

# Telomeres and Telomerase Group

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Scientific Report 2010 **cnio**



Maria A. Blasco

## Group Leader

Maria A. Blasco (Alicante, 1965) obtained her PhD in 1993 for her research at the *Centro de Biología Molecular "Severo Ochoa"* under the supervision of M. Salas. That same year, Blasco joined the Cold Spring Harbor Laboratory in New York (USA) as a Postdoctoral Fellow under the leadership of C. W. Greider. In 1997 she returned to Spain to start her own research Group at the *Centro Nacional de Biotecnología* in Madrid. She joined the CNIO in 2003 as Director of the Molecular Oncology Programme and Leader of the Telomeres and Telomerase Group and was appointed CNIO Vice Director in 2005.

Her major research achievements include: (1) Isolation of the core components of mouse telomerase and generation of the first knockout mouse for telomerase; (2) Generation of the first mouse with increased telomerase expression in adult tissues; (3) The finding that mammalian telomeres and subtelomeres have epigenetic marks characteristic of constitutive heterochromatin; (4) Discovery of telomeric RNAs, which are potent telomerase-inhibitors whose expression is altered in cancer; (5) Demonstration that telomerase activity and telomere length determine the regenerative capacity of adult stem cells; (6) Identification of the longest telomeres as a universal feature of adult stem cell niches; (7) The finding that telomerase over-expression in the context of cancer resistant-mice improves organismal fitness, produces a systemic delay in ageing and an extension in median life-span; (8) Discovery that telomeres rejuvenate after nuclear reprogramming; (9) Identification of the molecular mechanisms by which short telomeres/DNA damage limit nuclear reprogramming of defective cells; (10) Discovery that telomeric protein TRF1 can act as both a tumour suppressor and as a factor in ageing prevention.

Blasco has received the Josef Steiner Cancer Research, *Rey Jaime I*, Körber European Science, *Alberto Sols* and *Fundación Lilly* Preclinical Research, Awards. She has also been the recipient of the Spanish National "*Santiago Ramón y Cajal*" Research Award in Biology (2010). Blasco has also been awarded the EMBO Gold Medal and has served on its Council since 2008.

## Summary

We study the mechanisms by which tumour cells are immortal and normal cells, mortal. The immortality of cancer cells is one of their most universal characteristics, which is sustained by telomerase activity. The enzyme telomerase is present in more than 95% of all types of human cancers and not present in normal cells in the body. Telomeres are nucleoprotein complexes at the ends of chromosomes that are essential for chromosome protection and genomic stability. One of the many factors leading to ageing is the progressive shortening of telomeres associated with organism ageing. When telomeres are altered (in their length or their integrity) adult stem cells have an impaired regenerative capacity.

Telomere length defects are associated to cancer and ageing processes and have a profound effect on stem cell behaviour. We aim to determine the role of genetic and epigenetic telomere regulators in cancer and ageing by generating new mouse models and studying the role of these factors in stem cell biology.

## Strategic Goals

- Study the biology of telomeres and telomerase: generate mouse models to study the role of telomeres and telomerase in cancer and ageing
- Assess the interplay between telomeres and DNA repair pathways
- Characterise telomeric heterochromatin
- Elucidate the role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to induced Pluripotent Stem (iPS) cells



**Staff scientists:** Isabel López de Silanes, Rosa M. Marión, Paula Martínez, Agueda M. Tejera and Elisa Varela. **Post-doctoral fellows:** Fabian Beier (since March), Bruno M. Bernardes, Luigia de Bonis, J. Alejandro Palacios, Martina Stagno and Gerdine J. Stout (until October). **Graduate students:** María García, Ianire Garrobo (since August), Ralph Schneider, Antonia Tomás (until February) and Elsa Vera. **Technicians:** Oscar Aparicio (until September), Mercedes Gallardo and Rosa M. Serrano.

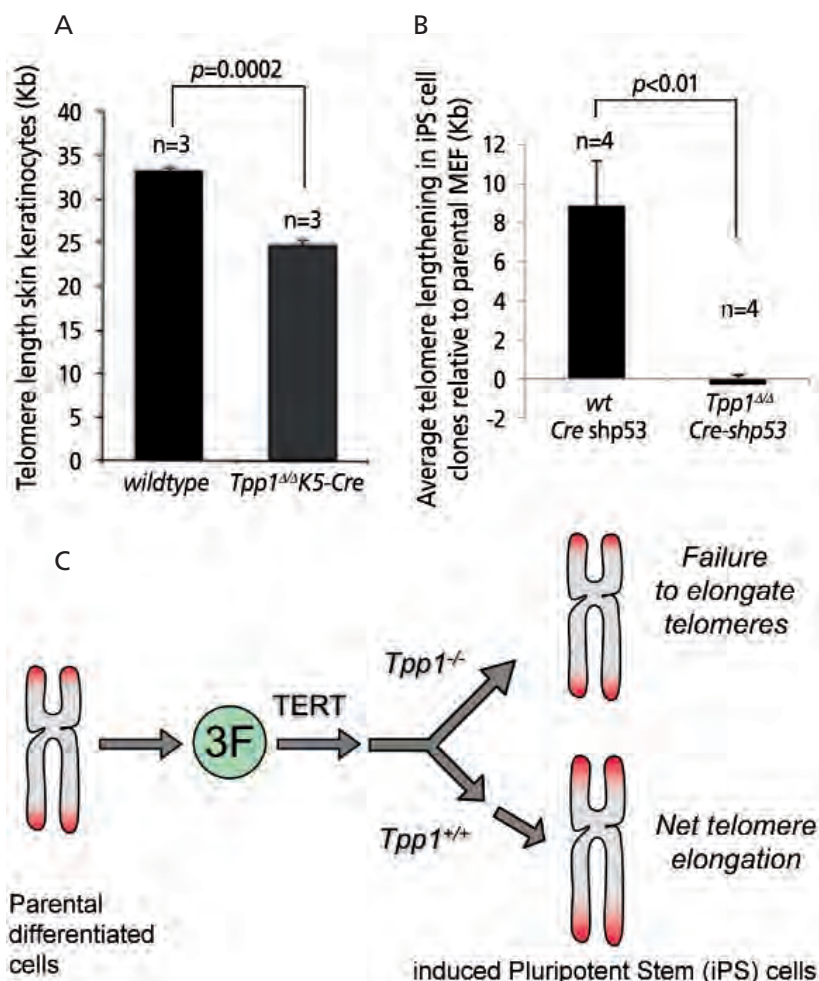
## Highlights

### The roles of TPP1 in TERT recruitment, telomere elongation and skin development

TPP1 is part of the shelterin complex bound to mammalian telomeres. Previous studies suggested that TPP1 is important for telomerase regulation at the ends of chromosomes as well as the binding of telomeric proteins TRF1 and TRF2 at telomeric repeats. Further understanding of the role of TPP1 in telomere regulation *in vivo* and in mouse development and disease had not been achieved due to the lack of mouse models with complete TPP1 abrogation.

We generated *Tpp1*-deficient mouse embryonic fibroblasts (MEFs) as well as mice with targeted *Tpp1* deletion to stratified epithelia. Both MEFs and mice deleted for *Tpp1* show induction of telomere damage and cell-cycle arrest, demonstrating that TPP1 protects telomeres from eliciting a DNA damage response (DDR). *Tpp1* null mice die perinatally and show severe skin hyperpigmentation and defective hair follicle morphogenesis. *Tpp1* deletion results in decreased TERT (the telomerase catalytic subunit) binding to telomeres and accelerated telomere shortening both in MEFs and mice (Figure 1).

These results showed that TPP1 is the essential element connecting telomerase with telomeres. In the absence of TPP1 telomerase is no longer able to efficiently bind and elongate telomeres and, as a consequence, stem cells are not able to



**Figure 1:** TPP1 is required for telomere maintenance *in vivo* and for net telomere elongation during nuclear reprogramming. (A) Telomere shortening in skin keratinocytes from *Tpp1*<sup>ΔΔ</sup>K5-Cre newborn mice. Mean telomere length (kb) and standard error values are shown per genotype. n, independent mice used for the analysis. The Student's t test was used for statistical calculations; p values are indicated. (B) Average lengthening of iPS clones when compared to their corresponding parental MEF, expressed in Kb. *Tpp1*<sup>+/+</sup> iPS clones show a net elongation of telomeres that is abolished in iPS cells derived from *Tpp1*<sup>ΔΔ</sup> MEFs. n, total number of iPS clones analysed. Statistical significance was determined by Student's t test. (C) While nuclear reprogramming of parental differentiated TPP1-proficient cells results in net telomere elongation, reprogramming of TPP1-deficient cells fails to elongate telomeres.

regenerate tissues and the mice develop degenerative pathologies. The discovery that TPP1 is essential for the "anti-ageing" activity of telomerase was confirmed using nuclear reprogramming experiments.

Nuclear reprogramming to induced pluripotent stem (iPS) cells from differentiated cells involves telomere elongation by telomerase. TPP1-deficient iPS cells are not able to elongate telomeres (Figure 1), indicating that TPP1 is essential for telomere elongation *in vivo*. Our results suggest a telomere-capping model whereby TPP1 not only prevents the induction of a DNA damage response at telomeres by preventing fusions and telomere breakage but is also required for telomere elongation by telomerase.

### The roles of Rap1 in telomere function and gene expression

Rap1 is a component of the shelterin complex at mammalian telomeres. Human hRap1 was identified by homology with budding-yeast scRap1. scRap1 binds to telomeres and controls telomere length and subtelomeric silencing. In addition, scRap1 acts as a transcriptional regulator controlling the expression of glycolytic enzymes and ribosomal genes. Human hRap1 is recruited to the telomeres by TRF2 and its overexpression in cells leads to continuous telomere lengthening. The roles of hRap1 in transcriptional regulation and subtelomeric (S) gene silencing are unknown.

We studied the *in vivo* role of mouse Rap1 in telomere biology and its impact on gene expression programmes and organismal viability by generating cells and mice conditionally deleted for Rap1 in a tissue-specific manner. We found that mammalian *Rap1* is dispensable for telomere capping but it prevents telomere recombination and fragility. In contrast to analogous mouse models for TRF1 and TPP1 deficiency, which have 100% perinatal mortality, targeted *Rap1* deletion to stratified epithelia does not affect mouse viability but these mice show shorter telomeres and develop premature hyperpigmentation of the skin.

The exclusive location of shelterins at the ends of chromosomes has remained undisputed. We have now revealed that Rap1 is not only present at the telomeres but also all along the arms of chromosomes.

This finding has been made possible thanks to ChIP-Seq technology which allows for the genome-wide determination of *in vivo* Rap1 binding sites in chromatin.

We found that Rap1 binds to both telomeric and extratelomeric sites through the (TTAGGG)<sub>2</sub> consensus motif (Figure 2) which is present at the promoter regions of some genes as well as in inter-genomic regions. Our studies have demonstrated that Rap1 is not only an atypical shelterin protein because of its presence in parts of the chromosome other than the telomeres, but also, the absence of Rap1 results in changes in the expression patterns of genes involved in cancer, cell adhesion and metabolism, without affecting telomere capping.

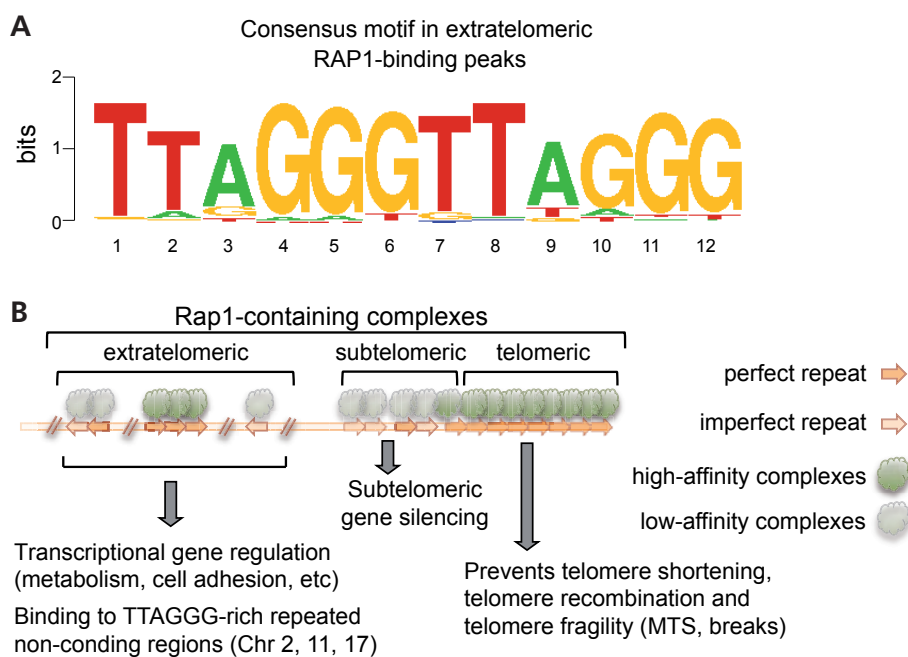
Our findings place a telomere protein at the interface between telomere function and transcriptional regulation (Figure 2), bringing the complex subject of shelterins a step closer to gene transcriptional regulation and opening avenues for research on the relationship between Rap1-dependent transcriptional programmes and the cancer and ageing processes.

### Telomeric RNAs are bound by a complex array of RNA-binding proteins

Despite the long established belief that telomeres are transcriptionally inactive because of their compact and protective structure, telomeres are transcribed by the DNA-dependent RNA polymerase II giving rise to UUAGGG repeat-containing telomeric transcripts known as TERRA.

TERRA are novel structural components of telomeres; they are heterogeneous in size and their abundance is modulated by a number of biological processes including telomere length and telomeric chromatin status, cellular stresses, as well as during nuclear reprogramming, ontogenesis and cancer. Importantly, TERRA act as potent inhibitors of telomerase activity *in vitro* and are likely to negatively-regulate telomerase activity *in vivo*. However, the molecular mechanisms and factors that control TERRA levels are still largely unknown.

Using biotin pull-down assays followed by LC-MALDI TOF/TOF mass spectrometry we have identified a set of RNA-binding



**Figure 2:** *De novo* identification of a consensus Rap1-binding site. (A) Rap1 binding matrix predicted by the Weeder algorithm represented as a sequence logo. (B) Summary of results. Rap1 binds to both telomeric and non-telomeric chromatin, thereby exerting a dual role in telomere function (control of telomere length and prevention of telomere fragility and recombination) as well as transcriptional gene regulation, including S-gene silencing. Rap1 also binds to non-coding regions enriched in TTAGGG repeats, where it may also have a role in preventing fragility and recombination.

proteins which endogenously bind and regulate TERRA in the context of primary mouse embryonic fibroblasts. Some of the TERRA-bound identified proteins had previously been proposed to act as molecular bridges or linkers to recruit telomerase to the telomeres.

We have now found that these proteins affect the levels and cellular localisation of TERRA as well as the ability of telomerase to extend telomeres. A model in which telomeres are subjected to additional levels of regulation with some of the TERRA-associated proteins having roles in TERRA stability, telomere protection and telomere length regulation can now be proposed. Our findings anticipate an impact of TERRA-associated ribonucleoproteins on telomere biology and telomere diseases such as cancer and ageing.

## Publications

Martinez P, Thanasoula M, Carlos Ana R, Gómez-Lopez G, Tejera AM, Schoeftner S, Dominguez O, Pisano DG, Tarsounas M, Blasco MA (2010). Mammalian Rap1 controls telomere function and gene expression through binding to telomeric and extratelomeric sites. *Nat Cell Biol* 12, 768-780.

*News & Views commentary for this article:*

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Tejera AM, Stagno d'Alcontres M, Thanasoula M, Marión RM, Martínez P, Liao C, Flores JM, Tarsounas M, Blasco MA (2010). TPP1 is required for TERRA recruitment, telomere elongation during nuclear reprogramming, and normal skin development in mice. *Dev Cell* 18, 775-789.

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Varela E, Blasco MA (2010). 2009 nobel prize in physiology or medicine: telomeres and telomerase. *Oncogene* 29, 1561-1565.

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Flores I, Blasco MA (2010). The role of telomeres and telomerase in stem cell aging. *Febs Lett* 584, 3826-3830.

López de Silanes I, Stagno d'Alcontres M, Blasco MA (2010). TERRA transcripts are bound by a complex array of RNA-binding proteins. *Nat Commun* 1, 1-9.

Blagosklonny MV, et al. (2010). Impact papers on aging in 2009. *Aging (Albany)* 2, 111-121.

## Awards and Recognition

"Santiago Ramón y Cajal" National Research Award in Biology 2010, Spain

Lilly Foundation Biomedical Research Award 2010 for best preclinical research, Spain

Member, *Agenda Ciudadana de Ciencia e Innovación* Expert Panel, Spain

Member, Scientific Advisory Board, *Fundación Príncipes de Girona*, Spain

Editorial Board Member, *Aging & Disease*