

Immunoisolation and Partial Characterization of Fanconi Anemia Protein Complexes in *Xenopus* Egg Extracts

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The Fanconi Anemia (FA) core complex contains at least eight proteins, and each is required for monoubiquitination of FANCD2. The current model for FA proposes that the FA core complex acts as an E3 ubiquitin ligase assembly to monoubiquitinate FANCD2 during DNA replication and in response to DNA damage in human cells and in *Xenopus laevis* cell-free extracts. In human cells, the biochemical characterization of one FANCA-containing complex led to the identification of at least three new FA genes (Meetei, 2003). These studies also demonstrated that the FA core complex is part of a larger complex of proteins, termed BRAFT, which contains BLM, TopoIII3B1, and RPA, proteins that may function with the FA core complex to maintain genomic stability. An understanding of the FA protein complexes and subcomplexes, and how the complexes might be biochemically and functionally different, may continue to enhance our understanding of FA.

To enable characterization of FA protein complexes we used a cell-free system based on extracts derived from *Xenopus* eggs (Sobeck, 2006). Important advantages of this approach include the precise natural synchronization of the extract, the ease of biochemical manipulations, and the concentrated store of proteins in *Xenopus* eggs. Egg extracts are an outstanding source of soluble nuclear factors because although the egg contains only one cell equivalent of nuclear DNA, it stockpiles enough nuclear proteins to support the formation of thousands of new nuclei during early development.

We performed gel filtration chromatography of egg extracts to resolve native protein complexes. The fractions were analyzed by immunoblot to obtain a profile of complexes containing FA proteins. In addition to analysis by size fractionation, complexes were immunoisolated using highly specific affinity-purified antisera against xFANCA, -G or -M. The immunoisolated proteins were analyzed by tandem mass spectrometry (LC/MS/MS).

We found that distinct xFANCA, xFANCG and xFANCM complexes exist in *Xenopus* egg extracts. The largest xFANCM complex (xFAM-1) contains xFANCM, xFANCA, xFANCC, xFANCF, and several other proteins. In a second, smaller complex (xFAM-2), xFANCM associates with xFANCG and xFANCA, and a different set of proteins. In *Xenopus* egg extracts, xFANCA and xFANCA-containing complexes are abundant, whereas BRAFT is not detected suggesting it is only a minor complex. In comparison, human cells appear to have similar complexes, but in different proportions. We propose that incorporation of unique subunits might enable functional specialization of distinct

FA complexes.

Translational applicability: Identifying functionally important proteins and protein complexes in the FA pathway will help us further develop cell-free screens for small molecules that could translate into therapeutics for FA patients.