Cell Reports
Report

Modularized Functions of the Fanconi Anemia Core Complex

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http://dx.doi.org/10.1016/j.celrep.2014.04.029

SUMMARY

The Fanconi anemia (FA) core complex provides the essential E3 ligase function for spatially defined FANCD2 ubiquitination and FA pathway activation. Of the seven FA gene products forming the core complex, FANCL possesses a RING domain with demonstrated E3 ligase activity. The other six components do not have clearly defined roles. Through epistasis analyses, we identify three functional modules in the FA core complex: a catalytic module consisting of FANCL, FANCB, and FAAP100 is absolutely required for the E3 ligase function, and the FANCA-FANCG-FAAP20 and the FANCC-FANCE-FANCF modules provide nonredundant and ancillary functions that help the catalytic module bind chromatin or sites of DNA damage. Disruption of the catalytic module causes complete loss of the core complex function, whereas loss of any ancillary module component does not. Our work reveals the roles of several FA gene products with previously undefined functions and a modularized assembly of the FA core complex.

INTRODUCTION

Fanconi anemia (FA) is a complex genetic disorder encompassing 16 tumor suppressor genes that act together to protect cells against genotoxic stress, particularly complexed DNA lesions such as DNA interstrand crosslinks (Bogliolo et al., 2013; D'Andrea, 2010) and potentially DNA-protein crosslinks created by endogenous metabolites (Langevin et al., 2011; Rosado et al., 2011). Classical manifestations of FA include pancytopenia, chromosomal abnormalities, congenital abnormalities, and a high predisposition to a broad spectrum of cancers. Despite the identification of genetic defects in patients with FA, the molecular mechanism underpinning FA pathway functions remains unclear.

A group of classical FA genes is connected by a DNA damage-induced monoubiquitination reaction in the nucleus (Garcia-Higuera et al., 2001; Smogorzewska et al., 2007; Taniguchi et al., 2002). Monoubiquitination of the FANCD2/I complex has the presumed functions of recruiting DNA lesion-processing endonucleolytic activities (Knipscheer et al., 2009; Kratz et al., 2010; Liu et al., 2010; MacKay et al., 2010; Smogorzewska et al., 2010) and transcriptional activation of tumor suppressor genes (Park et al., 2013). The E3 ligase activity of this reaction resides in the FA core complex consisting of seven FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, and FANCL) and two FA-associated *p*roteins (FAAP20 and FAAP100), with the RING domain protein FANCL bearing the E3 ligase activity (Alpi et al., 2008; Meetei et al., 2003). Aside from FANCL and FAAP20, most other components of the core complex have neither recognizable motifs nor clearly defined functions as to how they contribute to the DNA damage-mediated FANCD2/I monoubiquitination.

Studies of protein-protein interactions within the FA core complex have suggested the existence of three subcomplexes (Figure 1A). FANCA, FANCG, and FAAP20, form a subcomplex (A-G-20) (Ali et al., 2012; Garcia-Higuera et al., 1999; Kruyt et al., 1999; Reuter et al., 2000; Waisfisz et al., 1999). The UBZ domain of FAAP20 is suggested to bind to ubiquitinated histone (Leung et al., 2012; Yan et al., 2012). FANCG contains seven TPR repeats and is considered a possible scaffold for the subcomplex (Blom et al., 2004; Léveillé et al., 2004). The FANCB-FANCL-FAAP100 subcomplex (B-L-100) contains the E3 ligase FANCL (Ling et al., 2007; Medhurst et al., 2006). Given that FANCL alone acts sufficiently in reconstituted ubiquitination reactions (Alpi et al., 2008; Longerich et al., 2009; Sato et al., 2012), whether FANCB and FAAP100 contribute to the E3 activity is unclear. A third subcomplex is formed by FANCC, FANCE, and FANCF (C-E-F). FANCF has been shown to interact with FANCM (Deans and West, 2009) and was also suggested to act as an adaptor protein (Léveillé et al., 2004). Despite these observations, the

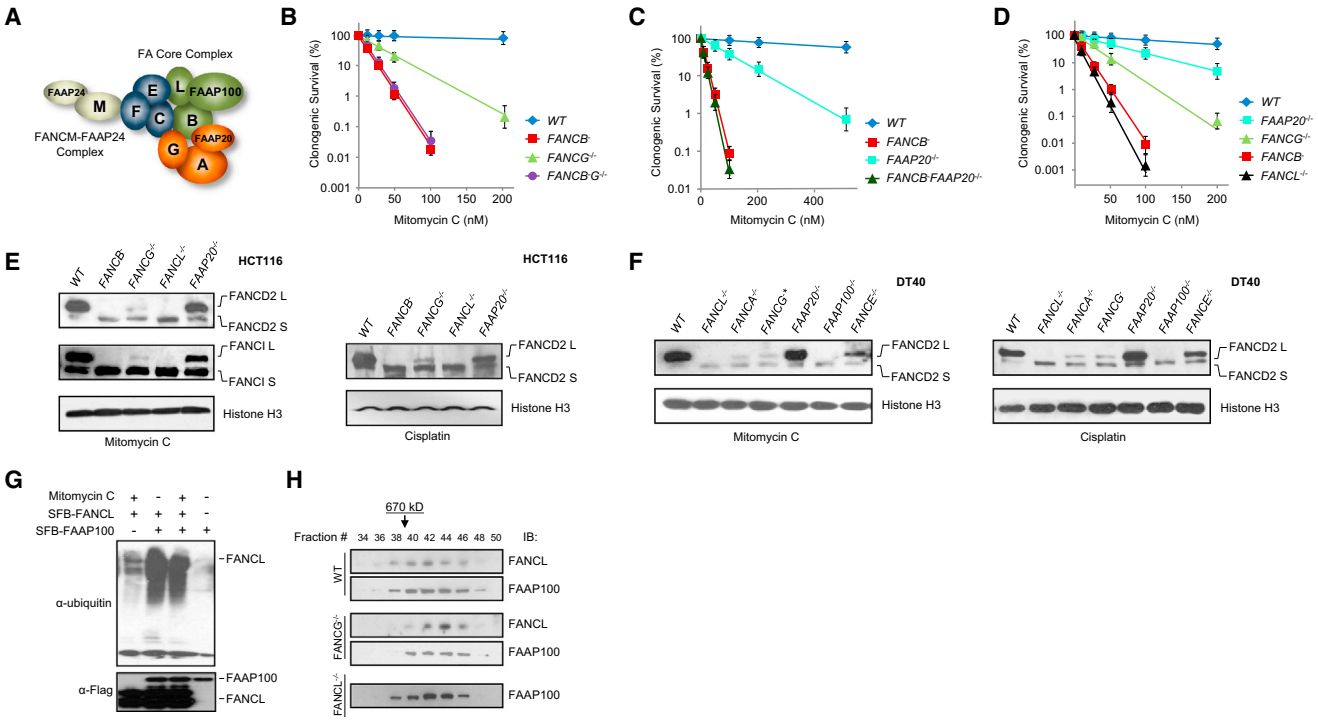

Figure 1. Various Sensitivities of FA Knockout Mutants Identify the FA Core Complex Catalytic Module

(A) Depiction of three protein interaction modules within the FA core complex and the FANCM-FAAP24 complex. See also Figures S1A–S1C.

(B) Clonogenic survival of wild-type HCT116 (*WT*), *FANCB*$^{-}$, *FANCG*$^{-/-}$, and *FANCB*$^{-}$*G*$^{-/-}$ mutants treated with mitomycin C. See also Figures S1D, S1E, and S2.

(C) Clonogenic survival of wild-type HCT116 (*WT*), *FANCB*$^{-}$, *FAAP20*$^{-/-}$, and *FANCB*$^{-}$*FAAP20*$^{-/-}$ mutants treated with mitomycin C. See also Figure S2.

(D) Clonogenic survival of wild-type HCT116 (*WT*), *FAAP20*$^{-/-}$, *FANCG*$^{-/-}$, *FANCB*$^{-}$, and *FANCL*$^{-/-}$ mutants treated with mitomycin C.

(E) Immunoblots detecting MMC- or cisplatin-induced monoubiquitination of chromatin-bound FANCD2 and FANCI in wild-type HCT116 cells (*WT*) and the indicated knockout mutants. See also Figures S3A and S3B.

(F) Immunoblot detecting MMC- or cisplatin-induced monoubiquitination of chromatin-bound FANCD2 in wild-type chicken DT40 (*WT*) and indicated knockout mutants. *FANCG gene localizes in the single Z chromosome and only one allele exists in DT40 cells. See also Figures S3C and S3D.

(G) Immunoblots detecting FANCL auto ubiquitination in protein extracts prepared from 293T cells stably expressing SPB-FAAP100 and/or SFB-FANCL with or without MMC treatment. Protein extracts from indicated cell lines were subjected to S beads pull-down and immunoblotted by antiubiquitin or Flag antibodies.

(H) Superose 6 gel filtration profiling of the FA core complex in HCT116 *WT*, *FANCG*$^{-/-}$, and *FANCL*$^{-/-}$ mutants. Nuclear extracts were fractionated by Superose 6 gel filtration, and the indicated fractions were immunoblotted (IB) with FANCL or FAAP100 antibodies. The arrow indicates the elution position of the FA core complex (670 kDa). Fraction 21 marks the void.

Error bars in survival curves were derived from SDs from four to six independent experiments with triplicates.

function of each subcomplex and how they integrate together in the context of the FA core complex remain largely unclear.

Because that conservation of the classical FA pathway is primarily within vertebrates, typical genetic platforms such as yeast or *Drosophila* could not be employed to reveal the interaction among FA genes. In this study, we undertook an epistatic analysis approach with mammalian and chicken DT40 cells to elucidate the functions of the FA core components. By generating a series of isogenic loss-of-function single and double mutants of key core complex genes, we find that loss of different FA core components gave rise to variable impacts on the activation of the FA pathway and correspondingly variable cellular sensitivities to crosslinking reagents. Here, we present evidence that the differential sensitivities reflect distinct functions of the FA core complex components, suggesting a modularized functional assembly of the core complex that enables it to carry out the spatially defined monoubiquitination reaction upon DNA damage.

RESULTS

Differential Damage Sensitivities of FA Core Component Mutants

FA core complex components form three protein interaction modules: A-G-20, B-L-100, and C-E-F, as suggested by several groups and us (Léveillé et al., 2004; Ling et al., 2007; Medhurst et al., 2006; Yan et al., 2012) (Figures 1A and S1A–S1C). To address the functionality of each protein interaction module in cellular survival against DNA damage, we created *FANCG*$^{-/-}$, *FANCB*$^{-}$, and a *FANCG*$^{-/-}$*FANCB*$^{-}$ double-knockout mutants via homologous replacement targeting (Figures S2A, S2B, and S2D) in the HCT116 parental cell background. The strict isogenicity among these knockout mutants allows direct comparison of cellular survival outcomes as a direct reflection of protein function. Using clonogenic survival assay, we found that the *FANCG*$^{-/-}$ mutant was significantly more resistant than