Identification of a Novel Partner Protein for FANCM

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Objective: A multisubunit protein complex, termed the " FA (Fanconi Anemia) core complex", plays a critical role in maintaining genomic stability in human cells and in replicating *Xenopus laevis* cell-free extracts. FANCM is a member of the FA core complex and is one of two FA core complex proteins with evolutionarily-conserved domains. FANCM was originally immunoisolated as a member of the BRAFT (for BLM, RPA, FA, and Topo IIIα) complex. BRAFT contains several proteins associated with BLM, the protein defective in Bloom syndrome, as well as the FA core complex (Meetei et al., 2003). The object of this study was to identify protein partners of FANCM. *Xenopus laevis* egg extracts were used for these experiments because they provide a powerful biochemical platform for dissecting the functional roles of proteins that influence genomic stability and the DNA damage response.

Methods: We identified *Xenopus* FANCM (xFANCM) and created antibodies specific for its C-terminal region. We analyzed egg extracts by size exclusion chromatography and immunoprecipitation with antibodies directed against several FA core complex proteins. Proteins in xFANCM-containing protein complexes were identified by mass spectrometry analysis, and antibodies were generated against candidate FANCM-interacting proteins. DNA shift assays were performed using defined DNA minisubstrates in egg extracts similar to those reported previously for FANCD2 and Mre11 (Sobeck et al., 2007 and Costanzo et al., 2001). Chromatin binding assays were performed in replicating extracts in the presence and absence of FA core complex proteins.

Results: A novel protein-binding partner of xFANCM (termed xMIP-1, for xFANCM Interacting Protein 1) was immunoisolated from egg extracts. The interaction was

confirmed by reciprocal coimmunoprecipitation and immunoblot with an antibody specific for xMIP-1. Cofractionation demonstrated that xFANCM and xMIP-1 eluted in a protein complex of ~900 kDa. Recruitment of xMIP-1 to replicating chromatin was dependent on xFANCM but was xFANCA-independent. xMIP-1 exhibited a DNA-stimulated mobility shift in egg extracts in response to DNA double strand breaks.

Conclusion: We identified xMIP-1 as a new interactor of xFANCM. xMIP-1 recruitment to chromatin is dependent on xFANCM but does not require the core complex protein xFANCA. Like FANCD2 and Mre11, xMIP-1 is modified in response to DNA double strand breaks. Taken together, our data supports a role for the xFANCM/xMIP-1 interaction in maintaining genomic stability.

Translational Applicability: Identification and analysis of FANCM interacting proteins could provide novel insights into the function of the FA core complex and new protein targets for drug design. Analysis of FA proteins in *Xenopus* cell free extracts can lead to the identification of activating and inhibitory small molecules and their targets.