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Ubiquitin and Fanconi Anemia

Ioannis Drikos and Alexandros Sachinidis

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<http://dx.doi.org/10.5772/intechopen.77123>

Abstract

Fanconi anemia (FA) is an inherited disease distinct from the failure of bone marrow, growth disturbance, predisposition to cancer and concomitant chromosomal abnormalities. FA is associated with genes involved in DNA replication and DNA repair processes. More than 20 proteins have been identified to be related with FANC pathway operation. Necessary prerequisite for activation and regulation of FA pathway is the monoubiquitination of heterodimer FANCD2-FANCI by core proteins of Fanc complex. The monoubiquitination of FANCD2-FANCI is crucial for nuclear localization of heterodimer, binding to chromatin and regulation of DNA repair procedure. Mutations of genes of FANC complex proteins associated with deficiency of DNA repair pathways affected cellular and genome instability. The interaction between proteins and ubiquitination affected genomic integrity and stability.

Keywords: Fanconi anemia, DNA repair, ubiquitination, FANC proteins, FANCL, FANCD2

1. Introduction

Fanconi anemia is a rare recessive human genetic disorder first described by pediatrician Guido Fanconi in 1927. Patients with Fanconi anemia characterized by insufficient bone marrow regulation, developmental abnormalities and predisposition of cancer. Abnormal cell cycle progression, production of inflammatory cytokines and chromosomal instability also considered to be characteristics of the syndrome. Developmental abnormalities occur in 70% of patients, 40% develop defects of the skin and rare disorders such as renal deficiencies have been reported in less than 10% [1, 2].

The development of FA associated with DNA repair processes and regulation of cell cycle control. Nowadays, more than 20 genes and proteins have already been identified which

are crucial for FA development (**Figure 1**). Except these genes, patients with mutations in RAD51C hitherto display bone marrow failure even if RAD51C seems to have a crucial role in FA development. In the majority of patients appeared, FA biallelic mutations inherited from each parent. FANC proteins interact through intracellular signaling pathway during cell cycle progression and FANC/BRCA complex demonstrate the genome stability [3–5].

Intracellular FA’s evolution pathway involved eight key proteins such as FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM forming a complex which monoubiquitylates the FANCD2/FANCI. The monoubiquitylated ID complex interacts with FAN1 (anemia Fanconi Associated Nuclease 1) appearing endonuclease and exonuclease activity in vitro. According to several studies, FA intracellular pathway involved FANCD1 and BRCA2 has been already revealed. These molecules are necessary for homologous recombination process via interaction with helicase BACH1 [5–7].

The FANCI interacts with BRCA1 and protein complex of FANCP/SLX4. The SLX4 connecting with multiple proteins such as XPF, MUS81, and SLX1 responsible for Holliday intersections. The ubiquitylation of FANCD2 and FANCI is absent in cells with mutations in crucial proteins of the FA pathway. FANCL considered to be an essential part of the FA complex

FANC proteins	Molecular Weight (kDa)	Chromosome localisation	Function	Modification
FANCA	163	16q24.3	FANC Core	Ubiquitin SUMO
FANCB	95	Xp22.31	FANC Core	–
FANCC	63	9q22.3	FANC Core	–
FANCD1/BRCA2	380	13q12.13	Homologous Recombination	–
FANCD2	162	3p25.3	DNA damages signalling Replication forks Transcription factor	Ubiquitin SUMO
FANCE	60	6p21.22	FANC Core	–
FANCF	42	11p15	FANC Core	–
FANCG	68	9p13	FANC Core	Ubiquitin
FANCI	140	15q25.16	DNA damages signalling Replication forks	Ubiquitin SUMO UBL5
FANCI/BRIP1	140	17q22-q24	Helicase	–
FANCL	43	2p16.1	FANC Core E3 ubiquitin ligase	–
FANCM	250	14q21.3	Anchor the FANC Core to chromatin translocase	–
FANCN/PALB2	130	16p12.1	Homologous Recombination	–
FANCO/RAD51C	42	17q25.1	Homologous Recombination	–
FANCP/SLX4	200	16p13.3	Endonuclease Transcription factor Potential E3 SUMO ligase	SUMO
FANCO/XPF	101	16p13.12	Endonuclease	SUMO
FANCR/RAD51	37	15q15.1	Homologous Recombination	–
FANCS/BRCA1	207	17q21.31	E3 Ubiquitin ligase	SUMO
FANCT/UBE2T	23	1q32.1	Homologous recombination E2 conjugating Enzyme	Ubiquitin
FANCU/XRCC2	32	7q36.1	Homologous Recombination	–

Figure 1. The FANC proteins according to chromosome location in the human genome and main cellular function [6].

and represents an E3 ligase activity, which is required for coupling ubiquitin to lysine 561 of human FANCD2 and lysine 523 of FANCI [8, 9].

FANCL has three distinct structural regions such as region DRWD and RING domain. Functionally, DRWD domain coordinates the substrate binding and is essential for the interaction with E2 protein. The C-terminal region (RING) is required for adequate binding of the FANCL to UBE2T. The importance of RING domain reflected in the evolutionary conservation of FANCL homologous proteins. The UBE2T considered as crucial enzyme in ubiquitin E2 ligase function and is essential for FANCL-mediated ubiquitylation of FANCD2 *in vivo* [9–12].

2. Ubiquitylation and deubiquitylation of FANC pathway

Ubiquitylation is an important event for activation of ID complex and ICLs repair. Except ubiquitylation are also important molecules deubiquitylation. This process is crucial for recycling of the ID molecules while the ubiquitylated and nonubiquitylated forms are necessary for normal cellular function. USP1 seems to be enzyme involved in deubiquitylating procedure [13, 14].

FANCD2 and FANCI have been identified as targets of FANCL monoubiquitylation. *In vitro* studies of FANCD2 have already increased the understanding of the operation of FANC complex and the regulation of signaling pathway. As already mentioned, FANCD2 is a 160 kDa protein which can be monoubiquitinated at lysine 561 (K561) [15, 16].

Recent studies revealed that ubiquitylation of FANCD2/FANCI regulated by the FANCL, FANCB, FAAP100, and FANCT/UBE2T which participate in reconstitution of E2-E3 ligase complex. Mutants of proteins involved in FANC complex lead to destabilization and significant reduction of ubiquitin action of FANCD2/FANCI [17].

In DT40, cell line mutations of FANC complex proteins lead to inactivating of FANCC and USP1 without affecting FANCD2 ubiquitylation. These results suggesting FANC complex proteins may have a significant role in the DNA damage response and other cellular functions interpedently of FANCD2/FANCI ubiquitylation [13, 14].

Proper monoubiquitination of FANCD2 and FANCI also requires several other proteins such as PTMs, ATM, ATR, and CHK1, and other basic proteins of FANC complex important for optimal monoubiquitylation of FANCD2 and FANCI [15, 16].

It is also crucial that the phase of cell cycle monoubiquitylation of FANCD2/FANCI occurred. The complex of FANC proteins is considered active only during S phase assessed by the monoubiquitylation of FANCD2. Additionally, FANCD2 foci located on chromatids during G2 and M phases.

FANCD2 and FANCI are known as direct targets of ubiquitin ligase FANC complex [2]. Proteins of the complex demonstrated ligase E3 ubiquitin action. Additionally, UBE2T may act as E2 ligase while *in vitro* experiments UBE2T undergo monoubiquitylation in K91 lysine. Ubiquitylation is required for binding of the dimer to the

chromatin. In order to be effective the process of DNA repair both proteins must undergo ubiquitination/deubiquitination.

USP1 is crucial for deubiquitination demonstrated complex with UAF1. The USP1-UAF1 cluster locates FANCI into the SIM area and causes deubiquitination of both FANCI and FANCD2 [18]. The deubiquitination signal allows the precision and track replication [12, 18].

Another important checkpoint of complex FANCD2/FANCI related to structure of chromatin. In vitro studies revealed that the chromatin structure affects the FANCI dependent FANCD2 ubiquitination. It has been reported that phosphorylation of histone H2AX is necessary to connect FANCD2 in chromatin after MMC or UVC damage responses. In cases of monoubiquitination, deficiencies of FANCD2 may be activated two other protein molecules with E3 ligase activity such as BRCA1 and RAD18 interacting with FANCD2 in order to detect FANCD2 in proper position [19].

Recent studies have shown that FANCD2 may act independently of the ubiquitination as a modulator of the NF- κ B transcriptional activity. D'Andrea et al. proposed this activity be amended under stress and expression of the regulated genes such as FANCD2 activated after DNA damage responses.

It is widely accepted that the function of proteins of FA pathway involved in DNA repair by promoting homologous recombination (HR) rather than nonhomologous (NHEJ). Molecular failure during the operation of FANCD2 pathway leads to deficiency of HR processes increasing the NHEJ [19, 20].

Cloning of the FANCD2 gene increased our understanding of the operation of FANCD2 complex proteins [20]. The FANCL is a protein consisting of a characteristic region with ubiquitin-ligase E3 action. Mutations in ubiquitination region of FANCL affected the ligase E3 action on FANCD2 without affecting the interaction with other proteins of the complex. Recent studies have shown that it is necessary to connect the FANCD2/FANCI ubiquitination with FANCT/UBE2T for reconstitution of the E2-E3 ligase activation [21, 22] (**Figure 2**). This activation is unaffected by the FANCL, FANCB, and FAAP100.

Disturbance of any core proteins of FANCD2 complex leads to destabilization and significant reduction of ubiquitination of FANCD2/FANCI and FANCD2 inactivation [19, 21, 22].

The FANCD2 complex monoubiquitinates protein FANCI at lysine 523 (K523) [10]. The monoubiquitination of FANCD2 and FANCI also requires the activity of several other proteins. ATM, ATR, and CHK1 cause phosphorylation of FANCD2 and FANCI and several core proteins of FANCD2 complex crucial for the monoubiquitination of FANCD2 and FANCI [23].

Additionally, FANCD2/FANCI monoubiquitination is also dependent for the activity of Rad18. Rad18 and phosphorylated H2AX (Z-H2AX) lead FANCD2 and FANCI in subcellular regions [23].

Apart from the breakdown processes, the phase of the cell cycle occurred FANCD2/FANCI monoubiquitination is also important. The FANCD2 complex proteins are active during the S

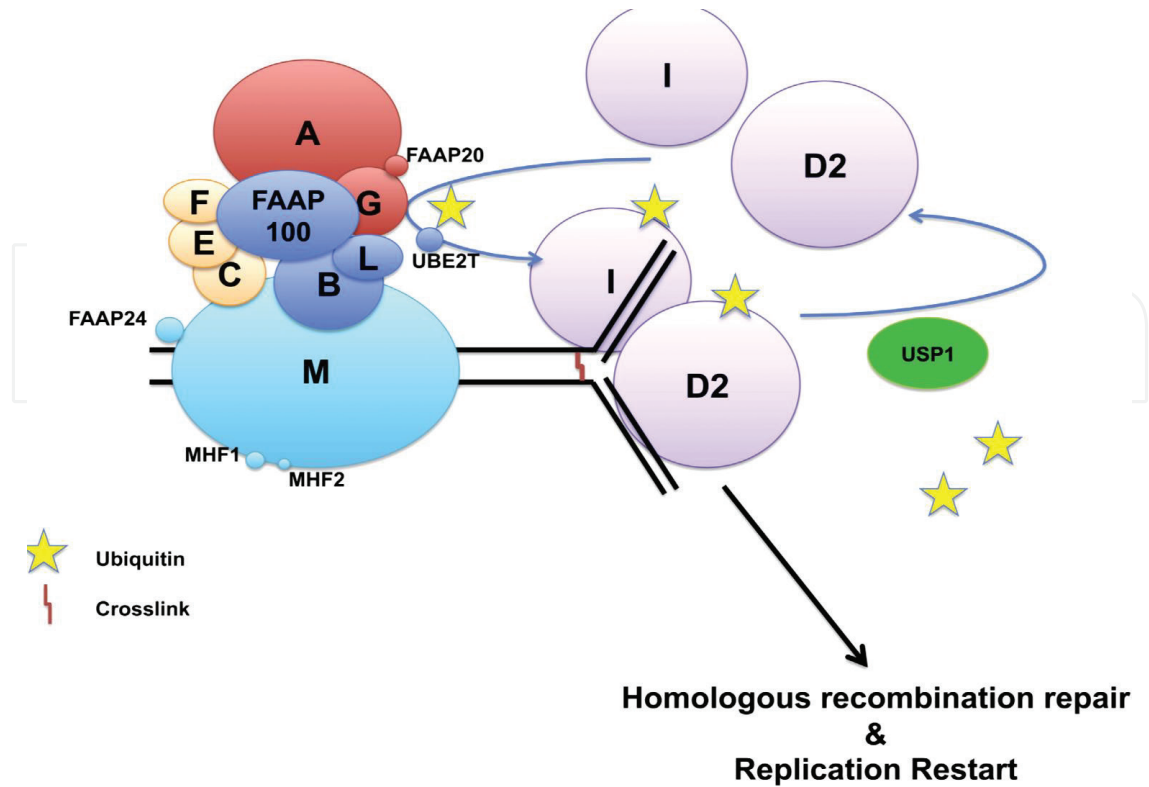


Figure 2. The FANCD1 core complex is an E3 ubiquitin ligase targeting FANCD2 and FANCI to coordinate DNA repair. In response to stalled replication forks by DNA lesions (here a crosslink), the FANCD1 core complex is recruited by FANCD1-FAAP24 and MHP1 and MHP2 [6].

phase according to FANCD2 monoubiquitination [23, 24], while the presence of FANCD2 foci in sister chromatids also referred during G2 and M phases [25].

Therefore, when the cell stress processes occur and the cells are in S/G2 phase monoubiquitinated FANCD2 and FANCI identified in DNA repair area. These two monoubiquitinated proteins form a heterodimer complex [13]. The binding of FANCD2 with histone H2B reveals that ubiquitination is crucial of complex identification to chromatin and DNA repair process [26, 27].

The UBE2T protein is constitutively present in the core of the complex of FANCD proteins, in contrast with the other proteins detected to cytoplasm. Mutations of UBE2T display FA considered as an important member of FANCD complex proteins [28, 29]. Except the UBE2T, UBE2W appears to have ligase E2 activity regulated monoubiquitination of FANCD2/FANCI after UV exposure [30–32] (**Figure 2**).

Additionally, FANCD2/FANCI complex ubiquitination is required for chromatin regulation. As already mentioned, the effective regulation of these proteins induced after DNA damage not only undergoes ubiquitination but also regulates deubiquitination effectively [33–35].

FANCD2 can act independently of ubiquitination as a regulator of NF- κ B activity, particularly through the action of the TNF- α promoter [35, 36]. D'Andrea et al. demonstrated the expression of the FANCD2 gene may be altered by DNA damage caused by UV exposure via SLX4/

FANCP ubiquitination of FANCD2. Besides, the FANCD2, FANCI, FANCA, and FANCG are also ubiquitinated. The FANCA is ubiquitinated and regulates the activity of proteasome [34].

The FANCG is not necessary for ubiquitination of FANCD2 and FANCI but is required for the interaction with RAP80-BRCA1 complex [35]. It seems that the interaction of FANCG-RAP80-BRCA1 affects the regulation of FANCD2 complex proteins on HR/NHEJ procedures [36–38].

Recent studies have shown RAD18 E3 ubiquitin ligase activity, being crucial of monoubiquitylation of PCNA on replication forks [39, 40]. Monoubiquitylation of PCNA in the lysine-164 by the RAD18 and RAD6 activates the polymerase switching [40]. Apart from its role in the mechanism of regulation of the polymerase, the RAD18 associated with monoubiquitylation of molecules of DNA repair procedure such as 53BP1 and also interact with the DNA repair protein WRNIP1 [41].

RAD18 protein is also important in the process of homologous recombination independent of ubiquitylation [41]. Also in recent studies, it appears to be decisive in the RAD6 ubiquitylation of FANCD2. Experimental data by immunoprecipitation reveal that RAD18-FANCD2 binding takes place both in the presence or in absence of damage DNA. The RAD18 affected monoubiquitylation of FANCD2 and FANCI after treatment with various factors of DNA cross without depending on PCNA modification [40, 41]. The limited response of RAD18 leads to hypersensitivity after MMC and cisplatin treatment [40, 41].

The data indicate an essential role of E3 ligase for RAD18 identification FANCD2 and FANCI into chromatin, while the ubiquitylation process observed in phase S. The RAD18 regulates monoubiquitylation of FANCD2 and FANCI in FANCL independent manner [42–44].

Other recent reports indicated that RAD18 may coordinate events of homologous recombination. Huang et al. have shown that the RAD18 bound to DNA damage sites attracting other proteins in DNA repair process. This function is independent of its role as an E3 ubiquitin ligase [45].

Huang et al. demonstrated that RAD18 is important for attracting RAD51C. Cells with RAD51C deficiency revealed increased sensitivity and radial configuration in response to treatment with ICL [45]. Geng et al. reported that RAD18 participates in FANCD2 deubiquitylation and ubiquitylation of ID complex in ICLs repair process. Although the significance of ubiquitylation is equally important, deubiquitylation process may affect the normal cell cycle progression [42–44].

USP1-UAF1 complex causes deubiquitination both FANCI and FANCD2. This procedure is important for the proper function of DNA repair such as different procedure revealed [33]. Deubiquitination seems to regulate the proper DNA repair and replication procedure.

USP1 is regulated at a transcriptional level during phase S such as the molecule activity must be increased. USP1 levels during S phase are quite stable. There are unknown regulatory processes of chromatin related with deubiquitylation of FANCD2 [42–44]. USP1 activity is greatly enhanced by WD40 and UAF1. USP1 reveals to act as deubiquitylating enzyme for PCNA [45, 46].

3. The role of sort proteins in FANC complex operation

Considering both biochemical and functional criteria, FANC proteins are divided into three main groups [47]. According to immunoprecipitation experiments, major proteins of the first group constitute the main core of FANC complex [48, 49]. Proteins such as FANCA, B, C, E, F, G and L together with the FA-associated protein such as FAAP20 and FAAP100 detected during DNA repair processes on chromatin through FANCM complex. The complex binds FAAP24 and FANCM interacting histones through MHF1 and MHF2 [50].

Mutations in FAAP20, FAAP24, FAAP100 or MHF1, MHF2 deactivate DNA repair mechanisms through FANC proteins. The core proteins of FANC interact with UBE2T [51].

The UBE2T reveals E2 ubiquitin ligase activity monoubiquitinated FANCD2 and FANCI. The FANCD2 and FANCI, group II proteins are known targets of the ubiquitin ligase in the core of FANC complex. Experimental data have shown lack or improper function of FANC complex affects monoubiquitination of FANCD2 and FANCI [19, 52].

Ubiquitination of FANCD2 and FANCI is necessary for transfer of these proteins into the chromatin region interacting with proteins involved in homologous recombination process of DNA. Experimental results in mice revealed USP1 association in PCNA de-ubiquitination. USP-1 monoubiquitinate FANCD2/FANCI affecting the deterioration of DNA repair proteins [43, 44].

Proteins of the third group (peptides III) do not cause serious problems in ubiquitination of FANCD2 and FANCI. In this group, proteins related to the homologous recombination (HR) such as FANCD1/BRCA2, FANCI/BRIP1, FANCN/PALB2, FANCO/RAD51C, FANCR/RAD51, FANCS/BRCA1, FANCU/XRCC2 (Sawyer et al. 2014), and proteins with endonuclease action including FANCP/SLX4 (interacts with SLX1) and FANCC/XPF (interacts with ERCC1) [53].

The main proteins constituting core complex localized in the cytoplasm during cell cycle and contribute into nucleus after DNA damage display. Thus, proteins such as FANCA and FANCG are interacting with FAAP20, FANCB, FANCL, FAAP100, and FANCC. FANCL, FAAP100, and FANCB represent the catalytic subunit of the FANC complex [53].

Recent studies revealed the role of proteins of FANC complex in DNA damage control. FANCA, FANCC, and FANCD2 seem to have a crucial role in evolution of mitophagy that allows the degradation of damaged mitochondria [54, 55].

4. Evolutionary conservation of proteins in the Fanconi anemia pathway

Among the FA pathway, proteins such as FANCM, SLX4, and BRCA2 are most conserved in mammals [56–61]. This evolutionary conservation indicates the importance of these

proteins in cell cycle progression. Vertebrates, flies, worms, plants revealed recognizable orthologs of some of the components of the FANCL complex [62–64]. All organisms with FANCL also have FANCD2 and FANCI orthologs proven to be monoubiquitylated [62–64].

Author details

Ioannis Drikos^{1*} and Alexandros Sachinidis²

*Address all correspondence to: johndrikos@yahoo.com

1 Paediatric Clinic, Thrasio General Hospital of Elefsina, Athens, Greece

2 Second Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, Hippocraton Hospital, Thessaloniki, Greece

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