The Q Motif of Fanconi Anemia Group J Protein (FANCJ) DNA Helicase Regulates Its Dimerization, DNA Binding, and DNA Repair Function*

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Background: The conserved Q motif of RNA/DNA helicases has been structurally implicated in nucleotide binding; however, its biochemical functions are less certain.

Results: Mutagenesis of the Q motif in FANCJ DNA helicase perturbs dimerization, DNA binding, and catalytic function.

Conclusion: The Q motif is essential for FANCJ enzymatic activity and DNA repair.

Significance: The FANCJ Q motif is important for its biochemical and cellular functions.

The Q motif, conserved in a number of RNA and DNA helicases, is proposed to be important for ATP binding based on structural data, but its precise biochemical functions are less certain. FANCJ encodes a Q motif DEAH box DNA helicase implicated in Fanconi anemia and breast cancer. A Q25A mutation of the invariant glutamine in the Q motif abolished its ability to complement cisplatin or telomestatin sensitivity of a fancj null cell line and exerted a dominant negative effect. Biochemical characterization of the purified recombinant FANCJ-Q25A protein showed that the mutation disabled FANCJ helicase activity and the ability to disrupt protein-DNA interactions. FANCJ-Q25A showed impaired DNA binding and ATPase activity but displayed ATP binding and temperature-induced unfolding transition similar to FANCJ-WT. Size exclusion chromatography and sedimentation velocity analyses revealed that FANCJ-WT existed as molecular weight species corresponding to a monomer and a dimer, and the dimeric form displayed a higher specific activity for ATPase and helicase, as well as greater DNA binding. In contrast, FANCJ-Q25A existed only as a monomer, devoid of helicase activity. Thus, the Q motif is essential for FANCJ enzymatic activity in vitro and DNA repair function in vivo.

Helicases are molecular motors that couple the energy of nucleoside triphosphate (NTP) hydrolysis to the unwinding of structured DNA or RNA (1–3). Helicases are important in DNA metabolism and are implicated in the cellular processes of replication, DNA repair, recombination, transcription, chromosome segregation, and telomere maintenance (4, 5). The conversion of the energy derived from NTP hydrolysis into unwinding of double-stranded nucleic acids is coordinated by seven sequence motifs (I, Ia, II, III, IV, V, and VI), which are the feature motifs of superfamily-1 and -2 helicases (6). These motifs are usually clustered in a region of 200–700 amino acids called the helicase core domain. Because of the sequence of motif II (DEAD or DEAH or DEXH), the helicase family is also called the DEAD box (or DEAH or DEXH) protein family. Structural studies have demonstrated that the conserved helicase motifs are closely associated in the tertiary structure of helicases and are located in specific positions within the two so-called RecA-like domains coordinating ATP binding and hydrolysis to DNA unwinding (6–8).

The Q motif (Gly-Phe-Xaa-Xaa-Pro-Xaa-Pro-Ile-Gln) was first identified in DEAD box RNA helicases. It is located in the upstream sequence of motif I (i.e. Walker A box) and consists of a nine-amino acid sequence containing an invariant glutamine (Q) residue (9). Site-specific mutagenesis studies demonstrated that the Q motif controls ATP binding and hydrolysis in the yeast translation initiation factor elf4A, and in vivo analyses in yeast showed that the Q motif and upstream aromatic group are important for cell viability (9). The Q motif was also shown to be important for ATPase activity of a viral helicase, NS3 (10). Aromatic residues were proposed to aid in hydrophobic stacking interactions with the adenine (11). The Q motif not only regulates ATP binding and hydrolysis but also regulates the affinity of yeast translation initiation factor Ded1 for RNA substrates and its helicase activity (12). It was further proposed that the Q motif in elf4A and Ded1 RNA helicases functions as a molecular on-off switch for ATP hydrolysis and helicase activity (11, 12). A very recent study of the RNA helicase Hera examined the effect of the glutamic acid substituted for the invariant glutamine residue and the influence on the binding and hydrolysis of NTPs (13).
glutamine within the Q motif. This work suggested that the Q motif is responsible for sensing the nucleotide state of the helicase and establishing a stable interaction of the Walker A box (P-loop) with other helicase motifs, and this stabilization is required for catalytic competence (13).

The Q motif, also called motif O in RecQ helicases, is well known for being conserved among most SF1 and SF2 DNA helicases as well. Several crystal structures of DNA helicases have been determined that show that the conserved glutamine is structurally important for nucleotide binding. The crystal structures of the ATP-bound Escherichia coli UvrB (14), PcrA (15), RecQ (16), and UvrD (17) DNA helicases show that the conserved glutamine of the Q motif forms a bidentate hydrogen bond with the adenine base; however, its precise role(s) in the biochemical functions of DNA helicases is less well understood. For example, the Q motif of phage λ packaging motor was shown to be involved in DNA-motor interactions and governs its force-generating ability (18). Recently, the Q motif of the SWI2/SNF2 active DNA-dependent ATPase A domain was shown to be required for ATP hydrolysis but not for ATP binding (19).

Among the DNA helicases that contain a Q motif is FANCJ (also known as BACH1 or BRIP1), a member of the superfamily 2B DEAH box proteins (20). The identification of FANCJ mutations in early onset breast cancer patients (20, 21) and Fanconi anemia group J patients (22-24) implicates FANCJ as a tumor suppressor caretaker that ensures genomic stability. Although cellular evidence has begun to characterize the role of FANCJ helicase in human disease and DNA repair pathways (for review see Refs. 25, 26), its biochemical properties and mechanism of DNA unwinding remain to be thoroughly described. FANCJ is a DNA-stimulated ATPase, and mutation of the invariant lysine residue in the conserved motif I (Walker A box) in the helicase core domain of FANCJ abolishes its ATPase activity and DNA unwinding of simple partial duplex DNA substrates (27, 28). FANCJ requires a 5′ ssDNA tail to unwind both standard duplex (27, 28) and G-quadruplex (G4) DNA substrates (29, 30); however, the enzyme can also displace the invading strand of a D-loop DNA substrate in an ATP-dependent manner (28). FANCJ bears a conserved iron-sulfur domain (31, 32), and replacement of an alanine immediately adjacent to the fourth conserved cysteine within the Fe-S domain uncouples ATPase and translocase activities from unwinding of duplex or G-quadruplex DNA substrates (32). The functional importance of the other conserved motifs present in the FANCJ helicase core domain or its oligomeric state has not been experimentally evaluated.

In this work, we investigated the importance of the Q motif in FANCJ for its biochemical and cellular functions. Replacement of the invariant glutamine in the Q motif of FANCJ with an alanine impaired ATP hydrolysis, abolished FANCJ helicase activity, and eliminated its ability to catalytically strip protein bound to DNA. The FANCJ Q motif mutant was impaired for DNA binding but displayed ATP binding and a temperature-induced folding transition similar to wild-type FANCJ. The FANCJ Q mutant allele failed to rescue cisplatin or telomestatin sensitivity of a FA-J null cell line, and expression of the FANCJ Q mutant in a wild-type background exerted a dominant negative effect. Taken together, the biochemical and genetic analyses demonstrate that the Q motif in FANCJ is essential for its catalytic activity and DNA repair function in vivo.

**EXPERIMENTAL PROCEDURES**

**Plasmid DNA**—The Q motif mutations were generated by QuikChange site-directed mutagenesis kit according to the manufacturer’s instructions (Stratagene) using respective primers (supplemental Table S1) in the vectors of pVL1392 (27) and pEGFP-C2 (Clontech) that contain wild-type FANCJ or BLM. All desired mutations and constructs were confirmed by direct DNA sequencing using the purified plasmids.

**Recombinant Protein**—Baculovirus encoding FANCJ-WT, FANCJ-Q25A, or FANCJ-DQ with a C-terminal FLAG tag was used to infect High Five insect cells, and the recombinant FANCJ proteins were purified with modifications to a protocol previously described (27). Briefly, cell pellets were resuspended in buffer A (10 mM Tris HCl (pH 7.4), 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 10 mM NaPi, 10 mM NaPP). Cells were lysed in the presence of protease inhibitors (Roche Applied Science) for 45 min at 4 °C with mild agitation and centrifuged at 21,000 × g for 10 min at 4 °C. The supernatant was incubated with FLAG antibody resin (Sigma) for 2 h at 4 °C. The resin was washed twice with buffer B (50 mM Tris-HCl (pH 7.4), 500 mM NaCl, 0.5% Nonidet P-40) followed by buffer C (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% Nonidet P-40). FANCJ was eluted with 4 μM/ml 3× FLAG peptide (Sigma) in buffer D (25 mM Tris-HCl (pH 7.4), 100 mM NaCl, 10% glycerol, 0.1% Tween 20, 5 mM Tris (2-carboxyethyl) phosphine hydrochloride) (Sigma) for 1 h. FLAG-tagged FANCJ proteins were dialyzed for 2 h against buffer D using a dialysis tube with a 50-kDa molecular weight cutoff (Tuff-O-DIALYZERTM), and aliquots were frozen in liquid nitrogen and stored at −80 °C. The concentrations of wild-type and mutant FANCJ proteins were determined by Bradford (Bio-Rad) using bovine serum albumin (BSA) as a standard. Although there is protein-protein variability because protein assays detect protein-specific functional groups, the FANCJ protein concentrations determined by Bradford were in close agreement with those obtained from the 660 nm assay (Pierce) using BSA as a standard. Wild-type and mutant purified FANCJ proteins were analyzed for SYPRO Orange-based thermal unfolding (33) using a MX3005P Real time PCR cycler (Stratagene). The unfolding curves were recorded in triplicate from at least two different protein batches in their final storage buffer (see above).

**DNA Substrates**—PAGE-purified oligonucleotides used for the preparation of DNA substrates were purchased from Lofstrand Labs (Gaithersburg, MD) and are listed in supplemental Table S2. The forked duplex DNA substrate was prepared from the DC26 and TSTEM25 oligonucleotides as described previously (28). G4 DNA substrate was prepared from oligonucleotide TP (49-mer) as described previously (30).
Helicase Assay—Helicase assay reaction mixtures (20 μl) contained 40 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM MgCl2, 2 mM dithiothreitol, 2% glycerol, 100 ng/μl bovine serum albumin, 2 mM ATP, 10 fmol of the specified tetraplex or duple DNA substrate, and the indicated concentrations of FANCJ. Helicase reactions were initiated by the addition of FANCJ and incubated at 30 °C for 15 min unless otherwise indicated. Helicase reactions were terminated by addition of Stop buffer containing EDTA. Reaction products were resolved on non-denaturing 8 or 12% (19:1 acrylamide/bisacrylamide) polyacrylamide gels for the G4 and forked duplex substrates, respectively, and quantitated as described previously (30).

Electrophoretic Mobility Shift Assay (EMSA)—Protein/DNA binding mixtures (20 μl) contained the indicated concentrations of FANCJ and 0.5 nM of the specified 32P-end-labeled DNA substrate in the same reaction buffer as that used for helicase assays (see above) containing 2 mM ATP ±S or no nucleotide. The binding mixtures were incubated at 24 °C for 30 min after the addition of FANCJ. After incubation, 3 μl of Loading dye (74% glycerol, 0.01% xylene cyanol, 0.01% bromphenol blue) was added to each mixture, and samples were loaded onto native 5% (19:1 acrylamide/bisacrylamide) polyacrylamide gels and electrophoresed at 200 V for 2 h at 4 °C using 1 × TBE as the Running buffer. The resolved radiolabeled species were visualized using a PhosphorImager and analyzed with ImageQuant software (GE Healthcare).

ATP Hydrolysis Assay—ATP hydrolysis was measured using [γ-32P]ATP (PerkinElmer Life Sciences) and analysis by thin layer chromatography (TLC) on polyethyleneimine-cellulose plates (Mallinckrodt Baker). The standard reaction mixture (20 μl total volume) contained 40 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM MgCl2, 2 mM dithiothreitol, 2% glycerol, 100 ng/μl bovine serum albumin, 250 μM [γ-32P]ATP, 60 nM FANCJ protein and was incubated at 30 °C. The product was spotted onto a polyethyleneimine-cellulose TLC plate and resolved by using 0.5 M LiCl, 1 M formic acid as the carrier solvent. The TLC plate was exposed to a PhosphorImager cassette for 1 h and visualized using a PhosphorImager and analyzed with ImageQuant software.

For experiments to determine $K_{cat(ATP)}$, M13mp18 ssDNA was used at 2.1 nM; the concentration of ATP ranged from 31 to 8000 μM, and the reaction was incubated for 30 min. For determination of $k_{cat}$, the concentration of ATP was 8.5 mM, 5-μl aliquots were removed and quenched with 5 μl of 0.1 M EDTA at 0, 7.5, 15, 30, and 45 min, respectively. For determination of $K_{cat}$, the M13mp18 ssDNA concentration ranged from 0 to 10.6 nM, and the reaction was incubated for 30 min. The kinetic parameters were calculated by Enzyme Kinetics 1.3 (SigmaPlot, Systat Software Inc.) using the Michaelis-Menten equation. All experiments were repeated at least three times with S.D. indicated by error bars.

Size Exclusion Chromatography—Purified recombinant FANCJ protein (0.2 mg/ml, 0.5 ml) was applied to a 24-ml Superdex-200 size exclusion column (GE Healthcare) using an AKTA FPLC (GE Healthcare) that was equilibrated and eluted with 25 mM Tris-HCl (pH 7.5), 10% glycerol, 0.15 M NaCl, 1 mM EDTA, 0.5 mM DTT. The column was run at a rate of 0.1 ml/min, and 0.5-ml fractions were collected. Proteins were detected using a UV detector. Protein concentration was determined by the Bradford method using BSA as standard in a 96-well plate. Enzymatic activity of the FANCJ protein was examined with the standard protocol as described above. The column was calibrated using standard molecular mass markers containing blue dextran (2000 kDa), thyroglobulin (670 kDa), alcohol dehydrogenase (150 kDa), albumin (66 kDa), carbonic anhydrase (29 kDa), and aprotinin (6.5 kDa) (Sigma). The Stokes radii of FANCJ-WT and FANCJ-Q25A were determined from their elution profile. The $K_{av}$ for each protein was calculated from its elution profile and included and excluded volumes of the column. The Stokes radius of each protein was determined from a standard linear plot of log Stokes radius ($R_s$) versus $K_{av}$ using molecular weight markers.

Glyceral Gradient Sedimentation—An aliquot (0.1 ml) of purified FANCJ protein (0.2 mg/ml) was layered onto a 5-ml 15–35% glycerol gradient in a buffer containing 25 mM Tris-HCl (pH 7.5), 0.2 M NaCl, 1 mM EDTA, 1 mM DTT, 0.01% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride, 0.2 μg/ml aprotinin, 0.2 μg/ml leupeptin, 0.1 μg/ml antipain, 1 mM pepstatin A, and 1 mM benzamidine. After centrifugation at 45,000 rpm for 18 h at 4 °C in a Beckman SW 50.1 rotor, four drops were collected from the bottom of the centrifugation tube. The distribution of FANCJ proteins was determined by 10% SDS-PAGE that was subjected to a Western blot assay using anti-FLAG antibody. The protein concentration of the FANCJ peaks was determined by the Bradford method using BSA as standard in a 96-well plate. Helicase and ATPase activity of protein were examined with the standard protocol as described above. Thyroglobulin (669 kDa), ferritin (440 kDa), and catalase (232 kDa) were used as protein molecular markers (GE Healthcare). Sedimentation values of proteins were determined as described previously. The apparent molecular weight was calculated using the Monty-Siegel equation: $M_{app} = 6 \pi \eta N \rho s \text{Av}$ divided by $(1 - \nu \rho)$, where $\eta$ = viscosity of the medium, $N = \text{Avogadro’s number}$, $a = \text{Stokes radius}$, $s = \text{sedimentation coefficient}$, $\nu = \text{partial specific volume}$, and $\rho = \text{density}$ of the medium. The frictional ratio ($f/f_0$), which estimates the deviation of the protein from a globular structure, was determined by the equation $f/f_0 = a/(3\nu M/4\pi N)^{1/2}$.

Cell Lines—HeLa cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% L-glutamine at 37 °C in 5% CO2. For transient transfection, Lipofectamine2000 (Invitrogen) was used according provider’s protocol, and cells were used 48 h after transfection.

Chicken DT40 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 1% (v/v) chicken serum, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 1% (v/v) penicillin/streptomycin in a 5% CO2 incubator at 41 °C. Generation of fancj knock-out cell lines was described elsewhere (34). The DT40 cell lines were transfected with plasmids encoding GFP, GFP-FANCJ-WT, GFP-FANCJ-Q25A, and GFP-FANCJ-Q2Q and selected by G418 resistance using a procedure described previously (35).

Cell Survival Assay—Colony formation assays were carried out in medium containing 1.4% (w/v) methylcellulose and cisplatin or telomestatin at the dosages described previously (36).
**Immunofluorescence Studies**—Cells were fixed with formaldehyde (3.7%) at room temperature for 15 min. Fixed cells were washed four times with PBS and treated with 0.5% Triton X-100 solution (Sigma) at room temperature for 10 min. Cells were washed four times with PBS and blocked with 10% goat serum (Sigma) overnight at 4 °C. Indirect immunostaining was performed by first incubating cells with a mouse anti-γH2AX monoclonal antibody (1:500, Millipore) overnight at 4 °C. Following four washes in PBS with 0.1% Tween 20, cells were incubated with Alexa Fluor 488 goat anti-mouse IgG (1:400, Invitrogen) for 1 h at room temperature. Cells were washed four times with PBS containing 0.1% Tween 20 and coated with ProLong Gold anti-fade reagent containing DAPI (Invitrogen). Coverslips were placed on chamber slides, and cells were cured at room temperature in the dark for 24 h. Immunofluorescence was performed on a Zeiss LSM 510 META inverted Axiovert 200 M laser scan microscope with a Plan-Apochromat 63×/1.4 oil differential interference contrast objective. Images were captured with a CCD camera and analyzed using the LSM Browser software package.

**Statistical Analyses**—Statistical significance was calculated using a Student t test by assuming a two-sample equal variance with a two-tailed distribution.

**RESULTS**

**Sequence Alignment of the FANCJ Q Motif**—Although the functional importance of the conserved Q motif has been examined for a number of RNA helicases, its potential significance in DNA helicases has not been extensively studied. Inspection of the SF2 FANCJ DNA helicase in a variety of species reveals the presence of a Q motif upstream of motif I (supplemental Fig. S1A). A sequence alignment of this region of the FANCJ/Rad3 family of iron-sulfur domain helicases also demonstrated the presence of the conserved Q motif, marked by an invariant glutamine, a highly conserved upstream proline, and tyrosine (supplemental Fig. S1B). The RecQ family of SF2 DNA helicases has a Q motif with an invariant glutamine as well; however, the proline and tyrosine found in FANCJ-like proteins are absent in RecQ helicases (supplemental Fig. S1C). Interestingly, the Q motifs of FANCJ and UvrB, a prokaryotic SF2B helicase involved in nucleotide excision repair, are surprisingly similar and both contain the glutamine as well as the tyrosine and the proline at the same position in the Q motif (14). Clinical significance of the Q motif is evidenced by the identification of a Bloom syndrome-causing missense mutation of the invariant glutamine (Q672R) in BLM helicase (37).

**Genetic Characterization of FANCJ Q Mutant Alleles**—To characterize the functional importance of the Q motif in FANCJ, we replaced the glutamine with an alanine (FANCJ-Q25A) or deleted the nine amino acid Q motif (FANCJ-ΔQ) (Fig. 1A). The FANCJ-Q25A and FANCJ-ΔQ alleles were genetically characterized by assessing their ability to complement the loss of the wild-type protein in the response to certain agents that introduce DNA damage and replication stress. Previous studies have shown that FANCJ is required for resistance to DNA cross-linking agents via its role in DNA interstrand cross-link repair or a G-quadruplex-specific DNA-binding compound (telomestatin (TMS)) by its role in unwinding G-quadruplex DNA substrates. Because the functions of chicken and human FANCJ are conserved (38), we were able to perform genetic complementation assays with a deletion mutation in the fancj gene in DT40 cells to determine the ability of the FANCJ mutant proteins to function in these pathways. Plasmids encoding green fluorescence protein (GFP)-tagged FANCJ-WT, FANCJ-Q25A, or FANCJ-ΔQ were transfected into the chicken DT40 fancj−/− cell line. Western blot analysis demonstrated that the FANCJ Q mutant proteins were expressed at a similar level as FANCJ-WT in fancj null cells (Fig. 1B).

The results of cisplatin survival as measured by a colony formation assay demonstrated that the fancj−/− cells expressing FANCJ-Q25A or FANCJ-ΔQ were as sensitive to cisplatin as the fancj−/− vector cells (Fig. 1C). Compared with the fancj−/− FANCJ-WT cells, fancj null cells transfected with either empty vector or FANCJ Q mutations (Q25A and ΔQ) showed very similar reduction in percent survival as a function of cisplatin dose and were ~60-fold more sensitive at the highest concentration of cisplatin (1 μM). γH2AX foci, a marker of the double strand break, were elevated by ~3.5-fold in cisplatin-treated fancj−/− FANCJ-Q25A or fancj−/− FANCJ-ΔQ cells compared with the isogenic fancj−/− FANCJ-WT cells (Fig. 1, D and E). These results indicate that the FANCJ Q variant fails to render the cells resistant to the DNA cross-linking agent cisplatin as measured by cell viability and accumulation of double strand breaks.

Recently, we reported that FANCJ-depleted cells treated with the G4-interactive compound telomestatin (TMS) displayed impaired proliferation and elevated levels of apoptosis and DNA damage compared with siRNA control cells, providing evidence that G4 DNA is a physiological substrate of FANCJ (30). To assess the ability of the FANCJ-Q25A or FANCJ-ΔQ to function in the cellular response to TMS, we performed genetic complementation assays with the FANCJ-transfected cell lines used for the cisplatin resistance assays. As shown in Fig. 2A, expression of either FANCJ Q mutant protein in the fancj null cells failed to rescue TMS sensitivity as measured by a colony formation assay, whereas cells transfected with FANCJ-WT were resistant to TMS. γH2AX foci were elevated by ~3-fold in TMS-treated fancj−/− FANCJ-Q25A or fancj−/− FANCJ-ΔQ cells compared with the isogenic fancj−/− FANCJ-WT cells (Fig. 2, B and C), suggesting the accumulation of double strand breaks in cells expressing either FANCJ Q mutant protein.

**FANCJ Q Mutants Exert a Dominant Negative Effect when Expressed in a Wild-type Background**—Recently, our laboratory discovered that expression of a FANCJ mutant protein with a pathogenic A349P amino acid substitution in a wild-type background exerted a dominant negative effect on resistance to DNA cross-linking agents or TMS, indicating that the mutant protein interferes with normal DNA metabolism (39). Therefore, we examined if the FANCJ-Q25A or FANCJ-ΔQ mutant allele also exerted a dominant negative effect. Compared with the fancj+/− cells expressing GFP-tagged FANCJ-WT or transfected with empty vector, fancj+/− cells expressing FANCJ-Q25A or FANCJ-ΔQ showed reduced survival as a function of cisplatin dose and were ~10-fold more sensitive to the highest concentration of cisplatin (1 μM) (Fig. 3A). γH2AX foci were
elevated by ~4-fold in cisplatin-treated fancj-/- FANCJ-Q25A or fancj-/- FANCJ-ΔQ cells compared with the isogenic fancj-/- FANCJ-WT cells (Fig. 3, B and C). Similar results were observed when cells were treated with TMS (supplemental Fig. S2). We concluded that the FANCJ-Q mutants exert a dominant negative effect on cell survival or the accumulation of DNA damage after cisplatin or TMS treatment.

One possible mechanism for the dominant negative effect of FANCJ-Q25A is that the mutant protein competes with endogenous wild-type FANCJ for interaction with its protein partners (39). Because Western blot analysis demonstrated that GFP-tagged FANCJ-Q25A was expressed similar to FANCJ-WT, we wanted to determine whether the FANCJ-Q25A variant was associated with proteins known to interact with endogenous wild-type FANCJ. Co-immunoprecipitation experiments using nuclear extracts from HeLa cells demonstrated that TopBP1 and BRCA1, proteins previously shown to interact with FANCJ (20, 40–42), were pulled down by a GFP antibody from lysates...
of cells that expressed GFP-tagged FANCJ-WT or FANCJ-Q25A, but not GFP alone (Fig. 3D), suggesting that FANCJ-Q25A interacts with its protein partners in a manner similar to FANCJ-WT.

Consequences of Q Motif Mutations in FANCJ or BLM Proteins on DNA Damage-induced Foci Formation—Failure to restore cisplatin or telomestatin resistance in fancj<sup>-/-</sup> cells expressing either FANCJ Q mutant protein led us to explore reasons for the observed defects in vivo. Therefore, the ability of the mutant proteins to form DNA damage-inducible foci compared with FANCJ-WT was evaluated. Close inspection of individual DT40 cells by immunofluorescence microscopy demonstrated that GFP-tagged FANCJ-WT formed DNA damage-inducible foci when the corresponding transfected cells were exposed to a 1 μM concentration of the DNA cross-linking agent cisplatin, whereas GFP-tagged FANCJ-Q25A or FANCJ-ΔQ formed ~3- or 5-fold less foci, respectively, under the same conditions (Fig. 4, A and B).

To extend our observations made in chicken cells, we employed the human U2OS and HeLa cell lines for FANCJ localization studies. Both cell lines were transfected with plasmids encoding GFP, GFP-FANCJ-WT, GFP-FANCJ-Q25A, or GFP-FANCJ-ΔQ. Cells were treated with the DNA cross-linking agent mitomycin C (MMC) or the replication inhibitor hydroxyurea (HU). FANCJ-WT formed nuclear foci after either treatment (Fig. 4C), consistent with previous observations (32). Although FANCJ-Q25A or FANCJ-ΔQ mutant proteins localized to nuclei, they formed ~5-fold less foci compared with FANCJ-WT after treatment with MMC or HU (Fig. 4, C and D).

A Bloom syndrome-causing missense mutation (Q672R) has been identified in the conserved Q motif of BLM helicase (37).
To determine whether the Q motif of BLM is also important for localization, we generated two BLM missense mutations in the Q motif (BLM-Q672R and BLM-Q672A), and we expressed these GFP-tagged proteins in human U2OS and HeLa cells. GFP-BLM-WT formed large and bright foci after cellular exposure to HU; however, BLM-Q672R formed smaller sized foci, and BLM-Q672A formed foci similar to GFP-BLM-WT, but a significant fraction of the Q672R mutant was retained in nucleoli (Fig. 4E). These results suggest that a mutation in the conserved Q motif of BLM impairs BLM foci formation after replication stress, a finding that is consistent with that observed for FANCJ.

Biochemical Characterization of FANCJ Q Mutant Proteins—The inability of FANCJ-Q25A or FANCJ-ΔQ to substitute for wild-type FANCJ in the DNA damage response suggested that the mutant proteins were biochemically defective. To determine the nature of the defect(s) and gain insight into the role of helicase motif Q, the recombinant proteins were expressed using a baculovirus system and purified for characterization of their biochemical and physical properties. Both FANCJ-Q25A and FANCJ-ΔQ recombinant proteins expressed at a similar level in insect cells compared with FANCJ-WT as determined from quantitative Western blots. Similar amounts of FANCJ-Q25A/FANCJ-ΔQ protein compared with FANCJ-WT were

FIGURE 3. Expression of FANCJ Q mutant exerts a dominant negative effect on wild-type cells. A, cisplatin sensitivity of cells with indicated genotypes was evaluated by colony formation assay. B, γH2AX immunofluorescence staining of cisplatin-treated fancj+/+ cells transfected with plasmids encoding GFP, GFP-FANCJ-WT, GFP-FANCJ-Q25A, or GFP-FANCJ-ΔQ. DT40 cells were treated with 1 μM cisplatin for 12 h, followed by immunofluorescence detection. C, quantitative analyses of γH2AX foci shown in B. Data represent the mean of at least 100 cells counted with S.D. indicated by error bars. Using a Student’s T test for analysis of the γH2AX foci data, the differences in p values between fancj+/+ FANCJ-WT cells and fancj+/+ FANCJ-Q25A or fancj+/+ FANCJ-ΔQ were 0.004 and 0.003, respectively, indicating a significant difference (p < 0.01) for each. D, co-immunoprecipitation experiments using nuclear extracts from HeLa cells expressing GFP, GFP-FANCJ-WT, or GFP-FANCJ-Q25A and immunoprecipitated with GFP antibody, and the pulldown was detected with antibodies against TopBP1 and BRCA1.
found in the soluble fraction and could be purified in an identical manner as that used for FANCJ-WT (Fig. 5A). On average, similar yields of wild-type and mutant recombinant proteins (80 μg/1.2 × 10^8 insect cells) were obtained from the purifications. To rule out artifacts due to misfolding or instability, the purified recombinant FANCJ-Q25A protein was subjected to thermal unfolding studies and found to exhibit an unfolding transition similar to the wild-type protein (Fig. 5B). The melting temperatures (T_m (°C)) for FANCJ-WT and FANCJ-Q25A were 40.6 ± 1.3 and 41.8 ± 1.6, respectively.

Effect of FANCJ Q Motif Mutations on ATP Hydrolysis—We first examined the DNA-dependent ATPase activity of FANCJ-
Q25A and FANCJ-ΔQ compared with FANCJ-WT (Table 1). As a control, we included purified recombinant FANCJ-K52R, which was previously reported to be seriously compromised in its ATPase activity (27, 30, 41). Using covalently closed M13 single strand DNA as the effector molecule, we performed FANCJ ATPase assays as a function of ATP concentration (supplemental Fig. S3) and determined $K_m$ values of ATP hydrolysis for FANCJ-WT, FANCJ-Q25A, and FANCJ-ΔQ to be 0.89, 1.4, and 1.4 mM, respectively. Using an ATP concentration (8.5 mM) that was ~10-fold greater than the $K_m$ for FANCJ-WT, ATPase assays with FANCJ-WT, FANCJ-Q25A, and FANCJ-ΔQ yielded $k_{\text{cat}}$ values of 2636, 220, and 110 min$^{-1}$, respectively. For comparison, the $k_{\text{cat}}$ value for FANCJ-K52R was 5 min$^{-1}$, suggesting that both the Q mutant proteins retained a low but detectable level of ATPase activity. Based on these results, we suggest that FANCJ-Q25A and FANCJ-ΔQ bind ATP similar to FANCJ-WT but do not hydrolyze ATP as efficiently as FANCJ-WT. In the absence of DNA effector, ATP hydrolysis by FANCJ-WT was reduced by greater than 200-fold, consistent with the previous report that FANCJ is a DNA-stimulated ATPase (27). In the absence of DNA, FANCJ-Q25A displayed a $k_{\text{cat}}$ value similar to FANCJ-WT, whereas FANCJ-ΔQ had a slightly lower $k_{\text{cat}}$ that was comparable with FANCJ-K52R.

**Mutated Q Motif in FANCJ Abolishes Its Motor ATPase-dependent Functions**—We next examined DNA unwinding activity catalyzed by the FANCJ Q mutants and FANCJ-WT using a forked duplex DNA substrate (supplemental Table S1), which is efficiently unwind by FANCJ-WT (28). Neither the FANCJ-Q25A nor the FANCJ-ΔQ protein could unwind this type of substrate utilizing the conditions in which FANCJ-WT unwound the forked duplex DNA molecules to near completion (Fig. 6A).

Recently, we reported that FANCJ-WT unwinds 5’-tailed G-quadruplex DNA substrates even more efficiently than duplex DNA (30). Because G-quadruplex is a very different substrate than a forked duplex, we tested the ability of the FANCJ Q mutants to unwind a G-quadruplex (G4) DNA molecule and found that FANCJ-Q25A and FANCJ-ΔQ failed under conditions that FANCJ-WT efficiently unwound the G-quadruplex DNA molecule (Fig. 6B).

We also increased the incubation time (45 min) or protein concentration of FANCJ-Q25A or FANCJ-ΔQ up to 307 nM, but we failed to detect helicase activity on either forked duplex or G4 DNA substrates (data not shown). Thus, targeted mutation of the Q motif in FANCJ abolished its helicase activity on forked duplex or G4 DNA substrates, presumably due to the strong effect of the Q motif mutations on

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**TABLE 1**

| Protein       | $K_m$ (mM) | $V_{\text{max}}$ (μm/min) | $K_{\text{cat}}$ (μm/min) | $K_m$ (nM) | $K_{\text{cat}}$ (μm/min) |
|---------------|------------|---------------------------|--------------------------|------------|---------------------------|
| FANCJ-WT      | 0.89 ± 0.1 | 12 ± 2                    | 2600 ± 300               | 0.89       | 0.096 ± 0.04              |
| FANCJ-Q25A    | 1.5 ± 0.2  | 0.58 ± 0.2                | 220 ± 30                 | 0.58       | 2.5 ± 0.4                 |
| FANCJ-ΔQ      | 1.4 ± 0.2  | 0.58 ± 0.2                | 101 ± 30                 | 0.58       | 2.7 ± 0.6                 |
| FANCJ-K52R    | ND         | ND                        | ND                       | ND         | ND                        |

$K_m$ is the Michaelis constant, $V_{\text{max}}$ is the maximum velocity, and $K_{\text{cat}}$ is the catalytic constant. Values are expressed as the mean of triplicate experiments.

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FIGURE 5. Purity and thermal stability of recombinant FANCJ proteins. A, purified recombinant FANCJ-WT, FANCJ-Q25A, and FANCJ-ΔQ proteins were evaluated by their detected migration after SDS-PAGE on Coomassie-stained gels according to their predicted sizes. Two micrograms was loaded for each protein. M, marker. B, thermal stability of the FANCJ-Q25A mutant. Unfolding curves of FANCJ-WT and FANCJ-Q25A were analyzed in this study. Two independent preparations of FANCJ recombinant mutant or wild-type proteins were analyzed in triplicate. FANCJ-Q25A unfolds similar to FANCJ-WT as a function of temperature, indicating no negative influence on the protein fold. FANCJ-WT is colored in green; FANCJ-Q25A is colored in blue.

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DNA-stimulated ATPase activity. It has been suggested that the SF1 and SF2 helicases that lack this conserved glutamine might be nonspecific NTPases, as is the case with the viral helicase NS3 and the DEAH box helicases Prp2 and Prp16 (43). Thus, we tested different NTPs to assess if they might serve as an energy source for the helicase reaction; however, only ATP was utilized by FANCJ-WT, and none of the other NTPs were able to support helicase activity of FANCJ-Q25A or FANCJ-DQ (Fig. 6C).

Recently, we determined that FANCJ can use its motor ATPase function to disrupt protein-DNA interactions (60). The ability of FANCJ to displace protein bound to DNA can be experimentally tested by measuring streptavidin displacement from a biotinylated oligonucleotide in a reaction that is ATP-dependent (supplemental Table S2). We tested each FANCJ Q mutant to catalyze streptavidin displacement and found that neither mutant protein was active under conditions that FANCJ-WT efficiently removed streptavidin in a kinetic manner (Fig. 7A). We also tested different NTPs in the streptavidin displacement reaction, but we found that only ATP was utilized by FANCJ-WT, a result that was consistent with its helicase activity. Moreover, none of the other NTPs could support the ability of FANCJ-Q25A or FANCJ-DQ to disrupt the protein-DNA interaction (Fig. 7B).

**Mutation of the Invariant Glutamine in the Q Motif of FANCJ Adversely Affects Its DNA Binding**—The poor ability of the FANCJ-Q25A mutant protein to hydrolyze ATP in the presence of DNA and the failure to unwind DNA substrates might reflect an impairment of DNA binding activity. To address this issue, we performed gel mobility shift assays with FANCJ-Q25A and FANCJ-WT proteins using radiolabeled forked duplex DNA or single-stranded DNA. Qualitative and quantitative analyses of DNA binding to either the forked duplex (Fig. 8, A and B) or single-stranded 45-mer (Fig. 8, C and D) demon-
stratified that FANCJ-WT bound the DNA molecules in a protein concentration-dependent manner with apparent dissociation constant (Kd) values of 7.5 and 5.8 nM for the forked substrate and ssDNA, respectively; however, FANCJ-Q25A was compromised in its ability to bind the forked duplex or single-stranded DNA with apparent Kd values of 51 and 35 nM, respectively. The presence of ATP/H9253 (supplemental Fig. S4) did not enhance binding of either mutant protein to forked duplex DNA (A) or single-stranded DNA (B).

Effect of the Q25A Mutation on FANCJ Dimerization—Certain helicases may self-assemble to form dimers or higher order oligomers that can influence their catalytic or biological functions (1, 2, 6). To determine whether FANCJ can oligomerize and whether the Q motif mutation might affect oligomerization, the recombinant FANCJ proteins were analyzed by size exclusion chromatography (Fig. 9A) or glycerol gradient ultracentrifugation (Fig. 9B). By size exclusion chromatography, it was determined that catalytically active FANCJ-WT protein eluted from a Superdex200 HR column in two major peaks, fractions B9/10 and B6 (Fig. 9A). In contrast to FANCJ-WT, FANCJ-Q25A was found in fraction B6 (Fig. 9A). An additional peak of FANCJ-WT or FANCJ-Q25A was also detected in the void volume; however, the fraction lacked ATPase or helicase activity, suggesting an aggregated form of FANCJ. According to the molecular weight standards used to calibrate the size exclusion column, Stokes radius values were calculated for the FANCJ protein peaks. FANCJ-WT and FANCJ-Q25A from the B6 fractions were determined to have Stokes radius values of 4.56 ± 0.08 and 4.56 ± 0.15 nm, respectively. FANCJ-WT protein from the B9/10 fraction yielded a Stokes radius of 6.82 ± 0.09 nm. Similar results were obtained for multiple preparations of FANCJ-WT and FANCJ-Q25A.

The fractions obtained from size exclusion chromatography were further analyzed for helicase activity. Western blot analysis of the FANCJ-Q25A-B6, FANCJ-WT-B9/10, and FANCJ-WT-B6 fractions confirmed that equal amounts of protein, determined by Bradford analysis of the corresponding protein fractions, were used for biochemical assays (Fig. 9A). Protein titrations demonstrated that the FANCJ-WT B9/10 fraction unwound the 19-bp forked duplex DNA substrate much more efficiently than the FANCJ-WT B6 fraction. For example, there was a ~4-fold increase in DNA substrate unwound using 1.25 ng of the B9/10 fraction compared with the B6 fraction (Fig. 10, A and B). As expected, the FANCJ-Q25A B6 fraction lacked any detectable helicase activity (Fig. 10A).

FIGURE 7. ATP-dependent protein displacement activity of FANCJ wild-type and Q mutants. A, indicated concentrations of FANCJ protein were incubated with 2 mM ATP and DNA substrate (0.5 nM) with streptavidin bound to the covalently linked biotin moiety residing 52 nucleotides from the 5’ end of the radiolabeled oligonucleotide (supplemental Table S2). B, FANCJ proteins stripping assays using different nucleoside triphosphates. A phosphorimage of a typical gel for helicase assays with each substrate is shown. M, marker.
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FIGURE 8. DNA binding activity of FANCJ as detected by gel mobility shift assays. The indicated concentrations of FANCJ proteins were incubated with 0.5 nM forked duplex DNA substrate (A) or single-stranded DNA (C) at room temperature for 30 min under standard gel shift assay conditions as described under “Experimental Procedures.” The DNA-protein complexes were resolved on native 5% polyacrylamide gels. Quantitative analyses of gel mobility shift assays are shown in B and D for A and C, respectively. Data represent the mean of three independent experiments with S.D. indicated by error bars. Using a Student’s t test for analysis of the DNA binding data, the differences in p values between FANCJ-WT and FANCJ-Q25A for binding to the forked duplex or single-stranded 45-mer were all statistically significant (p < 0.05) with the exception of the 0.5 nM FANCJ concentration in B.

FANCJ-Q25A (B6) and FANCJ-WT (B6, B9/10) were examined for ATPase activity. In the presence of covalently closed M13 single strand DNA, the FANCJ-WT B9/10 fraction displayed a 7-fold greater kcat for ATP hydrolysis than the FANCJ-WT B6 fraction (Fig. 10C). The B6 fraction of FANCJ-Q25A displayed a significantly reduced kcat value (0.3 s−1) compared with FANCJ-WT B6 (2.2 s−1) or FANCJ-WT B9/10 (15 s−1) (Fig. 10C).

Analyses of FANCJ protein binding to forked duplex DNA (Fig. 10, D and E) demonstrated that the FANCJ-WT B9/10 fraction showed increased DNA binding compared with the FANCJ-WT B6 fraction throughout the protein titration. For example, FANCJ-WT B9/10 bound 65% of the DNA substrate compared with 30% DNA bound by FANCJ-WT B6 using the same amount of protein (10 ng). FANCJ-Q25A B6 poorly bound either forked duplex or single-stranded DNA molecules compared with FANCJ-WT B6. We also examined the ability of FANCJ-WT B6 and B9/10 fractions for binding to a shorter single-stranded 30-mer. FANCJ-WT B9/10 bound more efficiently than FANCJ-WT B6 to the 30-mer (supplemental Fig. S5A), a result that is consistent with the forked duplex substrate binding results. The FANCJ-Q25A B6 fraction bound the 30-mer very poorly (supplemental Fig. S5B).

To independently assess the effect of the Q motif mutation on the FANCJ assembly state, the purified recombinant FANCJ-WT and FANCJ-Q25A proteins were analyzed by glycerol sedimentation (Fig. 9B). From the glycerol gradient analysis, FANCJ-WT was found in two major peaks (fractions 25 and 33), whereas FANCJ-Q25A was found in a single peak (fraction 33) (Fig. 9B). According to the molecular weight standards used to calibrate the glycerol gradient sedimentation, the values of the sedimentation coefficients for FANCJ-WT and FANCJ-Q25A fraction 33 were 6.98 ± 0.64 and 6.93 ± 0.26 S, respectively. Combining the S value and Stokes radius n in the mass equation yielded a native monomer mass of 131 kDa for FANCJ-WT and 130 kDa for FANCJ-Q25A. The S value for FANCJ-WT (fraction 25) was 10.6 ± 0.86 S. Combining the S value and Stokes radius n in the mass equation, FANCJ-WT fraction 25 corresponded to a molecular mass of 298 kDa.

Results from helicase and ATPase assays, as well as gel-shift analyses for DNA binding demonstrated that the FANCJ-WT fraction 25 displayed greater activity than FANCJ-WT fraction 33 (Fig. 11, A–E). Moreover, FANCJ-Q25A fraction 33 was inactive as a helicase and hydrolyzed ATP or bound DNA very poorly (Fig. 11, A–E). These results confirm the findings from the size exclusion chromatography analysis, suggesting that FANCJ-WT exists in two populations distinguished by molecular weight and relative catalytic activity, whereas FANCJ-Q25A is found only in a lower molecular weight form that is devoid of helicase activity.

DISCUSSION

Previous work suggested that the Q motif in many RNA and DNA helicases plays an important role in nucleotide binding; however, our biochemical studies of the FANCJ-Q25A mutant protein indicate that deletion of the Q motif or replacement of the conserved glutamine with an alanine affected DNA binding in addition to ATP hydrolysis. Therefore, the importance of the FANCJ Q motif for binding nucleic acids may be shared by some other RNA and DNA helicases. Although the FANCJ-Q25A mutant is able to bind ATP as suggested by similar Km values compared with the wild-type protein, the mutant protein is clearly compromised in its ability to hydrolyze ATP. The reduced ATPase activity of FANCJ-Q25A may be partly attributed to its reduced ability to bind DNA (because it is a DNA-stimulated ATPase) and/or oligomerize, but it is likely to also reflect a perturbation of the nucleotide binding pocket/hydrolysis by the Q25A substitution. Although FANCJ-Q25A retains some ability to bind DNA and hydrolyze ATPase (albeit less efficiently compared with FANCJ-WT), it is completely inactive as a helicase on short duplex and G-quadruplex DNA sub-
strates suggesting that the Q motif plays an important role in DNA binding as well as its enzymatic functions.

Consistent with the notion that dimerization is important for the catalytic function of FANCJ, our biochemical data suggest that the FANCJ-WT dimer has a significantly higher specific activity for ATP hydrolysis or DNA unwinding compared with the FANCJ monomer. The increased catalytic activity of the FANCJ dimer is accompanied by a greater ability to bind ssDNA or partial duplex DNA molecules. The demonstration that the dimeric form of FANCJ displays greater catalytic activity and DNA binding is interesting in light of evidence that various DNA helicases have been reported to exist as monomers, dimers, or higher order oligomers (2, 44, 45). Although it is tempting to speculate that the effect of the Q25A substitution on FANCJ dimerization contributes to the negative effects of the mutation on its ATPase-driven helicase activity and protein displacement function, it should be kept in mind that the wild-type monomer is not nearly as impaired as the FANCJ-Q25A mutant protein, suggesting that the Q motif mutation affects multi-
ple aspects of the FANCJ protein, including its ability to bind DNA, efficiently hydrolyze ATP, and dimerize.

To our knowledge there have been no reports of higher order homo-oligomer formation for other members of the FANCJ family. However, interactions between FANCJ-like helicases and other proteins have been reported to exist. For example, the mammalian XPD helicase resides in a large TFIIH protein complex that also contains the XPB helicase (46). FANCJ has been reported to directly interact with BLM helicase both physically and functionally (47); however, the BLM interaction domain on FANCJ was mapped to a C-terminal portion of the protein beyond the conserved helicase core. The finding that FANCJ dimerization is important for optimal biochemical activity of the protein raises the question if related Fe-S domain helicases...

FIGURE 10. Biochemical analysis of selected FANCJ protein fractions from size exclusion chromatography. A, helicase reactions (20 μl) were performed by incubating the indicated FANCJ fractions with 0.5 mM DNA substrate at 30 °C for 15 min as described under “Experimental Procedures.” Triangle, heat-denatured DNA substrate control. B, quantitative analyses of helicase assays described in A. Using a Student’s t test for analysis of the helicase data, the differences in p values between WT-B6 and WT-B9/10 throughout the protein concentration range were statistically significant (p < 0.01). C, ATP hydrolysis (kcat) was determined for each fraction in the presence of covalently closed M13 single strand DNA (2.1 mM). Using a Student’s t test for analysis of the ATPase data, the difference in p value between WT-B6 and WT-B9/10 was statistically significant (p < 0.001). The difference in p value between WT-B6 and Q25A-B6 was 0.002, indicating that it was statistically significant (p < 0.01). D, indicated concentrations of FANCJ proteins were incubated with 0.5 mM forked duplex DNA substrate at 24 °C for 30 min under standard gel shift assay conditions as described under “Experimental Procedures.” DNA-protein complexes were resolved on native 5% polyacrylamide gels. E, quantitative analyses of gel mobility shift assays described in D. Using a Student’s t test for analysis of the DNA binding data, the difference in p values between WT-B6 and WT-B9/10 throughout the protein concentration range was statistically significant (p < 0.05).

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might also oligomerize. Because most if not all FANCJ-like helicases possess a Q motif, it will be of interest to determine whether mutations in the Q motif affect protein interactions in other helicases of this family and related SF2 families such as the RecQ helicases. The well characterized Rep helicase from *Escherichia coli* has been shown to dimerize much more effectively when bound to ssDNA (48), providing a precedent for this possibility.

Structural data of SF2 helicases suggest that the Q motif plays a role in nucleotide binding/hydrolysis. The N-terminal region of certain helicases may also be involved in oligomerization (see below); however, the details pertaining to the importance of the
Q motif in mediating protein-protein interaction between monomer subunits requires further study. The SF2 helicase SecA has been well characterized for its ability to homodimerize. Interestingly, a small deletion in the N-terminal domain prior to the conserved Q motif dramatically shifts the SecA dimer into a monomer, and such monomer-biased mutants are poorly functional biochemically (49, 50). The Vindigni laboratory reported that oligomerization of RECQ1 is mediated by both an N-terminal domain (residues 1–103) that bears the conserved Gln residue (amino acid 96) and a C-terminal Winged helix domain (51); however the structures of E. coli RecQ (16) and human RECQ1 (53) implicate the Q motif in nucleotide binding as well. The co-crystal structure of archaeal Hel308 with a partial duplex DNA molecule placed the Q motif on the edge of RecA domain 1 near the cleft that is formed with RecA domain 2 (54). The crystal structure of the SF2 UvrB helicase shows that the Gln residue is mainly interacting with the adenine base positioning it for catalysis. There are possible long range interactions between the carbonyl oxygen atoms from the P-loop via hydrogen bonding also suggesting a role in nucleotide binding and positioning (58). However, archaeal XPD does not form oligomers so the effect of the Q-motif cannot be rationalized utilizing the XPD structure.

Consistent with its effects on the biochemical functions of FANCJ, the FANCJ Q mutant alleles failed to rescue cisplatin or telomestatin sensitivity of an FA-J null cell line and exerted a dominant negative effect in a wild-type background cell line, as telomestatin sensitivity of an FA-J null cell line and exerted a FANCJ, the FANCJ Q mutant alleles failed to rescue cisplatin or nalidixic laboratory reported that oligomerization of RECQ1 is mediated by both an N-terminal domain (residues 1–103) that bears the conserved Gln residue (amino acid 96) and a C-terminal Winged helix domain (51); however the structures of E. coli RecQ (16) and human RECQ1 (53) implicate the Q motif in nucleotide binding as well. The co-crystal structure of archaeal Hel308 with a partial duplex DNA molecule placed the Q motif on the edge of RecA domain 1 near the cleft that is formed with RecA domain 2 (54). The crystal structure of the SF2 UvrB helicase shows that the Gln residue is mainly interacting with the adenine base positioning it for catalysis. There are possible long range interactions between the carbonyl oxygen atoms from the P-loop via hydrogen bonding also suggesting a role in nucleotide binding and positioning (58). However, archaeal XPD does not form oligomers so the effect of the Q-motif cannot be rationalized utilizing the XPD structure.

Consistent with its effects on the biochemical functions of FANCJ, the FANCJ Q mutant alleles failed to rescue cisplatin or telomestatin sensitivity of an FA-J null cell line and exerted a dominant negative effect in a wild-type background cell line, as detected by reduced cell survival or induction of γ-H2AX foci after exposure to cisplatin or TMS. The basis for the dominant negative phenotypes exerted by the FANCJ-Q25A mutant allele is of interest because it may lead to a better understanding of the role of FANCJ in DNA metabolism to preserve genomic stability and enable DNA repair or smooth replication progression to occur. Although it seems likely that FANCJ operates with other proteins of the Fanconi anemia pathway to repair DNA interstrand cross-links, emerging evidence suggests that the helicase performs some of its cellular functions (e.g. resolving G4 structures) outside the classic Fanconi anemia pathway (for review, see Refs. 25, 26). The dominant negative effect of FANCJ-Q25A on cisplatin or TMS resistance may be explained by the mutant protein interfering with the proper metabolism of DNA intermediates during replication or repair. Demonstration that FANCJ-Q25A was co-immunoprecipitated with its protein partners BRCA1 and TopBP1 suggests that protein hijacking may contribute to the dominant negative phenotypes. This and other mechanisms for the effects of helicase-inactivating mutations as a basis for dominant negative phenotypes is discussed in a recent review (39).

The BLM protein encoded by a Bloom syndrome patient-derived mutation Q672R (37) localized to the nucleus; however, there were a reduced number of foci after cellular exposure to mitomycin C or hydroxyurea. The BLM-Q672R mutation was previously shown to abolish the helicase activity and severely diminish the ATPase activity of the purified recombinant protein. However, the BLM-Q672R protein retained its normal DNA binding, leading the authors to propose that residue Gln-672 is involved in ATP binding (43). Although the Q25A mutation also impaired FANCJ catalytic function and DNA damage-induced localization, the effect of the mutation appears to be distinct from that of the BLM-Q672R mutation because BLM has been shown to form oligomeric ring-like structures in vitro (59), it would be of interest to examine the assembly state of the patient-derived BLM-Q672R mutant. Overexpression of BLM-Q672R in Bloom syndrome cells failed to correct the high sister chromatid exchanges in Bloom syndrome cells (52), demonstrating that the molecular defect of the mutant protein displayed phenotypic consequences.

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