm telomeres to two different poles (centromeric and telomeric) of the nucleus. This process may eventually lead to chromosome breakage within minor satellite sequences, elimination of p-arm telomeres and formation of Rb fusions. This possibility is currently being examined experimentally.

Robertsonian-like fusion without loss of telomeric sequences

Chromosomes of many vertebrate species exhibit ITS in their pericentromeric regions (Meyne et al. 1990). It has been suggested that these ITS occurred as a result of evolutionary Rb-like fusions (Holmquist and Dancis 1979) (Fig. 2, panel 3). The presence of telomeric sequences at Rb fusion sites is inconsistent with functional telomeres, suggesting that telomeres must have lost their function either partially or completely prior to the formation of Rb-like fusions. There are at least three explanations for the loss of telomere function without loss of telomeric sequences. First, the major factor determining telomere function is the presence of telomere-binding proteins (TP) at telomeric sequences (Zakian 1995). These proteins may become inactivated as a result of the presence of some other proteins in their close proximity, perhaps centromeric proteins, leading to the loss of telomere function. Rap1p, which is a major yeast TP, is present at many interstitial chromosome sites (Zakian 1995). This suggests that inactivation of TP is a relatively frequent phenomenon in eukaryotic cells. Also, the other vital component of the chromosome, the centromere, may sometimes become inactivated, for example in the case of viable dicentric chromosomes. Some Rb fusions are basically dicentric chromosomes in which one centromere must be inactivated.

Second, telomeres may lose their function as a result of changes in telomere chromatin structure. Telomeres in lower eukaryotes are highly condensed heterochromatic structures (Gilson et al. 1993). Although the state of telomere chromatin condensation in mammalian chromosomes is not known, indirect evidence suggests that it may be similar to that in yeast (Zakian 1995). In line with this possibility, it has been suggested that chromosome fusigenic potential in mammalian cells may be determined not only by telomere length but also by the status of telomere chromatin structure. For example, chromosomes originating from an embryonic Chinese hamster cell line were frequently involved in end-to-end chromosome fusions although the length of their telomeres was on average 20 kb (Slijepcevic et al. 1997b). In a large number of cases, chromosome fusion sites exhibited chromatin filaments indicative of chromatin decondensation (Slijepcevic et al. 1997b). Thus, telomere chromatin undercondensation may lead to end-to-end chromosome fusion in spite of relatively long telomeres. Similarly, frequencies of Rb fusion were extremely high in the murine scid (severely combined immunodeficiency) cell line, exhibiting average telomere length of about 30 kb (Slijepcevic et al. 1997b). In addition, at least three clonal Rb metacentrics exhibiting ITS at fusion sites were identified in the scid cell line (Slijepcevic et al. 1997b). Finally, there was a high frequency of so-called multibranched chromosomes in the scid cell line: several chromosomes joined together with their p-arm telomeres to form a multibranched configuration (see Slijepcevic et al. 1997b). Multibranched chromosomes are the hallmark of the human ICF (Immune deficiency, Centromeric instability, Facial abnormalities) syndrome, characterized by heterochromatin undercondensation (Maraschio et al. 1988; Sawyer et al. 1995), suggesting that scid cells may have a defect in chromatin organization that, in turn, may contribute to high frequencies of Rb fusion in spite of relatively long telomeres.

The third possibility is highly hypothetical and is based on recent experiments with $mTR^{-/-}$ mice. These experiments indicate that telomerase is not an essential enzyme in at least six generations of $mTR^{-/-}$ mice (Blasco et al. 1997), suggesting that its temporary inactivation may be tolerated. Thus, one cannot rule out the possibility that temporary inactivation of telomerase gene(s) in germ-line cells might have occurred by unknown mechanism(s) during evolution, leading to telomere shortening and partial loss of telomeric sequences. This partial loss might have been sufficient to initiate the chromosome fusion process in germ-line cells, leading to Rb metacentrics containing ITS at fusion points. Given that one centromere is inactivated, such Rb metacentrics might become stable and transmissible to future generations.

Although telomere inactivation seems a plausible explanation for the presence of ITS at Rb fusion points there is an alternative possibility. Degenerate telomere-like sequences may be a part of centromeric heterochromatin in some mammalian species (Shampay et al. 1995). These sequences may expand by a variety of amplification mechanisms (Slijepcevic et al. 1997a), including transposition (Cherry and Blackburn 1985). Thus, telomere-like DNA sequences that constitute part of centromeric heterochromatin in some species might not be distinguishable from normal telomeric sequences by most conventional techniques.

Conclusions

Telomeric sequence patterns in a variety of mammalian Rb fusions indicate that at least three mechanisms may be involved in their formation (Fig. 2). Only one of these mechanisms (chromosome breakage within minor satellite sequences, Fig. 2, panel 2) is clearly demonstrated to be operational in karyotype evolution (Garagna et al. 1995; Nanda et al. 1995). One of the remaining mechanisms (telomere inactivation, Fig. 2, panel 3) appears to be a possibility in karyotype evolution as evidenced by the presence of ITS in the pericentromeric regions of chromosomes from a number of vertebrate species (Meyne et al. 1990). However, the possibility cannot be ruled out that telomere-like sequences may be a part of the centromeric heterochromatin in some species. Finally, a recent report suggests that another mechanism, namely that of telomere shortening as a result of telomerase inactivation (Fig. 2, panel 1), may be responsible for Rb fusion in somatic cells (Blasco et al. 1997).

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