Functional Characterization of FANCA Complexes

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We have analyzed the FA pathway in the *Xenopus* cell-free system. We identified sequences of *Xenopus* homologs of human FA genes, and created antibodies that enabled us to specifically immunodeplete egg extracts in order to dissect the role of these proteins in replicative DNA synthesis and in the DNA damage response. We found that xFANCA accumulates on chromatin in both a DNA-damage dependent and replication-dependent manner. Depletion of the core complex protein xFANCA from replicating egg extracts abrogated the loading of xFANCD2-L on chromatin and resulted in the accumulation of chromosomal breaks during unperturbed replication.

Taken together with previous findings, these results suggest that the FA proteins may assemble in a step-wise manner on chromatin during replication, however the underlying mechanism and timing is unknown. Three FANCA subcomplexes have been previously isolated from asynchronous HeLa nuclear extracts (Meetei *et al*. 2003). To examine whether FANCA-containing complexes are constitutive, cell cycle dependent, and/or DNA dependent we analyzed *Xenopus* egg extracts, which are naturally and precisely synchronized in M or S phase of the cell cycle, and are essentially free of DNA. The identity of xFANCA in a size-fractionated native complex was confirmed by mass spectrometry. Interestingly, we found xFANCA in only one complex of molecular mass 500 kDa, whereas human FANCA isolated from asynchronous HeLa cells has previously

been found in three complexes of approximately 500 kDa, 900 kDa, and 1.5 to 2.0 MDa (BRAFT). We found xFANCA present in the same size (500 kDa) complex in both M and S phase extracts. We are currently investigating if larger FANCA-containing complexes such as BRAFT assemble in replication competent extracts depending on the presence of either replicating chromatin or defined DNA structures.