Oxidative stress/damage induces multimerization and interaction of Fanconi anemia proteins

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ABSTRACT

Fanconi anemia (FANC) is a heterogeneous genetic disorder characterized by a hypersensitivity to DNA-damaging agents, chromosomal instability, and defective DNA repair. Eight FANC genes have been identified so far, and five of them (FANCA, C, E, F, G) assemble in a multinuclear complex and function at least in part in a complex to activate FANCD2 by monoubiquitination. Here we show that FANCA and FANCG are redox-sensitive proteins that are multimerized and/or form a nuclear complex in response to oxidative stress/damage. Both FANCA and FANCG proteins exist as monomer under non-oxidizing conditions, while they become multimers following H$_2$O$_2$ treatment. Treatment of cells with oxidizing agent not only triggers multimeric complex of FANCA and FANCG in vivo, but also induces the interaction between FANCA and FANCG. N-ethylmaleimide treatment abolishes multimerization and interaction of FANCA and FANCG in vitro. Together, we conclude that FANCA and FANCG uniquely respond to oxidative damage by forming complex(es) via intermolecular disulfide linkage(s), which may be crucial in forming FANC complex and their function.
INTRODUCTION

Fanconi anemia (FANC) is an autosomal recessive disorder characterized by chromosomal instability and defective DNA repair, and FANC-deficient cells exhibit extreme sensitivity towards oxygen and DNA crosslinking agents such as diepoxybutane and mitomycin C (1-3). The gene products of eight complementation groups of FANC have been identified and cloned (FANCA, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, and FANCL) (1-3). Mutations in any of eight different genes lead to FANC disease, a degree of genetic heterogeneity comparable to that of other DNA repair disorders, suggesting that each group represents a distinct protein.

FANCA and FANCG proteins are part of a large nuclear protein complex required for their function, and the disruption of this complex results in the specific cellular and clinical phenotype common to most FANC complementation groups (4). FANCA gene encodes a 162-kDa phosphoprotein and its phosphorylation correlated with FANCA/FANCC protein accumulation in the nucleus (5). FANCA mutant cells isolated from a FANC patient was defective in its phosphorylation and failed to bind to FANCC. Furthermore, a mutant FANCA protein failed to complement the MMC sensitivity of FANCA/-/- cells, suggesting that FANCA phosphorylation may be involved in FANCC interaction, nuclear localization of FANCA, or its function in crosslink repair. FANCG gene encodes a 65-kDa protein and was identified as human XRCC9. XRCC-9 (FANCG) complements the Chinese Hamster Ovary (CHO) mutant UV-40 cell line that is hypersensitive to UV, ionizing radiation, simple alkylating agents, and DNA cross-linking agents (6, 7). The mutant cells also show a high level of spontaneous chromosomal aberrations that can be fully corrected by introduction of XRCC9 cDNA transformants (7). A possible
involvement of FANC proteins in DNA repair was strengthened by recent findings on the interaction of FANCD1 with BRCA1 following DNA damage (8). FANCD1 is identical to BRCA2 gene and is unique among FANC genes in that it is essential for the formation of Rad51 foci in response to ionizing radiation (9), suggesting that it may be involved in homologous recombination-mediated strand break repair.

Cells lacking FANC gene showed hypersensitive phenotype following H2O2 treatment, suggesting a role for FANC proteins in redox signaling and repair of oxidative DNA damages (10-13; data not shown). Interaction between FANCA and FANCG was well established by communoprecipitation, cellular localization, and yeast two hybrid analysis (4, 14-18). Although the detailed functions of FANC proteins have yet to be determined, there is a growing consensus on the role for FANC proteins in DNA repair and damage signaling pathways. In this study, we investigated interaction of key FANC proteins following oxidative damage(s). We found that FANCA and FANCG are multimerized and/or form a complex via intermolecular disulfide linkage(s) in response to oxidative damage. Nuclear localization as well as FANCA-FANCG interaction occurs concomitantly with monoubiquitination of FANCD2 upon H2O2 treatment, suggesting that FANCA-FANCG interaction may be an early response to oxidative damage that plays a crucial role in DNA repair and damage signaling pathways.
MATERIALS AND METHODS

Antibodies and chemicals

Anti-FANCA and -FANCG antiserum (polyclonal) were generated in rabbits using immunogens peptides using FANCA residues [525-544 (ENMGLYEDLSSAGDITEPHS) and 1230-1249 (HFAIQVREENDRLKLD)] and FANCG residues [101-120 (ERVLETQEQQGPRL EQGLRE) and 604-622 (DRDAFLEERTSLKPSCDL)]. Anti-flag and anti-myc antibodies were obtained from Sigma Chemical Co. (St. Louis, MO), and anti-FANCD2 antibody (monoclonal antibody NB100-316) was from Novus Biologicals. N-ethylmaleimide (NEM), β-mercaptoethanol, and mitomycin C (MMC) were from Sigma, and DTT was from Roche Molecular Biochemicals. H₂O₂ was obtained from Fisher Scientific Co.

Cloning of FANCA and FANCG genes

The cDNA for human FANCG was subcloned into pcDNA6.2-DEST (Invitrogen) by PCR using the forward primer 5’-CACCACCATGGAGCAGAAGCTTATTTCGGAGGAAGACCTATCC CGCCAGACCACCTCTCTGTGGGC-3’ and the reverse primer 5’-TCACAGGTCACAATTGGG-3’. Both primers contain a CACC sequence for recombination into pcDNA6.2-DEST and the forward primer also encodes a myc tag. Positive clones were isolated and confirmed by DNA sequencing. Human FANCA cDNA was subcloned into pFlag (Kodak) at NotI and HindIII by PCR using the forward primer 5’-TCTGCGGCCGATGTCCGACT CGTGGGTCCCG-3’ and reverse primer 5’-TCTAAGCTTTCAGAAGAGATGAGGCTCCTG-3’. Positive clones were confirmed by restriction enzyme analysis and DNA sequencing. Positive clones (plasmids) were transfected into COS cells for expression. For GST-FANCG expression, human FANCG was subcloned into pAcG2T (PharMingen) at EcoRI by PCR using the forward primer 5’-CGGGAATTCATTCCATTCCGCAGACCACC-3’ and the reverse primer 5’-
CGGGAATTCCTACAGGTCACAAGAC-3’. Positive clones were confirmed by restriction analysis and DNA sequencing.

**Cell cultures & Preparation of cell extracts**

COS cells and HeLa cells were grown in Dulbecco's modified Eagle's Medium (DMEM) and Dulbecco's modified Eagle's media-F12 (DMEM/F12, GIBCO-BRL), respectively, which were supplemented with 10% fetal calf serum (GIBCO-BRL), penicillin (10 unit/ml, Sigma), and streptomycin (0.1 mg/ml, Sigma). For all experiments in this study, cells were in exponential growth phase. Cells were treated with H2O2 or MMC for 2 h at the indicated concentrations at 37 °C. After reagent exposure, cells were washed with phosphate-buffered saline and lysed directly on the 100 mm plates by adding 500 µl of cold lysis buffer (1% NP-40 in PBS in the presence of 1 mM Na3VO4, 1 mM NaF and Mammalian Protease Inhibitor Cocktail) to each and scraping rapidly with a cell scraper. The cell lysates were centrifuged for 30 min at 20,000 × g, and the supernatant (the NP-40 soluble protein fraction) was harvested. The pellet (the NP-40 insoluble protein fraction) was washed once with lysis buffer and dissolved with SDS- sample loading buffer. These soluble and insoluble proteins were immediately analyzed by electrophoresis/immunoblotting or immunoprecipitation.

**Protein Purification**

Anti-FLAG M2 agarose was used in purifying Flag-FANCA protein and used according to the manufacturer's (Sigma) instructions. The supernatants from transfected COS cell lysates were incubated at 4°C for at least 4 h with anti-FLAG M2 affinity gel (Sigma) that had been preequilibrated in PBS buffer. The beads were then washed three times with 1% NP-40 in PBS buffer. In the final step, the M2 column was eluted with 3 column volumes of 500 µg/ml FLAG
peptide. GST-FANCG proteins were expressed in Sf9 insect cells following recombinant baculovirus infection (19) and purified by glutathione-Sepharose resin (Amersham Biosciences) and by FPLC on a 1-ml HiTrap Q column (Amersham Biosciences) (20).

**SDS-PAGE and Western blot analysis**

Cell lysates (25 µg per lane) or purified proteins were were resolved on 6 to 8% SDS-polyacrylamide gels under reducing conditions (10 mM DTT or 1% β-mercaptoethanol) or under non-reducing conditions. Proteins were then transferred to polyvinylidene difluoride membrane, probed with an anti-FANCA or -FANCG antibody (a rabbit polyclonal IgG), -FANCD2 antibody (monoclonal mouse IgG, Novus) followed by horseradish peroxidase-conjugated secondary antibody. Proteins were visualized by using the ECL system (Amersham Biosciences).

**Immunoprecipitation**

Cell lysates were clarified by centrifugation in a microcentrifuge for 10 minutes at 8,000 x g at 4 °C. The supernatants were removed and incubated with ANTI-FLAG® M2 antibody for 2 hour at 4 °C. The mixture was then added to a 60 µl packed gel volume of protein G agarose affinity beads (pre-washed and equilibrated in lysis buffer) and incubated for an hour with mixing at 4°C. The beads were collected by centrifugation for 2 min at 2,000 x g and the supernatants were removed by aspiration. The pellets were washed three times with 1 ml of lysis buffer per wash and collected by centrifugation as described. After washing, the affinity-bead pellets were each suspended in the presence of 30 µl of 2X Laemmli sample buffer (21), and analyzed by SDS- PAGE and immunoblotting.
RESULTS

Multimerization of FANC proteins under oxidizing conditions

Increasing evidence points to a role for FANC proteins in redox signaling and the repair of oxidative damages (22-24). As with this, cells lacking FANC genes showed hypersensitivity to H₂O₂ treatment (13; data not shown). Since several FANC proteins (FANCA, FANCC, and FANCG) contain unusually high number of cysteines, we wondered whether some of these residues were directly involved in redox regulation. To examine for redox regulation of FANC proteins, insect cells were transfected with recombinant baculovirus expressing GST-FANCG. Purified FANCG protein was analyzed on SDS-PAGE following a brief air oxidation and addition of reducing agent. Purified GST-FANCG migrated as multimers (>250-kDa in size) under oxidizing condition, while it migrated as a monomer with increasing amount of DTT (Figure 1A). Transition from monomer to multimer likely occurred through the formation of intermolecular disulfide linkage(s) on FANCG not the GST portion of the fusion protein since purified GST did not form multimers regardless of its redox status (Figure 1B). It is interesting to note that GST-FANCG (90-kDa) migrated more than one form of multimers in a range from 180-kDa to a much larger in size under oxidizing conditions (Figure 1A & 1C).

Redox-mediated transition of monomer/multimers of FANCA and FANCG expressed in COS cells

We next examined whether a redox potential affects monomeric/multimeric change of FANC proteins. COS cells were transfected with either a mammalian vector encoding FANCA, FANCG, or cotransfected with both FANCA and FANCG vectors. Cell lysates were examined for expression of FANCA and FANCG by Western blotting. A stable expression of FANCA was observed regardless of the presence of FANCG (Figure 2A lanes 1 & 2). On the other hand,
FANCG protein was stably expressed only in the presence of FANCA and was hardly detected without FANCA (Figure 2A, lane 3 vs lane 4), suggesting that FANCA is necessary for stable expression of FANCG in mammalian cells (25). Similar to an in vitro study with purified GST-FANCG (Figure 1A), FANCA multimers were converted to a monomer following incubation with increasing amount of DTT (Figure 2B), suggesting that multimerization of FANCA occurs through intermolecular disulfide linkage(s). FANCA and FANCG in COS cells exist as monomers under reduced condition (lane 1, Figure 2C), but converted to various sizes of multimer(s) under non-reducing condition (lane 2, Figure 2C).

**Treatment of cells with mitomycin C also induces multimeric complex of FANCA and FANCG in vivo**

FANC cells are highly sensitive to MMC, a DNA damaging agent that primarily induces interstrand crosslink DNA damage although it can cause oxidative damage as well as other types of DNA damages (intrastrand crosslink and monoadduct) (24, 26). We therefore examined whether MMC treatment also induces multimeric complex of FANC proteins *in vivo*. COS cells expressing both FANCA and FANCG were treated for 2 hours with either H₂O₂ or MMC and examined for multimerization of FANC proteins. Similar to H₂O₂ treatment, cells treated with MMC induced multimerization of FANCA (Figure 3A, top panel). FANCA multimers were converted back to monomer when the samples were subjected to SDS-PAGE in the presence of DTT (Figure 3A, middle panel). Concommitant analysis of FANCG also showed multimerization of FANCG in response to MMC treatment (Figure 3B), suggesting that multimerization of both FANCA and FANCG are induced by MMC.

**FANCA-FANCG interaction is significantly enhanced in response to H₂O₂ or MMC**
Since redox potential affects multimerization of both FANCA and FANCG, we examined whether it also influences FANCA-FANCG interaction. A stable interaction between FANCA and FANCG was observed in COS cells transfected with flag-FANCA and myc-FANCG (Figure 4A). Compared to non-treated control cells, COS cells treated with H$_2$O$_2$ induced multimerization of FANCA (Figure 4B; top panel, lanes 2-4 vs. lanes 5-7) as well as interaction between FANCA-FANCG, the latter was evidenced by significantly higher amount of FANCG co-precipitated with FANCA in a flag-antibody pulldown assay (Figure 4B; bottom panel, lanes 2-4 vs. lanes 5-7).

Since MMC treatment of cells induced multimerization of FANC proteins (Figure 3), we also examined whether MMC treatment affected the FANCA-FANCG interaction of (Figure 5). COS cells expressing flag-FANCA and myc-FANCG were treated with either H$_2$O$_2$ or MMC, and examined for co-precipitation of FANCG following immunoprecipitation of flag-FANCA using an anti-flag antibody (Figure 5). Association of myc-FANCG with flag-FANCA was significantly increased following treatment of cells with either H$_2$O$_2$ or MM-C (Figure 5A, lane 4 vs lanes 5-6). Most, if not all, of the FANCG co-precipitated with FANCA was in multimerized form of FANCG that migrated as >250 kDa in size in the absence of DTT (Figure 5B, lanes 5 & 6). We also observed that N-ethylmaleimide treatment abolishes multimerization and interaction of FANCA and FANCG in vitro (data not shown). Together, our results suggest that the FANCA-FANCG interaction may occur through intermolecular disulfide linkage(s) in a oxidative damage-dependent manner.
DISCUSSION

FANC cells exhibit a defect in damage-induced cell cycle arrest, a high level of chromosomal aberrations, and hypersensitivity to DNA crosslinking agents. FANC mutant cells from groups A, B, C, E, F, and, G were defective in forming the FANC protein complex (4, 14, 27) and, FANCG (XRCC9) protein is required for binding of the FANCA and FANCC proteins (4, 14), suggesting that FANC proteins form a large multiprotein complex which may be necessary for its function in damage signaling and repair. In this study we examined the interaction of FANC proteins in response to oxidative stress. Our study showed that oxidative DNA damage(s) induced multimerization and interaction of FANC proteins that concomitantly occurred with monoubiquitination of FANCD2.

Most redox reactions occur at the thiol group of cysteine, the prominent redox-active amino acid in proteins. FANCA and FANCG possess unusually high number of cysteines (38 and 16, respectively), while they do not have any cysteine-mediated motifs such as zinc finger, ring finger, etc. All three FANC proteins formed various multimers under oxidizing conditions in both native and denaturing PAGE, suggesting that formation of FANC complex is induced upon redox change via intermolecular disulfide linkage(s) between FANC proteins. Many transcription factors and DNA binding proteins harboring zinc-finger motif are regulated by redox-mediated change at intramolecular disulfide linkage(s) (28, 29). On the other hand, intermolecular disulfide linkage(s) also attributes the control of protein function through oxidation state of conserved cysteines. Glutathione forms a dimer via disulfide linkage upon redox change and is an important cellular defense against oxidative stress (30). Fos/Jun (31, 32) and NF-Y complex (33) are the transcription factors that form heterodimer for their function in
response to oxidative stress. It should be pointed out however some of the complexes involving disulfide linkage(s) such as gp70-Pr15E interaction may not be regulated by redox change since the complexes are resistant to treatment with reducing agent(s) (34). Given the fact that redox change facilitates physical interaction of FANCA with FANCG, the FANCA-FANCG interaction is likely controlled by intermolecular disulfide linkage(s). Identification of cysteine sites on FANCA and FANCG would be necessary to clarify the roles for the FANCA-FANCG complex in damage signaling and DNA repair.

Multimerization of FANC proteins or the FANCA-FANCG interaction is induced by either H$_2$O$_2$ or MMC treatment. H$_2$O$_2$ not only oxidizes protein, but also directly attacks DNA to oxidize bases on chromosomal DNA; the latter is usually repaired by base excision repair (22, 35). MMC however mostly induces inter- and intrastrand DNA crosslinks, although it can cause oxidative damage on non-DNA components. Resistance to MMC was shown to require direct interaction between FANCA and FANCG in the nucleus (15). In addition, cytoplasmic localization of FANCC is important for correction of MMC sensitivity in the FANCC phenotype, suggesting that MMC sensitivity may also be coupled to cytoplasmic defense mechanism against oxidative damage (36). It is not clear however whether multimerization of FANC proteins or the FANCA-FANCG interaction is limited to certain oxidative DNA damage(s), or a response to various DNA damaging agents.

Oxidative damage not only facilitates the FANCA-FANCG interaction, but also induces multimerization of FANC proteins (FANCA, FANCC, and FANCG) through disulfide linkage(s) (Figure 1 & data not shown). Although we do not know whether the remaining FANCA proteins (FANCE and FANCF) of the complex also form multimers under non-reducing conditions, a
possibility exists that the entire FANC complex may occur through disulfide linkage(s) following redox change. Functional implication of FANC multimers in response to oxidative stress is not clear, however, the earlier study suggested that FANCA dimerization is required for formation of FANC complex (18). It is possible that multimers of FANCG compared to the FANCG monomer preferentially interacts with FANCA (Figure 5B). Alternatively, multimerization of the FANC proteins has a unique role following oxidative damage that is different from those for the FANC complex.

FANCA-FANCG interaction is not only crucial for stability of FANCG (25; Figure 2A), but also essential for their function in the nucleus. FANCA-FANCG interaction concomitantly occurred with monoubiquitination of FANCD2 following oxidative damage (data not shown). Previous study showed that MMC treatment induced the FANC proteins in nuclear matrix and chromatin fractions. This induction occurs in wild-type cells and mutant FANCD2 cells but not in mutant FANCA cells (37). Cells lacking FANCA compared to wild-type cells showed a significantly lower level of FANCL (38). FANCL with its E3 ubiquitin ligase activity is likely a catalytic subunit required for monoubiquitination of FANCD2 (38). FANCA may play an essential role in monoubiquitination of FANCD2 via stabilization of FANCL and its E3 ubiquitin ligase activity in the nucleus. Both FANCA and FANCD2 are phosphoproteins and their phosphorylation appears to be induced by DNA damage (5, 39). It would be interesting to see whether the FANCA phosphorylation also affects interaction between FANCA and FANCG.

FANCA and FANCG, once forming a complex, likely serve as a core to facilitate the formation of a larger FANC complex containing at least five FANC proteins (A, C, E, F, and G) following oxidative damage (Figure 6). FANC complex stabilizes FANCL (formally known as PHF9) (38), a new
member of FANC essential for monoubiquitination and redistribution of FANCD2 to nuclear foci containing BRCA1 following DNA damage (38). FANC complex also interacts with BRCA2 (FANCD1), a protein involved in recombinational repair pathway through regulation of Rad51 activity (9, 40, 41). In addition, FANC complex is associated with Bloom Syndrome complex, a large complex containing RecQ helicase, replication protein A, and topoisomerase III (42). Further studies would be necessary to see whether the FANCA-FANCG interaction via disulfide linkage(s) is essential for functions of the FANC complex in damage-induced cell cycle checkpoint and DNA repair described above (Figure 6).
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FIGURE LEGENDS

Figure 1. Redox-mediated transition of FANCG between monomer and multimers.  
A. Purified GST-FANCG was oxidized on air at 4°C for overnight with opening the tube cap. Increasing amount of DTT was added to the samples before subjecting to SDS-PAGE and Western blot analysis.  
B. Purified GST protein was oxidized on air overnight at 4°C either in the absence (lane 2) or presence (lane 3) of 10 mM DTT and visualized by Coomassie staining following SDS-PAGE.  
C. Purified GST-FANCG in a buffer containing 1 mM DTT was incubated with various amounts of H₂O₂ and/or 2-mercaptoethanol (2-ME) followed by SDS-PAGE and Western blot using an anti-FANCG antibody. Monomer, dimer, and trimer positions were indicated by arrow according to their molecular weights.

Figure 2. Multimerization of FANCA and FANCG expressed from COS cells under non-reducing conditions.  
A. COS cells were transfected with mammalian vector (pCDNA) expressing flag-FANCA (lane 1), myc-FANCG (lane 3), or both (lanes 2 & 4). Cell extracts (60 µg) were prepared from each transfected cells and loaded onto SDS-PAGE and analyzed by Western blot using either an anti-FANCA (lanes 1-2) or –FANCG (lanes 3-4) antibody.  
B. Transition of FANCA multimers into monomer in the presence of DTT. Cell extracts from COS cells transfected with FANCA-expressing vector were oxidized on air overnight prior to addition of increasing amount of DTT. Samples were then analyzed by SDS-PAGE and Western blot using an anti-FANCA antibody.  
C. Transition of multimers of FANCA/FANCG proteins into monomer in the presence of DTT. Cell extracts were prepared from COS cells cotransfected with FANCA and FANCG. Extracts were incubated at 4°C for overnight either in the presence
(lane 1) or absence (lane 2) of 10 mM DTT prior to loading onto SDS-PAGE and Western analysis. Ku70 was used as an internal control for FANCA and FANC G in cell extracts.

**Figure 3.** COS cells treated with either H\textsubscript{2}O\textsubscript{2} or a DNA damaging agent (MM-C) induced multimeric complex of FANCA and FANC G \textit{in vivo}. COS cells co-transfected with flag-FANCA- and myc-FANC G-expressing vectors were treated with increasing amount of either H\textsubscript{2}O\textsubscript{2} or MMC for 2 hrs. After harvesting cells, extracts were prepared and analyzed for multimerization of FANCA (panel A) or FANC G (panel B) by SDS-PAGE. Where indicated (middle panel), 10 mM DTT was added before subjecting to SDS-PAGE. Ku70 in the bottom panel was used as an internal control.

**Figure 4.** Interaction between FANCA and FANC G \textit{in vivo} was significantly stimulated by H\textsubscript{2}O\textsubscript{2} treatment. Panel A. Cell extracts (500 µg) prepared from COS cells co-transfected with flag-FANCA and myc-FANC G-expressing vectors were incubated with an anti-flag antibody for 2 hrs for immunoprecipitation using protein A-Sepharose beads. Proteins bound to the beads were eluted with flag peptide, and analyzed in the presence of 10 mM DTT for FANCA and FANC G by SDS-PAGE and silver-staining (lane 1) and Western blot using anti-FANCA and -FANC G antibodies (lane 2). M represents the lane with protein markers. Panel B. COS cells transfected with flag-FANCA and myc-FANC G-expressing vectors were either mock-treated (lanes 1-4) or with 1 mM H\textsubscript{2}O\textsubscript{2} (lanes 5-7) for 2 hrs. Cell extracts (50 µg) were incubated with an anti-flag antibody for immunoprecipitation. Total immunoprecipitates (lanes 2 & 5) were further eluted with flag peptide to separate bound fraction to the beads (lanes 3 & 6) and the eluant (lanes 4 & 7). T, B, and E stand for total immunoprecipitates (T), bound fraction to the beads (B), and eluant/unbound fraction (E), respectively. Samples were analyzed by SDS-PAGE and
Western blot using an anti-FANCA (top panel) or anti-FANCG antibody (bottom panel). In the bottom panel, DTT (10 mM) was added to the sample prior to SDS-PAGE to fairly quantify myc-FANCG.

**Figure 5.** Interaction between FANCA and FANCG is induced *in vivo* upon treatment with either H$_2$O$_2$ or MMC. COS cells co-transfected with expression vectors for flag-FANCA and myc-FANCG, and treated for 2 hrs with none (lanes 3-4), 1 mM H$_2$O$_2$ (lane 5), or 1 µM MMC (lane 6). Cell extracts (200 µg) were incubated with an anti-Flag antibody for 2 hrs prior to addition of protein A-Sepharose beads for immunoprecipitation. Lanes 1 & 2 contained no cell extracts. Following immunoprecipitation, samples were analyzed by SDS-PAGE and Western blot using an anti-FANCG antibody (panels A & B) or an anti-FANCA antibody (panel C). For analysis, samples were treated with 10 mM DTT (panel A) or 0 mM DTT (panels B & C) for 1 hr just before subjecting to SDS-PAGE. In panel B, ** represent unidentified band that did not match to monomer form of myc-FANCG.

**Figure 6.** A proposed model for formation of FANC complex in response to oxidative stress/damage.
Figure 1B

DTT (10 mM)  -  +

1  2  3

(kDa)
30  50

GST
Figure 1C

| H₂O₂ (mM) | - | 1.0 | 50 | 100 | 100 | 100 | 100 |
|------------|---|-----|----|-----|-----|-----|-----|
| 2-ME (mM)  | - | -   | -  | -   | 15  | 50  | 100 |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |

(kDa)

FANCG (multimers)

FANCG (monomer)
Figure 2B

The figure shows a gel electrophoresis with different concentrations of DTT (mM) ranging from 0 to 100. The gel contains markers in kilodaltons (kDa) and bands labeled as flag-FANCA (multimers) and flag-FANCA (monomer).
Figure 3B

- DTT

- DTT

+ DTT

mhc-FANCG

Ku70
Figure 4A
Figure 4B

1 mM H₂O₂  

Flag Ab   -  +  +  +  +  +  +  

T  T  B  E  T  B  E  

1  2  3  4  5  6  7  

flag-FANCA (multimers)  
flag-FANCA (monomer)  
myc-FANCG (monomer)
Figure 5A & 5B

A. (+ DTT)

| Flag-Ab | none | - | + | - | + | H₂O₂ | MMC |
|---------|------|---|---|---|---|------|-----|
| 1       | -    |   |   |   |   |      |     |
| 2       | +    |   |   |   |   |      |     |
| 3       | -    |   |   |   |   |      |     |
| 4       | +    |   |   |   |   |      |     |
| 5       | -    |   |   |   |   |      |     |
| 6       | +    |   |   |   |   |      |     |

B. (- DTT)

| Flag-Ab | none | - | + | - | + | H₂O₂ | MMC |
|---------|------|---|---|---|---|------|-----|
| 1       | -    |   |   |   |   |      |     |
| 2       | +    |   |   |   |   |      |     |
| 3       | -    |   |   |   |   |      |     |
| 4       | +    |   |   |   |   |      |     |
| 5       | -    |   |   |   |   |      |     |
| 6       | +    |   |   |   |   |      |     |

(kDa): 250 160 105 75

**myc-FANCG**
Figure 5C

C. (- DTT)

| Flag-Ab | none | H$_2$O$_2$ | MMC |
|---------|------|----------|------|
| 1       | -    | +        | +    |
| 2       | +    | -        | -    |
| 3       | -    | +        | -    |
| 4       | +    | +        | +    |
| 5       | +    | +        | +    |
| 6       | +    | +        | +    |

(kDa)

flag-FANCA
Figure 6

Oxidative stress/damage

FANC core

Other FANC proteins

FANC complex

Repair factors

BRCA1
Oxidative stress/damage induces multimerization and interaction of fanconi anemia proteins
Su-Jung Park, Samantha L. M. Ciccone, Brian D. Beck, Byoung-Hoon Hwang, Brian Freie, D. Wade Clapp and Suk-Hee Lee

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