FancJ Acetylation as a DNA Damage Response

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ABSTRACT

BRCA1 was linked to tumor suppression through its interactions with multiple proteins, which included FancJ. When phosphorylated at Ser990, FancJ was seen to repair DNA lesion via homologous recombination. Other than phosphorylation, acetylation at Lys1249 was observed after DNA interstrand crosslinks (ICLs) induction. The cell’s ability to survive ICL due to camptothecin or mitomycin C was not interfered by preventing FancJ acetylation; however, FancJ’s function in the G2/M checkpoint maintenance during cellular division was disrupted by both preventing and mimicking FancJ acetylation, allowing cells to undergo mitosis directly after serious DNA damage. Thus, FancJ acetylation is believed to play a critical role in the cell’s DNA damage response of halting cellular division when deemed insecure.
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BACKGROUND of DNA DAMAGE RESPONSE

Approximately 20,000 DNA lesions are generated spontaneously in a human cell per day (Vilenchik and Knudson, 2000). These lesions constantly challenge the genomic stability of the cell, interfering with its regular process of DNA replication and transcription. Thus, it is essential for the cell to both identify and process such lesions in order to maintain genomic stability. Mammalian cells have developed a complex network of DNA damage responses (DDR) for recognizing DNA damages, initiating checkpoints during the cell cycle, activating repair pathways, and/or triggering apoptosis. Unprocessed DNA damages, on the other hand, can evolve into chromosomal changes and gene mutations. Although only 2% of the estimated 3.2 billion base pair long human genome codes for proteins to carry out cellular activities, DNA mutations in such a small margin can produce drastic results from altering the protein’s original function to inactivating the protein completely (Bejerano et al, 2004). Nevertheless, mutations occurring at the non-protein coding region are not absolutely ineffective to the cell’s overall performance, especially if the change occurred on the transcriptional factors for protein regulation. Unsurprisingly, cancer susceptibility is strongly linked to mutations in the DDR genes that are either inherited or acquired during one’s lifetime. For the following project, acetylation of FancJ (also known as BACH1 and BRIP1) is proposed to be a critical modification of the DDR network.

The DDR network is activated upon the presence of DNA damage, which may be a result from both endogenous and exogenous sources. DNA damages from endogenous sources are often involved with the byproducts of cellular metabolic processes, including the deamination and hydrolysis of nucleotide bases and the oxidation and alkylation of DNA from reactive
radicals (Marnett, 2000). Physical and chemical mutagens are also omnipresent in the surrounding with nearly unavoidable ultraviolet radiation from the sun and ionizing radiation from both the commercial industries and other individuals. In addition, the genotoxic chemicals ingested from one’s diet and emitted in the air have been accumulating in every individual’s body, further increasing the rate of DNA damage (Hakem, 2008). One of the most lethal damage includes breaks in DNA, especially if the single break occurred on both strands of the double helix DNA structure. On a microscopic scale, such break on a gene can result in its loss of function; while on a macroscopic scale, the broken fragment of DNA may be translocated onto another chromosome or be deleted from the cell and its offspring (Negritto, 2010).

In response to such DNA damages, DDR proteins can arrest cells in the G1/S, intra-S, G2/M, intra-M, or G0 phase of the cell cycle, allowing the cell to undergo damage repair or apoptosis. The G1/S checkpoint, also called the restriction point, determines whether the cell should enter the synthesis phase of DNA replication after the initial enlargement of the cell during the G1 phase. If the cell decides to go into the S-phase, DNA undergoes replication and any detected errors can activate the intra-S checkpoint in order to stall the replication fork, preventing the replication fork from copying damaged DNA or from collapsing upon contact with DNA obstructs. When DNA replication is completed, the cell prepares to divide and runs the G2/M checkpoint for quality control of the replicated DNA and cell growth (Kaufmann and Paules, 1996).

**BRCA1 and FancJ in DDR and Repair**

Breast cancer susceptibility protein 1 (BRCA1) was the first gene identified to be associated with hereditary breast and ovarian cancers. Mutated BRCA1 protein is computed to
increase the risk of such cancer by 50-60% (Levy-Lahad and Friedman, 2007). This gene is located on the long arm of chromosome 17, in the interval of 17q12-21, and codes for 1863 amino acids (Grushko et al, 2002). It has a nuclear localization and export signal, a DNA binding site, and two BRCA1 C-Terminus (BRCT) domains. Each BRCT repeat is approximately 100 amino acids long and acts as a loading dock for phosphoproteins (Yu et al, 2003). By itself, BRCA1 mainly functions as a sensor to recognize DNA lesion and sends other repair factors a signal to either mend the lesion or kill the cell. Other than the mutations on BRCA1, mutations on the proteins that directly act along with it have contributed 40% of the heredity cases for cancer development. One particular partner protein, FancJ, have been found to contribute in an error-free DNA repair pathway upon BRCA1’s recognition of double-strand breaks (Walsh and King, 2007).

FancJ was first identified as a DEAH helicase used to unwind nucleic acids in the 5’ to 3’ direction (Cantor et al, 2001). This 1249 amino acids long protein binds directly to the BRCT domain of BRCA1 and is phosphorylated at serine 990 in order to promote homologous recombination on sites of DNA breaks during the late S/G2 phase of the cell cycle (Peng et al, 2006). Along with FancJ, there are at least 12 other Fanconi Anemia (FA) proteins (FancA/B/C/D1/D2/E/F/G/I/L/M/N) aiding the BRCA-FA pathway in DNA repair. Eight FA proteins (FancA/B/C/E/F/G/L/M), a FancM-interacting protein, and FAAP100 form a nuclear FA-core complex with E3 ligase activity. Subunit FancL directly interacts with the E2 ubiquitin conjugating enzyme UBE2T. Together with the Bloom protein (BLM), replication protein A (RPA), and topoisomerase III(alpha), the FA-core complex becomes a larger multisubunit complex called BRAFT. Double-strand DNA break, along with BRCA1, recruits BRAFT to the site of
damage, where it monoubiquitinates FancD2 at lysine 561 and Fancl. Due to the monoubiquitination of both FancD2 and Fancl, FancJ is drafted into the nucleus, where it forms a complex with BRCA1 at the lesion site. BRCA-FancJ unwinds the DNA while the MRN complex (MRE11, Rad50, and NBS1) containing 5’-3’ exonuclease activity chews the broken end of a DNA strand, creating a 3’ overhang on the opposite complementary strand. The flanking ends are stabilized by RPA and Rad52 while Rad51 invades a sister chromatin strand, making a DNA bubble. The BRCA-FancJ complex brings the 3’ overhangs into the bubble and starts synthesizing DNA by using the sister strand as a template. The loop will eventually crossover and the sister chromatin exchanges strands. The FA-induced HR pathway will be off upon FancD2 deubiquitination by ubiquitin specific peptidase 1 (USP1). (Litman et al, 2008; Kee and D’Andrea, 2010; please refer to Figure 1 in the Appendix for a general scheme DBS repairing with Homologous Recombination)

**Defects in BRCA-FA pathway and Alternative to Repairing Double-Strand Breaks**

Defects in both BRCA and FA proteins do not only jeopardize the early onset of breast and ovarian cancer but also increase the chances of developing Fanconi Anemia (FA), a recessive blood disorder. FA patients may not have enough blood cells to support the body while they continue to synthesize defective blood cells in the bone marrow. Children undergoing development may be prone to retardation and/or irregular body growth. Ultimately, faulty blood production may cause more serious blood disorder such as leukemia. (Kee and D’Andrea, 2010)

Since the homologous recombination pathway is not possible, the cell may utilize nonhomologous end joining when double-strand break occurs. Unlike homologous
recombination, no sister chromatins are used as template; rather, both the ku10/80 heterodimer and DNA-PK subunit bind to the free ends of the DNA break. Autophosphorylation of DNA-PK takes place in order to process the ends, which may modify or delete nucleotide bases to further make the ends compatible if non-complementary overhangs are present before ligation. This mechanism of break repair is inherently mutagenic, relying on chance pairing while presenting a chance of having overhangs modified for joining. (Helleday et al, 2007)

**Double-Strand Break as an Intermediate to BRCA-FancJ Homologous Recombination**

Other than creating DNA breaks, carcinogenic sources may also cause nucleotides to covalently bond with each other on the same strand (intrastrand crosslink) or with a displaced nucleotide on the opposite strand (interstrand crosslink), inducing cellular arrest by preventing the DNA template from being read and/or the unwinding of the double-stranded DNA during replication. For such damage, human cells will usually utilize the nuclear excision repair pathway (NER), which can later be partnered up with the BRCA-FancJ repair machinery.

For intrastrand crosslinks, FancJ can directly unravel the unconventional covalent bonds like the G-quadruplex among the nucleotides of the same strand (Wu et al, 2008); however, interstrand crosslinks require a little more work. Normally, the Xeroderma Pigmentosum Complementation Group-C (XPC) and hHR23B complex detect damaged DNA in the genome during replication (Boer and Hoeijmakers, 2000); during transcription, however, the ERCC8/ERCC6 complex stalls RNA polymerase II around the site of damage in the place of the XPC complex to activate the NER pathway (Kwei et al, 2004). The replication fork is stalled before it reaches the lesion and the leading strand is cleaved off. The multisubunit TFIIH
complex containing DNA helicases XPD and XPB unwinds the DNA around the lesion, creating a repair bubble with XPA and RPA stabilizing the temporal single-strands of DNA. Subsequently, the initiating complex XPC/hH23B or ERCC8/ERCC6 leaves the lesion. A DNA strand within the repair bubble is later incised by the newly recruited endonuclease XPF/ERCC1, which cleaves DNA at the 5’. As a result, an oligonucleotide of 30 to 50 bases, containing the lesion, is flanked off with all its nucleotides – except for that of the crosslink – unbounded to its complementary strand. Using the uncut DNA strand – which still contains the crosslink – as a template, new nucleotides are synthesized to fill in the gap along with the previously replicated lagging strands. Exonucleases XPF/ERCC1 and XPG are recruited again to make a final cut on the complementary strand containing the crosslink and new nucleotides are synthesized once again to replace it. Although the interstrand crosslink is gone, a break is made previously with the leading strand during replication and this break can then undergo homologous recombination as previously mentioned with the BRCA-FancJ complex and the crosslink-free sister chromatin. (Al-Minawi et al, 2009; Handa et al, 2006; Niedernhofer et al, 2004; please refer to Figure 2 in the Appendix for a general scheme of NER and HR partnership by inducing DSB as an intermediate)

**FancJ Acetylation**

Jenny Xie, who had previously worked in the Cantor lab, discovered a recent modification on FancJ after inducing DNA damage. Aside from being phosphorylated at serine 990 by BRCA1, FancJ was also found to be acetylated using mass spectrometry. By Immunoblot analysis using the pan-acetyl antibody, they found that acetylation of FancJ required amino acids 1239 to 1249, which contained 3 lysine residues at the 1240, 1442, and 1249 position. Through transductions of FancJ mutants, converting lysine into arginine for each of the lysine
candidates, lysine 1249 was determined to be the dominant site for FancJ acetylation. They further discovered that this acetylation was due to CREB-binding protein (CBP) and that histone deacetylase (HDAC) class I, II, and III were responsible for reverting the modification. Inhibition of HDAC class I and II by trichostatin-A (TSA) and class III by nicotinamide (NAM) retained the acetylation of FancJ (Figure 3). However, studies on what possible functions FancJ acetylation uphold were lacking. As a follow up, this acetylation of FancJ was hypothesized to be a modification done as a DNA damage response and a necessity for FancJ to be activated during DNA repair via homologous recombination. Through immunofluorescence analysis and Western blotting of both wild type and mutant FancJ, the acetylation on FancJ had demonstrated to play a role in the G2/M checkpoint.
MATERIALS AND METHODS

All experiments after cell line preparation/maintenance and plasmid construction were performed for at least three times to eliminate the probability of an event due to random chances.

Cell line preparation and maintenance

Hela and FA-J (EUFA30-F) cells were incubated in a 37°C chamber with 5% carbon dioxide and were grown in DMEM media supplemented with 10% and 15% fetal bovine serum, respectively, and 100U/mL penicillin/streptomycin. To insert FancJ and its mutants into the FA-J cells, 10ug of pOZ vectors containing nothing, FancJ-WT, FancJ-K1249Q, or FancJ-K1249R, along with 5ug of both gag pol and env, were transfected into 293TD cells. The growth medium containing the newly synthesized viruses was collected on the third day. The virus supernatant was applied on the FA-J cells with 5ug/mL polybrene. After a day, stable FA-J pOZ cell lines were generated by sorting pOZ-infected cells with anti-IL-2 magnetic beads (Dyna Beads), selecting for IL-2 positive cells to expand. A Western blot was carried to observe quality of transduction.

Plasmid Construction

The K1249Q and K1249R pOZ vector were generated with the QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) by using the myc-FancJ-WT-pOZ as template and the following primers:

(K1249R-pOZ-forward) 5’GGCATGTTTTCTGGTTTTAGGGCGGCCGCTGGAGGAGCA3’
(K1249R-pOZ-reverse) 5’GTCTCCTCCAGCGCCGCTGGAGGAGGAGCA3’
(K1249Q-pOZ-forward) 5’GGCATGTTTTCTGGTTTTAGGGCGGCCGCTGGAGGAGCA3’
(K1249Q-pOZ-reverse) 5’GTCTCCTCCAGCGCCGCTGGAGGAGGAGCA3’
**Immunoprecipitation and Western Blot Assays**

Cells were harvested, lysed, and processed for Western blot analysis using a 500mM NETN lysis buffer (20mM Tris, 150mM NaCl, 1mM EDTA, and 0.5% NP-40) containing 10mM NaF and 1mM NaVO3; before running on a 4% Bis-Tris gel (MOPS SDS running buffer at 200V, both from NuPage), the lysate was diluted to 150mM NETN with 25mM NETN lysis buffer. For acetylation detection, the NETN buffer was supplemented with 10uM TSA and 5mM NAM. Transfer from the gel onto the nitrocellulose paper was done via electric current at 35V for 90 minutes. The membrane was then washed with 5% nonfat milk before applying antibodies. Antibodies used for myc-FancJ immunoprecipitation (incubated with 50uL of protein A beads, along with primary, for 4 hours at 4C) and Western blot assays included FancJ polyclonal Abs E67, N-myc-FancJ polyclonal H-50 (Santa Cruz; used 2uL for IP), BRCA1 monoclonal MS110, gamma-H2AX monoclonal S139 (Millipore), polyclonal pan-acetylated lysine (Cell signaling), and beta-actin monoclonal A2228 (Sigma). These primaries were diluted 1:1000 with TBS; for secondary antibodies, 1:5000 dilution was made with TBS for either horseradish peroxidase-conjugated anti-rabbit (polyclonal) or anti-mouse (monoclonal). Primaries and secondaries were incubated separately with the paper membrane for 1 hour before being washed with TBS 3 times. Expression of protein was detected by chemiluminescence (Amersham).

**Drug Treatment**

Cells were left untreated or treated with either 250nM mitomycin C (MMC) or 1uM camptothecin (CPT) for an hour. During specific hours after post-treatment, cells were collected and were lysed for detection of FancJ, BRCA1, gamma-H2AX, or acetylation on a Western blot as previously described.
Immunofluorescence Microscopy

Stable FA-J cell lines or Hela cells were seeded onto 6-well plates with a glass cover slip in each well and were incubated overnight before being treated. During specific hours post-treatment, TBS washed cells were fixed with either 3% paraformaldehyde/2% sucrose (for gamma-H2AX) or 95% ethanol/5% acetic acid (for phosphorylated H3) for 10 minutes at room temperature. Permeabilization was done with 0.5% (for gamma-H2AX) or 0.1% (for phosphorylated H3) Triton X in 20nM HEPES for 5 minutes. After 3 TBS washes, primaries for FancJ (myc-tagged or wt), gamma-H2AX, or anti-phospho-Histone H3 Ser10 monoclonal 3H10 (Millipore) was applied on the glass slip as recommended by their company provided product information sheet. Three washes of 5 minutes were done with TBS and recommended dilution of secondary fluorescent antibodies (red for mouse and green for rabbit) were applied for 30 minutes in the dark. Similar washes were made afterward; the glass slips were mounted onto glass slides with Dapi and were sealed with clear nail polish. The glass sides were examined under a fluorescent microscope for species of interest.

Mitotic Index

Seeded FA-J cells on cover slips were either untreated or treated with MMC. The mitotic index was calculated by dividing the number of cells with phosphorylated H3 foci by the total number of cells. Cells were incubated with nocodazole to a final concentration of 100ng/mL before performing immunofluorescence as previously described at 24 hours post-treatment.
RESULTS

FancJ acetylation is enhanced by DNA damage

Given that certain protein modifications were probably induced by the network of DNA damage response, FancJ was monitored for signs of acetylation in both Hela and FA-J cell lines before and after DNA damaging treatments. Mitomycin C and camptothecin were used on Hela cells to induce either interstrand crosslinks or DNA breaks. Subsequently, FancJ was immunoprecipitated from untreated, MMC treated, and CPT treated Hela cells, all of which showed about equal expression of endogenous FancJ with relatively similar intensities from the Western blot of Figure 4A. More importantly, acetylation of the immunoprecipitated FancJ was seen faintly on the untreated Hela cells and that this state of acetylation was magnified slightly after 12 hours post-treatment with either MMC or CPT on the Western blot of Figure 4A, hinting a consequence of experiencing DNA damages. Yet, neither the DNA damage nor the difference in FancJ acetylation was seen to affect FancJ’s ability to bind to BRCA1 – which was co-immunoprecipitated along with FancJ – since matching intensity was detected throughout the Hela cells from Figure 4A. To better understand FancJ’s progression of acetylation after DNA damage, Hela cells were once again treated with MMC and FancJ was immunoprecipitated from the cell for traces of acetylation at specific hours post-treatment. From untreated to all the way towards 24 hours post-treatment, the intensity indicating acetylation of FancJ was observed to increase over time since treatment in Figure 4B, further backing up the possibility of a DNA damage response modification.

Switching over from endogenous expression of FancJ in Hela cells to exogenous expression of FancJ in FA-J cells, whether the phenomenon of FancJ acetylation was cell line
specific or endogenous restricted, happening on only innate FancJ, could be answered. Likewise, FA-J cells treated with MMC were immunoprecipitated over time for detection of FancJ acetylation. While the same progression of FancJ acetylation in Hela cells was seen in both FA-J WT and K1249Q cells as indicated by the increasing intensity of acetylation detection over time under equal amount of precipitated FancJ (Figure 4C), that from FA-J K1249R had demonstrated otherwise. From untreated to 16 hours post-treatment, the FA-J cells carrying the K1249R mutant – which prevented acetylation – showed little to no detections of acetylation on the precipitated FancJ in Figure 4C. Nevertheless, acetylation could be done on FancJ regardless of being innately endogenous in Hela cells or genetically exogenous in FA-J cells as long as the 1249 residue permits acetylation.

**FancJ acetylation mutants are functional**

Knowing that FancJ acetylation could be done in both Hela cells and FA-J cells, the hypothesis on whether this DNA damage induced modification facilitates the function of FancJ in DNA repair was taken to test. Addressing this potential, FA-J cells that originally lacked FancJ were complemented with nothing, pOZ-vector only, myc-tagged FancJ wild type (WT), myc-tagged FancJ lysine to glutamine (K1249Q) mutant, or myc-tagged FancJ lysine to arginine (K1249R) mutants. Upon the expansion of stabilized FA-J cell lines, FancJ was blotted for in the newly inserted FA-J cells and Hela cells, most of which expressed comparable level of FancJ when the intensity of the beta-actin loading control were alike. However, since the FA-J untreated and pOZ was not given the construct for FancJ, no expression was seen on their respective lanes in Figure 5A. From the same figure, no gamma-H2AX was detected, indicating no DNA break damages were done to the cell during the transduction of the vector, FancJ-WT,
or FancJ mutants (K1249Q and K1249R). When both Hela cells and FA-J cell lines were treated with MMC and harvested directly after treatment, the previously nonexistent gamma-H2AX was clearly detected in all the cell lysate as seen in Figure 5B. The strength of these gamma-H2AX bands could alternatively be used to quantify the amount of DNA breaks within the cell and from the figure, all the cells shared roughly the same amount of gamma-H2AX in relevant to their loading control, beta-actin. For instance, both FA-J pOZ and Hela in figure 5B displayed a fainter signal for gamma-H2AX but their concentration of beta-actin was also lower; vice versa for that seen in FA-J Q1249R, where greater loading control detection led to a stronger signal in gamma-H2AX.

With DNA damage being induced and gamma-H2AX acting as an indicator for double-strand DNA breaks, the kinetics of damage was monitored by immunofluorescence staining for gamma-H2AX in cells over time. In Hela cells, gamma-H2AX foci were localized within the nucleus of over 90% of the cells during the first hour post-treatment. At the same time, the co-stained FancJ was recruited into the nuclei as well, appearing in approximately 30% of the treated Hela cells. As FancJ continued to be recruited until the 6th hour post-treatment, where an average of 73% of the cells displayed it, gamma-H2AX foci in the nucleus was seen to drop continuously since its spike during the early hour. By the 18th hour post-treatment, the percentage of cells with gamma-H2AX was close to zero. With gamma-H2AX gone and DNA breaks repaired, the concentration of FancJ foci was also seen to decrease after the 6th hour post-treatment, slowly returning to normal levels by the 24th hour. The flow of FancJ and gamma-H2AX after MMC treatment was graphed in Figure 5C.
To determine whether DNA break repair was functional in FA-J cells, a similar immunofluorescence staining for gamma-H2AX and FancJ was conducted. Treated cells during the early hour and late hours were examined. The nucleus of the cell was stained with blue Dapi in Figure 5D to ensure that these observations were made in the presence of a cell rather than randomly stained background. Gamma-H2AX was stained with a red fluorescent dye while FancJ was stained with a green fluorescent dye. At 4 hours post-treatment, the FA-J cells were brought up to the microscope and co-localization of both red gamma-H2Ax and green FancJ were observed in all the FA-J cell lines, except for that of the FA-J pOZ cells due to the absence of FancJ. Thus, FA-J pOZ cells was only seen with red gamma-H2AX stains. At a later time point of 24 hours post-treatment, the FA-J cells were observed again; however, both red gamma-H2AX and green FancJ were not seen in the nucleus of the cells. The FA-J pOZ cells was the only exception with traces of red gamma-H2AX left over, meaning the damage was not repaired for lacking the FancJ protein. Yet, DNA damage from both FA-J cells with the wild type and mutant were eliminated over time.

**FancJ acetylation plays a role in checkpoint maintenance**

Since FA-J cells with FancJ mutants did not affect their ability to remove double-strand breaks, FancJ’s function in the G2/M checkpoint maintenance was putted on the line for investigation. Defects in checkpoint maintenance were evaluated by comparing the mitotic index (24 hours post-treatment) of both untreated and MMC treated FA-J cells with nocodazole stalling the cell cycle in the M-phase. No significant difference was seen in the mitotic index of most untreated FA-J cells, which had a mitotic index around 8%. The mitotic index of the untreated FA-J pOZ cell, on the other hand, was counted to be almost twice of that compared
to the other FA-J cells. Again, no great differences were seen when the FA-J cells were treated with MMC; the average mitotic index of FA-J pOZ and K1249R mutant experienced a 3% and ~1% drop, respectively, while the FA-J K1249Q mutant had a raise of 2% in its mitotic index. Surprisingly, the mitotic index of the WT-FancJ FA-J cell had experienced a 7% drop, a decrease by a factor of more than 8 compared to the untreated FA-J WT cells (Figure 6). In other words, the FancJ-WT FA-J cells were not entering mitosis after the repair of induced DNA damages. Unlike the FA-J WT, the mimic FancJ acetylation mutant (K1249Q) failed to maintain the checkpoint, showing H3 phosphorylation by 24 hours after treatment.
DISCUSSION

Although the true extent of FancJ’s acetylation at lysine 1249 remained unclear, this modification was shown to be a critical regulator for FancJ’s function during the cell cycle, especially after serious DNA damage. Expression of two FancJ mutants that mimicked either the constitutive deacetylated FancJ K1249R or the acetylated FancJ K1249Q protein isoforms were analyzed throughout the experiments. While the mutants functioned similar to that of endogenous WT-FancJ in being acetylated (K1240Q only) and removing gamma-H2AX recruited by double-strand DNA breaks after MMC treatment (both K1249Q and K1249R), the mutants were found to be distinct from the wild type in maintaining the G2/M checkpoint. Notably, a lower percentage of cells undergoing mitosis was seen in FA-J cells expressing the WT-FancJ after serious DNA damage while those with the mutant proceeded almost immediately into the M-phase of the cell cycle after damage. Since the FancJ K1240Q mutant failed to maintain the checkpoint, despite having the same initial DNA damage response of being acetylated as in the wild type, there must have been some other aspects of checkpoint signaling perturbing in the FA-J cells that express the acetylation mimic. Perhaps this mutant failed to mediate a protein interaction or act upon a DNA substrate of importance for checkpoint maintenance. Instead, FancJ acetylation could serve as a switch, in which acetylation and deacetylation is essential to maintain such checkpoint.

In summary, experimental findings indicate that both endogenous and exogenous FancJ has the ability to potentiate homologous recombination but conserved lysine 1249 is essential for proper checkpoint maintenance. It is not surprising that regulators of FancJ acetylation state, HDACIII, SIRT1, and CBP have roles in DNA repair and genomic stability; it remains to be
determined, however, whether associated repair defects are related to failures to regulate FancJ acetylation. Complicating this analysis, HDACIII, SIRT1, and CBP have many other histone and non-histone protein substrates that also have roles in DNA repair. Whether HDAC or CBP associated defects derive from the failure to regulate FancJ acetylation will be an important question for future studies.
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APPENDIX

Figure 1: General Scheme of Double-Strand Break Repair via Homologous Recombination
Figure 2: General Scheme NER and HR Partnership by Inducing Double-Strand Break as an Intermediate
Figure 3: Enhancement of FancJ acetylation with TSA and NAM inhibiting Histone Deacetylase when cells are treated with 1μg of CBP
Figure 4: (A) Hela cells were left untreated or treated with MMC (250nM, 1 hour) or CPT (1μM, 1 hour) before being harvested at 12 hours post-treatment while (B) showed FancJ acetylation over time after MMC treatment in Hela cells. (C) FA-J cells (myc-WT and myc-K1249R) were immunoprecipitated for expression and/or acetylation of myc-FancJ over time after MMC treatment, with the latter greatly preventing such modification.
Figure 5: (A) Expression of FancJ in Hela cells and FA-J cells, complemented with either nothing, pOZ vector, or myc-tagged FancJ (WT, K1239Q, K1249R), were blotted along with gamma-H2AX. (B) Gamma-H2AX, used to stabilize broken ends, was observed after MMC treatment (250nM, 1 hour) on cells from part A. (C) Kinetic graph of both FancJ and gamma-H2AX foci localization over time in Hela cells after MMC treatment (250nM, 1 hour). (D) FA-J cells were seeded onto 6 well plates and were incubated overnight before MMC treatment (250nM, 1 hour). Immunofluorescence was performed hours after post-treatment (blue-Dapi, red-gamma-H2AX, green myc-FancJ).
Figure 6: FA-J cells were seeded onto 6-well plates and were incubated for 48 hours before MMC treatment (250nM for 1 hour). The cells were then incubated with nocodazole to a final concentration of 100ng/mL for 24 hours before performing immunofluorescence, staining for phosphorylated-Serine 10 on Histone H3 during mitotic activities. The mitotic index (cells with phosphorylated-Ser10 H3 over total number of cells) was then computed on both MMC treated and untreated FA-J cells to determine the percentage of cells undergoing mitosis. Unlike the other mutated FancJ, the WT-FancJ greatly hindered the cell from entering mitosis after DNA damage from MMC.