

## A Novel Component of Fanconi Anemia Core Complex is Essential for Activation of the FA-associated DNA Repair Pathway

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**Objective:** The Fanconi anemia (FA) core complex plays a critical role in the DNA damage response pathway involving FA and breast cancer proteins, BRCA1 and BRCA2. This complex consists of eight FA proteins (FANC A, B, C, E, F, G, L, M) and two FA-associated proteins (FAAP100 and FAAP24), which are all required for monoubiquitination of FANCD2 in response to DNA damage. The objective of our study is to identify additional components of the FA core complex and understand their roles in the FA pathway. We will also screen FA patients for mutations in these proteins.

**Methods:** The discovery of new components of FA core complex has been achieved through immunopurification and subsequent identification by mass spectrometry. High quality antibodies to these new proteins have been generated from rabbits. Co-immunoprecipitation and chromatography were performed for verification. The essential roles in FA pathway were tested by siRNA depletion. Immunoblotting and sequencing were performed to screen FA patients with mutations. The nullizygous mutations were generated by gene-targeting disruption in chicken DT-40 cells.

**Results:** We have identified several new candidate components in the FA core complex. Coimmunoprecipitation and gel-filtration chromatography have demonstrated that at least one of them is an integral component of FA core complex, and essential for the stability and function of the complex. Depletion of this protein by siRNA revealed that it is required for the monoubiquitination of FANCD2 and FANCI in DNA damage response. However, no mutations have been found in FA patients. The structure of this protein implies that it could be important for targeting the FA core complex to DNA.

**Conclusions:** We have discovered a new integral component of FA core complex, and found that it is an essential for activation of the FA pathway.

**Translational Applicability:** The identification of novel components of the FA core complex raised the possibility that these proteins could be defective in patients of new complementation groups. Characterization of the function of the new components should also facilitate the understanding of the mechanism of action by the FA core complex, which should be helpful for development of the treatment for FA patients.