

# Telomeres and Telomerase *Group*

## Summary

Telomeres are nucleoprotein complexes at the ends of chromosomes that are essential for chromosome protection and genomic stability. Telomeric chromatin is enriched in epigenetic marks characteristic of constitutive heterochromatin, which act as negative regulators of telomere length.

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 cells

## Maria A. Blasco *Group Leader*



Maria Blasco, born in Alicante in 1965, obtained her PhD in 1993 for research on DNA polymerases at the *Centro de Biología Molecular* under the supervision of M. Salas. That same year, Blasco joined the Cold Spring Harbor Laboratory, New York, USA, as a Postdoctoral Fellow under the leadership of C. W. Greider. During this time, Blasco cloned one of the mammalian telomerase genes and generated the first telomerase knockout mouse.

In 1997 she returned to Spain to start her own research Group at the *Centro Nacional de Biotecnología* in Madrid, where she continued her work on the development of mouse models for the study of the role of telomerase in cancer and ageing. She joined the CNIO in 2003 as Director of the Molecular Oncology Programme and Leader of the Telomeres and Telomerase Group.

Blasco has received the Swiss Bridge Award for Research in Cancer, the Josef Steiner Cancer Research Award, the EMBO Gold Medal, the *Fundación Carmen and Severo Ochoa* Award for Molecular Biology, the *Rey Jaime I* Basic Research Award, the Körber European Science Award, and the *Alberto Sols* Biomedical Research Award. She also serves on the Editorial Board of several scientific journals and is an elected EMBO Member, a Young Global Leader (the World Economic Forum), and a Member of the *Academia Europaea*.

Maria belongs to the Faculty 1000 (stem cells and regeneration) and was appointed to the EMBO Council in 2008. She has authored more than 150 papers and made major contributions to the field of telomeres and telomerase and their role in ageing, cancer, and the reprogramming of differentiated cells.



**Staff scientists:** Ignacio Flores (until June), Isabel López de Silanes, Rosa M. Marión, Paula Martínez, Elisa Varela (since February). **Post-doctoral fellows:** Bruno M. Bernardes (since April), Maria Luigia de Bonis (since October), Carolyn J. Mcnees (until October), J. Alejandro Palacios, Stefan Schöftner (until July), Martina Stagno D'Alcontres, Gerdine J. Stout, Katerina Strati (until June), Alessandra Strom (until June), Agueda M. Tejera. **Graduate students:** Raquel Blanco (until September), María García (since June), Ralph P. Schneider, Irene Siegl-Cachedenier (until February), Antonia Tomás, Elsa Vera. **Technicians:** Oscar Aparicio, Rosa M. Serrano.

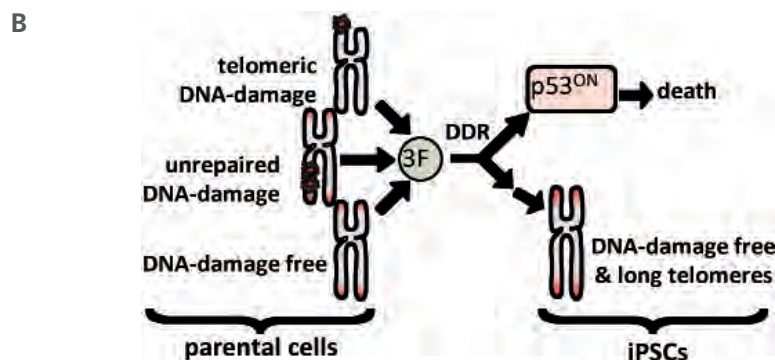
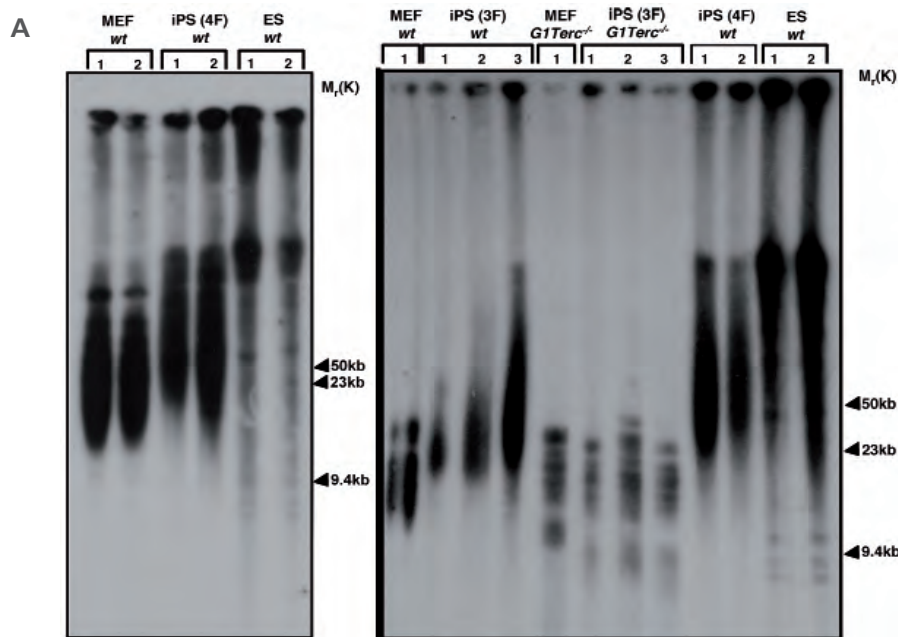
## Highlights

### Telomeres are rejuvenated in induced pluripotent stem (iPS) cells

Generation of iPS cells from differentiated cells can be achieved by over-expression of four transcription factors, namely Oct4, Sox2, Klf4 and c-Myc.

Telomeres shorten with increasing age and contribute to organismal ageing by limiting the proliferative capacity of adult stem cells.

We showed that telomeres are efficiently elongated in iPS cells compared to the parental differentiated cells from both young and aged individuals, demonstrating that telomeres efficiently rejuvenate during nuclear reprogramming (Figure 1). Telomere elongation is usually mediated by telomerase and iPS telomeres acquire the epigenetic marks of embryonic stem (ES) cells. Cells with short telomeres cannot be reprogrammed despite their normal proliferation rates; a minimum telomere length is necessary for iPS cell generation. Cells derived from telomerase-deficient mice failed to elongate telomeres, and telomeres continued to shorten during iPS cell generation. This resulted in the inability of these cells to form chimeras, highlighting the importance of telomerase activation during reprogramming for the quality of the resulting iPS cells. The observed



**Figure 1:** Reprogramming of mouse differentiated cells to iPS cells. (A) Telomerase-dependent telomere elongation. Telomeres are longer in wild-type 3F and 4F iPS cells than in parental MEF. *G1 Terc<sup>-/-</sup>* iPS cells show shorter telomeres than parental MEF. Two to three independent cell cultures per cell type. (B) p53 is a main barrier to reprogramming of cells with increased DNA damage by preventing that they become iPS cells.

dramatic decrease in reprogramming efficiency of cells derived from increasing generations of telomerase-deficient mice can be restored by telomerase reintroduction, indicating that accumulation of critically short telomeres is effectively limiting reprogramming.

### **p53 limits reprogramming to ensure iPS cell genomic integrity**

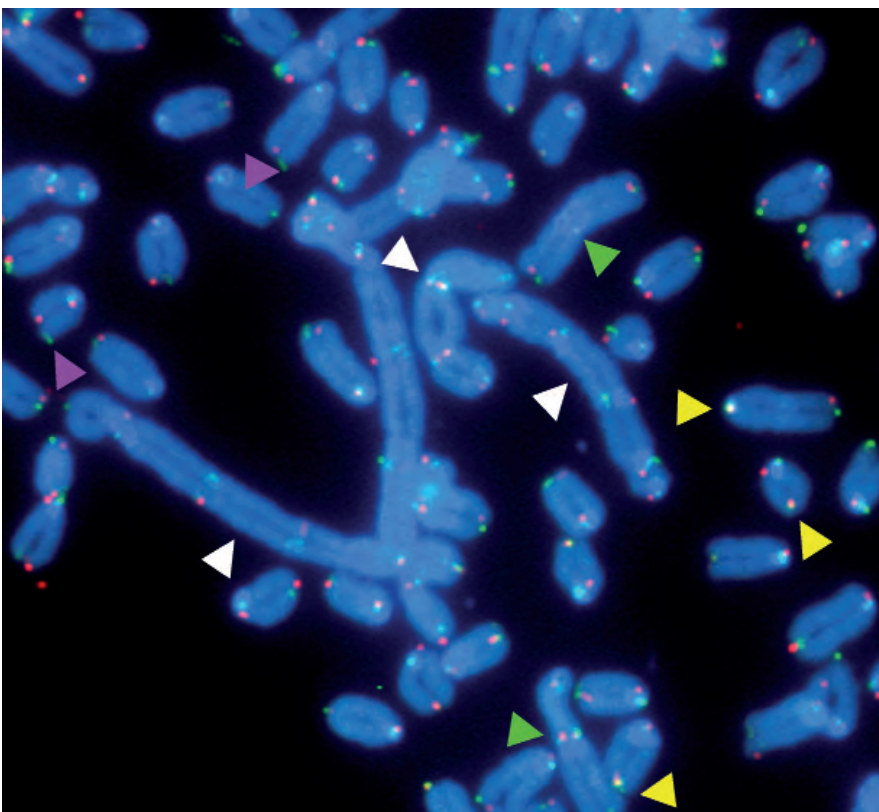
Our data support the existence of “reprogramming barriers” that abort the reprogramming of sub-optimal cells with uncapped telomeres. An explanation for the low efficiency of reprogramming might relate to the presence of DNA damage in cells undergoing reprogramming. We showed that p53 is critical in preventing the reprogramming of cells carrying various types of DNA damage: short telomeres, DNA repair deficiencies or exogenously-inflicted DNA damage (irradiated cells). Reprogramming in the presence of pre-existing – yet tolerated – DNA damage

is aborted by the activation of a DNA damage response and p53-dependent apoptosis. Abrogation of p53 allows efficient reprogramming in the face of DNA damage and the generation of iPS cells carrying persistent DNA damage and chromosomal aberrations. Thus, during reprogramming, cells increase their intolerance to different types of DNA damage and p53 is critical in preventing the generation of iPS cells from suboptimal parental cells. Interestingly, these results indicate that p53 is critical to control the spreading of damaged cells in both reprogramming and malignant transformation (Figure 2).

### **Impact of TRF1 deficiency in degenerative pathologies and tumorigenesis**

To address the role of TRF1 in the context of a mammalian organism we generated tissue-specific TRF1 conditionally deleted cells and mice. TRF1-deleted mouse embryonic fibroblasts show rapid induction of senescence, abundant telomeric  $\gamma$ -H2AX foci, phosphorylation of checkpoint kinases CHK1 and CHK2, as well as cell cycle arrest. TRF1-depleted telomeres are breakage-prone, show abundant telomere fusions and multitelomeric signals (Figure 2). TRF1-deleted mice die perinatally and show an early onset of degenerative pathologies associated with induction of telomere-instigated DNA damage, activation of the p53/p21 and p16 pathways, and cell cycle arrest *in vivo*.

Abrogation of p53, rescued mouse survival, hair growth defects, and skin hyperpigmentation; it also led to epithelial abnormalities associated with human telomere-related pathologies including increased cancer. These results suggest that dysfunction of a telomere binding protein is enough to produce severe telomeric damage and loss of telomere capping in the absence of telomere shortening, resulting in premature tissue degeneration, acquisition of chromosomal aberrations and development of neoplastic lesions. This is the first mouse model for a telomere protein leading to increased



**Figure 2:** TRF1 deletion induces chromosomal aberrations. Representative image of metaphase spreads from TRF1<sup>-/-</sup>-LT-Cre MEF. Examples of chromosome concatenation (white arrowheads), dicentric chromosomes (green arrow heads), chromatid fusions (yellow arrowheads), and multitelomeric signals (purple arrowheads) are indicated.

cancer and degenerative phenotypes in the absence of telomere shortening and any generational lag.

### Short telomeres are a main source of damage leading to organismal ageing

We have used telomerase-deficient TRF2-overexpressing mice to provide evidence that progressive telomere shortening is linked to global deregulation of the mammalian transcriptome and loss of maintenance of epigenetic silencing mechanisms, exemplified by the re-expression of an Xi-linked transgene upon telomere shortening. Indicative of induction of a stress response, we found down-regulation of genes promoting cell cycle progression and up-regulation of the mTOR and Akt survival pathways. Strikingly, cells with critically short telomeres showed down-regulation of

various DNA repair pathways.

Our findings suggest that progressive telomere shortening and the accumulation of dysfunctional telomeres with age may constitute a unique source of DNA damage sufficient to induce global alterations in genome regulation.

## Publications

Li H, Collado M, Villasante A, Strati K, Ortega S, Cañamero M, Blasco MA, Serrano M (2009). The *Ink4/Arf* locus is a barrier for iPS cell reprogramming. *Nature* 460, 1136-1139.

Marión RM, Strati K, Li H, Murga M, Blanco R, Ortega S, Fernández-Capetillo O, Serrano M, Blasco MA (2009). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* 460, 1149-1153.

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Ferrón SR, Marqués-Torrejón MA, Mira H, Flores I, Taylor K, Blasco MA, Fariñas I (2009). Telomere shortening in neural stem cells disrupts neuronal differentiation and neurogenesis. *J Neurosci* 29, 14394-14407.

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Muñoz P, Blanco R, de Carcer G, Schoeftner S, Benetti R, Flores JM, Malumbres M, Blasco MA (2009). TRF1 controls telomere length and mitotic fidelity in epithelial homeostasis. *Mol Cell Biol* 29, 1608-1620.

Flores I, Blasco MA (2009). A p53-dependent response limits epidermal stem cell functionality and organismal size in mice with short telomeres. *PLoS ONE* 4, e4934.

García-Lavandeira M, Quereda V, Flores I, Saez C, Díaz-Rodríguez E, Japon MA, Ryan AK, Blasco MA, Dieguez C, Malumbres M, Alvarez CV (2009). A GRFa2/Prop1/stem (GPS) cell niche in the pituitary. *PLoS ONE* 4, e4815.

Stout GJ, Blasco MA (2009). Genetic dissection of the mechanisms underlying telomere-associated diseases: impact of the TRF2 telomeric protein on mouse epidermal stem cells. *Dis Model Mech* 2, 139-156.

## Awards and Recognition

Alberto Sols Award for Excellence in Research, Spain

Member, Scientific Advisory Board, Spanish National Research Council (Consejo Superior de Investigaciones Científicas – CSIC), Spain

Senior Editor, *Cancer Research*

Editorial Board Member, *Stem Cell Reviews and Reports*

Editorial Advisory Board Member, *Epigenomics*