Fanconi Anemia Complementation Group A (FANCA) Protein Has Intrinsic Affinity for Nucleic Acids with Preference for Single-stranded Forms*

Received for publication, October 19, 2011, and in revised form, December 13, 2011 Published, JBC Papers in Press, December 21, 2011, DOI 10.1074/jbc.M111.315366

Fenghua Yuan‡1, Liangyue Qian‡1, Xinliang Zhao‡, Jesse Y. Liu‡, Limin Song‡, Gennaro D’Urso§, Chaitanya Jain§, and Yanbin Zhang‡2

From the Departments of‡ Biochemistry and Molecular Biology and §Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, Florida 33136

The Fanconi anemia complementation group A (FANCA) gene is one of 15 disease-causing genes and has been found to be mutated in ~60% of Fanconi anemia patients. Using purified protein, we report that human FANCA has intrinsic affinity for nucleic acids. FANCA binds to both single-stranded (ssDNA) and double-stranded (dsDNA) DNAs; however, its affinity for ssDNA is significantly higher than for dsDNA in an electrophoretic mobility shift assay. FANCA also binds to RNA with an intriguingly higher affinity than its DNA counterpart. FANCA requires a certain length of nucleic acids for optimal binding. Using DNA and RNA ladders, we determined that the minimum number of nucleotides required for FANCA recognition is ~30 for both DNA and RNA. By testing the affinity between FANCA and a variety of DNA structures, we found that a 5′-flap or 5′-tail on DNA facilitates its interaction with FANCA. A patient-derived FANCA truncation mutant (Q772X) has diminished affinity for both DNA and RNA. In contrast, the complementing C-terminal fragment of Q772X, C772–1455, retains the differentiated nucleic acid-binding activity (RNA > ssDNA > dsDNA), indicating that the nucleic acid-binding domain of FANCA is located primarily at its C terminus, where most disease-causing mutations are found.

Fanconi anemia (FA)3 is an autosomal recessive or X-linked disorder characterized by bone marrow failure, developmental abnormalities, predisposition to cancer, and hypersensitivity to cross-linking agents (1–10). Thus far, 15 distinct genes have been identified to cause the deadly disease (6, 11–14). Eight of them are components of the FA core complex (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) that monoubiquitinates both FANCD2 and FANCI, a key event that initiates interstrand cross-link (ICL) repair (6, 8, 9, 15). Downstream of the FANCD2 and FANCI monoubiquitination are double-strand break repair proteins (i.e. FANCD1/BRCA2, FANCI/BRIP1, and FANCN/PALB2, FANCO/RAD51C, and FANCP/SLX4) that are critical to achieving successful ICL repair (6, 11–14, 16, 17). Although a deficiency in each FA gene shows similar clinical and cellular phenotypes, ~85% of FA patients present defective FANCA (~60%), FANCC (~15%), or FANCG (~10%) genes, and ~15% of them have defects in the other FA genes (8, 9, 18).

FANCA physically interacts with FANCG and the transcription factor HES1 within the FA core complex (19–25), which has been found to be localized to chromatin (26, 27). FANCA was also found to be involved in psoralen ICL-induced mutagenesis and in spontaneous and UV light-induced base substitution mutagenesis in human cells, implying its involvement in the mutagenic translesion synthesis of DNA damage (28, 29). Additionally, FANCA was shown to be required for recruiting FANCO/RAD51 and FANCD1/BRCA2 into mitomycin C-induced nuclear foci, indicating its role in the homologous recombination repair of ICLs (12, 30, 31). These data imply that FANCA may play multiple roles in DNA metabolism and transactions. However, because FANCA is not evolutionarily conserved and lacks identifiable domains/motifs, it remains largely unknown how FANCA is involved in these biological processes.

In this study, we report that purified human FANCA binds to nucleic acids with strong preference for single-stranded forms. This novel property of FANCA supports its role in DNA damage repair and should be helpful in understanding how FANCA and the whole FA core complex contribute to the maintenance of genomic stability.

**Background:** The FANCA (Fanconi anemia complementation group A) gene is mutated in ~60% of Fanconi anemia patients, but no biochemical activity has been identified.

**Results:** Purified FANCA binds to nucleic acids of a certain length through its C terminus.

**Conclusion:** FANCA has an intrinsic affinity for nucleic acids with a strong preference for single-stranded forms.

**Significance:** Unveiling the biochemical activity of FANCA is critical for understanding its functions in DNA repair.

---

†1 To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Gautier Bldg., Rm. 310, 1011 NW 15th St., Miami, FL 33136. Tel.: 305-243-9237; Fax: 305-243-3955; E-mail: yzhang4@med.miami.edu.

‡1 Both authors contributed equally to this work.

‡2 To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Gautier Bldg., Rm. 310, 1011 NW 15th St., Miami, FL 33136. Tel.: 305-243-9237; Fax: 305-243-3955; E-mail: yzhang4@med.miami.edu.

§ This work was supported, in whole or in part, by National Institutes of Health investigator research grant from the Florida Biomedical Research Program (to Y. Z.).

© 2012 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.

*This work was supported, in whole or in part, by National Institutes of Health Grant R01 HL105631 (to Y. Z.). This work was also supported by a new investigator research grant from the Florida Biomedical Research Program (to Y. Z.).

The abbreviations used are: FA, Fanconi anemia; ICL, interstrand cross-link; RPA, replication protein A; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; ssRNA, single-stranded RNA.

This is an open access article under the CC BY license.
FANCA Binds to Nucleic Acids

EXPERIMENTAL PROCEDURES

Expression and Purification of Human FANCA Proteins—cDNA for human FANCA was obtained by PCR amplification from a universal cDNA pool (BioChain Institute, Inc.). The full-length open reading frame of FANCA was sequenced and found to exactly match NCBI Reference Sequence NM_000135. Over-expression of hexahistidine-tagged FANCA was achieved in insect High Five cells using the Bac-to-Bac expression system (Invitrogen). Truncation mutants were produced through a PCR-based method (32). Expression of FANCA and its mutants was confirmed by Western blot analysis using a Pierce ECL kit. Antibodies against FANCA were kindly provided by the Fanconi Anemia Research Fund. Monoclonal antibody THE against the His6 tag (GenScript, Piscataway, NJ) was also used to confirm expression and subsequent purification. Upon expression of the recombinant proteins in insect cells, the cells were homogenized using a Dounce homogenizer to prepare extracts.

Wild-type FANCA and various truncation mutants were purified using a Hitrap Q-Sepharose Fast Flow column; a 5-ml Hitrap Blue column; a Mono S, Mono Q, and/or Superdex 200 gel filtration column (GE Healthcare); and/or a 2-ml high-resolution hydroxylapatite column (Calbiochem) and by tracing FANCA protein through SDS-PAGE and Western blotting. Protein concentration was determined using the Coomassie (Bradford) protein assay reagent (Pierce). The purified proteins were stored at −80 °C in aliquots. Purified replication protein A (RPA) was prepared as described previously (33).

EMSA—Oligonucleotides that were used to create single-stranded DNA (ssDNA; 61-mer), double-stranded DNA (dsDNA; 61 bp), the 5′-tail (30-mer for the single-stranded part and 31 bp for the double-stranded part), the 3′-tail (30 bp for the double-stranded part and 31-mer for the single-stranded part), the splayed arm (30 bp for the double-stranded part and 31-mer for the single-stranded part), the 5′-flap (with a 31-mer flap), the 3′-flap (with a 31-mer flap), the static fork (all arms are 30 bp), and the static Holliday junction (all four arms are symmetrically 30 bp) were adopted from a design by Gari et al. (34) with the same sequences. It should be noted that there is a 1-base 5′-overhang, originally designed to label the 3′-end of the substrates, on the double-stranded area of the 5′- and 3′-tails, splayed arm, and 5′- and 3′-flaps and on one arm of the static fork and Holliday junctions. Annealing was carried out in a water bath within ~5 h by slowly cooling from 85 °C to 20 °C. The quality of annealing was monitored by native gel electrophoresis. Proper annealing was verified by the mobility of a corresponding substrate, e.g. the static Holliday junction moves slowest because of its largest size. RNA was chemically synthesized by Integrated DNA Technologies, Inc. using the same sequence as the 61-mer ssDNA.

DNA binding EMSA analysis was performed as described previously (35) in a 10-μl reaction containing 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM EDTA, 1 mM DTT, 6% glycerol, 1 mM 5′-32P-labeled oligonucleotide substrates, and the indicated amounts of protein. The reactions were incubated at 18 °C for 45 min, followed by the addition of 4 μl of 50% (w/v) sucrose. The reaction mixtures were resolved by electrophoresis through a 4% nondenaturing polyacrylamide gel in 40 mM Tris acetate (pH 7.6) and 10 mM EDTA with 6% glycerol using the Owl P9DS electrophoresis system (Thermo Scientific). The setting was 100 V (~1.5 watts/gel) for 40 or 90 min as indicated. DNA substrates and shifted bands were visualized by autoradiography. Quantitation of the bands was performed using NIH ImageJ software.

Determination of Dissociation Constant of FANCA for Nucleic Acids—At steady state (equilibrium), Kd can be determined through the following equation: Kd = [A][B]/[AB], where [A], [B], and [AB] are the concentrations of FANCA, nucleic acids, and the FANCA-nucleic acid complex, respectively. Because the concentration of nucleic acids ([B]) was very low in our EMSA experiments (1 nM), the FANCA protein concentration ([A]) that shifted 50% of nucleic acids ([AB] = [B], thus [B]/[AB] = 1) was used to estimate Kd.

Determination of Minimum Length of Nucleic Acids Required for FANCA Binding Using DNA and RNA Ladders—DNA ladders (10-, 20-, 30-, 40-, 50-, 61-, 71-, and 99-mers) were created by mixing oligonucleotides with different sequences and labeling with 32P. RNA ladders (10-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, and 100-mers) was created as recommended by the manufacturer (Ambion) and labeled with 33P. 10-mer, 15-mer, and 30-mer oligonucleotides were the 3′-truncated forms of a 61-mer oligonucleotide: GACGCTGCCGAATTCTACCAGTTCCCTTGCTAGGACATCTTTGCCACCTGCAGGTTCA-CCC. Corresponding dsDNAs were prepared by annealing with the complementing oligonucleotides.

RESULTS

Purified Human FANCA Efficiently Interacts with Nucleic Acids—To study its biochemical properties, we overexpressed wild-type human FANCA protein in insect cells using the Bac-to-Bac expression system. As shown in Fig. 1B, we purified WT FANCA to near homogeneity. Purified WT FANCA migrated to a position corresponding to its calculated molecular mass of 164 kDa on SDS-polyacrylamide gel, indicating that it was the full-length protein. The identity of FANCA was further confirmed by Western blotting using a FANCA-specific antibody and an anti-His6 antibody (Fig. 1B).

Because FANCA has been shown to be involved in many steps of DNA repair, we reasoned that FANCA is likely to interact directly with DNA. Indeed, EMSA analysis by incubating increasing amounts of purified FANCA with 32P-labeled ssDNA or its dsDNA counterpart showed that FANCA bound to both ssDNA and dsDNA in a concentration-dependent manner (Fig. 1C). Intriguingly, FANCA had significantly greater affinity for ssDNA than for dsDNA. Using the paired t test, we determined the statistical significance of shifts between ssDNA and dsDNA (Fig. 1D). The p values for 16, 32, and 64 nM FANCA between ssDNA and dsDNA were 0.013, 0.031, and 0.014, respectively, which indicates a significant difference (p < 0.05). Furthermore, the Kd determined by protein titration showed that FANCA bound to ssDNA ~4-fold better than to dsDNA (11.1 nM for ssDNA and 42.3 nM for dsDNA) (Table 1).

Because FA is also a developmental disease and FANCA is involved in the regulation of gene expression (24, 25, 36), it is conceivable that FANCA could somehow be involved in RNA transactions, e.g. RNA stability, transcription, or translation, to
carry out its functions. When FANCA was incubated with a single-stranded RNA (ssRNA) oligonucleotide with the same sequence as the ssDNA, we indeed observed that FANCA possessed affinity for RNA. In fact, its affinity for ssRNA determined by \( K_d \) was significantly better than for ssDNA (2.8 nM for ssRNA and 11.1 nM for ssDNA) (Table 1). The p values determined by paired t test for 4 and 8 nM FANCA between ssDNA and ssRNA showed a significant difference (0.041 and 0.033 for 4 and 8 nM FANCA, respectively) (Fig. 1D).

**FANCA Requires Large Area of Nucleic Acids for Optimal Binding**—The preferential binding of FANCA to ssDNA resembles that of RPA, a well known ssDNA-binding protein that is involved in DNA replication, damage signaling, recombination, and repair (37–39). EMSA analysis of purified RPA (33) with ssDNA and dsDNA of different lengths indicated that RPA is very specific for ssDNA and forms protein-DNA filaments with increasing amounts of RPA and increasing sizes of ssDNA (multiple shifted bands in Fig. 2A, right panel). A high
TABLE 1

| Nucleic acid        | \( K_d \) (nM) |
|---------------------|-----------------|
| ssRNA               | 2.8 ± 0.49      |
| Splayed arm         | 10.2 ± 0.07     |
| ssDNA               | 11.1 ± 1.77     |
| 5'-Tail             | 12.6 ± 0.49     |
| 5'-Flap             | 12.6 ± 0.50     |
| 3'-Tail             | 16.9 ± 4.38     |
| 3'-Flap             | 17.2 ± 1.63     |
| Holliday junction   | 27.0 ± 6.29     |
| Static fork         | 35.2 ± 2.33     |
| dsDNA               | 42.3 ± 3.89     |

\( K_d \) was calculated based on the following equation: \( K_d = [A][B]/[AB] \) (see “Experimental Procedures”). Different nucleic acids are listed in order based on their \( K_d \) values.

FANCA Binds to Nucleic Acids

FANCA Binds to Nucleic Acids

**FA and RNA oligonucleotides in Fig. 2**.

- Different nucleic acids are listed in order based on their \( K_d \) values.

**Comparison**

- The dissociation constant (\( K_d \)) of FANCA for ssDNA was calculated as \( K_d = [A][B]/[AB] \), where [A] and [B] are the concentration of RNA and DNA, respectively.

**Stability**

- The stability of replication forks (40, 41) was maintained by FA proteins, which are involved in maintaining the stability of replication forks (40, 41).

**Interaction**

- FA proteins are involved in maintaining the stability of replication forks (40, 41), and they can interact with dsDNA through their DNA-binding domains.

**Conclusion**

- The DNA-binding activity of FA proteins is involved in maintaining the stability of replication forks (40, 41).

**DISCUSSION**

- FA proteins have been generally believed to be involved in the repair of DNA ICLs that block replication and transcription (1–9). Although there are at least 15 FA disease-causing genes, \( \sim 60 \% \) of the disease is caused by a deficiency in FANCA (8, 18). Thus far, the established DNA-interacting components...
(FANCM, FANCI, FANCD2, FANCJ, FANCO, and FANCP) account for only ~5% of FA, an observation that does not seem to support the role of FA proteins in DNA repair (9). Discovery of the robust nucleic acid-binding activity of FANCA is important because it explains not only how FANCA localizes to chromatin but also provides solid biochemical support for its role in DNA repair.

An intriguing observation of this study is the preferential binding activity of FANCA for ssDNA over dsDNA. It has been known that recruitment of the FA core complex to chromatin relies strictly on replication (40, 41). Therefore, it is likely that FANCA recognizes the exposed ssDNA in the stalled replication forks, which look like a 5'-flap structure or a splayed arm, and contributes to assembly of the FA core complex on the stalled forks. It would be interesting to further investigate whether and how the DNA-binding activity of FANCA facilitates repair of cross-links through damage recognition, translesion synthesis (29), and homologous recombination events (30).

Thus far, FANCM-FAAP24 is the only identified DNA-binding component in the FA core complex (42, 43). FANCM can remodel stalled replication forks through fork reversal and branch migration, thus stabilizing the stalled replication forks and providing temporal and spatial access for the damage to be repaired (34, 44). FANCM appears to be responsible for recruitment of the FA core complex to chromatin (21, 42, 43, 45–48). The monoubiquitinated FANCI-FANCD2 complex may also be recruited to chromatin through a FANCM-dependent mechanism (15, 49–51). However, unlike other factors in the core complex, FANCM is not required for the formation of the eight-subunit (but not the 10-subunit) core complex (45), and FANCM−/− cells are only partially deficient in damage-in-
duced FANCD2 monoubiquitination (52, 53). Fancm−/− knock-out mice further support that FANCM may have a stimulatory but not essential role in monoubiquitinating FANCD2 (54). Additionally, a direct interacting partner for FANCM-FAAP24 in the FA core complex has not been identified thus far, although FANCM-FAAP24 was originally identified through protein association in a FANCA-specific immunoprecipitation assay (5, 43, 55). FANCM−/− cells are sensitive to camptothecin, a topoisomerase inhibitor. Susceptibility to camptothecin is a unique feature identified only for FANCD1/BRCA2 and FANCN/PALB2 but not for components of the FA core complex (52). These observations suggest that FANCM may act downstream of FANCD2, and therefore, the upstream FA core complex may be recruited to DNA through other mechanisms, such as the DNA-binding activity of FANCA. Based on our observations, FANCA seems to be capable of recruiting the FA core complex to stalled replication forks through its ssDNA-binding activity and its preferential recognition of 5′-flap and splayed arm structures.

Another interesting insight to emerge from these studies is that FANCA has a higher affinity for ssRNA than for ssDNA. There is currently limited information to explain how the RNA-binding activity of FANCA could be linked to its functions. However, we think this activity may be physiologically relevant to RNA-related processes, such as transcription, translation, and RNA stability. First, the FA core complex has been reported to be involved in regulating gene expression through transcriptional (25) and post-transcriptional (56) mechanisms. Second, besides the nucleus, FANCA does localize to the cytoplasm (57–59), which supports a possible function in RNA metabolism. Third, FANCA has been shown to functionally interact with PKR (protein kinase regulated by RNA), a critical factor in translational control as well as regulation of cell proliferation and apoptosis (60). We speculate that, through its RNA-binding activity, FANCA may actively participate in these important biological processes. Further investigation into this issue should help us understand the unusually disproportional contribution of FANCA to FA.

The nucleic acid-binding domain of FANCA is located at its C terminus, where an imperfect leucine zipper and an ATR phosphorylation site are found (Fig. 1A) (61). It would be interesting to test whether the partial leucine zipper and the phosphorylation site have any effect on nucleic acid binding. It is very intriguing that, by analyzing the FANCA variants (1380 public entries as of March 23, 2011) available in the Fanconi Anemia Mutation Database, we found that ~90% of the reported disease-causing point mutations of FANCA are located at the C terminus (from amino acids 772 to 1455), where the nucleic acid-binding domain is identified, further supporting the idea that FANCA is likely to exert its functions through its affinity for nucleic acids.

Acknowledgments—We thank Drs. Murray Deutscher and Mary Lou King (University of Miami) for reagents. We are grateful to Dr. Wei Yang (NIDDK, National Institutes of Health) and Dr. Murray Deutscher for critical comments and discussion.

REFERENCES
1. de Winter, J. P., and Joenje, H. (2009) The genetic and molecular basis of Fanconi anemia. Mutat. Res. 668, 11–19
2. Joenje, H., and Patel, K. J. (2001) The emerging genetic and molecular basis
of Fanconi anemia. *Nat. Rev. Genet.* 2, 446–457
3. Kutler, D. I., Singh, B., Satagopan, J., Batish, S. D., Berwick, M., Giampietro, P. F., Hanenberg, H., and Auerbach, A. D. (2003) A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood* 101, 1249–1256
4. Niedernhofer, L. J., Lalai, A. S., and Hoeijmakers, J. H. (2005) Fanconi anemia cross-linked to DNA repair. *Cell* 123, 1191–1198
5. Thompson, L. H., and Hinz, J. M. (2009) Cellular and molecular consequences of defective Fanconi anemia proteins in replication-coupled DNA repair: mechanistic insights. *Mutat. Res.* 668, 54–72
6. Wang, W. (2007) Emergence of a DNA-damage response network consisting of Fanconi anemia and BRCA proteins. *Nat. Rev. Genet.* 8, 735–748
7. D’Andrea, A. D. (2010) Susceptibility pathways in Fanconi anemia and breast cancer. *N. Engl. J. Med.* 362, 1909–1919
8. Moldovan, G. L., and D’Andrea, A. D. (2009) How the Fanconi anemia pathway guards the genome. *Annu. Rev. Genet.* 43, 223–249
9. Yuan, F., Song, L., Qian, L., Hu, J. J., and Zhang, Y. (2010) Assembling an orchestra: Fanconi anemia pathway of DNA repair. *Front. Biosci.* 15, 1131–1149
10. Alter, B. P. (2003) Cancer in Fanconi anemia, 1927–2001. *Carcinogenesis* 24, 1680–1694
11. Keen, Y., and D’Andrea, A. D. (2010) Expanded roles of the Fanconi anemia core complex. *Hum. Mol. Genet.* 19, 425–440
12. Vaz, F., Hanenberg, H., Schuster, B., Barker, K., Wiek, C., Erven, V., Nevel, K., Endt, D., Kesterton, L., Autore, F., Fraternali, F., Freund, M., Hartmann, L., Grimwade, D., Roberts, R. G., Schaai, H., Mohammed, S., Rahman, N., Schindler, D., and Mathew, C. G. (2010) Mutation of the RADS1 gene in a Fanconi anemia-like disorder. *Nat. Genet.* 42, 406–409
13. Kim, Y., Lach, F. P., Desetty, R., Hanenberg, H., Auerbach, A. D., and Smogorzewska, A. (2011) Mutations of the SLX4 gene in Fanconi anemia. *Nat. Genet.* 43, 142–146
14. Cybulska, K. E., and Howlett, N. G. (2011) FANCP/SLX4: a Swiss army knife of DNA interstrand cross-link repair. *Cell Cycle* 10, 1757–1763
15. Smogorzewska, A., Matsuoka, S., Vinciguerra, P., McDonald, E. R., 3rd, Hurov, K. E., Luo, J., Ballif, B. A., Gygi, S. P., Hofmann, K., D’Andrea, A. D., and Elledge, S. J. (2007) Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell* 129, 289–301
16. Crossan, G. P., van der Weyden, L., Rosado, I. V., Lanegetic, F., Gaillard, P. H., McIntyre, R. E., Sanger Mouse Genetics Project, Gallagher, F., Ketten, M. L., Lewis, D. Y., Brindle, K., Arends, M. J., Adams, D. J., and Patel, K. J. (2011) Disruption of mouse Slek, a regulator of structure-specific nucleases, phenocopies Fanconi anemia. *Nat. Genet.* 43, 147–152
17. Stoepker, C., Hain, K., Schuster, B., Hillhorst-Hoostee, Y., Rooimans, M. A., Steltenpool, I., Oostra, A. B., Eirich, K., Korthof, E. T., Nieuwint, A. W., Jaspers, N. G., Bettecken, T., Joenje, H., Schindler, D., Rouse, J., and de Winter, J. P. (2011) SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype. *Nat. Genet.* 43, 138–141
18. Auerbach, A. D. (2009) Fanconi anemia and its diagnosis. *Mutat. Res.* 668, 4–10
19. Park, S. J., Ciccone, S. L., Beck, B. D., Hwang, B., Freie, B., Clapp, D. W., and Lévesque, G., and Carreau, M. (2008) HES1 is a novel interactor of the Fanconi anemia core complex. *Blood* 112, 2062–2070
20. Tremblay, C. S., Huang, F. F., Habib, O., Buard, V., Levesque, G., and Carreau, M. (2008) HES1 is a novel interactor of the Fanconi anemia core complex. *Blood* 112, 2062–2070
case FANCM.

45. Kim, J. M., Kee, Y., Gurtan, A., and D’Andrea, A. D. (2008) Cell cycle-dependent chromatin loading of the Fanconi anemia core complex by FANCM/FAAP24. *Blood* 111, 5215–5222.

46. Ciccia, A., McDonald, N., and West, S. C. (2008) Structural and functional relationships of the XPF/MUS81 family of proteins. *Annu. Rev. Biochem.* 77, 259–287.

47. Medhurst, A. L., Laghmani el, H., Steltenpool, J., Ferrer, M., Fontaine, C., de Groot, J., Rooimans, M. A., Scheper, R. J., Meetei, A. R., Wang, W., Joenje, H., and de Winter, J. P. (2006) Evidence for subcomplexes in the Fanconi anemia pathway. *Blood* 108, 2072–2080.

48. Ling, C., Ishiai, M., Ali, A. M., Medhurst, A. L., Neveling, K., Kalb, R., Yan, Z., Xue, Y., Oostra, A. B., Auerbach, A. D., Hoatlin, M. E., Schindler, D., Joenje, H., de Winter, J. P., Takata, M., Meetei, A. R., and Wang, W. (2007) FAAP100 is essential for activation of the Fanconi anemia-associated DNA damage response pathway. *EMBO J.* 26, 2104–2114.

49. Mosedale, G., Niedzwiedz, W., Alpi, A., Perrina, F., Pereira-Leal, J. B., Johnson, M., Langevin, F., Pace, P., and Patel, K. J. (2005) The vertebrate Hef ortholog is a component of the Fanconi anemia tumor suppressor pathway. *Nat. Struct. Mol. Biol.* 12, 763–771.

50. Garcia-Higuera, I., Taniguchi, T., Ganesan, S., Meyn, M. S., Timmers, C., Hejna, J., Grompe, M., and D’Andrea, A. D. (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell* 7, 249–262.

51. Montes de Oca, R., Andreassen, P. R., Margossian, S. P., Gregory, R. C., Taniguchi, T., Wang, X., Houghtaling, S., Grompe, M., and D’Andrea, A. D. (2005) Regulated interaction of the Fanconi anemia protein FANCD2 with chromatin. *Blood* 105, 1003–1009.

52. Singh, T. R., Bakker, S. T., Agarwal, S., Jansen, M., Grassman, E., Godthelp, B. C., Ali, A. M., Du, C. H., Rooimans, M. A., Fan, Q., Wahengbam, K., Steltenpool, J., Andreassen, P. R., Williams, D. A., Joenje, H., de Winter, J. P., and Meetei, A. R. (2009) Impaired FANCD2 monoubiquitination and hypersensitivity to camptothecin uniquely characterize Fanconi anemia complementation group M. *Blood* 114, 174–180.

53. Rosado, I. V., Niedzwiedz, W., Alpi, A. F., and Patel, K. J. (2009) The Walker B motif in avian FANCM is required to limit sister chromatid exchanges but is dispensable for DNA cross-link repair. *Nucleic Acids Res.* 37, 4360–4370.

54. Bakker, S. T., van de Vrugt, H. J., Rooimans, M. A., Oostra, A. B., Steltenpool, J., Delzenne-Goette, E., van der Wal, A., van der Valk, M., Joenje, H., te Riele, H., and de Winter, J. P. (2009) Fancm-deficient mice reveal unique features of Fanconi anemia complementation group M. *Hum. Mol. Genet.* 18, 3484–3495.

55. Meetei, A. R., Sechi, S., Wallisch, M., Yang, D., Young, M. K., Joenje, H., Hoatlin, M. E., and Wang, W. (2003) A multiprotein nuclear complex connects Fanconi anemia and Bloom syndrome. *Mol. Cell. Biol.* 23, 3417–3426.

56. Li, Y., and Youssoufian, H. (1997) MxA overexpression reveals a common genetic link in four Fanconi anemia complementation groups. *J. Clin. Invest.* 100, 2873–2880.

57. Näf, D., Kupfer, G. M., Suliman, A., Lambert, K., and D’Andrea, A. D. (1998) Functional activity of the Fanconi anemia protein FAA requires FAC binding and nuclear localization. *Mol. Cell. Biol.* 18, 5952–5960.

58. Kruyt, F. A., Waisfisz, Q., Dijkmans, L. M., Hermsen, M. A., Youssoufian, H., Arwert, F., and Joenje, H. (1997) Cytoplasmic localization of a functionally active Fanconi anemia group A-green fluorescent protein chimera in human 293 cells. *Blood* 90, 3288–3295.

59. Du, W., Li, J., Sipple, J., Chen, J., and Pang, Q. (2010) Cytoplasmic FANCA-FANCC complex interacts and stabilizes the cytoplasm-dislocated leukemic nucleophosmin protein (NPMc). *J. Biol. Chem.* 285, 37436–37444.

60. Zhang, X., Li, J., Sejas, D. P., Rathbun, K. R., Bagby, G. C., and Pang, Q. (2004) The Fanconi anemia proteins functionally interact with the protein kinase regulated by RNA (PKR). *J. Biol. Chem.* 279, 43910–43919.

61. Collins, N. B., Wilson, J. B., Bush, T., Thomashevski, A., Roberts, K. J., Jones, N. J., and Kupfer, G. M. (2009) ATR-dependent phosphorylation of FANCA on serine 1449 after DNA damage is important for FA pathway function. *Blood* 113, 2181–2190.