Telomeric RNA enters the game

Béatrice Horard and Eric Gilson

Two studies report expression of telomeric-repeat-containing RNAs in vertebrates. This discovery challenges the long-standing notion that telomeres are transcriptionally inert.

One of the major questions in telomere research is how telomeres protect the extremities of linear chromosomes from being recognized and processed as double-strand breaks. The current view is that the protective functions of telomeres rely on telomere-specific DNA conformations, higher-order chromatin organization, as well as telomere-associated proteins (reviewed in ref. 1). Vertebrate telomeres consist of TTAGGG repeats, and the G-rich strand extends beyond its complement to form a 3′ overhang named the G-tail, which is a substrate of telomerase (a telomere-specific reverse transcriptase that compensates for the replicative erosion of telomeric DNA) (Fig. 1a). The G-tail may also adopt particular DNA conformations at the very end of chromosomal DNA by invading the telomeric duplex DNA to form the so-called t-loop, and by folding into intra- or intermolecular guanine-quadruplexes called G4 (reviewed in ref. 2). However, the function of t-loop and G4 structures in telomere protection remains unknown.

Telomeric chromatin is organized, at least in part, into tightly packed nucleosomes exhibiting marks characteristic of heterochromatin (reviewed in ref. 3). Moreover, human telomeres exert a position effect on the expression of subtelomeric genes, suggesting that they can initiate the formation of transcriptionally silenced chromatin at subtelomeres[4,5]. Vertebrate telomeres also bind selectively to proteins involved in a network of homo- and heterotypic interactions. Telomeric proteins are essential in regulating telomerase activity and in preventing checkpoint activation and telomere fusion (reviewed in ref. 6).

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Two independent groups now report — one of page 228 of this issue — the identification of a set of transcripts derived from telomeric DNA[7,8] (Fig. 1a). Previous studies by Lingner and colleagues had revealed unexpected links between telomere factors and nonsense-mediated mRNA decay (NMD)[9], stimulating their interest in telomeric transcription. Further studies by this team has now led to the discovery of a set of transcripts that they termed telomeric-repeat-containing RNA or TERRA[7]. In independent studies investigating the possibility that telomeric heterochromatin is regulated by non-coding RNA, Schoeftner and Blasco[8] have identified the seemingly identical transcripts, which they termed TelRNA. Here we use the term TERRA in referring to these transcripts.

TERRA molecules were identified in human, mouse and fish, although they are probably not restricted to these vertebrate species. They can be found in different cell types, including embryonic stem (ES) cells, primary somatic cells, immortalized and cancer cells. They are heterogeneous in length, ranging from 100 bases up to at least 9 kilobases[7,8]. Their sequences contain mainly UUAGGG repeats and their synthesis seems to be initiated in subtelomeric regions[7,8] (Fig. 1a).

TERRA molecules display two hallmarks of DNA-dependent RNA polymerase II (PolII) transcripts: they are polyadenylated and their synthesis is sensitive to α-amanitin[7,8] Furthermore, chromatin immunoprecipitation experiments have shown a substantial enrichment of PolII at telomeres[8]. Interestingly, PolII co-immunoprecipitates with the telomeric DNA-binding protein TRF1[8]. Depletion of TRF1 causes levels of TERRA to decrease, but does not affect that of PolII at telomeres[8]. These results, together with the possible subtelomeric location of TERRA-initiation sites, suggest that TRF1 is not required to initiate TERRA transcription, but rather facilitates TERRA elongation or stability, or both (Fig. 1a). Moreover, the conserved size distribution of TERRA molecules among cells exhibiting various mean lengths of telomeres, and the apparent short half-life of the transcripts (2–3 h), suggest the existence of unique mechanisms of elongation, termination, processing and degradation, which remain to be elucidated[7,8].

TERRA molecules are found in nuclear fractions, where they colocalize with various telomeric components in interphase nuclei, and are also found at the chromosome tips during metaphase[7,8]. Together, these findings indicate that TERRA is associated with chromosomal ends at different stages of the cell cycle. The nuclear localization suggests that TERRA is not translated, although a rapid transit through the cytoplasm cannot be excluded. If TERRA were indeed translated, the two possible frames would generate $(Arg\text{-}Val)_n$ and $(Gly\text{-}Leu)_n$ polypeptides in vertebrate species.

Schoeftner and Blasco[8] have shown that TERRA levels are affected by different factors, including developmental stage, cellular stress, telomere length, tumour stage and telomeric chromatin structure; however, it remains unclear how synthesis of TERRA and its levels in the cell are regulated. Interestingly, TERRA levels are increased in cells deficient in the histone-methyl transferases (HMTases) Suv39h and Suv4-20h[8]. Conversely, TERRA levels are decreased in cells lacking DNA methyl transferase (DNMTase) or Dicer activity[8]. Deficiency of HMTases at telomeres correlates with decreased heterochromatin marks, whereas cells lacking DNMTase or Dicer activity display an increased density of these

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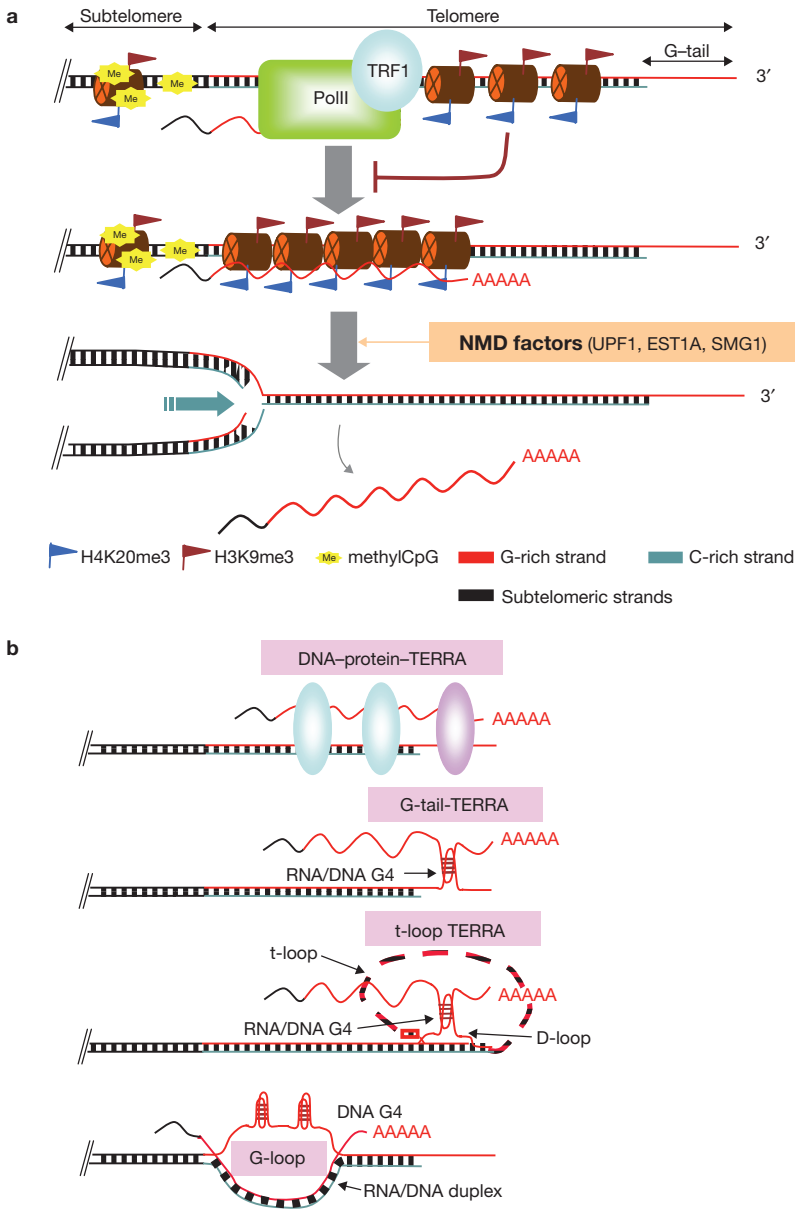

Figure 1 Models for telomeric repeat-containing RNA (TERRA) synthesis and telomere association. (**a**) Vertebrate telomeres consist of TTAGGG repeats, with the G-rich strand extending beyond its complement to form a 3′ overhang named the G-tail. The duplex region is bound by a specific set of proteins, including the TTAGGG-DNA-binding protein TRF1. Additionally, the duplex is enriched in chromatin modifications characteristic of constitutive heterochromatin, such as trimethylation of H3K9, H4K20 and HP1 binding. It is notable that in the absence of CpG in the telomeric track, only the subtelomeric region is a potential substrate for DNA methyltransferases. TERRA molecules seem to originate from the C-rich strand, starting from the subtelomeric domain and proceeding into the telomeric track. These RNA molecules are poly(A)-tailed and localized to chromosome ends, and may be synthesized by PolII. TRF1 probably interacts with PolII but is not required to initiate transcription; instead, it may facilitate elongation and/or stability of the transcripts. Heterochromatin seems to negatively regulate TERRA abundance. However, TERRA is a key element of heterochromatin assembly at telomeres. This would create a negative feedback loop regulating the level of telomeric heterochromatin. Several NMD components (UPF1, EST1A and SMG1) seem to control the displacement of TERRA from telomeric chromatin. (**b**) Possible modes of TERRA–telomere association. Proteins exhibiting affinity for double- (blue) and single- (pink) stranded nucleic acids may trap nascent transcripts or processed transcripts at telomeres. The G-rich 3′ overhang of telomeres can fold into four-stranded structures called G4, composed of stacked G-quartets, or into t-loops, after invasion of the 3′ overhang into the duplex DNA. The G-rich transcript TERRA might tether the G-tail or the displaced strand at the base of the t-loop by forming intermolecular G4-RNA/DNA structure. Alternatively, a G-loop containing a co-transcriptional RNA/DNA duplex and a G4-DNA structure on the non-template strand can form on transcription of G-rich regions such as telomeres. Persistence of these RNA/DNA hybrids may promote genome instability.

marks[3]. These results suggest that telomeric heterochromatin represses TERRA formation at telomeres, for example, by inhibiting transcriptional elongation. As TERRA transcription seems to originate from subtelomeric regions, it seems counterintuitive that subtelomeric DNA de-methylation in DNMTase-deficient cells does not cause de-repression of the TERRA promoter and thereby increased TERRA expression. It is possible that de-repression of the subtelomeric region or disruption of the RNAi pathway increases the level of a putative antisense transcript of TERRA, which causes TERRA degradation. Additionally, in accordance with the co-transcriptional gene-silencing model of heterochromatin formation[10], a low level of TERRA synthesis may be required for the establishment of telomeric heterochromatin (Fig. 1a). Consistently, Schoeftner and Blasco[8] observed large TERRA foci in the vicinity of inactive X-chromosomes decorated by the *Xist* RNA. Interestingly, the NMD machinery, which regulates the association of TERRA with telomeres (see below), seems to be also involved in X-inactivation[11], suggesting that there are unexpected connections between TERRA, NMD and telomeric heterochromatin.

Various NMD effectors are enriched at telomeres and their depletion increases TERRA signals and triggers telomere damage, including complete telomere loss[7]. Despite the presence of one UAG stop-codon in each telomeric repeat sequence, the role of the NMD factors is probably not to stimulate TERRA degradation, but rather, to reduce its association with telomeres[7]. If the increased level of TERRA at telomeres is the cause of the telomere de-protection observed in NMD-deficient cells, this would indicate that TERRA is detrimental for telomere stability. Alternatively, if NMD-depletion triggers telomere damage independently of an effect on TERRA, the increased association of TERRA with telomere could be stimulated by the cell in an effort to reinforce telomere protection. Similarly, the increased levels of TERRA observed with thermal shock may help to protect telomeres against stress-induced damage[8], as has been proposed for the heat-induced transcription of human satellite III found in pericentromeric heterochromatin[12].

TERRA may also be involved in the regulation of telomerase. Indeed, the NMD factor EST1A interacts physically with telomerase[9,13] and *in vitro*, TERRA inhibits telomerase — probably by RNA duplex formation in the template region of the telomerase RNA component (Terc)[8]. The

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effect of TERRA on telomerase remains to be tested *in vivo*. It is worth noting that the NMD effector UPF1 interacts with the DNA polymerase δ, suggesting additional links between NMD, TERRA and telomere replication[7] (Fig. 1a).

A crucial question is how TERRA is specifically associated with telomeric chromatin. *A priori*, two non-exclusive modes can be envisaged: interaction with telomeric proteins and hybridization with single-stranded telomeric DNA (Fig. 1b). Although any telomere-bound protein should now be examined for their putative ability to tether TERRA to telomeres, the ability of nucleolin and of heterogeneous nuclear ribonucleoproteins (hnRNPs) to bind to both telomeric RNA and DNA makes them attractive candidates[14]. Alternatively, TERRA may be involved in intermolecular G4 with either the G-tail or the displaced strand of the D-loops formed at the base of t-loops. Support for this hypothesis comes from the finding that a 22mer oligonucleotide composed of UUAGGG repeats can fold into G4 structures *in vitro*[15]. However, intermolecular G4 involving DNA–RNA hybrids has not yet been reported. Another interesting possibility involves co-transcriptional pairing of TERRA to the C-rich strand DNA templates. Indeed, such structures, named G-loops, can be formed when G-rich DNA templates, including telomeric DNA, are transcribed *in vitro* (reviewed in ref. 2; Fig. 1b). Interestingly, such DNA–RNA duplexes are highly recombinogenic and various factors associated with RNA processing (such as THO/TREX and ASF2/SF2) assist in their resolution, thereby preventing genomic instability[2]. Hence, it may be anticipated that not only NMD, but also other RNA-processing pathways are important in TERRA turnover and telomere association.

Recent studies have shown that transcription occurs throughout the genome (including repetitive DNA regions), that a substantial portion of polyadenylated transcripts is non-coding, and that PolII is associated with silent regions[16–18]. In view of these findings, the observation that telomeres are transcribed is not so surprising. Thus, it is possible that TERRA is merely the product of a general mechanism governing repetitive DNA transcription, without an essential function at telomeres. On the other hand, given the results discussed here[7,8], TERRA may prove to be a key component of the telomere machinery. Functional organization of telomeres should now be revisited and, undoubtedly, future studies will reveal intriguing new connections between telomere protection, epigenetic regulation and RNA metabolism.

The finding of RNA molecules at telomeres is not without clinical relevance in the treatment of cancer, as it may contribute to the telomeric alterations accompanying malignant transformation[8]. Thus, TERRA may be a valuable target for anti-cancer agents directed against telomeres. In this regard, it will be of great interest to evaluate the capacity of known DNA G4 ligands to bind to equivalent structures in TERRA.

Last but not least, TERRA may be part of the transmissible information determining telomere state. Consistent with recent work showing that RNA can transmit epigenetic information between generations[19], one can imagine that TERRA belongs to the telomeric heritage that we received from our parents.

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Outsourcing CREB translation to axons to survive

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Nerve growth factor induces sensory neuron survival via retrograde signalling from the axon to the cell body. Local translation of the transcription factor CREB in the axon, followed by its transport to the nucleus, is involved in this process.

During development, the nervous system often produces more neurons than it eventually requires. These neurons then compete for limiting amounts of 'survival' factors, or neurotrophins, and those that do not get enough die; for example, dorsal root ganglion (DRG) sensory neurons compete for nerve growth factor (NGF) released by the cells they target. It is generally thought that binding of NGF to TrkA (its receptor) induces the phosphorylation and endocytosis of TrkA and the retrograde transport of 'signalling endosomes' carrying NGF–pTrkA complexes together with downstream effectors such as the MAP kinase Erk5 (ref. 1) . When they reach the cell body, these signalling endosomes induce the phosphorylation and activation of cAMP response element binding protein (CREB), a transcription factor that promotes DRG neuron survival[2]. This model has assumed that a pre-existing pool of CREB in the cell body is phosphorylated following NGF signalling. On page 149 of this issue, Cox *et al.*[3] report that NGF induces the local translation of CREB in axons and that, surprisingly, this axonally synthesized pool of CREB is required

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