Telomeric position effect: From the yeast paradigm to human pathologies?

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Review

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Abstract

Alteration of the epigenome is associated with a wide range of human diseases. Therefore, deciphering the pathways that regulate the epigenetic modulation of gene expression is a major milestone for the understanding of diverse biological mechanisms and subsequently human pathologies. Although often evoked, little is known on the implication of telomeric position effect, a silencing mechanism combining telomere architecture and classical heterochromatin features, in human cells. Nevertheless, this particular silencing mechanism has been investigated in different organisms and several ingredients are likely conserved during evolution. Subtelomeres are highly dynamic regions near the end of the chromosomes that are prone to recombination and may buffer or facilitate the spreading of silencing that emanates from the telomere. Therefore, the composition and integrity of these regions also concur to the propensity of telomeres to regulate the expression, replication and recombination of adjacent regions. Here we describe the similarities and disparities that exist among the different species at chromosome ends with regard to telomeric silencing regulation with a special accent on its implication in numerous human pathologies.

Keywords: Telomere; Chromatin; Epigenetics; Human diseases

1. Introduction

The organization of genomic DNA has greatly evolved from unicellular to complex organisms. Despite the increasing complexity of the information carried by the DNA sequence throughout evolution and subsequently, the chromatin architecture that controls the folding of the genome within the nucleus, shared mechanisms orchestrate the epigenetic regulation of chromosome organization and its influence on genome functions. Indeed, it is now evident that chromatin structure plays an important role in regulating gene transcription by providing the proper subnuclear environment to ensure spatial and temporal gene expression. Eukaryotic chromatin is organized into two distinct and interconvertible states, euchromatin and heterochromatin. Each chromatin state can be defined by its level of compaction, the positioning and the spacing of the nucleosomes, its histone code, the covalent modification of the underlying DNA, its non-histone binding factors, the spatial localization within the nucleoplasm and its dynamics during cell cycle.

Heterochromatin was originally described as a portion of the genome deeply stained from metaphase to interphase associated with the pericentric regions, telomeres and some interstitial domains. In higher eukaryotes, constitutive heterochromatin is enriched in methylated DNA, histone H3K9 di- and trimethylation, HP1 binding and can spread over genomic regions inducing thereby the silencing of other sequences. A classical example of silencing is known as position effect variegation (PEV) and occurs when a gene is juxtaposed to heterochromatin. The nature of telomeric and subtelomeric chromatin differs from global constitutive heterochromatin due to the specificity of its DNA sequences, the particular structure of its nucleosomal

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fiber and the binding of specific factors. However, despite these structural differences, most telomeres and subtelomeres from *Saccharomyces cerevisiae* to *Homo sapiens* repress natural or artificially inserted neighboring genes by a mechanism named *T*elomeric *P*osition *E*ffect (TPE) (Fig. 1). Such a widespread conservation of telomeric silencing among eukaryotes suggests that is fundamental for telomere function and consequently chromosome integrity.

The mechanisms of TPE are well documented in *S. cerevisiae* and this review will focus on the cross talks between telomere structure and silencing in different species, with a special emphasis on the emerging role of TPE in human cells and a large spectrum of pathologies.

2. TPE discovery and definition

TPE has been extensively studied in baker's yeast, although it was revealed in this model organism five years after its discovery in *Drosophila melanogaster* [1−3]. Unlike Drosophila, telomeres in *Saccharomyces cerevisiae* are constituted of stretches of highly repetitive telomerase-added repeats and thus resemble most of the eukaryotic telomeres therefore constituting a powerful genetic system for the study of TPE.

TPE in yeast was first demonstrated by insertion of a construct containing a *URA3* marker next to an array of telomeric repeats. Integration of this construct at the subtelomeric *ADH4* locus, close to the VII-L telomere, deletes the terminal 15 kb of the chromosome and positions the *URA3* promoter 1.1 kb from the newly formed telomere, termed truncated VII-L. Expression of the *URA3* gene allows growth of the cells on plates lacking uracil. However, on plates containing a drug toxic for cells expressing *URA3* (5-fluoro-orotic acid or 5-FOA), 20−60% of the cells were still able to grow, suggesting that the *URA3* was silenced in the vicinity of the telomere [4]. Some of the features of TPE were concomitantly described, such as the stochastic reversibility, since the same cells plated onto a medium without uracil can still grow without the amino acid; or the promoter independence and expression variegation, since expression of the *ADE2* gene is also repressed in the same context and colonies obtained present white (ade2+) and red (ade2−) sectors.

TPE has been thoroughly investigated in yeast and its classical definition was first established in this model as a silencing effect originating from the telomere and consisting in an inward Sir-dependent heterochromatin spreading. Since then numerous results (that we will discuss below) have been reported from different organisms on the requirements, the modulations, or the chromosomal and nuclear contexts of this silencing effect. This will lead us to broaden the definition of TPE and to consider it at the level of functional genomic and nuclear organization.

3. What is required for TPE?

Experiments on TPE in *S. cerevisiae* were mostly performed at the truncated VII-L chromosome [4] but also at several other truncated [5] and natural telomeres [6], allowing the identification of more than 50 proteins that can modulate TPE. However, among deletion mutants of these different proteins, only a few exhibit a specific and complete suppression

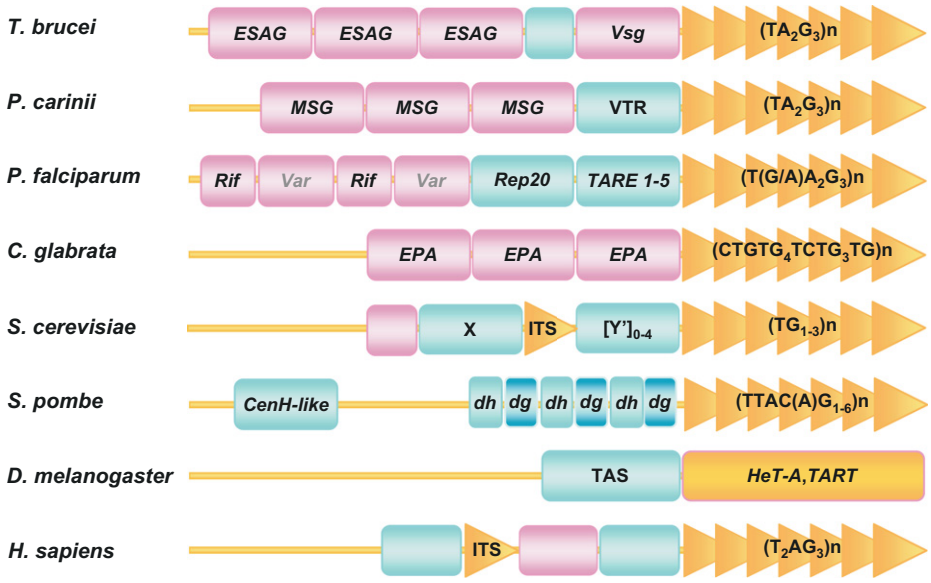

Fig. 1. Comparison of chromosome ends in various eukaryotic organisms known to exhibit telomeric position effect. In eukaryotes, the subtelomeres are patchworks of genes (pink rectangles) interspersed within repeated elements (blue rectangles). At least in baker's yeast and human, large polymorphic blocks of repeated sequences are distributed between the different chromosomes and subtelomeres contain genes. The simple repeats that constitute the telomeric DNA of many organisms and that are synthesized by the telomerase enzyme are represented by triangles and the specific sequence is indicated for every organism. The end of chromosomes of *D. melanogaster* is not synthesized by a telomerase enzyme but is formed through the retrotransposition of the non-LTR retroptransposons *HeT-A* and *TART* (yellow rectangle). VTR, *V*ariable *T*andem *R*epeat; TAS, *T*elomere *A*ssociated *R*epeat; ITS, *I*nterstitial *T*elomeric *R*epeats; ESAG, *Trypanosoma brucei E*xpression *S*ite *A*ssociated *G*ene; Vsg, variant coat protein; MSG, *Pseudomonas carinii* surface glycoprotein gene. Var, Rif, *Plasmodium falciparum* subtelomeric genes; *Rep20*, *TARE 1−5*, subtelomeric repeats. EPA, *Candida glabrata* adhesin gene. Dh, dg, *Schizosaccharomyces pombe* subtelomeric repeats.

of telomeric silencing [7]. Among them, Sir-complex proteins (Sir2p, Sir3p and Sir4p for *S*ilent *I*nformation *R*egulators), [8], Ku heterodimer components (yKu70p and yKu80p), [9,10] and C-terminal domain of Rap1p [11] are absolutely required for TPE. These proteins have several functions but they are bound to the telomeres and their deletion abrogates telomeric silencing [12−14]. Rap1p and the Ku complex can both bind telomeric DNA and Sir4p [15,16]. In the current model for TPE establishment and linear spreading, Rap1p and Ku are responsible for the initiation/nucleation step. They recruit the Sir-proteins at the telomere and thereby initiate the formation of an heterochromatin complex that will propagate toward the subtelomeres through the interaction of the Sir-complex with histone tails [15−18]. This spreading requires the Sir2p NAD-dependant histone deacetylase activity that permits Sir3p and Sir4p binding [19,20] and distributes along the DNA sequence by sequential deacetylation of the histone tails and binding (Fig. 2).

In other organisms, most of the few factors mediating TPE identified so far are functional homologs of *S. cerevisiae* proteins. Thus, in *Schizosaccharomyces pombe*, the telomeric repeats-binding protein Taz1p recruits spRap1 (homologous to Rap1p in *S. cerevisiae* [21,22] at the telomere, and both proteins are required for TPE [23,24]. Nevertheless in this model, no link could be established between Ku and TPE. Although they have no homolog in *S. cerevisiae*, HP1 proteins are

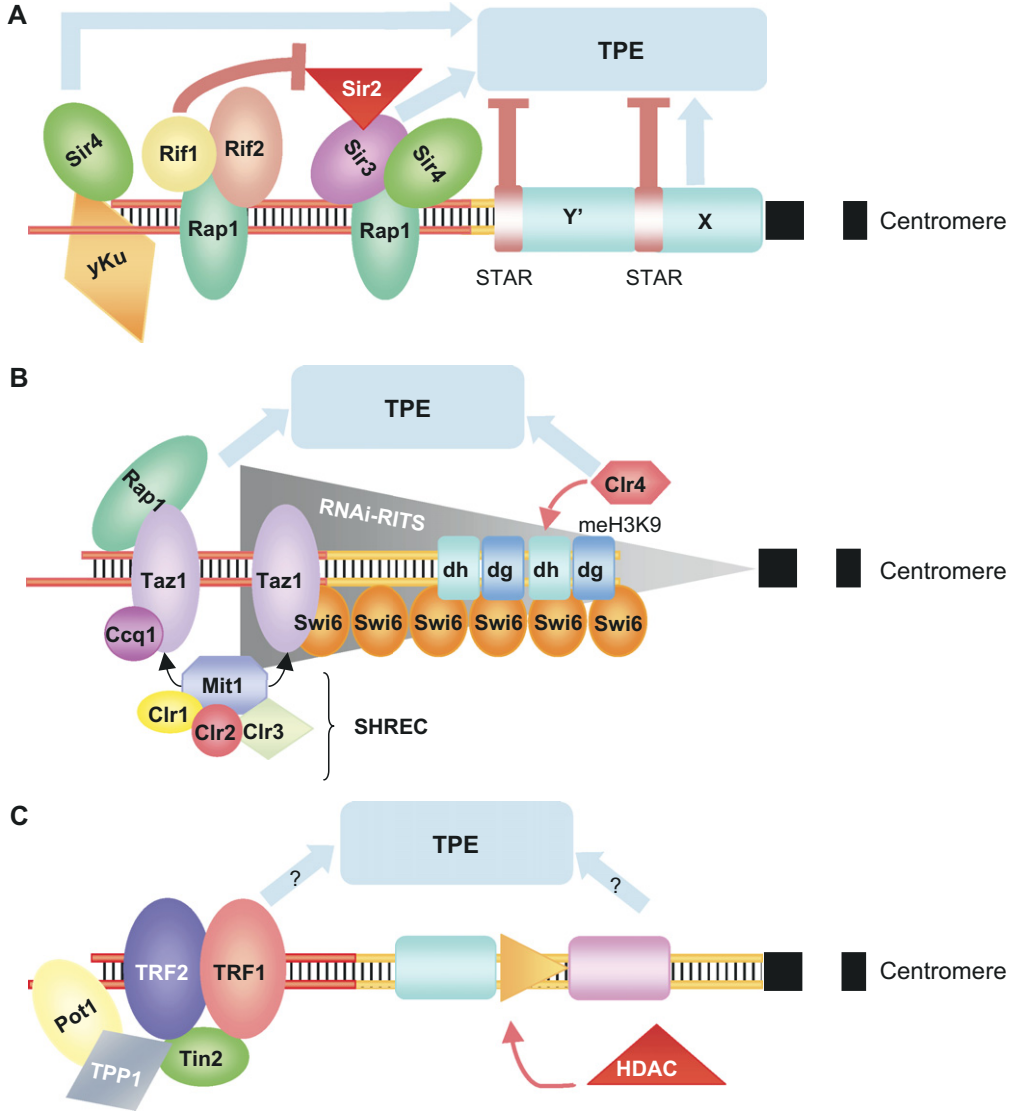

Fig. 2. Implication of the telomeres and subtelomeres in the regulation of telomeric position effect. (A) In *Saccharomyces cerevisiae*, distinct DNA binding factors cooperate for the spreading of the silencing along the telomeric and the subtelomeric region. At natural telomeres, the X element reinforces the silencing while Y′ element acts as a boundary. (B) In *Schizosaccharomyces pombe*, the telomeric protein Taz1 and methylation of histone H3 K9 residues by the Clr4 histone methyltransferase recruit Swi6 to the telomeric associated sequences and spread silencing toward the centromere to cover the subtelomeric region over 45−75 kb in cooperation with the RNAi-RITS machinery. The SHREC complex containing the Clr3 histone deacetylase and the Mit1 chromatin remodeling factor associates with Ccq1 and Swi6 and cooperates with the Taz1 and RITS pathway to facilitate chromatin condensation and telomeric silencing. (C) In human cells, the telomeric position effect may involve the cooperation of telomere binding proteins such as TRF1 and classical chromatin remodeling factors. As described in different species, the identity of the subtelomeric regions might influence TPE and explain different pathologies associated with the rearrangement of these regions.

involved in TPE in Drosophila (on chromosome 4 [25]) and fission yeast [22,26] where they could play similar roles of the budding yeast Sir3p and Sir4p in the spreading of heterochromatin.

4. Telomere length and telomeric silencing

Increasing the length of telomeres improves TPE [19,27,28]. A plausible explanation is that longer telomeres bind more Rap1p that subsequently recruit more Sir-complexes and thereby facilitate the formation of a heterochromatin complex able to polymerize and spread within the subtelomeric regions. However, the interplay between TPE and length regulation might be less direct. For instance, inactivation of the Rif proteins improves TPE and increases telomere length by alleviating the competition with Sir proteins for access to telomere-bound Rap1p [29,30].

Insertion of a stretch of telomeric repeats at an internal chromosomal site can induce silencing, certainly through Rap1p binding, but this effect is not as strong as telomeric silencing, which can account for Ku participation in the recruitment of silencing factors [31] and for a proper subnuclear localization within heterochromatin compartments enriched in Sir proteins [32,33]. In conclusion, the influence of telomere length on TPE is not merely the length by itself but concomitant changes in the recruitment of silencing factors (Fig. 2).

5. Modulation by the subtelomere: importance of the telomere identity

As pointed out earlier, most of the studies on TPE in *S. cerevisiae* have been performed on truncated telomeres that all exhibited a variable silencing effect depending on the telomere analyzed [4]. TPE is also present at baker's yeast natural telomeres but its strength can vary widely [6]. Indeed, each of the 32 yeast chromosomes has a different composition in subtelomeric elements that can modulate TPE and polymorphisms in these regions are also found in the different strains [34,35]. In *S. cerevisiae*, these modulators include two types of subtelomeric repeats: X and Y′ (Fig. 1).

The X subtelomeric element contains a ''core X'' element consisting in an ARS consensus sequence (ACS) and an Abf1p site present at all yeast telomeres [35]. This core X sequence behaves as a protosilencer, i.e. it does not act as a silencer by itself but reinforces silencing when located in the proximity of a master silencer [36,37]. At some chromosome ends, the core X element can be associated to strong boundary elements dubbed STAR for *S*ub*T*elomeric *A*ntisilencing *R*egions consisting of binding sites for Tbf1p and Reb1p [38].

The Y′ element is found on 50−70% of yeast telomeres, in 1−4 copies inserted between the X element and the telomere [39]. It contains two open reading frames (ORF), an ARS that can also bind the ORC complex and a STAR element immediately abutting the telomeric repeats, which similarly to the X STAR sequences consists of Tbf1 binding sites [38].

Different combinations of STAR and protosilencer at native telomeres are likely to contribute to their respective behaviors with regard to TPE. In general terms, X-only telomeres usually exhibit a strong silencing [6] even if two of them do not seem to propagate silencing or bind Rap1p [40]. Mutations in the ''core X'' that either impair Abf1p binding or recruitment of ORC to the X-ACS sequence strongly reduce telomeric silencing at XI-L chromosome [6] suggesting that both binding sites contribute to telomeric silencing. Sir1p, that does not seem to have any effect on truncated telomeres [8], participates in silencing at the XI-L telomere [6,38], possibly through interaction with the ORC complex [41,42]. The Y′ element possesses anti-silencing properties linked to the boundary activity of the STAR sequence and limits the extend of spreading toward the subtelomeric regions [38]. On truncated VII-L, the level of telomeric silencing decreases exponentially and continuously with distance, but on some native telomeres, combination of X and Y′ elements results in variable levels of gene silencing with regions protected from TPE by the STAR boundaries and resumed silencing at a distance from the telomere by protosilencers suggesting that telomeric position effect can be a discontinuous mechanism relayed from place to place by silencers and protosilencers [6,38]. In agreement with this hypothesis, functional interactions between a protosilencer and a silencer are not hampered by the presence of a subtelomeric insulator or an intervening insulated domain [37,38]. Such a discontinuity in silencing argues in favor of a coalescence model in which silencers and protosilencers form complex interactions leading to an apparent spreading of silent chromatin that might be further facilitated by nuclear positioning.

6. TPE and histones modifications

The post-translational modifications of the core histone (H2A, H2B, H3 and H4) provide heritable information that orchestrates transcription, replication and chromatin organization through cell divisions. TPE is induced by such modifications converting euchromatin into repressive chromatin. In baker's yeast, the first clues were given by mutations in histone H4 N-terminal tail residues that suppress TPE at the truncated VII-L telomere [8]. Furthermore, deletions of N-terminal tails of histones H3, and H2A also impede telomeric silencing [43−45] by altering histone acetylation and Sirp recruitment [18,46]. Indeed, among the different residues that can be modified, lysine can bear several modifications such as acetylation, methylation, phosphorylation, ubiquitination or ADP-ribosylation with a preponderant role for acetylation in TPE modulation [8,44]. Moreover, TPE induced by heterochromatin spreading is dependant upon Sir2p HDAC activity [20] and can be counteracted by Sas2p-dependant acetylation of H4-K16 [47]. Deletion mutants of *Sas2* have an increased TPE and Sir3p spreading extents from 3 to 15 kb at the truncated VI-R [48]. Same type of extended telomeric silencing and Sir3p spreading has recently been reported in deletion and unacetylable mutants of the H2A variant, H2A.Z, enriched at subtelomeric regions [49,50] and in catalytic mutants of its acetyl-transferase, Nu4A [51]. In conclusion, acetylation seems to act as an important boundary against the propagation of telomeric silencing.

To the present knowledge in *S. cerevisiae*, histone methylation only affects some lysines on H3: Lys4, Lys36 and Lys79 [52] that are methylated by specific methyltransferases (Set1p, Set2p and Dot1p respectively [53] and can also be demethylated (Lys4 by Jhd2p, Lys36 by Jhd1p and Rph1p, [54]) except for Lys79, located within the histone core. Methylation of these residues displays anti-silencing properties at the telomeres, mainly by preventing Sirp association to histone H3 tail [55]. Noteworthy, deletion mutants of *Set1p* and *Dot1p* exhibit reduction in telomeric silencing level due to the limiting amount of Sir3p that is displaced toward euchromatic regions that are no longer protected by H3 methylation (Fig. 3).

The interpretation of this code can be affected by another adjacent modification on the same histone tail and the primary function of a particular modification might be modulated by another adjacent modification. Thus, the combinatory use of different modifications participates in the function of the chromatin fiber and gives enormous potential for the variability of the biological response. For example H2B ubiquitination is necessary for H3-K4 and H3-K79 methylation which prevent Sir4p binding, whereas Sir4p recruits Ubp10p/Dot4p that deubiquitinate H2B and Sir2p that deacetylates H4-K16, both favoring establishment of telomeric silencing [56].

In *Schizosaccharomyces pombe*, the chromatin changes associated with the TPE machinery are more closely related to that of Drosophila and mammals. Firstly, it requires the methylation of H3K9 residues by the Clr4 histone methyltransferase [57], which allow the binding of Swi6 (the ortholog of HP1). Secondly, specialized repeats (*dg* and *dh*) present at subtelomeres and other heterochromatin loci contribute to telomeric silencing through the formation of the RITS complex. This RNAi-induced transcriptional silencing machinery uses siRNAs and specific factors (such as Argonaute) to initiate the targeting of H3K9 methylation and heterochromatin assembly at repetitive DNA and stabilize the compaction of higher-order chromatin structure to silence specific chromosome regions [58]. Recently, a new complex termed SHREC and composed of Clr1, Clr2, the Clr3 histone deacetylase and the SNF2 chromatin-remodeling factor homolog Mit1 has been identified [59]. At telomeres, SHREC interacts with Ccq1, a telomere binding protein capping and protecting telomeres from degradation [60] that along with Taz1 (the homolog of mammalian TRF1/2) and Swi6/HP1 act in parallel to the RNAi pathway to restrict polymerase II and stabilize heterochromatin structures (Fig. 2).

7. Telomeric position effect and nuclear periphery: The reservoir model

In budding yeast, the 32 telomeres are clustered into 4−6 foci which are primarily associated with the nuclear envelope [61]. This peripheral localization of telomeres is dependent on redundant pathways [62]. One acts through Ku and the second through Sir4 [63,64]. Neither Ku nor Sir4 possess a transmembrane domain and their recruitment to the nuclear envelope depends upon other factors. Sir4 is tethered to the nuclear periphery through interaction with Esc1, which is localized at the edge of the nucleus between nuclear pores independently of silent chromatin [63]. The anchoring of Ku also depends on the presence of Esc1 but only during S phase [63].

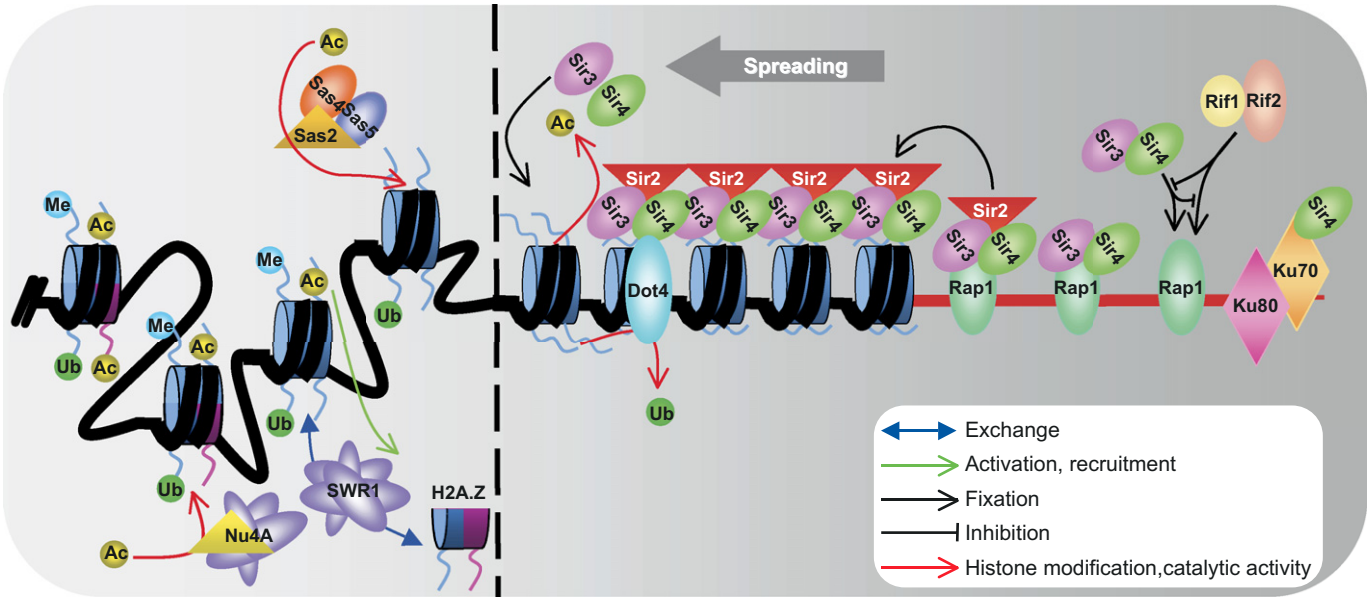

Fig. 3. Telomeric position effect is regulated by different levels of chromatin compaction in *Saccharomyces cerevisiae*. Telomeric position effect is regulated by proteins bound to the telomeres and the subtelomeres. The Sir complex is recruited to the telomere by Rap1. The Sir2 histone deacetylase recruited at telomeres sequentially removes the acetyl residues along the subtelomeres. This spreading of repressive chromatin is facilitated by the sequential recruitment of Sir3 and 4 along the chromatin fiber leading to the variegated and heritable repression of telomere proximal genes. The Dot4 protein is recruited by Sir4 and removes ubiquitin residues from H2B impairing thereby methylation and preventing Sir4p binding and the spreading of telomeric silencing. The Sas2-associated complex counteracts silencing by modification of Lysine 16 at histone H4 tail. The incorporation of acetylated H2A.Z histone variants at subtelomeric region act as a boundary that limit the spreading of silencing (represented by a dashed line) toward the centromeric regions.

The factors responsible for the peripheral localization of Ku during G1 have not yet been characterized.

Mating-type silencers require telomere proximity to be fully functional [65] and tethering of a silencer-flanked reporter to the nuclear periphery facilitates its repression [65]. However, relocation to this peripheral nuclear compartment probably does not cause repression *per se* [66] and silencing can be maintained without perinuclear anchoring [67]. All of these data, together with microscopy analyses, converge toward a reservoir model where telomere clusters act as a subnuclear compartment concentrating key silencing factors like the Sir proteins [65]. Consequently, although the perinuclear location is not strictly required to maintain the silent state, silencers and telomeres would need to be somehow associated to this compartment to be in a local microenvironment containing enough silencing factors to shut-down gene expression. In agreement with this model, close interactions between *HML* silencers and telomeres have been observed by using a methyltransferase targeting assay [68].

Consistent with previous work showing that insulator activity in various species correlates with a particular spatial arrangement in the nucleus [69—72], the STAR boundary elements antagonize the peripheral localization of telomeres, suggesting a mechanism by which they block TPE spreading [73]. Therefore, the individual organization of native subtelomeres is likely to directly influence telomere subnuclear localization and TPE capacities.

Nuclear periphery can also confer an optimal environment for transcriptional activation through the binding of genes to the nuclear pore complex [74]. Promoting a stronger association with the nuclear envelope improves silencing in non-inductive conditions but increases expression in inductive conditions, accounting for an ambivalent role of nuclear rim association [75]. Since telomeres are mainly localized between pores [76], one can predict that silent telomeres and activated genes distribute to different subdomains at the nuclear envelope.

8. TPE and the 3Rs: Replication, recombination and repair

Besides their effect on transcriptional regulation, telomeres also exert position effect on others DNA transactions.

In budding yeast, the telomeres replicate at the late stage of the S-phase [77,78] mainly because they inactivate or delay most of the origins present in the subtelomeric X and Y′ elements [4,79—82]. Reminiscent of TPE, short telomeres can replicate earlier than long telomeres [83], Sir3 inactivation leads to a premature activation of Y′ origins [80] and tethering of Sir proteins near an origin can reset replication timing from early to late S-phase [84]. These results are in favor of a model in which the Sir-mediated silent chromatin emanating from telomeres blocks replication initiation in the subtelomeric regions. However, the telomeric position effect on replication timing extends over a distance (∼35 kb) that is well beyond the 6—8 kb seen for the Sir-dependent gene repression [6,85]. This suggests that Sir-independent chromatin-mediated mechanisms can also contribute to the late activation of telomere-associated origins.

Subtelomeric domains are cold-spots for meiotic recombination in a variety of organisms. For example, the crossovers are rare in the telomeric regions of grasshopper chromosomes [86] and the double-strand breaks that initiate meiotic recombination in yeast are quasi-absent from sequences within 50 kb from telomeres in a Sir4-independent manner [87]. However, it is worth noting that meiotic recombination can occur at an elevated rate near some human telomeres and can have both advantageous and pathological consequences in human biology [88]. In mitotic yeast cells, telomere proximity represses the homologous recombination between two internal stretches of telomeric DNA but not between two non-telomeric sequences [89]. Interestingly, similarly to the TPE on meiotic recombination, this anti-recombination effect does not rely on a Sir-dependent mechanism, revealing that Sir and non-Sir dependent mechanisms are responsible of various classes of TPE. The spatial localization of telomeres in yeast cells contributes to the regulation of TPE. In addition, the anchoring of the telomeres to the nuclear pores is essential for efficient repair of Double Strand Breaks at subtelomeric zones, likely by protecting the chromatin structure [90].

9. Biological functions of yeast TPE

Determining the biological relevance of TPE is difficult due to its link to the composition of individual telomeres and the discontinuity in regions of genes exposed to telomeric silencing. Genome-wide studies of transcription levels in *S. cerevisiae* could determine that about 267 genes located less than 20 kb from the telomeres display a mean expression level that is roughly 20% of non-telomeric gene expression [85]. Among this small number of genes, less than 10% are repressed in Sir-dependent pathway whereas one half is derepressed upon H4 depletion, which is about three times more than for non-telomeric genes [85]. Genome-wide mapping of deacetylase activity identified a lysine deacetylase specific for H2B and H3 that is involved in the Sir-independent repression of genes clusters gathered in domains 10—25 kb from telomere, termed HAST (*H*da1-*A*ffected *S*ub*T*elomeric) [91].

There are different explanations for the paucity of meiotic recombination initiation events within the subtelomeric regions. First, breaks fails to occur in subtelomeres merely as a consequence of the heterochromatin nature of chromosome ends. However, this process is Sir-independent, suggesting that the anti-recombination properties of subtelomeric regions have been actively selected during evolution because of detrimental effects on chromosome stability. Indeed, subtelomeric recombination initiations are expected to favor unequal crossovers and to promote homolog disjunction, as observed for instance for human chromosomes 16 or 21 with crossovers near the telomeres [92,93].

Recently, a genome comparison of closely related Hemiascomycetes species revealed that subtelomeric gene families are in general specific for the different species and do not

exhibit the same characteristics in terms of copy number and subtelomeric distribution [94]. The expression of these genes is influenced by TPE in several yeast species [95,96]. Nevertheless, a subtelomeric enrichment of genes related to stress response and metabolism in non-optimal growth conditions appears to be a conserved feature in many yeast species [91]. Most of these genes are silenced under optimal growth conditions. In budding yeast, many different types of stress-like nutrient starvation, heat shock or chemical treatment can induce a hyperphosphorylation of Sir3p and a consequent decrease in TPE at the truncated VII-L [97]. This also leads to an increase in the expression of natural subtelomeric genes such as the *PAU* genes which are involved in cell wall constitution and drug resistance [98]. Gene of cell wall proteins are also submitted to Sir-dependent TPE in *Candida glabrata* [99]. In *S. cerevisiae*, subtelomeric HAST domains also contain clusters of normally silenced genes that are involved in neoglucogenesis or stress response [91]. For example, the *FLO* genes, involved in cellular adherence, are mostly contained within these domains and are silenced in a Sir-independent way which is however dependent upon Sir2p homologs, Hst1p and Hst2p [100]. In *S. pombe*, many genes involved in response to nitrogen starvation are also clustered in subtelomeric regions and silenced by Hda1p ortholog, Clr3 [101].

In summary, clustering stress response genes at subtelomeres seems to be an evolutionary conserved strategy that allows their reversible silencing and a fast response to changes in environmental conditions (this will be further discussed in the parasite section). One can imagine that a single mutation or epimutation altering TPE would allow subtelomeric gene expression to proceed at full rein, increasing the chances for the cell to express a gene that would be important for adaptation [102]. Indeed, telomeres of budding yeast carrying a template mutation in the telomerase RNA gene replacing the yeast telomeric repeat sequence by the human-type sequence appear stable but have lost their capacity to silence [103].

10. TPE is conserved at the unusual telomeres of Drosophila

Unlike many organisms, *Drosophila* species lack telomerase but maintain their telomeres by the transposition of the retrotransposons *HeTA* and *TART* to chromosome ends [104] (Fig. 1). Proximal to the terminal transposon array, Drosophila telomeres carry several kilobases of conserved complex satellites termed *T*elomere *A*ssociated *S*equences (TAS) (reviewed in [105]). Despite the structural differences of telomeres in other species, *Drosophila melanogaster* exhibits telomeric silencing as observed when reporter genes are inserted at a telomeric position [1]. All the telomeric transgenes subjected to variegation lie adjacent to TAS, suggesting that these sequences also contribute to telomeric silencing as for subtelomeric elements in other organisms [106]. Interestingly, genetic modifiers of position effect variegation (PEV) display little or no effect on TPE, suggesting the existence of specialized mechanisms. TPE at the second and third chromosomes is sensitive to the dose of Polycomb group genes [107], whereas TPE at the fourth chromosome or at a terminally deleted minichromosome is sensitive to the dose of HP1 [25,108−110]. HP1 is a stable component of all telomeres in Drosophila cells and its absence in mutant *Su(var)2−5* cells causes multiple telomere-telomere fusions [111]. Interestingly, chromosome protection and telomeric transcriptional repression appear as separable mechanisms associated with two types of HP1 binding [112]. HP1 caps the telomere by direct binding while it contributes to telomeric silencing by interacting with trimethylation of Lysine 9 at the histone H3 tail [112,113]. Mutation in the gene for HP1 also increases the abundance of *HeT-A* and *TART* RNA and their frequency of transposition to broken telomeric ends [114]. In contrast, mutations of *Ku80* or *Ku70* strongly increase transposition but do not affect expression of *HeT-A* [115] (Fig. 1). The remarkable capacity of yeast and fly telomeres to uncouple telomere protection from TPE functions might provide a unique ability of rapid adaptation through changes in the subtelomeric transcriptional program and gene shuffling without altering the integrity of the rest of chromosomes.

The ATM kinase that plays conserved roles in DNA repair and telomere function is specifically required for normal levels of HP1 and HOAP (Heterochromatin protein 1/ORC2 associated protein) at telomeres, but not at centric heterochromatin. In addition, *atm* mutations may suppress TPE, by affecting normal telomere chromatin structure [116]. ATM is specifically required for localization of HP1 to telomeres but not centromeric or euchromatic sites and loss of *atm* suppresses silencing by telomere-associated sequences but not at euchromatic sites [116]. Moreover, mutants of *gpp,* the fly ortholog of the *Saccharomyces cerevisiae Dot1* gene, dominantly suppress silencing by telomeric, but not centromeric heterochromatin. [117]. Analysis of *gpp* mutants indicates that, like Dot1 [118], GRAPPA functions as a methyltransferase and is required for the methylation of lysine 79 of histone H3 (H3meK79) suggesting that, as observed in yeast, telomeric position effect at Drosophila telomeres is associated with classical modifications of the histone code [117]

In ovaries and oocytes, *HeT-A* and *TART* are partially regulated by the RNAi machinery. Indeed, mutation in the RNA helicase gene*, Spindle-E* (*spnE*) encoding a DEAD-box helicase and the argonaute gene *aubergine* (*aub*) necessary for the assembly of the RNA-induced silencing complex (RISC), increases the rate of *HeT-A* and *TART* transcription and is accompanied by more frequent transpositions to chromosome ends [119].

Thus, despite their unique and distinct composition and maintenance process, Drosophila telomeres share many of the features of telomeres in other species with regard to the heterochromatization of the telomeric and subtelomeric regions.

11. Telomeric position effect in higher eukaryotes

The heterochromatin nature of mammalian telomeres and their capacity to induce position effect have been controversial for many years. The first example of telomeric position effect

in vivo came from the analysis of replication timing of human chromosome 22 carrying chromosomal abnormality. Deletion of chromosome termini is frequently observed in pathologies such as cancer or genetic diseases. Various processes that result in the addition of a new telomere can stabilize these broken chromosome ends. One of these pathways is the process in which the broken chromosome acquires a telomeric sequence from another chromosome, homolog or sister chromatid called telomere capture. An alternative is *de novo* telomere addition where the end of a broken chromosome is stabilized by telomerase dependent addition of telomeric repeats named telomeric healing. Telomere healing following the deletions of subtelomeric elements changes the replication timing of chromosome 22, which is shifted from early S phase to later time [120]. This delayed replication is not associated with differences in DNA methylation status, condensation of the chromatin structure of the region or silencing of some subtelomeric genes located 50 kb from the telomere suggesting that the large distance between the telomere and the genes may protect from the spreading of telomeric silencing [120]. However, other studies imply that human telomeres neither modulate the expression of nearby genes nor affect the homeostasis of telomeres [121,122].

Compelling evidence for transcriptional silencing in the vicinity of human telomeres was provided experimentally by using transgenes inserted adjacent to telomeres, similar to the approach used with yeast after telomere fragmentation [123,124]. By a telomere seeding procedure, natural telomeric regions have been replaced by artificial ones containing a reporter gene. Using this method, reporter genes in the vicinity of telomeric repeats were found to be expressed on average ten-fold lower than reporter at non-telomeric sites. Overexpression of the human telomerase reverse transcriptase (*hTERT*) in the telomeric clones resulted in telomere extension and decrease in transgene expression [123] while overexpression of *TRF1*, involved in telomere length regulation, lead to the re-expression of the transgene [124], indicating the involvement of both the telomere length and architecture in TPE as observed in yeast. In addition, the treatment of cells with Trichostatin A, an inhibitor of class I and II histone deacetylases antagonizes TPE. In human cells, TPE is not sensitive to DNA methylation [124] while hypermethylation of the transgene appears as a secondary effect in TPE in mouse ES cells [125].

In mammals, all three HP1 paralogs are found at telomeres, and loss of histone H3 methyltransferases leads to reduced levels of HP1 proteins at telomeres [124,126,127]. In human cells, there is a correlation between HP1 delocalization and TPE alleviation by TSA treatment [124]. Taken together, these data suggest that in mammals, like in other simpler eukaryotic organisms, classical heterochromatin factors cooperate with telomere-associated proteins in the remodeling of the telomeric and subtelomeric regions and the propagation of the silencing at chromosome ends (for review see [128]). By comparison to position effect variegation, TPE might thus be an alternative and specialized silencing process acting for instance through the interaction between the chromatin remodeling factor SALL1 and TRF1 [129] or the telomeric sheltering component TIN2 and HP1 [130] (Fig. 2).

12. Telomeric position effect and human pathologies: Evidence and case reports

We have described in the first part of this review the importance of subtelomeric elements in the regulation of TPE in yeast and in fly. Very little is known on the function of human subtelomeres in the regulation of the diverse roles of telomeres in cellular homeostasis. However, these regions prone to recombination and rearrangements are associated with genome evolution but also human disorders. Indeed, the involvement of TPE has been evoked to explain the etiology of several developmental diseases although the molecular mechanisms remain hypothetical.

Unlike the reporter genes artificially inserted immediately adjacent to the telomere, the most distal unique region of the chromosomes and telomeres are separated by different types of subtelomeric repeats varying in size from 10 to up to 300 kb in human cells and contain repetitive sequences of different types (reviewed in [105]). In the human population the subtelomeric regions are highly polymorphic and the rate of recombination at chromosome ends is higher than in the rest of the genome. Such rearrangements participate in the genome variability and the length of variation may be up to hundreds of kilobases among the different haplotypes (Fig. 1). Although the coverage of chromosome ends has not been fully achieved, available sequences allowed the representation of a detailed paralogy map showing that several blocks of sequences are shared by different human subtelomeres [131,132]. Various tandemly repeated units called *T*elomere-*A*ssociated *R*epeats (TAR1), short native telomeric arrays and numerous degenerate telomere-like repeats are also located at variable distance from the telomere and subtelomeres contain members of 25 small families of genes encoding potentially functional proteins [131,132]. Interestingly, many of them are involved in the adaptation to the environmental changes like in other species suggesting that the plasticity of chromosome ends is likely to play a key role in genome evolution and that abnormalities or dysregulation of these genes may have phenotypical consequences. Thus, telomere length-mediated transcriptional regulation of natural telomeric genes in human cells is likely to operate through the telomeric heterochromatin structure, involving long and variable stretches of subtelomeric sequences. Therefore, a broad range of natural telomeric gene expression level is likely to be found from individual to individual as described in yeast [6] and renders analysis of telomeric position effect in human pathologies challenging. The only naturally occurring situations wherein telomeric repeats are adjacent to unique sequences are those that occur in patients with truncated chromosomes ends that have been repaired by the process of telomeric healing or that lead to the formation of ring chromosomes. However, the molecular pathogeneses associated with these rearrangements have never been investigated.

Thus, TPE may play a direct role in human diseases as a result of repositioning of active genes near telomeres or subtelomeric sequences following such chromosome rearrangements and subtelomeric element may either participate in the spreading of TPE or shelter genes from this silencing. In addition, TPE might be indirectly involved in human health through the regulation of adaptation genes in protozoan parasites.

13. Idiopathic mental retardation

The importance of subtelomeric rearrangements, chromosome-end truncations and telomere healing in idiopathic mental retardation is a well-established issue [133−135]. These abnormalities were first observed by standard chromosome banding and fluorescent *in situ* hybridization (FISH) testing. About 5−10% of the clinical cases with severe to mild mental retardation show relatively small subtelomeric abnormalities of chromosomes arms with exception of the p-arm of the acrocentric chromosomes. During the recent years, the development of high resolution genetic analysis techniques allowed a better characterization of the genotype of patients affected with such developmental delays and lead to the identification of different genes disrupted by these subtle terminal deletions [136−138]. Nevertheless, gene deletion does not always explain the pathological manifestations. Among the hundreds of patients analyzed, the size of the subtelomeric region disrupted may be accompanied by variable degrees of chromatin condensation and explain the penetrance of the clinical manifestations in patients [137].

Thus, it is conceivable that genes residing in close proximity to healed telomeres become epigenetically inactivated contributing to the phenotype. However, characterization of the rearrangement's effect on gene expression is still needed to prove that mental retardation is caused by modification of the chromatin architecture at telomeric and subtelomeric loci.

14. Ring chromosome

Hundreds of patients have been reported with various combinations of malformations, minor abnormalities and growth retardation usually associated with mental retardation linked to the formation of a ring chromosome [139,140]. Ring chromosomes are thought to be formed by deletion near the end(s) of chromosomes followed by fusion at breakage points and have been described for all human chromosomes. The resulting phenotypes vary greatly depending on the size and the nature of the deleted segments. Most ring chromosomes are formed by fusion of the deleted ends of both chromosome arms coupled with the loss of genetic material. However, in a few cases, the rings are formed by telomere-telomere fusion with little or no loss of chromosomal material and have intact subtelomeric and telomeric sequences suggesting that the ''ring syndrome'' might be associated with the silencing of genes in the vicinity of a longer telomere. The formation of intact ring caused by telomere−telomere fusion and associated with putative telomeric position effects has been reported for different autosomes [141−143]. For instance, a severe seizure disorder with features of non-convulsive epilepsy is a characteristic of ring chromosome 20. In this pathology, the formation of ring chromosome is generally associated with a breakage in each chromosome arm and the subsequent fusion of the broken ends with the loss of the telomere, subtelomeric regions or *CHRNA4* and *KCNQ2,* two well-known epilepsy genes. In a patient with a typical severe epilepsy, classical cytogenetic methods, chromosome and quantitative FISH showed that the ring had a longer telomere than either of the 20p or 20q telomere end suggesting that telomeric position effect silences the *CHRNA4* and *KCNQ2* genes [144].

Strikingly, most of these genetic diseases associated with mental retardation and different malformations are either linked to terminal deletion or fusion raising the hypothesis of a major contribution for telomere and subtelomere integrity in development. However, attempts to make genotype−phenotype correlations with specific anomalies have been difficult because of the paucity of reported cases and the variability in the size of the terminal deletion. In addition these chromosomal abnormalities are often mosaic, and the occurrence of sister chromatid exchange complicates the description of these heterogeneous developmental disorders and the precise classification of the genetic alterations.

Ring chromosomes have also been described in other species such as *S. pombe*, which survives in the absence of telomerase and telomeric repeats by rendering chromosomes circular [145−147]. In these ring chromosomes, Taz1p binding, which is highly specific to telomeric repeats [21], telomeric associated structure and function are stably inherited [148]. Therefore, the existence of another factor epigenetically associated with the subtelomeres and forming a functional complex with the telomeres was hypothesized and one can speculate now that the recruitment and spreading of Swi6, SHREC and RITS machinery may participate in this epigenetic maintenance of telomeric function [22].

The probable conservation of mechanisms from simple to complex organisms evoked throughout this review may thus help to understand the etiology of these large and heterogeneous groups of genetic abnormalities.

15. Facio-scapulo humeral dystrophy as a model of telomeric position effect pathology?

One of the best characterized human genetic diseases potentially linked to TPE is the Facio-Scapulo-Humeral Dystrophy (FHSD). This puzzling pathology is associated with the deletion of repeated elements at the 4q35 locus. Normal 4q35 chromosome termini carry 11−100 copies of a 3.3 kb repeated element named *D4Z4* while in FSHD patients the pathogenic allele has only 1−10 repeats. This autosomal dominant disorder is the third most common myopathy clinically described by a progressive and asymmetric weakening of the muscles of the face, scapular girdle and upper limb. The pathogenic alteration does not reside within the gene responsible for the disease but is rather related to an epigenetic mechanism. Several hypotheses have been proposed to explain this enigmatic pathology [149,150]. Evidence for the binding of

a repressor complex to *D4Z4* that might regulate the expression of the nearby genes was provided [151]. However, the most popular hypothesis to explain this pathogenesis is the involvement of PEV or TPE [152]. *D4Z4* shares the properties of heterochromatic sequences and it was postulated that *D4Z4* and surrounding sequences would be packed as heterochromatin leading to the silencing of nearby genes. In patients, the partial loss of the *D4Z4* repeat would lead to local chromatin relaxation and to the transcriptional upregulation of genes [153,154]. However, the analysis of the chromatin structure of this locus either in normal individuals or in FSHD patients does not support this hypothesis [155]. Alternatively *D4Z4* may act as an insulator, separating heterochromatic telomeric sequences distal to *D4Z4* from euchromatic sequences upstream [152]. Interestingly, the 4q35 subtelomeric region appear as a mosaic of regulatory elements that either shield or propagate TPE depending on the number of D4Z4 elements (Ottaviani, Gilson and Magdinier, unpublished observations) and the understanding the cross talks between *D4Z4* and the telomere would provide insights in the deciphering of this complex epigenetic disease and the involvement of the *D4Z4* subtelomeric element in transcriptional activity or replication timing of 4q35 chromosome end.

16. Senescence and aging

In mammals, aging is associated with a multitude of gene expression changes and increasing evidence supports the hypothesis of a link between senescence or aging and modification of chromatin. The architecture of the telomeric and subtelomeric regions is also remodeled during these two processes suggesting a putative connection between telomeric gene expression and age-related disorders.

Data from a mouse model invalidated for the telomerase show that the heterochromatin status of the telomeres and subtelomeric regions are decondensed upon telomere size reduction suggesting that the length of the distal TTAGGG repeats influence the epigenetic status of subtelomeric chromatin [156]. Loss of heterochromatin marks at telomeres and subtelomeres after abrogation of Suv39 histone methyltransferase correlates with extremely elongated telomeres [126]. Consequently, the formation of heterochromatin might appear as a negative signal for telomere elongation. The progressive modification of the histone code at telomeres and subtelomeres upon telomere shortening in the mouse raises the possibility of a control of telomere lengthening that can also influence the expression of subtelomeric genes, as was observed experimentally in an artificial system [124].

The occurrence of telomeric position effect during senescence was recently investigated in human fibroblasts maintained in culture for an extended period of time [157]. A total of 34 telomeric genes and the length of the corresponding telomeres were analyzed in young and senescent cells. Despite a differential expression for 17 out of these 34 genes, telomere length alone is not sufficient to determine the expression status of telomeric genes.

Also, the analysis of eight telomeric genes on a single chromosome end showed that telomere shortening influence gene expression through the local alteration of chromatin structure. This observation fits the model proposed in yeast where TPE is influenced by the proteins bound to the telomeres rather than the telomere length *per se* [157]. Despite efforts to understand the differences between quiescence and senescence at the molecular level, only a few markers can distinguish these two states. Also, different genes found variably expressed in the different cells are implicated in the regulation of the cell cycle and the pathways controlling senescence or apoptosis, suggesting also that the differential expression might have secondary effects on cell cycle regulation.

Although loss of TPE might not be the basis of senescence, it could be responsible for the progressive changes in gene expression associated with aging. Furthermore, viewing replicative senescence as one of the protective mechanisms against tumor formation, it is plausible that senescence-associated genes play significant roles in tumorigenesis repression. At this point, further studies are needed to elucidate the respective biological function of genes differentially expressed in senescent cells and cells suffering from telomere dysfunction.

17. Telomeric silencing and parasitic infection

Antigenic variation is a highly efficient survival strategy employed by various pathogens to bypass the eradication by the immune response of the host. A set of genes responsible for such challenges called contingency genes is subject to spontaneous mutations resulting in pathogen diversity [158]. In all these widely different species, variant gene families are within or just upstream of patchworks of subtelomeric repeats adjacent to telomeres and can be regulated by a telomeric position effect mechanism. Such regulation has been described for *Leishmania major*, *Trypanosoma brucei* and *cruzi*, *Palsmodium falciparum* and related species, *Candida glabrata* and *Pneumocystis carinii* (Fig. 1).

Antigenic variation can be achieved by at least two distinct mechanisms facilitated by telomeres. In *T. brucei* and *P. carinii*, exclusive expression of a single *vsg* or *MSG* gene respectively occurs from a unique ‘‘expression site’’ into which silent genes are sequentially rearranged near to a telomere [159,160]. By contrast *P. falciparum* and *C. glabrata* control their variant gene families by default subtelomeric silencing similar to what was described in yeast.

The African trypanosomes, *Trypanosoma brucei* and related species, are protozoan parasites responsible for diseases of cattle and for the human sleeping sickness. While in the bloodstream and tissues spaces of the host, *T. brucei* escapes elimination by the immune system by undergoing antigenic variation and sequentially express a large family of variant surface glycoprotein genes (*vsg*) from one of the 20 subtelomeric expression sites (*ES*). Indeed, the expressed *vsg* is invariably located adjacent to a telomere in a polycistronic transcription unit that might be regulated by telomere-specific mechanisms. However, it is uncertain whether telomere-induced repression can spread to the *ES* promoters, which

can be as far as 50 kb upstream of the telomere (reviewed in [161]).

P. carinii has approximately 100 subtelomeric genes encoding major surface glycoproteins (*MSG*s) expressed in an exclusive manner, which facilitate adhesion and immune evasion in the lung causing thereby lethal pneumonia in immune-compromised individuals [162−164].

The malaria parasite *Plasmodium falciparum* undergoes allelic variation through switching expression of variant surface proteins pfEMP1 encoded by the *var* gene family. Evidence for the epigenetic regulation at *P. falciparum* was obtained experimentally by the insertion of a human marker gene into the end of chromosome 3 [165]. Expression of the marker gene and natural *var* genes are subject to reversible gene silencing. The *P. falciparum* genome encodes a SIR2 homolog (*pfSir2*) that is involved in this silencing and the knock-out of *pfSir2* simultaneously derepresses many subtelomeric genes, including *var* genes and *rifin* genes. Subtelomeric genes are placed 40 kb from the telomere itself and Sir2 binding and concomitant TPE can spread to such a distance. Furthermore, *var* gene activation correlates with the repositioning into a location that may be permissive for transcription and changes in histone acetylation [166−168]. Reminiscent of *S. cerevisiae*, silent *var* genes on clustered telomeres are heterochromatinized and localized at the nuclear periphery in the vicinity of silencing factors. Upon activation, one telomere might move toward a permissive area of the nucleoplasm and boundary elements prevent transcription of the adjacent genes. The *var* gene that was expressed moves out of the active area and relocalizes with the other silent telomeres. The involvement of boundary elements remain hypothetical but likely play a role in the switching of the *var* genes upon parasitic infection.

In the pathogenic fungus, *Candida glabrata*, TPE can repress the expression of subtelomeric virulence genes [96]. SIR3 and RIF1 are required for subtelomeric silencing and RIF1 regulates telomere length. The deletion of these two genes leads to a hyperadherent phenotype after the derepression of subtelomeric genes encoding adhesins [96,169].

18. Conclusions

Through the description of the numerous pathologies and phenomenon that could be linked to telomeric silencing, it appears that TPE on natural chromosomes depends on the nature and the size of the subtelomeric regions that might directly act on the topology of chromatin. Consequently, a number of factors that can influence directly or indirectly the telomere length will likely affect the expression of subtelomeric sequences by changing telomere conformation and maintenance and *vice versa*. Given the parallels between yeast and human telomeres, the yeast model provides useful insights for the understanding of TPE in human cells and the deciphering of numerous pathologies. Altogether, a more complete knowledge of telomeric silencing in human cells, the identification of subtelomeric rearrangements and their consequences on the epigenetic regulation of telomeres would likely provide important insights into the role of chromosome ends in numerous pathologies such as cancer, developmental disorders and mental retardation, infertility and spontaneous recurrent miscarriages, age-related diseases or endemic parasitic infections. In addition the deciphering of the molecular mechanism sustaining these pathologies would provide a new avenue for the development of therapeutic approaches aimed at correcting the molecular defects caused by inappropriate modification of telomeric silencing.

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