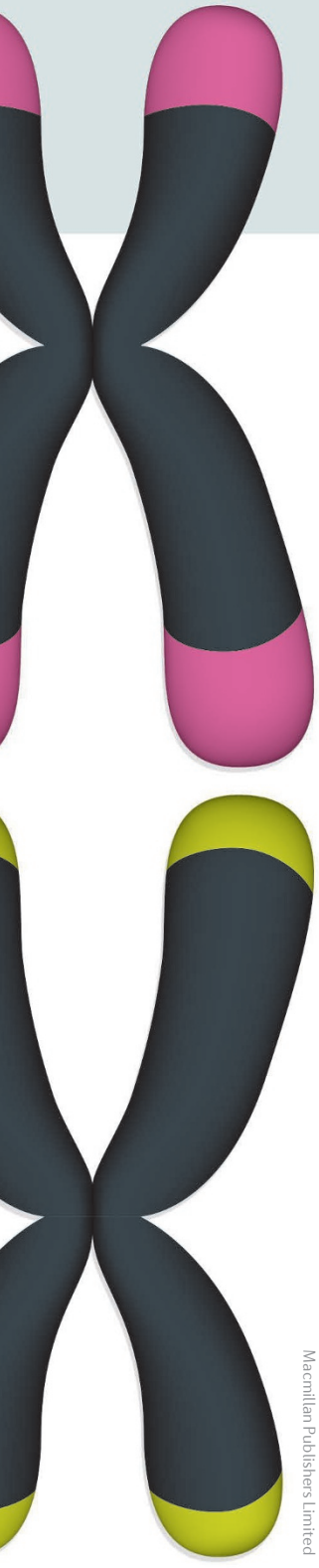
Nature Reviews Genetics | Published online 17 Jul 2017; doi:10.1038/nrg.2017.58



Macmillan Publishers Limited

NON-CODING RNA

A protective role for TERRA at telomeres

From yeast to humans, telomeric DNA is transcribed by RNA polymerase II into the long non-coding (lnc) telomeric repeat-containing RNA (TERRA), but the exact functions of this RNA and its mode of action have remained poorly understood. Two studies in *Cell* now reveal a protective role for TERRA at chromosome ends.

Telomeric DNA is composed of long repetitive sequences with a single-stranded 3′ overhang several hundred nucleotides in length. The length of telomeres, which shorten with every cell division, is maintained by the ribonucleoprotein complex telomerase, the lack of which leads to progressive telomere shortening and activation of the DNA damage response, resulting in irreversible cell cycle arrest (senescence) or cell death.

To map chromatin regions bound by TERRA, Chu *et al.* combined two established methods, chromatin isolation by RNA purification (ChIRP) and capture hybridization analysis of RNA targets (CHART), to develop a new approach they call CHIRT-seq, which identified thousands of binding sites in mouse embryonic stem (ES) cells. RNA fluorescence *in situ* hybridization (FISH) also showed that TERRA was not restricted to telomeres.

Functional effects of TERRA were assessed through antisense oligonucleotide-mediated degradation of the lncRNA *in vivo*, and successful depletion was confirmed by RNA-FISH and northern blot analysis. Transcriptomic analysis showed marked changes in gene expression resulting from TERRA depletion.

Using an *in vivo* proteomic approach, the authors used identification of direct RNA interacting proteins (iDRiP) to determine the protein interactome of TERRA. The analysis identified 134 enriched proteins belonging to several functional groups, including chromatin and transcription factors, cell cycle regulators and DNA replication proteins, as well as proteins involved in alternative lengthening of telomeres (ALT), a mechanism that relies on homologous recombination-mediated DNA copying to counteract telomere shortening.

> “marked changes in gene expression resulting from TERRA depletion”

ALT has previously been associated with mutations in the chromatin remodeller α-thalassaemia/mental retardation syndrome X-linked (ATRX) both *in vitro* and in several human cancers, where it is activated to promote telomere lengthening in the absence of telomerase. Chu *et al.* observed that ATRX colocalized with TERRA *in vivo*. Moreover, the proteins were found to share a subset of target genes, displaying antagonistic effects on gene expression, with TERRA promoting and ATRX suppressing gene expression of both telomeric and non-telomeric targets. *In vitro* and *in vivo* analyses showed further that TERRA modulates ATRX distribution and competes with telomeric DNA for ATRX binding.

Finally, depletion of TERRA led to increased telomerase RNA levels and activity in mouse ES cells, concomitant with an increase in telomere dysfunction and defects, indicating a role for TERRA in maintaining telomere integrity. The authors propose that TERRA expression may promote ALT in cancer cells by specifically suppressing ATRX and telomerase in this context.

In a second study, Graf *et al.* focused on yeast cells, in which one critically short telomere is sufficient to trigger senescence. It was known that in the absence of telomerase, homology-directed repair (HDR) between sister chromatids can partially compensate telomere shortening to prevent early-onset senescence.

Using quantitative PCR (qPCR) and DNA–RNA hybrid immunoprecipitation (DRIP), the authors could show that TERRA accumulates at critically short telomeres, forming RNA–DNA hybrids (R-loops). TERRA levels and TERRA R-loops were found to be tightly regulated through the cell cycle, forming in the S phase before telomere replication, before being degraded. Upon telomere shortening, however, the accumulation of TERRA R-loops leads to the activation of the DNA damage response, which promotes HDR, thereby protecting against premature senescence.

Taken together, the two studies provide pivotal insights into the regulatory function of TERRA in mice and yeast. Given the essential role of telomeres for maintaining genome integrity, and the detrimental impact of telomere crisis (a state of extensive genome instability) in cancer, it will be interesting to determine how far these novel findings translate to humans.

Linda Koch

ORIGINAL ARTICLES Chu, H.-P. *et al.* TERRA RNA antagonizes ATRX and protects telomeres. *Cell* **170**, 86–101 (2017) | Graf, M. *et al.* Telomere length determines TERRA and R-loop regulation through the cell cycle. *Cell* **170**, 72–85 (2017)