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FANCM-FAAP24 and FANCI: FA proteins that metabolize DNA

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Abstract

Fanconi anemia (FA) is a rare autosomal recessive or X-linked disorder characterized by aplastic anemia, cancer susceptibility and cellular sensitivity to DNA crosslinking agents. Eight FA proteins (FANCA, -B, -C, -E, -F, -G, -L and -M) and three non-FA proteins (FAAP100, FAAP24 and HES1) form the FA nuclear core complex that is required for monoubiquitination of the FANCD2-FANCI dimer upon DNA damage. The other three FA proteins, FANCD1/BRCA2, FANCI/BACH1/BRIP1 and FANCD3/PALB2, act in parallel or downstream of the FANCD2-FANCI dimer. Despite the isolation and characterization of several FA proteins, the mechanism by which these proteins protect cells from DNA interstrand crosslinking agents has been unclear. This is because a majority of the FA proteins lack any recognizable functional domains that can provide insight into their function. The recently discovered FANCD1 (Hef) and FANCI (BRIP1/BACH1) proteins contain helicase domains, providing potential insight into the role of FA proteins in DNA repair. FANCD1 with its partner, FAAP24, and FANCI bind and metabolize a variety of DNA substrates. In this review, we focus on the discovery, structure, and function of the FANCD1-FAAP24 and FANCI proteins.

Keywords

Fanconi anemia; FANCD1; FAAP24; FANCI; Helicase; DNA repair

1. Introduction

Fanconi anemia (FA) is a rare recessive disease with both autosomal and X-linked inheritance [1–3]. FA is a bone marrow failure syndrome with patients showing a high incidence of aplastic anemia, myelodysplastic syndrome and acute myeloid leukemia [1–3]. FA patients also show various developmental defects and are prone to solid tumors, especially squamous cell carcinomas of the head and neck [1–3]. Cells derived from FA patients are highly sensitive to DNA crosslinking agents, such as mitomycin C (MMC), cisplatin and diepoxybutane [1]. FA cells show an abnormal cell cycle distribution, with a larger number of cells having 4N DNA content, particularly following treatment with DNA crosslinking agents. FA cells also show high degree of genomic instability, including increased levels of spontaneous and interstrand crosslink (ICL)-induced chromosome breakage, and formation of radial chromosomes.

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FA is a genetically heterogeneous disease and, to date, 13 genes and their corresponding protein products have been identified (FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M and -N) [1]. Eight FA proteins (FANCA, -B, -C, -E, -F, -G, -L and -M) and three non-FA proteins (FAAP100, FAAP24 and HES1) form the FA core complex that is required for the monoubiquitination of the FANCD2-FANCI dimer upon DNA damage [1,4].

Complementation groups deficient for any of the core complex components show a complete absence of monoubiquitinated FANCD2. The remaining three FA proteins, FANCD1/BRCA2, FANCI/BRIP1/BACH1 and FANCG/PALB2, act in parallel or downstream of FANCD2.

Collectively, these proteins work in a common pathway that has been termed the FA-BRCA pathway. Both FANCM and FANCI possess helicase domains, a property that suggests direct involvement of the FA-BRCA pathway at DNA repair sites. This review focuses on the FANCM-FAAP24 dimer and FANCI proteins.

2. FANCI

2.1. Discovery and nomenclature

FANCI was originally identified as a 130 kDa protein by far-Western blotting in a screen for proteins that interact directly with the BRCT domain of BRCA1 [5]. The 130 kDa protein was subsequently detected in immunoprecipitates using monoclonal antibodies against BRCA1 [5]. It was finally purified from HeLa cell nuclear extract incubated with GST-BRCT fusion protein, identified by mass spectrometry, and named BACH1 (BRCA1-Associated C-terminal Helicase 1) [5]. BACH1 was later renamed BRIP1 (BRCA1-Interacting Protein C-terminal helicase 1), since a transcription factor with a HUGO approved symbol, BACH1 (BTB and CNC homology 1), was already in use. The name BRIP1 was approved by the human genome nomenclature committee (HGNC ID 20473). Mutations in this gene are observed in patients belonging to the FA-J complementation group, and consequently, BRIP1 is also known as FANCI [6–8].

2.2. Structure and Homologs of FANCI

FANCI is a 1249 residue polypeptide. Its N-terminal 888-residue structure shows strong homology with the catalytic and nucleotide binding domain of DEAH helicase family members, including XPD and human CHL1 [5] (Fig. 1). Helicases belonging to the DEAH (Asp-Glu-Ala-His) family are processive, meaning the protein moves or translocates continuously and directionally along nucleic acids and displaces the paired strands or proteins in their path [9]. FANCI contains seven helicase-specific motifs that are characteristic of DEAH helicases and shows 48% homology to the helicase domain of human CHL1 protein [5]. Similar to other members of the DEAH family, FANCI contains a nuclear localization signal in its helicase domain from position 158–175 (Fig. 1) [5]. FANCI contains an Fe-S (iron-sulfur) domain in the helicase domain between Walker A and B boxes (Fig. 1) [10]. The Fe-S domain in XPD is essential for helicase activity. An A349P mutation, present in the Fe-S domain of a patient from the FA-J complementation group, is predicted to abolish helicase activity [10].

The C-terminal region of FANCI shows 39% homology with synaptonemal complex protein 1, a component of the transverse filaments of developing meiotic chromosomes [5]. The BRCA1-interacting region of FANCI extends from residues 976–1006 [5,11].

FANCI homologs from several other species have been isolated and studied. Chicken FANCI (chFANCI) and *C. elegans* FANCI (DOG-1) are 54% identical (68% similar) and 31% identical (50% similar), respectively, to human FANCI, with greatest similarity in the seven conserved helicase domains [12,13]. However, both proteins lack the Ser-X-X-Phe motif required for interaction with BRCA1 [12,13]. The FANCI homolog isolated from Chinese

hamster ovary (CHO) cells is a partial clone, named SUV1 (S-phase UV-inducible), that shows strong homology with the FANCD1 helicase domain [14,15].

2. 3. Biochemical functions of FANCD1

FANCD1 is a nuclear protein. Immunofluorescence microscopy using FANCD1 antibodies revealed a punctuate nuclear pattern in multiple human cell lines [5]. FANCD1 and BRCA1 foci co-localize in asynchronous cells. The intensity of FANCD1 foci is reduced, but not completely abolished, in BRCA1 null cells (HCC1937), suggesting that BRCA1 is required for efficient localization of FANCD1 [5].

FANCD1 contains both DNA-dependent ATPase and helicase activities, and FANCD1 helicase activity is dependent on its ATPase activity [11,16]. FANCD1 is a 5'-3' helicase [11,16]. Recombinant FLAG-tagged full-length FANCD1 protein purified using a baculovirus expression system shows ATPase activity in the presence of calf thymus DNA, circular single-stranded M13 DNA, and supercoiled pcDNA 3.0 [11]. Mutation of a conserved lysine at position 52 (K52R) of FANCD1 abrogates its *in vitro* ATPase and helicase activities [11]. FANCD1 DNA-dependent ATPase activity is diminished during the G1 phase of the cell cycle and is reactivated as cells enter S-phase [17].

FANCD1 purified from insect cells and HeLa cells can unwind partial duplexes. Single-stranded circular M13 DNA containing an annealed ³²P-labeled 19-mer complementary DNA fragment is efficiently displaced by FANCD1 [11]. RNA:DNA hybrids, known as R-loops, can be formed in cells due to transcription. A failure to dissociate such structures ahead of the replication fork can result in a block to replication [18]. FANCD1 dissociates the RNA:DNA hybrid *in vitro*, suggesting that it may have a role in clearing these hybrids *in vivo*. FANCD1 cannot dissociate RNA:RNA hybrids, *in vitro* [11]. FANCD1 has the ability to recognize certain DNA structural elements, many of which are present at replication forks or as intermediates of homologous recombination (HR) (Fig. 2). FANCD1 preferentially binds to, and unwinds forked duplex substrates, as compared to a duplex with a 5' ssDNA tail [16]. The minimum 5' tail required for unwinding is ~15 nucleotides (optimal length ~35) in the absence of other factors [16]. Replication protein A (RPA), which co-localizes and immunoprecipitates with FANCD1, is one of the regulatory factors that stimulates FANCD1 helicase activity and helps it unwind longer duplexes [19]. Addition of BRCA1 *in vitro* has no effect on FANCD1 helicase activity [11]. FANCD1 can unwind 5' flap substrates, a type of D-loop, as efficiently as it unwinds forked substrates [16]. Initiation of unwinding of a 5' flap substrate requires a 5' ssDNA tail. D-loops are formed during HR, when single-strand DNA invades duplex DNA. In fact, under similar conditions, FANCD1 unwinds all D-loop substrates efficiently, but not Holliday junction structures, which are present as an intermediate of HR (Fig. 2) [16]. Also, FANCD1 unwinds bubble junctions poorly [16]. These results suggest that FANCD1 has the ability to recognize and unwind DNA structures that arise during normal DNA metabolism such as DNA replication or repair.

FANCD1 tracks along DNA during unwinding [20]. Requirements for backbone continuity in order for FANCD1 to track along DNA were assessed on different structures [16,20]. Any perturbations within 6 nucleotides of the ssDNA-dsDNA junction, following initial binding of FANCD1, reduces unwinding activity, suggesting that FANCD1 requires nucleic acid continuity in the 5' ssDNA tail of forked duplexes [16]. Discontinuity in the 3' ssDNA has no effect on unwinding activity [16]. However, FANCD1 requires backbone continuity in both strands of the duplex as it tracks along DNA and unwinds it, suggesting that FANCD1 has the ability to sense both strands of the DNA duplex during the unwinding reaction [20]. This requirement for backbone continuity can be overcome by increasing the amount of 5' ssDNA tail used in loading the enzyme, suggesting that a distinct mechanism for loading FANCD1 is necessary to unwind damaged DNA molecules [20]. FANCD1 is sensitive to backbone perturbations by both

polyglycol linkage or abasic sites [20]. The sensitivity to backbone perturbation arose from sequestration of FANCD1 protein [20].

2.4. Structure-function analysis of FANCD1

Mutational analysis revealed the importance of several amino acids in FANCD1 function. The lysine residue at position 52 (K52) of FANCD1 is conserved among numerous ATPases and helicases, and a substitution renders FANCD1 incapable of hydrolyzing ATP [5]. The K52R mutant of FANCD1 acts as a dominant negative [5]. Two missense mutations, P47A and M299I, were observed in the helicase domain of FANCD1 in two different breast cancer patients that were negative for mutations in BRCA1 and BRCA2 [5]. The P47A mutant is important for FANCD1 stability, shows no detectable ATPase activity [5,11], and has only weak helicase activity on forked duplex structures [20]. The M299I mutant of FANCD1 shows an elevated ATPase activity *in vitro*, but its helicase activity towards M13 DNA that contains both single and double-stranded DNA is similar to that of wild-type FANCD1 [11].

A nuclear localization signal from residues 158-175 is essential, since deletion of this motif results in defective nuclear localization [21]. Further, a rare R173C polymorphism observed in the nuclear localization signal impairs FANCD1 translocation to the nucleus [21]. Phosphorylation of FANCD1 at Ser-990 is important for its interaction with BRCA1 [22]. Adjacent amino acids, Pro-991 and Phe-993, are also essential for binding to BRCA1 and are required for phosphorylation of FANCD1 at Ser-990 [22]. Lysines 141 and 142 are required for direct interaction of FANCD1 with MLH1 [23].

2.5. Role of FANCD1 in interstrand crosslink repair

FANCD1, like other FA proteins, works to resolve ICLs. Human cells that either completely lack, or which have reduced FANCD1 protein as a result of siRNA knockdown, have increased sensitivity to DNA-crosslinking agents, such as MMC [6,8,23]. DT40 cells that lack FANCD1 are extremely sensitive to both cisplatin and MMC [12]. Similarly, *dog-1* mutants of *C. elegans* are sensitive to ICL-inducing agents like ultraviolet A-activated trimethyl psoralen (TMP plus UVA), nitrogen mustard, and cisplatin [13]. The ICL repair function of FANCD1 is independent of BRCA1, since a human FANCD1 that lacks the BRCA1-interacting domain corrects the cisplatin sensitivity of DT40 cells [12]. Also, S990A mutants of FANCD1, which do not interact with BRCA1 when expressed in FA-J cells, correct the sensitivity to MMC [23]. The ICL repair function of FANCD1 is MLH1-dependent [23]. FANCD1 is physically linked to MutLα complex (a heterodimer of MLH1 and PMS2). The helicase domain of FANCD1 binds directly to MLH1, and PMS2 contributes to the FANCD1-MLH1 interaction *in vivo* [23]. How MutLα cooperates with FANCD1 in ICL repair is unknown? It has been proposed, however, that the MutLα complex facilitates the repair process by mobilizing or regulating FANCD1 helicase activity, and thus unwinding of DNA, in the vicinity of damaged DNA [23].

It is unclear whether FANCD1 acts in the same genetic pathway with the FA core complex proteins and FANCD2 to repair ICLs. The loss of FANCD1 has no effect on FANCD2 monoubiquitination in any of the species examined, which suggests that FANCD1 acts in a manner either parallel to, or downstream from, FANCD2 in the FA pathway. *Fancj;fancc* double mutants in DT40 cells are more sensitive than either of the single mutants, and this places FANCD1 in an additional pathway that is independent of the core complex [12]. However, the *fcd-2/dog1* double mutant shows similar sensitivity to single mutants in *C. elegans*, placing FANCD1 downstream of FANCD2 in the same pathway [13]. These observed differences could be a consequence of using different genes (FANCC vs. FANCD2) or cell systems (DT40 vs. *C. elegans*), or FANCD1 may simply function in ICL repair by two different pathways in DT40 cells but not in *C. elegans*.

ICLs are highly toxic lesions that are usually detected during replication as a result of stalling of the replication fork. While it is clear that FANCD1 functions in ICL repair, the specific role has not been defined. It has been proposed that ICLs are unhooked by the MUS81-EME1 and XPF-ERCC1 endonucleases. This unhooking requires unwinding of parental DNA to create a substrate for incision. FANCD1 may help unwind parental DNA and create the substrate, or FANCD1 may unwind the short adducted oligonucleotides after incision [24]. Alternatively, FANCD1 might promote loading of a translesion synthesis polymerase to stalled replication forks by displacing unwanted DNA or proteins [1].

2.6. Role of FANCD1 in BRCA1-dependent double-strand break repair

Several lines of evidence suggest that FANCD1 plays a role in DNA double-strand break (DSB) repair in mammalian cells that is dependent on BRCA1. First, FANCD1 contains a BRCA1 interacting region from residues 976-1006 that harbors a Ser990-X-X-Phe motif required for interaction with BRCA1. BRCA1 is a major player in the repair of DSBs [5,22]. Second, overexpression of the FANCD1 K52R mutant acts as a dominant negative and results in delayed DSB repair [5]. Third, using an I-SCE assay that measures repair of defined genomic DSBs, cells depleted of FANCD1 with a siRNA have defects in DSB repair [8]. Fourth, cells expressing FANCD1 shRNA show a delay in the resolution of DSBs as evidenced by a delay in clearing the ionizing radiation (IR)-induced γ -H2AX foci that are formed in response to DSBs [25]. Fifth, FANCD1 is phosphorylated and remains bound to BRCA1 after the generation of DSBs using IR [25]. Sixth, in response to IR, BRCA1 foci are diminished and delayed in FANCD1-deficient cells, but FANCD1 overexpression corrects the defect [25]. However, the functions of FANCD1 and BRCA1 differ with respect to Rad51 foci. BRCA1-deficient cells show loss of Rad51 foci, while FANCD1-deficient cells show intact Rad51 foci [8].

The evidence presented above suggests a role for FANCD1 in DSB repair in mammalian cells. However, studies of chicken FANCD1 (chFANCD1) and *C. elegans* FANCD1 (DOG-1) show little evidence to support a role for FANCD1 in BRCA1-dependent DSB repair [12,13]. Both chFANCD1 and DOG-1 lack the Ser990-X-X-Phe motif required for BRCA1 binding [12,13]. In fact, DOG-1 fails to bind BRC-1 (the *C. elegans* homolog of BRCA1) in either yeast two-hybrid or pull-down assays [13]. Further, chicken DT40 cells that lack FANCD1 and DOG-1 mutants in *C. elegans* do not show sensitivity to IR [12,13]. Also, using an I-SCE assay to measure defined DSB repair, it was shown that chFANCD1 is not required for BRCA1-dependent repair of double-strand breaks in DT40 cells [12].

2.7. Role of FANCD1 in resolving G4 DNA

Mono G tracts are guanine-rich regions in nucleic acids that have the potential to form G-quadruplex DNA, also known as "G4 DNA" [26–29]. The human genome contains more than 300,000 predicted G4 sites [26,27]. G4 sites have the potential to block DNA replication and are mutagenic [26,30]. Cells that are deficient in FANCD1 accumulate deletions at genomic sequences with a G4 DNA signature [29]. Also, deficiency of dog-1 in *C. elegans* shows fragile G4 DNA sites [26,30]. Human FANCD1 efficiently unwinds G4 DNA in a 5'-3' direction in an ATP hydrolysis-dependent manner *in vitro*, and also unwinds a variety of G4 DNA structures such as those observed in telomeres and Fragile X triplet repeats [28,29]. FANCD1 also unwinds two-stranded G2 tetraplex structures observed at the telomeres of some species. The unwinding of G4 DNA by FANCD1 is stimulated by RPA, but is inhibited by MSH6 and telomestatin [28]. FANCD1-depleted cells are sensitive to telomestatin and show elevated DNA damage and apoptosis [28]. Based on these findings, it appears that replication stalls when the replication fork approaches a G-rich region that has a propensity to form G4 DNA. In such cases, FANCD1 unwinds the G4 DNA, thus allowing the replication fork to proceed. In the absence of FANCD1, the G4 structure is not resolved and this can lead to deletions. Thus, FANCD1 functions to preserve genomic stability through the resolution of G4 secondary structures formed in

genomic G/C tracts. This function of FANCI, to maintain genomic G-rich tracts, appears to be independent of its function in the FA pathway to repair ICLs [26,29].

2.8. Role of FANCI during the cell cycle

FANCI is expressed at stable levels throughout the cell cycle [17,22]. FANCI is phosphorylated at Ser-990 from S to G2-M phase, but not in G1, and its interaction with BRCA1 is dependent on phosphorylation at Ser-990 [31]. On the other hand, FANCI is phosphorylated at yet unidentified residues during G1, and this inactivates its ATPase activity. FANCI purified from G1 phase and treated with lambda phosphatase shows enhanced ATPase activity [17]. Further, FANCI is required for the timely progression of cells through S phase [17]. HeLa cells that are depleted of FANCI with a siRNA show a delay in entry into S phase following release from synchronization with aphidicolin. The FANCI-BRCA1 complex remains tightly associated with chromatin during S phase progression [17]. The DNA-dependent ATPase activity of FANCI is activated as cells enter S phase, suggesting that the ATPase activity of FANCI may be required for timely progression through S phase.

The G2-M checkpoint controls the entry of cells from G2 to mitosis. Normal cells treated with IR arrest in G2 phase and do not enter mitosis until repair is completed [22]. A549 cells depleted of FANCI, using a siRNA, fail to arrest in G2 but progress to mitosis, suggesting that FANCI is required for the IR-induced checkpoint [22]. Also, the interaction of FANCI with BRCA1 is required for the activation of this checkpoint [22]. The requirement for FANCI in this G2 checkpoint may account for the IR sensitivity of FA-J cells [25]. In contrast to the G2 checkpoint defect in response to IR in cells treated with siRNA, cells derived from patients that are deficient for FANCI protein show increased accumulation in G2/M when treated with DNA ICL agents [23]. The dog-1 mutant of *C. elegans*, and Chicken DT40 cells lacking FANCI, are not sensitive to IR, but their treatment with DNA interstrand crosslinking agents leads to cell cycle arrest and/or apoptosis [12,13]. The discrepancy observed between mammalian FANCI and Chicken/*C. elegans* FANCI could result from the absence of the BRCA1-interacting domain in Chicken and *C. elegans* FANCI homologs, since the interaction of FANCI with BRCA1 is necessary to activate the checkpoint in mammalian cells.

In summary, FANCI is a DEAH helicase which possesses 5'-3' DNA:DNA and DNA:RNA helicase activity. It can bind and unwind forked DNA and 5' flap structures that arise during DNA replication and repair. FANCI works with MutL α in the FA pathway to repair ICLs. The function of FANCI in mediating resistance to ICLs is conserved in *C. elegans*, and in chicken DT40 and mammalian cells. FANCI also resolves G4 DNA. Thus, FANCI appears to repair or resolve a variety of structures that block the replication fork.

3. FANCM and FAAP24

3.1. Discovery and nomenclature

FANCM was first isolated as the Fanconi anemia A-associated polypeptide with a mass of 250 kDa (FAAP250) using an antibody to FANCA protein, and was identified by mass spectrometry [32]. FAAP250 was subsequently found to be mutated in a patient with Fanconi anemia and was renamed FANCM [33]. FANCM is also known as HEF because of its homology to an archaeobacterial protein, helicase-associated endonuclease for fork-structured DNA (Hef) [34]. FAAP24, Fanconi anemia A-associated polypeptide of 24 kDa mass, was discovered by a database search for proteins with homology to the C-terminus of FANCM [35].

3.2. Structure and Homologs of FANCM and FAAP24

FANCM and FAAP24 are two novel members of the XPF-MUS81 family [33,36]. FANCM possesses two enzymatic domains: one homologous to a superfamily 2 (SF2) DEAH-box helicases, or DNA-stimulated ATPases, and the other homologous to the endonuclease domain of ERCC4 (XPF) [33,36] (Figure 1). FANCM and ERCC4 (XPF), an endonuclease essential for nucleotide excision repair, are the only two proteins in the human genome with a helicase-ATPase domain at the N terminus and an endonuclease domain in the C-terminus [33]. FANCM closely resembles the archeal protein Hef, since it has both helicase and endonuclease domains and can resolve stalled replication forks [33,34]. The helicase-ATPase domains of FANCM are highly conserved compared with other members of the SF2 family of helicases. However, the endonuclease domain is degenerate at several residues essential for its function as compared to other endonucleases, such as ERCC4 and the Mus81 family [33]. FAAP24 is a 253 amino acid protein and shares 20% identity (31% similarity) with the C-terminal 300 amino acid region of FANCM (Figure 1). Both FANCM and FAAP24 harbor two helix-hairpin-helix (HhH) motifs in tandem in their C-terminal regions [35]. The HhH is a domain of approximately 20 amino acids that is involved in sequence-nonspecific DNA binding and which are found in proteins that are essential for the repair of DNA structure [37]. Similar to the ERCC4 domain of FANCM, the FAAP24 nuclease domain is degenerate at several residues essential for endonuclease function [35].

3.3. Biochemical activity of FANCM and FAAP24

Full-length FANCM shows single-stranded DNA (ssDNA)- and double-stranded DNA (dsDNA)-stimulated ATPase activity *in vitro*. Mutation of a conserved lysine at position 117 (K117R) results in loss of dsDNA-stimulated ATPase activity *in vitro* [33,38]. The N-terminal (N₁₋₈₄₀) fragment of FANCM shows ATPase activity that is not dependent on the ssDNA, dsDNA, or fork shaped-DNA (fsDNA). Mutation of a conserved glycine in Walker box ATP-binding site at position 116 (G116A) also results in loss of ATPase activity *in vitro* [34]. FANCM has the ability to recognize certain DNA structural elements (Fig. 2). FANCM-N₁₋₈₄₀ binds strongly to ssDNA, but only weakly to fsDNA and does not bind dsDNA at all *in vitro* [34]. FANCM-C₁₇₂₇₋₂₀₄₈ shows little DNA binding activity for ssDNA, dsDNA, or splayed-arm DNA [35]. Also, full-length N-terminal FLAG-tagged FANCM purified from insect cells does not show high affinity for ssDNA or dsDNA, or 5' or 3' recessed dsDNA [39]. Full-length FANCM, or FANCM-N₁₋₇₅₄, shows strong specificity and affinity for branched DNA structures like Holliday junctions and replication forks [33,38,39]. FANCM-N₁₋₇₅₄ has higher affinity for Holliday junctions than for fsDNA [38]. FANCM can possibly translocate along duplex DNA, dissociate DNA triplexes, and promote branch migration of Holliday junctions and replication forks *in vitro* [33,39,40]. FANCM is able to dissociate D-loops which are free of, or bound with, RPA [40]. These results suggest that FANCM is a translocase that specifically recognizes and processes DNA substrates that mimic replication and repair intermediates.

The FAAP24-FANCM-C₁₇₂₇₋₂₀₄₈ heterodimer can bind ssDNA, dsDNA, fsDNA and shows a preference for the splayed-arm DNA [35]. The ability of FAAP24-FANCM to bind structures that arise during replication or repair suggests that FANCM and FAAP24 may act as a sensor of the blockage of DNA replication or of DNA damage. The FAAP24-FANCM-C₁₇₂₇₋₂₀₄₈ heterodimer does not exhibit any nuclease activity on splayed-arm, 3' flap DNA or fsDNA, suggesting that these domains are inactive [35].

3.4. Structure-function analysis of FANCM and FAAP24

SiRNA-mediated depletion of FANCM in HeLa cells does not result in defects in the stability or assembly of the FA core complex, but the FANCM deficient patient cell line, EUFA867, shows reduced stability of FANCA and FANCG and loss of interaction between FANCA and

FANCL [33,41]. Several reasons were proposed for these observed differences. Among these is the fact that an epithelial cell line (HeLa) was used in one study, and a lymphoblast cell line (EUFA867) was utilized in the other. Another possible reason for the observed differences is that FANCM was transiently inactivated in one study using a siRNA, while EUFA867 cells have long term loss of FANCM [41]. It was also proposed that FANCM may not be the only gene defective in EUFA867 cells [41]. Consistent with this possibility, our unpublished results suggest that another FA gene, in addition to FANCM, may be deficient in EUFA867 cells.

EUFA867 show markedly defective FANCD2 monoubiquitination, either with or without treatment with MMC or hydroxyurea (HU) [33]. Similarly, depletion of FANCM in HeLa cells, using a siRNA, results in reduction of FANCD2 monoubiquitination [33,38,41]. Considerable evidence suggests that the FA core complex participates in DNA repair reactions, apart from its role in the monoubiquitination of FANCD2 [38,42]. The helicase domain of FANCM is the only module within the core complex that has enzymatic activity on DNA. The N-terminal region of FANCM, which contains the helicase domain, has two functions. First, it is required for the monoubiquitination of FANCD2 [38]. Second, the helicase domain of FANCM plays an enzymatic role in DNA repair subsequent to the monoubiquitination of FANCD2. The K117A mutant of FANCM is proficient for FANCD2 monoubiquitination, but is associated with sensitivity to MMC [38]. Speculatively, the ATP-dependent translocase activity of FANCM may allow the core complex to sense and locate damaged DNA. The endonuclease domain of FANCM interacts with FAAP24, which is required for efficient monoubiquitination of FANCD2 and for the repair of ICLs [35]. Moreover, FANCM and FAAP24 are required for the stability of the other and for the other to be targeted to chromatin [41]. FANCM is constitutively associated with chromatin, either with or without exposure to MMC or to blockage of replication [41]. The localization of FAAP24, and other FA core complex proteins, to chromatin is defective in cells depleted of FANCM, suggesting that FANCM is required for chromatin localization of FA core complex proteins [41].

3.5. Role of FANCM-FAAP24 in DNA repair at stalled replication forks

FANCM has been proposed to work at stalled replication forks [38]. Yeast cells defective in MPH1, an ortholog of FANCM, are deficient in the HR-dependent error-free bypass pathway. DT40 cells lacking nuclease domain, or which lack full-length FANCM, are sensitive to the DNA intersstrand crosslinking agent, cisplatin [34]. These cells show higher chromosome breakage when exposed to another crosslinker, MMC [34]. The observation that double mutants of FANCM and FANCC in chicken DT40 cells show similar sensitivity to crosslinking agents as either of the single mutants suggests that FANCM works in the same pathway as FANCC and other FA proteins [34]. Like FANCM, FAAP24 is also predicted to repair stalled replication forks. FAAP24-depleted cells are sensitive to both MMC and cisplatin, and show a reduced level of monoubiquitinated FANCD2.

3.6. Role of FANCM-FAAP24 in ATR/Chk1 checkpoint signaling

The FANCM-FAAP24 dimer plays an important role in ATR/Chk1-mediated checkpoint signaling [43]. HCKL2, interacts with ATR and Chk1, has also been shown to interact with FANCM-FAAP24. Cells that are depleted of FANCM or FAAP24 show a phenotype similar to cells that lack ATR, HCKL2, or Chk1 [44]. This function of FANCM-FAAP24 in ATR/Chk1-mediated checkpoint signaling is independent of the FA core complex [44].

3.7. Phosphorylation of FANCM

FANCM is a phosphoprotein that becomes hyperphosphorylated in cells treated with MMC, HU, or cisplatin [33]. The kinase responsible for the phosphorylation of FANCM has not been identified, but ATR has been proposed as a candidate [33]. Phosphorylation of FANCM increases its binding affinity for chromatin [41]. The phosphorylation of FANCM is

dynamically regulated during the cell cycle [41], and is independent of the assembly and ubiquitin ligase activity of the FA core complex [33]. FANCM is moderately or extensively phosphorylated during S phase and mitosis, respectively, but is dephosphorylated after mitotic exit [41]. FANCM seems to negatively regulate the E3 ubiquitin ligase activity of the FA core complex, since there is no detectable FANCD2 monoubiquitination when phosphorylation of FANCM is maximal [41]. Since FANCM activity is regulated by phosphorylation/dephosphorylation events, FANCM has been proposed as a signal transducer that regulates the activity of the FA core complex [33,41].

In summary, like FANCI, FANCM is a DEAH family helicase. It possesses DNA translocase and branch point translocation activity, which may function to remodel stalled replication forks. The FANCM-FAAP24 heterodimer possesses intrinsic DNA-binding activity for structures that mimic stalled/processed replication forks. This dimer also acts to target the FA core complex to DNA, and the phosphorylation of FANCM regulates this activity. Finally, FANCI also has a role in ICL repair, but may function to process the DNA lesion at a distinct step downstream of FANCM-FAAP24.

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References

1. Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat Rev Genet* 2007;8:735–748. [PubMed: 17768402]
2. Patel KJ, Joenje H. Fanconi anemia and DNA replication repair. *DNA repair* 2007;6:885–890. [PubMed: 17481966]
3. Levitus M, Joenje H, de Winter JP. The Fanconi anemia pathway of genomic maintenance. *Cell Oncol* 2006;28:3–29. [PubMed: 16675878]
4. Tremblay CS, Huang FF, Habi O, Huard CC, Godin C, Levesque G, Carreau M. HES1 is a novel interactor of the Fanconi anemia core complex. *Blood* 2008;112:2062–2070. [PubMed: 18550849]
5. Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA, Livingston DM. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* 2001;105:149–160. [PubMed: 11301010]
6. Levitus M, Waisfisz Q, Godthelp BC, de Vries Y, Hussain S, Wiegant WW, Elghalbzouri-Maghrani E, Steltenpool J, Rooimans MA, Pals G, Arwert F, Mathew CG, Zdzienicka MZ, Hiom K, De Winter JP, Joenje H. The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. *Nat Genet* 2005;37:934–935. [PubMed: 16116423]
7. Levran O, Attwooll C, Henry RT, Milton KL, Neveling K, Rio P, Batish SD, Kalb R, Velleuer E, Barral S, Ott J, Petrini J, Schindler D, Hanenberg H, Auerbach AD. The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nat Genet* 2005;37:931–933. [PubMed: 16116424]
8. Litman R, Peng M, Jin Z, Zhang F, Zhang J, Powell S, Andreassen PR, Cantor SB. BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCI. *Cancer cell* 2005;8:255–265. [PubMed: 16153896]
9. Pyle AM. Translocation and unwinding mechanisms of RNA and DNA helicases. *Annu Rev Biophys* 2008;37:317–336. [PubMed: 18573084]
10. Rudolf J, Makrantonis V, Ingledew WJ, Stark MJ, White MF. The DNA repair helicases XPD and Fanci have essential iron-sulfur domains. *Mol Cell* 2006;23:801–808. [PubMed: 16973432]
11. Cantor S, Drapkin R, Zhang F, Lin Y, Han J, Pamidi S, Livingston DM. The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations. *Proc Natl Acad Sci U S A* 2004;101:2357–2362. [PubMed: 14983014]

12. Bridge WL, Vandenberg CJ, Franklin RJ, Hiom K. The BRIP1 helicase functions independently of BRCA1 in the Fanconi anemia pathway for DNA crosslink repair. *Nat Genet* 2005;37:953–957. [PubMed: 16116421]
13. Youds JL, Barber LJ, Ward JD, Collis SJ, O'Neil NJ, Boulton SJ, Rose AM. DOG-1 is the *Caenorhabditis elegans* BRIP1/FANCD1 homologue and functions in interstrand cross-link repair. *Mol Cell Biol* 2008;28:1470–1479. [PubMed: 18086896]
14. Menichini P, Linial M. SUV1 and BACH1: a new subfamily of mammalian helicases? *Mutat Res* 2001;487:67–71. [PubMed: 11595410]
15. Viaggi S, Gallerani E, Molina F, Nuesse M, Fronza G, Ottaggio L, Campomenosi P, Abbondandolo A, Menichini P. Partial characterization of SUV1, a new mammalian gene induced by UV-C and expressed during the S phase of the cell cycle. *Environ Mol Mutagen* 2001;37:76–84. [PubMed: 11170244]
16. Gupta R, Sharma S, Sommers JA, Jin Z, Cantor SB, Brosh RM Jr. Analysis of the DNA substrate specificity of the human BACH1 helicase associated with breast cancer. *J Biol Chem* 2005;280:25450–25460. [PubMed: 15878853]
17. Kumaraswamy E, Shiekhatar R. Activation of BRCA1/BRCA2-associated helicase BACH1 is required for timely progression through S phase. *Mol Cell Biol* 2007;27:6733–6741. [PubMed: 17664283]
18. Shin JH, Kelman Z. The replicative helicases of bacteria, archaea, and eukarya can unwind RNA-DNA hybrid substrates. *The Journal of biological chemistry* 2006;281:26914–26921. [PubMed: 16829518]
19. Gupta R, Sharma S, Sommers JA, Kenny MK, Cantor SB, Brosh RM Jr. FANCD1 (BACH1) helicase forms DNA damage inducible foci with replication protein A and interacts physically and functionally with the single-stranded DNA binding protein. *Blood*. 2007
20. Gupta R, Sharma S, Doherty KM, Sommers JA, Cantor SB, Brosh RM Jr. Inhibition of BACH1 (FANCD1) helicase by backbone discontinuity is overcome by increased motor ATPase or length of loading strand. *Nucleic Acids Res* 2006;34:6673–6683. [PubMed: 17145708]
21. Lei H, Vorechovsky I. BACH1 517C→T transition impairs protein translocation to nucleus: a role in breast cancer susceptibility? *Int J Cancer* 2003;104:389–391. [PubMed: 12569564]
22. Yu X, Chini CC, He M, Mer G, Chen J. The BRCT domain is a phospho-protein binding domain. *Science* 2003;302:639–642. [PubMed: 14576433]
23. Peng M, Litman R, Xie J, Sharma S, Brosh RM Jr, Cantor SB. The FANCD1/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. *EMBO J* 2007;26:3238–3249. [PubMed: 17581638]
24. Thompson LH. Unraveling the Fanconi anemia-DNA repair connection. *Nat Genet* 2005;37:921–922. [PubMed: 16132046]
25. Peng M, Litman R, Jin Z, Fong G, Cantor SB. BACH1 is a DNA repair protein supporting BRCA1 damage response. *Oncogene* 2006;25:2245–2253. [PubMed: 16462773]
26. Kruisselbrink E, Guryev V, Brouwer K, Pontier DB, Cuppen E, Tijsterman M. Mutagenic Capacity of Endogenous G4 DNA Underlies Genome Instability in FANCD1-Defective *C. elegans*. *Curr Biol* 2008;18:900–905. [PubMed: 18538569]
27. Maizels N. Dynamic roles for G4 DNA in the biology of eukaryotic cells. *Nat Struct Mol Biol* 2006;13:1055–1059. [PubMed: 17146462]
28. Wu Y, Shin-ya K, Brosh RM Jr. FANCD1 helicase defective in Fanconi anemia and breast cancer unwinds G-quadruplex DNA to defend genomic stability. *Mol Cell Biol* 2008;28:4116–4128. [PubMed: 18426915]
29. London TB, Barber LJ, Mosedale G, Kelly GP, Balasubramanian S, Hickson ID, Boulton SJ, Hiom K. FANCD1 is a structure-specific DNA helicase associated with the maintenance of genomic G/C tracts. *J Biol Chem*. 2008
30. Maizels N. Genomic stability: FANCD1-dependent G4 DNA repair. *Curr Biol* 2008;18:R613–614. [PubMed: 18644339]
31. Shiozaki EN, Gu L, Yan N, Shi Y. Structure of the BRCT repeats of BRCA1 bound to a BACH1 phosphopeptide: implications for signaling. *Mol Cell* 2004;14:405–412. [PubMed: 15125843]

32. Meetei AR, de Winter JP, Medhurst AL, Wallisch M, Waisfisz Q, van de Vrugt HJ, Oostra AB, Yan Z, Ling C, Bishop CE, Hoatlin ME, Joenje H, Wang W. A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat Genet* 2003;35:165–170. [PubMed: 12973351]
33. Meetei AR, Medhurst AL, Ling C, Xue Y, Singh TR, Bier P, Steltenpool J, Stone S, Dokal I, Mathew CG, Hoatlin M, Joenje H, de Winter JP, Wang W. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nat Genet* 2005;37:958–963. [PubMed: 16116422]
34. Mosedale G, Niedzwiedz W, Alpi A, Perrina F, Pereira-Leal JB, Johnson M, Langevin F, Pace P, Patel KJ. The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. *Nat Str Mol Biol* 2005;12:763–771.
35. Ciccia A, Ling C, Coulthard R, Yan Z, Xue Y, Meetei AR, Laghmani el H, Joenje H, McDonald N, de Winter JP, Wang W, West SC. Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. *Mol Cell* 2007;25:331–343. [PubMed: 17289582]
36. Ciccia A, McDonald N, West SC. Structural and Functional Relationships of the XPF/MUS81 Family of Proteins. *Annu Rev Biochem* 2008;77:259–287. [PubMed: 18518821]
37. Doherty AJ, Serpell LC, Ponting CP. The helix-hairpin-helix DNA-binding motif: a structural basis for non-sequence-specific recognition of DNA. *Nucleic Acids Res* 1996;24:2488–2497. [PubMed: 8692686]
38. Xue Y, Li Y, Guo R, Ling C, Wang W. FANCM of the Fanconi anemia core complex is required for both monoubiquitination and DNA repair. *Hum Mol Genet* 2008;17:1641–1652. [PubMed: 18285517]
39. Gari K, Decaillet C, Stasiak AZ, Stasiak A, Constantinou A. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. *Mol Cell* 2008;29:141–148. [PubMed: 18206976]
40. Gari K, Decaillet C, Delannoy M, Wu L, Constantinou A. Remodeling of DNA replication structures by the branch point translocase FANCM. *Proc Natl Acad Sci U S A* 2008;105:16107–16112. [PubMed: 18843105]
41. Kim JM, Kee Y, Gurtan A, D'Andrea AD. Cell cycle-dependent chromatin loading of the Fanconi anemia core complex by FANCM/FAAP24. *Blood* 2008;111:5215–5222. [PubMed: 18174376]
42. Matsushita N, Kitao H, Ishiai M, Nagashima N, Hirano S, Okawa K, Ohta T, Yu DS, McHugh PJ, Hickson ID, Venkitaraman AR, Kurumizaka H, Takata M. A FancD2-monoubiquitin fusion reveals hidden functions of Fanconi anemia core complex in DNA repair. *Mol Cell* 2005;19:841–847. [PubMed: 16168378]
43. Collis SJ, Ciccia A, Deans AJ, Horejsi Z, Martin JS, Maslen SL, Skehel JM, Elledge SJ, West SC, Boulton SJ. FANCM and FAAP24 function in ATR-mediated checkpoint signaling independently of the fanconi anemia core complex. *Mol Cell* 2008;32:313–324. [PubMed: 18995830]
44. Collis SJ, Barber LJ, Clark AJ, Martin JS, Ward JD, Boulton SJ. HCLK2 is essential for the mammalian S-phase checkpoint and impacts on Chk1 stability. *Nat Cell Biol* 2007;9:391–401. [PubMed: 17384638]

Figure 1. Domain structure of human FANCD1, FANCD2 and FAAP24

(A) FANCD1 contains an ATPase/helicase domain in its N-terminus. There is a nuclear localization signal (NLS) between helicase motifs I and Ia, and an iron-sulfur (Fe-S) domain between helicase motifs Ia and II. The S-X-X-F motif in the C-terminus is required for interaction with BRCA1. The MutL α interaction region overlaps with the helicase domain. (B) FANCD2 contains an active ATPase/helicase domain in its N-terminus and a degenerate ERCC4-like domain in its C-terminus. FANCD2 interacts with FAAP24 via its C-terminal endonuclease domain. FANCD2 contains two HhH domains in its c-terminus. (C) FAAP24 contains an ERCC4-like domain and interacts with FANCD2. FAAP24 also contains two HhH domains in its c-terminus.

Figure 2. Substrate preference of FANCI and FANCD1

Both FANCI and FANCD1 can bind to forked DNA and D-loop structures. FANCI does not bind Holliday junctions but FANCD1 can bind and unwind Holliday junctions *in vitro*.