

The Fanconi Anemia Protein xFANCD2 Binds to Chromatin in a Replication and DNA Damage-Dependent Manner in *Xenopus* Cell Free Extracts

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Fanconi anemia (FA) is a rare cancer susceptibility syndrome that is characterized by variable developmental defects, a tendency to develop specific malignancies including acute myeloid leukemia, and bone marrow failure. A majority of the Fanconi proteins lack recognizable motifs, although it is known that they function together in a pathway composed of a "core complex" that is required to trigger the DNA-damage dependent monoubiquitination of the downstream protein, FANCD2. A new member of the FA core complex, FANCL, was recently identified. FANCL is a novel ubiquitin ligase and probable catalytic entity of the FA core complex. The function of the FA proteins is poorly understood because of their novelty and because tractable *in vitro* functional assays have not been available.

Because of these considerations, we have developed a new approach using the *Xenopus laevis* model system. *X. laevis* is a classical model for developmental studies and for biochemical evaluation of the functional roles of proteins influencing DNA replication and repair. To use the unique advantages of this model system we cloned the homologs for the known FA genes in *X. laevis*. The human and *Xenopus* sequences share significant homology throughout, while FANCL is the most highly conserved with overall homology of approximately 80%. All of the FA genes are expressed during *Xenopus* development.

One of the key indicators of FA pathway function is the DNA damage dependent appearance of the monoubiquitinated form of FANCD2 (FANCD2-L). In a *Xenopus* cell line treated DNA damaging agents FANCD2-L was induced, suggesting that the FA pathway is functionally conserved in *Xenopus*.

Cell-free extracts from *Xenopus* eggs offer a unique advantage for functional studies of proteins involved in DNA damage response. When DNA is added to activated egg extracts, a nuclear membrane forms and the DNA is replicated *in vitro*, complete with assembly of chromatin. We find that xFANCA, xFANCF, xFANCL and xFANCD2 become associated with the nuclear fraction during replication. xFANCD2-L binds to chromatin during the course of replication. In extracts treated with DNA damaging agents, chromatin binding of xFANCD2-L occurs earlier and increases during replication compared with untreated extracts. These results suggest a direct role for xFANCD2 in the repair of DNA damage recognized in the context of replication and describe the first cell-free functional assay for FA protein function during DNA replication.