

## The Fanconi Anemia Pathway Plays a Critical Role in Recombinational Telomere Maintenance in ALT-immortalized Human Cells

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**Background:** Fanconi anemia (FA) proteins are implicated in genetic recombination, a process that plays key roles in DNA repair and in telomere maintenance by Alternative Lengthening of Telomeres (ALT) pathways. ALT telomere replication and recombination are thought to occur in sub-nuclear PML bodies.

We find that FANCD2, FANCA, and FANCG localize to telomeric foci and PML bodies in ALT, but not in telomerase-positive or primary human cells. Co-IP experiments indicate ALT-specific *in vivo* interactions between FANCD2, the BLM DNA helicase, and the telomeric protein TRF2. FANCD2 localization to ALT telomeric foci is independent of ATM or ATR, but requires BLM expression and monoubiquitination by the FA core complex.

**Objective:** To understand the function of the FA pathway in ALT telomere replication and recombination.

**Methods:** RNA interference is used to assess the effects of FA protein depletion on the behavior of ALT and telomerase-positive human cells in culture.

**Results:** Depletion of FANCD2 leads to ALT-specific increases in telomere DNA synthesis, telomere entanglements, severely altered nuclear morphology and cell death. The PML bodies of FANCD2-depleted ALT cells contain unusually high amounts of DNA repair factors (RAD51, RPA, BLM, MRE11), telomere binding proteins and telomeric DNA. Analysis of interphase FANCD2-depleted ALT cells show striking increases in extrachromosomal telomeric DNA, a putative recombination byproduct, as well as frequent telomeric DNA fibers running between PML bodies and telomeric foci. Analysis of prometaphase and metaphase ALT cells support increased telomere and chromosomal entanglements after FANCD2 depletion. Interestingly, mitotic spreads not only contain simple telomeric fusions typical of telomere uncapping, but also show complex abnormalities including chromosome clumping, rereplicated chromosomes, and loss of centromere cohesion. Abnormally shaped and sized nuclei with large holes, bridging, giant nuclei, micronuclei, and multi-lobed nuclei appear 3-5 days after FANCD2 depletion in ALT cells. The above abnormalities occur at only low levels in telomerase-positive cells and can be partially suppressed by expression of telomerase in VA13 ALT cells.

**Conclusions:** We hypothesize that the telomere entanglements and/or fusions that occur following depletion of FANCD2 in ALT cells are the proximal cause of a loss of proper microtubule/kinetochore attachment which leads to mitotic failure. Continued replication after mitotic failure results in the occurrence of supernumerary centrosomes, aneuploidy, endoreplication and cell death. Our data supports a model in which the FA pathway plays a critical role in limiting telomeric recombination and/or resolving recombinational events in ALT cells.

**Translational Applicability:** The human ALT pathway serves as a useful model system for studying the function of FA proteins in DNA recombination, replication and repair. By determining the nature of telomere abnormalities in FANCD2-depleted ALT cells, we may gain

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key insights into the endogenous function of the pathway, thus aiding our understanding of the pathogenesis of the disease.