Review

Chromatin regulation and non-coding RNAs at mammalian telomeres

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ABSTRACT

In eukaryotes, terminal chromosome repeats are bound by a specialized nucleoprotein complex that controls telomere length and protects chromosome ends from DNA repair and degradation. In mammals the "shelterin" complex mediates these central functions at telomeres. In the recent years it has become evident that also the heterochromatic structure of mammalian telomeres is implicated in telomere length regulation. Impaired telomeric chromatin compaction results in a loss of telomere length control. Progressive telomere shortening affects chromatin compaction at telomeric and subtelomeric repeats and activates alternative telomere maintenance mechanisms. Dynamics of chromatin structure of telomeres during early mammalian development and nuclear reprogramming further indicates a central role of telomeric heterochromatin in organismal development. In addition, the recent discovery that telomeres are transcribed, giving rise to UUAGGG-repeat containing TelRNAs/TERRA, opens a new level of chromatin regulation at telomeres. Understanding the links between the epigenetic status of telomeres, TERRA/TelRNA and telomere homeostasis will open new avenues for our understanding of organismal development, cancer and ageing.

Contents

1. Introduction

Telomeres are nucleoprotein structures that protect the ends of linear chromosomes from degradation and from being detected as DNA double strand breaks (DSB) [1,2]. Vertebrate telomeres consist of TTAGGG tandem repeats that are bound by the multiprotein complex shelterin that mediates crucial functions in telomere control [1]. Located adjacent to telomeric repeats, repeat rich, gene-poor and recombinogenic subtelomeres (also referred to as telomere associated sequences, TAS) spread up to several hundreds of kilobases towards the centromere [3–5]. Telomere capping is dependent on a minimal length of telomeric repeats and on

shelterin binding, and may involve the formation of higher order DNA conformations, such as the T-loop structure [6]. Incomplete DNA replication of telomeres results in progressive telomere shortening that can eventually lead to telomere uncapping and the elicitation of a DNA damage response (DDR), which results in cell cycle arrest/senescence [7,8]. Telomere attrition is antagonized by telomerase, a reverse transcriptase that can add telomeric repeats onto chromosome ends [2,9]. In mammals, telomerase activity is downregulated soon after birth and consequently not sufficient to prevent progressive telomere attrition during the lifetime of an organism, thus providing a molecular basis for organismal ageing [10–13]. This model is supported by augmented stem cell dysfunction and premature loss of tissue regeneration in mice with accelerated telomere shortening due to telomerase deficiency [14–20]. Alternative lengthening of telomeres (ALT), a process based on homologous recombination events at telomeric and sub-

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telomeric repeats can compensate for telomerase deficiency in a number of human immortal cell lines and human cancers [21–24].

A highly compact chromatin structure is a common feature of telomeric repeats from yeast to man and thought to play an important role in telomere function [5]. Telomeric heterochromatin can spread and silence reporter genes inserted into subtelomeric regions, a phenomenon also referred to as "*telomere position effect*" or TPE [5]. In contrast to yeast telomeres, which are devoid of nucleosomes, vertebrate and *D. melanogaster* telomeric repeats are organized into a heterochromatic chromatin template carrying specific posttranslational histone modifications and chromatin associated proteins [5,25–29]. In addition, subtelomeric DNA is highly methylated in mouse cells and human somatic cells [29–31]. Importantly, mouse loss of function studies revealed that an impairment of telomeric and subtelomeric heterochromatin structure alters telomere length homeostasis and induces ALT [27,29,32]. The control of telomeric chromatin structure is dynamic and subjected to extensive remodelling during nuclear reprogramming. This suggests that telomeric chromatin structures could be under developmental but also tissue specific control [33,34]. Recently, telomeres were found to be transcribed, giving rise to UUAGGG-repeat containing, non-coding RNAs with an anticipated role in telomere length regulation and chromatin control (TERRA/TelRNA) [35,36]. Given the central role of non-coding RNAs in chromatin regulation across species, this anticipates an important role of non-coding RNAs and telomeric chromatin structure in telomere regulation [37].

In this review we first provide an overview on mammalian telomeres and telomeric chromatin structure and regulation. On this background we will present recent developments on telomeric chromatin dynamics and non-coding RNAs. Finally we discuss a possible impact of telomeric chromatin structure in human disease.

1.1. Telomeres and shelterin

Shelterin is a multiprotein complex that mediates major telomere functions such as chromosome end protection ("capping") and telomere length regulation. Shelterin is anchored to telomeric repeats via the DNA binding proteins POT1, TRF1 and TRF2 [1]. POT1 specifically binds to the single stranded telomeric G-strand overhang and interacts with TPP1 to control telomerase access and protect chromosome ends from eliciting an ATR kinase signalling-dependent DNA damage response [38–44]. Double stranded telomeric repeats are bound by the telomere repeat binding proteins TRF1 and TRF2, which in turn are connected to POT1 via TPP1 [1,45–47]. In addition, TRF2 was also reported to directly interact with POT1 [48]. TRF2 is a negative regulator of telomere length *in vivo* and has been demonstrated to be essential for telomere protection by inhibiting the activation of the ATM kinase pathway—supported by RAP1 [49–54]. A complex network of telomere regulation mechanisms depends on binding of TRF1 to telomeric repeats. TRF1 is a negative regulator of telomere length that interacts with TANK1 and TANK2 poly(ADP)-ribosylases (tankyrase1 and 2) which act as positive regulators of telomere length [55–58]. The TRF1 interacting nuclear factor 2 (Tin2) simultaneously interacts with TRF1 and TRF2 and negatively regulates telomere length by protecting TRF1 from inhibition by tankyrase 1 dependent poly(ADP-ribosyl)ation [59–62]. Extensive research on shelterin components and function has provided us with a complex network of protein interactions that synergistically control telomere length and chromosome capping. However, recent works focussed on the study of epigenetic marks at telomeric and subtelomeric chromatin, demonstrated that the basic telomeric chromatin template has an important role in the control of mammalian telomeres.

1.2. The signature of mammalian telomeric heterochromatin

Mammalian telomeric chromatin shares commonalities with the telomeric chromatin in yeast and flies, such as the silencing of reporter genes inserted into a subtelomeric position by TPE [26,63–65]. The findings that increasing telomere length augments TPE and histone deacetylation by treatment with Trichostatin A (TSA) releases reporter gene silencing, provided early evidence that telomere length and chromatin status modulate the repressive environment at mammalian telomeres [64,65]. Chromatin at mammalian telomeres is under-acetylated and share characteristic features of other repeat containing heterochromatic elements such as pericentric repeats [32,66]. The chromatin structure of these repeat elements is defined by the activities of Suv39h and Suv4-20h histone metyltransferases (HMTases) [67,68]. Similar to *D. melanogaster* and *S. pombe*, vertebrate telomeric and subtelomeric repeats are enriched for H3K9m3, mediated by the H3K9-specific Suv39h1 and Suv39h2 HMTases, mammalian homologs of *S. pombe Clr4* [28,67,69] (Fig. 1). Imposition of the H3K9me3 mark provides a high affinity-binding site for the recruitment of HP1 to telomeres [28,70,71]. In line with the sequential recruitment of constitutive heterochromatin, Suv4-20h1 and Suv4-20h2 HMTases are recruited though an interaction with HP1 and establish telomeric H4K20me3 [27,68,72]. Interestingly, the family of retinoblastoma (Rb) tumor suppressor proteins, consisting of Rb1, Rbl1 and Rbl2 was found to interact with the Suv4-20h HMTases to direct H4K20me3 to telomeric and centromeric repeats [73–77]. More recently, the mouse homolog of yeast Dot1 and *D. melanogaster grappa*, the HMTase Dot1L was found to mediate H3K79me2 at telomeres, which in turn enhances the establishment of H4K20me3 [78]. This suggests a model where Suv39h HMTases and Dot1L prepare a chromatin substrate suitable for the further chromatin compaction by HP1 and Suv4-20h HMTases [27,78] (Fig. 1). Except for a role of SIRT6 in H3K9 deacetylation at human telomeres, little is known about the enzymatic activities mediating the low acetylation levels at telomeric histones H3 and H4 [66]. Importantly, the telomeric histone code is not restricted to TTAGGG repeats, but also defines adjacent highly, repetitive and gene-poor, subtelomeric regions that can stretch up to several hundreds of kilobases towards the centromere [5,32]. However, to date it is not clear whether subtelomeric heterochromatin is formed *in cis* or results from the spreading of a telomeric heterochromatic "island" towards centromeres.

1.3. DNA methylation at subtelomeric repeats

Vertebrate TTAGGG DNA tandem repeats are organized into a heterochromatic structure but remain unmethylated due to the lack of methylate-able cytosine. In contrast, human and mouse subtelomeres were described to be heavily methylated through the action of the DNA methyltransferases DNMT1, DNMT3a and DNMT3b [29–31,79]. This additional layer of epigenetic regulation is thought to represent an additional mechanism enforcing TPE [29,80–83] (Fig. 1). HP1 and Suv39h1 were reported to have a role in recruiting DNMTs to pericentric repeats, however the establishment of DNA methylation at mouse centromeric and subtelomeric repeats occurs independently of Suv39h HMTases, suggesting a yet undefined alternative pathway for DNMT recruitment to subtelomeres [27,84,85]. The tight control of DNA methylation is crucial for telomere homeostasis as demonstrated by the existence of a cluster of miRNAs controlling the expression of DNMTs in mouse embryonic stem (ES) cells (Fig. 1) [86]. The murine miR-290 cluster tightly regulates the expression of the Rb family protein Rbl2, a potent transcriptional repressor of DNMTs. Absence of miR-290 cluster maturation in *Dicer 1* deficient ES cells results in increased Rbl2 levels and consequently reduced