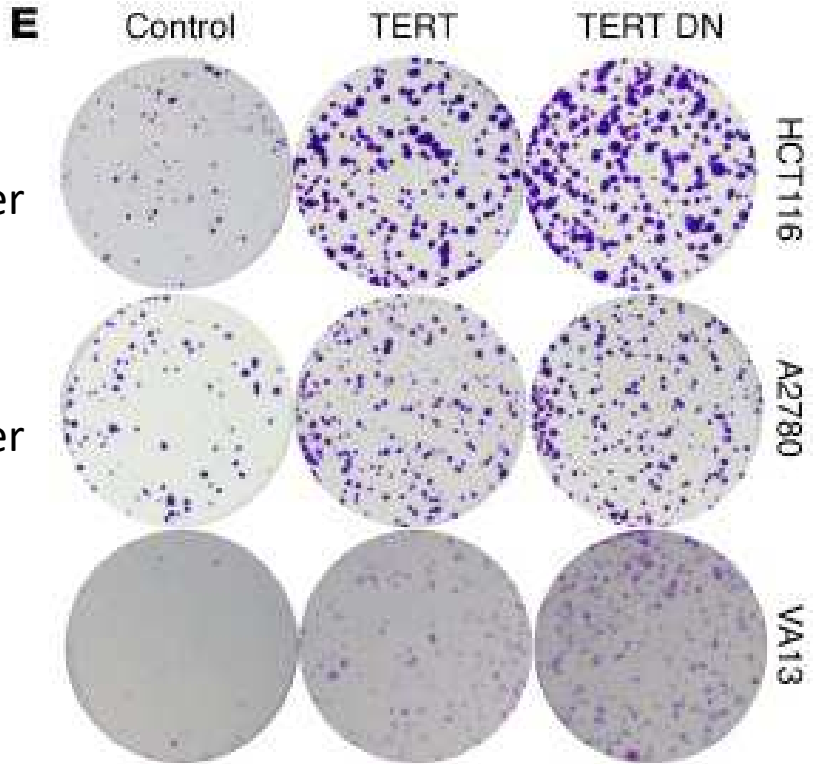
Extratelomeric functions of TERT

coupled to cancer cell dissemination and tumor formation.

Means to the ends: The role of telomeres and telomere processing machinery in metastasis
Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 2016,

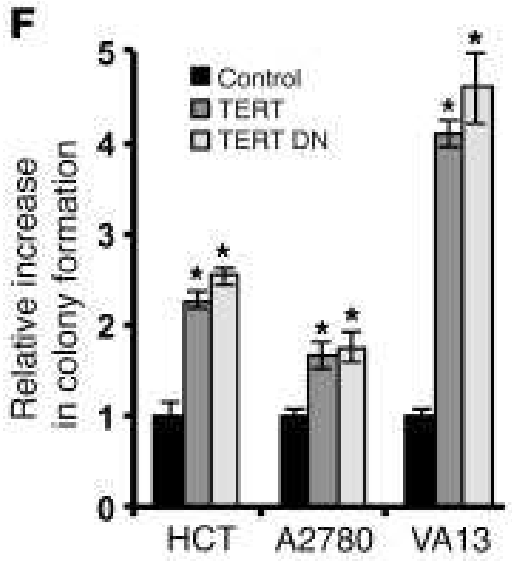
TERT also controls gene expression by acting as a transcription factor

Colony formation assay in tumor cells expressing TERT and TERT DN



Colon Cancer

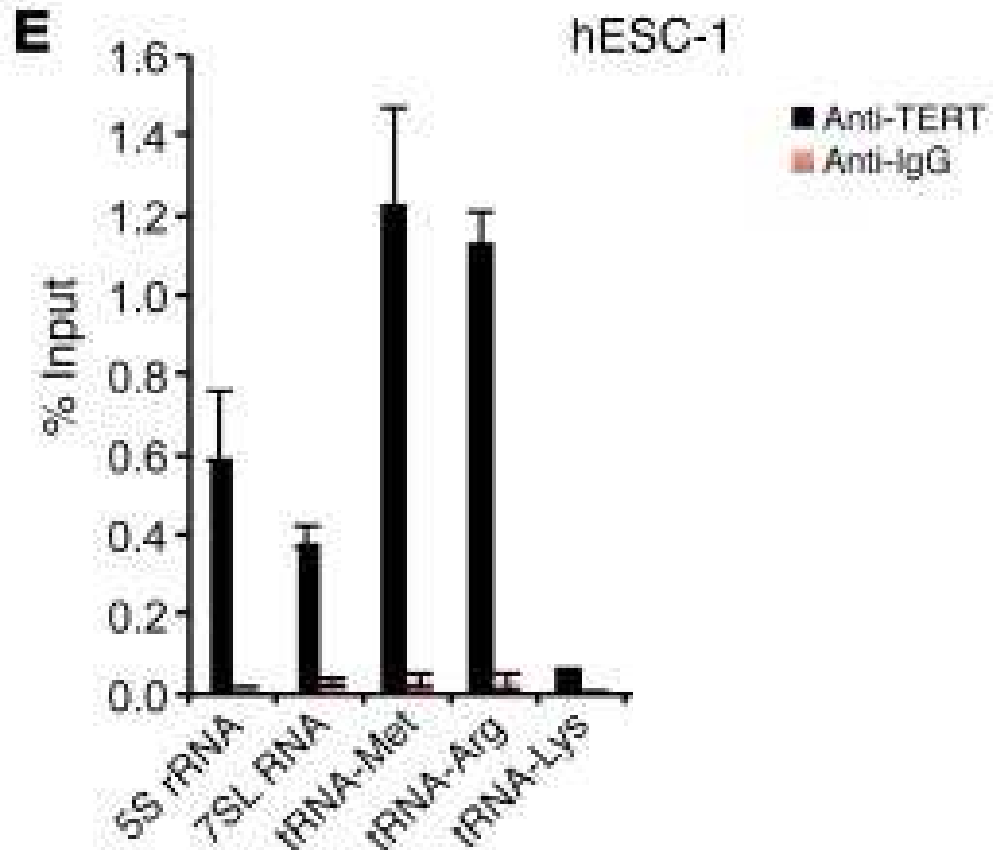
Lung Cancer



No TERT

catalytically inactive TERT (TERT DN)

Genome-wide binding of endogenous TERT reveals its association with genes transcribed by RNA polymerase III

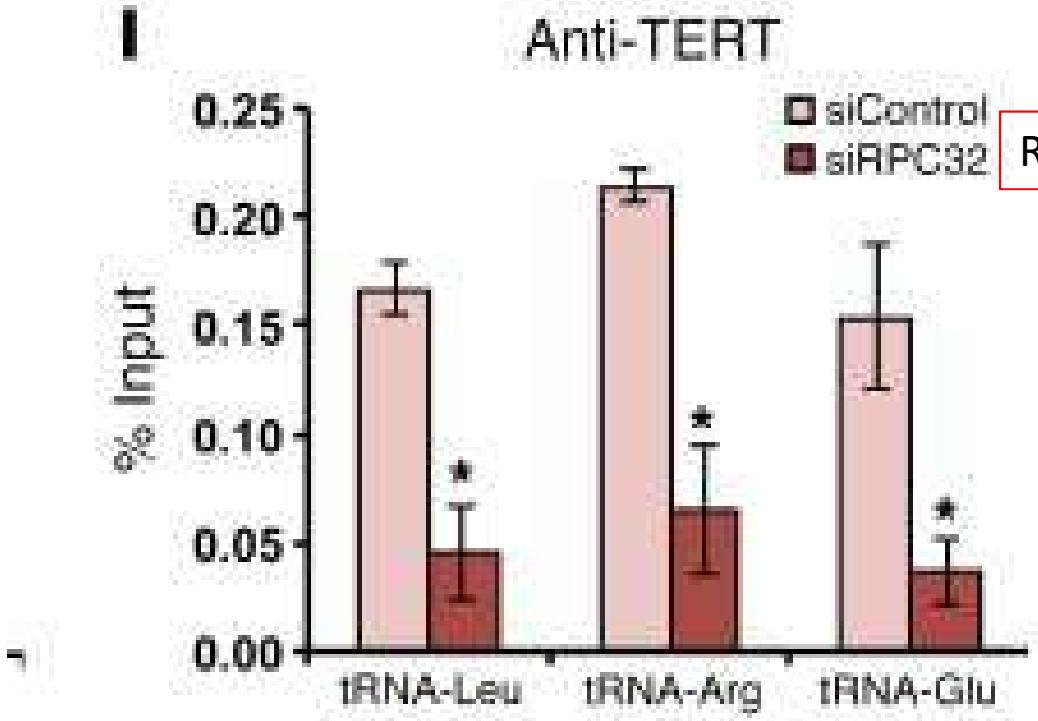


1 ChIP (chromatin immunoprecipitation) using TERT Ab and IgG Ab (control)

2 qPCR with primers specific for the indicated target regions

RPC32 is essential for activation of RNA pol III–driven promoters by TERT

ChIP



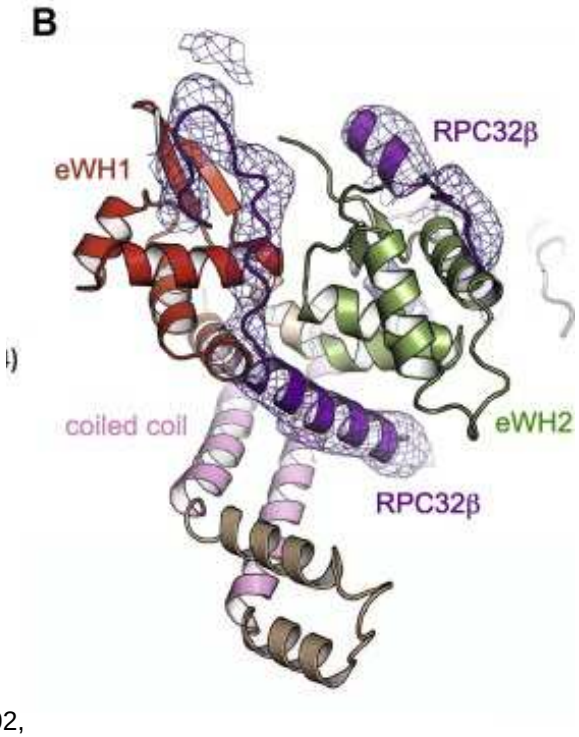
RNA pol III subunit RPC32

Pol III is the largest of the nuclear RNA polymerases with 17 subunits (in comparison to 12 and 14 subunits for Pol II and Pol I respectively)

RPC32 as a molecular bridge in between Pol III domains.
Bi-functional role for RPC32 through interactions with the largest Pol III subunit and through solvent exposed residues

Pol III is the largest of the nuclear RNA polymerases with 17 subunits (in comparison to 12 and 14 subunits for Pol II and Pol I respectively)

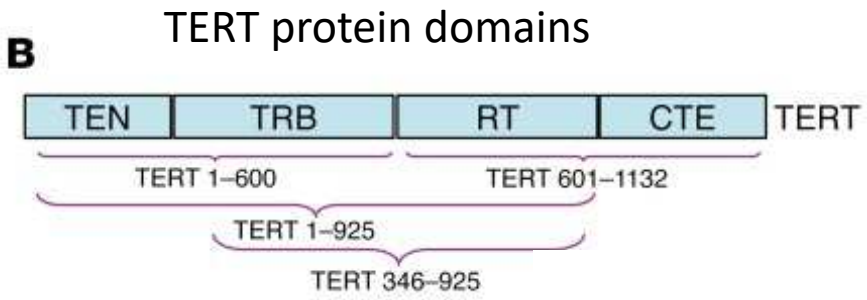
RPC32 as a molecular bridge in between Pol III domains.
Bi-functional role for RPC32 through interactions with the largest Pol III subunit and through solvent exposed residues



Structure of RPC62–RPC32β

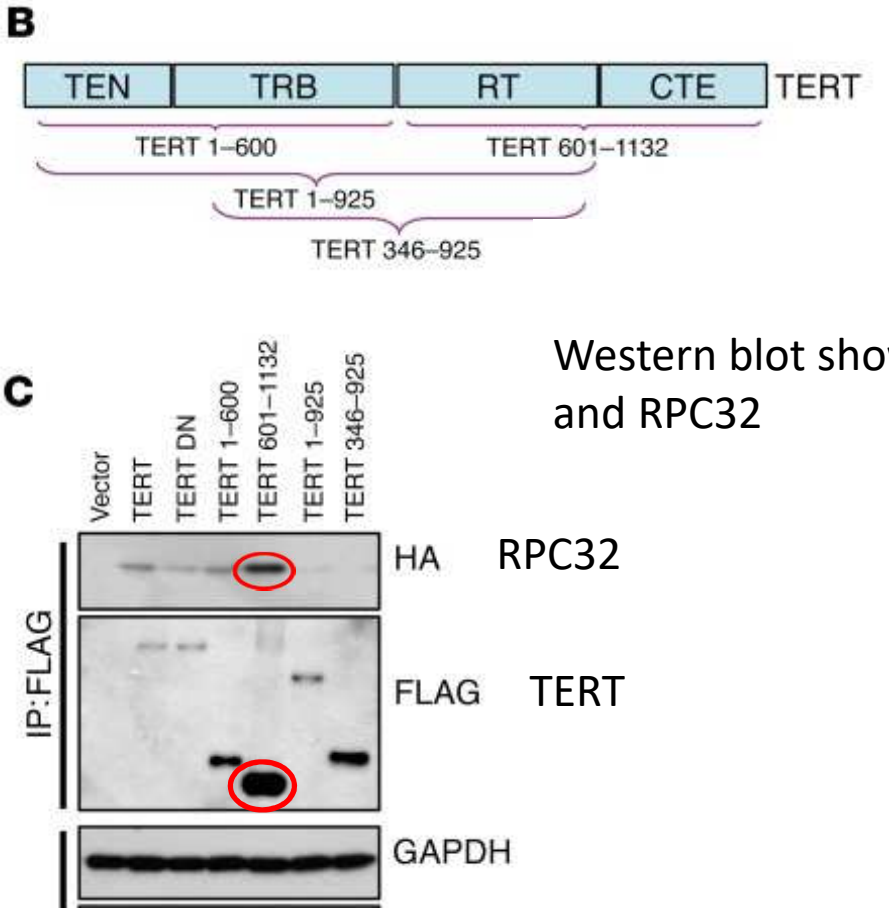
Journal of Structural Biology, Volume 192, Issue 3, 2015, 313–319

TERT deletion construct to investigate interaction of RPC32 with RNA pol III–driven promoters



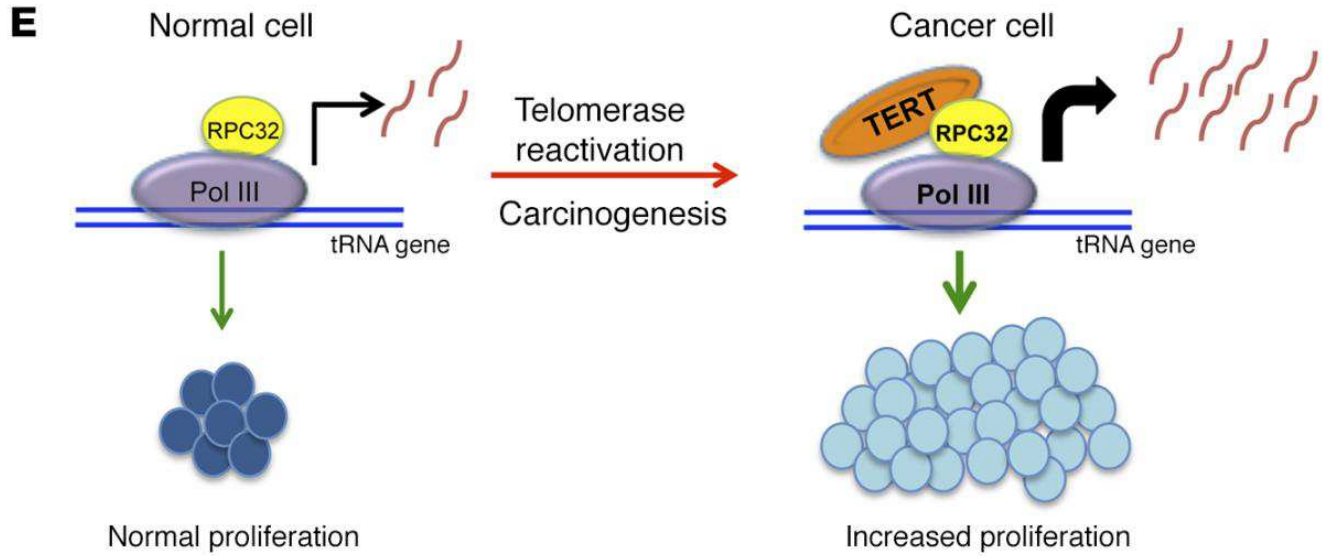
293T cells were transfected with Flag-tagged TERT, TERT DN and its deletion constructs along with HA-tagged RPC32

TERT activates RNA pol III–driven promoters by directly interacting with RPC32



Western blot shows co-IP between TERT and RPC32

TERT associates with the RPC32 subunit of RNA pol III and augments tRNA expression in breast cancer



J Clin Invest. 2016;126(10):4045-4060

Schematic representation of the metastatic cascade,
A) primary tumor formation,
B) endothelial cell (EC) recruitment and Angiogenesis
C) cancer cell migration and invasion into surrounding tissue,
D) cancer cell intravasation and survival within the system circulation,
E) extravasation and colonization of distant organs either as single cells/micrometastases or
F) overt metastatic lesions

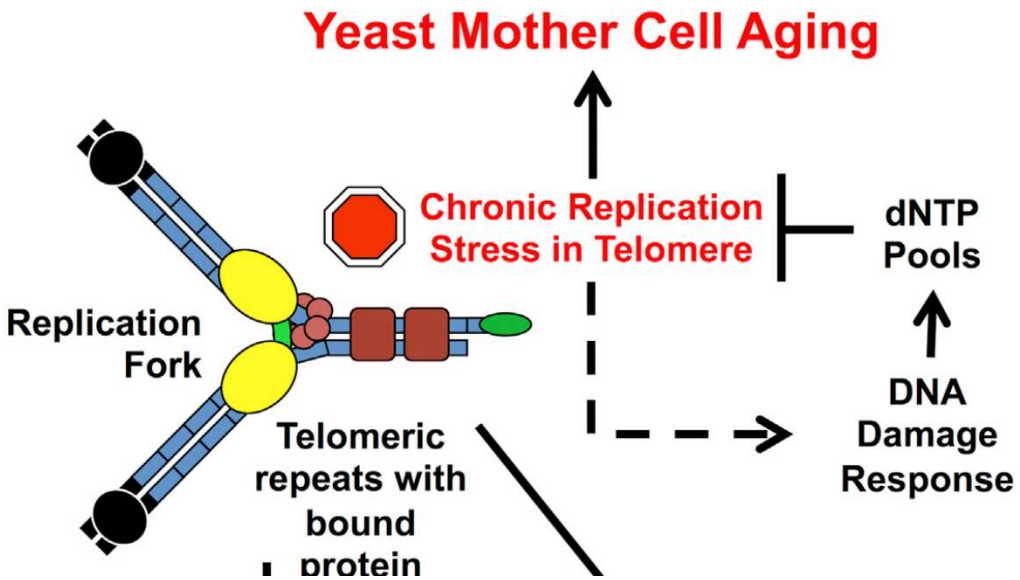
,

Means to the ends: The role of telomeres and telomere processing machinery in metastasis Nathaniel J. Robinson, William P. Schiemann Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 2016 Available online 18 October 2016

Extratelomeric functions of TERT.

Early after telomerase inactivation yeast mother cells show DNA damage response (DDR)

Before critical telomere shortening, telomerase is continuously required to respond to transient DNA replication stress in mother cells



after early telomerase inactivation yeast mother cells show transient DNA damage response (DDR)

before telomere shortening telomerase is continuously required to respond to transient DNA replication stress in mother cells

a lack of telomerase accelerates otherwise normal aging in yeast

TERRA

transcription of telomeric transcripts
TERRA (telomeric repeat-containing RNA)

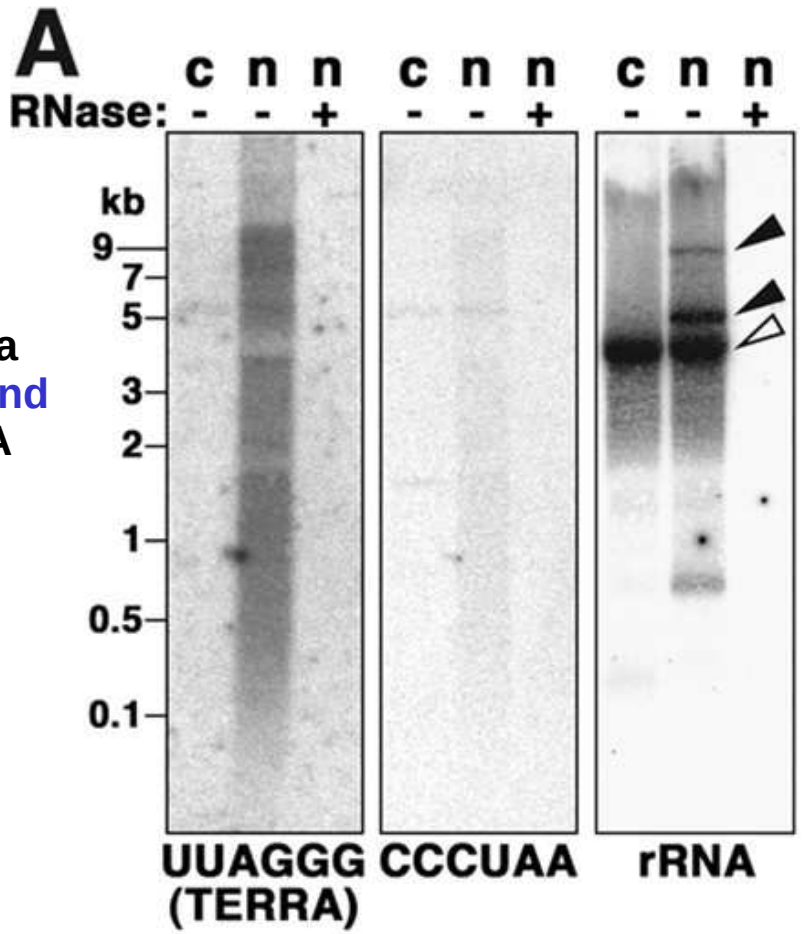
TERRA transcripts are nuclear long non-coding RNAs that are transcribed from the subtelomere towards the telomere.

They are transcribed by RNA polymerase II, giving rise to transcripts that contain UUAGGG-repeats.

They are heterogeneous in size (0.2–10 kb in humans and mice) as indicated by the smear detected in TERRA northern blots.

Identification of TERRA.
(A) obes.

Northern blot analysis of HeLa cytoplasmic (c) and nuclear (n) RNA



Probes:

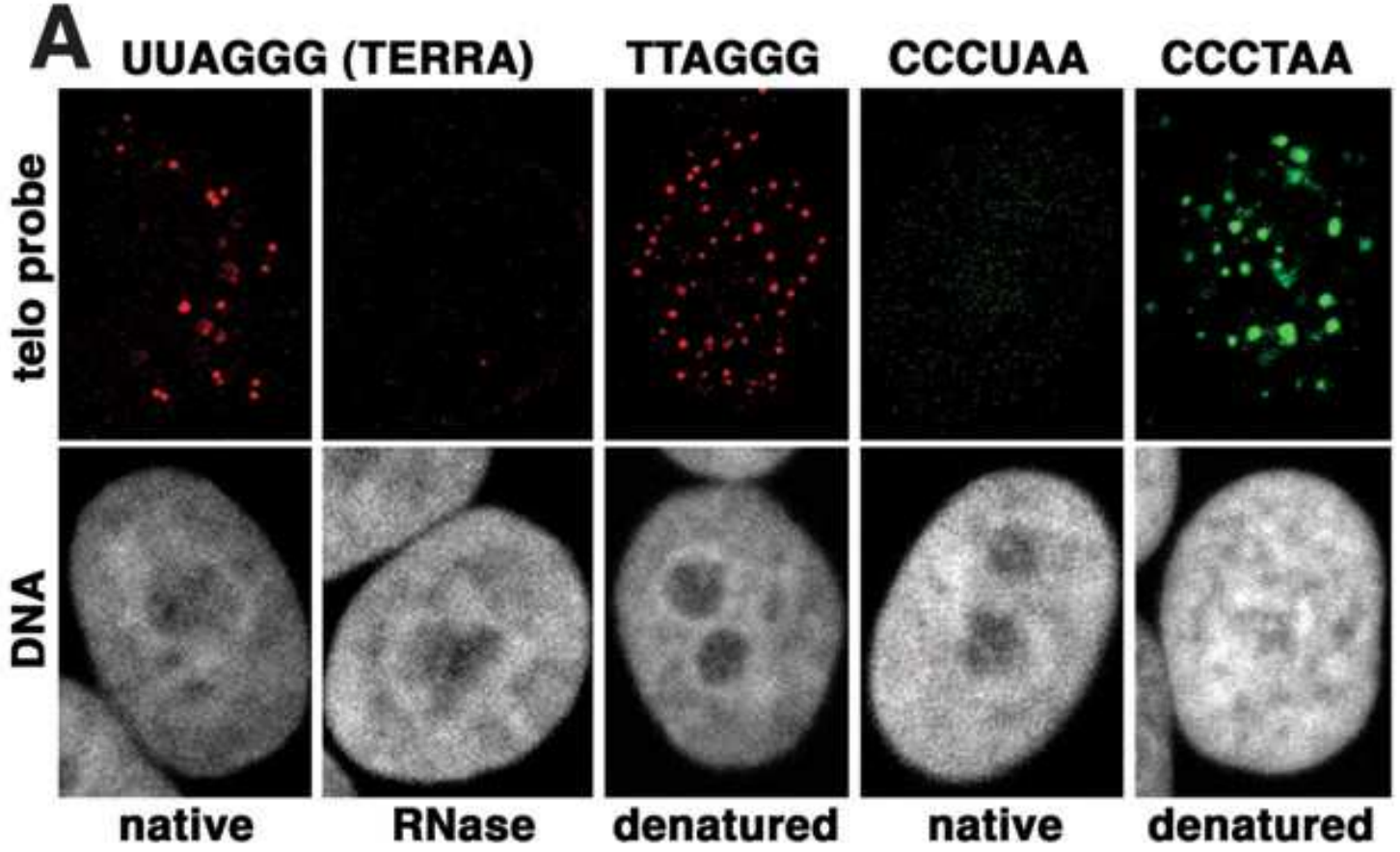
strand-specific telomeric

28S ribosomal RNA (rRNA)

Claus M. Azzalin et al. Science 2007;318:798-801

Fig. 2. Telomeric localization of TERRA. (A) RNA-FISH experiments with strand-specific telomeric DNA probes on HeLa cells.

Red and green signals correspond to the probes detecting CCCTAA-CCCUAA repeats or TTAGGG-UUAGGG repeats

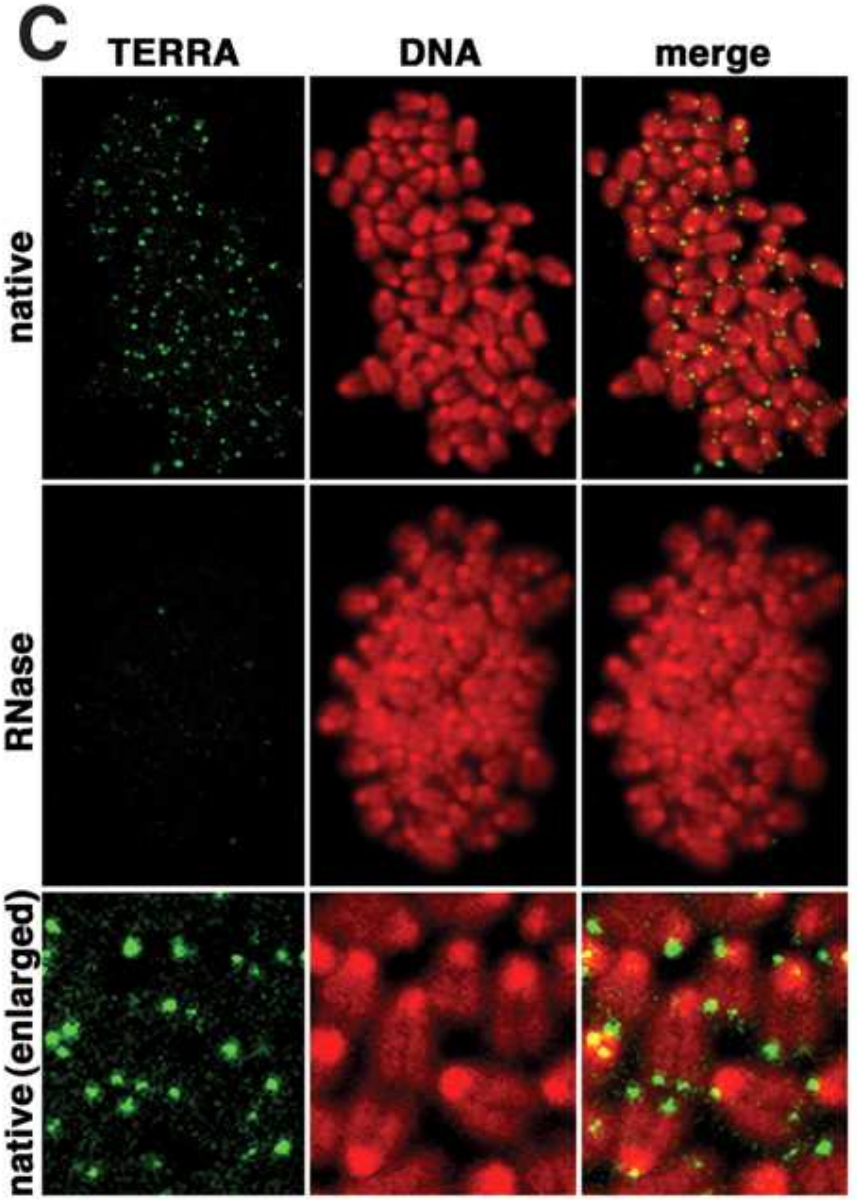


to detect DNA

to detect DNA

Claus M. Azzalin et al. Science 2007;318:798-801

Fig. 2. Telomeric localization of TERRA. (A) RNA-FISH experiments with strand-specific telomeric DNA probes on HeLa cells.



20 to 40 foci

Published by AAAS

Claus M. Azzalin et al. Science 2007;318:798-801

ARTICLE

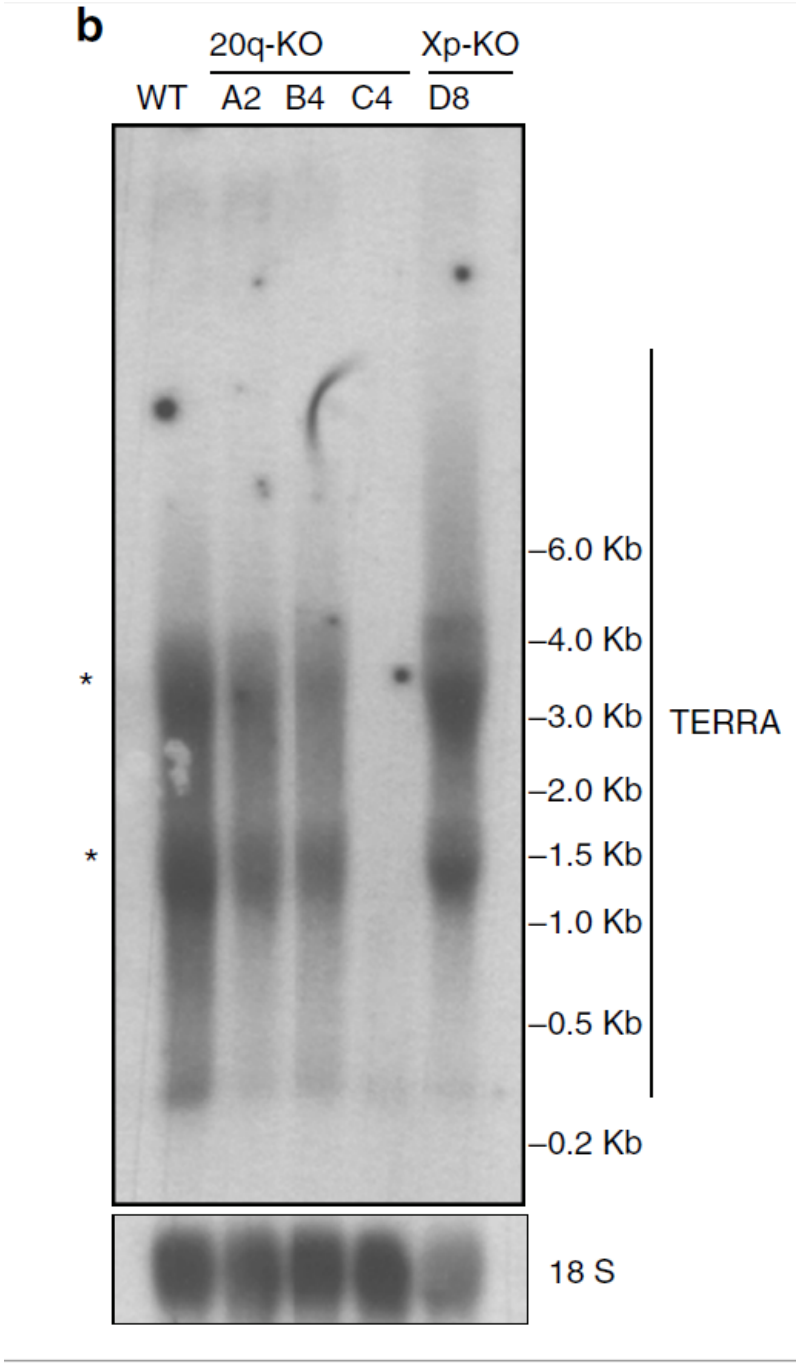
Received 19 Mar 2016 | Accepted 11 Jul 2016 | Published 17 Aug 2016

DOI: 10.1038/ncomms12534

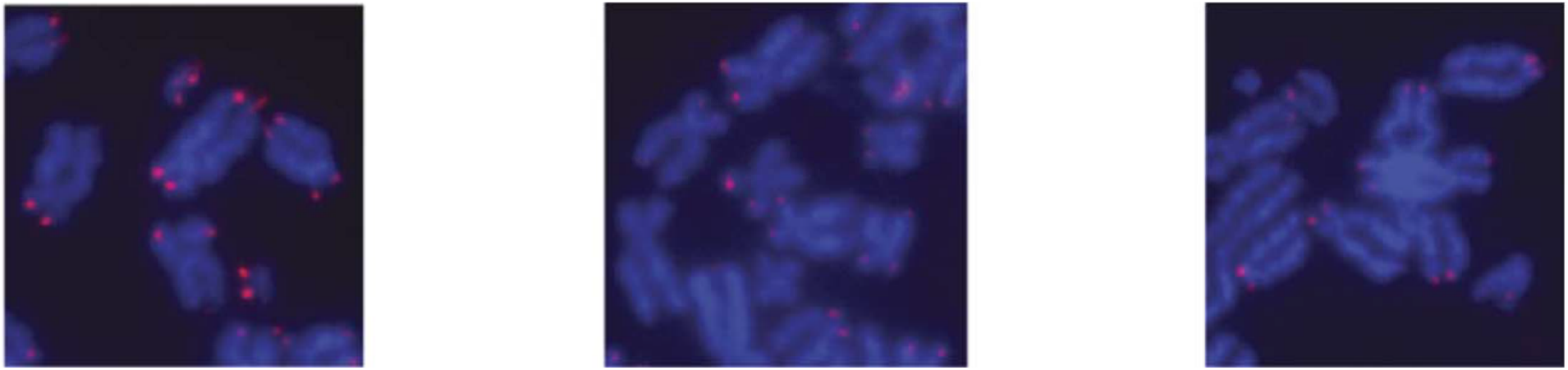
Telomeric RNAs are essential to maintain telomeres

Juan José Montero[1,*], Isabel López de Silanes[1,*], Osvaldo Graña[2] & Maria A. Blasco[1]

Telomeres are transcribed generating long non-coding RNAs known as TERRA. Deciphering the role of TERRA has been one of the unsolved issues of telomere biology in the past decade. This has been, in part, due to lack of knowledge on the TERRA loci, thus preventing functional genetic studies. Here, we describe that long non-coding RNAs with TERRA features are transcribed from the human 20q and Xp subtelomeres. Deletion of the 20q locus by using the CRISPR-Cas9 technology causes a dramatic decrease in TERRA levels, while deletion of the Xp locus does not result in decreased TERRA levels. Strikingly, 20q-TERRA ablation leads to dramatic loss of telomere sequences and the induction of a massive DNA damage response. These findings identify chromosome 20q as a main TERRA locus in human cells and represent the first demonstration in any organism of the essential role of TERRA in the maintenance of telomeres.


b
20q-KO
Xp-KO
WT
A2
B4
C4
D8
–6.0 Kb
–4.0 Kb
–3.0 Kb
–2.0 Kb
–1.5 Kb
–1.0 Kb
–0.5 Kb
–0.2 Kb
TERRA
18 S
*
*

WT KO for the Chr20q-TERRA locus

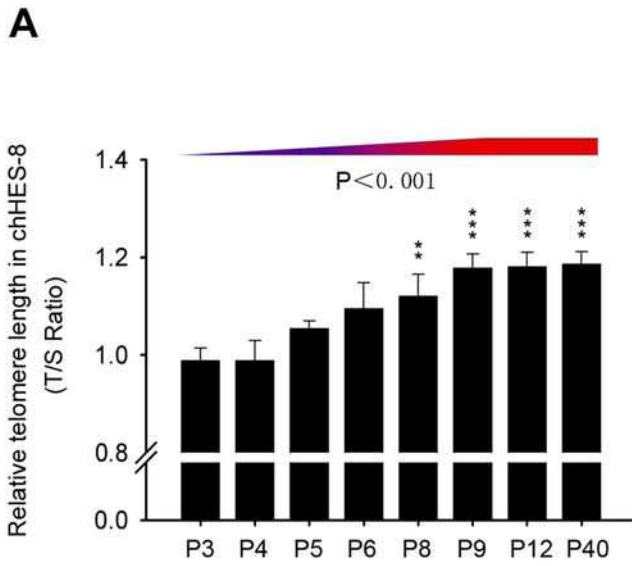


Q-FISH images

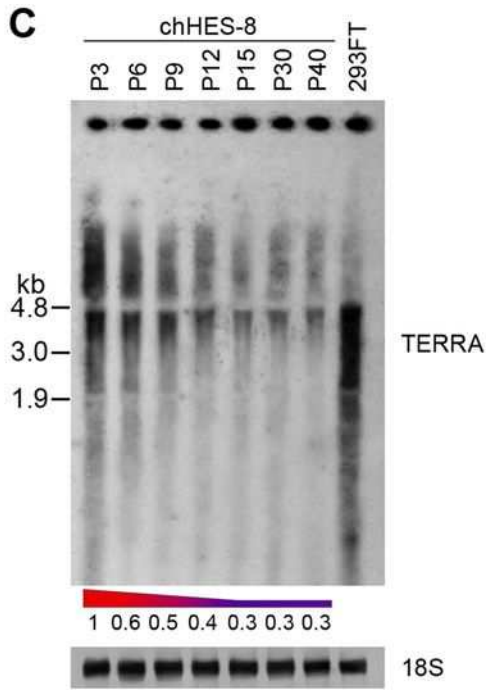
Deletion of the 20q-TERRA locus decreases telomere length

Decrease in TERRA levels was associated with telomere lengthening during the early expansion of hESCs.

human embryonic stem cell



Quantification of telomere length by real-time PCR



Northern blotting analysis of TERRA in total RNA extracted from passages

Sicong Zeng et al. FASEB J 2017;31:4783-4795

The FASEB Journal