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Mechanisms of the Evolutionary Chromosome Plasticity: Integrating the 'Centromere-from-Telomere' Hypothesis with Telomere Length Regulation

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Abstract

The 'centromere-from-telomere' hypothesis proposed by Villasante et al. [2007a] aims to explain the evolutionary origin of the eukaryotic chromosome. The hypothesis is based on the notion that the process of eukaryogenesis was initiated by adaptive responses of the symbiont eubacterium and its archaeal host to their new conditions. The adaptive responses included fragmentation of the circular genome of the host into multiple linear fragments with free DNA ends. The action of mobile genetic elements stabilized the free DNA ends resulting in the formation of proto-telomeres. Sequences next to the proto-telomeres, the subtelomeric sequences, were immediately targeted as the new cargo by the tubulin-based cytoskeleton, thus becoming proto-centromeres. A period of genomic instability followed. Eventually, functioning centromeres and telomeres emerged heralding the arrival of the eukaryotic chromosome in the evolution. This paper expands the 'centromere-from-telomere' hypothesis by integrating it with 2 sets of data: chromosome-specific telomere length distribution and chromomere size gradient. The integration adds a new dimension to the hypothesis but also provides an insight into the mechanisms of chromosome plasticity underlying karyotype evolution.

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Telomeres are essential functional elements of eukaryotic chromosomes that are required for their accurate segregation [de Lange, 2015]. According to an intriguing recent hypothesis, termed the 'centromere-from-telomere' hypothesis (CFTH), telomeres evolved as the first functional element of the eukaryotic chromosome. The other essential element, the centromere, gradually emerged from the telomere [Villasante et al., 2007a]. The CFTH predicts that eukaryogenesis started with engulfing of an α-proteobacterium by an archaeal host. This event was followed by massive invasion of the symbiont's mobile group II introns [Lambowitz and Zimmerly, 2004] into the genome of the host facilitating breakage of the host's circular chromosome into multiple linear fragments [McClintock, 1978]. The host's fragmented genome required stabilization of the newly emerging broken DNA ends, a process facilitated by non-LTR retrotransposons [Moore and Haber, 1996] eventually

generating proto-telomeres. Sequences positioned next to proto-telomeres, the subtelomeric sequences, were recognized immediately as the new cargo by the tubulin-based cytoskeleton thus turning subtelomeric regions into proto-centromeres [Villasante et al., 2007a]. This generated temporary genomic instability which finally disappeared through the emergence of properly functioning telomeres and centromeres (see below). Other events took place in parallel including the development of the nuclear membrane to separate the newly emerging fragmented genome from the rest of the cellular material [Martin and Koonin, 2006].

The key implication of CFTH is that telomeres and centromeres have a shared chromosome origin. This potentially means that telomeres and centromeres can also functionally interchange in the process of karyotype evolution [Ventura et al., 2004; Murphy et al., 2005]. Even though CFTH is not consistent with some other theoretical considerations, which envisage the independent origin of centromeres followed by the equally independent origin of telomeres [Cavalier-Smith, 2010], the potential explanatory power of the hypothesis from the perspective of karyotype evolution is refreshingly original. CFTH is compatible with the notion of chromosome plasticity which is evident from karyotype evolution studies. The term chromosome plasticity refers to the transforming potential of the chromosome material which works by exploiting the abilities of its functional elements, telomeres and centromeres, resulting in species-specific karyotypes accompanied by organismal phenotypes. A summary of chromosomal evolutionary changes occurring as a result of the chromosome plasticity is presented in figure 1.

The key implication of figure 1 is that chromosome maintenance is not a simple DNA sequence fidelity check like in the case of circular genomes. The chromosome integrity maintenance process is driven by the 2 functional elements, centromeres and telomeres, most likely regulated through epigenetic mechanisms, as both telomeres and centromeres are heterochromatic structures. In support of this view, numerous unbalanced chromosomal abnormalities with significant DNA sequence changes relative to the normal human genome have been reported with no phenotypic effects [Barber, 2005]. In all these cases functional centromeres and telomeres remained intact. This suggests that the processes regulating chromosome stability maintenance are more concerned with preserving the chromosome as one DNA molecule packed into the epigenetically regulated structure and less concerned with the restoration of the original sequence of the same molecule.

The aim of this paper is to review CFTH in the context of telomere length regulation mechanisms. The starting point is a brief overview of the known mechanisms for telomere length regulation in the light of CFTH. This will be followed by the discussion of 2 sets of data, namely telomere length analysis in individual chromosomes and chromomere size gradient. The analysis points to an interesting aspect of telomere biology which requires taking the evolutionary view for which the CFTH provides a suitable platform. The view emerging from this platform is the notion of evolutionary chromosome plasticity at the heart of which is the functional interchange between telomeres and centromeres.

Telomere Length Regulation in the Light of CFTH

There are 3 well-documented mechanisms for telomere length maintenance: the telomerase-based mechanism (TM) [de Lange, 2015], the homologous recombination (HR)-based mechanism also known as alternative lengthening of telomeres (ALT) [Pickett and Reddel, 2015], and the retrotransposon-based mechanism (RM) [Mason et al., 2008]. The usual assumption is that the most common mechanism is TM. This assumption is based on the observation that telomerase is remarkably conserved evolutionarily, leading to proposals that it could have coincided with the first functioning eukaryotic cell, or that it could have even preceded it [Nakamura and Cech, 1998]. HR is thought to be a relatively widespread mechanism observed in yeast, insects, and numerous other organisms but not as common as telomerase [Pickett and Reddel, 2015]. The least common mechanism is RM. It occurs only in organisms which lost telomerase, such as insects from the order Diptera [Mason et al., 2008].

How do these 3 mechanisms fit CFTH? The first assumption of CFTH is that eukaryogenesis was prompted by the adaptation of the bacterial symbiont and the archaeal host to their new conditions. As part of the adaptation process, the symbiont's class II introns, a class of retrotransposons, invaded the host's circular genome and caused its fragmentation into linear DNA molecules [Garavís et al., 2013]. The genome fragmentation resulted in free DNA ends which became opportunistic targets for mobile genetic elements from the host's genome, such as non-LTR retrotransposons, eventually leading to stabilization of free DNA ends and formation of proto-telomeres resulting in the formation of the first proto-eukaryotic linear chromosomes [Garavís et al., 2013]. Thus,
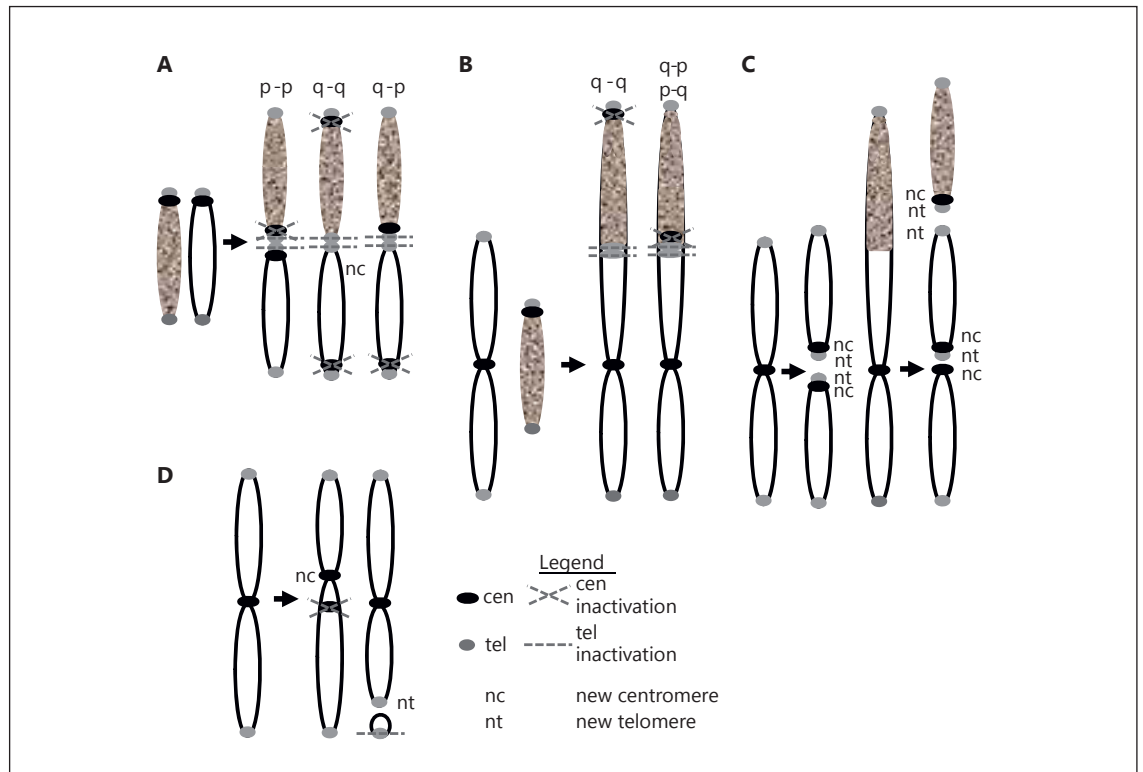
Fig. 1. The concept of chromosome plasticity involving 4 major mechanisms. The starting point in the process is the emergence of the first eukaryotic chromosome which was presumably telocentric [Villasante et al., 2007a]. The karyotype evolution started from the first eukaryote karyotype consisting of telocentric chromosomes. **A** Fusion of 2 telocentric chromosomes to produce the first metacentric chromosome. Each chromosome is shown in different pattern to illustrate possible interactions between p- and q-arms. One of the depicted events resembles Robertsonian fusion [Garagna et al., 1995; Nanda et al., 1995; Slijepcevic, 1998] demonstrated in higher organisms. **B** As the karyotype complexity increases, tandem fusions of 2 or more chromosomes including telocentric/acrocentric, metacentric, and submetacentric chromosomes are possible. The depicted event only involves interaction between metacentric and telocentric chromosomes. Published literature suggests that tandem fusion mostly involves telocentric chromosomes [Nanda et al., 2002; Hartmann and Scherthan, 2004; Chi et al., 2005; Tsipouri et al., 2008; Ropiquet et al., 2010]. **C** More complex karyotypes feature other types of rearrangements including centric fission of metacentric chromosomes [Perry et al., 2004]. **D** Finally, chromosome rearrangements which include de novo formation of centromeres [Ventura et al., 2004] or telomeres (chromosome healing) [Jankowska et al., 2015] are also possible. Please note that depicted events in **A–D** do not represent the full spectrum of possible events.

CFTH predicts that telomerase was not involved in the formation of proto-telomeres. It was engaged later when its biochemical properties enabled stabilization of proto-telomeres and their conversion into fully functioning telomeres [Villasante et al., 2007a]. Phylogenetic studies indicate that telomerase belongs to the same group of reverse transcriptases as non-LTR retrotransposons [Eickbush, 1997; Nakamura and Cech, 1998]. When the loss of telomerase occurs during evolution, like in *Drosophila* and other dipterans, telomere maintenance is taken over by non-LTR retrotransposons: *HeT-A*, *TART*, and *TAHRE* [Villasante et al., 2007b]. The gist of the argument is that the loss of telomerase forces affected cells to return to the evolutionary solution for stabilization of broken DNA ends preceding telomerase – non-LTR retrotransposons. Thus, from the perspective of CFTH, 2 seemingly different mechanisms, TM and RM, could represent either a single evolutionary mechanism that has different varieties or 2 closely related mechanisms, which share the evolutionary origin.

How does HR fit the CFTH scenario? It has been argued that the formation of the T-loop structure found at telomeres resembles the HR process in which the single-stranded G+T-rich telomeric overhang invades the DNA double helix to form the D (displacement) loop eventually leading to the T-loop [de Lange, 2004]. The G+T-rich overhang invasion can have 2 outcomes. If the overhang is coated with the POT-1 protein, part of the shelterin
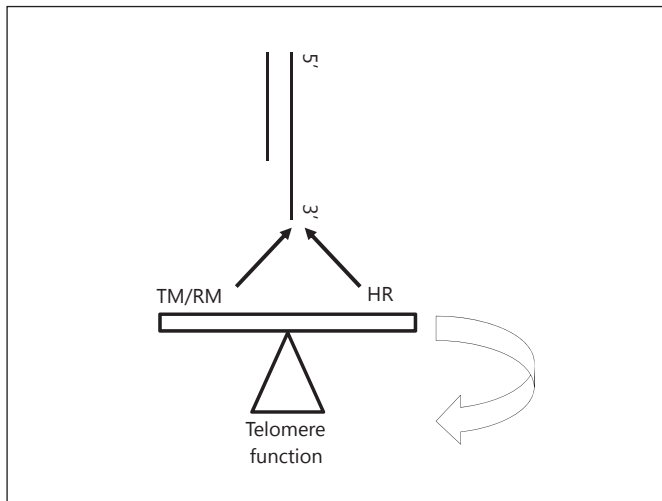
Fig. 2. Telomere length regulation in the form of a 'lever' on a 'fulcrum'. The 'lever' represents mechanisms responsible for telomere length regulation which have forms of opposing 'weights'. These compete for the same substrate: single stranded 5′-3′ telomeric overhangs. The 'fulcrum' represents telomere function (capping). The bended arrow symbolizes the mechanistic link between the 'fulcrum' and the 'lever': the capacity of the G+T-rich telomeric overhang in all eukaryotic organisms studied to form G-quadruplexes as the basis for either T-loop formation or the other end stabilizing structures which confer the capping function.

complex, this will lead to the formation of a T-loop structure signifying TM [Pickett and Reddel, 2015]. However, if the molecular coating switch occurs from POT-1 to the HR protein, RAD51, presumably via replication protein A, this will lead to HR [Pickett and Reddel, 2015]. Thus, TM and HR share the same substrate. This argues that telomere homeostasis may not be a game with 1 player only, TM or HR, but rather a balancing game in which both players, TM and HR, are involved simultaneously. In line with this possibility, it has been argued that HR represents a normal component of telomere maintenance [Pickett and Reddel, 2015]. This argument is based on observations that the HR-based ALT mechanism and telomerase coexist in mouse [Neumann et al., 2013] and human cells [Muntoni and Reddel, 2005]. This scenario also implies that the human cancer pathology represents a disbalance of the 2 mechanisms. The disbalance in some tumors is altered in favor of telomerase. For example, 85% of tumors screened for telomerase activity are positive [Kim et al., 1994]. In the remaining 15% of tumors, the disbalance is altered in favor of ALT.

The scenario in which telomere maintenance resembles a 'lever' balanced by 2 'weights', TM/RM and HR

(fig. 2), is not incompatible with CFTH. As in any lever, the fulcrum determines the balancing mechanism. In this scenario, the 'fulcrum' represents the telomere function (fig. 2). The function of telomeres is to resolve the problem of free DNA ends in linear chromosomes which must be stabilized. This is known as the capping function which prevents broken DNA ends from being targeted by repair mechanisms [de Lange, 2004]. The other function of telomeres is to resolve the end replication problem [de Lange, 2015]. However, it must be noted that TM, HR, or RM activities are not required in every cell cycle but only occasionally as modest telomere sequence loss is not reflected in the organismal phenotype [Harley et al., 1990]. In some cases telomere sequence loss is desirable. For example, in the case of human cells, telomere sequence loss may act as a tumor suppressor mechanism [Artandi and DePinho, 2000].

Thus, all 3 mechanisms for telomere length regulation fit well with CFTH. They may be interpreted as 'weights' on a 'lever' guided by the 'fulcrum' (fig. 2). Importantly, individual components of this balancing mechanism must be mechanistically linked including opposing 'weights': TM/RM and HR (fig. 2). As indicated above, TM and HR share the same substrate, the telomeric G+T-rich overhang (fig. 2). Remarkably, RM, which superficially resembles a fundamentally different mechanism from TM, is actually similar to TM: *Drosophila* telomeres generated by retrotransposons show the same strand bias as those generated by telomerase. The strand running 5′-3′ towards the chromosome end is G+T-rich in *Drosophila* as in other eukaryotes [Danilevskaya et al., 1998; Abad and Villasante, 1999]. This implies that the telomere capping function is heavily dependent upon the sequence composition of the 5′-3′ strand running in the direction of the chromosome end (fig. 2). It seems likely that the telomere capping function requires the formation of G-quadruplex DNA structure in all eukaryotes [Paeschke et al., 2005]. *Drosophila* is the same in this sense [Abad and Villasante, 1999].

It is important to stress that in the context of CFTH, telomere length regulating mechanisms acquire a new dimension: they must be integrated into the process of eukaryotic chromosome evolution. One of the most detailed studies of chromosome evolution suggests the active interplay between centromeres and telomeres in this process [Lima-de-Faria, 1983]. Thus, the key question is how TM, HR, and RM are integrated into the interplay with centromeres. Studies focusing on distribution of telomere length in individual chromosomes may provide useful clues as discussed in the next section.

Chromosome-Specific Telomere Length Regulation

It is generally assumed that TM, RM, and HR are regulated by local factors, proteins that are in close proximity to the chromosome ends. However, a recent study suggests that T-loops can interact with distant non-telomeric regions via the shelterin protein TRF2 [Wood et al., 2014]. These distant regions are interstitial telomeric sequences (ITSs). The interaction between T-loops and ITSs occurs over long distances, spanning megabases of DNA [Wood et al., 2015]. This observation is consistent with the possibility that TM, HR, and RM could potentially be affected by factors located far from chromosome ends. Remarkably, a set of results based on the analysis of telomere length distribution in individual chromosomes suggest that this may be true. The factor affecting telomere length may be the centromere's position.

What is known about TM, HR, and RM, including the regulatory mechanisms originates from molecular biology techniques which normally detect only average DNA sequence length and thus ignore distribution of telomere length in individual chromosomes. The advent of quantitative fluorescence in situ hybridization (Q-FISH) enabled length analysis of individual telomeres. The first Q-FISH systematic analysis reported significant differences between p-arm and q-arm telomeres in mouse [Zijlmans et al., 1997]. All mouse chromosomes are acrocentric: the p-arm telomeres are positioned very close to the centromeres. Telomeres closer to centromeres were significantly shorter than their counterparts more distant from centromeres. This observation has since been replicated many times in the case of mouse cells [e.g., Hande et al., 1999; Modino and Slijepcevic, 2002]. Interestingly, the first Q-FISH study in human cells reported a weak correlation between centromere position and telomere length [Martens et al., 1998]. Again, telomeres more distant from centromeres were longer. Further analysis revealed a significant positive correlation between individual chromosome arms and telomere length: longer arms had longer telomeres than shorter arms [Wise et al., 2009]. Thus, similar to mouse acrocentric chromosomes, human chromosomes which are predominantly sub-metacentric show longer telomeres at q-arms than at p-arms suggesting that centromere position may affect telomere length. In line with this possibility, analysis of telomere length in Chinese hamster (*Cricetulus griseus*) [Slijepcevic and Hande, 1999] and a plant, pear millet (*Pennisetum glaucum*) [Sridevi et al., 2002], revealed the same association between telomere length and centromere position.

Unfortunately, telomere length distribution in individual chromosomes remains under-investigated, thus precluding any generalization or establishing whether a causative relationship exists between centromere position and telomere length. Nevertheless, it is worth examining the potential effect of centromere position on telomere length in light of CFTH. For this, it is important to revisit the part of CFTH focusing on the emergence of centromeres in chromosome evolution.

How Did the Centromere Evolve?

The key CFTH argument is that centromeres evolved from telomeres. This argument has a solid experimental support [for details, see Villasante et al., 2007a]. In brief, after the formation of the first proto-telomere, the 'lever' scenario (fig. 2) was activated employing 2 mechanisms to maintain proto-telomere function, RM and HR. The result was the expansion of telomeric sequences by HR. After 1 or 2 rounds of amplification, newly generated telomeric sequences moved away from the chromosome end thus becoming subtelomeric sequences. Newly formed subtelomeric sequences were immediately targeted as new cargo by the tubulin-based cytoskeleton. Thus, not only telomeres, the end-stabilizing structures, but also centromeres, 'the chromosome transporters' in the cell cycle, represented a novelty in the evolution of the eukaryotic chromosome.

The centromeres' equivalent in circular genomes of prokaryotes is the partitioning locus, PAR, which provides the segregation function [Lin and Grossman, 1998]. In the newly emerging proto-eukaryotic linear chromosome, PAR sites were continued to be targeted by tubulin-based cytoskeleton resulting in pseudo-dicentric chromosomes prone to breakage. This caused a series of breakage-fusion-bridge cycles requiring the constant action of retrotransposons to stabilize broken DNA ends. This resulted in competition between PAR sites and newly formed subtelomeric regions for attracting tubulin. Eventually, subtelomeric regions transformed into functional centromeres through becoming stronger attractors of tubulin than PAR sites which lost the tubulin-attracting function. Similarly, proto-telomeres turned into properly functioning telomeres when telomerase evolved from a non-LTR retrotransposon reverse transcriptase [Villasante et al., 2007a]. It also seems reasonable to assume that the first functioning eukaryotic chromosome was telocentric (fig. 1A).

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The key points of CFTH described above have good support in the literature [Villasante et al., 2007a; Garavís et al., 2013]. However, the requirement for any scientific hypothesis is that it is testable. So, the next question is how can CFTH be tested? One way of testing it is to trace telomeric sequences in the karyotype evolution.

If one functional chromosome element (the centromere) can originate from the other element (the telomere) as CFTH implies, this transforming potential should continue throughout evolution of the eukaryotic chromosomes. In other words, the functions of telomeres and centromeres are interchangeable: a former centromere could become a new telomere and vice versa, presumably by epigenetic mechanisms which are at the heart of the chromosome plasticity underlying karyotype evolution (fig. 1). The support for this scenario is widespread [Villasante et al., 2007a]. Take *Drosophila*, for example, an organism considered to be a special case because it lacks telomerase. The analysis of the *D. melanogaster* Y chromosome revealed that *Het-A* and *TART*-related sequences normally found at telomeres are also present at centromeres [Abad et al., 2004]. This finding was further substantiated [Berloco et al., 2005] suggesting that centromeres and telomeres functionally interchange in *Drosophila* karyotype evolution. Is this form of functional interchange detectable in species other than *Drosophila*, in particular those using telomerase for telomere length regulation? If so, this possibility would also argue in favor of the notion that RM and TM are far more related than anticipated previously.

When FISH was invented in the late 1980's, one of the first phylogenetic studies was on the distribution of telomeric $(TTAGGG)_n$ sequences in vertebrate chromosomes [Meyne et al., 1990]. Remarkably, this study indicated that telomeric sequences are found outside telomeric regions, predominantly in centromeric or pericentromeric regions of chromosomes in 50% of the cases studied. Given that small ITS blocks are beyond resolution of FISH, it seems likely that the figure of 50% is an underestimate. In line with this possibility, ITSs, not visible by FISH, are identifiable in the human genome, the classical example being the centromeric region of chromosome 2 [Ijdo et al., 1991]. A random scanning of the cytogenetic literature reveals that since the initial study of Meyne et al. [1990], numerous studies have been undertaken to analyze the distribution of ITSs in the genomes of species for which this information is not available, with the trend continuing until the present day. It seems that the presence of ITSs in centromeric or pericentromeric regions of chromosomes is a rather widespread phenomenon. For example, a recent study of squamate reptile karyotypes revealed centromeric ITSs in 24 out of 68 species analyzed [Rovatsos et al., 2015]. ITSs are naturally prone to breakage following the exposure of cells to DNA damaging agents [Slijepcevic et al., 1996]. Furthermore, ITSs in human chromosome 2q14 behave as a common fragile site [Bosco and de Lange, 2012]. Analysis of the capacity of telomeric sequences to repair revealed that they are irreparable [Fumagalli et al., 2012] or behave as fragile sites [Sfeir et al., 2009]. This suggests that both ITSs and terminal telomeric sequences are naturally prone to breakage; this possibility is in line with the notion of chromosome plasticity, in particular centric fission (fig. 1C).

Taken together, these observations provide a suitable test for CFTH. Furthermore, the fact that ITSs are identifiable in centromeric regions of chromosomes in organisms that use either RM or TM as a mechanism for telomere length regulation suggests that RM and TM are far more evolutionarily related than usually anticipated.

## Integration of CFTH with Telomere Length Regulation in Individual Chromosomes

Having outlined, in brief, the CFTH stance on the origins of chromosome functional elements, centromeres and telomeres, the experimental support for CFTH, and suitable tests, the next step is to find out whether the telomere length distribution in individual chromosomes (see above) is compatible with CFTH. It is clear from the set of results originating from mouse, human, Chinese hamster, and pear millet cells that centromeres, or more precisely their position on the chromosome, affect telomere length (see above). While this scenario is of interest to CFTH, it will remain unknown whether it is compatible or not with CFTH unless the underlying mechanism is identified. The next part of this article will examine 2 independent lines of study which have potential for providing a mechanistic explanation for the specific distribution of telomere length in individual chromosomes outlined above and thus may add a new dimension to CFTH.

Plasmids are circular DNA molecules less complex than circular bacterial chromosomes. When telomeric or centromeric sequences are inserted into plasmids, they are stably propagated in yeast [Enomoto et al., 1994]. However, when plasmids contain both telomeric and centromeric sequences, they become unstable, a phenomenon known as TEL+CEN antagonism [Enomoto et al., 1994]. The same type of antagonism is observed in

short YACs [Enomoto et al., 1994]. It has been argued that TEL+CEN plasmids and short YACs are unstable because they are being pulled to opposite regions of the nucleus during mitosis – telomeres and centromeres usually assume a polarized configuration known as Rabl configuration. Importantly, TEL+CEN antagonism is regulated by yeast proteins that regulate telomere length (Rap1) and telomere position effect (Sir family members) [Enomoto et al., 1994]. These observations suggest that mechanisms behind telomere length regulation may be influenced by the relationship between telomeres and centromeres, thus providing some form of a mechanistic clue that could explain the observed Q-FISH data (see above). It seems likely that telomeres closer to centromeres must be somehow suppressed so that they do not interfere with the centromere function unlike their counterparts more distant from telomeres.

Interestingly, a series of cytological observations by Lima-de-Faria [1983] is consistent with this possibility. These observations demonstrated a remarkable regularity in chromosome organization involving the interplay between telomeres and centromeres. In prophase of meiosis, chromosomes differentiate into structural units known as chromomeres. Lima-de-Faria [1983] analyzed the distribution of chromomere size in >700 species. The pattern of distribution showed a highly specific gradient: the size of chromomeres decreased proportionally to the distance centromere-telomere (fig. 3). The largest chromomeres were always located in the vicinity of centromeres, whereas the smallest chromomeres were always located in the vicinity of telomeres. The existence of this regularity in a wide range of plant and animal species suggests its evolutionary conservation. In the context of TEL+CEN antagonism, the chromomere size gradient potentially acquires its molecular mechanism (fig. 3) at the heart of which is chromosome mechanics during cell division, in this case meiosis.

The function of the chromomere size gradient may be to facilitate polarization of centromeres and telomeres in preparation for chromosome segregation. Assuming that meiotic telomeres show similar size to mitotic telomeres, the polarization process implies that telomere size must be adjusted to suit chromosome mechanics in mitotic/meiotic segregation. For this reason, p-arm telomeres must be epigenetically suppressed so that they do not interfere with the centromere function. The potential sign of suppression is their smaller size relative to the q-arm counterparts. This scenario implies that both centromeres and telomeres are subject to the mechanical pressure during mitotic/meiotic segregation of chromosomes.
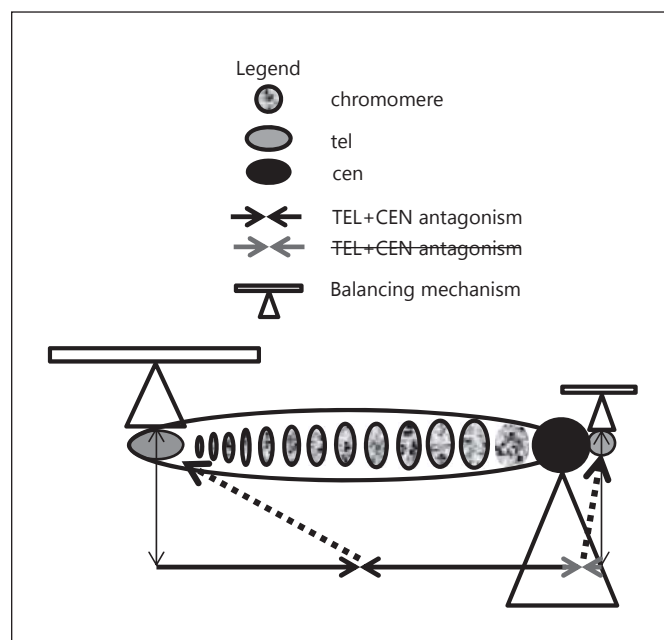

Fig. 3. Integration of TEL+CEN antagonism [Enomoto et al., 1994] and chromomere size gradient [Lima-de-Faria, 1983]. Only a telocentric chromosome is depicted. The p-arm telomere is not subjected to the TEL+CEN antagonism as a result of epigenetic suppression, possibly reflected in its shorter length relative to the q-arm telomere. The q-arm telomere acts as a counterpart of the centromere in the process of chromosome mechanics behind chromosome segregation as explained in figure 4. The function of the chromomere size gradient, the gradual reduction of chromomere size in the direction centromere-telomere, is to help in the processes underlying chromosome mechanics.

Given that there are 2 pairs of telomeres and only 1 centromere in each functioning chromosome, 1 pair of telomeres must be identified as the counterpart of the centromere in chromosome mechanics, and the other pair suppressed. If both pairs of telomeres are equally treated in this mechanic process, the consequence would be unsustainable chromosome instability as explained in figure 4.

In anaphase, the 2-chromatid chromosome is pulled in 2 opposite directions resulting in the immediate separation of centromeres (fig. 4A). This is followed by the gradual release of sister chromatids from the cohesion forces in the direction centromere-telomere and finally the separation of telomeres at the end of this process resulting in 2 new nuclei containing single-chromatid chromosomes [Lima-de-Faria and Bose, 1962; Kirk et al., 1997] (fig. 4A). Thus p-arm telomeres must behave like centromeres in the case of telocentric and acrocentric chromosomes: there must be no separate mechanical pressure on them

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Fig. 4. Principles of chromosome mechanics in anaphase based on Lima-de-Faria and Bose [1962] and Kirk et al. [1994]. **A** Metacentric, submetacentric and telocentric/acrocentric chromosomes are shown in the transition metaphase-anaphase. Chromosomes are subjected to 2 types of force: the centromere separating force (depicted as ←→), and the telomere holding forces (→←). The strength of the telomere holding force is indicated in grey boxes. Chromosomes start separating in anaphase. The first point of separation is the centromere. The gradual release of sister chromatids from cohesion forces follows in the direction centromere-telomere. Telomeres are released from holding forces only when the rest of the sister chromatids is fully separated. In the case of metacentric chromosomes, the process of separation of p-arms and q-arms is symmetric because of their equal lengths. In the case of submetacentric chromosomes, the process of separation of p-arms and q-arms is asymmetric because of unequal lengths. As a result, the shorter arm may be stretched to achieve temporary symmetry [Kirk et al., 1997] as indicated by dashed lines with 2 arrows. As soon as the arms are separated and the telomere holding force is released, the stretched p-arms return to their normal length. In the case of telocentric/acrocentric chromosomes, the telomere holding force for the p-arm is absent. **B** If the telomere holding force is applied on p-arm telomeres of telocentric/acrocentric chromosomes, DNA breakage may occur somewhere between the centromere and telomere.
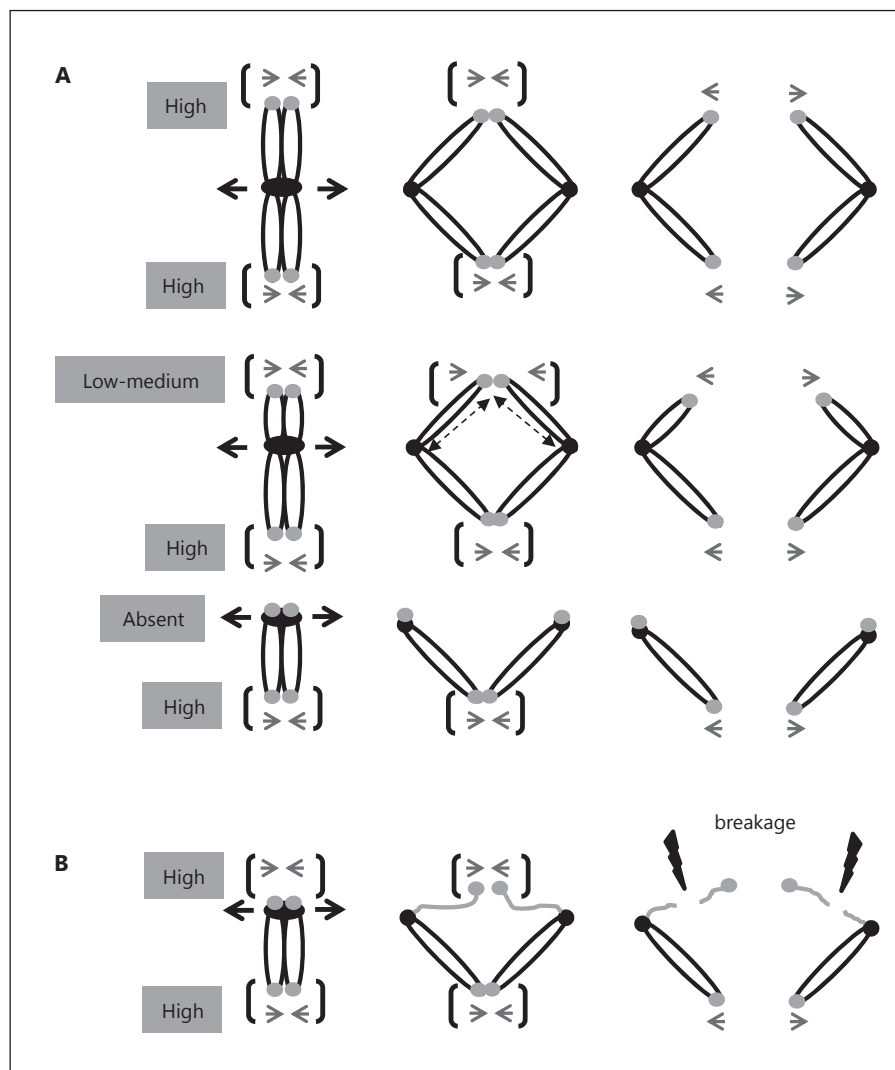
(fig. 4A). By contrast, q-arm telomeres in telocentric or acrocentric chromosomes, and p-arm and q-arms telomeres in metacentric and submetacentric chromosomes must be subjected to the opposite type of pressure. They must be held together until sister chromatids are completely released from the grip provided by cohesion forces (fig. 4A). Only when this grip is no longer active, the pressure on telomeres is released leading to their separation (fig. 4A). However, if p-arm telomeres in telocentric/acrocentric chromosomes are subjected to the equal mechanical force as q-arm equivalents on the same chromosomes, the result would be chromosome breakage with the breakpoints located somewhere between centromeres and p-arm telomeres with subsequent breakage-fusion-bridge type of instability (fig. 4B). For this reason, the

holding pressure is proportional to the arm size: (i) absent in the case of p-arm telomeres in telocentric/acrocentric chromosomes and in short chromosomes in general and (ii) variable in strength ranging from low to high depending on the size of the arm (fig. 4). Therefore, the entire situation outlined in figure 4 looks like a TEL+CEN antagonism on the scale of a eukaryotic chromosome which must be avoided: p-arm telomeres must be epigenetically suppressed. A possible manifestation of this suppression is chromosome-specific telomere length regulation in which p-arm telomeres are shorter than q-arm telomeres (fig. 3). This scenario would require a stringent experimental testing. Nevertheless, all elements of it are described making the testing process relatively straightforward. Interestingly, the analysis of mouse strains with re-

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arranged karyotypes indicated telomere length resetting in rearranged chromosomes relative to the normal counterparts [Modino and Slijepcevic, 2002] thus supporting the idea of telomere length readjustment in newly rearranged chromosomes.

Synthesis and Concluding Remarks

The merger of CFTH with mechanisms of telomere length regulation (fig. 2–4) presented above can now be used as the platform for explaining the mechanisms behind the evolution of chromosome plasticity (fig. 1). As outlined earlier, the term chromosome plasticity refers to the transforming potential of the chromosome material in which the key players are centromeres and telomeres. These chromosome functional elements possess the remarkable power of the functional interchange observable in the 4 major types of evolutionary chromosome rearrangements (fig. 1). The functional interchange is based on epigenetic mechanisms and may involve subtle rearrangements of telomeric and centromeric sequences.

The CFTH predicts that the first functioning eukaryotic chromosome was probably telocentric or acrocentric (fig. 1A). It seems reasonable to assume that the first fully functioning eukaryotic karyotype consisted predominantly of telocentric/acrocentric chromosomes. Acrocentric chromosomes contain the larger load of repetitive sequences between centromeres and p-arm telomeres than telocentric chromosomes. This load could be the result of rearrangements originating from the process of genomic instability that featured heavily in the transition from proto-telomeres and proto-centromeres to fully functioning equivalents. The constraints of chromosome mechanics during cell division required setting telomere length in a highly specific fashion to enable stable segregation of the genetic material (fig. 2–4). In the process of karyotype evolution, telocentric chromosomes started transforming by either p-arm telomere fusion (also known as Robertsonian fusions or centric fusions) or fusion between q-arm telomeres or p-arm and q-arm telomeres resulting in the emergence of metacentric chromosomes (fig. 1A). All processes involved inactivation of 2 telomeres (fig. 1A). However, centromeres were affected differently. In the case of p-arm to p-arm or p-arm to q-arm fusion one centromere was inactivated and the other one served as the functional centromere for the new chromosome (fig. 1A). In the case of q-arm to q-arm fusion, 2 centromeres were inactivated and telomeres at the fusion point transformed into the functional centromere

(fig. 1A). The process of centromere/telomere inactivation or telomere transformation into centromeres involved epigenetic mechanisms which are still not fully understood [Amor and Choo, 2002] but was accompanied by subtle rearrangements of repetitive subtelomeric/pericentromeric sequences or changes in telomere metabolism as argued by Slijepcevic [1998].

More complex karyotypes, gradually emerging in evolution, consisted of a mixture of telocentric, acrocentric, and metacentric chromosomes which could transform further by the process of tandem fusion involving 2 or more chromosomes accompanied again by the epigenetic process of telomere and centromere inactivation/reactivation (fig. 1B). The telomeres in new chromosomes probably required length resetting to satisfy requirements of chromosome mechanics (fig. 2–4). In line with this possibility, mouse strains with constitutional chromosome rearrangements show differences in telomere length between the rearranged chromosomes and their intact counterparts [Modino and Slijepcevic, 2002]. One of the best case studies of tandem fusions in karyotype evolution is the transformation of the ancestral muntjac karyotype (2n = 70) into the Indian muntjac karyotype (2n = 6 or 7) through the process of serial tandem fusions. It has been unequivocally shown that fusion sites involved either telomere-telomere or telomere-centromere fusions [Chi et al., 2005; Tsipouri et al., 2008] in line with CFTH.

Furthermore, the complex karyotypes that emerged with evolutionary time could be further reshaped by centric fission (fig. 1C). This process typically involves chromosome breakage at centromeric sites, reactivation of old telomeres, and reshaping the existing centromere which splits in two (fig. 1C) [Perry et al., 2004]. The fission could also occur at sites of silent centromeres requiring their reactivation (fig. 1C). Again, telomere length resetting is required to satisfy the constraints of chromosome segregation (fig. 2–4). The natural fragility of ITSs and the low repair capacity of telomeric sequences [Slijepcevic et al., 1996; Sfeir et al., 2009; Bosco and de Lange, 2012; Fumagalli et al., 2012] possibly reflect predisposition of metacentric chromosomes containing ITSs to centric fission.

Finally, the last mechanism that shaped the karyotype evolution is de novo formation of centromeres and telomeres (fig. 1D). This mechanism is still poorly demonstrated, but there is enough evidence in the literature to suggest that formation of neo-centromeres, or more precisely evolutionary centromere repositioning, is relatively widespread [e.g., Ventura et al., 2004]. Formation of new telomeres, also known as chromosome healing is poorly documented in karyotype evolution. It is possible that

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this mechanism is rare because it involves the loss of genetic material (fig. 1D). However, the mechanism is relatively well-documented in the case of holocentric chromosomes [Jankowska et al., 2015].

In conclusion, merging CFTH with the mechanisms underlying telomere length regulation, as outlined in this paper, provides a new platform for the study of the chromosome plasticity that typifies karyotype evolution.

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