

## FANCD2-mediated Regulation of PARP Activity Confers Telomere Maintenance

A. Lyakhovich<sup>1,2</sup>, A. Castellanos<sup>1,2</sup>, M. José.Ramírez<sup>1,2</sup>, M. Castellà<sup>1,2</sup>, A.M. Simons<sup>3</sup>, S. Smith<sup>4</sup>, J.D. Parvin<sup>3</sup>, J. Surrallés<sup>1,2</sup>

<sup>1</sup>Universitat Autònoma de Barcelona, Barcelona, Spain; <sup>2</sup>Centre for Biomedical Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Spain; <sup>3</sup>Department of Pathology, Harvard Medical School and Brigham and Women's Hospital, Boston, MA; <sup>4</sup>Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY

**Objectives:** We have recently reported the involvement of PARP family member Tankyrase-1 into telomere maintenance mediated by FANCD2. Here we study whether FANCD2 suppression of TRF1 polyADP-ribosylation (PARsylation) may deprotect telomere DNA leading to telomere elongation by recombination.

**Methods:** We utilized *in vitro* models to show FANCD2-mediated suppression of PARP1- or TRF1- PARsylation. We then performed immunostaining analyses to compare Tankyrase-1 and TRF1 expression in FA cells with different genetic backgrounds. In order to monitor the disturbance of TRF1:telomere DNA complexes in FANCD2 deficient and proficient cells, we designed a ChIP-footprint assay and studied TRF1-dependent deprotection of telomere DNA. We then determined expression of PARP1/2, TRF1/2, Tankyrase1/2 and Caspase-3 by Western blotting and applied colorimetric assay to monitor caspase-3 enzyme activity in a panel of FA cells. We also applied Q- and Flow-FISH to measure telomere length upon FANCD2 RNAi depletion. We then studied recombination events by 2D Southern.

**Results:** We were able to show FANCD2-dependent inhibition of PARP family members PARP1 and Tankyrase-1 *in vitro* and proved that FANCD2 affects PARP activity in living cells. We demonstrated that FANCD2 deficient cells have an abnormal cleavage of PARP1 mediated by caspase-3 activity. Moreover, absence of FANCD2 deprotects telomere DNA by decreasing the level of TRF1-bound DNA, although signals from telomere DNA in FANCD2<sup>-/-</sup> cells were stronger than in FANCD2<sup>-/-</sup> corrected ones. This fact could be due to the differences in telomere lengths. Indeed, we demonstrated telomere lengthening in FANCD2 deficient or depleted cells. When PARP inhibitor was added, we recognized equal signals of telomere DNA and similar degrees of telomere DNA protection in both cell types suggesting that FANCD2 somehow mimics the effect of PARP inhibition. Since telomere elongation upon FANCD2 depletion was also observed in the ALT-cells having alternative telomere maintenance mechanism, we assumed that it was caused by recombination. Since one of the evidences of recombination is presence of extrachromosomal structures, we then compared 2D Southern blots probed for telomere DNA in FANCD2 deficient and corrected cells.

**Conclusions:** These data suggest that FANCD2 suppression of PARP enzyme Tankyrase-1 leads to deprotection of telomeric DNA by disturbing its binding with TRF1. This led us to the model where cells deficient in FANCD2 have activated caspase-3 level leading to extensive cleavage of PARP and consecutive activation of Tankyrase-1. In turn, increased TRF1 PARsylation results in deprotection of telomere DNA following telomere elongation caused by recombination. Consistent with this view, in normal cells, FANCD2 modulates PARP activity and keeps Tankyrase1-mediated PARsylation of TRF1 in balance thus allowing efficient protection of telomere DNA.

*Lyakhovich (continued)*

**Translational Applicability:** This new model of FANCD2-mediated PARP inhibition opens important issue for searching new targets for treatment of FA and better understanding biochemical pathways that may lead to FA development.