The epigenetic regulation of mammalian telomeres

María A. Blasco

Abstract | Increasing evidence indicates that chromatin modifications are important regulators of mammalian telomeres. Telomeres provide well studied paradigms of heterochromatin formation in yeast and flies, and recent studies have shown that mammalian telomeres and subtelomeric regions are also enriched in epigenetic marks that are characteristic of heterochromatin. Furthermore, the abrogation of master epigenetic regulators, such as histone methyltransferases and DNA methyltransferases, correlates with loss of telomere-length control, and telomere shortening to a critical length affects the epigenetic status of telomeres and subtelomeres. These links between epigenetic status and telomere-length regulation provide important new avenues for understanding processes such as cancer development and ageing, which are characterized by telomere-length defects.

Dyskeratosis congenita
A condition that is characterized by bone marrow failure, genetic instability, elevated cancer risk and other abnormalities. Mutations in telomerase components have been described in some cases.

Aplastic anaemia
A condition that results from a peripheral deficiency of all bone-marrow-derived haematopoietic lineages, such as red blood cells, platelets and leukocytes. There are many causes of this potentially fatal clinical syndrome. Mutations in telomerase components have been described in some cases.

Telomeres and Telomerase Group, Molecular Oncology Programme, Spanish National Cancer Centre (CNIO), 3 Melchor Fernández Almagro, Madrid E-28029, Spain.
e-mail: mblasco@cnio.es
doi:10.1038/nrg2047

Telomeres are nucleoprotein structures that protect the ends of chromosomes from DNA repair and degradation[1,2]. In vertebrates, telomeres do not contain genes and consist of TTAGGG repeats that are bound by a specialized multiprotein complex, known as 'shelterin' or 'the telosome', which has fundamental roles in the regulation of telomere length and protection[1,3]. The subtelomeric regions located adjacent to telomeres are also enriched in repetitive DNA, and contain a low density of genes. Proper telomere functioning requires both a minimum length of TTAGGG repeats and the integrity of the shelterin complex[1,2]. Telomere length is maintained by telomerase, a reverse transcriptase that adds telomeric repeats *de novo* after each cell division, counteracting the end-replication problem in those cell types in which it is expressed[2,4]. Alternative ways to maintain telomere length have also been described, such as ALT (alternative lengthening of telomeres), which relies on homologous recombination between telomeric sequences[5,6]. In mammals, the length of telomeres differs between individuals and species and is under complex genetic control[7,8].

Most adult cells, including stem cells, progressively lose telomeres during cell division and tissue renewal, and it has been proposed that this telomere shortening is rate limiting for human lifespan and contributes to the development of age-related pathologies[4,9]. Strong support for this idea comes from the study of the telomerase-deficient mouse model[10–12], and from some cases of dyskeratosis congenita and aplastic anaemia, which show premature loss of tissue renewal associated with decreased telomerase activity and short telomeres[4,13]. By contrast, the vast majority of human cancers have aberrantly elevated telomerase levels or activated ALT mechanisms, and are able to maintain telomeres and divide indefinitely[5,14].

Until recently, little was known in mammals about what has long been known in yeast and *Drosophila melanogaster* to be an important aspect of the regulation and functioning of telomeres and subtelomeric regions — their chromatin structure. Like yeast and fly telomeres, mammalian telomeres are thought to have properties that are characteristic of heterochromatin, as indicated by the fact that they can transcriptionally silence nearby genes[15,16]. Unlike yeast, in which only subtelomeres have nucleosomes, telomeric chromatin in humans contains nucleosomes that show a slightly altered spacing compared with non-telomeric chromatin[17,18]. However, the molecular details of the structure of mammalian telomeric heterochromatin remained unknown. Recent studies have shown that mouse telomeric and subtelomeric chromatin contains histone modifications that are commonly found in heterochromatin[19–21], and that subtelomeric DNA can be methylated[18,22–23]. Moreover, increasing evidence indicates the existence of functional links between these epigenetic marks and telomere-length homeostasis[19–21,24]. Alterations of histone modifications in telomeric chromatin or of DNA methylation in subtelomeric regions correlate with telomere-length deregulation[19–21,24], pointing to a higher-order structure at telomeres that is epigenetically regulated and is important for length control[25].

Here I begin by providing a brief overview of telomere structure, function and general maintenance mechanisms, followed by a summary of the well understood role of epigenetic modifications in the regulation of yeast telomeres. On this background, I then discuss the epigenetic features of mammalian telomeres and the mounting evidence that histone modifications and DNA methylation at telomeric regions have a central role in the regulation of telomere length. I conclude by discussion the implications of these findings for our understanding of the roles of telomeres in human disease and ageing.

Structure and function of telomeric regions

The structure of telomeric regions is generally conserved among organisms from yeasts to humans with some exceptions, including flies (see below). Telomeres consist of double-stranded G-rich repeats ending in a single-stranded 3′ overhang (the G-strand overhang), which provides the substrate for telomerase[2] (FIG. 1). The G-strand overhang can also fold back and invade the double-stranded region of telomeres, forming a protective structure known as the T-loop[26,27].

Telomere repeats are bound by sequence-specific factors, known as telomere-repeat-binding factors. In budding yeast, double-stranded TG_{1-3} telomeric repeats are bound by Rap1[28] (repressor–activator protein 1), whereas Cdc13 (cell division control protein 13) binds to the G-strand overhang[29]. In turn, Rap1 recruits to budding yeast telomeres the silent information regulator proteins Sir2, Sir3 and Sir4, and Rap1-interacting factors Rif1 and Rif2, forming the so-called telosome[30] (FIG. 1a). The number of Rap1–Rif complexes has been shown to count and autoregulate telomere length[31]. Both Rap1 and Rif proteins act as negative regulators of telomere length, and mutations that affect these proteins result in abnormally elongated telomeres[32–34]. By contrast, disrupting the function of the Sir3 and Sir4 proteins results in telomere shortening[35]. In the fission yeast, *Schizosaccharomyces pombe*, the Myb-domain containing Taz1 protein is the major telomere-repeat-binding factor that has been shown to negatively regulate telomere elongation[36], whereas a protein known as Pot1 (protection of telomeres 1) binds and protects the G-strand overhang[37] (FIG. 1b). Similar to the budding yeast Rap1 protein, Taz1 abrogation leads to aberrantly elongated telomeres and loss of telomere silencing[36]. Homologues of budding yeast Rap1 and Rif1 proteins have been also described at *S. pombe* telomeres, where they are recruited through interaction with Taz1 and also regulate telomere length and telomeric silencing[38].

In an analogous way to yeast, mammalian TTAGGG telomere repeats are also bound by a multiprotein complex known as the telosome or shelterin[1,3] (FIG. 1c). Shelterin contains orthologues of yeast proteins that bind to either the G-strand overhang, such as the POT1/TPP1 heterodimer[37,39,40], or to double-stranded repeats, such as the Myb-domain-containing telomere binding factors TRF1 and TRF2 and their interacting proteins RAP1 (an orthologue of budding yeast Rap1) and TIN2 (TRF1-interacting nuclear factor 2)[1,3]. TRF1 also interacts with the TANK1 and TANK2 poly(ADP)-ribosylases (also known as tankyrases)[41]. TRF1 and the TRF1-interacting proteins have been proposed to act as negative regulators of telomere length in an analogous manner to the budding and fission yeast Rap1 and Taz1 proteins, respectively, probably by controlling the access of telomerase to the telomere[1]. TRF2 and POT1 seem to have additional roles in telomere protection as they prevent end-to-end chromosome fusions[42,43], probably through their ability to interact with DNA-damage signalling and repair factors[44–48]. A putative role for mammalian shelterin components in disease is unclear, although they are upregulated in some human carcinomas[49–53] and mice with increased *Trf2* expression show increased epithelial carcinogenesis[51,54]. Interestingly, *Trf2* conditional deletion in adult hepatocytes has no effect on their viability[55].

Telomere maintenance mechanisms

Maintenance by telomerase. In both yeast and mammals, the G-strand overhang is the substrate for telomerase, which consists of a reverse transcriptase (TERT) that is able to synthesize telomeric repeats *de novo* and add them to chromosome ends using an associated RNA molecule (TERC) as a template[2]. In contrast to yeast, humans show telomere attrition with ageing, which is thought to result from limiting amounts of telomerase activity in the adult organism, which cannot compensate for the progressive telomere shortening that occurs as cells divide during tissue regeneration[4,25]. In this regard, telomerase has been found to be crucial for stem-cell function and proliferative potential, providing a possible basis for the known roles of telomerase in cancer and ageing. Whereas short telomeres that arise owing to telomerase deficiency result in impaired stem-cell functionality[56], defective tissue regeneration and decreased tumorigenesis[25], telomerase overexpression has the opposite effects[56,57].

In contrast to yeast and mammals, *D. melanogaster* lacks telomerase activity and, in this species, telomeres do not consist of telomerase-generated simple telomere repeats, but of two main classes of retrotransposons, HetA and TART[58], which are involved in telomere-length maintenance. *D. melanogaster* also lacks the direct telomere-repeat-binding factors that have been described in yeast and mammals.

Alternative lengthening of telomeres. In the absence of telomerase activity, both yeast and mammals are able to maintain or elongate their telomeric or subtelomeric DNA through the ALT mechanism. This phenomenon was first described in budding yeast strains that were deficient for the *EST1* gene[59,60] (ever shorter telomeres 1), and was later reported in telomerase-deficient human cells[5,6]. In both yeast and mammals, ALT has been shown to involve homologous recombination between telomeric sequences, and has been associated with the formation of extrachromosomal circles[5,6]. In yeast, two independent ALT pathways are defined by the Rad50 and Rad51 group genes[61], as well as being defined according to their telomere length[62]. In humans, ALT-positive cells are characterized by heterogeneous telomere length, with

Heterochromatin
Chromosomal material that is tightly coiled and generally inactive in terms of gene expression.

Nucleosome
The fundamental unit into which DNA and histones are packaged in eukaryotic cells. It is the basic structural subunit of chromatin and consists of ~200 bp of DNA wrapped around an octamer of histone proteins.

Histone modifications
Histones undergo post-translational modifications that alter their interaction with DNA and nuclear proteins. In particular, the tails of histones H3 and H4 can be covalently modified at several residues. Modifications of the tail include methylation, acetylation, phosphorylation and ubiquitination, and affect several biological processes, including gene expression, DNA repair and chromosome condensation.

DNA methylation
DNA methylation occurs predominantly in repetitive genomic regions that contain CpG residues. DNA methylation represses transcription directly by inhibiting the binding of specific transcription factors, and indirectly by recruiting methyl-CpG-binding proteins and their associated repressive chromatin-remodelling activities.

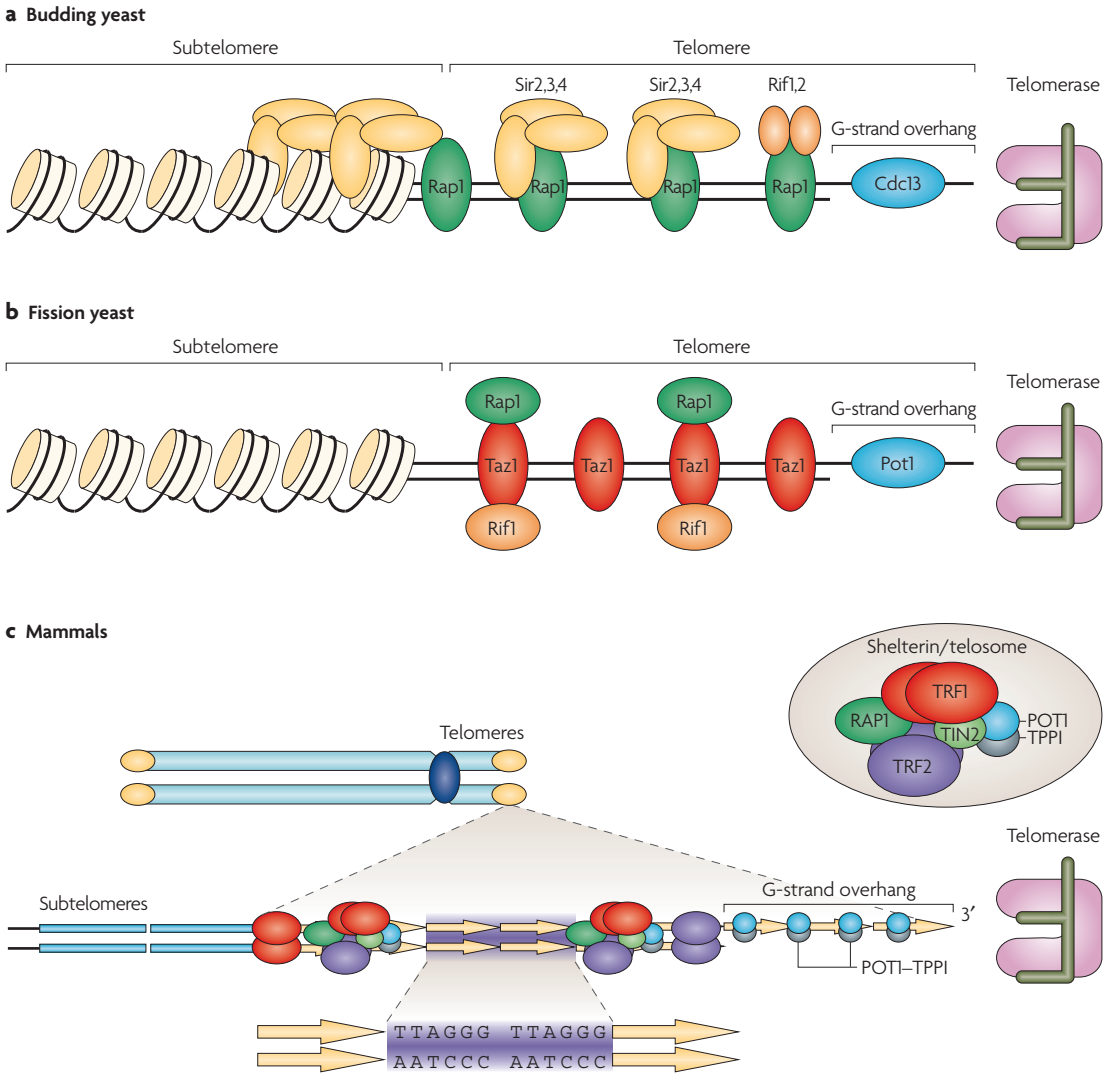

Figure 1 | **Structure of telomeres and subtelomeres in yeast and mammals. a** | Budding yeast telomeres. Double-stranded telomeric repeats are bound by the telomere-repeat-binding factor repressor–activator protein 1 (Rap1), which in turn recruits the silent information regulator proteins Sir2,3,4 and the Rap1-interacting factors Rif1,2. The G-strand overhang is bound by the single-stranded telomere-binding protein cell division control protein 13 (Cdc13). Both Rap1 and Rif proteins act as negative regulators of telomere length, and mutations that affect these proteins result in abnormally elongated telomeres. By contrast, disrupting the function of the Sir3 and Sir4 proteins results in telomere shortening. **b** | Fission yeast telomeres. Double-stranded telomeric repeats are bound by the telomere-repeat-binding factor Taz1, which in turn recruits homologues of budding yeast Rap1 and Rif1 proteins. The G-strand overhang is bound by the single-stranded telomere-binding protein protection of telomeres 1 (Pot1). Taz1 acts as negative regulator of telomere length. **c** | Mammalian telomeres. Double-stranded telomeric repeats are bound by a multiprotein complex known as 'shelterin' or the 'telosome', which comprises the Myb-domain-containing telomere binding factors TRF1 and TRF2, TIN2 (TRF1-interacting nuclear factor 2), RAP1 (orthologue of budding yeast Rap1), POT1 (orthologue of yeast POT1) and TPP1 (also known as POT1- and TIN2-organizing protein). The G-strand overhang is also bound by the POT1–TPP1 heterodimer. Telomerase is able to recognize the 3′ end of the G-strand overhang to elongate telomeres. In telomerase-negative cells, telomeres can be maintained by mechanisms that involve homologous recombination between telomeric repeats, the so-called alternative lengthening of telomeres (ALT).

PML body
Subnuclear compartments that are defined by the presence of the PML (promyelocytic leukaemia) protein. They have been associated with diverse nuclear functions including transcription, DNA repair, viral defense, stress, cell-cycle regulation, proteolysis and apoptosis.

the presence of both unusually short and long telomeres, and by the co-localization of telomeres with a specific type of PML body, the so-called ALT-associated PML bodies (APBs)[5,6].

In mammals, the telomerase-deficient mouse model has been instrumental in demonstrating telomere maintenance in the absence of telomerase, in both cultured mouse embryonic fibroblasts (MEFs)[63,64] and embryonic stem (ES) cells[65], and *in vivo* in the context of B-cell development, indicating that ALT takes place in situations other than immortalized cell lines[66]. However, although ALT can rescue the viability of telomerase-deficient yeast strains[59], it cannot rescue the viability of telomerase-deficient mice, suggesting that ALT

mechanisms are not sufficient for telomere maintenance in multicellular organisms.

In both yeast and mammals, proteins that are involved in the homologous recombination DNA-repair pathway have been implicated in ALT[5,6,59–62,67]. More recently, components of the shelterin complex, such as POT1 and TRF2, have also been suggested as potential regulators of ALT, as they can influence telomere recombination[54,68].

Epigenetic regulation of yeast telomeres

Chromatin at telomeres has several characteristics that are similar to those of heterochromatin at pericentromeric regions, such as the ability to silence nearby genes. This phenomenon is known as 'telomere position effect' (TPE), and was first described in *D. melanogaster*[69] and later reported in budding yeast[70] and fission yeast[71].

In budding yeast, TPE mainly involves Rap1[72] and the Sir proteins[73], although it can be influenced by many factors[30]. A model for heterochromatin assembly and spreading at budding yeast telomeres suggests that Sir proteins are recruited to telomeres through their interaction with Rap1, and then spread to subtelomeric regions, where they interact with the amino (N)-terminal tails of histones H3 and H4, promoting their hypoacetylation and leading to transcriptional repression[74,75]. Rap1 binds directly to telomeric repeats, which in yeast are devoid of nucleosomes[76], and interacts with Sir4, which recruits the other Sir proteins (Sir2 and Sir3). The NAD-dependent histone deacetylase activity of Sir2 can deacetylate histone H4, which creates the binding of Sir3 and Sir4 to histone tails at subtelomeric regions, promoting heterochromatin formation and the spreading of telomere silencing towards more centromeric regions[77]. Telomeric heterochromatin can be further stabilized by two higher-order events: the folding back of the telomeres[78,79], allowing additional Rap1–Sir and Sir–Sir interactions, and the association of the telomere with the Sir-rich nuclear periphery[80]. The extent of subtelomeric spreading of Sir proteins and of telomere silencing can be controlled by several factors that define chromatin domain boundaries[81,82]. Finally, proteins that are involved in ubiquitination have also been described as having an important function in telomere silencing and telomere protection in budding yeast[83] and in flies[84], respectively, probably through modification of telomere regulators, although formal demonstration for this is still pending.

The involvement of chromatin factors in yeast telomere-length regulation is well documented. Strikingly, several mutations that disrupt telomeric silencing also decrease the length of telomeres[35,85–87], suggesting that telomeric silencing positively regulates telomere length in budding yeast. The Rap1 counting pathway seems to be indirectly regulated by the Sir proteins[31]. The Rif proteins are thought to have a direct *cis*-acting role in telomerase inhibition[34], and in decreasing the formation of telomeric heterochromatin by competing with the Sir proteins for binding to the carboxy (C)-terminal domain of Rap1 (REF. 35). Chromatin factors might also be more directly involved in telomere-length regulation. In particular, the anchoring of telomeres to the nuclear periphery is achieved, at least in part, through silent chromatin[88], and seems to regulate telomere length in cells that are compromised for the Rap1 counting pathway[89,90]. Notably, deletion of Rif2 can also lead to recombination-dependent telomerase-independent telomere elongation[91], suggesting a link between telomeric chromatin and recombination.

The silencing machinery in the fission yeast *S. pombe* is much more closely related to that of mammals than is the budding yeast silencing machinery. The proper functioning of *S. pombe* telomeres requires the SET-domain-containing Clr4 histone methyltransferase[92], which is also involved in the methylation of H3K9 at pericentric heterochromatin, and which in turn creates a binding site for Swi6 (the orthologue of the *D. melanogaster* heterochromatin protein HP1). Furthermore, the pathway of heterochromatin formation that is mediated by small interfering RNAs (siRNAs) has been shown to regulate telomere silencing in this species[93,94]. In particular, both the telomere-repeat-binding protein Taz1 and the siRNA machinery have been shown to be important for the establishment of telomeric heterochromatin in fission yeast[94].

Regarding telomere-length regulation in fission yeast, various mutations that alleviate telomeric silencing and heterochromatin formation have no or limited effects on telomere length, including the inactivation of genes encoding Swi6, Clr4 and other components of the RNAi machinery[95–97]. By contrast, results from *D. melanogaster* show that the heterochromatin HP1 protein and components of the RNAi machinery negatively regulate the transposition-based mechanism of telomere elongation[98,99], suggesting that the role of heterochromatic activities in telomere-length regulation is not necessarily conserved during evolution. Finally, in fission yeast, deletions of *set1*, which encodes a histone modifier that is involved in gene activation, and of the SUMO E3 ligase gene *pli1p* alleviate telomere silencing and result in telomerase-mediated telomere elongation[100–102]. These results raise the question of whether absence of histone H3 lysine 4 (H3K4) methylation and sumoylation cause telomere elongation by directly modifying telomere structure or by modulating the expression of telomere-length regulators. All together, these findings suggest the existence of complex links between telomere components, chromatin factors and telomere-length regulation, which might involve both direct and indirect effects.

The chromatin structure of mammalian telomeres

Mammalian telomeric chromatin has several general commonalities with that of yeast. Mammalian telomeres also have the ability to silence subtelomeric genes through TPE[15,16] and, similar to yeast, TPE in human cells is influenced by telomere length and involves histone hypoacetylation, as it can be disrupted by treatment with the deacetylase inhibitor TSA[15,16]. Mammalian telomeres and subtelomeres also have similarities with pericentromeric regions in terms of sequence composition and gene content: both are characterized by a high content of DNA repeats and, whereas telomeres do not contain genes at all, subtelomeres, like pericentromeric

Pericentric heterochromatin
The late-replicating, gene-sparse, transcriptionally inactive, condensed chromatin regions that are rich in repeated sequence and occur near the centromeres of chromosomes.

Small interfering RNA
A non-coding RNA (~22 nucleotides long) that is derived from the processing of long dsRNA during RNAi. siRNAs direct the destruction or translation repression of mRNA targets that they hybridize with.

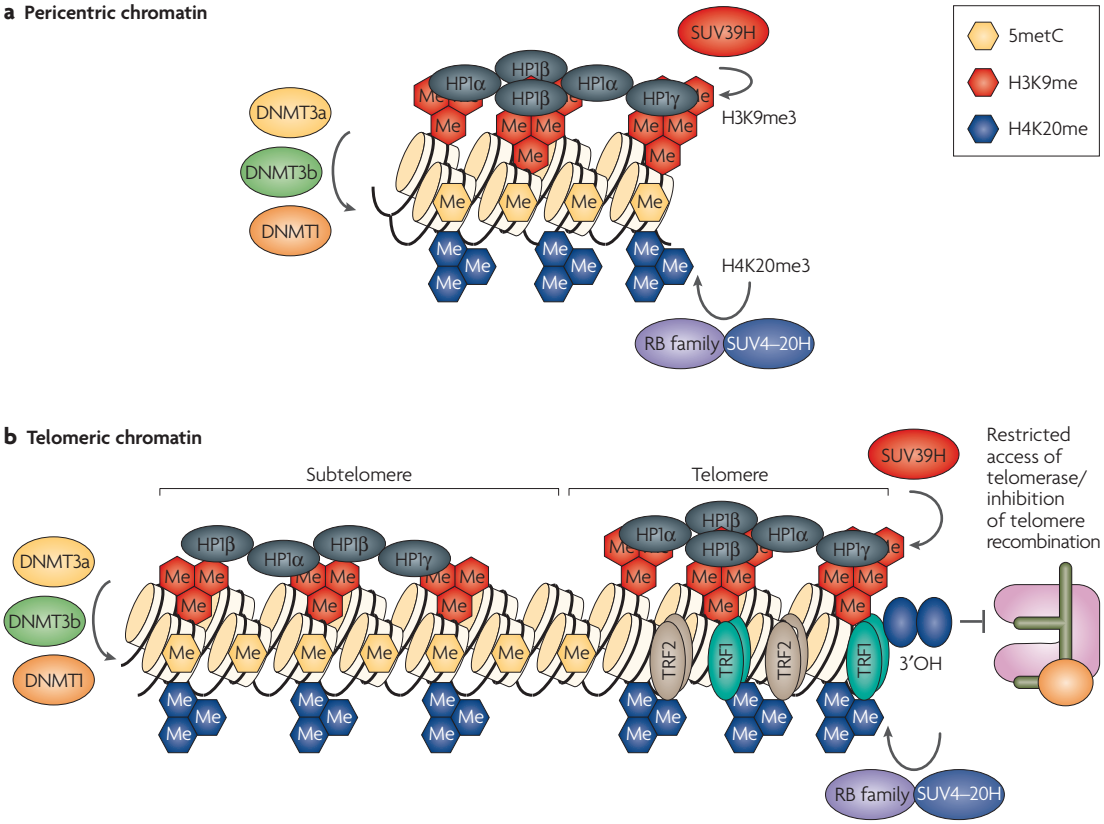

Figure 2 | **Epigenetic modifications at mammalian pericentromeric and telomeric regions. a** | Pericentromeric repeats are enriched in chromatin modifications that are characteristic of constitutive heterochromatin domains, such as trimethylation of histone H3 at lysine 9 (H3K9me3) and of histone H4 at lysine 20 (H4K20me3), and binding of isoforms of heterochromatin protein (HP1). The two histone modifications are carried out by the SUV39H and SUV4-20H HMTases, respectively. In addition, the retinoblastoma (RB) family of proteins has been shown to interact with the SUV4-20H HMTases to efficiently trimethylate H4K20. The DNA at pericentric regions is heavily methylated. **b** | Both telomeric and subtelomeric chromatin regions are also enriched in trimethylated H3K9 and H4K20, and HP1 isoforms. In addition, subtelomeric DNA is heavily methylated by the DNMT1, DNMT3a and DNMT3b enzymes. Both histone trimethylation and DNA methylation have been shown to independently act as negative regulators of telomere length. In addition, DNA methylation inhibits telomere recombination. 5metC, DNA methylation at 5-methylcytosine.

regions, are gene-poor. However, unlike yeast, in which only subtelomeric repeats contain nucleosomes[76], both mammalian telomeres and subtelomeres contain nucleosomes, and these show a slightly altered spacing compared with the non-telomeric chromatin[17,18].

Consistent with the observations of TPE in human cells, many marks that are usually found in heterochromatin can be found in mammalian telomeres. In particular, similar to mammalian pericentromeric regions (FIG. 2a), trimethylation of H3K9 and H4K20 have recently been identified at mammalian telomeric and subtelomeric domains[19–21] (FIG. 2b). These histone modifications are carried out by 'suppressor of variegation' histone methyltransferases. In the case of H3K9, the enzymes responsible are SUV39H1 and SUV39H2, which are homologues of the yeast Clr4 HMTase, whereas H4K20 trimethylation is carried out by SUV4-20H1 and SUV4-20H2 (REFS 103–106) (TABLE 1). Proteins of the retinoblastoma family of tumour suppressors (RB, p107 and p130; also known as RB1, RBL1 and RBL2) interact with SUV420H1 and SUV4-20H2 to maintain trimethylation of H4K20, at both telomeric and pericentric chromatin[20]. Human and mouse telomeres and subtelomeres can also be found to be enriched in HP1 (REFS 16,19–21). These proteins are recruited to chromatin through their affinity for trimethylated H3K9 residues and are important for chromatin compaction at both regions[19–21,98,106]. In addition, mammalian telomeric and subtelomeric repeats are characterized by low levels of acetylated H3 (AcH3) and H4 (AcH4)[107].

The control mechanisms that regulate TPE and the status of epigenetic marks at mammalian telomeres remain to be discovered. The observations that human TPE is reversed when the TRF1 protein is overexpressed[16] and enhanced when telomeres are overelongated[15] suggest that shelterin components might affect the epigenetic status of telomeres by their ability to regulate telomere length. Indeed, interactions between TRF1 and the HP1-interacting developmental regulator SALL1 (REF. 108), as well as between TIN2 and HP1 (REF. 109), have been reported, although their *in vivo* significance remains to be established.

Table 1 | **Epigenetic modifications at mammalian telomeres and subtelomeres**

Chromatin marks or proteins	Activity responsible	Location	Roles	Human disease	References
HP1α, HP1β, HP1γ	-	Telomeres, subtelomeres, pericentric chromatin	Chromatin compaction	Unknown	19–21,103, 104,106,107
Trimethylated H3K9	SUV39H1, SUV39H2	Telomeres, subtelomeres, pericentric chromatin	Chromatin compaction, silencing	Altered in cancer	19–21,103, 104,107
Trimethylated H4K20	RB1, RBL1, RBL2; SUV4-20H1, SUV4-20H2	Telomeres, subtelomeres, pericentric chromatin	Chromatin compaction, silencing	Altered in cancer	20,21,22, 104,105, 107
Acetylated H3 and H4	HATs, HDAC, Sirt	Telomeres, subtelomeres, pericentric chromatin	Active chromatin	Altered in cancer	22,107
DNA methylation	DNMT1, DNMT3a,3b	Subtelomeres, pericentric chromatin	Silencing, repressing recombination	Altered in cancer; Altered in ICF Syndrome	21,23,110, 115–118

DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; H3K9, histone 3 lysine 9; H4K20, histone H4 lysine 20; ICF, immunodeficiency–centromeric instability–facial anomalies syndrome; RB, retinoblastoma protein; RBL, retinoblastoma-like protein; HP, heteochromatin protein; Sirt, sirtuin (silent mating-type information regulation 2 homologue); SUV, suppressor of variegation.

In contrast to budding yeast, which lack DNA methylation, this epigenetic mark is an important chromatin modification in mammals[110], with key roles in transcriptional regulation and the definition of chromatin domains by regulating the accessibility of DNA-binding factors to DNA and the control of chromatin-remodelling activities. In mammals, highly repetitive regions such as pericentric chromatin are heavily methylated, and this methylation has been proposed to be important in preventing the high level of homologous recombination that would otherwise be expected to take place at these domains[111–113]. In mice and humans, DNA methylation of the subtelomeric regions might act as a secondary mechanism to reinforce TPE[114,23], whereas telomere-specific chromatin changes might be primary events in establishing telomeric silencing.

Mammalian telomere repeats (TTAGGG) cannot be methylated because they lack CpG sequences, which are the substrates for mammalian DNA methyltransferases (DNMTs). However, mouse and human subtelomeric sequences can be methylated[18,21,23,115] (FIG. 2b). There are three main DNMTs in mammalian cells: DNMT1, which functions as a maintenance DNMT that copies parental strand DNA methylation onto the daughter strand after DNA replication, and DNMT3a and DNMT3b, which are thought to function as *de novo* DNMTs[116,117] (TABLE 1). Cells that lack either DNMT1 or the DNMT3a,3b isoforms show a marked reduction in DNA methylation across the genome, including pericentric heterochromatin[116–118] and subtelomeric domains[21] (TABLE 1). Interestingly, decreased subtelomeric DNA methylation does not result in a decreased abundance of heterochromatic histone-methylation marks (trimethylated H3K9 and H4K20) at subtelomeric chromatin and the density of these marks slightly increases at telomeric repeats[21]. This suggests that DNA methylation is not required to direct heterochromatic histone modifications at telomeric and subtelomeric domains. This finding is not surprising given that it has previously been shown that DNA methylation does not direct histone modifications at pericentric heterochromatin domains[116–118].

Human telomeres do not exhibit all the features that have previously been described for constitutive pericentric heterochromatin. In particular, the proximity of genes to mammalian telomeres does not always result in DNA hypermethylation and gene silencing[119–121]. Moreover, human telomeric DNA is not late replicating, in contrast to both yeast telomeres and mammalian pericentric domains[122]. Overall, it seems that mammalian telomeres are organized into a unique chromatin structure, showing some, but not all, of the classical attributes of pericentric heterochromatin domains.

Epigenetic regulation of mammalian telomeres

The role of histone modifications. Disruptions of histone and DNA modifications at pericentric heterochromatin have been shown to result in chromosome segregation defects, which have been suggested as having a role in tumour development[103]. Interestingly, the same epigenetic defects at telomeres correlate with loss of telomere-length control[19–21,24]. In particular, cells that lack the SUV39H1 and SUV39H2 HMTases show decreased levels of H3K9 trimethylation at telomeres, concomitant with aberrant telomere elongation[19]. A similar deregulation of telomere length is seen in cells that lack all three members of the retinoblastoma family and show decreased levels of H4K20 trimethylation at telomeres[20,24].

These telomere-length changes might result from the altered functioning of telomere-length regulators as a consequence of heterochromatin disruption. In this regard, unlike yeast, in which only subtelomeric domains contain histones, mammalian telomere repeats contain both shelterin proteins and histones[1,17,18], and these histones carry heterochromatic marks[19–21]. It is possible that the *cis*-acting inhibition of telomerase activity by the shelterin proteins TRF1 and POT1 (REFS 123,124) and the effect of TRF2 on telomere recombination[54,125] are positively controlled by heterochromatin factors. Moreover, it has been proposed that TRF1 and tankyrase 1 might have a role in sister chromatid cohesion at telomeres[126], which in turn could be associated with the regulation of telomere structure.

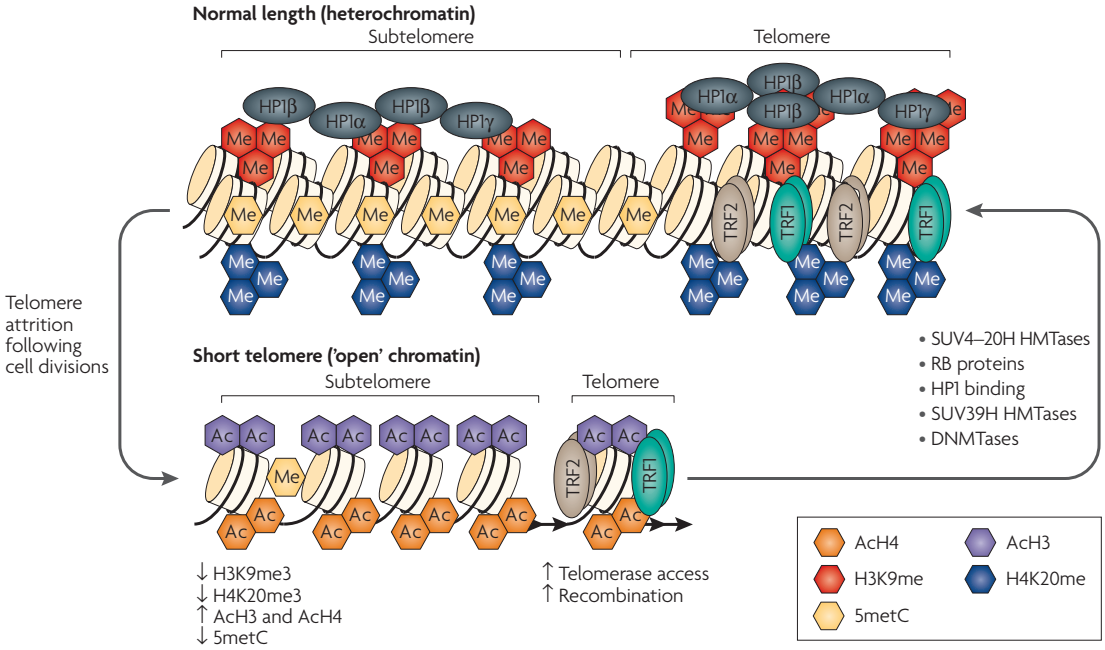

Figure 3 | **A model for the role of epigenetic modifications in telomere-length control.** Normal-length telomeres have features of constitutive heterochromatin, such as subtelomeric DNA hypermethylation, hypermethylation of histone H3 at lysine 9 (H3K9) and histone H4 at lysine 20 (H4K20), hypoacetylation of histones H3 and H4, and heterochromatin protein HP1 binding at both telomeres and subtelomeres. This suggests that they have a compacted and 'closed' conformation, which is not accessible to telomerase and that represses recombination between telomeric repeats. As telomeres become shorter with increasing cell divisions, these heterochromatic marks are decreased from telomeres and subtelomeres, concomitant with increased histone actelylation. This leads to a more 'open' chromatin state, which allows a greater accessibility of telomere-elongating activities (by telomerase and proteins that are involved in telomere recombination, which can lead to elongation by alternative lengthening of telomeres (ALT)). Once telomeres are sufficiently elongated, they can be assembled into heterochromatin by the activity of the SUV39H and SUV4-20H HMTases, as well as other heterochromatinizing activities (such as that of retinoblastoma (RB) family proteins, HP1 binding and DNA methyltransferases). Ac, acetylation mark; Me, histone-methylation mark; 5metC, DNA methylation at 5-methylcytosine.

The role of subtelomeric DNA methylation. Decreases in DNA methylation, both globally and specifically at subtelomeric regions, are accompanied by dramatically elongated telomeres, even when there is no loss of heterochromatic histone-methylation marks[21]. These results suggest that DNA methylation represents an additional way to control telomere length, independently of histone methylation.

The demethylation of subtelomeric regions in cells that lack DNMTs is also concomitant with increased homologous recombination between telomeric sequences[21]. Reintroduction of DNMT3a,3b into these cells results in the re-methylation of subtelomeric domains and decreased telomeric homologous recombination[21]. DNA methylation at subtelomeric repeats is therefore implicated as an important repressor of homologous recombination at telomeres, raising the possibility that DNA-methylation levels might regulate ALT.

As described above, histone methylation at telomeres and subtelomeres seems to be independent of DNA methylation. Interestingly, however, cells that are deficient for the SUV39H HMTases or the retinoblastoma family of proteins, which show defective histone methylation at telomeres, also show a global decrease in DNA methylation[20]. This effect would be expected to be recombinogenic, although formal demonstration of this is still pending. Although budding yeast lack DNA methylation, a connection between telomere heterochromatin structure and recombination has been established. In particular, Rif2 has been found to be a repressor of telomerase-independent telomere elongation by homologous recombination[92].

Of note, no link between the DNA-methylation status at mammalian subtelomeres and telomere-binding proteins has been established in mammals. However, such a connection can be expected to exist, as altered expression of some telomere-binding proteins has been shown to result in deregulated recombination frequencies at telomeres[54,68].

Telomere shortening affects epigenetic status

It has previously been shown in yeast that the length of telomere repeats can influence heterochromatin assembly at telomeres and telomere silencing. In particular, telomere elongation increases TPE[72,127]. However, some strains with long telomeres can have reduced TPE when telomere elongation is due to loss of telomere-bound silencing proteins[32,128]. In agreement with the situation in yeast, human TPE increases upon telomere elongation[15], suggesting a conserved link between telomere length and the epigenetic status of telomeres and subtelomeres.

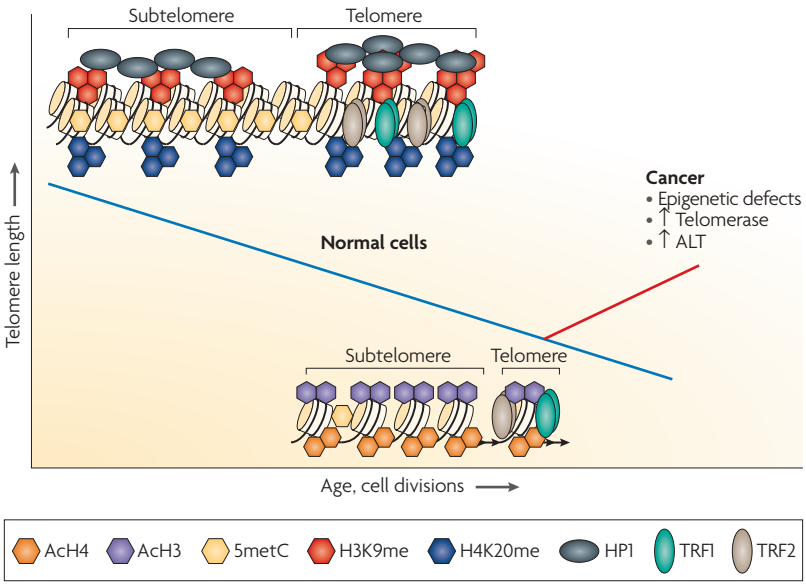

Figure 4 | **Epigenetic defects at telomeres and human disease.** Progressive telomere shortening with increasing cell divisions and ageing might lead to several epigenetic defects, both at telomeric and subtelomeric chromatin, which might in turn favour the activation and selection of telomere-elongation mechanisms. The activation of these mechanisms in the context of cancer cells might favour tumour growth by providing the cells with an indefinite proliferative potential. Ac, acetylation mark; ALT, alternative lengthening of telomeres; HP1, heterochromatin protein 1; Me, histone-methylation mark; TRF, Myb-domain-containing telomere binding factor; 5metC, DNA methylation at 5-methylcytosine.

In the context of the telomerase-deficient mouse model, it has recently been shown that progressive telomere shortening leads to epigenetic changes, both in telomeric and subtelomeric chromatin[107]. In particular, short telomeres have a decreased density of heterochromatic histone marks, such as trimethylated H3K9 and H4K20, at telomeric repeats, as well as decreased binding of HP1 (FIG. 3). They are also distinguished by an increased density of histone marks that are characteristic of more 'open' or active chromatin domains, such as increased histone H3 and H4 acetylation[107]. In addition to these epigenetic changes at the TTAGGG repeats, subtelomeric regions show decreased histone methylation, increased histone acetylation and a decrease in subtelomeric DNA methylation, suggesting that the length of the distal TTAGGG repeats influences the epigenetic status of subtelomeric chromatin[107]. The fact that telomeres and subtelomeres become hyperacetylated as the consequence of telomere shortening suggests a concomitant loss of transcriptional silencing at subtelomeric regions, in agreement with previous data obtained in both yeast and humans that shows that TPE is associated with hypoacetylation of H3 and H4 (REFS 15,74).

Similar to the findings described above for DNMT-deficient cells, the decreased DNA methylation that is seen at subtelomeric regions as a consequence of telomere shortening is also accompanied by increased telomere recombination[107]. This provides further support for a role of DNA methylation as a negative regulator of recombination at these regions, and might also provide an explanation for the fact that ALT is activated in the context of short telomeres in telomere-deficient cells and mice[63–66].

The fact that short telomeres lead to a change in the epigenetic status of telomeric and subtelomeric chromatin might also explain the preferential elongation of short telomeres by telomerase. In particular, telomerase has been shown to act specifically on the shortest telomeres in both budding yeast[129,130] and mammals[131,132], raising the possibility that short telomeres have specific chromatin marks that are recognized by the telomerase complex. The precise nature of these marks is still unknown, but might include increased H3 and H4 acetylation, as these marks are increased with telomere loss[107].

A model for mammalian telomere regulation

Loss of heterochromatic marks at telomeres and subtelomeres correlates with extremely elongated telomeres, in agreement with the idea that these marks act as negative regulators of telomere elongation. In particular, abrogation of SUV39H HMTase activity or loss of the retinoblastoma family of proteins results in decreased histone H3K9 and H4K20 trimethylation marks globally, as well as at telomeres, and these changes are concomitant with increased telomere length. This suggests that the maintenance of heterochromatic histone marks at telomeres is important for telomere-length regulation, such that when the amount of H3K9 and H4K20 trimethylation decreases below a critical level owing to attrition that occurs with consecutive cell divisions, telomere length is increased and these heterochromatic marks are re-established. This is in contrast to what occurs in budding yeast, in which telomere heterochromatinization antagonizes the binding of Rap1 to *cis*-acting negative regulators of telomere elongation.

Loss of subtelomeric DNA methylation that occurs independently of histone methylation in *DNMT*-deficient cells also results in aberrant telomere elongation[21]. This raises the possibility that loss of DNA methylation is an important additional mechanism for telomere-length control, as cells with defective H3K9 and H4K20 trimethylation also show DNA-methylation defects[20,103,104]. Importantly, this telomere-control mechanism is not active in budding yeast, as this species lacks DNA methylation. The fact that telomere length determines the abundance of histone heterochromatic marks at telomeres in the case of short telomeres (decreased density of histone trimethylation marks) suggests that a minimum number of telomere repeats is necessary for the assembly of telomeric heterochromatin. However, it is not known what determines a critical length for heterochromatin assembly and how this minimum length is sensed by heterochromatinizing activities.

Implications for human ageing and disease

Alterations of DNA methylation and histone modifications are common in human cancers[22,110]. Given that chromatin structure clearly affects the regulation of mammalian telomeres, these epigenetic changes might be an important link between telomere deregulation

ICF
Immunodeficiency–centromeric instability–facial anomalies syndrome (ICF) is an extremely rare autosomal recessive disease that is characterized by profound immunodeficiency. Many ICF patients carry mutations of the *DNMT3B* gene, leading to DNA-methylation defects.

Rett syndrome
An X-linked dominant neurological disorder that mainly affects girls and is one of the most common causes of mental retardation in females. Typical Rett syndrome is due to a mutation in the *MECP2* gene (methyl-CpG-binding protein 2) resulting in decreased DNA methylation.

and cancer development. In particular, tumours that show DNA hypomethylation or decreased H3K9 and H4K20 trimethylation at telomeric regions might be predicted to favour the activation of telomere-elongation mechanisms (telomerase and ALT), which in turn might sustain tumour growth in the absence of telomerase. DNA-methylation defects have also been associated with other human diseases, such as ICF and Rett syndrome[133,134], although so far there is no evidence that telomere length is deregulated in these patients.

The finding that epigenetic changes at telomeric and subtelomeric chromatin are associated with critically short telomeres[107] suggests that the shortening of telomeres that is seen in normal ageing and age-related pathologies, as well as premature ageing syndromes[4,13,25], might result in epigenetic defects at telomeres. This could in turn favour cancer development by activation of telomere maintenance mechanisms such as telomerase and ALT (FIG. 4), although this remains to be demonstrated. Furthermore, the fact that telomere shortening leads to increased H3 and H4 acetylation at telomeres and subtelomeres indicates a potential mechanism by which abnormal gene-expression changes at subtelomeric genes might arise, owing to the loss of the silent heterochromatin state, as suggested by previous TPE experiments in yeast and humans[15,16,30,74,77]. Finally, a number of environmental factors (such as smoking, obesity and stress) have been shown to accelerate the rate of telomere shortening, and might therefore also have an impact on chromatin modifications at telomeres and the expression of subtelomeric genes[135–137]. Altogether, a more detailed understanding of the consequences of disrupting the normal epigenetic regulation of telomeres is likely to provide important insights into the role of telomeres in a range of human pathologies.

Conclusions

Increasing evidence suggests that there is an important role for histone and DNA methylation in regulating mammalian telomere length and integrity, and an important role of telomere length in dictating the assembly of heterochromatic domains at telomeres. Future studies are needed to determine whether additional chromatin-remodelling activities participate in the assembly and regulation of mammalian telomeric chromatin. In this regard, yeast telomere biology indicates that there are several protein activities that might be important for mammalian telomere length. For example, studying the potential roles of the mammalian homologues of yeast Sir proteins[138–140] and sumoylation[141,142] proteins, which have key functions in determining the chromatin structure of yeast telomeres, will be of great interest. Similarly, given the role of the siRNA-mediated pathway for heterochromatin formation in fission yeast[93,94], determining whether telomere transcripts are produced in mammals[143], in a similar way to production of transcripts from pericentric chromatin, and investigating a potential role of the siRNA machinery will also be an important line for future investigation.

Finally, the mechanisms by which epigenetic alterations lead to telomere-length deregulation and increased homologous recombination must be elucidated. In particular, it will be of great interest to address whether telomerase has a higher affinity for telomeres that contain the chromatin marks that are characteristic of short telomeres, and look at the possible interplay between these chromatin modifications and the DNA-damage response that is associated to critically short telomeres[1]. Answering these questions will shed light on both ageing and diseases such as cancer, in which both epigenetic alterations and aberrant telomere functioning are involved.

1. de Lange, T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev.* **19**, 2100–2110 (2005).
A must-read review on the protein composition of mammalian telomeres and their role in the regulation of telomere length and telomere capping.
2. Chan, S. W. & Blackburn, E. H. New ways not to make ends meet: telomerase, DNA damage proteins and heterochromatin. *Oncogene* **21**, 553–563 (2002).
3. Liu, D., O'Connor, M. S., Qin, J. & Songyang, Z. Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins. *J. Biol. Chem.* **279**, 51338–51342 (2004).
4. Collins, K. & Mitchell, J. R. Telomerase in the human organism. *Oncogene* **21**, 564–579 (2002).
5. Muntoni, A. & Reddel, R. R. The first molecular details of *ALT* in human tumor cells. *Hum. Mol. Genet.* **14**, 191–196 (2005).
6. Dunham, M. A., Neumann, A. A., Fasching, C. L. & Reddel, R. R. Telomere maintenance by recombination in human cells. *Nature Genet.* **26**, 447–450 (2000).
7. Slagboom, P. E., Droog, S. & Boomsma, D. I. Genetic determination of telomere size in humans: a twin study of three age groups. *Am. J. Hum. Genet.* **55**, 876–882 (1994).
8. Zhu, L. *et al.* Telomere length regulation in mice is linked to a novel chromosome locus. *Proc. Natl Acad. Sci. USA* **95**, 8648–8653 (1998).
9. Flores, I., Cayuela, M. L. & Blasco, M. A. Effects of telomerase and telomere length on epidermal stem cell behavior. *Science* **309**, 1253–1256 (2005).
10. Blasco, M. A. *et al.* Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* **91**, 25–34 (1997).
11. Lee, H.-W., Blasco, M. A., Gottlieb, G. J., Greider, C. W. & DePinho, R. A. Essential role of mouse telomerase in highly proliferative organs. *Nature* **392**, 569–574 (1998).
12. Herrera, E. *et al.* Disease states associated to telomerase deficiency appear earlier in mice with short telomeres. *EMBO J.* **18**, 2950–2960 (1999).
13. Mason, P. J., Wilson, D. B. & Bessler, M. Dyskeratosis congenita — a disease of dysfunctional telomere maintenance. *Curr. Mol. Med.* **5**, 159–170 (2005).
14. Shay, J. W. & Wright, W. E. Telomerase therapeutics for cancer: challenges and new directions. *Nature Rev. Drug Discov.* **5**, 577–584 (2006).
15. Baur, J. A., Zou, Y., Shay, J. W. & Wright, W. E. Telomere position effect in human cells. *Science* **292**, 2075 (2001).
16. Koering, C. E. *et al.* Human telomeric position effect is determined by chromosomal context and telomeric chromatin integrity. *EMBO Rep.* **3**, 1055–1061 (2002).
References 15 and 16 provide conclusive evidence that TPE (or 'silencing' of genes near the telomeres) operates in mammalian cells.
17. Makarov, V. L., Lejnine, S., Bedoyan, J. & Langmore, J. P. Nucleosomal organization of telomere-specific chromatin in rat. *Cell* **73**, 775–787 (1993).
18. Tommerup, H., Dousmanis, A. & de Lange, T. Unusual chromatin in human telomeres. *Mol. Cell. Biol.* **14**, 5777–5785 (1994).
19. Garcia-Cao, M., O'Sullivan, R., Peters, A. H., Jenuwein, T. & Blasco, M. A. Epigenetic regulation of telomere length in mammalian cells by the SUV39H1 and SUV39H2 histone methyltransferases. *Nature Genet.* **36**, 94–99 (2004).
20. Gonzalo, S. *et al.* Role of the RB1 family in stabilizing histone methylation at constitutive heterochromatin. *Nature Cell Biol.* **7**, 420–428 (2005).
21. Gonzalo, S. *et al.* DNA methyltransferases control telomere length and telomere recombination in mammalian cells. *Nature Cell Biol.* **8**, 416–424 (2006).
This work shows that mammalian subtelomeric DNA is heavily methylated, and that this epigenetic modification acts as a negative regulator of telomere length and telomere recombination independently of histone methylation.
22. Fraga, M. F. *et al.* Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nature Genet.* **37**, 391–400 (2005).
23. van Overveld, P. G. *et al.* Hypomethylation of D4Z4 in 4q-linked and non-4q-linked facioscapulohumeral muscular dystrophy. *Nature Genet.* **35**, 315–317 (2003).
24. Garcia-Cao, M., Gonzalo, S., Dean, D. & Blasco, M. A. A role for the Rb family of proteins in controlling telomere length. *Nature Genet.* **32**, 415–419 (2002).
25. Blasco, M. A. Telomeres and human disease: ageing, cancer and beyond. *Nature Rev. Genet.* **6**, 611–622 (2005).
26. Griffith, J. D. *et al.* Mammalian telomeres end in a large duplex loop. *Cell* **97**, 503–514 (1999).
27. de Lange, T. T-loops and the origin of telomeres. *Nature Rev. Mol. Cell Biol.* **5**, 323–329 (2004).
28. Conrad, M. N., Wright, J. H., Wolf, A. J. & Zakian, V. A. Rap1 protein interacts with yeast telomeres *in vivo*: overproduction alters telomere structure and decreases chromosome stability. *Cell* **63**, 739–750 (1990).
29. Nugent, C. I., Hughes, T. R., Lue, N. F. & Lundblad, V. Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. *Science* **274**, 249–252 (1996).

30. Tham, W. H. & Zakian, V. A. Transcriptional silencing at *Saccharomyces* telomeres: implications for other organisms. *Oncogene* **21**, 512–521 (2002).
A must-read review on budding yeast telomeric heterochromatin and its roles in controlling telomere length and telomeric silencing.
31. Marcand, S., Gilson, E. & Shore, D. A protein-counting mechanism for telomere length regulation in yeast. *Science* **275**, 986–990 (1997).
32. Kyrion, G., Boakye, K. A. & Lustig, A. J. C-terminal truncation of RAP1 results in the deregulation of telomere size, stability, and function in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**, 5159–5173 (1992).
33. Krauskopf, A. & Blackburn, E. H. Control of telomere growth by interactions of RAP1 with the most distal telomeric repeats. *Nature* **383**, 354–357 (1996).
34. Levy, D. L. & Blackburn, E. H. Counting of Rif1p and Rif2p on *Saccharomyces cerevisiae* telomeres regulates telomere length. *Mol. Cell. Biol.* **24**, 10857–10867 (2004).
35. Palladino, F. *et al.* SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. *Cell* **75**, 543–555 (1993).
36. Cooper, J. P., Nimmo, E. R., Allshire, R. C. & Cech, T. R. Regulation of telomere length and function by a MYB-domain protein in fission yeast. *Nature* **385**, 744–747 (1997).
37. Baumann, P. & Cech, T. R. POT1, the putative telomere end-binding protein in fission yeast and humans. *Science* **292**, 1171–1175 (2001).
38. Kanoh, J. & Ishikawa, F. spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast. *Curr. Biol.* **11**, 1624–1630 (2001).
39. Ye, J. Z. *et al.* POT1-interacting protein PIP1: a telomere length regulator that recruits POT1 to the TIN2/TRF1 complex. *Genes Dev.* **18**, 1649–1654 (2004).
40. Liu, D. *et al.* PTOP interacts with POT1 and regulates its localization to telomeres. *Nature Cell Biol.* **6**, 673–680 (2004).
41. Smith, S., Giriat, I., Schmitt, A. & de Lange, T. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. *Science* **282**, 1484–1487 (1998).
42. Celli, G. B. & de Lange, T. DNA processing is not required for ATM-mediated telomere damage response after *TRF2* deletion. *Nature Cell Biol.* **7**, 712–718 (2005).
43. Hockemeyer, D., Daniela, J. P., Takai, H. & de Lange, T. Recent expansion of the telomeric complex in rodents: two distinct POT1 proteins protect mouse telomeres. *Cell* **126**, 63–77 (2006).
44. Zhu, X. D., Kuster, B., Mann, M., Petrini, J. H. & Lange, T. Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. *Nature Genet.* **25**, 347–352 (2000).
45. Samper, E., Goytisolo, F. A., Slijepcevic, P., van Buul, P. P. & Blasco, M. A. Mammalian KU86 protein prevents telomeric fusions independently of the length of TTAGGG repeats and the G-strand overhang. *EMBO Rep.* **1**, 244–252 (2000).
46. Tarsounas, M. *et al.* Telomere maintenance requires the RAD51D recombination/repair protein. *Cell* **117**, 337–347 (2004).
47. Karlseder, J. *et al.* The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. *PLoS Biol.* **2**, e240 (2004).
48. Bradshaw, P. S., Stavropoulos, D. J & Meyn, M. S. Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. *Nature Genet.* **37**, 193–197 (2005).
49. Oh, B.-K., Kim, Y.-J., Park, C. & Park, Y. N. Up-regulation of telomere-binding proteins, TRF1, TRF2, and TIN2 is related to telomere shortening during human multistep hepatocarcinogenesis. *Am. J. Pathol.* **166**, 73–80 (2005).
50. Matsutani, N. *et al.* Expression of telomeric repeat binding factor 1 and 2 and TRF1-interacting nuclear protein 2 in human gastric carcinomas. *Int. J. Oncol.* **19**, 507–512 (2001).
51. Muñoz, P. *et al.* XPF nuclease-dependent telomere loss and increased DNA damage in mice overexpressing *TRF2* result in premature aging and cancer. *Nature Genet.* **10**, 1063 (2005).
52. Nakanishi, K. *et al.* Expression of mRNAs for telomeric repeat binding factor (TRF)-1 and TRF2 in atypical adenomatous hyperplasia and adenocarcinoma of the lung. *Clin. Cancer Res.* **9**, 1105–1111 (2003).
53. Bellon, M. *et al.* Increased expression of telomere length regulating factors TRF1, TRF2 and TIN2 in patients with adult T-cell leukemia. *Int. J. Cancer* **119**, 2090–2097 (2006).
54. Blanco, R., Muñoz, P., Klatt, P., Flores, J. M. & Blasco, M. A. Telomerase abrogation dramatically accelerates TRF2-induced epithelial carcinogenesis. *Genes Dev.* **21**, 206–220 (2007).
55. Lazzerini Denchi, E., Celli, G. & de Lange, T. Hepatocytes with extensive telomere deprotection and fusion remain viable and regenerate liver mass through endoreduplication. *Genes Dev.* **20**, 2648–2653 (2006).
56. Flores, I., Cayuela, M. L. & Blasco, M. A. Effects of telomerase and telomere length on epidermal stem cell behavior. *Science* **309**, 1253–1256 (2005).
57. Sarin, K. Y. *et al.* Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature* **436**, 1048–1052 (2005).
58. Mason, J. M. & Biessmann, H. The unusual telomeres of *Drosophila*. *Trends Genet.* **11**, 58–62 (1995).
59. Lundblad, V. & Blackburn, E. H. An alternative pathway for yeast telomere maintenance rescues *est1*- senescence. *Cell* **73**, 347–360 (1993).
60. Lundblad, V. Telomere maintenance without telomerase. *Oncogene* **21**, 522–531 (2002).
61. Le, S., Moore, J. K., Haber, J. E. & Greider, C. W. RAD50 and RAD51 define two pathways that collaborate to maintain telomeres in the absence of telomerase. *Genetics* **152**, 143–152 (1999).
62. Teng, S. C. & Zakian, V. A. Telomere–telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **19**, 8083–8093 (1999).
63. Hande, M. P., Samper, E., Lansdorp, P. & Blasco, M. A. Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *J. Cell Biol.* **44**, 589–601 (1999).
64. Chang, S., Khoo, C. M., Naylor, M. L., Maser, R. S. & DePinho, R. A. Telomere-based crisis: functional differences between telomerase activation and ALT in tumor progression. *Genes Dev.* **17**, 88–100 (2003).
65. Niida, H. *et al.* Telomere maintenance in telomerase-deficient mouse embryonic stem cells: characterization of an amplified telomeric DNA. *Mol. Cell. Biol.* **20**, 4115–4127 (2000).
66. Herrera, E., Martinez, A. C. & Blasco, M. A. Impaired germinal center reaction in mice with short telomeres. *EMBO J.* **19**, 472–481 (2000).
67. Laud, P. R. *et al.* Elevated telomere–telomere recombination in WRN-deficient, telomere dysfunctional cells promotes escape from senescence and engagement of the ALT pathway. *Genes Dev.* **19**, 2560–2570 (2005).
68. Wu, L. *et al.* Pot1 deficiency initiates DNA damage checkpoint activation and aberrant homologous recombination at telomeres. *Cell* **126**, 49–62 (2006).
69. Levis, R., Hazelrigg, T. & Rubin, G. M. Effects of genomic position on the expression of transduced copies of the white gene of *Drosophila*. *Science* **229**, 558–561 (1985).
70. Gottschling, D. E., Aparicio, O. M., Billington, B. L. & Zakian, V. A. Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. *Cell* **63**, 751–762 (1990).
71. Nimmo, E. R., Cranston, G. & Allshire, R. C. Telomere-associated chromosome breakage in fission yeast results in variegated expression of adjacent genes. *EMBO J.* **13**, 3801–3811 (1994).
72. Kyrion, G., Liu, K., Liu, C. & Lustig, A. J. RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae*. *Genes Dev.* **7**, 1146–1159 (1993).
73. Aparicio, O. M., Billington, B. L. & Gottschling, D. E. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* **66**, 1279–1287 (1991).
74. Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S. M. & Grunstein, M. Histone H3 and H4 N-termini interact with Sir3 and Sir4 proteins: a molecular model for the formation of heterochromatin in yeast. *Cell* **80**, 583–592 (1995).
A molecular model for how heterochromatin is formed at budding yeast telomeres is first proposed here.
75. Moretti, P. & Shore, D. Multiple interactions in Sir protein recruitment by Rap1p at silencers and telomeres in yeast. *Mol. Cell. Biol.* **21**, 8082–8094 (2001).
76. Wright, J. H., Gottschling, D. E. & Zakian, V. A. *Saccharomyces* telomeres assume a non-nucleosomal chromatin structure. *Genes Dev.* **6**, 197–210 (1992).
77. Luo, K., Vega-Palas, M. A. & Grunstein, M. Rap1–Sir4 binding independent of other Sir, yKu, or histone interactions initiates the assembly of telomeric heterochromatin in yeast. *Genes Dev.* **16**, 1528–1539 (2002).
78. Strahl-Bolsinger, S., Hecht, A., Luo, K. & Grunstein, M. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. *Genes Dev.* **11**, 83–93 (1997).
79. de Bruin, D., Kantrow, S. M., Liberatore, R. A. & Zakian, V. A. Telomere folding is required for the stable maintenance of telomere position effects in yeast. *Mol. Cell. Biol.* **20**, 7991–8000 (2000).
80. Maillet, L. *et al.* Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression. *Genes Dev.* **10**, 1796–1811 (1996).
81. Fourel, G., Revardel, E., Koering, C. E. & Gilson, E. Cohabitation of insulators and silencing elements in yeast subtelomeric regions. *EMBO J.* **18**, 2522–2537 (1999).
82. Pryde, F. E. & Louism, E. J. Limitations of silencing at native yeast telomeres. *EMBO J.* **18**, 2538–2550 (1999).
83. Emre, N. C. *et al.* Maintenance of low histone ubiquitylation by Ubp10 correlates with telomere-proximal Sir2 association and gene silencing. *Mol. Cell* **17**, 585–594 (2005).
84. Cenci, G. *et al.* UbcD1, a *Drosophila* ubiquitin-conjugating enzyme required for proper telomere behavior. *Genes Dev.* **11**, 863–875 (1997).
85. Greenwell, P. W. *et al. TEL1*, a gene involved in controlling telomere length in *S. cerevisiae*, is homologous to the human ataxia telangiectasia gene. *Cell* **82**, 823–829 (1995).
86. Porter, S. E., Greenwell, P. W., Ritchie, K. B. & Petes, T. D. The DNA-binding protein Hdf1p (a putative Ku homologue) is required for maintaining normal telomere length in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **24**, 582–585 (1996).
87. Nislow, C., Ray, E. & Pillus, L. SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. *Mol. Biol. Cell* **8**, 2421–2436 (1997).
88. Gartenberg, M. R., Neumann, F. R., Laroche, T., Blaszczyk, M. & Gasser, S. M. Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. *Cell* **119**, 955–967 (2004).
89. Berthiau, A. S. *et al.* Subtelomeric proteins negatively regulate telomere elongation in budding yeast. *EMBO J.* **25**, 846–856 (2006).
90. Hediger, F., Berthiau, A. S., van Houwe, G., Gilson, E. & Gasser, S. M. Subtelomeric factors antagonize telomere anchoring and Tel1-independent telomere length regulation. *EMBO J.* **25**, 857–867 (2006).
91. Teng, S. C., Chang, J., McCowan, B. & Zakian, V. A. Telomerase-independent lengthening of yeast telomeres occurs by an abrupt Rad50p-dependent, Rif-inhibited recombinational process. *Mol. Cell* **6**, 947–952 (2000).
92. Nakayama, J., Rice, J. C., Strahl, B. D., Allis, C. D. & Grewal, S. I. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* **292**, 110–113 (2001).
93. Volpe, T. A. *et al.* Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* **297**, 1833–1837 (2002).
94. Kanoh, J., Sadaie, M., Urano, T. & Ishikawa, F. Telomere binding protein Taz1 establishes Swi6 heterochromatin independently of RNAi at telomeres. *Curr. Biol.* **15**, 1808–1819 (2005).
95. Ueno, M. *et al.* Fission yeast Arp6 is required for telomere silencing, but functions independently of Swi6. *Nucleic Acids Res.* **32**, 736–741 (2004).
96. Ekwall, K. *et al.* Mutations in the fission yeast silencing factors clr4 + and rik1 + disrupt the localisation of the chromo domain protein Swi6p and impair centromere function. *J. Cell Sci.* **109**, 2637–2648 (1996).
97. Hall, I. M., Noma, K. & Grewal, S. I. RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. *Proc. Natl Acad. Sci. USA* **100**, 193–198 (2003).
98. Perrini, B. *et al.* HP1 controls telomere capping, telomere elongation, and telomere silencing by two different mechanisms in *Drosophila*. *Mol. Cell* **15**, 467–476 (2004).
99. Savitsky, M., Kwon, D., Georgiev, P., Kalmykova, A. & Gvozdev, V. Telomere elongation is under the control of the RNAi-based mechanism in the *Drosophila* germline. *Genes Dev.* **20**, 345–354 (2006).
100. Xhemalce, B., Seeler, J. S., Thon, G., Dejean, A. & Arcangioli, B. Role of the fission yeast SUMO E3 ligase Pli1p in centromere and telomere maintenance. *EMBO J.* **23**, 3844–3853 (2004).

101. Xhemalce, B. *et al.* Role of SUMO in the dynamics of telomere maintenance in fission yeast. *Proc. Natl Acad. Sci. USA.* **104**, 893–898 (2007).
A role for SUMO proteins in telomerase-mediated telomere elongation is first shown here.
102. Kanoh, J. *et al.* The fission yeast spSet1p is a histone H3-K4 methyltransferase that functions in telomere maintenance and DNA repair in an ATM kinase Rad3-dependent pathway. *J. Mol. Biol.* **326**, 1081–1094 (2003).
103. Peters, A. H. *et al.* Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107**, 323–337 (2001).
104. Schotta, G. *et al.* A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev* **18**, 1251–1262 (2004).
105. Kourmouli, N. *et al.* Heterochromatin and tri-methylated lysine 20 of histone 4 in mammals. *J. Cell Sci.* **117**, 2491–2501 (2004).
106. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120 (2001).
107. Benetti, R., Garcia-Cao, M. & Blasco, M. A. Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. *Nature Genet.* **39**, 243–250 (2007).
Shows for the first time that telomere shortening to a critical length in mammals results in loss of histone and DNA methylation at mammalian telomeres and subtelomeres, concomitant with increased histone acetylation.
108. Netzer, C. *et al.* SALL1, the gene mutated in Townes–Brocks syndrome, encodes a transcriptional repressor which interacts with TRF1/PIN2 and localizes to pericentromeric heterochromatin. *Hum. Mol. Genet.* **10**, 3017–3024 (2001).
109. Kaminker, P. *et al.* Higher-order nuclear organization in growth arrest of human mammary epithelial cells: a novel role for telomere-associated protein TIN2. *J Cell Sci.* **118**, 1321–1330 (2005).
110. Jones, P. A. & Baylin, S. B. The fundamental role of epigenetic events in cancer. *Nature Rev. Genet.* **3**, 415–428 (2002).
111. Dominguez-Bendala, J. & McWhir, J. Enhanced gene targeting frequency in ES cells with low genomic methylation levels. *Transgenic Res.* **13**, 69–74 (2004).
112. Maloisel, L. & Rossignol, J. L. Suppression of crossing-over by DNA methylation in *Ascobolus*. *Genes Dev.* **12**, 1381–1389 (1998).
113. Bender, J. Cytosine methylation of repeated sequences in eukaryotes: the role of DNA pairing. *Trends Biochem. Sci.* **23**, 252–256 (1998).
114. Pedram, M. *et al.* Telomere position effect and silencing of transgenes near telomeres in the mouse. *Mol. Cell. Biol.* **26**, 1865–1878 (2006).
115. Steinert, S., Shay, J. W. & Wright, W. E. Modification of subtelomeric DNA. *Mol. Cell. Biol.* **24**, 4571–4580 (2004).
116. Okano, M., Bell, D. W., Haber, D. A. & Li, E. DNA methyltransferases DNMT3a and DNMT3b are essential for *de novo* methylation and mammalian development. *Cell* **99**, 247–257 (1999).
117. Okano, M., Xie, S. & Li, E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nature Genet.* **19**, 219–220 (1998).
118. Chen, T., Tsujimoto, N. & Li, E. The PWWP domain of Dnmt3a and Dnmt3b is required for directing DNA methylation to the major satellite repeats at pericentric heterochromatin. *Mol. Cell. Biol.* **24**, 9048–9058 (2004).
119. Ofir, R., Wong, A. C., McDermid, H. E., Skorecki, K. L. & Selig, S. Position effect of human telomeric repeats on replication timing. *Proc. Natl Acad. Sci. USA* **96**, 11434–11439 (1999).
120. Jiang, G. *et al.* Testing the position-effect variegation hypothesis for facioscapulohumeral muscular dystrophy by analysis of histone modification and gene expression in subtelomeric 4q. *Hum. Mol. Genet.* **12**, 2909–2921 (2003).
121. Bayne, R. A. L. *et al.* Sandwiching of a gene within 12 kb of a functional telomere and alpha satellite does not result in silencing. *Hum Mol Genet*. **3**, 539–546 (1994).
122. Wright, W. E., Tesmer, V. M., Liao, M. L., & Shay, J. W. Normal human telomeres are not late replicating. *Exp. Cell Res.* **251**, 492–499 (1999).
123. Ancelin, K. *et al.* Targeting assay to study the *cis* functions of human telomeric proteins: evidence for inhibition of telomerase by TRF1 and for activation of telomere degradation by TRF2. *Mol. Cell. Biol.* **22**, 3474–3487 (2002).
124. Loayza, D. & De Lange, T. POT1 as a terminal transducer of TRF1 telomere length control. *Nature* **423**, 1013–1018 (2003).
125. Wang, R. C., Smogorzewska, A. & de Lange, T. Homologous recombination generates T-loop-sized deletions at human telomeres. *Cell* **119**, 355–368 (2004).
126. Dynek, J. N. & Smith, S. Resolution of sister telomere association is required for progression through mitosis. *Science* **304**, 97–100 (2004).
127. Buck, S. W. & Shore, D. Action of a RAP1 carboxy-terminal silencing domain reveals an underlying competition between HMR and telomeres in yeast. *Genes Dev.* **9**, 370–384 (1995).
128. Wiley, E. A. & Zakian, V. A. Extra telomeres, but not internal tracts of telomeric DNA, reduce transcriptional repression at *Saccharomyces telomeres*. *Genetics* **139**, 67–79 (1995).
129. Marcand, S., Brevet, V. & Gilson, E. Progressive *cis*-inhibition of telomerase upon telomere elongation. *EMBO J.* **18**, 3509–3519 (1999).
130. Teixeira, M. T., Arneric, M., Sperisen, P. & Lingner, J. Telomere length homeostasis is achieved via a switch between telomerase-extendible and -nonextendible states. *Cell* **117**, 323–335 (2004).
This work shows that budding yeast telomerase activity preferentially acts on the shortest telomeres.
131. Samper, E., Flores, J. M. & Blasco, M. A. Restoration of telomerase activity rescues chromosomal instability and premature aging in *Terc*$^{-/-}$ mice with short telomeres. *EMBO Rep.* **2**, 800–807 (2001).
132. Hemann, M. T., Strong, M. A., Hao, L. Y. & Greider, C. W. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* **107**, 67–77 (2001).
References 131 and 132 show that mammalian telomerase activity preferentially acts on the shortest telomeres.
133. Xu, G. L. *et al.* Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* **402**, 187–191 (1999).
134. Amir, R. E. *et al.* Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature Genet.* **23**, 185–188 (1999).
135. Cawthon, R. M. *et al.* Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* **361**, 393–395 (2003).
136. Valdes, A. M. *et al.* Obesity, cigarette smoking, and telomere length in women. *Lancet* **366**, 662–664 (2005).
137. Epel, E. S. *et al.* Accelerated telomere shortening in response to life stress. *Proc. Natl Acad. Sci. USA* **101**, 17312–17315 (2004).
138. Haigis, M. C. *et al.* SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic β-cells. *Cell* **126**, 941–954 (2006).
139. Mostoslavsky, R. *et al.* Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **124**, 315–329 (2006).
140. Chua, K. F. *et al.* Mammalian SIRT1 limits replicative life span in response to chronic genotoxic stress. *Cell Metab.* **2**, 67–76 (2005).
141. Roth, W. *et al.* PIASy-deficient mice display modest defects in IFN and Wnt signaling. *J. Immunol.* **173**, 6189–6199 (2004).
142. Nacerddine, K. *et al.* The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. *Dev. Cell.* **9**, 769–779 (2005).
143. Marciniak, R. A. *et al.* A novel telomere structure in a human alternative lengthening of telomeres cell line. *Cancer Res.* **65**, 2730–2737 (2005).

Acknowledgements
I am indebted to E. Gilson (Ecole Normale Supérieure de Lyon, France) for useful discussions and critical reading of the manuscript. M.A.B.'s laboratory is funded by the Spanish Ministry of Education and Culture (MCyT), by the Regional Government of Madrid, the European Union and the Josef Steiner Cancer Research Award 2003.

Competing interests statement
The author declares no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Cdc13 | *EST1* | Pot1 | Rap1 | Rif1 | Rif2 | SALL1 | Sir2 | Sir3 | SUV39H1 | SUV39H2 | SUV4-20H1 | SUV4-20H2 | TERT | TIN2
UniProtKB: http://ca.expasy.org/sprot
DNMT1 | DNMT3a | DNMT3b

FURTHER INFORMATION
Spanish National Cancer Research Centre (CNIO): http://www.cnio.es
Access to this links box is available online.